Automated Dielectrophoresis With Microwell Chips

by

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Dielectrophoresis (DEP) is a physical effect that generates a force on a polarisable particle when it experiences a non-homogeneous electric field. It has been shown in earlier research that its measure allows finding the electrical properties of that particle, including the electric permittivity and conductivity. Prior approaches have applied DEP-based techniques to measure the electric properties of one or several cells at a time. However, the protocols they usually use suffer from several problems, among which are the poor statistical significance due to the small number of cells monitored, the relatively high level to noise ratio, the duration of the measurement or the loss of information during the data processing.

This thesis presents the work I have achieved in an effort to solve part of these problems. The approach was two-sided: part of the work was dedicated to the development of a new automate that measures the DEP force on a large population of cells with a low level of noise, and another part was dedicated to the development of new theoretical approaches in order to obtain lower bias and higher signal to noise levels from the data processing. For these purposes, the protocol and material used previously in the measurements of DEP force were studied, with particular attention to the microwell-based technique used at the University of Surrey from which an automatic procedure was developed in a series of consecutive improvements. The automate and software obtained at the end of this work were able to quickly measure the DEP spectrum and study it in a way that provided novel or more accurate information about the cells electrical properties.
Declaration of Originality:

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.
Acknowledgements

A Thesis is a long, and sometimes painstaking experience (sometimes only?). But it is also a privileged moment for a researcher, not only because it is the beginning of a carrier, but also because it leaves several years to focus on a chosen project without many disturbances. It is an opportunity to discover and extend the knowledge of the PhD student in whatever he or his supervisor finds helpful, or simply interesting, for the research. But doing a PhD in a foreign country is an even more challenging and rewarding experience, for it leaves so many occasions to discover new cultures and new people. I would like to thank Dr Mike Hughes for giving me the opportunity to live such a life-changing experience when he accepted me back in 2003 in the Biomedical Engineering group, and for all he did to help me since then, not only for my research but also for the many other things that makes him more than just a supervisor. I will never forget the welcoming to Guildford with the last-minute phone call to announce my arrival. Thanks a lot, Mike!

I am also grateful to everyone in the Biomedical Engineering department of the University of Surrey for the many good moments we shared during these three years in Guildford, with special thoughts to Karla (her diets made me put on weight. Strange...), Henry (shall I thank him for the hangovers?), Sonia (fantastic food, fantastic music... what more do we need?), Miguel (unbeatable when it comes to electronics and tennis, but in both case you need good legs to follow him) and Fatima (my PhD really started when I worked with you!). I also have a special thought for Montse and Himat for their patience during so many years sharing the house (I know, I am suuuuch a nice flatmate!), and of course for my dear Morgiane (I’m sure she will have a conference on the moon one of these days).

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Enfin, merci à mes parents pour m’avoir toujours encouragé à aller plus loin. A présent je suis au Royaume-Uni, ça a plutôt bien marché !

Elvis has left the building.

iii
# Contents

Abstract

i

Acknowledgements

iii

List of Figures

x

List of Tables

xv

Abbreviations

xvi

Physical Constants

xvii

Symbols

xviii

1 Introduction to Dielectrophoresis: research and problems

1.1 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 1
1.2 Discovery and developments of dielectrophoresis . . . . . . . . . . . . . . 2
1.3 Outline of dielectrophoresis theoretical developments . . . . . . . . . . . . 4
1.4 Previous methodologies . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8
  1.4.1 Presentation of several experimental designs . . . . . . . . . . . . . . 8
  1.4.2 Protocols for DEP force measurement . . . . . . . . . . . . . . . . . . 13
  1.4.3 Electrorotation . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 15
1.5 Aims and structure of this thesis . . . . . . . . . . . . . . . . . . . . . . . 16

2 Analysis of the pre-existing protocol for DEP measures with the microwell electrode

2.1 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 18
2.2 Light absorption measure: initial method and instruments . . . . . . . 19
  2.2.1 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19
  2.2.2 The microwell electrode and the manual procedure . . . . . . . . . . 19
  2.2.3 Experimental methods . . . . . . . . . . . . . . . . . . . . . . . . . . 21
2.3 Identification and characterisation of the sources of noise . . . . . . . . 22
  2.3.1 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 22
  2.3.2 Microflows and AC-electro hydrodynamics . . . . . . . . . . . . . . 23
  2.3.3 Conductivity of the suspending medium . . . . . . . . . . . . . . . . 24
  2.3.4 Filtering effects . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 25
## Contents

2.3.5 Quality of the light beam .................................................. 25  
2.3.6 Operator variability ......................................................... 26  
2.4 Data processing and introduction of bias ................................. 27  
2.5 Performances of the manual protocol for DEP measure ................. 29  
2.6 Conclusion ............................................................................ 34  

3 Developments in theoretical fields ............................................. 36  
3.1 Introduction ........................................................................... 36  
3.2 Microwell optimisation ........................................................... 37  
3.2.1 Study of the initial problem ............................................... 37  
3.2.2 The FEM model of the microwell ....................................... 38  
3.2.3 Results and theoretical optimisation .................................... 41  
3.3 Principle of the measure of the DEP force from a series of images of the microwell ......................................................... 43  
3.3.1 Introduction ...................................................................... 43  
3.3.2 Analysis of the cell dispersion in the microwell ..................... 44  
3.3.3 Model-based analysis of the image ...................................... 48  
3.4 Theoretical validation of the data processing algorithm ................ 53  
3.4.1 Methods .......................................................................... 53  
3.4.2 Analytical expression of the electric field ............................. 54  
3.4.3 Creation of simulated data .................................................. 60  
3.4.4 Results and limitations ...................................................... 66  
3.5 Analysis of the multishell model and thin-membrane approximation .. 70  
3.5.1 Introduction ...................................................................... 70  
3.5.2 Thin membrane approximation .......................................... 71  
3.5.2.1 Principle ...................................................................... 71  
3.5.2.2 Validation and limitations of the thin-membrane approx- imation ................................................................. 73  
3.5.3 Segmentation of the spectrum: analytical study of the single- shelled model ................................................................. 76  
3.5.4 Curve fitting and initial guess .............................................. 85  
3.5.5 Conclusion ........................................................................ 88  
3.6 Multiple populations analysis ............................................... 89  
3.7 Conclusion ............................................................................ 91  

4 Description of the apparatus and data processing ....................... 92  
4.1 Introduction ........................................................................... 92  
4.2 Structure of the automate ....................................................... 93  
4.2.1 Introduction ...................................................................... 93  
4.2.2 General presentation of the automate ................................. 93  
4.2.3 Preparation of an automated experiment ............................ 96  
4.2.3.1 Preparation to the measurement .................................. 96  
4.2.3.2 Performing the measurement ....................................... 98  
4.2.3.3 After the measurement ................................................ 106  
4.2.4 Liquid handling ................................................................. 106  
4.2.4.1 Principle ...................................................................... 106
## Contents

4.2.4.2 Fluid transmission .................................................. 108  
4.2.4.3 Information transmission .......................................... 111  
4.2.5 The measuring system ................................................ 115  
4.2.6 Generation of DEP effects ........................................... 118  
4.3 Structure of the program ................................................ 122  
4.3.1 Introduction .......................................................... 122  
4.3.2 Synchronisation of the device during the data acquisition .......... 124  
4.3.2.1 Synchronisation of the transmission between the computer and the devices .................................................. 124  
4.3.2.2 Synchronisation of data acquisition ................................ 125  
4.3.3 Acquisition and transfer of the data .................................. 129  
4.3.4 Data processing algorithms ......................................... 130  
4.3.4.1 Principle .......................................................... 130  
4.3.4.2 Application of the circular averaging ............................ 130  
4.3.4.3 Application of the curve fitting .................................. 133  
4.4 Conclusion .............................................................. 135  
5 Results .............................................................................. 137  
5.1 Introduction ............................................................... 137  
5.2 Results from the optimisation of the prototype ......................... 138  
5.2.1 Introduction .......................................................... 138  
5.2.2 Acquisition time ....................................................... 138  
5.2.3 Amplitude of the electric field ...................................... 140  
5.2.4 Experimental time ..................................................... 142  
5.2.5 Light path and focusing .............................................. 143  
5.2.6 Bubbles and tubing length .......................................... 144  
5.2.6.1 Effect of bubbles .................................................. 144  
5.2.6.2 Solutions found .................................................... 145  
5.2.7 Other improvements ................................................... 147  
5.2.8 Other attempts ......................................................... 148  
5.3 Performance of the final prototype ...................................... 150  
5.3.1 Introduction .......................................................... 150  
5.3.2 Noise study ........................................................... 151  
5.3.3 Bias study ............................................................. 154  
5.3.4 Repeatability study .................................................... 155  
5.3.5 Validation by comparison with the literature ...................... 157  
5.4 Effects observed and additional experiments .......................... 159  
5.4.1 Introduction .......................................................... 159  
5.4.2 AC electrohydrodynamic flows and heating ...................... 159  
5.4.3 Alignment of the particles .......................................... 161  
5.4.4 High-concentration effects ......................................... 163  
5.4.4.1 Introduction ....................................................... 163  
5.4.4.2 Methods .......................................................... 163  
5.4.4.3 Results ........................................................... 164  
5.4.4.4 Interpretation ..................................................... 166  
5.4.4.5 Conclusion ......................................................... 167  
5.5 Conclusion ............................................................... 167
6 Evaluation of Machine Performance 168
  6.1 Introduction ........................................... 168
  6.2 Analysis of the results obtained .......................... 168
  6.3 Comparison with manual experiments ......................... 170
    6.3.1 Introduction ........................................ 170
    6.3.2 Level of noise ....................................... 171
    6.3.3 Experimental duration ............................... 173
    6.3.4 Complexity of the manipulations ...................... 176
    6.3.5 Quantity of information available from the measurement 177
  6.4 Limitations ............................................. 177
    6.4.1 Introduction ........................................ 177
    6.4.2 Detection limits ..................................... 177
    6.4.3 Frequency band ...................................... 179
    6.4.4 Diffraction by the aperture of the microwell .......... 180
    6.4.5 Bubbles ............................................. 180
    6.4.6 Bias ................................................ 181
  6.5 Conclusion ............................................. 181

7 Conclusions and further work 182
  7.1 Introduction ............................................. 182
  7.2 Review of the objectives ................................ 182
  7.3 Improvements of the hardware ............................ 184
    7.3.1 Microcontroller ...................................... 184
    7.3.2 Signal generator .................................... 185
    7.3.3 Oscilloscope ........................................ 185
    7.3.4 Microscope ........................................... 186
    7.3.5 Power supply ........................................ 187
    7.3.6 Light source .......................................... 188
    7.3.7 Camera ................................................ 188
    7.3.8 Syringe pump ......................................... 188
    7.3.9 Valve ................................................ 189
    7.3.10 Tubing ............................................... 189
    7.3.11 Microwell chip ....................................... 190
  7.4 Improvements of the software ............................ 191
    7.4.1 Automated guesses ................................... 191
    7.4.2 Automated multiple populations calculation ........... 191
    7.4.3 microcontroller-based data processing ................ 192
    7.4.4 Inclusion of additional models ....................... 192
    7.4.5 Extension of the model of concentration evolution to the non-linear zones ............................................. 193
    7.4.6 Diminution of the number of curve fitting parameters 193
  7.5 Microflow reduction .................................... 193
    7.5.1 FEM model ............................................ 194
    7.5.2 Experimental data ..................................... 200
    7.5.3 Conclusion for the microflow reduction ................ 202
  7.6 Conclusion ............................................. 202
Contents

Bibliography 204

A Elements of cell physiology 1

B Ill-conditioned problems 5

C User’s manual 7
  C.1 Devices ........................................ 7
    C.1.1 The microscope ................................ 8
    C.1.2 The syringe pump .............................. 8
    C.1.3 The signal generator .......................... 9
    C.1.4 The oscilloscope .............................. 10
    C.1.5 The microchip ................................ 10
  C.2 Data Acquisition ................................ 10
  C.3 Data processing .................................. 16
  C.4 Device control ................................... 19
    C.4.1 Signal generator panel ......................... 19
    C.4.2 Pump panel .................................... 19
    C.4.3 Camera panel .................................. 20
  C.5 Curve fitting .................................... 20

D Electronic data 21
List of Figures

1.1 Compendex and Inspec indicative of DEP-related publications since 1969. 3
1.2 Charge distribution for different configurations of permittivity. 5
1.3 Left: scheme of the modelled shelled sphere. Right: equivalent sphere. 7
1.4 Castellated electrodes with yeast cells experiencing positive DEP (taken at the University of Surrey). 9
1.5 Example of hyperbolic electrode profile (taken at the University of Surrey). 10
1.6 a) zipper electrode profile, (b) same electrode during a DEP experiment (from the University of Surrey). 11
1.7 Principle of dot measurement. The DEP force pushes the particles inward or outward according to the situation. With time, the system reaches a homogeneous concentration and can be re-used. From (Fatoyinbo et al., 2008), with the kind permission of the authors. 12
1.8 Result of a simulation of planar a electrode with normalised dimensions, using a difference of potential of 2 V. 13
2.1 Scheme of the microwell structure. 20
2.2 Side-view scheme of a microwell used in the manual procedure. 21
2.3 Typical map of ACEO flows around surface electrode (from simulations, see Chapter 7 for details). 23
2.4 Bode diagram of the microship used in manual experiments in 2005 measured within the automate developed in this Thesis. The dashed lines measure the -3dB cut-off frequency and give a value close to 4 MHz. 25
2.5 Results provided from a DEP experiment with irregular illumination after data processing. The changes in light intensity are interpreted by the software as variations of the DEP force. 26
2.6 Areas of analysis in the manual experiments. The areas of interests for the data processing appear in light color. (from an actual experiment) 28
2.7 Spectrum of yeast cells measured by manual experiments. The red line is a fit using the 1-shell model developed in Chapter 3. 30
2.8 Histogram of the noise measured in yeast spectra, in manual experiments. The noise is measured by subtraction between the data and the fit 30
2.9 Spectrum of K562 measured by manual experiments. The red line is a fit using the 1-shell model developed in Chapter 3. 31
2.10 Histogram of the noise measured in K562 spectra, in manual experiments. The red line is a Gaussian fit. 31
2.11 Evolution of the spectrum of Jurkat cells over time. 33
2.12 Measures of different properties of the Jurkat cell lines from the spectrum, using the fitting technique presented in Chapter 3. 33
3.1 Scheme of the model used for the DEP simulation. On the left: scheme of the 3D microwell structure; on the right: cross section extracted for the FEM. ................................................. 40
3.2 Extraction of the smallest element from the radial section. .................. 41
3.3 Post processing image of the FEM solution of the element of well. ........... 42
3.4 Range of detection of the DEP force field in a 350 µm diameter microwell for different thicknesses of insulator and electrode layers. ......................... 43
3.5 Picture of a microwell manufactured from the optimised parameters. ...... 44
3.6 Image of a microwell filled with distilled water taken with infinite focal length, with (right) and without (left) the light diffuser between the light bulb and the microwell. ...................................................... 49
3.7 Example of an image before and after normalisation. .......................... 49
3.8 Image obtained after application of a threshold on Figure 3.7 .................. 50
3.9 Superimposition of image 3.3.3 with a disk centred at the centre of gravity of the well. .......................................................... 51
3.10 Mask obtained for 25 zones from image 3.3.3 ............................... 52
3.11 Electric potential at the surface of the wall in function of the height. Y-axis: electric potential in Volts; X-axis: height in m. .............................. 56
3.12 Wall potential reconstructed by the Fourier transform, as measured at the wall of the simulated FEM structure. The arc length is the distance over the z-axis. ......................................................... 59
3.13 Electric field from Equation 3.23 in a portion of microwell after recon-struction of the Fourier series. ........................................ 61
3.14 Screenshot of the model selection window of Comsol 3.4 .................... 62
3.15 Screenshot of the physics menu window for diffusion analysis ................. 63
3.16 Screenshot of the result of diffusion analysis for negative DEP ............... 64
3.17 Map of the concentration obtained for negative DEP. The red line is an example of integration path used for the creation of images. .................. 65
3.18 Images created from the results of the FEM simulation. Left: initial image at time 0. Right: image obtained after 3s. ................................. 66
3.19 Measures obtained for the CMF on simulated data, for different values of the evolution time. ..................................................... 68
3.20 Surface response of the level of non-linearity in % in function of the evolution time and the DEP force amplitude. Each level represent one percent error. ................................................................. 69
3.21 Typical distribution ........................................................................ 75
3.22 Results of the performance analysis of the thin-membrane approximation, using Monte-Carlo methods. ................................................. 75
3.23 Spectrum obtained from the one-shell model with the parameters given in legend. ........................................................................ 77
3.24 Results obtained from the Monte-Carlo analysis of Equation 3.39 on page 79 for a set of 10000 parameters selected according to Table 3.1 on page 74. K_L, K_m and K_h stand for the levels of the low, medium and high frequency plateaus respectively. The red dots stand for degenerated spectra, i.e. spectra where the transition frequencies are less than one decade further. ............................... 81
3.25 Results obtained from the Monte-Carlo analysis of Equation 3.39 on page 79 (continued). ω_l and ω_h stand for the low and high transition frequency respectively. The degeneracy clearly affects the precision of the calculus of the transition frequencies. 82

3.26 Example of a degenerated 1-shell spectrum: here, the mid-frequency plateau does not appear. 83

3.27 Sequences followed by the Monte Carlo program written in Maple for the determination of the error in the resolution of the system of equations 3.39. 83

3.28 Error produced on the measure of the scaling factor according to Equation 3.39 by the addition of white noise on the data. The X-axis stands for the standard deviation of the white noise added to the spectrum, the Y-axis are the measure of the error in %. 84

3.29 Evolution of the curve fitting after the optimisation process. The dashed line is a first, coarse approximation of the data (in blue) and is refined by the algorithm to give the plain red line. 86

4.1 Functional diagram of the automatic experiment 95
4.2 Front view of the automate. 97
4.3 Side view of the automate. 97
4.4 GUI for the data acquisition. 99
4.5 File system created by the program. The different files appear progressively as the data processing is completing. 100
4.6 Scheme of the fluid distribution system. 107
4.7 Detail of the machine, around the sample. 107
4.8 Detail of the machine, around the syringe pump. 108
4.9 Scheme of the capsule. 110
4.10 Capsules used in the machine. Left: the two parts that drive the fluid through the microwell. Right: an encapsulated microchip. 110
4.11 Scheme of the electronic board. 112
4.12 Grafcet of the microcontroller program. 114
4.13 Scheme of the optical system used in the automate. 116
4.14 Principle of the additional lens on the optical path. 117
4.15 Lens used in addition to the microscope. Left: mounting system. Right: lens used. 118
4.16 Fourier spectrum obtained from a 10-periods 20MHz sine wave generated by the signal generator used in the machine without the connection to the microchip. 119
4.17 Fourier spectrum obtained from a 10-periods 20MHz sine wave generated by the signal generator used in the machine with the connection to the microchip. 120
4.18 Scheme of the peak detector. 121
4.19 Structure of the different units of the program. 123
4.20 Scheme of the procedure for the exchange of data between the computer and a device via RS232 protocol. 125
4.21 Organigram of the data acquisition program. 126
4.22 Data processing GUI. 131
4.23 Structure of the variable ‘results’. 132
4.24 Curve fitting GUI. 134
5.1 Measures of the level of noise over time, for different samples of yeast cells at different concentrations. .................................................. 139
5.2 Evolution of the level of noise in the data with time, at different amplitudes of electric field. .......................................................... 141
5.3 Evolution of the decrease rate of the noise against the amplitude of the electric field. ................................................................. 141
5.4 Level of noise recorded on yeast cells DEP spectra with finite (red) and infinite (blue) focal length. ................................................. 143
5.5 Effect of a bubble in a yeast spectrum. ..................................................... 144
5.6 Surface response obtained by the study of evolution of the level of noise with the signal amplitude and the cell concentration. .................. 152
5.7 Isocontours corresponding to the surface above. ................................. 152
5.8 Histogram of the noise measure from yeast cells spectra. ...................... 153
5.9 Evolution of the bias induced in the yeast spectrum over time. ............... 154
5.10 Repeatability test on a single sample. Each curve have been acquired independently, after 5min interval. ................................. 155
5.11 Spectrum of yeast cells obtained from an average of 4 spectra. The error bars represent the standard deviation. ............................... 156
5.12 Evolution of the average level of light of images of yeast cells taken during the application of the DEP force. ................................. 162
5.13 Evolution of the average level of light of images of Bacillus atrophaeus Nakamura taken during the application of the DEP force. ............ 162
5.14 Spectra obtained from several suspensions of yeast cells at different concentrations. ................................................................. 164
5.15 Results obtained from the fit. ............................................................. 165
6.1 Comparison between distribution of noise in manual and automated experiments. ................................................................. 172
6.2 Different inhomogeneities in a microwell after manual resuspension. ........ 174
6.3 Comparison between the experimental duration of manual and automated procedures. ............................................................. 175
6.4 Discrepancies between the fit and the data over a wide variety of experiments 179
7.1 Scheme of a simplified and compact optical system to replace the microscope. ................................................................. 186
7.2 Diagram of a possible fluid distribution system with a 5-ways distribution valve. ................................................................. 190
7.3 Geometry of the model used for the simulation of the ACEO flow generated by two planar electrodes. ............................................ 195
7.4 Geometry of the modified model: two obstructions have been added on each electrode to cover their edges. ................................. 196
7.5 Geometry of the optimised model: several obstructions were placed along the surface of each electrode. ........................................ 196
7.6 Solution of the model presented on Figure 7.3. .................................. 197
7.7 Detail of the figure above, around the right-hand corner of the left electrode. .............................................................................. 197
7.8 Solution of the model presented on Figure 7.4. .................................. 198
7.9 Detail of the figure above, around the right-hand corner of the left electrode. 198
7.10 Solution of the model presented on Figure 7.5. ................................. 199
7.11 Detail of the figure above, around the right-hand corner of the left electrode. 199
7.12 Scheme of the planar low-microflow test electrode. ................................. 200
7.13 Image taken by the camera in the region between the 50 µm and 20 µm spaced bands. ................................................................. 201
7.14 Image taken by the camera in the region between the 20 µm and 10 µm spaced bands. ................................................................. 201

A.1 Scheme of a typical bacteria cell. (By Mariana Ruiz Villarreal, public domain copyrights) ................................................................. 2
A.2 Scheme of a typical plant cell. (By Mariana Ruiz Villarreal, public domain copyrights) ................................................................. 3
A.3 Scheme of a typical animal cell. (By Mariana Ruiz Villarreal, public domain copyrights) ................................................................. 3

B.1 Ill-conditioned case of inverse problem for an arbitrary function. ............ 6
C.1 The syringe pump module ................................................................. 9
C.2 Data acquisition GUI ....................................................................... 11
C.3 Data processing GUI ....................................................................... 17
C.4 Curve fitting GUI ........................................................................... 20
List of Tables

3.1 Table of the extrema values of the parameters used in the Monte-Carlo analysis of the thin-membrane approximation limitations. 74
3.2 Threshold of noise in the spectrum corresponding to 10% maximum error in the measure of the cell properties. 87
4.1 Instruction set used by the PC to control the operation of the microcontroller. 115
5.1 Rate of decrease of the level of noise in the data presented on Figure 5.1 at time t=1s, for the different yeast concentrations. 140
5.2 Cell viability before and after experiment, for two cell lines, with two measures for each. 157
5.3 Comparison between the measurements found and the data from the literature for yeast cells *Saccharomyces cerevisiae*. 159
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Alternating Current</td>
</tr>
<tr>
<td>ACEO</td>
<td>Alternating Current Electro-Osmosis</td>
</tr>
<tr>
<td>CMF</td>
<td>Clausius Mosotti Factor</td>
</tr>
<tr>
<td>DEP</td>
<td>DiElectroPhoresis</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite Element Modelling</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>IR</td>
<td>Infra Red light</td>
</tr>
<tr>
<td>hXX:</td>
<td>Hexadecimal number with digits XX</td>
</tr>
</tbody>
</table>
Physical Constants

Boltzmann’s constant \( k \approx 1.3806504 \times 10^{-23} \text{ J.K}^{-1} \)

Dynamic viscosity of water \( \eta \approx 1.003 \times 10^{-3} \text{ Pa.s at 20°C} \)

Permittivity of vacuum \( \epsilon_0 \approx 8.854187818 \times 10^{-8} \text{ F.m}^{-1} \)
# Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
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<tr>
<td>$a$</td>
<td>Cell radius</td>
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<td>$E$</td>
<td>Electric field</td>
<td>$V.m^{-1}$</td>
</tr>
<tr>
<td>$F$</td>
<td>DEP force field</td>
<td>$Kg.m.s^{-2}$</td>
</tr>
<tr>
<td>$K$</td>
<td>Clausius-Mossoti factor</td>
<td>1</td>
</tr>
<tr>
<td>$P$</td>
<td>power</td>
<td>W ($J.s^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Permittivity</td>
<td>$\varepsilon_0$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Conductivity</td>
<td>$S.m^{-1}(A^2.s^3.m^{-3}.kg^{-1})$</td>
</tr>
<tr>
<td>$\omega$</td>
<td>angular frequency</td>
<td>$rad.s^{-1}$</td>
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Dedicated to my father.
Chapter 1

Introduction to Dielectrophoresis: research and problems

1.1 Introduction

Cell biology is an area of research that has grown very quickly over the past decades, pushed by the pharmaceutical industry in an effort to understand diseases such as cancer, diabetes or neuronal diseases. Techniques used to study cells, such as flow cytometry, patch-clamp or microscopy have inherent practical problems associated with limitations like cost, ease of use, rapidity, ability to handle large number of cells or possibility to re-use the sample after measurement. That is why other methods are being investigated that can palliate these problems so to extend the possibilities of cell measurements. This Thesis presents the developments that have been made using a physical effect called dielectrophoresis (DEP) to measure some electrophysiological properties of a population of cells.

DEP has previously been investigated as a new solution to find more efficient ways to manipulate cells and analyse their electric properties at low cost and with simple protocols. Much research about DEP can be found in the literature and several advances have been made since its discovery, but the techniques developed so far has remained at the stage of laboratory exploration for different reasons. Before DEP could be used on a large scale, the physical principle underlying DEP effects had to be understood and the measurement stages had to be developed and optimised. Over the years, these
requirements have been partially met and the last years has seen different research groups proposing DEP-based devices for cell measurement. However, the devices developed so far suffer from several problems that prevent them from meeting the level of accuracy and ease of use that is needed to start considering their development on a larger scale. This chapter presents the main lines of the development process that occurred after the discovery of DEP and proposes an analysis of the problems encountered by the actual protocols exposed in the literature. This is the starting point of the conception and development of the device exposed in the next chapters.

1.2 Discovery and developments of dielectrophoresis

Dielectrophoresis is a physical phenomenon occurring on any polarisable particle under the influence of an inhomogeneous electric field. According to its electric properties, a particle under such conditions moves either in the direction of the electric gradient or in the opposite direction. Both situation arise from the same physical effect called dielectrophoresis (DEP), which by definition is called positive and negative respectively. This effect can drag a particle over a distance from a few micrometers to several millimeters depending on the electrodes used. H. A. Pohl reported the observation of that phenomenon and first developed a theory for the DEP using DC electric fields (Pohl, 1951, 1958). The formulas he developed initially were verified (Reynolds and Hough, 1957) using dielectric formulas developed by Clausius (Clausius, 1879) and mathematical results from Mosotti (Mosotti, 1850). This gave its name to the Clausius-Mosotti factor (CMF), which is the parameter that appears in the theory of DEP and expresses the specific dielectrophoretic properties of the particle. This CMF is what experimenters aim to measure in a typical DEP experiment because it can provide enough information to resolve the electric properties of the particle, as shown in Chapter 3 (page 36). It can be noted that, even though the first experiments with DEP were emphasising its mechanical properties, DEP had soon been used as a new method to measure the dielectric constant of millimetre-scale samples of materials (Roberts, 1961; Sher, 1968), which was a very difficult task to perform with the methods available then.

After its discovery, DEP started to be used more and more frequently. The idea quickly arose to use DEP on living cells, and several early works can be found that measure the behaviour of cells experiencing DEP forces (Jindrak, 1952; Ting et al., 1971)
Chapter 1. Introduction to Dielectrophoresis

or the capabilities of cell manipulation by DEP (Pohl and Hawk, 1966). Subsequently the number of uses of DEP started increasing over the years: a research in the Compendex and Inspec citation databases provides a good indication of the development of DEP-based techniques. Figure 1.1 on page 3 shows the number of publications per year since 1969 \(^1\), with a rapid rise since 2003 due among others to the publication of a new method to sort carbon nanotubes using DEP (Krupke et al., 2003). This evolution is the result of the regular gain of interest of researchers to DEP over the years, which bring new usages of this effect in various research fields. Among the different uses of DEP, we can cite carbon nanotube sorting (Mureau et al., 2006), cancer cell detection (Gascoyne et al., 1997), apoptosis detection (Labeed et al., 2006; Pethig and Talary, 2007b), microcomponent assembly (Lee and Bashir, 2003), or colloid experimentation (Lenglet et al., 2002). This list is far from exhaustive, but already shows how different are the applications already found for DEP.

This increasing interest for DEP-based techniques shows a great potential, and many efforts have been made to push the experimentation up to an industrial scale (Gascoyne et al., 1994, 1997; De Gasperis et al., 1998; Fatoyinbo et al., 2005). However, most application are restricted by the small size of the sample analysed and by the duration of the measures, which prevent their use on larger volumes. This is a general context in DEP research at the time being: much efforts are directed towards automatisation, reduction of the duration of the measure and increase of the size of the sample.

\(^{1}\)Source: www.engineeringvillage.com.
1.3 Outline of dielectrophoresis theoretical developments

An analysis by DEP is model-dependent. Contrary to methods like patch-clamp or measure by micro-electrodes, an analysis by DEP does not provide a direct measure of the conductivity or the permittivity of the measured particles. The data collected from an experiment has to be compared to a model that depends on the geometry of the particle in suspension. Different geometries have been studied but the most common one remains the sphere model (Kao, 1961).

The phenomenon of DEP can be thought as a dipolar interaction. How can an electric field induce a motion on a neutral material? The physical principle of DEP for an electrically neutral particle immersed in water was explained first by Pohl (1951). This triggered theoretical research that could predict the force exerted on particles of given shapes: in 1957, J.A. Reynolds reported the calculation of the DEP force for various homogeneous geometries of suspensoid particles (Reynolds and Hough, 1957), two years later the single-shell model was developed (Pauly and Schwan, 1959) that describes the DEP force exerted on a sphere surrounded by a shell and is still in use nowadays to predict the behaviour of simple living cells. Later, one single global theory was developed that unifies DEP and dielectrorotational forces by describing these phenomena as the interaction between the electric field and the particles equivalent dipole (Wang et al., 1994).

Figure 1.2 describes the mechanisms that generate the physical phenomenon of DEP according to the model developed by Pohl. The black arrow indicates the direction and gradient of electric field, $\varepsilon$ stands for the relative permittivity of a material, the circle represents a spherical homogeneous particle and the $\pm$ signs represent the local electric charges. According to Gauss law the differences in electric properties, together with the inhomogeneous electric field, generate an unbalanced partition of charges around the sphere. In such a situation, there are three possibilities, noted (a), (b) and (c) on the figure. In case (a), the sphere permittivity is lower than the mediums: this creates a charge configuration that opposes the electric field, this corresponds to a negative DEP. Case (b): there are no inhomogeneities of electric properties so no charge is generated, so there is no net force. Case (c) is the contrary case of case (a): the charge configuration around the sphere is aligned in the direction of the electric gradient, which corresponds
Figure 1.2: Charge distribution for different configurations of permittivity.

to a positive DEP. These cases still hold for a time-varying electric field, i.e. for complex values of permittivity.

The charge distribution created around the sphere by the electric field is complex, but if the spatial variations of the electric field are smaller than the size of the particle, then it can be approximated quite well by a dipole-like distribution. Therefore, the spherical distribution considered initially can be reduced to a 2-point distribution. The analytical expression of the magnitude of this equivalent dipole is called the Clausius-Mossotti Factor (CMF): it is the reduction of the particle geometry to a dipole and so it depends on the particle’s shape, but in the case of an AC electric field this factor also depends on the frequency of the electric field as explained later. Generally, a DEP experiment aims to measure the value of this CMF at different frequencies, its frequency representation is referred to as the DEP spectrum of the particle measured.

Just as a dipole experiences Coulomb forces under the influence of an electric field, so the sphere experiences similar forces under the same conditions. The force exerted on the particle by the DEP effect can then be approximated by the interaction between a dipole, modelled by the CMF, and the electric field. This is widely developed in the
literature cited above and the expression of the resulting force, called the DEP force, is commonly expressed as:

$$\vec{F}_{DEP} = 2\pi a^3 \varepsilon_m \varepsilon_0 \text{Re}(K(\omega)) \nabla(\vec{E}^2)$$  \hspace{1cm} (1.1)$$

Where $\vec{F}_{DEP}$ is the DEP force experienced by the particle, $a$ is the radius of the particle, $\varepsilon_m$ is the relative electric permittivity of the surrounding medium, $\varepsilon_0$ is the electric permittivity of free space, $\omega$ is the frequency, $\nabla(\vec{E}^2)$ is the gradient of the electric field squared, $\text{Re}$ is the real part operator and $K$ is the CMF that describes the magnitude of the particles equivalent dipole.

The common model used for living cells is the multi-shell model. As stated above, the CMF depends strongly on the particle geometry. The most common geometries are the sphere and the multi shelled-sphere, the last one describing a sphere enveloped by one or more layers. The sphere model gives the following expression of the CMF:

$$K_{\text{sphere}} = \frac{\varepsilon_c^s - \varepsilon_m^s}{\varepsilon_c^s + 2\varepsilon_m^s}$$  \hspace{1cm} (1.2)$$

where $\varepsilon_c^s = \varepsilon_c + j\sigma_c/\omega$ and $\varepsilon_m^s = \varepsilon_m + j\sigma_c/\omega$, $j$, $\varepsilon_c$, $\sigma_c$, $\varepsilon_m$ and $\sigma_c$ stand for the complex number $\sqrt{-1}$, the inner sphere relative permittivity and conductivity and the surrounding medium relative permittivity and conductivity respectively.

Figure 1.3 on page 7 presents a shelled sphere of radius $R_1$ with a layer of thickness $R_1 - R_2$ representing the cell membrane. This membrane is usually very small compared to the radius (see A on page 1). Considering that the inner sphere can be modelled by a single-sphere model with the layer as the surrounding medium, the particle can be replaced by an equivalent particle of relative permittivity $\varepsilon_{eff}$. The analytical expression is the following:

$$\varepsilon_{eff} = \varepsilon_m \frac{\alpha + 2K_{\text{sphere}}}{\alpha - K_{\text{sphere}}}$$  \hspace{1cm} (1.3)$$

$$K_{\text{shell}} = \frac{\varepsilon_{eff}^s - \varepsilon_c^s}{\varepsilon_{eff}^s + 2\varepsilon_c^s}$$  \hspace{1cm} (1.4)$$
where $\varepsilon_m^*$ is now the complex permittivity of the membrane, $\varepsilon_s^*$ the complex permittivity of the suspension medium and $\alpha = (R_1 - R_2/R_1)$. Equation 1.3 is known as the shell model and is the most common model to approximate the behaviour of a cell experiencing DEP. From here, it is easy to re-iterate the process and to add another layer, we just need to consider that the core is modelled by the expression above and the new layer is the new membrane. This process can be repeated indefinitely, according to the number of layers one wants to model. This multi-shell model forms the basis of the work described in this thesis.

**This model works for a variety of cases but also has its limitations.** The shell model presented above was proposed by Reynolds and Hough (Reynolds and Hough, 1957) and has been investigated widely in the 70s and 80s with numerous works on protoplasts, mitochondria or living cells (Ting et al., 1971; Asami and Irimajiri, 1984). It appeared from those works that the behaviour of the cell membrane and cytoplasm were reasonably well described by a single-shell model. However, the cell cytoplasm is not a homogeneous medium and the protoplasts, vesicles or nucleus it contains can be considered as sub-shells. The membrane can also be covered with a cell wall. So several works use models with more layers in order to fit the data closer (Gimsa et al., 1991). However, the more shells the model has, the harder it is to justify the analysis by curve...
fitting, as explained in Chapter 3 on page 36. Recent work has also shown that these models are limited to the micron-scale and that, for smaller objects, the double-layer can also affect the particle behaviour, so other models were proposed (Hughes et al., 1999; Huang et al., 2003) but will not be considered here because they are aimed to describe the behaviour of nanoparticles and are not relevant to the micron-scale cells used in this study. Ultimately, the shape of the DEP spectrum obtained experimentally determines which model is relevant (i.e. double shell, triple shell and so on).

1.4 Previous methodologies

1.4.1 Presentation of several experimental designs

The measure of the DEP force can be performed experimentally using different instruments. A typical DEP setup is composed of two or more electrodes that generate the electric field, and an apparatus that provide a measure of the DEP force. The geometry of the electrodes is a critical parameter because it determines the electric gradient and therefore the geometry and magnitude of the DEP force, as modelled in section 1.3 on page 4. Many families of electrode geometries have been created and tested experimentally but only the most frequently used are presented below.

1. Needle electrodes

The needle electrodes consists of two needles facing each other, separated by a given distance, usually around 100 µm. This apparatus has been used to investigate the changes in the physiology of the cancerous cell line K562 in several works (Labeed et al., 2003, 2006). Other works use only one needle and a ground plane, or other variations of needle electrodes, according to the need (Holzel, 2002).

2. Castellated and interdigitated electrodes

Figure 1.4 on page 9 shows a castellated electrode: this pattern consists of two planar electrodes with square-wave like edges, facing each other with an inter-electrode spacing of 100 µm approximately. These have been widely studied and a model of the electric field they generate has also been developed. The interdigitated electrodes are similar to the castellated electrodes but with longer
dents and a much thinner gap between the electrodes. Some works propose an analytical solution of the electric field in such a configuration (Chang et al., 2003).

3. Hyperbolic electrodes

One particular shape is the hyperbolic electrode, which has the particularity to create a homogeneous gradient of electric field, so that the DEP force has a constant magnitude. This makes it is easier to analyse the data, but such electrodes are not easy to manufacture. Some hyperbolic electrodes are shown on Figure 1.5 on page 10: they are constituted of four electrodes (in dark on the figure) where two opposite ones are grounded, and the two others experience the same electric potential, so that the electric field at the center of the device is null by symmetry.

The homogeneity of the resulting gradient of electric field make this electrode very common in a wide panel of applications, from ion trapping to DEP (Huang et al., 1992). They are usually made by etching a conductive layer deposited on a glass substrate. The name ’hyperbolic’ comes from the hyperbolic equation that defines the electrode contour: this contour shape is found by solving the Laplace equation of the electric potential with a constant DEP force imposed. The calculus is included in the CD along with some other shapes of electrodes for linear and parabolic electric fields (Maple codes/Hyperbolic electrode.mws).
4. **Zipper electrodes**

A zipper pattern is presented on Figure 1.6 on page 11. This example was developed at the University of Surrey on latex beads (Hoettges et al., 2003a), and other work reports its use on micrococcus (Price et al., 1988). The aim of this design is to increase the surface of DEP and to make use of electro osmotic flows in order to localise zones of strong DEP signal into well defined zones. The white, circular band seen on image a) separates two electrodes made of ITO deposited on a glass substrate, connected to a signal generator. Image b) was taken with UV illumination on fluorescent-marked latex beads. The area of strong DEP signal appears very clearly with this sort of electrodes, and the data obtained is easier to analyse than with castellated or interdigitated electrodes.

5. **Dot electrode**

The dot electrode has been developed at the University of Surrey, UK. It is an electrode optimized to make use of the widest space available so that the DEP signal is stronger than with a traditional planar electrode. A scheme of its principle...
Chapter 1. Introduction to Dielectrophoresis

Figure 1.6: a) zipper electrode profile, (b) same electrode during a DEP experiment (from the University of Surrey)

is presented on Figure 1.7: the chamber is delimited by the two electrodes; the top one is made of a thin deposition of ITO on glass and is transparent; the bottom electrode is a surface made of gold on glass substrate, etched on small circular surfaces called ‘dots’ that create the field inhomogeneity. This configuration has the advantage to make use of the entire height of the chamber, is able to measure the DEP spectrum of suspensions at high concentration (up to $10^8$ cells/mL for yeast cells) and usually focuses down to 100 to 200 µm field of view so that it monitors a large population of cells (Fatoyinbo et al., 2005, 2008).

This apparatus is similar to the one used for the measure with microwell, as explained in Chapter 2, and has a great potential for larger-scale uses. The main differences between the dot and microwell electrodes come from their geometries: the first is planar and non-conservative, because the particle can escape the field of view in the case of negative DEP, whereas the later is tubular and keeps the cells within its volume. The difference of shape also makes them adapted to different practical uses: a dot electrode could be more adapted to a surface, whereas a microwell would fit better in a tubing or another confined system. Finally, the field of view used by microwell electrodes is generally wider than for dots electrodes.
Planar electrodes generate strong local electric fields but use only a small fraction of the available volume. The planar electrodes, such as the castellated, the zipper or the interdigitated electrodes, consists of a thin conductive layer deposited on a substrate much wider than the layer thickness, so that they look like thin sheets of conductor between the cell suspension and the substrate. Such electrodes can be qualified as ‘two dimensional’ electrodes, because their electric field can be accurately compared with the one of theoretical surface electrodes. The electric field generated by a 2-D configuration has a high gradient around the electrode edges, which means that such a planar electrode generates a high DEP force around the edges of the conductive. However, solving the Laplace equation of the electric potential with such electrodes shows that the DEP force they generated decreases exponentially with the distance from the plane of the electrode (Chang et al., 2003). Therefore the region of high DEP signal is a film at the surface of the electrode, as shown on Figure 1.8, which makes those electrodes ideal for monitoring a confined region with a small number of cells, but not adapted for the measure on a large volume of sample with a large cell population.

Because deep electric fields are required for this work, 3D electrodes were
used instead of planar electrodes. As this work targets cell populations, a 3D electrode is more adapted to its purpose. The electrode choose here is named the ‘microwell’ electrode and has been developed at the University of Surrey and has the ability to perform DEP on a much larger volume than planar electrodes, for the same field of view of the measuring instrument (Hoettges et al., 2003b). More details about this electrode are given in Section 3.2 on page 37.

1.4.2 Protocols for DEP force measurement

Cell electrophysiological parameters are derived using DEP by fitting the data collected with a multi-shell model. Experimentally, the DEP force is measured with the electrodes described above by monitoring the displacements of the cells at different frequencies of the electric field. The different positions of the cell provide their speed, and at the micron-scale the viscosity of water is so high that the speed is proportional to the force exerted, mainly the DEP force. Once the speed of a cell is calculated,
Chapter 1. Introduction to Dielectrophoresis

it is then compared to a theoretical model of the DEP force like the shelled-sphere model introduced in section 1.3 on page 4, which predicts the cell behaviour according to the electric properties. Then, by comparing the model to the data, the electric properties of the cell monitored are retrieved. Several works use this model-based approach to measure the cells properties. In practice, the measurement of cellular electric properties by DEP is degraded by the fact that the model used does usually not take advantage of the totality of the information from the experiment, or the data covers only partially the model, and in addition there are different sources of noise. Below are detailed some of the techniques found in the literature to measure the DEP force, along with their advantages and inconvenients.

1. The measurement of the crossover frequency

This technique is simple but uses only one point of the DEP spectra, and sometimes cannot be applied. The DEP spectrum generally presents one or two zeros, which correspond to frequencies where the DEP force is null. These points are called the crossover frequencies. This is experimentally easy to measure as it just needs the experimenter to adjust the frequency until no cell motion is observed, and compare the value found to the theoretical value (Broche et al., 2005). However, this cannot be employed systematically because some cell lines do not present any crossover frequency, and others have a crossover out of range of the frequency band available for the experimenter. Moreover, this method does not use all the information that can be extracted from the experiment, such as the amplitude of the DEP force at away from the crossover frequencies.

2. The use of an auxiliary force that balances the DEP force

This technique is very accurate but difficult to perform in practice, and cannot be used on a large number of cells. Another technique consists in using a measurable external force that balances the DEP force so the experimenter can measure it when no net motion is observed. This can be achieved by using microflows, optical tweezers or other calibrated force that can handle a cell. A typical example is DEP levitation where DEP is balanced by the gravitational force (Qian et al., 2002). This method is relatively accurate but it measures one cells at a time, which makes it difficult to obtain statistically significant results. It can also be relatively complex to set in practice.
3. Cell tracking and cell counting

This technique is commonly used for DEP measures but cannot monitor a large number of cells. Techniques using cell tracking are useful for monitoring a number of cells without focusing on an entire population. Cell counting consists in counting the number of cells collected at the surface of an electrode after a given time. Cell tracking starts from the same principle but uses a camera and computer that monitors the cells and processes their displacement automatically (Pethig and Talary, 2007a). It is experimentally relatively easy to achieve and it has the possibility to detect individual behaviours among a small population of cell but it needs a robust tracking program and cannot monitor a high number of cells for reasons of visibility.

4. The measurement by light absorption

This technique focuses on a cell population and therefore the individual cells are not necessarily visible to the experimenter. The cell suspension is placed in the path of a light beam and the outgoing light intensity is monitored. The movement of cells causes the intensity to change and the variations of light are analyzed and treated to give the DEP spectrum. As this does not need focusing down to the cell scale, this technique can measure the electric properties of the whole population of cells present in the suspension, and can use 3-D electrodes which make use of bigger samples and then provide more statistically significant data. The difficulty arises from the additional theory of cell diffusion under a DEP force field, which has not been found in the literature so far, mainly because few works used this technique (Burt et al., 1989; Talary and Pethig, 1994; Huebner et al., 2005). Because of its relative experimental simplicity and its ability to handle large number of cells, this technique is the one that was retained for this work. The precise methods and instruments used are presented in the next chapter.

1.4.3 Electrorotation

Electrorotation is an effect occurring on polarisable particles submitted to a gradient of phase of electric field. Such a situation generates a torque in the particle, which induces its rotation in space. This effect is closely linked to dielectrophoresis: a common theory
has been developed that explain both effects from a common mathematical treatment (Wang et al., 1994).

The typical apparatus for electrorotation needs at least four electrodes that generate AC electric fields, but each one has a specified phase delay in order to produce a gradient with its neighbours. Generally, a four-electrodes system uses a phase delay of $\pi/4$ between two adjacent electrodes in order to obtain a sine wave profiled gradient. It is possible to calculate numerical results for such electric fields, as shown in previous publications (Hölzel, 1993; Hughes et al., 1994), as well as the accuracy of the results provided (Gascoyne et al., 1995).

Several publications present the usefulness of this method, especially for biological purposes such as yeast spectrum measurement or parasite detection (Hölzel, 1997; Dalton et al., 2001). Because the electrorotation effect is so closely linked to dielectrophoresis, we can see that the uses made of these two phenomenon are similar, and sometimes both are combined in a same study (Gimsa et al., 1991; Huang et al., 1992). Also, automated measures setups have been created that use cell-tracking algorithms (De Gasperis et al., 1998). However, just as for cell-tracking dielectrophoresis, this method is restricted to a measure on small number of cells, so that the experimenter has to repeat the measure numerous times before obtaining statistically significant results. The other restriction of electrorotation is the technical difficulty of the apparatus, which require an accurate de-phasing between the electrodes regardless of the frequency used. These two restrictions are the reasons why this effect has not been used here.

1.5 Aims and structure of this thesis

**DEP measurements are time-consuming and have difficulties following a population of cells.** Previous works, presented in more details in section 1.4 on page 8, shows that using DEP to measures cells behaviour is possible but the protocols used so far suffer from a variety of problems. Firstly, few of them can measure the behaviour of a large population of cells; secondly they require a long experimental time which makes it difficult to justify that the cells keep a constant physiology through the experiment; thirdly some of them does not use all the information collected from the experiment. The aim of the present thesis is to show that it is possible to conduct rapid DEP measure
on living cells and to obtain reliable data from it. This is performed by optimising both
the theoretical models and the experimental protocol. The models of multi-shelled cells
has been analysed and a method of data extraction has been develop that makes use of
the totality of the information present in the data collected. In parallel, an instrument
has been developed that can conduct DEP measures with 20 data points in under 5
minutes in a repeatable way, instead of 40 minutes for the previous manual method, and
with lower noise.
Chapter 2

Analysis of the pre-existing protocol for DEP measures with the microwell electrode

2.1 Introduction

The first generation of microwell electrodes was developed at the University of Surrey in 2003 (Hoettges et al., 2003b), and at the start of this thesis they had been used in several research projects with the aim to assess the possibilities of 3D electrodes technology (Huebner et al., 2005). The promising results of these studies showed that the technique of DEP measure by microwell was providing sensible data, generating a need to optimise the associated protocol and apparatus.

In order to optimize a process, one must start by assessing the performance of its initial state. In the present case, the measures of DEP by microwell as performed in 2005 were partly automatic and partly manual, and there was clearly room for improvement from different points of view. Introducing automated procedures as far as reasonably possible could diminish the variability due to the experimenter; the first generation of microelectrodes was making use of a relatively small portion of the microwell volume and re-designing it could increase this volume; several sources of noise from the apparatus or its surroundings were damaging the DEP signal and could be reduced by different means; the data processing was only using a few percents of the signal acquired. All these points
led to losses of signal-to-noise ratio in the process, which had to be quantified in order to prioritize the efforts according to the potential benefits.

This chapter presents the result of this procedure, and helps understanding the reasons of the choices presented later that had been made during this thesis. First is explained the technique of measurement by light absorption together with a presentation of the methods and instruments used in the initial protocol. Then the sources of noise that have been found in that protocol are presented with an explanation of their origin and the results they have on the signal. The data processing used initially is also detailed, and the assessment of the performances of the apparatus initially used is presented. At final, a set of objectives is set that has been followed during the development of the prototype.

2.2 Light absorption measure: initial method and instruments

2.2.1 Introduction

Light absorption techniques have been developed and used at the University of Surrey by the Biomedical Engineering department since 2002. A series of chips containing 3D electrodes were built by the Biomedical Engineering unit with the purpose to develop a lab-on-a-chip device that could analyse a population of cells by DEP and determine its electrophysiological properties. These electrodes, described below, were used manually and the experimenter had to place the sample to be analysed on the chip with a syringe and perform the data acquisition with the help of dedicated devices. The data acquired were processed in order to provide a DEP signal, and several works were conducted to determine how this signal could help the detection of changes in cell properties. Below are presented the methods of mounting the electrodes and the protocol used in the manual experiments.

2.2.2 The microwell electrode and the manual procedure

The initial protocol used to conduct the experiments of DEP measurements used a 3D electrode geometry named a microwell chip. A generic microwell is made from a hole
in several sheets of copper isolated by layers of polyimide. The geometry of the layers varied according to the purpose of the chip: the thickness of the sheets of copper and polyimide could vary between 35 \( \mu \text{m} \) and 125 \( \mu \text{m} \), but in total a chip did not exceed 1.5mm thickness. The standard chip used at the University of Surrey in 2003 was made of 12 layers of 100 \( \mu \text{m} \)-thick sheets of copper separated by 100 \( \mu \text{m} \)-thick layers of polyimide, and the well drilled through the layers had a diameter of 700 \( \mu \text{m} \) so that it remained much larger than the diameter of the cells analysed. During a DEP experiment, the microwell chip was connected to a signal generator (FG100, Digimess) and an oscilloscope (IDS710, Iso-tech); it was glued on a glass slide and monitored by a Nikon microscope equipped with a camera (Photonic Science 80104/P). Once the microscope was correctly positioned and focused, the experimenter injected some cell suspension in the well with a syringe. The well was then covered by a thin cover slip that flattened the interface between the sample and the air to avoid distortions of the light beam. Then, depending on the nature of the experiment, the signal generator was switched on for 30 to 180s , at 10 V to 20 V peak to peak and at a frequency chosen within the spectrum required. The activity in the microwell was observed through the microscope by the camera with regular frame acquisitions (1 frame every 2 to 5 s) which were recorded on a PC for a period of 30 to 180 s. When finished, the cover slide was removed, the liquid in the well was aspired with the syringe and replaced in the original suspension. This suspension was re-homogenised and a drop of it was then placed into the well again. Then the procedure was repeated with a different frequencies of the

![Figure 2.1: Scheme of the microwell structure.](image)
2.2.3 Experimental methods

This section presents the experimental methods followed in order to prepare the material used in the manual protocol described above. It includes the preparation of conductive medium, a description of the microwells, the methods for yeast culture and preparation of YPD Broth medium and the rinsing procedure prior to a DEP experiment.

1. **Yeast culture** Yeast is a eukaryote cell, which is fairly easy to cultivate because it is very resistant to contamination or variations of temperature. The cell line used here is *Saccharomyces cerevisiae*. Its DEP spectrum was measured in many previous works, so that it is often used as a reference to test the reliability of a protocol. It is cultivated in suspensions of YPD Broth (Y1375, Sigma-Aldrich) at a concentration of 50 grams in 1 litre deionised water, prepared prior to culturing.

![Side-view scheme of a microwell used in the manual procedure.](image-url)
2. **Conductive medium** DEP experiment requires to resuspend the cells into a low conductive medium. This medium must be prepared carefully in order to have a precisely defined conductivity, and also keep the cells alive during the time of the experiment. For yeast cells, this medium is a solution of 51g/L manitol to control the osmolarity with a phosphate buffer to control the conductivity. The phosphate buffer is a standard PBS solution and is added progressively with a constant monitoring by a conductivity meter (HI8733, HANNA instruments) until the desired conductivity is reached.

3. **resuspension protocol** The transfer of the cells from the high-conductivity culture medium to the low-conductivity resuspension medium is performed through a series of centrifugations. Some cell lines are more resistant to spinning than others, so the protocol varies according to the cells used. Hence the details of the resuspension are provided with each experiment. As an indication, the typical scheme of resuspension consists in 3 spinnings of 150s each, at $380 \times g$. After each spinning the cells are resuspended in the low-conductivity medium in order to rinse them from the culture medium.

### 2.3 Identification and characterisation of the sources of noise

#### 2.3.1 Introduction

Several important sources of noise were identified in the manual procedure. As any measuring technique, the measure of cell conductivity and permittivity by DEP includes several sources of noise that deteriorate the signal-to-noise ratio. The reduction of the level of noise in the final data is part of the optimisation process that has been performed in this thesis. In order to lower the noise, one must first find the sources, understand their nature and measure their relative importance. There are several sources of noise arising in the manual DEP procedure described in the previous section, the main one that has been identified are the microflows, the error of conductivity, the high-frequency resonant effect, the quality of the light beam, the pollution from external light sources and inter experiment variations. Each of these sources of noise are detailed in this section with their effect on the data.
2.3.2 Microflows and AC-electro hydrodynamics

The DEP force appears from the use of electric fields in a conductive medium, which in turn generate electric currents, and hence joule heating. Both electric and temperature gradients can raise the medium into motion causing local micron-scale flows of liquid, or ‘microflows’. Several kinds of microflow arise during a DEP experiment that disturbs the cell trajectory. They can be categorised according to their origin, velocity and dimensions. A review was published (Castellanos et al., 2003) that described the different sorts of microflows observed during a DEP experiment and analysed the amplitude of each one according to the scale of the system, the medium conductivity, the signal frequency and the voltage. According to that review, the microflows of importance in the microwells used in this work are the AC electro-osmotic flow for frequencies lower than 100 kHz and the electrothermal flow above that frequency.

AC electro-osmotic flows originate in the electronic double-layer formed at the electrode

Figure 2.3: Typical map of ACEO flows around surface electrode (from simulations, see Chapter 7 for details).
surface. When the electric field is applied, the ions present in that region experience Coulomb force which follows the direction of the electric field. In the case of an infinite planar electrode, the electric field is perpendicular to the electrode surface so no motion could occur, but the presence of an edge bends the electric field, which is equivalent to say that the field is locally decomposed into a two components, a perpendicular and a parallel to the electrode surface. Hence the parallel component drags the ions along the electrode surface, and a net motion of ions appears around the electrode edges which is transmitted to the fluid (Reppert and Morgan, 2002). This is a very local motion and it extends 50 µm from the electrodes edges, as experimentally measured in the microwell.

The electrothermal motion occurs when the electric current produces a significant temperature gradient. As the medium permittivity and conductivity are temperature-dependent, the gradient of temperature generates a gradient of electric properties. This puts the fluid to motion by the influence of the electric field (Fuhr et al., 1991). This flow extends deeper than the electro-osmotic flow and can affect the entire microwell but is usually less important in term of intensity so it has not been observed to disturb the measure of DEP significantly.

Because the microflows profiles are curled their effects are expected to make the concentration locally homogeneous. Since they take place in the regions of high electric gradient in the microwell, the data is affected in specific areas in the data captured. This is analysed in more details with the experimental data on Chapter 5 on page 159.

### 2.3.3 Conductivity of the suspending medium

The difference between the expected value of suspension conductivity and the true value may reach 15% after 15 min. A manual experiment lasts between 30 and 90 minutes. By that time the medium conductivity can vary because of the cell activity and exchanges of CO₂ between the medium and the atmosphere. A simple test has been performed to measure this effect: a sample of cells has been resuspended following the protocol explained in 2.2.3 on page 21, and the conductivity has been measured after the last resuspension and 15 minutes later. For a cell concentration of 10⁶ cells/ml, the conductivity was initially 30 µS/cm and shifted to 35 µS/cm after 15 minutes. Hence the results are biased with the duration of the experiment. This effect can only be reduced by diminishing the duration of an experiment.
Chapter 2. Analysis of the pre-existing protocol

2.3.4 Filtering effects

The original microwell chip behaves electrically like a low-pass filter so the voltage drops above the cut-off frequency of 4 MHz. When using the standard microwell chips presented in section 2.2.2 on page 19, the voltage from the signal generator starts to drop as the frequency increases above 4 MHz. This is due to the capacitive and resistive behaviour of the microchip, creating a low-pass filter with a cut-off frequency around 4 MHz. Therefore the maximum frequency that can be reached by this chip is limited by its design. This is a problem for the data processing because the high-frequency region plays an important role in the measure of the cell electric properties, as seen in section 3.5.3 on page 76, and 5 MHz is very often too low.

2.3.5 Quality of the light beam

The irregularities of the microscope light source can be significant compared with the variation from the DEP signal. The concentration of cells in the microwell during an experiment is measured with a light beam. Usually, the difference of light
intensity measured between the start and the end of the experiment varies between 1 and 20%, depending on the absorbency of the cell line, the sample concentration and the length of the microwell. However, during several experiments, some variations in the level of light from the light bulb have been detected in the data, probably due to electric interference circulating in the mains from other laboratory equipments. In several experiments, the resulting variations of light was high enough to perturb the measure and, after the normalising procedure explained in section 3.3 on page 43, the error on the measure of the DEP force reached 100%, as shown on Figure 2.5 on page 26. This shows that the light absorbency protocol is very sensitive and caution must be taken with the power supply of the light source.

2.3.6 Operator variability

Manual experiments depend strongly on the ability of the operator to perform in a repeatable fashion, which is difficult to assess. A protocol based on manual manipulation requires the operator to perform smoothly and regularly. The quality of the data obtained depends directly on these abilities. However, the manual

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{dep_data_with_noise.png}
\caption{Results provided from a DEP experiment with irregular illumination after data processing. The changes in light intensity are interpreted by the software as variations of the DEP force.}
\end{figure}
procedure for DEP measure is very sensitive and, as seen with the variations of light intensity above, even small variations during the experimentation can create an important noise after data processing. Unfortunately, there are several ways in which the operator has been found to introduce noise in the data during a DEP measure.

**Re-filling the microwell manually changes the length of the light path.** The light absorbency method uses the Beer Lambert law of light absorption by the medium and so requires a constant light path to give repeatable and comparable measurements. The manual procedure suffers from irregular amounts of fluids in the well so the cover slide is never twice at the same place and so, between two data acquisitions, the length of the light path varies. This variation has been estimated to be 10% to 50% of the average light path and is likely to generate noise in the results. Also, each time the operator fills the microwell with the suspension medium, a part of the fluid flows out of the chip so that, by the end of the experiment, an important quantity of cell suspension is lost. Hence, 1 ml of medium is usually needed to conduct a manual experiment.

**The speed of the fluid delivery together with a narrow needle can damage the cells by shear forces.** The use of a needle syringe is suspected to damage the cells by shear forces when the operator homogenises the medium: during an experiment of 45 minutes on capricornum algae, the cell concentration was found to vary by 20%. In the worst cases the concentration was diluted by a factor 10 between the beginning and the end of an experiment, and the missing cells were found to accumulate in the syringe, around the outlet. Hence the operator has to use the syringe smoothly in order to avoid any damage to the cells or any precipitation of the colloid in trapped turbulences.

It can also be noticed that a manual DEP measure is difficult to learn and fatigue, boredom or distraction can be a significant source of variation in the protocol, so a sources of noise in the results.

### 2.4 Data processing and introduction of bias

The initial data processing method use only a portion of the available data. The data processing used in 2005 with the manual protocol consisted in an averaging on selected zones of the images. The zones used for averaging are presented on Figure 2.6, it is a series of squares disposed along a band, from the center to the edge of the
Figure 2.6: Areas of analysis in the manual experiments. The areas of interests for the data processing appear in light color. (from an actual experiment)

image of the microwell. In practice, the band was divided into 10 squares. The data processing that follows consists in averaging the level of light in each square, and to select the area where the largest signal is recorded. Then the results are interpreted as a DEP spectrum.

Non-linearities appear in the data, with some differences between positive and negative DEP. As shown in section 3 on page 9 and in the Maple files on the CD in appendix, only a very special geometry of electrode can generate a linear evolution of the cell concentration over time. These electrodes are called ’hyperbolic electrodes’ because their shape is described exactly from a parabolic equation (Huang and Pethig, 1991). Because the electric field generated by a microwell is not hyperbolic but is a sum of Bessel functions (see 3.4.2 on page 54), the variations of concentration are not linear with the time, and not linear with the force amplitude either. The time non-linearity can be verified directly from the experiment; the non-linearity with the force amplitude is visible when one compares data from negative DEP with data from positive DEP. In the first case, the cells are repelled from the electrode within 5 to 10 seconds, so after
10 seconds the signal monitored becomes stationary. However, in the case of positive DEP, the cells are attracted towards the electrodes so the signal becomes stationary only when all the cells in the microwell are trapped around the edges, which can take several minutes. Therefore, after around 10 sec, the evolution of the light intensity recorded with negative DEP shows a net decrease whereas with positive DEP, the decrease is much slower. This eventually leads to a distortion in the final spectrum because the parts of the spectrum that shows negative DEP is likely to squeezed compared to the positive parts.

2.5 Performances of the manual protocol for DEP measure

Several cell lines have been investigated by manual measures of DEP in several series of experiments. The data used in this study have been recorded during manual experiments performed by Yvonne Huebner, who worked with manual DEP measures between 2004 and 2007. This choice has been made because of the skills of this operator ensure that the data that presented here correspond to a good quality standard and correspond to a good signal-to-noise ratio for manual experiments. This makes it a significative set of results, which can be compared later to datasets from automated experiments.

The cell investigated here are the yeast cells *Saccharomyces cerevisiae*, the Jurkat human T-lymphona cell line and the K562 human myelogenous leukemia cell line. These cells were grown and provided by Yvonne Huebner from her PhD work. All of them has been measured in different conditions and after different treatments for the purpose of the another study. However, we will only consider the measures performed with standard healthy conditions for the sake of comparability.

Performing a manual experiment requires a relatively high number of replicates before obtaining a meaningful level of signal to noise. The results obtained for the different cell lines are presented on the figures below. Figure 2.7 on page 30 presents a typical DEP spectrum of yeast at a concentration of $10^7$ cells/ml and for a medium conductivity of $30 \mu S/cm$, with a fit that uses the multishell model (see Chapter 3 for details). This fit gives a good approximation of the signal, and allows measuring

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1 PhD student in the Department of Microfluics from 2004, supervised by M.P. Hughes
Figure 2.7: Spectrum of yeast cells measured by manual experiments. The red line is a fit using the 1-shell model developed in Chapter 3.

Figure 2.8: Histogram of the noise measured in yeast spectra, in manual experiments. The noise is measured by subtraction between the data and the fit.

the level of noise by subtraction. This figure puts forwards the level of noise that can be found in the data: this can be observed better on Figure 2.8 on page 30, which present a histogram of the noise with 155 points taken from several experiments.

The spectra measured on K562 cells are presented below: Figure 2.9 on page 31 presents a spectrum of K562 cells acquired from a manual experiment, and Figure 2.10 on page
Figure 2.9: Spectrum of K562 measured by manual experiments. The red line is a fit using the 1-shell model developed in Chapter 3.

Figure 2.10: Histogram of the noise measured in K562 spectra, in manual experiments. The red line is a Gaussian fit.

31 presents the histogram of the noise measured on several spectra of K562 cells from different manual experiments. In both histograms, the noise spectrum shows a Gaussian-like shape, and the Gaussian fit in red on Figure 2.10 seems relatively close to the data, even if there is an important scattering.
Several experiments have also been performed on Jurkat cells. One of them is of particular interest: it measured the spectrum of three samples of Jurkat cells at three different times after they were last resuspended in the conductive medium. The samples used had a concentration of $3 \times 10^7$ cells/ml and the resuspension medium had a conductivity of $3 \text{mS.m}^{-1}$. The results are presented on Figure 2.11 on page 33: this figure presents three spectra, each one corresponding to a measure of the sample after a given time shown in the legend. It can be noticed that the time provided is the delay between the date of the last resuspension of the sample and the start of the manual acquisition. The acquisition itself was lasting between 40 and 60 minutes, depending on the ability of the operator. It can be seen that the results are contradictory: it seems like there is a significant change in the first crossover frequency when the cells has been incubated for 45 minutes in the medium, but this change is not present after 120 minutes. This illustrate the variability that appears in the manual procedure, and when using different samples of cells.

Finally, Figure 2.12 presents the measure of the electric parameters of the samples of Jurkat cells mentioned above, measured by the fitting technique presented in Chapter 3, for each spectrum at different times. Here the level of noise appears in the error bars: all the error bars exceed 100% of the measure, sometimes by far like on the top right hand figure. It is clear that the signal-to-noise ratio is much too low to allow a correct measure of the different parameters. This is mainly a problem of inverse resolution, as explained in Appendix B.

It is clear now that the recordings from manual protocols usually present a low signal-to-noise ratio, which has been observed to diminish quickly with a decrease of concentration, so that below a given threshold of concentration (typically below $10^6$ cells/ml for yeast cells) it becomes a matter of guessing the data among the noise. However, the performances of the measure can be increased in two ways: by increasing the amount of signal, which means working at high cell concentration (typically $10^8$ cells per ml for yeast cells), or by diminishing the amount of noise, which means averaging a number of replicates in order to diminish the amount of noise. In practice, some cell lines cannot be harvested at a concentration high enough to use the first method, so these ones have to be measured several times before obtaining a satisfying level of noise.

**Performing many replicates makes the experiment longer up to a point when**
Chapter 2. Analysis of the pre-existing protocol

Figure 2.11: Evolution of the spectrum of Jurkat cells over time.

Figure 2.12: Measures of different properties of the Jurkat cell lines from the spectrum, using the fitting technique presented in Chapter 3.
it becomes less reliable. The consequences of repeating an experiment are multiple. First, it increases the experimental time. One data acquisition takes from 40 to 90 minutes, so having to repeat 8 times the same experiment takes an entire day, which is an important problem when one considers that everything is performed manually. The operator is more likely to make a mistake after such a long time. Second, the repetition of the measure requires a repetition of the sample. If the operator chooses to change the sample being measured between each acquisition, he then needs to harvest a bigger quantity of cells, so he has to culture several flasks of a given cell line. But using different flasks has the effect to increase the variability between the populations being harvested, so it can lead to a variability in the results. Another possibility is to use the same sample for all the measurements in order to avoid this variability problem. However, the experiment time taking so many hours, it is difficult in that case to justify that the cells remain in the same state during the entire experiment.

2.6 Conclusion

The measure of DEP by microwell is a promising method but needs to be faster and more accurate. The method of measure of DEP by microwell as explained in this chapter has the potential to become a lab-on-a-chip technique: it could use little amounts of sample, requires relatively small areas of interaction so that it can be miniaturised, and it provides meaningful data. However, two main problems exist: its signal-to-noise ratio is dramatically low and its experimental time is too long to be of use in practice in a laboratory.

The solution that is proposed in this thesis is to automatise this protocol, as explained in the next chapters. This is guided by three main objectives:

1. Improvement of the signal-to-noise ratio up to a level that allows the use of curve fitting techniques
2. Reduction of the experimental time down to a duration comparable to the time required to wash the sample in the low-conductive medium (in general 10 minutes)
3. Reduction of the interactions between the operator and the measure down to the non-automatisable tasks
The methods used to meet these objectives are described in the next two chapters, first for the advances in the theoretical field and next for the technical improvements.
Chapter 3

Developments in theoretical fields

3.1 Introduction

The measure of DEP forces as described in Chapter 2 provides many information about the cell population. It not only allows measuring the properties of the particles in suspension, but can also provide information about the presence of multiple populations within the suspension. However, this information cannot be accessed directly from the raw signal obtained by the measure of the DEP force on the sample, and in practice a mathematical model of the experiment is needed in order to retrieve such data. In addition to this, the results obtained by the measure are very noise-sensitive. Therefore, the amount of signal available from the microchip, the quality of the image processing and the precision of the model used all have a great influence on the results, so they have been particularly studied in the frame of this work. The first section of this chapter on page 37 explains how the microchip can be optimised in order to provide a greater level of signal. The second section on page 43 presents the methods used in the image processing in order to retrieve the DEP spectrum from a series of images. The next section on page 53 details the validation methods that have been used in order to check the linearity of the image processing algorithm. The fourth section on page 70 explains some modifications that have been performed on the mutli-shell model presented on Chapter 1 on page 4 in order to simplify and increase the accuracy of the measure, especially at low frequency. Finally, the last section on page 89 proposes an analytical approach for the detection of multiple populations in DEP spectra.
3.2 Microwell optimisation

3.2.1 Study of the initial problem

The microwell used in the final version of the prototype is the result of an optimisation process. At the start of the present work, the measurements of DEP spectra were conducted with a manufactured microwell such as presented in Chapter 2 on page 19. The chip that held the microwell presented a problem at high frequency so that the high-frequency part of the spectra were not reliable. Therefore, an optimised design of these chips had been developed as part of this work. The initial chips were analysed by Finite Element Modelling (FEM), the relevant parameters were extracted and studied, and an adapted chip had been designed and manufactured using the theoretical results obtained.

The performances of the microchip depends on its geometry. The DEP force increases with the gradient of electric field, so it is easier to observe this phenomenon in the regions of high electric gradient. It is therefore important to shape the electrodes in a way that maximises the electric gradient in the region observed. In the case of the microwells, this means that the electric field must extend as far as possible into the centre of the well, where the signal is easy to visualise. In practice, the DEP force generated by the initial microchips presented on page 19 was typically observable in a 40 µm-wide band around the edge of the well, which corresponds to about 22% of the total surface observed by the camera.

In order to investigate the effect of the thickness of the electrode and insulator layers, a series of microchips was built manually with thicker electrodes. The dimensions selected were 100 µm-thick layers of copper and 100 µm-thick layers of insulator, in order to have a geometry clearly different from before. Such chips gave a signal measurable 100 µm further from the edge in the same conditions, which corresponds to about 50% of the surface observable. This test showed that it was worth investigating the effect of the geometry of the microchip on the amount of signal observed in order to optimise the dimensions of the layers.

The measure of the performances was performed by FEM. The behaviour of the microchips with different thicknesses of layers is difficult to conduct in practice because of the small dimensions of the layers. Therefore, a theoretical approach is preferred here:
the electric field is modelled by finite element modelling analysis (FEM analysis) and optimised to obtain a high electric gradient in the inner part of the well. FEM analysis consists of creating a computer model of the system in order to study and to simulate its behaviour, using the laws of physics to describe the evolution of the system. Several FEM programs exist, each one being more or less specialised in certain areas of physics. The software used in this study is Comsol 3.3a (Comsol AB) because of its versatility which makes it possible to simulate several physical phenomena at the same time, so that the model built here is re-used later on for the analysis of concentration effects on section 3.4.3 page 60.

The FEM simulation of a system depends on several parameters: the shape of the object, also called its ‘geometry’, the materials that constitute the object with their physical properties of interest, the laws of physics that describe the evolution of the system and the behaviour of the system at the boundaries of the geometry. Once these are set, the geometry is numerically segmented into tetrahedrons, or ‘elements’, and the software solves the equations from the physical laws on each element, and the solution can then be analysed. In order to solve the problem quickly, the geometry modelled must be as simple as possible so the number of elements generated by the segmentation remains small enough to fit the computer capabilities. However, those elements should not be too coarse because the precision of the result decreases if the size of the segments is too large. Therefore the geometry drawn initially has to be as simple as possible to allow a compromise between a small number of elements and a high accuracy. That is why most finite element analysis makes use of symmetries of the geometry in order to model only a part of it, the result is then retrieved in the whole object by using the axis and planes of symmetry.

3.2.2 The FEM model of the microwell

The microwell used in practice in the experiments is a 1.2mm-long cylindrical hole of 350 $\mu$m in radius. Because this model is to be used for more complex analysis, this tubular structure has been simplified into an infinitely long cylinder, with a similar layered structure along its wall. This geometry neglects the edges effect but, because the electric potential around the edges weakens rapidly with the distance, the DEP force in these regions is expected to be negligible at a short distance away from the electrode
and therefore is not observed in practice. It can also be argued that the radius of the microwell is about 4 times smaller than its length, so the distortion of the electric field is expected to remain confined around the layers situated at the extremities of the cylinder. This space accounts for 8% of the volume inside the microwell, so all in all the total contribution of the edges of the well to the DEP signal should be negligible.

The FEM model of the microwell can be reduced to a 2D element that includes half an electrode and half an isolation layer. The cylinder used here to simulate the microwell is a hole in a layered structure constituted by alternated layers of insulator and conductor. The conductive layer is 75 $\mu$m thick and is assumed to be perfectly conductive, so there is no potential loss across the electrode, and the insulator layer is 125 $\mu$m thick and is assumed to be perfectly isolating so there is no current passing through the insulator. As explained earlier, FEM analysis take advantage of the symmetries of the geometry: the microwell being a cylindrical structure, it possesses a vertical axis of symmetry. Therefore, as shown in Figure 3.1, only a radial section of the well needs to be modelled and the axial symmetry can regenerate the solution in the whole space.

It is possible to simplify the model further as shown in Figure 3.2: inside the microwell, the multilayered structure is periodic so the electric field is expected to follow a periodic pattern. Hence only a solution over a half-period of the structure is needed, the rest can be found by symmetry and periodicity.

Figure 3.2 shows the position of the rectangular element extracted from the radial section of the microwell. The orange square stands for the electrode, the grey one for the insulator and the blue for the suspending medium containing the cells. The physical laws relevant for this study concern the equations of the AC electric field for a frequency band of 100 Hz to 100 MHz. Therefore, the minimum electric wavelength is $\lambda_{\text{max}} = \text{frequency/speed of light} \simeq 33\text{cm}$, which is about 100 times longer than the radius of the microwell. In this situation the quasi-static approximation can be used to describe the electric field: the amplitude of the AC electric field to simulate can be calculated from the equations of a DC electric field with the difference that the material conductivity $\varepsilon$ is changed into a complex permittivity $\varepsilon^*$ that also include the material conductivity $\sigma$ and the frequency $\omega$, as follows:
Figure 3.1: Scheme of the model used for the DEP simulation. On the left: scheme of the 3D microwell structure; on the right: cross section extracted for the FEM.

\[ \varepsilon^* = \varepsilon - j \frac{\sigma}{\omega} \]  

(3.1)

\( j \) standing for the complex number. It is now possible to associate the equations of electrostatics to the geometry in the program. The material used for the electrode is copper (\( \sigma = 610^7 \text{ S/m}, \varepsilon = 1 \)), polyimide for the insulator (\( \sigma = 4.310^{-11} \text{ S/m}, \varepsilon = 3.4 \)) and distilled water in the well (\( \sigma = 2 \text{ mS/m}, \varepsilon = 80.2 \) at 20°C). The electrode external boundaries is set to 20 V, which corresponds to the amplitude peak to peak of the AC signal, the Z-axis is set to axis of central symmetry, the bottom boundary is set to symmetry plane (corresponding to an isolated state), the top boundary is set to antisymmetry plane (corresponding to a grounded state), and the insulator external boundaries is set to isolating condition.
3.2.3 Results and theoretical optimisation

The computer used for this study was equipped with an Intel Celeron 2.4 GHz microprocessor and 480MB of RAM memory and supported a maximum of 40,000 2-dimensional elements, with a decomposition at the second order. Figure 3.3 presents the result of the FEM analysis of the model presented above. As expected, the gradient of the electric field is more important around the edges of the electrode, which is a difficult region to observe with a microscope because of the light diffusion by the aperture.

As shown by the theory developed in section 3.3.2 on page 44, the measure of the DEP force is related to the average of the electric field along vertical lines. That is because the observation is performed by a camera that observes the microwell along the Z-axis, and cannot discriminate between two vertically aligned points. Therefore the parameter used to measure the performance of a simulated microwell is the average along the Z-axis of the DEP force generated on a homogeneous 1 $\mu$m diameter sphere, with a CMF of 1. This value was calculated for different thicknesses of electrode and insulator layers, the values being selected in given lists (17 $\mu$m, 35 $\mu$m, 70 $\mu$m, 100 $\mu$m and 125 $\mu$m.
Figure 3.3: post processing image of the FEM solution of the element of well.

for the electrode; 51 \( \mu m \), 96 \( \mu m \), 126 \( \mu m \) and 171 \( \mu m \) for the insulator). The range of the DEP force is defined by the distance from the electrode where the average DEP force calculated equals 1pN, which is the order of magnitude of the force exerted by the Brownian motion on the particle. The results are presented in Figure 3.4.

It appears from that study that the detection of the DEP force is improved when the thickness of the copper and insulator layers is increased. However, thicker electrodes have a greater surface of contact with the fluid, which decreases the resistance of the chip and decreases the value of the high-frequency cut-off. It can also be seen that the gain of detection range is not linear with the thickness of the layers, so that it is easy to extend the range of thin electrodes, but thicker ones requires more variations of thickness to extend their range. Therefore a compromise was selected using a well of 6 layers, with 70 \( \mu m \) copper and 120 \( \mu m \) insulator, for a total volume of 0.44mm\(^3\) and a high frequency cut-off estimated at 60 MHz. It can also be noted that this new microchip is designed in a way that reduced the surface of electrode that overlaps between the ground and the signal tracks, which is another way to lower the capacitance of the chip and so to higher
the cut-off frequency.

### 3.3 Principle of the measure of the DEP force from a series of images of the microwell

#### 3.3.1 Introduction

The high level of noise appearing in the manual protocol can be reduced by using more adapted image processing. Part of the problems observed in the manual experiments come from the image processing used, which only used a portion of the signal available and did not insure the linearity of the results (see section 2.4 on page 27 for more details). This has been solved by developing a model-based image processing and by increasing the amount of signal used on the images. The idea is to develop a physical model of the migration of a colloid exposed to DEP, and to use the images acquired to fit to the model. This section explains the model that has been developed to interpret the data and the treatments performed on the images acquired for the extraction of data.
3.3.2 Analysis of the cell dispersion in the microwell

The DEP force can be measured from the images acquired by the camera by using the equation of diffusion under a force field and the equation of light absorption, with the appropriate approximations. The variations of light observed in the microwell by the camera during an experiment are due to the movement of particles in the microwell: if the particles are less transparent to light than the surrounding water, which is the case for living cells, then their accumulation in a region appears darker. Conversely, when the same particles are repelled from a region, it appears brighter. Therefore the approach used here to model these variations of light consists of combining the equation that describes the evolution of the colloid concentration with the equation that predicts the behaviour of the light intensity, in order to link the level of light measured in a region with the cell concentration in that region at the moment the image is taken.

The evolution of the concentration is described by the equation of diffusion under a force field. Let us consider the situation of a volume of suspended cells under the influence of the DEP forces. Because the microwell has the shape of an empty cylinder, we use here the cylindrical space coordinates system with variables $r$, $z$ and $\theta$. Considering that a microwell is invariant by rotation around the $z$-axis, the system
is expected to be invariant over \( \theta \) so we can restrict the description of space to the two variables \( r \) and \( z \) only. As we are interested in the cell motion, we also need the time variable \( t \). Let us note \( c(r, z, t) \) the variable describing the cell concentration in space and \( F(r, z) \) the static vector field that describes the DEP force generated by the electric field in the microwell. The concentration is related to the force field by the equation of diffusion under a constant force. In the present case, this equation can be written as follows:

\[
\frac{\partial c(r, z, t)}{\partial t} = D \Delta c - \frac{1}{2\pi \eta a} \text{div}(c(r, z, t).F(r, z)) \tag{3.2}
\]

where \( \Delta \) is the Laplace operator, \( \text{div} \) is the divergence operator, \( a \) is the cell radius, \( \eta \) is the medium dynamic viscosity and \( D \) is the diffusion coefficient of the cells. This equation is very general and no analytical solution has been found in the case of a microwell geometry. However, it is possible to greatly simplify it if we consider the small-time approximation: this approximation considers the behaviour of the system at the moment when the force is applied, so the concentration is quasi-homogeneous over the time period considered, which makes the diffusion forces negligible. Hence Equation 3.2 simplifies into the following:

\[
\frac{\partial c(r, z, t)}{\partial t} \bigg|_{t=0} = -\frac{c_0}{2\pi \eta a} \text{div}(F(r, z)) \tag{3.3}
\]

where \( c_0 \) is the initial concentration. The force considered is the DEP force, so we can substitute \( F(r, z) \) by the analytical expression of the DEP force presented on Chapter 1, on page 6. We get the following result:

\[
\frac{\partial c(r, z, t \to 0)}{\partial t} = -\gamma \text{Re}(K).\text{div}(\nabla E^2(r, z)) \tag{3.4}
\]

And \( \gamma = a\varepsilon\varepsilon_0 c_0/3\eta k_{cell}, \varepsilon_0 \) being the relative permittivity of the medium and \( \varepsilon \) the electric permittivity of free space. This expression is used below to model the variations
of the light intensity over time as a function of the force field.

**The variations of light intensity are described by the Beer-Lambert law of absorption.** Empirically, for a reasonably low concentration, the absorption of light by cells is well described by the Beer-Lambert law, written as follows:

\[
I(r, \theta) = I_0(r, \theta) \exp(-k_{cell} l c)
\]  

(3.5)

where \(I\) is the light intensity collected by the camera, \(I_0\) is the light intensity that would be collected from a medium free of cells, \(k_{cell}\) is the linear coefficient of light absorption for the cell line, \(c\) is the concentration of cells in the medium and \(l\) is the length of the light path. The threshold of linearity for this law depends on the cell line and is usually relatively high. For instance, the yeast cell line used in the experiments has a linearity threshold above \(10^8\) cells/ml. When the cells experience DEP force, they move at different velocities according to the divergence of the electric field surrounding them, as expressed by Equation 3.4. Equation 3.5 above can then be re-written with the space variables as follows:

\[
I(r, \theta, t) = I_0(r, \theta) \exp(-k_{cell} l \int_0^l c(r, z, t) \, dz)
\]  

(3.6)

For the sake of clarity we introduce the Z-averaging operator noted \(< . >_Z\), so that the average of \(c(r, z, t)\) over \(z\) is noted \(< c >_Z (r, t)\). Equation 3.6 is then rewritten as follows:

\[
I(r, \theta, t) = I_0(r, \theta) \exp(-k_{cell} l < c >_Z (r, t))
\]  

(3.7)

It is experimentally time-consuming to measure \(I_0\) because it requires an additional measure with a cell-free medium. In order to remedy this, \(I_0\) is removed from Equation 3.7 by a normalisation. Experimentally, this normalisation consists of measuring the light collected when the concentration is homogeneous, just after the cell resuspension,
before the electric field is applied. An image taken at that moment is expressed as follows:

\[ I(r, \theta, t = 0) = I_0(r, \theta) \exp(-k_{cell}l) \]

(3.8)

By dividing Equation 3.7 by Equation 3.8, one obtains:

\[ \frac{I(r, \theta, t)}{I(r, \theta, t = 0)} = \exp(-k_{cell}l(<c>_Z(r, t) - c_0)) \]

(3.9)

And finally, by re-arranging Equation 3.9 we obtain the following:

\[ <c>_Z(r, t) = c_0 - \frac{1}{k_{cell}l} \ln \left( \frac{I(r, \theta, t)}{I(r, \theta, t = 0)} \right) = L(r, t) + c_0 \]

(3.10)

By combining Equations 3.4 and Equation 3.10, the relationship between the cell concentration and the light intensity appears in a form that can be used later for signal processing. Combining Equations 3.4 and Equation 3.10 provides the following result:

\[ \frac{\partial (L(r, t \to 0) + c_0)}{\partial t} = -\gamma \text{Re}(K). <\text{div}(\vec{E})>_Z(r) \]

(3.11)

And then:

\[ \text{Re}(K) = \frac{\frac{\partial (L(r, t \to 0))}{\partial t}}{-\gamma. <\text{div}(\vec{E})>_Z(r)} \]

(3.12)

Hence the measure of the variation of the light intensity at the beginning of an experiment provides a measure of the CMF force at any position in the well. This model
is the one used for the data processing explained next. It can be noticed that when the divergence of the electric field $\vec{E}$ tends towards 0, the right-hand term in Equation 3.12 diverges because the model reaches the limit of absence of signal. Therefore this equation is expected to work better in the regions of high electric gradient. This is generally the case at the neighbourhood of the electrodes, at the perimeter of the well, and has been optimised in a work explained in section 3.2 on page 37. It is also important to remember that Equation 3.12 is only valid in the small time approximation, which means that it only works in a reduced period of time after the application of the electric field. After this time limit, the linearity starts to deteriorate with the time. This time limit is also studied in section 3.4 on page 53.

### 3.3.3 Model-based analysis of the image

The data can be analysed by applying the model developed in Section 3.3.2 on page 44 to the data captured by the camera. This section presents how the model of cell diffusion under a force field presented by Equation 3.12 on page 47 is used to retrieve a DEP spectrum from a sequence of greyscale images. The processing is performed in three stages: a normalisation of each image, an averaging over circular regions defined by a pre-established mask, and a logarithmic processing step. These three stages correspond to the calculation of the function $L$ in Equation 3.10 on page 47, which is supposed to provide a linear measure of the DEP force.

An image of the microwell is presented on Figure 3.3.3 on the left. Because the microscope focal point is set to infinity, the circular aperture of the well appears as a slightly blurred spot on both images. However, the blurry region only affects the edges of the microwell: this can be seen on the figure on the right, where the roughened glass that hides the filament of the light bulb has been removed. In that case the filament appears neatly inside the well and the blurred region only affects about 20% of the apparent surface of the well, in a circular band around the edges. This could lead to artefactual results, but this region is not taken into account in the data processing for non-linearity reasons and so does not affect the results.

Equation 3.10 states that the concentration in the well varies linearly with the logarithm of the light intensity. The images taken from the camera give a measure of the light intensity, hence the function $L$ in Equation 3.10 can be found by dividing the light levels
Figure 3.6: Image of a microwell filled with distilled water taken with infinite focal length, with (right) and without (left) the light diffuser between the light bulb and the microwell.

Figure 3.7: Example of an image before and after normalisation.

on an image acquired at a given time by the reference frames acquired at time 0 and/or prior to the application of the signal in the microwell, when the cell concentration is still homogeneous. The resulting image is then processed again by a logarithm operation, and averaged over the regions defined by the mask. Two images obtained before and after the normalisation procedure are presented in Figure 3.7. The raw image of the microwell is on the left, the image treated with a normalisation and logarithm appears on the right. The zones of high and low concentration appear clearly on the normalised image on the right, and make it possible to distinguish between positive or negative DEP.

The camera is usually set to obtain a bright region in the well and a dark region elsewhere, so the image has a good contrast and the well can be easily detected by applying a threshold. This is useful for providing a mask of the well for the circular averaging,
which is used in the data processing to find the average of the function $L$ over $\theta$. The mask created for this purpose is constituted of 25 concentric shapes of equal surface, centred in the middle of the microwell, as shown in Figure 3.10 on page 52. These shapes should ideally be circular to fit the edges of the microwell; however the camera used is not ideal and can induce a slight distortion by stretching or compressing the X-axis of the image compared to the Y-axis. As a result, the well can appear slightly elliptic. In order to compensate this, the program that creates the mask detects the ellipticity and create the corresponding concentric areas. It must be noted that the diameters were selected such that the surface area of each concentric band is kept constant. That is because the value provided by an average does not have the same meaning if the size of the sample changes: a smaller surface does not have an equally statistical significance than a larger surface, which makes it difficult to compare the result provided by regions of different sizes. By keeping the surface area constant, one can compare the results between two bands and keep the statistical significance. The operation for creating the mask is performed in four steps:

- Detection of the well: the well is found by applying a threshold.
- Detection of ellipticity: the ellipticity of the image is processed by comparing the mean and the momentum along the X and Y-axes. The well is compared to an ellipse of equation $(\eta \times x)^2 + y^2 \leq R^2$, where $\eta$ is the ellipse ratio and $R$ its radius. $\eta$ is found by comparing the dispersion over the Y and X-axes of the points in the
well. The formulae used is $\eta = \bar{x}/\bar{y}$, where $\bar{x}$ and $\bar{y}$ are the standard deviations of $x$ and $y$ respectively. Then the radius is found by using the moment of inertia of the ellipse, which is defined by the following:

$$\sigma^2 \triangleq \int \int (x-x_0)^2 + (y-y_0)^2$$

$$\sigma^2_{\text{ellipse}} = \frac{1}{3} \frac{R^4}{\eta^5} (3(\eta^2 - 1)^{1/2} \eta^2 - 2(\eta^2 - 1)^{3/2} + 3\eta^4 \arcsin(1/\eta))$$

Hence the moment of inertia can be calculated numerically, with the first equation, the second equation serves to isolate the radius $R$ as a function of $\eta$ and $\sigma^2$. This is performed by the file `Find_mask.m` in the DEP program.

- Segmentation into bands: the concentric bands used for the averaging are generated using a function that has the geometric properties to divide the ellipse into rings of equal areas. This procedure is performed in a few tenths of seconds by the program developed in this thesis and only has to be run once for each experiment, which is fast enough to be negligible compared to time taken by the rest of the data processing of one experiment (typically 5 minutes). Figure 3.10 presents a mask obtained by this mean.

The model developed in Section 3.3.2 states that the average of a normalised image over one band of the mask obtained is proportional to the average cell concentration in the well.
corresponding area of the microwell, and so the DEP spectrum can be found from its
time-variations according to Equation 3.12 on page 47. The final stage of the image
processing is the averaging of the normalised images obtained and the calculation of the
average time-variation in the inner bands.

In practice, the division of two images required by the normalisation procedure men-
tioned previously takes a relatively long time and it is preferable to use simpler operations
such as addition and subtraction, which are optimised to work at a very low level on a
computer so to work much faster. In order to do this, the function $L$ can be processed
according to Equation 3.14:

$$L(r,t) = \frac{1}{-k_{cell}}(<ln(I(r,\theta,t=0))>_{\theta} - <ln(I(r,\theta,t))>_{\theta})$$  \hspace{1cm} (3.14)$$

where $\frac{1}{-k_{cell}}$ is an experimental scaling factor. That way, the treatment of the logarithm
is performed first on the raw images, then the images are averaged over the regions
defined by the mask and finally the normalisation is performed by a simple subtraction.
This makes the processing much faster: 12 images of dimension 800x600 can be processed
in 2 seconds with a Celeron(R) 2.20 GHz microprocessor instead of 7 seconds in the case
of a normalisation by division on the same computer. It must be remarked that the
experimental scaling factor was not measured experimentally because the total light

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{mask.png}
\caption{Mask obtained for 25 zones from image 3.3.3}
\end{figure}
path has to include the thickness of the evacuation zones around the microwell, which are not easily measurable. However this quantity does not vary from one measurement to another so the scaling factor remains constant for one cell line, and the data acquired at different frequencies are comparable.

3.4 Theoretical validation of the data processing algorithm

The analysis shown in the previous section avoids the presence of a bias in the result. The analysis exposed in the previous section demonstrates that the DEP force can be measured linearly from the data acquired by the machine. However, the linearity can only be assumed for a small time compared to the diffusion process, so there is a time limit above which the data starts presenting non-linearities. This section presents a method that has been developed to measure this limit, and to test the validity of the data processing in order to check if the data processing algorithm is consistent with the theory.

3.4.1 Methods

The validation of the data processing was first performed with simulated data. The image processing procedure outlined in the previous section needed to be validated before being used experimentally, in order to test its linearity, make sure there is no major error in the code, and check that the processing is consistent with the theory. In practice, it has not been possible to obtain a sample that corresponds to the geometry of a living cell, and for which the electric conductivity and permittivity were perfectly known. Therefore the validation was performed numerically, by simulating the data obtained by a perfectly know sample and by processing the data obtained with the algorithm presented above. A set of data has been generated from the theory explained above by numerical simulations. This generated a set of images, which have been processed by the methods described in the previous section in order to check if the results obtained were coherent with the numerical values selected initially. The simulation of data has been performed partly by Maple v.10 and partly by Finite Element Methods (FEM) with the software Comsol v.3.4 (Comsol AB.). The method consisted of simulating the migration of punctual particles in a microwell electric field according to
the DEP law, and to observing how the concentration of these particles varies over time. From these results, it was possible to obtain images of what would be the observation by a microscope. These images can then be processed in the same way as experimental data by the software developed for this purpose.

The different steps of this work are explained below: the model of the electric field was processed analytically with Maple v.10; the analytical solution obtained to describe the electric field generated by a microwell was then implemented into the simulation of cell migration in the DEP well in order to provide a simulation of the DEP migration; these simulations were analysed further in order to obtain a set of images; finally the images were analysed and the results were compared with the initial conditions used to simulate the colloid. It must be noted that the purpose of this method is not to check if the theory fits to the data, which has been done previously in the literature as explained in Chapter 1, but to check the validity between the data processing algorithm and the theory as well as determining the limitations of this method.

3.4.2 Analytical expression of the electric field

Section 3.2 on page 37 presents the FEM of the electric field that was developed in order to analyse the geometry of the microchip. This model was accurate enough to provide the results expected, but FEM data has an intrinsic problem of discontinuity. The reason for that comes from the technique of FEM itself, which makes the approximation that the field to calculate is a sum of polynomials defined in restricted portions of space. This creates two problems: firstly, the spacial restrictions create a discontinuity at the border of each element; secondly, a finite polynomial cannot be derived infinitely so it generates problems when the solution found is re-used into an equation were it is derived.

Because the aim here is to simulate the migration of the particles due to DEP, and because this motion is described by differential equations that uses the derivation of the electric field, the problem cited above appear when one try to use the FEM solution of the electric field found previously. In practice, this means that the calculation of the evolution of the concentration does not converge, especially at the boundaries between elements where the electric field is discontinuous. Therefore it was necessary at this point of the work to develop an analytical expression of the electric field generated in
the microwell: this approach prevents the divergences in FEM resolutions and provides more accurate data. The resolution of this analytical problem is presented below.

**Using reasonable approximations, it is possible to obtain an analytical expression of the electric field generated inside the microwell.** The work presented here uses the cylindrical model of the microwell presented earlier, on page 38 of this Chapter. The geometry of the electric field in the microwell dictates the evolution of the cell concentration over time. Hence it is the first step to model the migration of the cells in the microwell. This section presents the analytical solution of the electric field generated in a multilayered microwell. It uses the technique of resolution by Fourier transform in 2D axisymmetric geometry. Two approximations have been made to simplify the problem so that it can be solved: the first one is the approximation of a dilute system, the second one is the approximation of quasi-static electric field.

**The colloid is considered as dilute if its concentration is below a given threshold.** It is assumed here that the presence of colloid does not modify the electric field, which is a reasonable approximation in the condition of a dilute suspension, where the average distance between the particles that constitute the colloid is much greater than the diameter of the particles $d$. Hence this condition is valid when the concentration of particles is below a threshold $c_m = 1/d^3$ that depends on the radius of the colloid. For yeast cells, average radius is about $6 \, \mu \text{m}$, which gives a threshold of $1 \times 10^9 \, \text{cells/ml}$. This threshold can also be observed experimentally in Chapter 5, but most of the experiments conducted are below this limit so the model developed here still holds.

**The electric field can be accurately modelled by the quasi-static approximation if the frequency is lower than 3 GHz.** As mentioned in Chapter 4 on page 38, the electric field can be modelled accurately by a DC electric field if the wavelength of the signal is much greater that the dimension of the structure. For a microwell structure such as the one described here, the maximum wavelength is about 100mm, which corresponds to a frequency of 3 GHz. This is much greater than the maximum frequency of 20 MHz used in practice, so this approximation is valid.

**The problem depends on the wall potential, which has to be determined first.** The electric field generated by this structure is found by solving the Laplace equation if the boundary conditions of the domain to solve for are known. These boundary
conditions correspond here to the electric potential at the surface of the well wall. This is the wall potential, and it must be found first before solving the problem.

The quasi-static approximation used to model the electric field uses a DC voltage at the electrode, therefore the potential is set to zero at the grounded electrodes, and to Vrms at the others. Vrms has been set to 1 for the simulation, the result can be scaled after the processing because the problem is linear. So the electric potential is set at the surface of the electrodes, but it still has to be found at the surface of the insulator. For that, it must be remembered that the insulator is supposed to be perfectly insulating, which means that no current passes through it. This means that the current flows tangentially to the surface of the insulator, so the current density is constant at the surface of the insulator. Therefore the potential drop is linear over the surface of the insulator. The shape of the electric potential in function of the height at the surface of the wall is shown on Figure 3.11.

**The Laplace equation of the electric potential cannot be solved directly, but has to be solved via a Fourier transform of the wall potential.** The electric field generated by certain electrodes can be found by a direct resolution of this equation, that is the case for the hyperbolic electrode for instance as shown by Maple file Hyperbolic electrode.mws in the folder ‘Maple codes’ on the CD. But in general, it is not possible

![Figure 3.11](image.png)

**Figure 3.11:** Electric potential at the surface of the wall in function of the height. Y-axis: electric potential in Volts; X-axis: height in m.
to solve the Laplace equation directly from an electrode of complex geometry such as the microwell. In such situations, there are techniques that allow a resolution by indirect calculation, usually using Fourier transforms. By decomposing the wall potential into a Fourier series, it becomes possible to solve the Laplace equation for each harmonic of the series, and therefore to obtain an analytical resolution of the overall by superimposing the solution for each harmonic. The main stages of the calculation are shown below, but for more details the entire calculus has been included on the CD in the Maple file Electric field in a microwell-exact solution.mws in the ‘Maple codes’ folder.

1. **Decomposition of the wall potential:** the first step of the resolution is to decompose the wall potential into Fourier coefficients, which means finding the different coefficients of the series. Let us call $a_n$ the coefficient of the $n^{th}$ term of the cosine part of the series and $b_n$ the $n^{th}$ term of the sine part. For a wall potential such as described above, we obtain:

$$a_n = 2 \cos(\omega_n l_e/2) - \cos(\omega_n (l_e/2 + l_i))$$

$$b_n = 0$$

where $l_e$ and $l_i$ are the thicknesses of the electrode and insulator layers respectively, $n$ is an integer and $\omega_n = \frac{n\pi l_e + l_i}{l_e}$ is a spatial frequency defined by the periodic arrangement of layers that constitute the microwell. The $b_n$ coefficients are null because the wall potential is a symmetric function, this has been chosen to simplify the calculation.

2. **Resolution of the Laplace equation for a generic term:** Now that we have the expression of a generic term of the Fourier series of the wall potential, we can solve the Laplace equation for it to obtain the contribution from that term to the final electric potential in the microwell. The Laplace equation in axisymmetric geometry is given by Equation 3.17:

$$\frac{1}{r} \frac{\partial V_n(r, z)}{\partial r} + \frac{\partial^2 V_n(r, z)}{\partial r^2} + \frac{\partial^2 V(r, z)}{\partial z^2} = 0$$

It can be shown that the function of two variables $V_n(r, z)$ that are solutions of this equation are a product of two sub-functions, one of variable $V_{rn}(r)$ and the other of $V_{zn}(z)$. This is known as a variable separation technique and provides
two separate differential equation, one for each sub-function.

\[
\frac{dV_{r_n}(r)}{dr} + r \frac{d^2V_{r_n}(r)}{dr^2} = rC V_{r_n}(r) \\
- \frac{d^2V_{z_n}(z)}{dz^2} = CV_{z_n}(z)
\]

where \(C\) is a constant common to both equation. This system of equations can be solved for a given harmonic of the wall potential as boundary condition.

\[
V_{r_n}(r) = \frac{I_0(\omega z_n r)}{I_0(\omega z_n R)} (3.20)
\]

\[
V_{z_n}(z) = a_n \cos(\omega z_n z) (3.21)
\]

\[
V_n(r, z) = V_0 V_{r_n}(r) V_{z_n}(z) (3.22)
\]

Equation 3.20 is the exact solution for the electric field generated by the \(n^{th}\) harmonic of the wall potential in the microwell: \(V_0\) corresponds to the rms value of the AC signal used in the experiment, \(a_n\) is the \(n^{th}\) coefficient of the Fourier series of the wall potential as defined by Equation 3.15, \(I_0\) is the modified Bessel function of the first kind at order \(\nu = 0\) and \(R\) is the radius of the microwell. At this stage, we can already see a major difference between planar and tubular electrodes: as mentioned earlier, planar electrodes provide exponential-shaped electric field that create a film of electric gradient, and therefore cannot be used efficiently at long distances, whereas tubular electrodes such as the microwell structure provide first kind Bessel-shaped electric fields, which decrease over longer distances than exponential ones and therefore reach longer distances. This validates the choice of microwell electrodes for this study.

3. Reconstruction: because electric potentials add up, the sum of the contribution of each harmonic to the total potential is the sum of the potentials created by each harmonic. Therefore, the total electric potential in the microwell is given by the following:

\[
V(r, z) = V_0 \frac{l_e}{l_e + l_i} + \sum_{n=1}^{\infty} V_n(r, z)
\]

The series in Equation 3.23 does not collapse into an obvious function, so it can only be approximated numerically for a given number of harmonics. It can be noted that the \(a_n\) coefficients are zero for even values of \(n\), so the numeric evaluation of
the series is relatively fast. The accuracy of the reconstruction can be checked by measuring the electric field at the wall of the microwell and by comparing it to the theory presented on Figure 3.11.

Equation 3.20 is of great interest to understand how the electric field extends inside the microwell and is a reference for the optimisation of the chip. As mentioned earlier, the total potential is a product of two sub-functions, one that depends on $r$ and the other that depends on $z$. The $z$-subfunction is a simple cosine function, which is linked to the Fourier transform and is the representation of the shape of the harmonic is the electric field. It can only be modified in amplitude by changing the ratio between the thickness of the different layers, but it remains a cosine-shaped function. However, the $r$-subfunction is more interesting: it is a modified Bessel function of the first kind that mainly depends on $\omega z_n$, the spacial frequency of the harmonic. A modified Bessel function is similar in shape to an exponential function: it is continuously increasing or decreasing, with a progressive slope. The gradient of the slope depends on $\omega z_n$, it is the equivalent of the 'time
constant’ for an exponential model. This means that as $\omega z_n$ increases, the extent of $Vr_n$ diminishes in the well. Therefore, the higher the frequency, the shorter the range. This also means that choosing thin layers of electrode or insulator leads to a higher gradient of electric field but also to a shorter range of action. The best compromise is when the electrode and insulator layers have the same thickness, which minimises the amplitude of the high-frequency harmonics and increases the one of lower ones.

It can also be seen that the maximum extent of the electric field into the well is given by the first harmonic of $Vr_n$, which corresponds to the fundamental frequency given by $\omega_1 = \frac{\pi}{l_e + l_i}$ and so depends only on the thickness of the layers, and not on the radius of the microwell as one could expect. Therefore, the radius of a microwell should match the thickness of the electrode and insulator layers in order for the electric field to reach a significant portion of space in the well. A radius which is too large leads to a wide unused space, and so to a poorly optimised design, whereas inversely a radius that is too thin does not make use of the fundamental frequency and so is not optimal either.

3.4.3 Creation of simulated data

The expression of the electric field is used in the FEM model of cell migration. Now that the electric potential in the microwell is known, it is possible to implement it in a FEM software and to process the evolution of the concentration of particles over time induced by the DEP force.

The model of electric field above was used with Comsol for finite element modelling for stability reasons. The numerical methods used to solve a diffusion problem tend to diverge quite easily if the problem is not defined accurately. At first, the calculation of the concentration was performed using an approximation of the electric field calculated by FEM methods, but doing so resulted in a problem that could not converge towards a stable solution. The reason is that simulating the electric field by FEM induces some artefactual discontinuities in the potential between the elements of the mesh, which cause major problems of stability in the convergence of the solution when solving the time-dependent evolution of the concentration. That is why only the analytical solution presented in the previous section is used here: this solution had the
advantage of being continuous and so provided the convergence and gives reliable data as shown in the next section.

The model of diffusion used here is the one developed on section 3.3.3 on page 48, plus the diffusion term $D \Delta c$ that was added in order to check that it really is negligible. Therefore, the form used is the following:

$$\frac{\partial c}{\partial t} = D \Delta c - \frac{d^2 \varepsilon_m \varepsilon_0 \text{Re}(K(\omega))}{3\eta} \text{div}(c \nabla (E^2)) \quad (3.24)$$

Equation 3.24 is the equation of diffusion used by Comsol for the FEM resolution, where $\Delta c$ is the Laplacian of the concentration, $\varepsilon_m$ is the relative permittivity of the medium, $\varepsilon_0$ is the permittivity of vacuum, $\omega$ is the frequency of the electric field and $\text{Re}(K(\omega))$ is the real part of the Clausius-Mossoti factor, as explained in Chapter 1.

The first term, the one containing the Laplace operator, is due to the thermal diffusion and expresses the fact that the random motion of the particles due to thermal agitation tends to homogenise the concentration. The second term is constituted by a constant
ratio containing the different experimental constants such as particle diameter, viscosity, and so on, and the divergence of the concentration times a function of the electric field. This last term is what makes this equation difficult to solve, because it depends on two separate entities, the concentration and the electric field, which cannot be separated or solve independently. It can be noticed that no analytical solution has been found for that equation during this research, so it has to be solved numerically by FEM.

The software used for solving the equation is Comsol multiphysics 3.4 (Comsol AB.) using the 'convection and diffusion' mode. This mode is designed for modelling the convection phenomena with transport of matter by fluid flow. The values of diffusion and flows can be entered in a GUI that summarises the physical properties of the problem. However, the problem presented above is not completely a water-flow problem because the force term does not derive from an incompressible field. For instance, an increase of concentration can be seen as a compression of the amount of particles. This means that an extra term appears in addition of the flow rates asked by the GUI. This extra term is entered as a reaction rate on a dedicated space of the GUI.

This reaction rate-like term is found by decomposing the term of DEP force into a flow-like term and a residual term that is used as a reaction rate.
The first term is a flow-like term which can be implemented as such in Comsol by placing the DEP drag as a flow field. The second term appears because the DEP force does not derive from an incompressible field, and so its divergence is non-null. This is the term that appears in the reaction rate in Comsol.

The model implemented in Comsol provides a grid-independent solution. Once the model is implemented as an FEM model, it can be solved numerically. The solution must be compared at different mesh density in order to make sure that it is not mesh-dependent, which would indicate poor accuracy of the solution. The problem has therefore been solved with different meshes using an increasing number of elements, until no significant evolution in the result is observed. The results presented in Figure 3.4.3 are the mesh-independent results obtained from this process for a negative DEP force on a 10μm diameter spherical particle, at 10 V peak-to-peak voltage, for $Re(K) = -1/2$, in water. The colour presents the concentration of particles after 100s of application of the electric field, the horizontal and vertical axes standing for the $r$ and $z$-axes respectively in cylindrical coordinate system. The dynamic result can be seen on the file ‘Video-simulated negative DEP’ in the folder ‘Videos’ on the CD, along with another film that
shows the simulation of a positive DEP force in the same conditions, 'Video - simulated positive DEP'.

The simulations presented above have been used to simulate the experimental data for the purpose of comparison. The FEM simulation presented in the previous section makes it possible to simulate an experiment of cell migration by DEP in a known environment. Hence it is possible to simulate the experimental data acquisition with a given set of CMF values for the purpose of testing the image processing protocol presented on page 43. The creation of an image from the FEM simulation uses the Beer-Lambert law of absorption to model the interaction of the light beam with the concentration in the well:

\[ I_{\text{out}} = I_{\text{in}} \exp(- \int_{z=0}^{Z} k c(z) dz) \]  

(3.26)

where \( I_{\text{out}} \) and \( I_{\text{in}} \) are the intensities of the light beams arriving in and going out of the well respectively, \( z \) is the height of the point considered along the light beam, \( Z \) is the thickness of the well, \( k \) is the absorption coefficient of the particle mass and \( c \) is the concentration of particle. This equation can be used numerically on the simulated data to produce an image of the well simulated observed under a homogeneous light. This
Figure 3.17: Map of the concentration obtained for negative DEP. The red line is an example of integration path used for the creation of images.

was performed by using equation 3.26 along vertical lines as presented on Figure 3.17, using a homogeneous level of light $I_{out}$ from the bottom of the well. This calculation model the absorption of light along such a path and create an image similar to that observed by the camera. However, it does not take into account the diffraction of the light beams around the edges of the well, but as mentioned earlier on page 49 this zone corresponds to a small portion of the surface of the well on the image and is not used in the data processing, so this simplification does not affect the result.

For each linear path selected, a circular band was plotted on the simulated image at the corresponding radius. The result obtained by such an operation is presented in Figure 3.18. According to the Beer-Lambert law, this graph represents the level of light that comes out of the microwell at a given time after the application of the electric field.

This procedure was repeated with the simulated data for different values of time, and gave a series of images comparable to the one obtained from an experiment so that it could be analysed by the program developed in section 3.3.3 on page 48. Such an analysis provided the DEP force as measured by the data acquisition program, which could be compared to the value used by the simulation in order to check the presence of
bias. By repeating this procedure for different values of the DEP force, one can measure the limitation of the algorithm used in the data processing program. The results of this analysis are presented in the next section.

### 3.4.4 Results and limitations

#### 3.4.4.1 Introduction

The **images obtained from the simulation can provide information about the limitations of the data processing**. The technique presented above makes it possible to simulate the data obtained from an experiment with given DEP spectrum and particles. Hence it is possible to process a set of simulated data with known values of the CMF and known particles and check that the result obtained by the data processing algorithm corresponds with the data that has been used initially for the simulation. This procedure is a way of checking the linearity of the data processing algorithm and verifying the extent of its domain of validity.

However, the linearity time limit is likely to vary with one or several parameters. We must then investigate the parameters of importance for this limit in order to orientate the study. A way of isolating these parameters of importance is to consider the origin of this time limit, which is explained in section 3.3.2 on page 44: this limit comes from the small-time approximation used to simplify the equation of diffusion 3.3 on page 45. However, this equation can be re-written as follows:

![Figure 3.18: Images created from the results of the FEM simulation. Left: initial image at time 0. Right: image obtained after 3s.](image-url)
\[
\frac{\partial c(r, z, t)}{\partial t}_{|t=0} \times \frac{1}{c_0} = \alpha \times \text{div}(\vec{F}_n(r, z))
\] (3.27)

where \( F_n \) is the normalised DEP force field and \( \alpha = -\frac{1}{2\pi \eta a} \times F_0 \) is a value that only depends on the frequency, \( F_0 \) standing for the scaling factor coming from the normalisation of the DEP force. If one replaces the amplitude of the DEP force by the expression in Equation 1.1 on page 6, one obtains the following:

\[
\alpha = -\frac{1}{2\pi \eta} V_0^2 \pi a^2 \varepsilon_m \varepsilon_0 Re(K(\omega))
\] (3.28)

It appears then that the evolution if the concentration only depends on the geometry of the force field, which is constant, and on its intensity, which is a combination of the particle radius, the CMF and the voltage. Therefore, the only cause for a variation of the linearity time limit in a microwell-based measure is the variation of the intensity of the DEP force. In the simulations, this intensity varies by changing the CMF before each simulation, the reason being that \( \alpha \) is proportional to the CMF. That is the reason why the values of the CMF used below exceed the real values.

**The 2% limit of linearity for the data processing is typically around 2s at 20 Vpp, for \( K = 1 \), on a 10 \( \mu \)m-radius particle.** The simulations performed model the results obtained from a population of homogeneous spherical particles of 10 \( \mu \)m radius submitted to an AC voltage of 20 Vpp. The different simulations have been performed with linearly increasing values of the CMF, from \(-2\) to \(2\), and have given the series of curves presented on Figure 3.19. It can be seen on that figure that, even if it has not been explicitly implemented in the FEM simulation, the small-time approximation appears here in the fact that the data is very linear during the first seconds of evolution, and starts being distorted after.

Figure 3.20 presents the surface response of the error in the measure in function of time and amplitude of the DEP force (selected via a linear variation of the CMF). This result is valid in the conditions of the simulation, i.e. for a particle of 10 \( \mu \)m radius, for a voltage of 20 Vpp. But the results can be scaled to adapt to other experimental
Chapter 3. *Developments in theoretical fields*

Figure 3.19: Measures obtained for the CMF on simulated data, for different values of the evolution time.

conditions via the parameter $\alpha$ presented in Equation 3.27. Because $\alpha$ is proportional to the measurement, it is hence proportional to the error and can be considered as a scaling factor for the Y-axis of Figure 3.20. Hence, the results presented in this figure can be used for any experiment, provided that the Y-axis is scaled according to the changes of values of the cell radius and signal amplitude.

Let us illustrate this result with an example: several experiments have been conducted using yeast cells, which have a radius of $3 \mu m$ approximately, at an amplitude of 20 Vpp. Hence the ratio between the radius of the particle simulated and the yeast cells is 0.3, which, according to Equation 3.28, divide by a factor 0.09 the value of $\alpha$ from the simulation. The voltage being the same as simulated, the results provided by Figure 3.20 have to be scaled along the Y-axis by a factor 0.09 to be apply to this experiment. This means that the data obtained for yeast spectra remain within 1% linearity during the first 5s at most after the application of the electric field.

This study does not validate all the data obtained but only the data processing algorithm. The result presented above is important as it confirms that the
data processing algorithm does not have an intrinsic problem and that is can be used in theory. The limitation of this work is that the validation does not come from real data, so it is possible that other effect occurs that can damage the linearity. In practice, the most significant problem observed is the presence of microflows at low frequency, which distort the data as presented later in Chapter 5. However, this problem only affected a restricted portion of the spectra acquired and can easily be compensated, as explained later.
3.5 Analysis of the multishell model and thin-membrane approximation

3.5.1 Introduction

The measure of a DEP spectrum contains a great deal of information. In order to explore the possibilities of the measurement of the DEP force, it is essential to know what information can be extracted from an analysis. This section is therefore dedicated to the analysis of the DEP spectrum itself and the information that can be provided by the multi-shell model. It has been found that the DEP spectrum of a cell line can provide much information about that cell, but also has some restrictions: the signal-to-noise ratio in the data must be relatively high, and some information must be known about the cell such as the radius and, in the case of the normal multi-shell model, the thickness of the different layers. This section explains the nature of the information that can be extracted from the data using a multi-shell model to fit a DEP spectrum, and studies the signal-to-noise threshold necessary for the accuracy of the measure. That way, it is possible to set the system requirements in term of signal-to-noise ratio before making the efforts in the development of the apparatus, which is developed in the next chapter.

The DEP spectrum itself does provide information about the electric parameters of a cell, and the extraction of these parameters is complex. Two approaches has been observed in order to extract the cell parameters from the data. The first one is an original method developed as a part of the thesis and uses a set of analytical equations related to the model. The other one is inspired from the traditional curve fitting method commonly found in the literature, but it has been found that the method as presented in the literature is very dependant upon the thickness of the cell membrane, which is difficult to measure accurately. Therefore a part of the thesis has been dedicated to improve the curve fitting method and to propose a second method of data extraction that does not depend on the membrane thickness. Both method are complementary, as it is presented below.
3.5.2 Thin membrane approximation

3.5.2.1 Principle

It is possible to greatly simplify the expression of the CMF for the single shell model by using simple geometric considerations. The theoretical DEP spectrum for the shelled-sphere model is calculated in 3 steps, described earlier in Chapter 1 on page 6. First, the CMF of the inner sphere, $K_{\text{sphere}}$, is calculated as stated in Equation 1.2. Then the equivalent complex permittivity of the shelled sphere, $\varepsilon_{\text{eff}}^*$, is retrieved from $K_{\text{sphere}}$ as detailed in Equation 1.3. Finally the CMF of the shelled sphere is found from $\varepsilon_{\text{eff}}^*$. These equations include 7 independent parameters, $\varepsilon_c$, $\varepsilon_m$, $\varepsilon_s$, $\sigma_c$, $\sigma_m$, $\sigma_s$ and $\alpha$, which stand for the permittivity of cytoplasm, membrane and suspension medium respectively, the conductivity of cytoplasm, membrane and suspension medium respectively and $\alpha = \left(\frac{\delta + r^3}{r}\right)^3$, $\delta$ being the membrane thickness and $r$ the cell radius.

However, the cell membrane thickness is generally around 10nm for a normal cell, whilst the cell radius is usually bigger than 1µm, so $\alpha$ normally varies between 1.0001 and 1.001. Even with a very low noise in the experimental data, this very narrow range of values makes it difficult to solve $\alpha$ accurately and it is usually impossible to estimate the error on its measurement. However, the analysis of the single-shell spectrum revealed a way to remove the parameter $\alpha$ from the equations by using an appropriate approximation. Since $\alpha$ is experimentally very close to 1, it can be simplified into the first order term of its Taylor series:

$$\alpha = \left(\frac{\delta + r^3}{r}\right) \simeq 1 + 3\frac{\delta}{r} \quad (3.29)$$

This is the small-membrane approximation. It has the advantage of replacing a 3rd order polynomial by a linear term, which is going to simplify the calculus of the CMF. By substituting Equation 3.29 in Equation 1.3 and after simplifications, one obtains the following:

$$\varepsilon_{\text{eff}}^* = \frac{\varepsilon_c^* \varepsilon_m^* \varepsilon}{\varepsilon_c^* + \varepsilon_m^* \frac{\varepsilon}{\delta}} \quad (3.30)$$
The detail of the calculus can be found on the m-file `Equations in the thin-membrane approximation.mws` in the ‘Maple codes’ folder on the CD. We can see on Equation 3.30 that the factor $\frac{r}{\delta}$ is systematically associated with $\varepsilon_m^*$, so it is possible to group these two terms into one single variable: $\varepsilon_m^{*'} = \varepsilon_m^* \frac{r}{\delta}$. So we can define a modified conductivity and permittivity as follows:

$$\varepsilon_m' = \varepsilon_m \frac{r}{\delta} \sigma_m' = \sigma_m \frac{r}{\delta}$$

(3.31)

Therefore one obtains the following:

$$\varepsilon_{eff}^* = \frac{\varepsilon_* \varepsilon_m^{*'}}{\varepsilon_*^* + \varepsilon_m^{*'}}$$

(3.32)

$$K_{1\text{shell}} = \frac{\varepsilon_{eff}^* - \varepsilon_*^*}{\varepsilon_{eff}^* + 2\varepsilon_*^*}$$

(3.33)

Equation 3.32 is the expression of the CMF for the single shell model in the thin-membrane approximation. This expression can also be used to construct multilayered models, as the result can be used just like a normal CMF. The same technique can also be used for any other membrane, it only needs to exchange $\varepsilon_c^*$ with the CMF obtained from a multi-shell model with one less membrane.

The thin-membrane approximation makes it easier and more precise to obtain the surface capacitance and conductance from the measure. We can go further and exploit the expression of $\varepsilon_m^{*'}$ to find some interesting properties. $\varepsilon_m^{*'}$ is constituted by $\varepsilon_m$, the modified membrane permittivity, and $\sigma_m$, the membrane conductivity, and so similarly $\varepsilon_m^{*'}$ is constituted by $\varepsilon_m' = \varepsilon_m \frac{r}{\delta}$ and $\sigma_m' = \sigma_m \frac{r}{\delta}$. This makes it easier and more precise to calculate the membrane surface capacitance $C_S$ and surface conductance $S_S$:

$$C_S = \frac{\varepsilon_0 \varepsilon_m}{\delta} = \varepsilon_0 \varepsilon_m' \frac{r}{\delta}$$

(3.34)

$$S_S = \frac{\sigma_m}{\delta} = \sigma_m' \frac{r}{\delta}$$

(3.35)
Using this approximation allows finding the membrane capacitance and conductance per unit area without having to measure the thickness of the membrane, so the only error in the result is the error coming from the measure on the DEP spectrum. Of course, if one needs to find the values of $\varepsilon_m$ and $\sigma_m$ from a measure using the thin-membrane approximation, one needs to find out the thickness of the membrane $\delta$.

The thin-membrane approach is curiously not the current direction of research in DEP nowadays. Instead, several publications propose more complex models in order to account for the thickness of the membrane (Washizu and Techanum, 2008) or the anisotropy of membrane properties (Sukhorukov et al., 2001). Such research are complementary and could be merged with the thin-membrane approximation, but this would be another direction of research.

### 3.5.2.2 Validation and limitations of the thin-membrane approximation

The thin-membrane approximation being an approximation has necessarily some limitations. The thin-membrane approximation comes from the fact that the thickness of a membrane in a cell is much smaller than the cell radius for most cell types (see Appendix A for details), but it does not hold for thicker shells. Therefore the question arises to know what is the limit of the ratio between the thickness of a shell and the radius of the particle beyond which the thin-membrane approximation start being significantly wrong. The limit of this approximation can be found numerically by comparing the spectrum given by the normal CMF with the one given by the thin-membrane approximation for different values of membrane thickness. The difference between the two spectra can be measured and is compared to the 10% error threshold presented on Table 3.5.4 on page 87, so a maximum shell thickness can be sorted out of the models.

The discrepancies introduced by the thin-membrane approximation are measured by Monte-Carlo methods. The CMF of a single-shell sphere is a function of 8 parameters: the permittivities of the core, shell and surrounding, the conductivities of the core, shell and surrounding, the radius of the core and the thickness of the shell. These are too many parameters to allow a systematic study of the differences between the original model and its approximation. One of the ways to approach this problem is to use Monte-Carlo methods, which is a numerical analysis of the differences between both
models using a number of randomly chosen sets of parameters. For a correct analysis by Monte-Carlo methods, the number \( n \) of random sets of parameters must be greater than \( 2^n \), which means that at least 256 sets should be analysed to obtain a significant analysis on the 8 parameters of the CMF model. This analysis has been performed with Matlab Test_approximation_thin_membrane.m with a range of values presented on Table 3.1 on 10 000 random sets of parameters.

Some of the parameters can vary over several orders of magnitude: this is a problem if one uses a normally distributed random number generator because such a generator favours the population of values in the highest order of magnitude. This is of great importance here because the variations of the CMF are due to these wide ranges of magnitude so ignoring it would lead to a useless simulation. This can be fixed by using a logarithmic-scaled normally distributed random number generator, which takes a normal distribution of numbers over a logarithmic scale and scatters the histogram over several the orders of magnitude. The program is called Test_approximation_thin_membrane.m and can be found on the CD in the folder ‘Matlab codes’.

Once these values are selected, they are used to process the spectra of a normal 1-shell model and a thin-membrane 1-shell model. The results are then compared by subtraction and the maximum difference is stored. Figure 3.22 presents the results obtained by repeating this process 10000 times, taking the random sets of parameters created previously. The scattered data points have been organised according to the ratio between the thickness of the membrane and the radius of the particle, along the X-axis, the Y-axis standing for the measure of the error between the spectra in % of the signal.
Chapter 3. Developments in theoretical fields

Histogram of the values taken by $\sigma_c$ for a Monte–Carlo analysis

![Histogram of the values taken by $\sigma_c$ for a Monte–Carlo analysis](image1)

**Figure 3.21:** Typical distribution

Result of the Monte–Carlo study of the differences arising from the thin–membrane approximation

![Result of the Monte–Carlo study of the differences arising from the thin–membrane approximation](image2)

**Figure 3.22:** Results of the performance analysis of the thin-membrane approximation, using Monte-Carlo methods.
This figure shows that the thin-membrane approximation gives very accurate results for ratios below $10^{-3}$, which corresponds to the case of biological membranes for cells. However, the error increases in a linear fashion with this ratio, and can eventually reach 10% for ratios above $5 \times 10^{-2}$. This corresponds to the domain of cell walls such as found around certain bacteria or fungi. Therefore, the thin-membrane approximation is valid in the case of the membrane but cannot be applied to simplify the behaviour of cell walls. However, the main purpose of this approximation is to remove the thickness of the membrane from the curve-fitting algorithm, because this parameter is difficult to measure in practice and cannot be fitted accurately either, so the performances measured in Figure 3.22 demonstrate that this approximation fulfils its purpose. As for cell walls, their thickness varies usually between 0.1 µm and 5 µm so they can be measured more accurately if required.

3.5.3 Segmentation of the spectrum: analytical study of the single-shelled model

Analysis of the DEP spectrum may provide a method to find a first guess automatically to the curve fitting procedure. As stated earlier, both literature and experiments show that the shelled sphere geometry is appropriate to model the behaviour of a cell experiencing DEP forces. In particular, the single-shell geometry provides an analytical expression that is relatively simple compared to models with higher number of shells. This expression can be studied further to provide a set of equations that can help finding a first guess in the curve fitting procedure directly from the dataset, so the final fit would be more significant and the convergence of the fitting would be faster. In section 1.3 on page 4, it was explained that the single-shelled geometry provides an analytical expression for the spectrum of the DEP force. This expression is as follows:

\begin{align}
\varepsilon^{*}_{\text{eff}} &= \varepsilon^{*}_{m} \frac{\alpha + 2K_{\text{sphere}}}{\alpha - K_{\text{sphere}}} \\
K_{\text{shell}} &= \frac{\varepsilon^{*}_{\text{eff}} - \varepsilon^{*}_{s}}{\varepsilon^{*}_{\text{eff}} + 2\varepsilon^{*}_{s}}
\end{align}

A DEP spectrum generated by the single-shell model can be segmented into five regions of interest, each one providing different information. A typical
The single-shell spectrum is shown on Figure 3.23. It generally consists of 3 flat parts with 2 transitions in between. Each of these parts is a segment, and so it can be described by one point and by the slope. For the flat segments, the slope is zero so only a point is necessary. For the transitions segment, their slope is fixed by the levels of the plateau they link and by their extent on the spectra, which is set by the model to one decade. Therefore, the slope of each transition is only set by the levels of the plateaus it links, and so it can be described by a point too. At final, the DEP spectrum of a single-shell model without noise can be summarised by five points: the three plateaus can be defined by their respective level, and the two transitions can be described by the frequency at which they happen.

It can be noted that some publications in the literature use crossover frequencies, i.e. the frequencies where the CMF equals zero, because they are easy to measure experimentally and are useful in particle sorting experiments because they give the frequency where the behaviour of the particle switches from positive to negative DEP. However these crossovers do not always exist or remains at frequencies beyond the range of the signal generator equipment, so the mid-rise frequencies were preferred for the present study to
describe the transitions. These frequencies are defined as the ones corresponding to the point where a transition reaches its half-way, and appear as a red dots on Figure 3.23.

The segments obtained can be described by analytical equations that can be used for parameter solving. Each point reported on the spectrum above corresponds to an equation, so the spectrum of a single-shell model provides five independent equations. Let us call $K_l$ the low-frequency limit of the spectrum, $K_m$ the value reached by the spectrum between the frequency transitions, $K_h$ the high-frequency limit of the spectrum, $\omega_l$ the low-frequency transition and $\omega_h$ the high-frequency transition. $K_l$, $K_m$ and $K_h$ correspond to the level of the first, second and third plateaus respectively and $\omega_l$ and $\omega_h$ correspond to the first and second transitions on Figure 3.23. This gives the following set of equations:

\[
\begin{align*}
K_l &= \lim_{\omega \to 0} K(\omega) \\
K_m &= K \left( \exp \left( \frac{\log(\omega_l) + \log(\omega_h)}{2} \right) \right) \\
K_h &= \lim_{\omega \to \infty} K(\omega) \\
K(\omega_l) &= \frac{K_l + K_m}{2} \\
K(\omega_h) &= \frac{K_m + K_h}{2}
\end{align*}
\] (3.38)

This set of equations can be used to resolve the values of five of the parameters of the single-shell model, including the scaling factor between the real values of the DEP spectrum and the values measured, which has not be mentioned yet in this section but is an experimental variable that appears at the measurement stage. Here again the thin-membrane approximation presented in the previous section is useful: if it can be used, then only five parameters in the models are unknown, namely the conductivity and permittivity of the core, the permittivity and conductivity of the shell and the scaling factor, so they can all be solved. If the shell is not thin enough to use this approximation then we have to add the thickness of the membrane to the list of unknowns, so one of the unknowns has to be guessed in order for the system to be solved. Generally the thickness of the membrane for a living cell is generally around 10nm, so $\delta$ is often used as a parameter in the model in order to reduce the number of degrees of freedom down to five when necessary.
As no exact equation has been found for Equation 3.38, an approximation has been used. Equation 3.38 has some non-linear terms and so cannot be solved directly. However it is possible to linearise it by using several approximations. By doing this, some limitations appear that has to be quantified but, as the next sections will show, the limits stay within the range of what is being measured experimentally. The approximations made are first-order approximations concerning the frequency, and help simplifying the expression of $K_m$, $\omega_l$ and $\omega_h$ in Equation 3.38. The details are presented in the Maple spreadsheet Equations in the thin-membrane approximation.mws in the folder ‘Maple codes’ on the CD and provide the system of Equations 3.39.

$$
\begin{align*}
K_l &= \frac{(\sigma'_m - \sigma_s)\sigma_c - \sigma'_m\sigma_s}{(\sigma'_m + 2\sigma_s)\sigma_c + 2\sigma'_m\sigma_s} \\
K_m &= \frac{(\sigma_c - \sigma_s)\varepsilon'_m - (\varepsilon'_s\sigma_c + \varepsilon_c\sigma_s)}{(\sigma_c + 2\sigma_s)\varepsilon'_m + 2(\varepsilon_s\sigma_c + \varepsilon_c\sigma_s)} \\
K_h &= \frac{(\varepsilon'_m - \varepsilon_s)\varepsilon_c - \varepsilon'_m\varepsilon_s}{(\varepsilon'_m + 2\varepsilon_s)\varepsilon_c + 2\varepsilon'_m\varepsilon_s} - \frac{1.46 \times 10^{-9}}{a} \\
\omega_l &= \frac{\sigma'_m + 2\sigma_s}{\varepsilon'_m + 2\varepsilon_s} \frac{\sigma_c}{\sigma_c + 2\sigma_s} \\
\omega_h &= \frac{\sigma_c + 2\sigma_s}{\varepsilon_c + 2\varepsilon_s} \frac{0.143(12\varepsilon_s + 7\varepsilon'_m)}{7\varepsilon'_m}
\end{align*}
$$

Each segment of the spectrum is linked to some specific parameters of the problem. It appears from Equation 3.39 that each segment of the spectrum depends on specific parameters. The low-frequency segments depends more on the conductivity of the shell and the surrounding medium, the high-frequency segments depends mostly on the permittivity of the core and medium, and the middle segment depends mostly on the permittivity of the shell, the conductivity of the core and the properties of the medium. This also appears on the experiments, as it is presented in Chapter 5, and can be observed on simulated data by using the Maple spreadsheet Effect of the parameters on the spectrum in the ‘Maple codes’ folder.

The system of equations obtained comes from several approximations, so its accuracy has been tested by Monte-Carlo methods. The precision of the system presented in Equation 3.39 has been analysed by Monte-Carlo methods, just like for the thin-membrane approximation as presented in section 3.5.2.2 on page 73. This test has been performed with the Matlab script test_equations.m available on the CD, and have been using a sample of 10000 random electric properties over a range of selection
Chapter 3. *Developments in theoretical fields*

presented in Table 3.1 on page 74. The result obtained from the equations has been compared to the numerical values obtained from numerically calculated spectra in order to measure the error due to the different approximations made to obtain Equation 3.39. The results are presented on Figure 3.24 on page 81 for the amplitudes and on Figure 3.25 on page 82 for the frequency.

The system of equation found is accurate at less than 2% error for most of the experimental situations. The results presented on Figures 3.24 and 3.25 show that the system of equation 3.39 is generally accurate up to a few percents of error for the range of values selected on Table 3.1, which include most of the situations for real data. It can be observed that all the discrepancies fall within 2% error bars, except when the DEP spectrum is degenerated, which means that the transition frequencies are so close that the mid-frequency plateau disappears. Figure 3.26 on page 83 shows a degenerated spectrum. In such a case, the error explodes in the calculus of the transition frequencies and the parameter $K_m$. Therefore, Equation 3.24 can only be used when the transitions of the spectrum are well determined. In practice, the threshold value that separate the normal and the degenerated datasets is 1.3 decades, which is equivalent to a factor 23 between $\omega_l$ and $\omega_h$.

The set of equations obtained by segmentation can be solved for low-noise non-degenerated spectra, but the accuracy of the solution provided decreases rapidly with the addition of white noise. The Monte-Carlo study of the system of equation 3.39 has shown that this system is valid for a wide variety of non-degenerated noise-free spectra. It can then be solved in order to provide a measure of $\sigma_c, \sigma'_m, \varepsilon_c, \varepsilon'_m$ and the scaling factor. Because it is a complex non-linear equation, no analytical solution has been found so far so the resolution has been performed numerically with Maple 10.

Once again, the quality of the solution of the equation has to be tested by Monte-Carlo method in order to check if the precision and accuracy of the numerical solution found is low enough to be reliable, and to measure the conditions of a correct level of accuracy. The code created for the Monte-Carlo analysis of the resolution of the system 3.39 can be found on the CD, in the ‘Maple codes’ folder, in the file *Numerical resolution of the thin-membrane approximation equations.mws*: it works by creating on a random set of cell parameters selected in the same conditions as for the previous analysis, by generating a 1-shell DEP spectrum from it, by adding a white noise of given amplitude,
Chapter 3. *Developments in theoretical fields* 81

Figure 3.24: Results obtained from the Monte-Carlo analysis of Equation 3.39 on page 79 for a set of 10000 parameters selected according to Table 3.1 on page 74. $K_l$, $K_m$ and $K_h$ stand for the levels of the low, medium and high frequency plateaus respectively. The red dots stand for degenerated spectra, i.e. spectra where the transition frequencies are less than one decade further.
by segmenting the spectrum in order to find $K_l$, $K_m$, $K_h$, $\omega_l$ and $\omega_h$, by solving Equation 3.39 on the segments found and by comparing the solution obtained with the initial set of variables selected to measure the error on the results induced by the noise. This process has been repeated on 300 sets of data, and shows that both the accuracy and precision deteriorates with the presence of noise. As an example, Figure 3.28 presents the result of this analysis for the resolution of the scaling factor.

The poor performances of the resolution of the system of equation 3.39, compared with its good precision and accuracy, can be explained by its ill-conditioned nature. It can seem surprising that the system of equations 3.39, which presented a good approximation of the behaviour of the DEP spectrum as showed in Figures 3.24 and 3.25, provide a solution that is very sensitive to noise and that cannot be used in practice. This behaviour shows that the problem of solving this system of equation is an ill-condition problem: this means that, even with very precise equations,
Figure 3.26: Example of a degenerated 1-shell spectrum: here, the mid-frequency plateau does not appear.

Figure 3.27: Sequences followed by the Monte Carlo program written in Maple for the determination of the error in the resolution of the system of equations 3.39.
the solution can only be retrieved for very low levels of noise. This phenomenon is explained further in Appendix B and corroborate with the analysis made on the behaviour of curve fitting algorithms using the multi-shells model made in previous works (Gascoyne et al., 1995).

The poor behaviour of the solution with the presence of noise makes it preferable to avoid using the analytical method for measuring the parameters of the problem from the DEP spectrum, because of its additional inaccuracies due to the different approximations made during the development of the analytical calculation. In addition to this, Equation 3.39 comes from the analysis of a one-shell spectrum, but the complexity of the system increases with the addition of extra layers so that this approach cannot be re-used for a two-shell model. Therefore a more simple approach has been developed by using a curve fitting technique, as presented in the next section together with a comparison between both technique. However, it is useful to remember that each segment of the spectrum is correlated to a given set of parameters, as it come to be a useful information when
trying to find an initial guess, as explained in the next section.

### 3.5.4 Curve fitting and initial guess

**Proceeding by curve fitting is a solution to obtain the measure of the particle properties but can lead to additional errors.** The ideal solution to solve an inverse problem is to solve it analytically in order to obtain a model that can be used directly on the data. The previous section showed that this solution is not possible in the present case. The strategy used instead is then a model-based analysis, which assumes the shape of the solution and finds the set of parameters that makes it fit best to the data: this is the curve fitting technique and is used in most of the data analysis of DEP spectra in the literature. This technique is very powerful as it allows us to solve complex systems of equations, but it also requires particular attention as for the way to use it as it can easily create artefactual results, as explained below.

A curve fitting is an iterative process that minimises the distance between the data measured and the values provided by the model. In section 3.5 the multishell model used in this work is developed. Once a DEP spectrum is acquired, the experimenter determines the number of shells to use in the multishell model and an approximation of the measure expected. The data, the model and the approximation are then passed on to the curve fitting algorithm, which calculates the distance between the curves provided by the data and by the model (the distance between two sets of points is the sum of the distances between each pair of points), and then find a set of parameters that diminishes this distance. This two-step process is then repeated until the algorithm finds a minimum, which is interpreted as the measure.

Because it requires a first guess to initiate the algorithm, this technique depends strongly on the accuracy of this guess. If it is close enough to the real values, the algorithm can converge quickly and provide a good measure with an estimation of the accuracy, but if the guess is too far from the real values then the result does not converge and provides a random measure. This is a relatively minor problem as it only increases the time needed for the resolution, and a non-converged solution is easy to recognise. However, there are more important problems associated with this technique. One of them is the need of an appropriate model, as it is at the base of the analysis, but the multishell model presented here is robust and has been validated in several works in the literature in the
Figure 3.29: Evolution of the curve fitting after the optimisation process. The dashed line is a first, coarse approximation of the data (in blue) and is refined by the algorithm to give the plain red line.

limits of measures on living cells Broche et al. (2005); Sukhorukov et al. (2001); Raicu et al. (1996). Another important problem is the adjustment of the number of degrees of freedom in the model to match the degrees of freedom of the data. This means that the model used should not use more parameters than there are in the data, or the resolution can be inaccurate. Here again, section 3.5.3 showed that the spectrum acquired by the measure can be segmented in order to give the number of parameters it depends on, so the model needs to be adapted to match this number.

For instance, in practice, a spectrum from K562 cells can be segmented into 5 segments, each one depending mainly upon one parameter. This means that the model used to fit it can only use 5 parameters. In the case of the single-shell model, the fitting could be performed on the permittivity of cytoplasm and membrane, the conductivity of cytoplasm and membrane and the scaling factor for instance. This means that the radius of the cell and the thickness of the membrane, which appear in the model, have
Table 3.2: Threshold of noise in the spectrum corresponding to 10% maximum error in the measure of the cell properties.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Maximum level of noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_l$</td>
<td>Low-frequency value</td>
<td>± 0.03 (absolute)</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Mid-spectrum value</td>
<td>± 0.03 (absolute)</td>
</tr>
<tr>
<td>$K_h$</td>
<td>High-frequency value</td>
<td>± 0.03 (absolute)</td>
</tr>
<tr>
<td>$\omega_l$</td>
<td>Low-frequency transition frequency</td>
<td>7% (relative)</td>
</tr>
<tr>
<td>$\omega_h$</td>
<td>High-frequency transition frequency</td>
<td>7% (relative)</td>
</tr>
</tbody>
</table>

The problem here is that the thickness of a cell membrane is not easy to measure and can introduce additional errors in the result. That is why the thin-membrane approximation has been developed, as explained in section 3.5.2 on page 71, and can be of great use here by reducing the error in the result.

The last problem of importance arising when using the curve fitting technique is that it loses accuracy when it extrapolates the data that have not been measured. This occurs in practice because of the limitation of the spectrum at high frequency: most of the spectra acquired have a missing part at the high-frequency end that cannot be measured because the signal generator reaches its limit at 20 MHz. However such spectra generally have enough information to extrapolate the whole spectrum, but the accuracy of their fit is diminished and the error bars on certain parameters measured is greatly increased, as shown in Chapter 5.

The robustness of this method has been measured by Monte-Carlo analysis and give satisfying results. A study of the error propagation has been conducted on the curve fitting technique. The protocol is very similar to the one presented in the previous section for the analysis of the robustness of the equation system. It uses a set of randomly generated data to create a series of DEP spectra made of 20 data points, in a frequency range of 1 kHz to 20 MHz, in order to fit with the experimental conditions. Then a white noise of random amplitude is added to each of the spectra and a fit is performed on the result. The data provided by the fit is then compared to the data used for the simulation in order to measure the error. From the list of errors measured, it is possible to find the noise threshold that corresponds to 10% error in the measure. The results are reported in Table 3.5.4 below, and show that the curve fitting method remains robust for a relatively high level of noise.
These results have a major importance in the design of the machine because they set the maximum level of noise for the analysis. If we consider the parameters $K_t$, $K_m$ and $K_h$, the maximum error of $\pm 0.03$ can be reported to the range of the DEP spectrum, typically 0.5, to give a maximum admissible error of 3% on the measure of the DEP spectrum. This error threshold is re-used in various phases of development, in the data processing and in the performance analysis in Chapter 5. The implementation of the curve fitting methods is presented in Chapter 4 on page 130.

3.5.5 Conclusion

Equation 3.39 is ill-conditioned and cannot be solved analytically. The system of equations 3.39 presented on page 79 is an analytical representation of the problem of measuring the electric properties of a particles solution by DEP. The analytical approach consists of solving this system in order to obtain an analytical expression that can provide the measure according to the different points on the spectrum. This is known as an ‘inverse problem’. However, the complexity of Equations 3.39 makes it very difficult (if not impossible) to find an analytical solution, so that it can only be solved numerically. This method has been investigated with Maple 10 because this software allows solving non-linear polynomial equations with great precision. Unfortunately, even with great accuracy, the error in the resolution of Equation 3.39 is still well over 100%, so it has not yet been possible to find an algorithm that can solve Equation 3.39 systematically with a low level of error. Therefore a technique of curve fitting has been adopted.

The curve fitting technique is robust, but only if certain conditions are met. The curve fitting technique presented above is more tolerant about errors on the measure, but it requires an initial guess close enough to the solution and looses its accuracy if the spectrum is not entirely measured. Experimentally, the apparatus developed in Chapter 4 does not achieve the measure of the DEP force above 20 MHz. Unfortunately, this is lower than the high transition frequency so part of the spectrum is missing, and there is not enough information to solve the whole system without extrapolating the value of $K_h$. This has a dramatic effect on the precision of the measure of $\varepsilon_c$, as explained in Chapter 5.

The analytical approach could be used to provide a first guess. The coarse analytical solution provided by Equation 3.39 could be used as a complementary method
for a curve fitting technique by producing an approximation of the parameters, which
could be used as a first guess for the fitting. This could be a way to fit the data
automatically, without using external data by the way of the initial guess, and could lead
to a gain of time in the process and confidence in the data. This has not be implemented
in the final version of the program because such an implementation requires a connection
between Maple 10 (which solves the equation system) and Matlab 7 (which performs
the curve fitting) that was not available in the frame of this work.

### 3.6 Multiple populations analysis

One of the possibilities offered by DEP is the detection of a the number of populations
in a given sample. This is possible by measuring the DEP spectrum of a mixed sample
and resolving the spectrum that is provided. A relatively simple method is presented
here to perform such an operation. Let us take the example of two populations of cells
of concentration $c_1$ and $c_2$ respectively and absorbency $k_1$ and $k_2$ respectively. Equation
3.8 is then modified into the following:

\[ I(r, \theta, t = 0) = I_0(r, \theta) \exp(-l(k_1c_1 + k_2c_2)) \quad (3.40) \]

Hence, the function $L$ in Equation 3.10 is modified into the following:

\[ k_1 \Delta c_1 + k_2 \Delta c_2 = -\frac{1}{l} \ln \left( \frac{I(r, \theta, t)}{I(r, \theta, t = 0)} \right) = L(r, t) \quad (3.41) \]

Where $\Delta c_i$ is the variation of concentration of the species $i$, at each frequency measured.
So the measure of $L$ provides information on the linear combination $k_1 \Delta c_1 + k_2 \Delta c_2$.
It has been explained in 3.3.2 on page 44 that the variation of the concentration is
interpreted by the data processing as being proportional to the DEP spectrum (under
certain conditions). So the spectrum measured on a sample containing two populations
is the sum of the spectra of each individual population, say $K_1$ and $K_2$, with weighting
factors for each population, which are the product of the absorption coefficient by the
initial concentration. The equation above could then be re-written as follows:

\[ \alpha K_1(\omega) + \beta K_2(\omega) = L(\omega) \quad (3.42) \]
Where $\omega$ is the frequency, $\alpha = k_1 c_1(t = 0)$ and $\beta = k_2 c_2(t = 0)$. There are two possibilities from here: either the spectra are known (and then it is possible to retrieve the coefficients $\alpha$ and $\beta$), or the coefficients $\alpha$ and $\beta$ are known (and then it is possible to retrieve the spectra $K_1$ and $K_2$).

Let us consider the case where the spectra are already known. This can happen if one wants to measure the proportion of known species, like for instance the proportion of dead cells among a population. In that case, the spectra of living and dead cells are known and the absorption coefficients are identical. So the measure of the term $L$ in Equation 3.42 provide a system of equations, each one being the measure on one given frequency:

\begin{align*}
\alpha K_1(\omega_1) + \beta K_2(\omega_1) &= L(\omega_1) \\
\alpha K_1(\omega_2) + \beta K_2(\omega_2) &= L(\omega_2) \\
\alpha K_1(\omega_3) + \beta K_2(\omega_3) &= L(\omega_3) \\
\vdots & \\
\end{align*}  

The system of equations above is hyperstatic so it is easily solved.

Let us now consider the case where the spectra are unknown but the coefficients are. In that case, it is not possible to solve the problem with only one experiment so we need two experiments with two different ratio of populations. In that situation, Equation 3.42 provide the following system:

\begin{align*}
\alpha_1 K_1(\omega) + \beta_2 K_2(\omega) &= L(\omega) \\
\alpha_2 K_1(\omega) + \beta_1 K_2(\omega) &= L(\omega) \\
\end{align*}

This system can be solved for each frequency $\omega$ and provides both spectra. It is possible to separate the spectra of several populations of cells mixed together if enough data is provided. It is also possible to find the ratio of several populations of cells mixed together if these cells has similar absorption coefficients and if their individual spectra are known.
3.7 Conclusion

This chapter has presented several techniques of optimisation and data processing used at different levels in a DEP experiment. Several techniques have been presented here that aim to increase the signal-to-noise and the level of confidence the experimenter can have in the data acquired by the microwell technique. In section 3.2 on page 37 is presented on optimisation of the chip, which increases the amount of signal visible by the monitoring system, and therefore improves the conditions of the data acquisition. The pre-processing of the data is also re-thought in section 3.3 on page 43 with a image processing method that takes advantage of a model-based data extraction, which is validated in section 3.4 on page 53. The DEP spectrum obtained at this point is treated by curve fitting, as explained in section 3.5.4 on page 85, with a simplified model that brings more precise information, as detailed in section 3.5 on page 70.

All these improvements contribute to increase the signal-to-noise ratio in the results, which is expected to provide a level of accuracy high enough to allow measuring the properties of a sample. However, an important part of the noise that appear in the data originates in the manipulation of the sample during the experiment. Therefore, an important part of the present work has been dedicated in improving the manipulation of the sample by an automated device. This is presented in the next chapter.
Chapter 4

Description of the apparatus and data processing

4.1 Introduction

The aim of this project was to develop a device able to measure rapidly and accurately the DEP spectrum of a sample of suspended particles and to provide the measurement of the permittivity and conductivity of the suspended particle component with satisfying precision and accuracy. The realisation of such a device has been performed in two parts: the first one is the development of the device with its hardware, connections and power supply; the second one is the development of the software with the controls, the data acquisition and the data processing. These two aspects of the machine are developed in this chapter, with a detailed explanation of the devices that compose the automate and of their interactions.

Both the hardware and the software have been developed extensively during the three years of this work in a try/measure/improve optimisation loop. This process has been largely influenced by the theory, as explained in Chapter 3, which has gives a direction of research. When appropriate, the text includes references to the theory that has been developed in parallel as a guide. The scripts developed can also be found on the CD attached at the end of this book, and a user manual comes in Appendix C that explains how to prepare the machine and to use the software for a data acquisition.
4.2 Structure of the automate

4.2.1 Introduction

The prototype has been built by successive stages. As mentioned in the introduction, the automate has been improved by successive steps. The first automated DEP device has been made in December 2005 with a simple prototype, which demonstrated the principle of functioning. The results provided were not good, because the tubing used were too large and the connections were leaking so a large sample had to be used, and many bubbles were generated during the measurement so the data acquired was very noisy. However, this attempt was successful in cutting the time of operation by two compared to the manual experiment. Since then, the problems observed were analysed and eventually solved by improved design so that it was possible to find new ways to increase the precision, accuracy and rapidity of the overall system. The solutions exposed in this section are the results of this optimisation process and appeared progressively during the three years-long progression.

4.2.2 General presentation of the automate

The task of the automate can be summarised in four points. The prototype created for the automated measurements is an assembly of different devices connected together and synchronised by a computer. The choice of the devices selected depends on their functions and on the needs of the measurement, as the purpose of the machine was to measure the DEP spectrum of a sample of suspended particles with a microwell, it therefore has to:

1. be able to store the sample and to bring it to the microwell without damaging it. This is referred to as the sample holding.

2. create the DEP effect in the microwell. This is referred to as the generation of DEP effects.

3. monitor the effect of the DEP force on the sample. This is referred to as the measuring.

4. be able to synchronise its components. This is referred to as the synchronisation.
Each of these tasks is performed by one or several simple units, which have been collected, bought or created for the occasion. These units can be listed according to their respective task:

1. Sample manipulation: pump, valve and tubing to drive the fluid; seal and Perspex capsule to hold the microwell in a water tight compartment; tube holder and magnetic stirrer to hold the sample tube; bin to evacuate the washing medium and eventually the sample once finished.

2. Generation of DEP effects: signal generator and microwell.


We can also add the external components that are the sample and the experimenter, and we obtain a description of the system required for the automated measurement of a DEP spectrum. The reasons of the choices of the different units listed above is discussed in this section, and a detailed presentation of their interaction within the prototype is presented in the scope to provide enough information to the reader to be able to fix or built such a machine.

This results in a two-pole system where the information circulates in four different ways. To get a first idea of the system, Figure 4.1 presents an coarse functional diagram of the relations between the main elements of the device. Each actuator of the experiment is represented by an ellipse, and each relation or mean of connection is represented by a connector with a description. It can be seen that the most connected element are the computer and the microchip, both having four connections. This reflects the fact that the most important points in the machine are the preparation of the microchip and the synchronisation of the overall. These two points are discussed in detail later. It can also be observed that the experimenter is left only with two tasks consisting in preparing the sample and launching the acquisition, which fulfils the requirements of minimising the interactions between the measurement and the operator.

Figure 4.1 also present an synthetic way of analysing the machine: if we consider the two poles that are the microchip and the computer, we can see that we can go from
one to the other by four distinct pathways, each one corresponding to a circulation of information. In a chronological order that follows the experimental protocol, the first one is the path microchip-valve-sample-computer. This path can be linked to the preparation of the machine by the experimenter, who has to take care that the sample is correctly inserted into the system (which includes the pump and microchip) and has to provide the different parameters to the computer. This is the preparation phase, which is the domain of the experimenter in the protocol and is detailed in section 4.2.3 on page 96. The second pathway is the path microchip-pump-microcontroller-computer, which is the path followed by the information related to liquid handling during the resuspension phase of the protocol. This is discussed further in section 4.2.4 on page 106. The third pathway is the path microchip-microscope-camera-computer, which is the path followed by the data during the data acquisition and therefore corresponds to the measuring. This is detailed in section 4.2.5 on page 115. Finally, the last two pathways left are the microchip-signal generator-computer and microchip-oscilloscope-computer, which are related to the generation of the DEP signal and its monitoring. In practice, these two informational paths are used synchronously in order to generate a
controlled DEP effect in the microwell. This is explained in section 4.2.6 on page 118.

Two views of the automate are presented on Figure 4.2 and 4.3, on page 97. All the units are not visible on these pictures, but it is possible to see the overall organisation described above. It can be remarked that Figure 4.2 shows a cover that prevents seeing the microchip. This cover is very useful to block the light from the ceiling, which can contaminate the measurement and contribute to the level of noise. A comparative study is presented in Chapter 5 to show the gain of performances.

4.2.3 Preparation of an automated experiment

4.2.3.1 Preparation to the measurement

Even if the automate performs most of the tasks during the data acquisition, the experimenter still has a major role in the preparation of the measurement. It is he who prepares the sample and chooses the parameters of the acquisition, both roles being of great importance. The preparation of the sample is explained in Chapter 2 on page 21 in the case of yeast cells. Most of it has not been changed between manual and automated protocols, except that, for automated measurements, the culture medium is systematically sterilised prior to the cell culturing.

The experimenter must prepare and place the sample carefully in the machine. Before the preparation of the sample, the system must be washed with the conductive medium. Hence a sample of conductive medium must be placed at the inlet of the system and the washing procedure must be launched several times. This procedure empties the tubing from the washing agent it contains and replaces it by the conductive medium used in the experiment in order to avoid a modification of the conductivity of the sample once it is inserted. Once the washing is finished, it is wise to start the calibration of the signal generator, as explained in section 4.2.6 on page 118. After the calibration, the sample loading procedure must be launched with the conductive medium in place of the sample. This procedure prepares the system for loading the sample.
Figure 4.2: Front view of the automate.

Figure 4.3: Side view of the automate.
4.2.3.2 Performing the measurement

When the sample is ready for the measurement it must be placed on the sample holder, above the magnetic stirrer. Heavy cells tend to sediment faster than the resuspension can homogenise them, so if the cell line used is particularly heavy the magnetic stirrer may be used. Then the system inlet must be inserted deep enough into the Eppendorf tube to touch its bottom. This is to prevent the case when the level of sample falls below the inlet. Once the sample is placed, the experimenter has to make sure it is correctly inserted into the machine. This is done by the sample loading procedure, which should be sufficient to prepare the machine at this stage. However, it is a good practice to check the presence of bubbles in the microscope before starting.

Finally, the experimenter has to select the parameters of the data acquisition according to the cell line he investigates. When the sample is correctly loaded in the system, the experimenter must select the parameters of the data acquisition that correspond to the cell line and to any specific condition he may want. This is done on a Graphic User Interface (GUI) on the computer. The program developed for the acquisition of the DEP spectrum and the measurement of the sample electric properties can be found on the CD, in the folder ‘DEP project’, in ‘Matlab codes’. This folder is to be saved in the path list of Matlab with its sub-directories. Once this is done, the program can be launched by typing the command \texttt{DEP} in the command window of Matlab. This opens on the screen the GUI presented on Figure 4.4, which is the main panel of the program. Closing it should close all the other GUIs that may have been opened from it and ends the experiment.

This data acquisition GUI is constituted of 6 panels:

1. **Directory and description:**
   
   This panel is at the top left corner and contains the directory browser and the tag editor. The browser allows the user to choose or create the directory where the file system will be saved. This system is a tree structure that classifies the data received and processed during the operation of the machine. It is represented in Figure 4.5, with the different files that are produced by the different scripts presented in section 4.3 on page 122. The tag editor, on the other hand, is a short description of the experiment performed; it is saved along with the data in the
different files and can appear in the figures as a legend, after data processing. It is a marker that makes it easier to trace the data.

2. Controls:

This is the top-right panel. It is the panel that contains all the links to actions that are not directly related to the experiment, i.e. data processing, device control, device checking and signal calibration. It contains four push buttons with different effects.

- **Device control:** Clicking on this item opens a secondary GUI that contains different options for a direct control of the devices. This includes the possibility to select the valve position, to regulate the speed of the motor, to fill the syringe with a given quantity of the sample, to set frequency and amplitude of the waveform, to switch the signal generator on and off and to visualise the image captured by the camera. It can be useful for removing trapped bubbles out of the microwell.

- **Calibration:** Clicking on this button launches the signal generator calibration procedure. This allows the program to detect any frequency effect of the microchip that could damage the AC signal during the experiment, and to compensate it as explained in section 4.2.6 on page 118. Before running the calibration the user must fill the system with the conductivity medium he
will use for the experiment in order for the microwell to have an impedance close to the experimental. The calibration is performed around the values of the amplitude and frequency selected by the user in the corresponding fields of the GUI. Ideally, the calibration should be repeated every time the conductivity is changed.

- **Data processing**: Clicking on this item launches the data processing GUI to extract the DEP spectrum and the measurement of the cell properties from the images acquired from the sample. This operation can be performed anytime after the data have been acquired, or can be performed on data acquired from previous experiments. More information can be found in section 4.3.4 on page 130.

- **Re-check devices**: Clicking on this item launches the auto-detection of the devices connected to the computer via serial port. This can be useful if the user forgot to switch on one of the devices before launching the program, or if a problem occurred during the initial detection. The auto-detection takes
a few seconds and the results are prompted in the message board, at the bottom of the GUI.

3. Procedures:

The procedure panel lists all the different procedures that appear in the experimental protocol. It is where the different actions are selected by the user. Usually, a DEP experiment uses the first four buttons, from top to bottom respectively.

- **Rinse the system**: This is the first step of an experiment. This procedure allows the user to rinse the system with a solution placed at the inlet of the system. Usually, when the system is not powered, the tubings are filled with a detergent that keeps the clean. This detergent has to be washed with a proper solution. Ideally, the conductive medium prepared for the experiment should be used. The procedure uses about 500 µL of solution, and should be repeated three times to make sure the detergent is completely removed. Once the sample of conducive medium is placed in the sampler holder and the inlet of the tubing touches the bottom of the sample tube, the procedure can be launched. A message appears then to make sure the sample is in place. After the system is rinsed, it is good practice to change the syringe if the previous solution was significantly different, as explained in section 4.2.4.2 on page 108. It can be noted that, if the liquid in the tubing has dried up after a long period without use, the residuals are quite difficult to wash, so a preliminary series of rinses with a solution of Triton X100 can be used to facilitate the cleaning.

- **Load the sample**: This procedure rinses the system with the conductive media and places the sample into the microwell so the machine is ready for data acquisition. First, a sample of 1.5mL of conductive medium must be placed on the sample holder, with the inlet of the tubing inside. Then the procedure can be launched: it displays a first message to ask if the solution is into place, and starts to pump the medium through the tubing three times. Once the system is clean, a second message appears that asks the user to place the conductive medium and to replace it with the sample to be analysed. The minimum amount of sample usable by the machine is 300 µL; less than that generates some bubbles in the microwell and damages the signal. Once the
sample to be analysed is in the sample holder and the tubing reaches the bottom of the tube, the user must select 'Ok' to finish the loading. After that, the sequence draws a quantity of liquid that corresponds to the volume required to fill the tubing up to the microwell. This volume can be changed in the field ‘Dead volume’ of the GUI, but the value pre-recorded should correspond to the volume required so no changes should be required. once finished, the system is ready for the data acquisition.

• Launch the acquisition: Clicking on this button starts the data acquisition. Before doing that, the user must make sure that the system is clean and filled with the sample, and that all the experimental parameters are set on the 'Experimental parameters' panel (see the 'Experimental parameters panel' section below for more details). The light source should be switched on now if it has not been done yet. When launching an acquisition, the user is asked how many repeat measurements he wishes to obtain. These are independent replications of a given measurement that will be performed successively by the machine on the sample. If the level of light is too bright or too dark for the camera, the program detects it and asks the user to modify it before it continues. Once the number of replications is set, the experiments begin and a progress bar appears on the screen that shows the time left before the completion of the current measurement. Closing this progress bar stops the data acquisition.

The data collected by this procedure are saved in the folder selected in the 'Directory and description' panel on the top-left corner. Each replicate generates a dedicated folder (i.e. 'Replicate1', 'Replicate2', and so on) in which a folder 'Data' is created. This is where the raw data collected during that phase is stored, as a series of Matlab files. This 'Data' folder can use between 50 and 500Mb of memory depending on the parameters set for the acquisition, but once the data processing has been performed it is safe to delete it if necessary. At the end of each measurement, the experimental conditions are stored in the file 'Experimental_conditions.mat' in the folder corresponding to that replication. This file can be opened in Matlab later and contains all the information present on the GUI, plus the order in which the frequency have been measured (variable ‘permutation’, see section 4.3.2 for details), the
date and hour of the beginning and end of the measurement, the time when each image was taken and the voltage peak to peak measured experimentally.

- **Wash and finish**: When all the experiments are finished, it is necessary to wash the system with a detergent so that it remains clean. The detergent used normally is a solution of Decon 5%, because it has a high cleaning power and can be rinsed away completely. 1.5mL of this solution must be placed in a tube on the sample holder, with the tubing inlet in it, before starting this procedure. Clicking on ‘Wash and finish’ displays a message that checks if the system is ready. Clicking ‘ok’ starts the procedure; it fills the tubing with the solution so that the devices can be switched off and the system can wait several days before another experiment.

- **Edit text**: Clicking on this button opens a text file and save it on the path selected in the ‘Directory and description’ panel. It is a model text file where the user can detail the experiment he is to perform. The model file can be changed in the DEP folder where the program is stored.

- **Check image**: Clicking on this button opens a preview of the camera and a histogram of the level of light recorded. This is useful if one wants to check the alignment of the microwell in the system, or the saturation of the camera. However, the machine contains an algorithm for the detection and regulation of the level of light so it should not be too sensitive to illumination levels. If required, the light source can be regulated directly from the power supply.

4. **Experimental parameters**:

This panel contains all the data related to the experimental conditions. It is split into two areas, ‘main’ and ‘advanced’ parameters, which concern the parameters that affect the measurement and devices respectively. The main parameters are the following:

- **Lowest frequency**: This is the value of the lowest frequency used by the signal generator, in Hz. When setting this parameter, one should consider that too low a frequency can lead to electrolysis in the microwell, which creates bubbles and modifies the pH in the medium so that it may become toxic for the cells. 1 kHz is the default value because it is does not cause damage in
media at conductivity used here, but according to the conductivity of the suspension medium one can go lower, with a minimum at 100 Hz.

- **Highest frequency**: This is the value of the highest frequency in the spectrum, in Hz. The maximum frequency reachable by the signal generator is 20 MHz, which is the default value.

- **Points/decade**: This is the number of points per frequency decade to measure in the DEP spectrum. For instance, an experiment with a minimum frequency of 1 kHz and a maximum frequency of 10 MHz with 5 points per decades measures the DEP force for 21 different frequencies selected regularly on a logarithm scale between 1 kHz and 10 MHz.

- **Voltage**: This is the amplitude of the signal provided by the signal generator during the experiment, after the correction by the calibration. The maximum is 20 Vpp but too high a voltage can cause problems, especially with microflows. The ideal voltage depends on the cell line, medium conductivity and frequency band, and has to be found experimentally.

- **Medium conductivity**: This is the conductivity of the suspension medium, in $\mu$S/cm. It is necessary for the signal processing and should be provided if the user wants to measure the permittivity and conductivity of the cells in the sample.

- **Cell concentration**: This is the concentration of the sample, in cells per mL. This parameter is not necessary for the data processing but is stored with the experimental conditions for reference.

5. **Advanced parameters:**

This panel contains all the parameters that affect the behaviour of the machine during an experiment. Generally, there is no reason to change these parameters between two experiments, as they have already be optimised.

- **Syringe volume**: This is the volume of the syringe in mL; it is used by the machine to calculate the motor speed, which controls the debit of fluid in the tubing during the resuspension phase.

- **Syringe length**: This is the length of the syringe in mm; it is also used by the machine to calculate the motor speed.
• Resuspended volume: This is the amount of sample in mL that passes through the microwell each time that it needs to be refreshed. A larger amount of sample for the resuspension provides a better homogeneity in the concentration before the application of the DEP field, but this improvement generally saturates around 100 µL so a default value of 130 µL has been selected.

• Images/points: This is the number of images taken by the camera for the measurement of each point of the DEP spectrum. As shown in the simulations in section 3.4.3 on page 60, the time scale of the evolution of the concentration when the DEP force is applied is of the order of magnitude of 2s. This value can vary between 0.5s and 10s, according to the cell properties and the voltage. The number of images should be selected accordingly in order to have enough data points during the evolution of the signal. The default value is 5 images/s and corresponds to the case of yeast cells, for a voltage of 15 Vpp.

• Blanks/points: Before starting the measurement of DEP, a number of images are taken in order to be used as a reference during the data processing. This sets their number.

• Time/points: This is the amount of time spent for the measurement of each point of the DEP spectrum, in seconds.

• Motor speed: This sets the speed of the pump, in % of the full scale.

• Dead volume: This corresponds to the volume between the microwell and the syringe, in mL.

• Well diameter: This is the diameter of the microwell, in µm.

6. Messages:

This panel displays the information about the processing. This can be the beginning and end of an experiment, the list of devices detected, the start and end of the rinsing procedure, and so on. The text displayed in this window is saved in the file Experimental_conditions.mat created after each measurement in the folder selected by the user in the first panel.
4.2.3.3 After the measurement

Once finished, the experimenter must remove the sample and wash the system. When the data acquisition is finished, the experimenter can choose between performing another acquisition, either on the same sample or on another, or finishing the acquisition. In the first case, if the sample is the same, the data acquisition can be re-launched directly. If not, the system must be rinsed with conductive medium before inserting the new sample, as in the preparation procedure explained above. Otherwise, if the system is to be switched off, it must first be cleaned with a proper cleaning agent, such as Decon 5%, in order to keep the microwell clean. This precaution prevents the formation of bubbles inside the microwell, and is performed automatically after the user has placed a sample of washing liquid at the inlet of the system. When the washing procedure is finished, the system can be switched off.

As it can be seen above, the role of the experimenter is limited to the preparation and the cleaning, before and after the experiment. However, the quality of the preparation is crucial for the quality of the data, so this role is of major importance in the protocol. A poor preparation of the sample leads to a poor repeatability of the experiment; an incorrect sample loading increases the probability of observing bubbles in the microwell, which increases the noise in the data; an incorrect washing leaves some detritus in the microwell that makes it more difficult to wash if a bubble is caught, and may contaminate the next samples. For all these reasons, loading and washing the tubings must be conducted with particular care. These effects are investigated further in Chapter 5.

4.2.4 Liquid handling

4.2.4.1 Principle

Several devices are used to handle the sample. After being loaded on the machine, the sample is manipulated with the help of several devices: the pump, the valve, the capsule, the tubing, the microcontroller and the computer. These devices are connected as described on Figure 4.7 and 4.8. Here again, the system can be decomposed to smaller circuits: one is dedicated to the transmission of the fluid, the other to the transmission of the information.
Chapter 4. Description of the apparatus and data processing

Figure 4.6: Scheme of the fluid distribution system.

Figure 4.7: Detail of the machine, around the sample.
4.2.4.2 Fluid transmission

This system has the purpose to carry the sample to the microchip for the experiment, and to re-suspend the microwell between each measurement in order to make sure that the cell concentration is homogeneous before each data acquisition, as explained in Chapter 3 in section 3.3.2 on page 44. It consists of two different branches, one for the measurement and the other for the evacuation of the fluid. The sample is aspirated through the tubing by the action of a syringe pump and a valve that routes the liquid pumped either to the sample or to the bin. The microwell sits in the measurement branch of the system and is automatically filled and resuspended when the pump is activated and the valve is positioned accordingly. The evacuation branch is used for cleaning the tubing before and after the data acquisition, so that the washing agent can be aspirated through the measurement branch and binned by the evacuation branch. This is a way to diminish the probability of injecting a bubble into the measurement section of the system.

The syringe pump itself comprises three elements: a linear stepper motor, a disposable syringe and a valve. The tubing that connects the different elements together is PEEK.
tubing with 1/16” outer diameter, 1/32” inner diameter (Supelco, Z226955). The valve, manufactured by Rheodyne (http://www.rheodyne.com/), is a 6-way, 2-position valve with stepper motor (MV series, Rheodyne) and a motor control card included (ref. PN 7900501, Rheodyne). It is powered on 0-24 V, and can be switched by a logic control 0-5 V connected to the microcontroller.

The linear stepper motor (RadioSpares, ref. 510-1114) displaces a 15cm-long screw linearly along its axis. This screw is attached to the piston of a 1mL syringe (polypropylene, BD Plastipak, Becton Dyckinson SA) so that the activation of the stepper motor actuates the syringe. This allows controlling the amounts of fluid with an increment of 2.5 µL per step. The motor is actuated by 4 logic signals of 12 V amplitude, generated by the microcontroller and amplified by a signal conditioning circuitry. More details of this can be found in the section 4.2.4.3 on page 111.

The disposable syringe can be removed from the system for replacement. This can be done by unfastening the four holding screws and the tubing connector. This operation should be done regularly to make sure that the syringe is clean.

Finally, the microchip is placed in a tight sealed Perspex capsule that allows both the fluid and the light to pass through the microwell. This system has been designed to reduce the amount of fluid necessary for a DEP experiment and to avoid the presence of bubbles trapped in the microwell.

The capsule is made of two pieces of Perspex placed on each side of the microchip. Each piece has been manufactured by microdrills so that it possesses channels that are used to conduct the fluid towards the vicinity of the microwell. A gasket has been designed that leads the fluid from the outlet of the capsule into the microwell. In order to avoid the leaks, all these parts are screwed together.

The volume of fluid required to fill the system from the inlet to the pump is 130 µL±10%, which defines the minimum volume of sample required by the device to conduct an experiment. This volume is relatively small for a sample of living cell in suspension so that it makes it easy to analyse suspensions of high concentrations of cells. This can be an important issue for cell lines that are difficult to grow, such as mammalian cells in general.
Chapter 4. *Description of the apparatus and data processing*

Figure 4.9: Scheme of the capsule.

Figure 4.10: Capsules used in the machine. Left: the two parts that drive the fluid through the microwell. Right: an encapsulated microchip.
Several arrangements of the tubing network were tried before the final version of the device presented above was established. The tubing selected initially had a 1/16” inner diameter and therefore required an important fluid volume in the system, and so bigger samples. A typical experiment used to require up to 1mL samples. Bigger tubing also increases the chance of bubbles being trapped in the tubes, which caused many problems when they reached the microwell, and increases the chances of leaks, which waste the sample and create artefactual flows in the microwell. All these issues have been fixed by using a thinner 1/32” Teflon tubing. A DC motor was also used initially to manipulate the syringe, but that was found to be difficult to control. Stepper motors are much more accurate and flexible, so a 12 V stepper motor was selected instead. Finally, several arrangements of the fluid distribution were tried and are detailed in the transfer report joined in the CD with this thesis (Broche, 2007). The one presented above has provided the best performance in terms of volume and probability of retaining/generating bubbles.

4.2.4.3 Information transmission

The microcontroller controls the different electromechanical units and is linked to a PC via an RS232 connection. The elements of the syringe pump are synchronised during their operation by a microcontroller. Its role is to operate the commands issued by the computer that concern the manipulation of fluid by the system. The connection between the microcontroller and the PC is an RS232 standard, using a baud rate of 19200 Bauds/sec, no parity, 8 data bits, 1 stop bit, no terminator, no flow control, and no request to send. The RS232 signal sent by the computer is converted by an integrated circuit (MAX232CPE+, Maxim) in order to provide a TTL signal readable by the microcontroller on pins 6 and 7 of port C. The stepper motor, valve and sensors are connected to the different ports of the microcontroller as shown on the diagram of the electronic board presented in Figure 4.11 on page 112. The datasheet of the different components is available on the CD in the folder ‘Documents’.

The valve is connected directly to the port C on pin 5, which is possible thanks to the signal conditioning board fitted with the valve. It is controlled by a simple logic command, where ‘0’ and ‘1’ correspond to the configuration syringe-to-sample and syringe-to-bin respectively.
Figure 4.11: Scheme of the electronic board.
The stepper motor is connected to port D on pins 0 to 3, one for each coil, and is controlled by synchronised pulse sequences. However, the power available from the microcontroller is 125mW maximum per pin, and each coil of the stepper motor requires up to 5W power. Therefore a power converter stage is required between the microcontroller and the coils. The power generated by the microcontroller being sufficiently high, a simple power diode is used here instead of the traditional Darlington diode. A resistor is included between the diode and the microcontroller to regulate the power supplied by the pin, and a free wheel diode between the coil pin maintains the continuity of the signal.

Two sensors are added on the pathway of the piston in order to detect the end of travel. This allows the microcontroller to stop operating the stepper motor if the course reaches its extreme. These sensors are connected to port B on pins 0 and 1 for the detection of the minimum and maximum position respectively.

The 4 MHz crystal necessary for the operation of the microcontroller is connected to pins 13 and 14. It is grounded to a wide ground plane via two 16pF capacitor in order to provide a clean signal.

A light control pin is also available on port C, pin 2, for the operation of a light source. It is a TTL modulated signal that can be used to control the amplitude of a light source via a capacitive circuit. This has been included for future works, in order to provide an automated control of the illumination of the microwell.

Finally, a reset switch has been added on pin 1 of the microcontroller in order to reset it in the event of a problem, and a photodiode is linked directly to the supply rail to indicate when the board is powered.

The transmission of the instructions from the PC is performed by a simple assembler code. The microcontroller is programmed with the assembler code provided on the CD in the folder ‘Assembler codes’. It follows a wait and test procedure as presented on the flow diagram 4.12.

Different instructions are sent by the PC to activate the corresponding program, according to the association presented on Table 4.2.4.3. Some instructions require parameters,
other don’t. When the parameter refers to a position, it is measured in number of revolutions of the stepper motor. For instance, the instruction M h01 h01 h1F moves the piston of the syringe:

- $X_1 = h01$: in the direction of an increase of the volume
- $X_2 = h01, X_3 = h1F$: by $1 \times 256 + 31 = 287$ tours.

When a program is finished, a report is sent back to the computer to indicate that the process can continue. This report is a character string constituted by the word Done followed by the character corresponding to the instruction. In the case of the example above, the report would be DoneM. This is a way to create a bidirectional exchange of
### Table 4.1: Instruction set used by the PC to control the operation of the microcontroller.

<table>
<thead>
<tr>
<th>Instruction</th>
<th>Parameters ($X_i$)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>Stop the automated resuspension launched by the command ‘R’.</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td>Start the calibration procedure to determine the initial position.</td>
</tr>
<tr>
<td>M</td>
<td>3 bytes</td>
<td>Moves the piston over a distance $X_{2-3}$ in the direction given by the first byte $X_1$.</td>
</tr>
<tr>
<td>P</td>
<td>2 bytes</td>
<td>Places the piston at the position $X$ along the axis of the syringe.</td>
</tr>
<tr>
<td>R</td>
<td>2 bytes</td>
<td>Starts a continuous resuspension using a course $X$ of the syringe.</td>
</tr>
<tr>
<td>S</td>
<td>1 byte</td>
<td>Set the velocity of the stepper motor to $X%$ of the maximum.</td>
</tr>
<tr>
<td>V</td>
<td>1 byte</td>
<td>Switch the valve according to the first bit of the parameter.</td>
</tr>
<tr>
<td>?</td>
<td>None</td>
<td>Send the characters ‘PIC16F877’ to the computer via the RS232 connection.</td>
</tr>
<tr>
<td>p</td>
<td>None</td>
<td>Send the position of the piston to the PC, coded on two bytes.</td>
</tr>
<tr>
<td>s</td>
<td>None</td>
<td>Send the speed of the stepper motor to the PC, coded on one byte, in % of the full scale.</td>
</tr>
<tr>
<td>v</td>
<td>None</td>
<td>Send the position of the valve to the PC, coded on one byte.</td>
</tr>
</tbody>
</table>

information in order to help the synchronisation of the devices and to correct the errors that can occur in the transmission.

### 4.2.5 The measuring system

The observation of the microwell requires three devices: the microscope, which prepares the image of the microwell; the camera, which records it and generate an electronic file; to the computer, were this file is transmitted and stored. Other elements have been included into this system to make it more reliable: an IR filter reduces the amount of IR received by the microchip, a lens on the microscope sets its focus to infinity and an external power supply has been connected to the light bulb of the microscope. It can be
noted that the IR filter does not reduce the luminosity of the image observed because the camera has its own IR filter.

The resulting system, which includes all these elements, is presented on Figure 4.13. The optics are what enables the measurement of the DEP signal, and therefore special care must be been taken for the design of this part. This is the reason why a microscope (Zeiss, ref. 65380) is used to observed the microwell. This makes the observation more reliable than by using a custom-made apparatus. A camera (Dolphin 16-bits, Allied Vision Technologies) observes the image obtained from a C-mount connection on top of the microscope and provide the data to a computer via FireWire connection. The light beam can also be filtered by an anti-IR element (Zeiss filters, ref. BG38) that cuts most of the IR radiation and prevents heating effects. This heating is the cause of local microflows that can appear during measuring as explained in section 2.3.2 on page 23, so filtering the IR can help reducing the microflows.

An important modification has been brought to the microscope by adding a lens (Thorlabs, CAY046) on the optical path between the microwell capsule and the microscope.
objective. This lens changes the trajectory of the light beam so as to obtain a measurement that corresponds with the theory developed in section 3.3.2 on page 44. According to theory, the light beam passes vertically through the microwell, which corresponds to observing an object infinitely far, which requires an infinite focal length. The principle of this operation is shown on Figure 4.14. The focal point of the additional lens is placed in the focal point of the microscope so that the system obtains an image of the parallel light beam passing through the sample instead of an image of the microscope focal plane. As a result, the final image is not only a measurement of the cells contained in the thickness of the focal plane (about 70 µm for a x2.5 objective) but it adds the contribution of all the cells contained in the microwell, so information is being used. This technique can help reduce the noise in the data, as detailed in Chapter 5.

The measurement also depends directly on the level of light recorded, as mentioned in section 3.3 on page 43, so the source of light must be particularly steady. The study of the level of noise in section 3.5.4 on page 85 sets the noise threshold to ±3%, so the variations of the light source are expected to be much lower. However, it has been reported in section 2.3.5 on page 25 that the intensity of the light source varies over
time, with an amplitude that can overcome the level of the signal. It was therefore necessary to investigate this effect. Replacing the light bulb by a new one had no effect on the result, so it was deduced that the effect observed was generated by a series of variations of the voltage from the mains occurring during the day, probably originating from other laboratories. The solution applied has been to use a steady power supply to power the light bulb. The power supply used (EA-PS 3016-10B, Elektro Automatik, Farnell) can provide 160W up to 16 V DC, with an accuracy of 8 mV maximum. The light bulb being powered at 6 to 12 V during a measurement, the final precision of the light source is $8 \times 10^{-3}/6 = 0.0013$, so 0.13% of error. This is more than satisfying and led to very good results, as shown later in Chapter 5.

4.2.6 Generation of DEP effects

The DEP effect can be observed by using an inhomogeneous AC electric field. Therefore two elements are required for generating DEP forces on a sample:

1. A gradient of electric field: this is created by the microchip, which has been optimised for that purpose as explained in Chapter 3 on page 37.

2. A generator of AC electric potential: this is performed by a dedicated device, as explained below.
The AC signal for the DEP is generated and monitored by two separated units. The AC signal used to generate the DEP force is created by a signal generator (FG100, Digimess) and sent to the microwell directly with a coaxial cable connection. The peak-to-peak amplitude of the signal can be varied from 0 to 20 V and its frequency can be varied between 0.1 Hz and 20 MHz. In practice, there are many difficulties arising for frequencies lower than 1 kHz, partly because of water electrolysis at low frequency if the amplitude of the electric field is greater than 1.23 V (standard potential for water electrolysis at 23°C) and partly because some physical effects generate different microflows that mask the DEP signal when the signal’s frequency is lower than 1 kHz. This is explained in more details in Chapter 5 on page 159.

In order to check the presence of harmonics, the level of distortion has been investigated by monitoring the waveform directly with the oscilloscope at high frequency. As shown on figure 4.2.6, no significant signal distortion or attenuation was observed at 20 MHz when the oscilloscope was not connected to the microchip: the amplitude of the 20 MHz frequency was 639 units and the amplitude of the most significant harmonic was 18.2
units; the ratio between the fundamental peak and the highest harmonic is then 2.85% of the amplitude, and 0.081% of the power. Figure 4.2.6 presents the FFT spectrum of the signal when the generator is connected to the microchip: the amplitude of the peak corresponding to the fundamental frequency is 646 units and the amplitude of the most significant harmonic is 35.1 units; the ratio between the fundamental peak and the highest harmonic is then 5.4% of the amplitude, and 0.3% of the power. Therefore the influence of the distortion in the wave has been neglected in the rest of the work.

Equation 1.1 on page 6 states that the DEP force is proportional to the gradient of the electric field squared, which means that it is proportional to square of the amplitude of the electric signal sent to the microchip. This in turn means that the accuracy of the measurement of the DEP signal depends directly on the accuracy of the signal generator, so that a drop in the signal amplitude with the frequency results in a drop of the DEP spectrum at this frequency. This can clearly lead to bias in the results, so an oscilloscope (IDS710, Iso-tech) was added to the system in order to monitor the amplitude of the signal provided by the generator.
For more accuracy, the AC signal is treated by a peak detector prior to be monitored. The oscilloscope is controlled by the computer via an RS232 connection. The measurements of the electric signal are taken systematically by the software during the application of the DEP force in the microwell. However, this oscilloscope uses different channels for different ranges of frequency and each channel has its own level of precision, so the precision of the result varies according to the frequency of the signal. This issue was solved by using a peak detector, shown on Figure 4.18, connected in parallel with the microwell chip. This unit converts the voltage from the signal generator into a DC signal before it is measured by the oscilloscope, so that only the low frequency channel is used for any frequency. The peak detector also provides the advantage that the measurement of the signal’s amplitude can be performed with an analogue to digital converter, which could be added to the microcontroller in future upgrades as explained in Chapter 7.

In order to avoid artefactual results, this peak converter must be tuned to the needs of the system. This means that the frequency of the RC circuit must be lower than the lowest frequency used during the experimentation so that the voltage read by the oscilloscope remains steady. In practice, DEP measurements cannot be easily performed below 100 Hz because at such frequencies the microchip acts like an electrolyser and generates bubbles. Hence \( RC < 100 \text{ Hz} \). It also means that the time constant of the RC circuit should be shorter than the time between two data acquisitions, so that the measurement from the previous one does not affect the following one. In practice, the system takes about 10s between two acquisitions, hence \( RC > 0.1 \text{ second} \). In practice, a value of 1 second has been selected. Finally, the value of the capacitor should be as small as possible in order to reduce its charging time when the diode is opened. Therefore the values selected are \( R = 10\text{M}\Omega \) and \( C = 0.1 \mu\text{F} \).

![Figure 4.18: Scheme of the peak detector.](image)
The signal generator is calibrated before each data acquisition. Usually, the amplitude of the signal generator measured with the oscilloscope via the peak detector presents some light variations with the frequency. Typically, one can measure a variation of 5% following a regular pattern in the spectrum. This is supposed to be due to impedance effects in the coaxial cables, in the microchip and in the internal circuitry of the generator. It can be compensated by calibration when the microwell has been filled with the conductive medium, prior to the measurement. This calibration procedure consists of measuring the amplitude of the signal over a range of amplitudes (1 to 10 Vpp) and frequencies (1 kHz to 20 MHz), and to compare the result obtained with the ideal values sent in the command. The ratio between the measurement obtained and the command is the correction factor, and is used during the measured to modify the commands sent to the signal generator in order to compensate the variations of amplitude. The result provide a variation smaller than the resolution of the oscilloscope, which is about 1%.

4.3 Structure of the program

4.3.1 Introduction

The previous section has presented the system and explained the role of its different parts in the acquisition of the measurement. However, this structure is only part of what is required for the measurement of the DEP spectrum, and the determination of the conductivity and permittivity of the different constituents of the cells analysed. There is also a need to synchronise the different devices for the measurement, store the data obtained and process them according to the theory developed in Chapter 3. This is performed by the computer and the microcontroller, with different programs organised to work together: all the operations of acquisition, synchronisation and processing are performed by programs that have been developed in parallel with the machine. This section explains how the computer and the microcontroller manage the different devices and the treatment of the information. The computer code can be found in the folder ‘DEP project’ on the CD, in the folder ‘Matlab codes’. It is composed of a main program \texttt{DEP.m} and several dozen of sub-programs; all of them have been coded using
Matlab r2007a and require a version of Matlab equal or higher than this to run. The microcontroller code can be found on the CD in the folder ‘Assembler codes’. 

The structure of the program developed for the machine is explained on Figure 4.19. It consists in several units organised in a tree-like hierarchy, with the starting program `DEP.m` at the top, the different data acquisition GUIs in the middle and the curve fitting GUI at the bottom. This hierarchy is a consequence of the logical order of the measurement, from the acquisition to the data processing, but can be short-circuited if the user wishes to process a dataset previously recorded. This is explained later.

A manual that explains how to use this program to perform a measurement with the automate can be found in Appendix C. Instead, the following sections are dedicated to the internal functioning of the different blocks of the diagram 4.19 from the point of view of the information, which can be organised around three themes:

1. The synchronisation of the different units of the machine
2. The acquisition and transfer of the data
3. The processing of the data

The programs developed to achieve this are explained in the next sections. However, the aim has not been to explain them at a deep level, as this would be time-consuming and not of great interest, but to explain the principles of functioning with enough details for one to be able to understand and modify the code if one needs to change it.

4.3.2  Synchronisation of the device during the data acquisition

4.3.2.1  Synchronisation of the transmission between the computer and the devices

The dialogue between the computer and the different devices is established in both direction. The lowest level of synchronisation concerns the connection between the different devices. Most of the devices are connected to the computer by RS232 protocol, the only exception being the camera that uses a FireWire connection that uses its own protocol and was driven directly by Matlab. The RS232 protocol is designed to fit custom needs and so is much more flexible, so a protocol of transmission has been defined that uses a feedback method.

The errors occurring during the transmission are detected and corrected. This method is currently used in data transmission and consists in verifying the data after transmission. It works as shown in diagram 4.20. This method is useful to correct an information that has been distorted by noise. This happens regularly in the laboratory where the experiment takes place, probably because of the many electronic devices that surround the apparatus: an average of one error usually occurs every twenty commands in one transmission line during the measurement. Therefore, when a command is damaged during transmission, the device cannot recognise it and sends an error report to the computer, which re-send the command. This correction protocol continues until the command has been recognised, but this usually happens only once.

This correction protocol is included in the different transmission procedures found in the folder ‘DEP project/programs/Acquisition’ on the CD.
4.3.2.2 Synchronisation of data acquisition

The synchronisation of the experiment is a higher level of interpretation than the synchronisation of the connections. It is performed by the order in which the commands appear in the programs, and the interactions between the devices. This is developed in this section.

The experiments starts with the command ‘DEP’ in Matlab. Using the command ‘DEP’ with Matlab initiates a detection of the different devices that should be connected to the computer, i.e. the oscilloscope, the signal generator, the microcontroller and the camera, with the program ‘DetectInstruments.m’. The result of this detection is listed in a structure named experiment, and the Graphical User Interface (GUI) for the data acquisition starts. This interface is a control window for the acquisition of the DEP spectrum where the different experimental parameters are given to the computer. Once the system has been prepared (see section 4.2.3 on page 96), the acquisition is launched by the user from the data acquisition GUI. In order to measure the DEP spectrum of
a sample, the measurement of the DEP force is repeated at different frequencies by the machine. Hence the procedure developed in the data acquisition program is iterative, as presented on Figure 4.21.

- **Initialisation**

  The initialisation phase uses the controls defined by the user to set the parameters of the data acquisition. The data acquisition depends on several inputs that are given by the experimenter to the computer, via the data acquisition GUI mentioned above, and which are retrieved by the program at the beginning of the data acquisition. The values provided in the different fields are stored in different variables with the script `GetParameters.m`:
  - `AcquisitionTime` is the duration of the data acquisition in seconds,
  - `PointPerDecade` is the number of data point per frequency decade in the final DEP spectrum,
  - `Voltage` is the amplitude and `Frequency` is the list of frequencies of the waveform to be generated by the signal generator,
  - `MotorVelocity` is the speed of the syringe course,
SnapshotsNumber is the number of images acquired per data point, BlankNumber is the number of blank images (i.e. images taken before the application of the electric field, which serve as references) and ResuspendedVolume is the volume of sample resuspended between each acquisition. Then, the list of frequencies used in the experiment is randomised, the oscilloscope window is configured to match the amplitude of the signal generator, the camera is prepared to acquire the number of snapshot required for the experiments, a list \( \Delta t \) of acquisition time is created for the acquisition of snapshots and the different variables used in the processing are initialised.

- **Preparation**

  The preparation phase set the different devices with the parameters selected by the user. During the preparation phase, the oscilloscope is set to a random frequency selected among the list created during the initialisation phase, the sample is resuspended in the microwell and the data transfers from the computer buffer to the hard disk are completed. Several variable are re-initialised to prepare the next cycle.

- **Acquisition of the reference**

  A series of images is acquired before the electric field is applied to obtain a reference. The data processing developed on next chapter requires a reference of light, so a phase is dedicated to acquire a series of images before the application of the electric field by the signal generator. The number of images taken can be changed by the user.

- **Acquisition of the data**

  The acquisition of data is timed according to the parameters set by the user. The data acquisition starts by initialising a timer, which is used to monitor the date of image acquisition. Almost simultaneously (in less than a microsecond) the signal generator is switched on and the first image is acquired. The next images are taken when the value of the timer matches the next value of time set on the snapshot time list created previously. When all the pictures are acquired the process continues to the next step.

- **Saving the data**

  The images are saved in group on the hard drive. After the acquisition, the
sample resuspension is launched and the data are transferred from the computer buffer to the hard drive in the form of a matrix, saved in a mat-file in a directory previously chosen by the user (see section 4.3.3 on page 129 for more details). If the experiment is finished, the program passes to the next step, else it goes back to the initialisation phase.

- **Finalising**

  At final, the devices are re-initialised and the different variables are saved in a mat-file named ‘experimental data.mat’. The machine is then ready for another data acquisition.

**This procedure has been optimised.** The different stages of the procedure presented here have been arranged in a particular order that minimises the waiting times so the measurement is faster. The method used to make the process faster is to parallelise the tasks performed by the computer and the microcontroller. Because the version of Matlab used here does not allow parallel processing, only the tasks that use the microcontroller can be run in parallel, which includes the operations of sample injections. This is why the re-injection of sample into the microwell is performed before the transfer of the images from the RAM to the hard drive and before the signal generator setup: it takes 5 to 6 seconds for the resuspension to complete and it only uses the microcontroller, so by that time the computer can save the frames in memory, which takes about 3 seconds for 10 images, and can start configuring the signal generator, which takes about 5 seconds. By the time the signal generator is configured, the resuspension has finished. This arrangement saves up to 50% of the total acquisition time.

Some delays have also been included in several parts of the procedure for different purposes. The system waits 1 second for the fluid to stabilise after the valve closes to make sure it is immobilised before acquiring the first images, and then the first reference images are taken. It also waits 1 second before the valve closes because, when the syringe has finished the injection, the pressure in the pump is slightly higher than the atmosphere so there is a little movement of fluid from the syringe to the outside. If the valve is switched to stop the flow, then this fluid is routed to the bin and is lost, which if repeated 20 times leads in a significant amount of wasted sample. Giving a little backward move to the syringe after the injection and waiting one second before switching the valve is enough to get rid of this problem.
Finally, it can be observed that the data is not processed at all during this stage, because Matlab cannot parallelise both the data processing and the data acquisition. It would add about one more minute to process the data during the acquisition, which means an increase of about 20% of the experimental time. This has not been considered necessary, and the data processing is done in common for all the experiments by the pre-processing program.

4.3.3 Acquisition and transfer of the data

In order to measure the electric properties of cells, the movement of the suspension must be monitored by the camera before and during the application of the electric field in the microwell. The images taken before the application of the electric field provide a reference that is used during the data processing as an initial state. The images taken during the cell migration are compared to the reference and provide the measurement of the DEP force field. Here is presented how this series of images is stored in the system and prepared for data processing. The purpose of the explanation provided here is to provide enough detail so that one can read, understand and modify the different files that contain the raw data.

The images are formatted and stored as a 3D matrix. In section 4.3.2.2 on page 125 is an explanation of how the images are acquired during the measurement: it is performed in two series of acquisitions for each frequency of the spectrum, one is the series of reference images taken before the application of the electric field and the other one is the measurement images taken during the application of the electric field.

When acquired, each image is automatically stored in a memory buffer on the computer. The two series of images obtained after one measure is transferred from the memory buffer to the Matlab workspace by the command `peekdata`, which creates a 4D matrix from the data. The result of this operation is then formatted according to the size of the images taken by the camera in order to obtain a 3D matrix of dimension $l \times L \times n$, where $l$ and $L$ are the dimensions of the images taken by the camera in pixel and $n$ is the number of images taken in the two series of acquisition.

Then the matrix is saved in a mat-file, in the folder ‘Data’ of the corresponding replicate (see Figure 4.5 on page 100 for an explanation of the folder tree). Each matrix is saved
in a different file that is named after the frequency of the electric field used during the acquisition. That way it is possible to access the data easily if one needs to check the images, if one needs to investigate a particular effect or to check the presence of bubbles for instance. The system is then ready for the data processing.

4.3.4 Data processing algorithms

4.3.4.1 Principle

The data processing is performed in two steps. First comes the treatment of the images as explained in section 3.3 of Chapter 3, on page 43. This stage corresponds to the measurement of the DEP force and the calculation of the DEP spectrum, and is completely automated. Then comes the curve fitting of the DEP spectrum explained in section 3.5.4 of Chapter 3, on page 85. This operation is semi-automated so it cannot be done in the continuity of the previous processing. That is why a dedicated GUI has been created for that purpose. The details of these two processes are explained in the next sections.

4.3.4.2 Application of the circular averaging

The principle of this operation has already been presented in section 3.3.3 of Chapter 3, on page 48. Instead of the principles, this section presents the organisation of the process so that one can know where the data can be found and modify if necessary.

The circular averaging is the first of the two steps of the data processing, so it is also named the ‘pre-processing’, because the results it provides must be processed further in order to obtain the measurement of the conductivity and permittivity of the sample. The pre-processing operation can be performed by the ‘Data processing GUI’, accessible from the main GUI, which appears on figure 4.22.

The left-hand box lists the experiments that are to be processed. The experiments that have been performed before opening this GUI appear automatically, and the experiments previously done can be loaded by pushing the ‘Add...’ button below the list and by selecting the file ‘experiment.mat’ corresponding to the experiment to process. The items listed here are processed one after the other when pushing the ‘Launch’ button.
Chapter 4. Description of the apparatus and data processing

Figure 4.22: Data processing GUI

The right-hand box lists the data that have been pre-processed and that can be used for the curve fitting algorithm. The result can be visualised directly by selecting ‘Plot raw data’ or can be processed further, as explained in the next section.

The acquired images are re-loaded and processed in order to provide a measurement of the DEP. When the pre-processing is launched from the left-hand panel, the computer loads the sequences of images acquired at the different frequencies of electric field and analyses them one by one. The analysis consists in a circular averaging on the image of the microwell together with a logarithm normalisation, as described earlier in Chapter 3, on page 44.

The result of the pre-processing is stored in a ‘.mat’ file. The analysis of the average level of light in the microwell is explained in Figure 4.23: the images are grouped by frequency of electric field; each group is composed of several images taken at different time of application of the field; each image is decomposed into concentric rings where the
measurement of the DEP force is averaged. Hence the result obtained is a 3D matrix, named ‘results’ in the program, formatted as follows:

\[ \text{results}(F,T,P) \]

where \( F \) is the index of the frequency among the list created at the beginning of the experiment, \( T \) is the index corresponding to the time when the picture has been taken starting from the application of the electric field and \( P \) is the index corresponding to the ring on which the data has been averaged out on the image. The corresponding lists of frequencies, acquisition times and ring position is provided by three other variables, namely \texttt{Frequency}, \texttt{Deltat} and \texttt{position} respectively, which give the numerical values from the indexes. These variables are all stored in the same file ‘Results.mat’ in the folder corresponding to the corresponding replicate at the end of the pre-processing.

The DEP spectrum is calculated out of the result of the pre-processing. Once the data has been pre-processed according to the theory exposed in Chapter 3 on page 44, the result obtained is expected to be proportional to the DEP force over each respective
band. Hence, the DEP spectrum is found by averaging the data obtained in the matrix results calculated previously over a given 3D area of interest that regroups the linear regions. The results of this operation is stored in the file ‘Spectrum.mat’. This contains the spectrum, the corresponding error bars and the list of frequencies, respectively named $K$, $errK$ and Frequency. It should be noted that the spectrum obtained by this calculus is theoretically proportional to the true value of the DEP force, but that the proportionality factor is unknown and is therefore one of the variables solved by the curve fitting.

4.3.4.3 Application of the curve fitting

The previous section shows how the images acquired are pre-processed and give the DEP spectrum. This spectrum provides the measurement of the permittivity and conductivity of the different parts of the cell, as explained in section 3.5.4 of Chapter 3, on page 85. The program developed in that aim is presented below.

**The DEP spectrum is loaded, and fitted with a multishell algorithm.** Once the images have been pre-processed, the user can launch the fitting GUI by pushing the ‘Fit the data’ button. The windows then appears then on the screen as shown on Figure 4.24. This GUI uses the multishell model presented in chapter 3 on page 85 to fit the data selected on the right-hand box of the pre-processing GUI. This procedure is not fully automatic because the program needs a guess a the initial values of permittivity and conductivity. That is why this GUI proposes several input boxes where the user can select the number of shells to use for the fit and the initial values for each of them. It is also possible to choose the thin-membrane approximation developed in Chapter 3 for the layer corresponding to the membrane; doing so modifies the value of the guess according to Equation 3.31 on page 72.

The fit is performed with the script ‘FitSpectrum.m’ that uses the model defined by the script ‘MultiShellModel.m’. The first one contains the code that performs the fit and, for that purpose, uses a program available from the Matlab fitting toolbox called by the command ‘fit’. The second one contains an algorithm that numerically processes the multishell model for a given number of layers with their respective properties, and is used at each iteration of the fitting procedure. Both scripts can be used outside the GUI, or can be placed in another program. The script of the model is called with the following command:
Figure 4.24: Curve fitting GUI

\[
\text{Spectrum} = \\
\text{MultiShellModel} (\text{Frequency}, \text{Permittivity}, \text{Conductivity}, \text{Dimension})
\]

where \text{Frequency} is a vector of the frequencies selected for the X-axis of the spectrum, \text{Permittivity} is a vector containing the values of the permittivity of the core, layers, and medium respectively, \text{Conductivity} is a vector containing the values of the conductivity of the core, layers, and medium respectively, and \text{Dimension} is a vector containing the values of the radius of the core, and the thickness of the layers. If the first layer has a thickness of zero then the script uses the thin-membrane approximation. \text{Spectrum} is the value of the spectrum corresponding to the list of frequencies provided.

The script that fits a DEP spectrum with the multishell model can be called with the following command:

\[
[\text{Measure, Error, Names, gof, Scale}] = \\
\text{FitSpectrum} (\text{Frequency, Spectrum, Guess, Vary, Dimension})
\]

where \text{Frequency}, \text{Spectrum} and \text{Dimension} have the same meaning as above, \text{Guess} is the vector of the initial guesses for the values of permittivity, conductivity and dimensions of the layers, provided by the user, \text{Vary} is a vector of logical values that equals 1 if the corresponding content of the \text{Guess} vector is supposed to vary during the fit, 0 if it is constant. \text{Measure} provides the numerical result of the fit for the permittivity and conductivity of the different parts of the cell, \text{Error} is the level of error on the measurement, \text{Names} is a list of names corresponding to each value of the vector \text{Measures} in order to remember their meaning, \text{Scale} is the measurement of the scaling factor and
gof is the fit object resulting from the use of the function fit in Matlab, which contains information about the accuracy of the fit.

The result of the fit is saved into different files. The curve fitting GUI allows the user to select up to five shells, with two or three guesses for each one. It must be noted that only the first shell can model a thin membrane because of a problem of implementation of the thin-membrane algorithm. When the button ‘Fit the data’ is selected, the GUI start to fit the data selected one after the other. The fitting provides two different types of information: information about the measurement (Measure, Error, Scale) and information about the fit itself (gof, which contains the fitted curve among others). For ease of use, the fitted curve is saved in the file Spectrum.mat that contains the pre-processed data, so that both can be plotted on the same graph from the same file. The other results are stored in the file Measure.mat: this file contains the values of the initial guesses provided by the user Guess, the parameters measured by the fit Measure, the corresponding error bars Error, the respective name of the parameters measured Names, the relevant information about the quality of the fit gof and the description label entered by the experimenter before the experiment TaskTag. The file Measure.mat is placed in the directory of the corresponding replicate.

The result of the processing can be presented directly into a normal plot, or into a cytogram-like plot. Once the fit has been performed, there are two options to visualise it directly: the direct plot or the cytogram plot. A direct plot creates one figure for each of the parameters measured, whereas a cytogram plots only one parameter as a function of another. According to the experiment, the user is asked which variable is assigned to each axis. For instance, if a series of experiments have been performed with increasing values of medium conductivity, the GUI asks if the user wants to plot the measurement as a function of the medium conductivity. In the case of a direct plot, the user has also the possibility to average the results acquired if there are repeated data points.

4.4 Conclusion

The system presented in this chapter has all the necessary features to conduct a DEP experiment in accordance with the theory. This chapter has explained
the functioning of the machine that has been developed to solve the problems that arise in the manual experimentation, as presented in Chapter 2. It has been possible to build a machine that can perform a DEP measurement automatically, but still the presence of the experimenter is necessary to prepare the sample and to initialise the curve fitting. However, it has not been possible to reduce these tasks to simple automated tasks for different reasons explained in the chapters 3 and 4, therefore the system obtained from this work is automated as far as is has been possible in the frame of this thesis.

The role of the experimenter has been reduced to the minimum, as set in the requirements. The only points left where the experimenter has to intervene are the cell-specific points where the machine has no mean to find the information otherwise. An attempt has been made to detect the number of transitions and plateaus of the DEP spectrum measured and to deduce the best adapted model, but this has proved unfruitful so far, mainly because the number of plateaus and transition frequencies is not always linked to the number of shells. On top of this, an attempt has also been made to find an initial coarse guess from the equations presented in section 3.5.3 of Chapter 3 on page 76, but the quality of the resolution was too poor to lead to a correct guess, and the method presented has not been generalised for any number of shells. These issues could be a topic for further work.

The overall performances of the machine have been tested and have improved. Even with these limitations, the performances of the machine are significantly improved since the manual procedure. The diminution of the acquisition time has been mentioned in this chapter, but the selection of the devices, their arrangement and the scripts developed to use the overall have largely increased the signal-to-noise ratio. This has been measured by several test, presented in the next chapter.
Chapter 5

Results

5.1 Introduction

Chapter 4 described the device as developed in this thesis, and Chapter 3 presented the models used by the data processing associated with the device. As explained in these two chapters, much care has been taken in different aspects of the work, both for the theory and the practice, in an effort to increase the signal-to noise ratio, the reliability of the final data and the accessibility of the software. The results of this work has been measured with a series of tests on the repeatability of the experiments and the levels of noise recorded on different samples. This chapter presents the results of these series of test and the different attempts that have been made to improve the machine. Two sorts of tests have been made:

1. Assessment of the improvement made by a particular change in the procedure or in the system. These are presented in section 5.2 on page 138.

2. Assessments of the performance of the final result, in the optimal configuration. These are presented in section 5.3 on page 150.

In addition to these results, some interesting data have been added concerning the effect observed in the microwell during the measurement and that have an effect on the quality of the data obtained. These are presented in section 5.4 on page 159.
5.2 Results from the optimisation of the prototype

5.2.1 Introduction

As explained in Chapter 4, the device has been developed by a series of refinements, such as the addition of a lens, the modification of the image processing algorithm, and so on. The effect of each change was checked experimentally to observe any improvements or deterioration in the data. According to the results obtained, the change was kept, modified or discarded. This section presents the results obtained during this optimisation process. The modifications that led to an improvement of the results are presented with the measurement of the improvement. However, the modifications that did not bring any improvements are also presented, with an explanation of the probable reasons of the failure.

Many improvements were tested to reduce the level of noise and the effect of each has to be measured. Several sources of noise were identified in 2.3 on page 22, and different solutions emerged to try to diminish them. These solutions had to be tested with a criterion of selection in order to keep the most effective. The criterion of interest here is the signal-to-noise ratio of the DEP spectrum obtained from a measurement of yeast cells, because yeasts samples are easy to obtain experimentally. The tests and their results for the different solutions retained are presented in this section.

5.2.2 Acquisition time

The reduction of the duration of the data acquisition affects the signal-to-noise ratio. The acquisition time is the duration of application of the electric field during the measurement of the DEP force, at each given frequency of the spectrum. During that time, the particle evolves by the effect of the DEP force and provides the signal to the camera. It seems then that the longer the acquisition time, the better the results. However, little is known yet about the evolution of the noise, therefore a measurement of the evolution of the noise with the acquisition time has been realised for a given strength of electric field.

Yeast samples were used at seven different concentrations from $1.3 \times 10^6$ to $1.3 \times 10^9$ cells/ml, at a medium conductivity of $50\mu$S/cm. Each sample was measured by the
machine with an acquisition time of 4 seconds and a voltage of 12Vpp. The data were processed normally and the curve fitting method explained in Chapter 4 was used to estimate the levels of signal and noise for each time point. However, the level of noise cannot be compared between two experiments because it depends of the amplitude of the signal, so it has been normalised by taking the noise-to-signal ratio, which is the inverse of the signal-to-noise ratio. The noise-to-signal ratio has therefore been processed for each time point and has been reported on Figure 5.1.

The concentration has an effect on the amount of signal that can be measured after a given time. This graph contains much information. First, it can be seen that, for a given amplitude of the electric field, the level of noise/signal shows the same rate of decrease, regardless of concentration. This means that there is a minimum duration of application of the electric field before the results obtained reach an acceptable level of signal-to-noise ratio. Second, it can be observed that the noise/signal ratio diminishes quickly in the first seconds but tends to stabilise after that. The level obtained here after 4 seconds depends on the concentration of the sample: the higher the concentration, the lower the noise in the data. Third, there does not appear to have much difference
between the evolution at $5.1 \times 10^6$ cells/ml and $5.1 \times 10^7$ cells/ml so the variability seems pretty high. However, if one compares the slope of the different curves at 1s, one find the results shown on Table 5.1. These show that the higher the concentration, the faster the attenuation of the noise, which means that for a given initial level of noise a sample at high concentration can reach a lower level of noise than one of lower concentration. However, in practice, it is difficult to obtain very high concentrations of cells. This is especially true for tissue cells, which grow on surfaces and therefore do not have much space to develop. Hence there is a compromise to be reached for each cell line between the high concentration and the experimental restrictions.

### 5.2.3 Amplitude of the electric field

The theory developed in Chapter 3 states that the evolution of the concentration at small time is proportional to the strength of the electric field. Therefore, the amplitude of the wave generator was expected to have an effect on the evolution of the signal/noise ratio. A series of experiments were performed on yeast cells, at a concentration of $3.5 \times 10^7$ cells/ml, for different amplitudes of electric field. The signal/noise ratio in the results was analysed the same way as presented previously. The results are presented on Figures 5.2 and show that the amplitude does have an effect on the evolution of the level of noise: the higher the amplitude, the faster the level of noise decreases and hence the higher the signal/noise ratio. However, it is not yet possible to determine if this effect is due to an increase of the signal or to a diminution of the noise, or both. This can be investigated further by considering the theoretical evolution of the signal.
Chapter 5. Results

Evolution of the noise measured on yeast cell spectra with time

Figure 5.2: Evolution of the level of noise in the data with time, at different amplitudes of electric field.

Effect of the voltage on the decrease rate of the noise level

Figure 5.3: Evolution of the decrease rate of the noise against the amplitude of the electric field.
The amplitude of the electric field has an influence on how fast the signal appears. According to Equation 3.4 on page 45, the evolution of the concentration should be proportional to the amplitude of the electric field squared, which means that the decrease rate of the noise level reported on Figure 5.2 should also be proportional to the amplitude of the electric field squared. Figure 5.3 shows the rate of decrease of the noise/signal ratio as a function of the amplitude of the electric field, for different amplitudes. The curve obtained can be fitted relatively well by a parabolic curve, which can be interpreted as being due to the evolution of the concentration (i.e. the signal), as predicted by Equation 3.4, with a constant level of noise, or as being due to a mix effect of the evolution of the signal and the noise. However, this second supposition is unlikely because there is no reason why the noise, which may have many sources, should depend so strongly on the amplitude of the electric field. Therefore, only the first proposition is likely to be valid.

It can also be seen on Figure 5.2 that the level of noise tends to 20% of the signal when given enough time, independently from the amplitude of the electric field. This means that the amount of signal measurable does not change with the amplitude, if given enough time of evolution. Therefore, the voltage does really act as a scaling factor on the time scale during the measurement and lowers the time needed to reach the best signal/noise ratio.

### 5.2.4 Experimental time

The experimental time is the time it takes for the machine to complete the acquisition of the measurement of a sample. This duration is the sum of the resuspension time, the acquisition time and the duration of the data transfers, the total being multiplied by the number of data points acquired. It has been shown in the previous section that the acquisition time should not exceed 10s. In addition, the data transfers and sample resuspensions take 20s in total, for a configuration using a very slow motor speed (10% of the full scale). In total, this gives a value of 30s per data point for a very slow configuration, so that the experimental time for a 10-point spectrum would be 5min. In general, the spectra acquired have between 15 and 30 data points and the experimental time varies from 3 to 15min, depending on the number of points required in the spectrum, the acquisition time and the resuspending time.
5.2.5 Light path and focusing

Section 4.2.5 on page 115 presented the optical system that was used for the acquisition of images during the measurement. The microscope was augmented with an additional lens that has the effect of focusing the light beam to infinity, so that the image recorded measures the concentration in the whole microwell. The addition of this lens is supposed to increase the level of signal in the data, but can also damage the quality of the image by adding more inhomogeneities in the background. To this end a comparison was made between recordings with and without this lens. Figure 5.4 compares the noise recorded in an experiment with and without additional lens: the results for yeast cells are not conclusive but tend to show that the lens configuration seems to provide better results above $10^8$ cells/ml, but worst below that threshold. However, as this configuration is closer to the theory developed in Chapter 3, it was used for the experiments in order to prevent possible artefacts.

![Figure 5.4](image_url)

**Figure 5.4:** Level of noise recorded on yeast cells DEP spectra with finite (red) and infinite (blue) focal length.
5.2.6 Bubbles and tubing length

5.2.6.1 Effect of bubbles

The presence of bubbles in the system creates several problems: because bubbles do contract when pressure is applied and expand when the pressure is removed, they prevents the fluid from stopping neatly after pumping and create a residual flow during the acquisition phase; they damage the quality of the image by diffracting the light; they modify the trajectory of the cells in the microwell during the application of the DEP force.

Figure 5.5 shows the effect of the presence of a bubble in the microwell during an experiment. Such an event creates a very important variation of the level of light, and therefore creates outlier points. Here the bubble has appeared at the frequency 310 kHz and the result measured is three orders of magnitude greater than the data expected. This makes it easy to filter the noise created by bubbles during a measurement, but it also destroys the data as there is no way to recover. More significantly, bubbles remain stuck in the microwell and deteriorate several points of the spectrum. Sometimes, the bubble can be attached so strongly inside the microwell that the stepper motor cannot create a
flow strong enough to detach it; in such a case, the acquisition has to be stopped and the experimenter has to detach the syringe from the pump and to use it manually in order to produce enough pressure to detach the bubble. This problem occurs occasionally, especially when the microwell is dirty from a previous experiment.

5.2.6.2 Solutions found

Several solutions have been found to prevent the appearance of bubbles in the system and to facilitate their evacuation from the microwell.

1. Thinner tubing

   Diminishing the diameter of the tubing helps decrease the probability of formation of a bubble. This is because thinner bubbles require more interfacial energy, and are therefore more difficult to create. In practice, the difference is very visible: the 1/8 inch tubing used in the first prototype was transparent and it was possible to see microbubbles trapped in the pipes and travelling through the microwell during the resuspensions; the 1/16 inch tubing is opaque but no bubble could be seen passing through the microwell during any phase of the measurement.

2. Shorter tubing

   Another problem that occurs from the use of tubing is its resistance to the flow. A high resistance to the flow is a problem for several reasons:

   - A high resistance means a high gradient of pressure through the tubing. Living cells can be very pressure-sensitive and can be damaged by high pressure gradients.
   - There is always a bubble that remains at the bottom of the syringe and acts like a pressure capacitance. With the tubing resistance, the system obtained is an RC circuit with a cut-off frequency that decreases with the resistance. This is a problem when the syringe pump is activated during the resuspension of the sample because it operates in a back-and-forth movement with a frequency of several seconds, according to the experimental parameters. Hence, if the cut-off frequency of the system falls below the frequency of activation of the pump, the efficiency of the resuspending is greatly diminished and the system
cannot obtain a homogeneous concentration before a measure, which damages the results.

- It increases the work load of the stepper motor. The stepper motor selected has a maximum linear force of 20N, which can be a limitation when using thick liquids that require more force to pass through the tubing.

The resistance of a tubing can be characterised by two parameters: its radius and its length. The thinner the tubing, the smaller the cross-section and so the bigger the resistance. The tubing also has a specific resistance per length unit, hence the longer the tubes the higher the resistance. However, it has been explained above that the radius should not be larger than 1/16”, so only the length of tubing can be modified. Therefore, the solution found has been to use Teflon tubing with the smallest possible length. The total length of tubing used in the final prototype is about 40cm. The cut-off frequency of the system has been measured roughly by observing the time it takes for the system to reach an equilibrium after a pressure step created by the syringe pump and has been evaluated at 2 Hz ±50%. A direct observation through the microscope during a resuspension phase shows that this cut-off frequency is high enough to allow a correct resuspension of the sample in the microwell.

3. **Filling the tubing at the end of the experiment**

One simple way to avoid the presence of bubbles in the system is to avoid the presence of air in the tubing. To this end, the machine is filled with a washing medium when the experiment is finished instead of being emptied. This also has the advantage to keep the system clean, so that the microwell is kept free of dirt, which further lowers the probability of trapping a bubble during a resuspension. The washing medium used is a solution of 5% Decon 90: this product has the property to dissolve a wide range of compounds and is completely washed by water so that it does not contaminate the sample after use.

4. **Slow flow rates**

As explained before, some bubbles may be trapped in the system. Provided that they do not appear in the microwell and that their size is reasonably small, this is not a problem. However, if a bubble is attached to a portion of tubing, it may detach during the resuspension phase if the flow applied is too high. Therefore,
using slow flow rates prevents this from occurring. It also diminishes the constrains of the cells and increases their survival rate. However, a lower flow takes longer to resuspend the sample in the microwell and thus increases the experimental time. In practice, a compromise of 20% of the full speed is used as a default value in the data acquisition GUI.

5. One-way rinsing flow

The rinsing procedure used to wash the system before and after the experiment has been designed to avoid the injection of bubbles into the microwell. It works by passing the washing medium only from the inlet to the syringe, and by emptying the syringe directly to the bin. Therefore, the flow only travels in one way through the microwell and the air surrounding the outlet cannot enter the tubing.

6. Good quality connectors

The connections between the different elements of the fluid system consisted at specially-designed elements, such as finger-tight connectors. This minimises the total volume required to fill the machine and to avoid leaks.

When followed, these measures can lead to a very low probability of bubble interference. The probability of observing a bubble in a measurement can be estimated by comparing the number of data points wasted by bubble noise to the number of data point acquired, over a large number of spectra. For the first prototype, the probability found is about $1/6 \pm 10\%$; it fell to $1/280 \pm 10\%$ with the last prototype. However, bubbles still happen occasionally, so the user must remain vigilant and should control the microwell before each measurement.

5.2.7 Other improvements

Other improvements were made during prototyping but their effects could not be measured in term of signal-to-noise ratio. However, they clearly improved the usage of the machine, so they have been listed in this section.

1. Sample volume

The last prototype required a minimum volume of sample of 350µl. Because of its thick and long tubing, the first prototype required a minimum volume
of sample of 1ml. Most of the sample was spilled by leaks, so that the sample could not be re-used for a second measure. The last prototype developed used thinner and shorter tubing together with better connections, and decreased the minimum volume necessary for a measurement to 350µl, without losses so that the same sample can be measured several times.

2. Decreased experimenter manipulations

The software manages most of the tasks, leaving the experimenter with what is essential. The last prototype developed use software that can handle all the non-essential tasks during the measurement of the DEP force. The role of the experimenter is to bring what is necessary to the automate, that is to say the sample, its information about the concentration and conductivity and a guess for the curve fitting.

3. Data communication

Error management made the last prototype much more reliable, so that it can run for several hours of experiment without interruption. The different prototypes developed had many problems conducting long experiments because of the errors of transmission in the RS232 cables, probably due to the noisy electromagnetic background of the laboratory. Even with shielded cables, some errors occurred and stopped the procedure because the program could not handle them correctly. The last version of the software identifies these errors and corrects them so that the experiment goes on in the event of a transmission problem. So far, no time limit has been observed during the data acquisition, even after three hours of use. However, because of the initial problems of stability, the software saves the data as soon as they are measured so that a problem in the measurement cannot delete what has been acquired.

5.2.8 Other attempts

Several approaches to improve the device did not work. The most important ideas have been reported here together with the reasons why they did not succeed, so that further work can eventually be carried out.
1. Signal modulation

The main microflows occurring during the application of the DEP field can be reduced by reducing the temperature gradient. The parasitic microflows appearing during the measurement of the DEP force are expected to damage the quality of the results by modifying the trajectory of the particles submitted to the DEP force. So, diminishing their velocity should increase the reliability of the measurement. As explained in Chapter 2 on page 23, the main microflows occurring during the measurement are the A.C. electro-osmotic and electrothermal flows. the later depends on the gradient of temperature, among others factors (Castellanos et al., 2003). Hence, diminishing the temperature gradient can lower the electrothermal microflow. This can be achieved by reducing the sources of heating, which are mainly due to the light and the electric field. The heating from light absorption are lowered by IR filtering as this wavelength is not recorded by the camera, but the electric field cannot be modified because it generates the DEP force and, as has been shown previously, the higher the voltage, the better the result.

The modulation technique reduces the temperature gradient but also reduces the DEP force beyond the measurement limit. It is possible to lower the heating by using pulsed electric field with a modulation technique. This can produce a high-amplitude signal with a low average power. A test has been performed with a square modulation of 100 Hz, with a duty ratio of 1/10. Unfortunately, the results were very poor because the signal decreased dramatically with the duty ratio, so this technique has not been developed further.

2. Light reference in a separate well

The instabilities of the light source add an important noise to the data. The data processing procedure explained in Chapter 3 explains that the DEP spectrum is obtained from a normalised set of data. This normalisation is a way to provide a measurement free from the influence of the light source, and is performed by dividing the images acquired by a reference image taken prior to the application of the electric field.

The light source can be normalised for each image with a second microwell, but this decreases the amount of signal measured by the camera. However, this method requires a stable source of light so that its luminosity does
not change during the application of the electric field. This was a problem with the initial light source that was unstable, as explained in section 2.3.5 on page 25. That is why a microchip has been created with two microwells close to each other, but with only one of them powered. That way, it was possible to find a reference of light intensity for each image and to immunise the data processing from the fast variations of the light source. However, this method requires to increase the field of view of the camera in order to observe the two microwells, which means that less area is used to measure the signal, which diminishes the signal-to-noise ratio. Instead of that, the solution kept is to use a very stable power supply for the light source.

3. **Impedance measurement by Wheatstone bridge** The detection of the DEP force is performed by measuring the light absorption of the sample. However, other works have reported a measuring technique using the variations of impedance of the microchip from the movements of the cells (Milner et al., 1998). This possibility has been investigated with the first prototype: such a measurement requires a great accuracy, so a Wheatstone bridge has been developed with four microchips, connected by pair. However, the results were very poor and the project has not been followed.

### 5.3 Performance of the final prototype

#### 5.3.1 Introduction

The machine, used together with the program that has been developed with it, is able to perform the data acquisition, processing and post-processing for a 20-points experiment in less than 8min for a single measurement or 6min per measurement if more than one is performed. Yet, the experimenter can control some essential parameters of the acquisition and curve fitting so that the calculation of the data includes the specificity of the cell line being measured.
5.3.2 Noise study

The level of noise in the system depends on several parameters. However, as explained in the first section of this chapter, only two of them are significant for this study; the concentration of the sample and the amplitude of the sine wave used to generate the DEP force. Therefore the analysis of the noise can be performed by a surface response analysis using these two parameters.

The quality of the data increases with the concentration and with the signal amplitude. The surface response analysis was performed with samples of yeast cells at three different concentrations ($5 \times 10^6$, $5 \times 10^7$ and $6.2 \times 10^8$ cells/ml), and using three different signal amplitudes (8, 13 and 18Vpp). For each couple of parameters, two DEP spectra were recorded in order to optimise the system, to detect any problem in the experiment and to evaluate the repeatability. Once obtained, the raw spectra were processed normally by the software and the curve fitting has provided an estimation of the signal for each dataset. This estimation has then been used to calculate the level of noise in the data by evaluating the difference between the data acquired and the estimation provided by the curve fitting. The average noise is then found by processing the standard deviation of the noise found in the signal.

The result of this analysis is presented on Figures 5.6 and 5.7 and shows clearly the relation between noise level, signal amplitude and concentration. The noise is greatly diminished at high concentration and tends to decrease with increasing amplitudes too. It can also be seen that the signal amplitude does not have a strong effect above 14V, but that weak DEP signals create a strong level of noise. This is related to the fact that the DEP signal cannot be measured at time $t = 0$ and that it takes some time to generate a measurable difference of light in the microwell. However, as explained in section 5.2.2 on page 138, it is necessary to diminish the experimental time as much as possible. Here, the duration of application of the DEP field is 5s, and Figure 5.7 shows that this is the smallest time usable for yeast cells in order to obtain noise-free data for relatively low concentrations.

It is important to know what is the type of noise present in the data, in order to check the presence of bias or other phenomenon. Figure 5.8 presents the histogram of that error from the dataset provided by the difference of four yeast spectra with their respective fits, at a concentration of $5.1 \times 10^8$cells/ml. This histogram indicates a noise...
Chapter 5. Results

Figure 5.6: Surface response obtained by the study of evolution of the level of noise with the signal amplitude and the cell concentration.

Figure 5.7: Isocontours corresponding to the surface above.
level under 3% for 42% of the data acquired. A significant part of this noise is due to the discrepancy between the data and the fit below 6 kHz, which is attributed to the effect of electrohydrodynamic flows, which are known to perturb DEP collection in that frequency range (Castellanos et al. 2003). If this low-frequency part is removed from the histogram of the noise, then 55% of the data acquired has a level of noise under 3%. However, several points appear above 10% of noise: these are the points at the edges of the spectrum. At low-frequency, the EHD effects introduce a noticeable drop of the DEP signal amplitude, leading to a difference with the curve fitting of 10 to 20%, depending on the cases. At high-frequency, the edge of the spectrum is bent because of the second frequency transition, but the curve fitting algorithm does not always find a good fit for that region due to inadequate number of data, and sometimes provide a bias of 10 to 30%, that also appear in the histogram. These edges effect contribute to higher the average noise in the spectrum, so most of the time the very low-frequency region should not be measured, and the high-frequency limit of the spectrum should be pushed further in order to acquire more data points for the curve fitting algorithm around the high frequency transition.
5.3.3 Bias study

The analysis proposed in Chapter 3 on page 48 uses the small time approximation to provide a minimum bias in the data. The limit of this approximation should be measured in order to limit the length of the data acquisition and to check the validity of the results obtained. The bias can be seen by comparing the spectra measured at different time, scaled to the same amplitude, on a given sample. This analysis has been performed on yeast cells at $5 \times 10^8$ cells/ml, with a medium conductivity of 5.3 mS/m and an electric field amplitude of 16Vpp. The result is presented on Figure 5.9 and shows a slight upwards shift of the low-frequency region as time goes on. However, this shift remains within the 3% error limit for the first 2.8s in this case. As the square of the amplitude of the electric field acts as a scaling factor over time, it is possible to extend this time limit as required by diminishing the voltage. However, it has been shown in the first sections of this chapter that the experimental time should be as short as possible, therefore a measurement over a short period of time is appreciable and so the electric field should be sufficiently high. In practice, the small-time limit is set to 3 to 4 seconds in order to leave enough time for the data acquisition, as the data acquired during the first second of application of the DEP force is not used in the data processing.
5.3.4 Repeatability study

In order to be measured accurately, the repeatability study should be isolated from the noise as far as possible. That is why this test has been performed on samples of yeast cells at very high concentration and high electric field amplitude, according to the results found in the previous sections. The concentration used here is $7 \times 10^8$ cells/ml, the amplitude is 16Vpp and the conductivity of the medium is 5 mS/m. Figure 5.10 shows the level of repeatability of the measurement between two spectra acquired over a period of time of 15min in total. It can be seen from this figure that no bias is introduced between two experiments, and that the only differences observed can be attributed to the normal level of noise under such conditions as defined in the previous section.

Another test was performed over several spectra in order to investigate the effect of time on the repeatability. Figure 5.11 shows a spectrum obtained from an average of four experiments on a sample of yeast cells at $5.1 \times 10^8$ cells/ml over a period of time of 30min, with the error bars standing for the standard deviation between the acquired
Chapter 5. Results

Comparison between an average spectrum of yeast cells and the corresponding fit

![Figure 5.11: Spectrum of yeast cells obtained from an average of 4 spectra. The error bars represent the standard deviation.](image)

 spectra. The dashed curve that appears on the figure represents the average fit using the double shell model with thin membrane approximation on the yeast spectra. The difference between the fitted curve and the data provides an estimation of the error in the data.

Compared with the previous result, we can observe that the error bars in the region of the low-frequency transition are slightly greater than expected. This can be attributed to very small changes in the conductivity of the suspending medium after 30min of preparation, because the cell concentration is high enough to give rise to significant variations. However, it should not be an issue at lower concentration, and in general the level of error still remains within the 3% threshold value. It can also be noticed that the low-frequency edge of the spectrum is slightly bent upwards. As mentioned earlier, this is due to EHD effects and is not very strong at high voltage such as 16V as used here. The curve fitting has been performed without these points for more accuracy.

Several tests have also been performed on the cell samples in order to check if the cell populations were surviving the pressure gradient and the shear forces in the tubing. One test has been made on a sample of yeast cells and another on K562 cells. Both were
using a motor velocity of 30% of the full scale, in a 10 minute long experiment using 30 data points. The ratio of living cells has been determined by Trypan blue test on the sample before and after the automated DEP measurement with the following procedure.

- The sample is prepared according to the protocol explained in Chapter 2.

- Once ready for the measurement, the sample is split into two. One part is used for the DEP measurement and the other is tested with Trypan blue in order to determine the ratio of living cells before the experiment.

- After the experiment, the sample used for the DEP measurement is tested with Trypan blue in order to determine the ratio of living cells after the experiment.

The cell count was performed after 5 minutes of application of the Trypan blue to the sample, the counting was performed on haematocytometer on a 1mm$^2$ surface composed of 16 squares. For visibility reasons, the initial sample was diluted one hundred times before counting. The results are presented on Table 5.2 and show a light decrease, which remains within the confidence bounds. This may show that the procedure damages the sample, but this action is too small to be detected on the time scale of the experiment so it should not affect the results.

5.3.5 Validation by comparison with the literature

Section 3.5 on page 70 of Chapter 3 presented a method of data processing by curve fitting. Using curve fitting on DEP spectra is not new and several researchers have used it to determine the properties of yeast cells (Raicu et al., 1996; Hözel, 1997; Fatoyinbo et al., 2008). However the analysis made in Section 3.5 showed that a curve fitting should be done with a restricted number of parameters according to the model selected,
and that the thin-membrane approximation can be used in that purpose. Section 3.5.2 on page 71 demonstrates that this approximation is numerically valid within the range of membrane thicknesses that can be observed in living cells, and that it can be used in the multishell model to fit a DEP spectrum. This develops a new approach that requires a validation by comparison with the literature.

This comparison was performed using yeast cells, which is widely used in Depas standard model which has been studied extensively with DEP in previous works as mentioned in Chapter 1. The sample was prepared according to the protocol presented in section 2.2.3, and measured by the machine using the program presented in section 4.3. The DEP spectrum was measured on 20 points between 1 kHz and 20 MHz with 4 acquisitions per point, using a conductive medium of 2.5 mS/m prepared accordingly to the protocol presented in Chapter 2.

The validation was performed by comparing the values found from the fit with the values provided by the literature. Table 5.3 presents the result of the curve fitting for the four yeast spectra measured here (left column) and the values found in the literature (right column). The values of the parameters obtained from the measurement have acceptable confidence bounds with regards to the literature, with the exception of the cytoplasm permittivity which cannot be accurately measured since the experimental setup does not cover the high frequency region far enough. The numerical values found for yeast cells fall into the bounds defined by the literature, even though the values of membrane conductance and wall capacitance are relatively high. However, the different measures of this conductance found in the literature reports that it varies over a broad range of values, which indicates that this parameter is likely to be sensible to the experimental conditions. The values found for the membrane capacitance and the wall capacitance and conductance are close to the values reported from the literature, the differences noted can be attributed to a difference in the cell physiology during the measurement.
### Results

#### Obtained results versus Published results

<table>
<thead>
<tr>
<th>Property</th>
<th>Obtained results</th>
<th>Published results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm conductivity</td>
<td>$400 \pm 50 \text{mS/m}$</td>
<td>$550 \pm 50 \text{mS/m}$ (Hölzel, 1997)</td>
</tr>
<tr>
<td></td>
<td>$140 \pm 40 \text{mS/m}$</td>
<td>$140 \pm 40 \text{mS/m}$ (Fatoyinbo et al., 2008)</td>
</tr>
<tr>
<td>Cytoplasm permittivity</td>
<td>$195 \pm 100$</td>
<td>$50.6 \pm 2.1$ (Raicu et al., 1996)</td>
</tr>
<tr>
<td>Membrane conductance</td>
<td>$1000 \pm 90 \text{S/m}^2$</td>
<td>$5.5 \pm 50 \text{S/m}^2$ (Hölzel, 1997)</td>
</tr>
<tr>
<td></td>
<td>$340 \pm 200 \text{S/m}^2$</td>
<td>$340 \pm 200 \text{S/m}^2$ (Fatoyinbo et al., 2008)</td>
</tr>
<tr>
<td>Membrane capacitance</td>
<td>$6.6 \pm 3.0 \text{mF/m}^2$</td>
<td>$7.03 \pm 0.11 \text{mF/m}^2$ (Raicu et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>$7.6 \text{mF/m}^2$</td>
<td>$7.6 \text{mF/m}^2$ (Hölzel, 1997)</td>
</tr>
<tr>
<td>Cell wall conductivity</td>
<td>$20 \pm 5 \text{mS/m}$</td>
<td>$0.6$ to $24 \text{mS/m}$ (Hölzel, 1997)</td>
</tr>
<tr>
<td>Cell wall capacitance</td>
<td>$2.7 \pm 0.7 \text{mF/m}^2$</td>
<td>$1 \text{mF/m}^2$ (Hölzel, 1997)</td>
</tr>
</tbody>
</table>

Table 5.3: Comparison between the measurements found and the data from the literature for yeast cells *Saccharomyces cerevisiae*.

### 5.4 Effects observed and additional experiments

#### 5.4.1 Introduction

Several effects were observed during the use of the machine that helped to understand the limitations of the machine, which informed the choices made in Chapter 4 in the data processing algorithm.

#### 5.4.2 AC electrohydrodynamic flows and heating

During an experiment, the cells are submitted to other forces than DEP, that are due to different flows generated in the microwell. Some micron-scale flows, or ‘microflows’, can appear during the measurement of DEP in a microwell and originate from different sources. As they affect the cell motion, these flows are likely to damage the results. The main ones are presented in section 2.3.2 on page 23, where it is explained that each different flow occurs at a specific power/frequency domain. The only microflow of importance in the case of the microwell with the protocol used here is the AC electro-osmosis, which affects the DEP collection when the frequency is below 20 kHz. Because its presence diminishes the signal-to-noise ratio, some experiments have
been conducted to investigate how to reduce these flows. Two techniques have been found, presented below.

The microflows distort the DEP spectrum at low frequency, with an enhanced effect at low amplitude of electric field. Figure 5.11 on page 156 presents a DEP spectrum of yeast cells acquired at high concentration and high electric field amplitude. A noticeable difference is visible between the fit from the theory and the data acquired for frequencies below 5 kHz, with the difference increasing as the frequency diminishes. This frequency band corresponds to the domain of A.C. electro-osmotic flows, which are strongly believed to be responsible for this discrepancy. With a finite focal length configuration, it is also possible to check the presence of this flow when the cell concentration is low enough to allow observation of individual cells. In such a situation, the observer can see the cells ‘bumping’ on the electrodes, which is caused by the circular motion induced by the local circular microflows. This behaviour can be observed with planar electrodes on the videos recorded on the CD in the folder Videos/Microflow study - 1 kHz. This effect is more pronounced at high conductivity, typically from 10 mS/m, and increases with the conductivity for higher values.

It is possible to immunise the results from the effects of microflows by limiting the experimental frequency range. The method used here to avoid the influence of the microflows is to remove the data points affected before performing the curve fitting. This is easy to do as the microflows only occur at low frequency, typically below 5 kHz. This technique is performed automatically by the curve fitting algorithm, even though it is possible to remove this filter from the curve fitting panel presented on Chapter 4.

Above 5 kHz, the AC electro-osmotic flow diminishes quickly with the frequency so that the main microflows left are the buoyancy flow and the electrothermal flow. The first is an effect of the gradient of density caused by the gradient of temperature, the other is caused by a gradient of electric conductivity and permittivity also caused by the gradient of temperature. As stated earlier, these flows do not affect the DEP motion significantly, however they have still to be prevented by adding an IR filter to the light source in order to cut the infrared light from the light bulb before the beam passes through the microwell.
5.4.3 Alignment of the particles

When the particles observed are not constituted by spherical objects, an effect was observed on the images that is supposed to correspond to the alignment of the particle along the electric field lines. This create an artefact in the data during the first second of each acquisition and had to be corrected. This effect has been observed on *Bacillus atrophaeus* Nakamura (ATCC, reference number 9372) at a voltage of 12Vpp, for 10s of application, at any frequency between 5 kHz and 20 MHz. When performing an experiment on such cells, the level of light monitored through the microwell presents a homogeneous, transitory and rapid evolution at the beginning of the application of the electric field.

Figures 5.12 and 5.13 show the evolution of the light intensity measured in the microwell with time for yeast cells and for *Bacillus atrophaeus* Nakamura. It can be observed that both present a clear decrease after 2s, which corresponds to a negative DEP. However, the data acquired on bascillae show a very strong positive slope in the first 500ms after the application of the electric field.

This effect is supposed to be due to the reorientation of the bascillaes in the direction of the electric field. In the absence of any force, the bascillaes are orientated in any direction in a random pattern, which provides an anisotropic absorbency to the sample. Once the electric field is applied, the bacteria act like dipoles and align in the direction of the electric field lines. Because this field has a radial and an axial components (see the simulations on chapter 3 on page 54) the bascillaes are being aligned in an ordered pattern by the electric potential applied in the well. This anisotropic configuration changes the absorbency of the sample and therefore changes the average light received.

This effect is fast compared to the DEP collection, so it can be compensated by removing the data acquired during the first second of the experiment. This is performed by default by the data processing program.
Chapter 5. Results

**Figure 5.12:** Evolution of the average level of light of images of yeast cells taken during the application of the DEP force.

**Figure 5.13:** Evolution of the average level of light of images of *Bacillus atrophaeus* Nakamura taken during the application of the DEP force.
5.4.4 High-concentration effects

5.4.4.1 Introduction

Increasing the particle concentration of a sample for DEP measures has the effect of lowering the average distance between the particles in suspension, which promotes particle-particle interactions. A theory has been developed to predict the effect of this interaction using multiple image method (Dong et al., 2004) and states that increasing the concentration should create a distortion in the high-frequency transition of the DEP spectrum, and should also slightly shift the spectrum towards lower values of CMF. A series of measures on high concentration sample has been performed in order to test these results with real data.

5.4.4.2 Methods

Yeast cells were prepared according to the protocols defined in Chapter 2. The final version of the automate was used to measure the DEP spectra of the different suspensions of yeast cells for several concentration, from $3.5 \times 10^6$ cells/ml to $7 \times 10^8$ cells/ml. A suspension of high concentration of yeast cells was obtained by collection of a cell culture by centrifugation, and washed four times in the conductive media following the protocol described in section 2.2.3. Then, cells were counted on a haematocytometer on a diluted part of the sample, and a concentration of $7.0 \times 10^8$ cells/ml was found. The lower-concentrated samples were obtained by dilution of this sample with conductive media.

The suspension medium used had a conductivity of 5 mS/m, the measurements have been performed at 13 frequency points from 1 kHz to 20 MHz and have been repeated 2 times. The data acquisition was performed with increasing values of concentration in order to reduce the possibilities of contamination from a sample at very high concentration to a lower one, thus modifying the average cell concentration in the sample. The spectra obtained were normalised for comparison purposes. The data obtained was treated, fitted and displayed with the software presented in Chapter 4 developed for that purpose.
Chapter 5. Results

5.4.4.3 Results

The DEP spectra measured from the protocol exposed above are presented in Figure 5.4.4.3. Each sample was measured twice so two spectra appear for each value of cell concentration. The results explained in section 5.3 can be seen here: the higher the concentration, the lower the noise in the spectrum obtained. The repeatability of the machine can also be seen in the fact that each pair of spectra follows the same pattern, especially at higher concentration.

These spectra were fitted with the algorithm provided by the data processing GUI. The results are reported in Figure 5.4.4.3: the measurements of the cytoplasm, membrane and wall properties are plotted on the first, second and third line respectively, and the measurements of permittivity and conductivity are plotted in the first and second column respectively. This shows several changes in the DEP spectrum measured by the apparatus when the cell concentration increases: the first noticeable effect is a downwards shift of the spectrum, the second one is an increase of the low mid-rise frequency. This goes with an evolution of the measurements of some of the yeast properties presented on Figure 5.4.4.3.
Unsurprisingly, the measurement of the cytoplasm permittivity (top-left graph) does not provide satisfying results because of the poor convergence of the fit due to a lack of information at high frequency. The other measures provide interesting information: the general tendency is a stable measurement throughout increasing concentrations, with the clear exception of the membrane conductance drop and the wall permittivity rise, both above $10^7$ cells/ml. It can also be noted that the membrane capacitance tends to drop above $10^8$ cells/ml.

One can also notice that for each graph, except the top-left one, most couple of points...
are relatively close compared to one or two others that are much further apart. This indicates the irregularity of the curve fitting technique, due to its particular sensitivity to the noise in the data and to the particular links between the constituents of the cell and the different parts of the spectrum. For instance, a noisy data point in the low-frequency transition of the spectrum is likely to prevent a good convergence of the curve fitting and to provide an inaccurate measurement of the membrane properties, but affects much less the measurements on the cytoplasm because these properties are linked to the high-frequency end of the spectrum (Huang et al., 1992).

5.4.4.4 Interpretation

Two explanations are proposed here to explain the behaviour of the effect observed in Figures 5.4.4.3 and 5.4.4.3.

1. **Residual culture medium:**
   The higher the cell concentration, the more difficult it is to rinse the sample with the suspension medium. Therefore, it is possible to interpret the results obtained by a difference in the conductivity of the suspending medium, originating from a contamination by the residual high-conductive culture medium in the high concentration sample. However, the model developed in section 3.5.3 predicts that a modification of the medium conductivity should only affect the low-frequency part of the spectrum by increasing the low transition frequency and lowering the CMF below this transition. Here, the differences we can also observe a shift at medium and high frequency that cannot be explained by this contamination.

2. **Dipole-dipole interaction:**
The second explanation is proposed by the literature (Dong et al., 2004) and interpret this behaviour by the predominance of dipole-dipole interactions between two particles at high concentration. As predicted by Dong et al., a shift towards lower value is observed, but more pronounced than expected. It is unfortunately not possible to verify the distortion of the high frequency transition because the measurement cannot reach a frequency high enough.
5.4.4.5 Conclusion

The changes observed in the spectra in Figure 5.4.4.3 are likely to be a combination of both effects. Unfortunately, it is difficult to determine quantitatively the contribution of each one from the results obtained by the DEP machine. Obtaining a measurement at higher frequencies could help determining the part of each effect and provide more significant results, and measuring the conductivity inside the microwell could provide a much better accuracy for its determination. Hence, the proportion of each effect could be determined by additional measurements.

5.5 Conclusion

This chapter has presented the results of the measurements performed during the optimisation process and during the assessment of the final prototype. Many sources of noise have been analysed and most of them have been reduced, such as the instability of the light source, the presence of bubbles, the repeatability of the signal generator, or the heating sources. Some other sources of noise are more localised and have been treated by removing their effect from the data acquired, such as microflows, the re-orientation effect or the bubbles trapped in the microwell. In total, the amount of noise in the data reaches typically around 5% of the signal, which is still too high to obtain a systematic accuracy of 10% from the curve fitting, but still provides a significant amount of data within the 3% limit. However, the limitations of the signal generator at high-frequency is a serious limitation for the measurement of the cytoplasmic permittivity, so the prototype developed cannot measure this parameter accurately. The comparison with the manual method together with the limitations of the automate are discussed further in Chapter 6.
Chapter 6

Evaluation of Machine Performance

6.1 Introduction

Chapter 5 provided information about the performance of the prototype that can help drawing some guidelines about DEP experimentation. This is described in the first section of this Chapter. The second section is dedicated to assessing how far the objectives set at the end of Chapter 2 have been met by the last version of the prototype: it is seen that the most important has been realised but some problem still persist. These are enunciated in the last section of this Chapter as the limitations of the device.

6.2 Analysis of the results obtained

In Chapter 5, on page 138, we saw that the results obtained from the last version of the prototype presented some interesting aspects concerning the evolution of the level of signal and noise. These results can be interpreted further and give some guidelines for the design of a DEP measure experiment.

The amplitude of the electric field does not increase the level of signal. Figure 5.2 on page 141 shows that an increase of the amplitude of the electric field decreases the convergence time of the signal-to-noise ratio towards a minimum, but that the value
reached does not change. It can be concluded that the maximum amount of information that can be measured in the experiment does not change with the amplitude of the signal. This makes sense, because the final state of the microwell is an equilibrium state reached when all the particles have finished migration, which does not depend on the strength of the DEP force but on the length of time the field has been applied.

**The amplitude of the electric field acts as a quadratic scaling factor over time.** We can investigate how the convergence behaves with a change of amplitude. The rapidity of convergence can be characterised by an objective criterion such as the gradient of the curves obtained in Figure 5.2 at a given time. The gradient of the different curves have been measured at time $t = 1.5s$, because the measurement of the slope at that time is relatively easy for all the data and the inaccuracy is relatively low. The results, presented on Figure 5.3 on page 141, show that this gradient depends on the amplitude of the electric field like a quadratic function.

This can be understood better by the analysis of the data. The signal-to-noise ratio increases as a quadratic function of time. However there is no particular reason why the amount of noise should vary over time in a repeatable quadratic fashion because the sources of noise reported in section 2.3 on page 22 depends mostly on external factors such as the conductivity or the light source, and the main source of noise that depends on the electric field is the AC electro-osmotic flow, which depends on the $4^{th}$ power of the amplitude (Castellanos et al., 2003) instead of the $2^{nd}$.

Therefore, this quadratic behaviour can only be explained by the variation of the level of signal while the amount of noise remains roughly constant over the different values of electric field amplitude. This observation validates the use of Equation 3.4 on page 45, which states that the variation of the concentration, and therefore the variation of the signal, depends on the amplitude of the electric field squared. This observation is important because it justifies the calculation made in Chapter 3 about the evolution of the concentration in the microwell, which is the basis of the data processing algorithm. Equation 3.4 can also be re-arranged as follows:

\[
\frac{\partial c}{\partial (V_0^2 \times t)} = -\gamma \text{Re}(K)\text{div}(\nabla E_n^2(r, z)) \tag{6.1}
\]
Where $V_0$ is the amplitude of the electric field and $E_n$ is the normalised electric field. Here, the amplitude appears as a scaling factor over the time axis, which means that an increase of the voltage of the signal generator has the effect to generate faster changes of concentration, but does not affect the amplitude of the signal.

**The level of signal is given by the concentration.** The effect of the concentration on the amplitude of the signal has also been investigated in Chapter 5. Figure 5.1 on page 139 presents the time evolution of the signal/noise ratio obtained from several measures of yeast cells suspensions at different concentrations, but with the same electric field amplitude. It shows that the evolution of the ratio remains is unperturbed with the concentration so that the noise-to-signal ratio takes always the same amount of time to stabilise. So increasing the concentration does not change the duration of the measurement. However, the figure also shows that the level reached by the noise-to-signal ratio decreases with an increase of concentration. This means that increasing the concentration has the effect on increase the signal-to-noise ratio.

The interpretation presented here shows that there is no need to measure the DEP force for longer than a few seconds, because the level of noise remains roughly constant and there is more chance that the signal reaches a saturation level at longer time. Moreover, the data points acquired during the first second contain an important level of noise and a low level of signal, therefore they can only contribute to add noise in the DEP spectrum. That is why the data points obtained from the measurement before 1s of application of the DEP field are systematically discarded from the data processing procedure. This is also a way to immunise the data processing from re-orientation effects, as explained in section 5.4.3 on page 161.

### 6.3 Comparison with manual experiments

#### 6.3.1 Introduction

Chapter 5 describes the performance of the prototype developed during this thesis, in order to improve the measurement of the DEP spectrum of a sample. This can be compared to the performance of the manual experiments as explained in Chapter 2,
which was the reference at the start of this work. This comparison can be made over several criteria:

1. Level of noise
2. Experiment duration
3. Complexity of the manipulations
4. Quantity of information available from the measurement

6.3.2 Level of noise

The level of noise in the data is measured in reference to the level of signal. In order to compare the noise generated in manual and automated experiments, one common criterion of measurement must be selected. In general, the criterion selected is the signal-to-noise ratio, but as we are more interested here about the level of noise, the criterion selected for this analysis is the noise-to-signal ratio. Because DEP spectra can sometimes cross zero, it could lead to problems to divide the noise measured by the signal because this operation could give infinite levels of noise-to-signal, which would not be significant. That is why the levels of signal taken in this study is the standard deviation of the DEP spectra, whereas the level of noise is measured by the residuals of the curve fitting.

The histogram of the noise in manual experiments has a mid-height value of 16%, against 4% in the case of automated experiments. Figure 6.1 presents the histograms of noise-to-signal ratio for 203 experiments using the manual protocol performed by a trained experimenter (above), and in 243 by automated experiments (below). As explained in chapter 2 on page 26, the level of noise in a manual experiment depends on the experimenter, that is why a skilled experimenter has been chosen here as a reference, with three years of practice in manual DEP measure. That way, the assessment of performance consider the best case and is a fair comparison.

The mid-height value provides an indication of the expectation of noise-to-signal ratio in the measurements. The figures present a mid-height value of 16% and 4% for the manual and automated procedures respectively. These results means that there is approximately 4 times less noise in measures taken automatically than in the manual case.
Figure 6.1: Comparison between distribution of noise in manual and automated experiments.
In addition to this, it can be seen that the extent of the histogram is more confined in the automated case than in the manual one: 85% of the noise-to-signal ratio remains within 10% absolute error in the first case, against about 50% in the second case.

Finally, the histogram of automated measures show a large peak at very low level of noise-to-signal ratio. This peak correspond to the experiments at very high concentration (above \(10^8\) yeast cells/ml), and is below 0.75% absolute error. It shows that it is possible to obtain very low levels of noise when the conditions are exceptionally good, which may not always be the case in real conditions, especially for cell lines that are difficult to grow.

**Manual injections of sample also create more inhomogeneities in the microwell than tubing injections.** The comparison of images of the microwell taken during the experiments show another difference between manual and automated experiments: the manual injection of a sample in the microwell tends to create bubbles and inhomogeneities inside the microwell, which is not the case for automated experiments. Figure 6.2 presents two types of inhomogeneities that appear in manual resuspensions. The upper figure shows the presence of bubbles at the bottom of the microwell, due to trapped air that cannot be evacuated because there is no escape route through the glass slide. The lower figure shows some inhomogeneities that appear during the application of the DEP field. In this case, these white patches have appeared after 20sec of application of a 16Vpp 7 MHz electric field in a 2.5 mS.m\(^{-1}\) conductive medium. These occur when the cells have not been separated correctly from the previous experiment and have formed clusters that distort the electric field, therefore creating local gradients of electric field and local DEP force fields. Such inhomogeneities does not occur in automated experiments, because the flow passes through the microwell instead of being injected.

### 6.3.3 Experimental duration

The experimental duration is the time required to complete the series of measures required for the calculation of the DEP spectrum of a sample. This time is recorded for each experiment, manual or automated, which allow comparing more precisely the gain of time between both procedure. Figure 6.3 presents the histogram of the experimental time taken to complete 203 manual experiments (top histogram), and 243 automated experiments (bottom histogram).
Figure 6.2: Different inhomogeneities in a microwell after manual resuspension.
Chapter 6. Evaluation of Machine Performance

Histogram of the duration of manual experiments on yeast cells

Histogram of the duration of automated experiments on yeast cells

Figure 6.3: Comparison between the experimental duration of manual and automated procedures.
The time taken to perform the measurement of a DEP spectrum has been divided by 13. The time taken for a manual experiment to measure a 21-point DEP spectrum is around 65min in average, depending on the different parameters of the measurement and on the skill of the experimenter. For the same 21-point DEP spectrum, the acquisition time of an automatic experiment in about 4min50: this means a reduction of more than x13. This short acquisition time increases the number of experiments that can be performed in a given time. For a given spectrum, a measure using the same acquisition time would lead to a reduction of the noise of $\sqrt{13} \approx 3.6$ times between the automated and manual experiment.

A short acquisition time also diminishes the effect of cell degradation over time due to exposure to the low-conductivity medium. This means that the measurement is performed over a more homogeneous sample, so the data measured is more significant. It also increases the number of times that a given sample can be analysed, which has been useful for the repeatability study in Chapter 5.

It can also be noticed that the histogram of manual experimental time follows a large distribution, whereas the automated one looks like a series of more discrete peaks. That is because the manual procedure depends on the experimenter, which does not perform as regularly as the machine and therefore cannot determine the length of the measurement in less than a 10min time window, whereas the machine preforms very regularly and provides a measurement in a 5s window. Therefore, a series of automated experiments conducted with the same acquisition parameters creates a narrow peak in the histogram. Here, several series of experiments have been reported in the histogram, each one with different acquisition parameters and different peaks.

6.3.4 Complexity of the manipulations

The procedure is simpler, so it generates less errors. The only manipulations necessary for the automated machine are the rinsing procedure, the introduction of sample into the system and the washing procedure when finished. All these procedures only consist of placing a container in the sample holder and placing the tubings in it, so it is a very basic manipulation. This makes it much more reliable in terms of repeatability of the experimenter, and in terms of additional error or data loss due to a wrong manipulation.
The system is easier to wash. A manual experiment uses a microwell glued on a glass plate, which means that the sample sits in a cup-shaped volume. This avoids leaks, but makes it difficult to rinse. So, once the experiment is finished, the remaining water dries out and leaves some of the components of the medium on the surfaces. These residues can contaminate the next sample when the well is used for another experiment. That is why the manual procedure requires the regular building of new microwells. This is not necessary with the machine, because the sample passes through the microchip so that no residue is left behind, and the washing is much more efficient.

6.3.5 Quantity of information available from the measurement

The automated procedure require more parameters than the manual one. These extra parameters concern the configuration of the resuspension rate, the delays, and others. Therefore it is possible to obtain more information about the experiment with a log of an automated procedure, allowing more reproducibility.

6.4 Limitations

6.4.1 Introduction

The automated system made several important advances in the measurement of the DEP spectrum compared to the manual procedure. However, the system is limited by several effects: EHD, high frequency generation, shear forces, minimum sample volume, data transfer delays, heating and user interface clarity. These limitations are presented below with some details about their causes. Next chapter presents some lines of research to continue to extend the limitations of the prototype.

6.4.2 Detection limits

One of the limitations of a measuring device is the range of detection it has compared to the signal it has to measure. In the present case, the DEP spectrum should provide important information up to several hundreds of MHz, according to the continuity of
the spectrum obtained for yeast cells. This range is limited by several experimental problems, exposed below.

1. **Concentration**

It is showed in Chapter 5 on page 150 that the concentration strongly affects the quality of the data obtained and that an increase of concentration provides an improvement in the measurement. This also means that, below a threshold, the concentration is too low to provide a measurable signal so that the DEP force cannot be detected. For yeast cells, for example, this threshold is typically $10^6$ cells/ml.

However, too high a concentration leads to a very high absorbency of the sample, so that the camera cannot detect the signal. This typically occurs at concentrations higher than $5 \times 10^9$ cells/ml for yeast cells.

Therefore the concentration of the sample should be selected in a way to set its absorbency inside the boundaries provided above. This can be a limitation if one wishes to conduct measures for concentrations higher or lower than permitted, but this problem can be solved by using thinner/thicker microwells in order to measure higher/lower concentrations respectively, so other microchips should be designed for such a purpose.

2. **Experimental time**

With the help of the machine, the acquisition time has been reduced to 4min50s for the measurement of a 21-point spectrum. This is a good performance compared to the manual procedure, but the analysis of how this time is spent shows a limitation in the procedure: each data point takes 5 seconds to measure giving a total measurement time of 1min45s; the 2min5s left is the time used by the machine for the resuspension and the transfer/storage of data. This limits the duration of the measurement but could be improved by changing the design of some parts of the device. This is explained further in Chapter 7.

3. **Convergence of the curve fitting algorithm**

Figure 6.1 on page 172 presents the histogram of the noise-to-signal ratio. This ratio is calculated by taking the standard deviation of the DEP spectrum as the level of signal, and the difference between the data and the fit as the level of noise.
However, this method requires a convergence of the fit in order to assume that it provides a correct evaluation of the signal. This convergence depends partly on the quality of the data, but mostly on the first guess given to the curve fitting algorithm. When the guess is inaccurate, the algorithm fails to converge and provide a poor fit, and therefore an incorrect measure of the signal. Figure 6.4 presents the same histogram as presented on Figure 6.1, but with non-converged data. It can be seen that the error reaches several hundreds of percents of the level of signal for non-converged data.

This effect also exists on manual data so it does not originate from the automation, but from the numerical method of curve fitting. It is a limitation in the utilisation of the data processing module, and can make it time consuming for the experimenter to find a correct guess for the fit. Fortunately, an unconverged fit does provide a very high error and so it can be detected easily.

6.4.3 Frequency band

The range of the DEP spectrum is limited at high frequency by the capability of the signal generator, and at low frequency by the microflows such as the AC electro-osmotic
flow. These limits come from the technology of the signal generator and of the microchip.

The high-frequency limit comes from the fact that the signal generator has a limited bandwidth, but also from the cut-off frequency of the microwell chip. The new microchip developed during this thesis has a cut-off frequency higher than the high frequency limit of the signal generator and does not limit the measurement by the prototype.

The low-frequency limit arises from the generation of electrolysis at the surface of the electrodes. This effect separates the water molecules into dihydrogen and dioxygen molecules in the gaseous form, therefore creating bubbles and modifying the ionic content of the solution in the microwell. This generally damages the measurements beyond any restoration capability.

6.4.4 Diffraction by the aperture of the microwell

The result of a measure with an infinite focal length is presented in Chapter 3 on page 48: it appears that a region of the image of the microwell is blurred by the diffraction of the light by the edges. This corresponds to a relatively thin circular region, around the microwell. This is not considered as being a problem here because the evolution of the concentration in this region is very non-linear, so this circular band is masked before the application of the data extraction algorithm. However, this can be a limitation if an improved algorithm is developed that could take into account the non-linear regions of the microwell.

6.4.5 Bubbles

Even if their frequency of appearance is relatively low (1 bubble every 250 measurements in average), the presence of a bubble is a real problem for the experimenter because they tend to remain stuck inside the microwell, and damage the measurement in a way that cannot be recovered. Moreover, they can be difficult to remove, and may require to stop the experiment. Even if the precautions explained in Chapter 5 are observed, bubbles remain a limitation to the system.
6.4.6 Bias

The analysis provided in Chapter 3 on page 44 uses the small-time approximation to find a measure of the DEP force from the evolution of the light received by the camera. This approximation works relatively well within the first seconds of application of the electric field but induces a bias as time goes by. This bias is transmitted to the DEP spectrum, and therefore to the measurement. This limits the accuracy of the system, but could be improved by developing the theory further.

6.5 Conclusion

The differences between the manual and automated protocols show clearly that the later provide better quality data, with higher signal-to-noise ratio and shorter experimental time. The objectives set in Chapter 2 have been partially met:

1. The signal-to-noise ratio is now low enough to start using curve fitting techniques in the case of a high-concentration sample, which is not always possible to obtain
2. The experimental time has been reduced by a factor 13, which is a great success but could be diminished even further
3. The number of interventions on the measurement by the experimenter has been dramatically reduced, but the data processing algorithm still require an initial guess for the curve fitting

In general, the use of microwell chips has been largely improved and the results already have enough quality to provide a good measure of the yeast cells electric parameters (as presented in Table 5.3 on page 159), but there still are many improvements that could be done more or less easily and that could improve the measurement further. Chapter 7 presents a list of suggestions for further developments of the prototype that covers all the limitations seen in this Chapter.
Chapter 7

Conclusions and further work

7.1 Introduction

Chapter 5 and 6 showed that the prototype developed makes a significant improvement from the previous manual experiments. It is now possible to obtain a level of precision and accuracy low enough to allow the measurement of the conductivity and permittivity of the different constituents of the cells monitored under reasonable conditions. However, the system still has several limitations; some are inherent to the use of DEP such as microflows, and others come from choices of design such as the minimum volume of sample usable by the machine. These limitations can be improved by a better design of the apparatus and/or software, and by new approaches of the problems. This section presents some suggestions that could be the base of future works in order to obtain a finite, manufacturable machine from the prototype.

7.2 Review of the objectives

In the end of Chapter 2, a list of three objectives was set for the orientation of the prototype development. These objectives were:

1. Improvement of the signal-to-noise ratio up to a level that allows the use of curve fitting techniques
2. Reduction of the experimental time down to a duration comparable to the time required to wash the sample in the low-conductive medium (in general 10 minutes)

3. Reduction of the interactions between the experimenter and the measurement down to the non-automatisable tasks

As shown in Chapter 6, the first of these has been partially met. The final version of the prototype provides high enough signal-to-noise ratio only at high concentrations of cells in the sample. Several recommendations are listed later that could lower this concentration threshold and extend the application of the machine to lower concentrations of cells.

It was also shown in Chapter 6 that the experimental time has been reduced dramatically from the manual procedure, and that the acquisition of a complete DEP spectrum can be done in less that 5 minutes. This meets the objective number two, yet this performance can be pushed further by better designs of the valve and tubing system, as explained in this chapter in section 7.3, which could increase the number of experiments performed per hour.

The protocol developed with the machine, explained in Chapter 5 and detailed in Appendix C, automated the tasks of resuspension and data processing. At the end of the project, the only interactions remaining are the preparation of the sample and the initialisation of the curve fitting. However, it should be possible to automated the later and to obtain a completely transparent data analysis. The strategy to accomplish this is also explained in this chapter, in section 7.4.1.

The objectives set at the start of this thesis have therefore been partially or totally met. Further development of the apparatus should include a more ambitious set of objectives, which could be the following:

1. Improvement of the signal-to-noise ratio up to a level that allows the use of curve fitting techniques for low concentrations of cells in the sample (below $1 \times 10^7$ in the case of yeast cells).

2. Reduction of total experimental time down to less than a minute

3. Transparent data processing
4. Autonomous device with possibility of remote control by any computer

In addition to the previous, revised objectives, it is now realistic to add to the list the need to obtain a standalone device, independent from its environment, that would only require a connection to the mains as a power supply and could perform the measurement, data processing and transmit the result to a printer or a computer. This could be done in the longer term by reducing the volume of the device and by simplifying its different modules. The prototype, as it has been developed during this thesis, is composed of several sub-units including general-purpose devices such as the oscilloscope, the signal generator, the microscope and the power supplies. These devices have been selected because of their performances so that they would not present a limitation during the development of the data acquisition procedure. However, they take a relatively large space and can not be transported easily. This is because each of these units has been designed for general purposes and is not used at its full potential. Therefore, it is possible to design an electronic board and an optical system that would perform only the necessary operations so that the machine obtained would be much more compact, and manufacturable.

The different suggestions that appear in this Chapter in order to meet these enhanced or new objectives are categorised in two sections: the improvement of the hardware, and of the software. These two categories are separated into the two sections below.

### 7.3 Improvements of the hardware

#### 7.3.1 Microcontroller

The microcontroller used for the control of the syringe pump has a great potential for controls and displays, or even for data processing. However, its use is restricted by the slow connection to the computer, which uses an RS232 protocol at 19200 bauds/sec. This transfer rate causes some delays in the data acquisition procedure, which adds to the experimental time and therefore restricts the minimum duration of an experiment.

The RS232 could be changed for a faster connection such as USB. Using a USB protocol should increase the transfer rate by up to 3 orders of magnitude. This
could help decrease the experimental time and could open new possibilities, such as microcontroller-driven image acquisition and processing, but this would also require a faster microcontroller. Using a USB connection also requires the purchase of appropriate control modules for Matlab in order for the program to detect and use the USB connections.

7.3.2 Signal generator

One of the most restrictive modules of the prototype is the signal generator, which has a limited bandwidth and cannot reach frequencies high enough for the complete measurement of a typical DEP spectrum. This bandwidth limitation comes from the fact that the electronic circuits in the generator cannot produce such a high frequency. In addition, the impedance of the microchip and the connection cables would make it difficult to reach a frequency higher than 100 MHz. However, it is possible to increase the frequency band and to reduce the impedance of the cables by creating a signal generator board that would be integrated into the prototype. Such a device is already under development in the Biomedical Engineering research group and is expected to provide a high-frequency limit higher than 150 MHz.

7.3.3 Oscilloscope

The oscilloscope helps to control the amplitude of the electric field generated at the microchip electrodes. This control helps to check any voltage drop due to impedance effects and is used for the calibration of the signal generator prior to a series of experiments. However, this monitoring function could be performed by the microcontroller. The model used in the prototype has eight built-in ADC inputs of 12 bits resolution, on port A. Only one is necessary for the monitoring of the sine wave produced by the generator, and the connection would be quite straightforward as it does not require any impedance matching. However, this has not been done so far for time restriction reasons: such a project would require a re-programming of both the microcontroller and the computer program, plus a testing phase, which is expected to take about two months for completion.
7.3.4 Microscope

The choice of using a microscope in the prototype was motivated by the need of high accuracy in the measurement in order to obtain the best conditions possible in a shorter time. However, it is the largest unit of the prototype after the computer, it cannot be transported easily and cannot be replaced without time-consuming modifications of the other modules, the tubings in particular.

A dedicated optical system could replace the microscope. It is possible to use a reduced optical system with minimal functionality instead of the microscope, which can take its role as a magnifier. Such a system can be bought in separate pieces from supplier such as Thorlabs that provide custom optical solutions. It would consist in the assembly of a light source, a collimation lens, an adjustable microwell holder, an objective with its support, a magnifier, a C-type mount for the camera and a base to assemble these modules altogether and place them accurately.
Another problem with the use of a microscope is the depth of its field of view, which does not permit the monitoring of a large portion of the microwell (80 µm for a x2.5 objective). This has been partly solved by the addition of a lens as explained in Chapter 3, but this adds an artefact in the images captured by blurring the edges of the microwell. It was shown in Chapter 5 that this does not affect the result of the measurement with the data processing used here, but further work could make better use of the signal and could provide more accurate data.

**A telecentric objective could be used.** A solution could be to replace the traditional infinitely-focused objective by a telecentric one. Telecentric objectives have the ability to provide large depth of view without distortion and are commonly used in robotics but can be relatively expensive (typically from 700 to 2000).

**Imaging several microwells at the same time could reduce the experimental time.** The prototype takes images of only one microwell because it makes use of the whole surface of the image, which is the best configuration in term of signal-to-noise ratio. However, a better system could require less demanding image quality so that several microwells could be imaged at the same time. This could be possible if the field of view of the optical device was greater, and could reduce the experimental time further by running several experiment in parallel or could increase the signal/noise ratio by averaging the data from different microwells.

### 7.3.5 Power supply

The prototype uses three sources of electrical power: one power supply for the microcontroller and syringe pump, another one for the light source and the mains for the camera. This is because the difference of power needed for these two systems is too large to be merged easily into one conditioning board, and the requirement of steadiness of the light bulb power supply is difficult to obtain. However, it should be possible to develop an electronic board that could distribute the electric power to the different units with the right voltages and currents. This can be investigated further in order to obtain a compact and independent design, but could prove difficult for the supply of the light bulb.
Chapter 7. Conclusions and further work

7.3.6 Light source

As mentioned above, driving the light source is a problem because of the level of steadiness required and of the 25W of power needed by the bulb. The use of power LEDs has been investigated, but has failed so far to produce a light bright enough to be detected through a sample.

The light source also appears to be a problem for samples that have a high light absorbency. In such cases, the amount of light collected by the camera is very low and eventually the microwell cannot be detected. This could be avoided if one uses a more powerful source of light, such as a laser. However, this would also require modification of the optics and imaging system, and could induce problems with interference, so this solution should be investigated separately before being implemented into the prototype.

7.3.7 Camera

The camera used in the prototype is a complete device that can be adapted to many uses. It makes it a good choice for the purpose of building a prototype because it works ‘out of the box’ and only requires standard connections. Moreover, it provides good quality images and its flexibility allowed the automated adjustment of the exposure during the data acquisition. However, it is powered through the mains so it cannot be neatly integrated to a finite product, it represents a significant cost in the machine and it takes a relatively large space around the microscope.

A custom solution could replace the general purpose camera for more compactness and more independence. It is possible to use a more compact design by changing this camera to an LCD electronic board driven by the microcontroller. This requires many changes in the architecture of the prototype and the programs, but it is one of the most critical task to accomplish in order to obtain an independent device that could be used in various environments.

7.3.8 Syringe pump

The syringe pump has been developed in order to fit to the specific use made in this project, especially the requirement of using an easy-to-clean system. For that purpose,
the syringe pump uses disposable syringes that can be replaced for each experiment. However, its design is quite simple and its body is physically separated from the optical system, which is good to avoid the propagation of vibrations during the measuring phase, but a complete device should integrate both part into one main body.

This aim can be fulfilled by using pre-fabricated syringe pumps. This requires the modification of the layout of the prototype, both for the tubing and for the arrangement of the different units, and also to adapt the microcontroller program in order to control the syringe motor. The syringe should have its outlet pointing downwards in order to use the gravity to keep the air away from its outlet, to prevent the injection of air bubbles in the tubing.

### 7.3.9 Valve

**A 5-way valve can improve the design of the device significantly.** The valve used in the prototype has 6 outlets and 2 positions. It has been selected because of its availability, high quality standards, low fluid retention, rapidity of operation and ability to be controlled easily by a TTL signal. However, the fact that it only offers 2 positions poses a strong restriction in the design of the fluid distribution system. Ideally, a 6-position distribution valve would have been of much greater use as it would allow an extra tubing line for the manipulation of the sample during the measurement of the DEP spectrum (which would decrease the experimental time), another line for the manipulation of suspension medium for automated rinsing, another for the manipulation of the washing liquid used at the end of the measurement, and a stop-flow position. Such a system would be much easier to use and less confusing for the experimenter.

### 7.3.10 Tubing

The tubing arrangement has been regularly improved during the evolution of the prototype: the radius of tubing has been reduced, the length of the segments has been minimised and the arrangement of the different parts has been simplified and optimised, all this in order to avoid the capture of bubble and to diminish the volume of sample sitting in the tubing.
However, it would still be possible to improve this system by adding bubble traps on the flow path. This could avoid bubbles travelling in the fluid and being captured by the microwell, which causes many problems during an experiment. In addition to this, a more compact system could further reduce the amount of tubing used to connect the different elements together and should therefore lead to a lower quantity of sample used by the system.

Finally, the use of a 6-position valve could open new possibilities for the spacial arrangement of the tubing and the fluid flow logic. A setup such as shown on Figure 7.2 could help reduce the experimental time by allowing the syringe to be loaded while the sample is being measured, so that the new sample could be injected into the microwell just after the measurement. Such an arrangement is expected to lower the residual time mentioned in Chapter 6 on page 177 down to a few tens of seconds.

### 7.3.11 Microwell chip

The microwell design presented in Chapter 3 was optimised to provide a large signal in a reduced volume, therefore its dimensions should not be changed unless the diameter of the microwell is to be modified. The chip used for the experiments made with the
Chapter 7. Conclusions and further work

Prototype has only one microwell because only one microwell was planned to be imaged in order to maximise the signal-to-noise ratio. As explained above, further experiments could use several electrically independent microwells in order to obtain several measures for each image acquired. This could dramatically reduce the experimental time but requires a new microwell chip design.

It could also be possible to monitor the impedance of the microwell in order to monitor the conductivity of the medium. This can help obtaining more reliable data as any change of medium conductivity by contamination would be measured and taken into account in the data processing. However, this amelioration would require a long development process in order to obtain a device reliable enough for the purpose of the measurement.

7.4 Improvements of the software

7.4.1 Automated guesses

It is explained in chapter 3 that the role of the experimenter is reduced to the minimum during an experiment using the prototype developed here. It is very difficult to automate the preparation of the samples and it would require complex robotics. However, the interaction of the experimenter during the curve fitting could be avoided if the program could find an acceptable first guess. This would make the data processing faster and more reliable.

This should be possible by using the equations presented in section 3.5.3 on page 76, but only for very low levels of noise and bias. The results obtained so far did not allow such a method, but further developments could lead to lower levels of noise, and therefore to an automated procedure of guess finding using the spectrum segmentation.

7.4.2 Automated multiple populations calculation

As explained at the end of Chapter 3, it should be possible to determine the presence of multiple populations in the sample when some information are known about them. This capability can be added relatively easily to the data processing algorithm for later study.
7.4.3 microcontroller-based data processing

The microcontroller used in the prototype has an external clock of 4 MHz, which is fast enough for the purpose of data transmission but is too low for data processing purposes. If the prototype is to be autonomous, the computer should ultimately be replaced by a microcontroller, which means that this chip should perform all the image and data processing. This is a long-term goal, but changing the microcontroller for a more powerful one will be a necessary step for the development of a fully independent device.

Without going so far, a faster microcontroller could also provide faster data transmissions and could manage extra devices such as the camera, which could be useful if a dedicated chip is to replace the camera used so far. This could be a 8-month project, including the construction of the camera and its integration in the prototype. It would also require to re-write the data processing program for the microcontroller, which could take an extra month.

7.4.4 Inclusion of additional models

The data processing algorithm proposed so far uses the multi-shell model, with an option to apply the thin-membrane approximation on the first layer. This is sufficient to spherical cells, but many cells are not spherical. Therefore it could be interesting to include more complex models such as elliptic shapes, in order to extend the possibilities of the device to more biological cases of cells.

Moreover, it could be useful to extend the thin-membrane approximation to other layers, in order to treat the case where organelles are modelled so that the cell membrane becomes the 4th layer. This has not been done so far because the study proposed here aims to validate the use of the automate, but further theoretical developments are required in order to extend the result of the thin-membrane approximation to any layer. This could potentially lower the number of unknowns to solve in the problem, which could provide a better curve fitting algorithm.
7.4.5 Extension of the model of concentration evolution to the non-linear zones

The evolution of the particle concentration in the microwell is modelled in Chapter 3 in the small time approximation. This allows simplifying the problem and finding an implementable mathematical formulae, but it rejects the non-linear zones and, ultimately, produce a time-dependent bias which limits the use of the algorithm. A more general solution of the evolution of the concentration should make use of more data, therefore increasing the reliability of the measurement. This should be achieved by re-considering Equation 3.4 on page 45 and solving it using more general approximations.

7.4.6 Diminution of the number of curve fitting parameters

The scaling factor of the spectrum measured remains an unknown of the curve fitting algorithm. This reduces the amount of data that can be recovered from this analysis, because only five parameters can be measured out of a spectrum. That is why the measurement of the scaling factor prior to an experiment should allow the user to exclude the scaling factor from the list of unknowns in the curve fitting algorithm, therefore increasing the amount and quality of the data obtained. One possibility could be to assess this scaling factor by comparing the changes of light intensity before and after the introduction of the sample in the microwell. This would require an analysis of the dependence of this factor with the different parameters of the problem, and it would require a large study in order to prove the repeatability of this technique.

7.5 Microflow reduction

In parallel with the development of the microwell, a study of the microflows was conduced using a planar electrode model. The aim was to study the generation of the flow that appear in the electronic double layer when using DEP, and to develop strategies that could lower their amplitude. This work has not been used in the development of the machine but can lead to a new generation of electrodes with more developments.

It is known that the surface of the electrodes is a cite that creates a flow during DEP experiments (Reppert and Morgan, 2002). Moreover, at the microchip electrodes scale
Chapter 7. Conclusions and further work

194

the water is very viscous and tends to create vortexes very easily around local sources of motion. Therefore, by placing some objects at the surface of the electrodes, the flows generated is expected to curl in such a way that the flow lines would be contained in a restricted volume at the vicinity of the objects, instead of expending far into the medium. This has been studied in more details, the results are presented below.

7.5.1 FEM model

The FEM model developed for this analysis simulated the behaviour of a planar electrode made of two parallel stripes of copper. Each stripe had an infinite length, a width of 1mm and a negligible thickness. This was close to the case of usual planar electrodes made of a thin deposition of ITO on glass. The microflow was modelled by defining a constant surface velocity of 100 µm/s on top of the electrodes, in the direction of the electric gradient. This is quite a coarse approximation as it does not take into account the velocity profile along the surface of the electrode, but it generates a net flow that has a similar effect to that of a more complex profile on the large-scale and the same order of magnitude as what is observed experimentally (Castellanos et al., 2003). It can also be argued that a surface velocity does not take into account the thickness of the double-layer, but in water the Debye length is of the order of magnitude of the nanometre, which can be neglected compared to the width of the electrode.

The geometry of the model used for this study is presented on Figure 7.3; the material used was water at a conductivity of 5 mS/cm, the solution in the case of a 10Vpp 1 kHz electric field is presented on Figure 7.6. The electric field appears in colour, the direction of the flow is given by the arrows and the amplitude of the flow is provided by the contour levels. It can be seen that the flow follows a circular motion, which agrees with the observations, and that the flow extent is comparable to the distance between the electrodes.

The idea suggested was to stop the propagation of the flow by placing some element at the edges of the electrodes. The model proposed for this goal is presented on Figure 7.4: the edges of the electrodes were covered by an isolative material (Nylon) that does not create an electric gradient. Therefore these elements boundaries were set to a null velocity. The results appear in Figure 7.8 and shows a change in the velocity map and
amplitude. The contour lines show a slight decrease of the velocity at large distance, and a closer look at the electrode corner shows that the flow is deviated by the object.

The next idea was to use this deflection effect together with the high viscosity of water at that scale to confine the flow in local vortexes. This has been modelled by placing several objects at the surface of the electrode with regular spacing. The layout is presented on Figure 7.5 and the solution on Figure 7.10. Here the reduction of the flow is very clear: the velocity drop was of 3 orders of magnitude in the medium. A closer look on Figure 7.10 shows that the flow went in the opposite direction compared to the previous simulations, even though the boundary conditions were the same. This can be understood by having a closer look at the surface of an electrode: Figure 7.11 is a close view of the right-hand side of the left electrode, and shows that the flow is expected to create closed loops between the deflectors. This was responsible for the reverse direction of the flow, as the main flow lines were generated by the friction with the flow loops. It can also be seen from these images that the electric field (the colormap information) was not significantly modified by these changes so the DEP force is expected to be unchanged.
Chapter 7. Conclusions and further work

Figure 7.4: Geometry of the modified model: two obstructions have been added on each electrode to cover their edges.

Figure 7.5: Geometry of the optimised model: several obstructions were placed along the surface of each electrode.
Figure 7.6: Solution of the model presented on Figure 7.3.

Figure 7.7: Detail of the figure above, around the right-hand corner of the left electrode.
Chapter 7. Conclusions and further work

Figure 7.8: Solution of the model presented on Figure 7.4.

Figure 7.9: Detail of the figure above, around the right-hand corner of the left electrode.
Chapter 7. Conclusions and further work

Figure 7.10: Solution of the model presented on Figure 7.5.

Figure 7.11: Detail of the figure above, around the right-hand corner of the left electrode.
by the presence of flow deflectors on the surface of the electrodes.

### 7.5.2 Experimental data

The simulations above were tested on a planar electrode made of ITO deposition on a microscope glass slide, covered with micrometre-wide polyester bands created by photoresist and lithography. A scheme is presented on Figure 7.12: the ITO electrodes had rectangular shapes in such a way to create two linear gaps of 100 µm in the direction of the slide corresponding to the model; four isolator patterns were designed, each one was filling an area with isolator bands parallel to the electrode gap and separated by a given distance. The isolator gaps used were 70 µm, 50 µm, 20 µm and 10 µm. The slide was covered by another microscope slide leaving a gap of about 200 µm between both.

An experiment was made with this electrode using yeast cells cultivated according to the procedure exposed in Chapter 1, suspended in a conductive medium of 3 mS/m at a concentration of $10^6$ cells/ml to leave a good visibility. The medium was injected between the slides and an AC current of 10Vpp, 1 kHz was connected to the electrodes.
Figure 7.13: Image taken by the camera in the region between the 50 µm and 20 µm spaced bands.

Figure 7.14: Image taken by the camera in the region between the 20 µm and 10 µm spaced bands.
The results were monitored by a camera and the videos obtained are presented on the CD; some images of interest are also reported on Figure 7.13. The results show that the cells that are in the region of the isolator layers are trapped in smaller flow loops than the cells that are not next to flow restrictors. The observations made on the different restrictor spacing show that the size of the loops tend to correspond with the restrictor spacing. However, it must be noticed that the electrodes were on the bottom slide, so that the yeast cells were accumulating at the bottom of the apparatus, between the restrictors, which may have helped to generate this effect. A less biased protocol should take into account a different orientation, such as placing the electrodes on the top slide.

7.5.3 Conclusion for the microflow reduction

The presence of deflective bands has an effect on the EHD microflows at 1 kHz and tends to decrease their long-range effect on the cell. This is showed by the theory and was partially confirmed experimentally. However, more study is needed to obtain more quantitative data and to transfer this technique to the microwells: because of their geometry, it would be difficult to deposit layers of flow restrictors inside a microwell. One possibility would be to etch the layers of copper over several microns in order to obtain a restrictive profile from the edges of the isolator layers. This would require testing and optimisations, at least on the acid solution and the etching time required to obtain a significantly lower EHD flow.

7.6 Conclusion

The results presented in this thesis show that the automated measurement of DEP using a microwell can provide very good quality data, and may be improved enough to provide a precise and accurate measure of the electric parameters of the different constituents of the cells, such as the permittivity and conductivity of the cell wall, membrane and cytoplasm. It also allows measuring these properties over a large number of cells at a time, providing statistically significant measures under 5 minutes and with a high signal-to-noise ratio. The method presented can measure the membrane properties more accurately than by previous works using DEP because the thin membrane approximation pushes the signal above the minimum threshold defined in the literature. (Gascoyne et al.,
Hence the error bars on the membrane conductance and capacitance are low enough to provide significant data. Such a system expands the potential applications of DEP within cell electrophysiology.

However, the system developed can be improved further in order to provide a fully autonomous device that could be capable of measuring the DEP spectrum of a sample of cells within 10% error, in laboratory conditions. This aim is now in the range of reach and can be attained by continuing regular improvements on the prototype presented here, such as presented in this chapter.
Bibliography


Appendix A

Elements of cell physiology

This section will focus on the links between the models proposed above and the anatomy of a cell. Cells present an incredibly large diversity of shape, size and composition so this work does not present a complete study but will focus on the aspect of importance for DEP experiments.

The models developed in the previous section use a simplified version of geometrical aspects of the cells: it describes the dipole generated by each object or layer, because each dipole contributes to migration and cells contain lots of vesicles and other sacs of different sizes. In addition to that, they are often enclosed in several layers. Each of these elements will have a contribution to the DEP force and will orientate the selection for a proper multi-shelled model. Therefore the cells will be categorised according to their structure, and the geometric orders of magnitude will be catalogued.

The cells can be separated into two categories: prokaryote or eukaryote. Among other differences, prokaryote cells do not have a nucleus contrary to the other ones who store their DNA in it. Prokaryote cells include bacteria and archaea; they have a size ranging between 0.1µm and 50µm and usually do not contain massive intracellular organelles. Their membranes are covered with a murein layer of 10 to 100nm thickness, called the ‘wall’. For the bacteria cells, the thickness of the wall mainly determines two sub-categories: Gram-positive (thick wall, 20-100nm) or Gram-negative (thin wall, 10nm). The wall is also covered by an additional layer called the ‘capsule’, generally made of polysaccharide, whose thickness can reach up to twice the size of the cytoplasm.
In contrast, the eukaryote cells size from 10\(\mu\)m to 500\(\mu\)m and contain bigger organelles. The size and type of organelle varies between the plant cells and the animal cells. Plant cells have a large space called ‘vacuole’ enclosed by a membrane, the tonoplast, which serves for several purposes like maintaining the Ph in the cytoplasm or change the cell’s shape. The vacuole space occupies 30\% of the cellular space but can reach up to 90\% of it sometimes. The chloroplast is another organelle of the plant cells; it contains the chlorophyll used for photosynthesis and measure 5 to 10\(\mu\)m long for 2 to 3\(\mu\) diameter.

The plant cell membrane is surrounded by a wall made of cellulose of 20nm thickness. Eventually, a secondary cell wall can be found, still made mainly of cellulose.

The animal cells do not contain chloroplasts and their vacuoles are not apparent when observing the whole cell. They do not show a wall and so are only enclosed by the double membrane of phospholipids.

Eukaryote cells also use a variety of organelles in common, like the mitochondria, the endoplasmic reticulum or the Golgi apparatus. The mitochondria are spheroids organelles used for the generation of ATP, and measure 3 to 5\(\mu\)m long for 0.5 to 1\(\mu\)m in diameter; they are surrounded by a bi-layer of phospholipids. The endoplasmic reticulum (ER) is the place where most proteins are generated and matured; this is divided in a rough ER where the ribosomes are attached to generate the proteins and a smooth ER where the proteins mature. Both are mainly composed of folded phospholipids membrane and take the form of flat sacs around the nucleus; each sac is around 100nm deep and 1 to 3 \(\mu\)m large. Some proteins are matured in other organelles, the Golgi vesicles, which
Appendix A. Elements of cell physiology

Figure A.2: Scheme of a typical plant cell. (By Mariana Ruiz Villarreal, public domain copyrights)

Figure A.3: Scheme of a typical animal cell. (By Mariana Ruiz Villarreal, public domain copyrights)

are made of folded membrane and measure 1 to 3 µm in diameter. A eukaryote cell can contain up to 100 Golgi vesicles.

Each kind of cell presents a particular morphology that will have to be modelled for the DEP experiments. Cell walls can be expressed by shelled models, and organelles can be modelled as many spheroids inside the cytoplasm. For a first approach, the shelled
model has been used to model the cytoplasm and its membrane, but further work will need to consider more complete models to account for the wall, capsule and organelle.
Appendix B

Ill-conditioned problems

Many systems can be considered as a ‘black box’, which transforms a signal at its input into another one at its output. In mathematics, such a ‘box’ can usually be defined as a system of equations, acting on a set of input variables, and providing an output set, which is generally the data of an experiment. The problems associated with such a system generally consists in finding what these input variables are, given the system of equations and its output. These problems are named ‘inverse problems’, and can be found in many domains of science.

For instance, let us consider the inverse problem of the equation: \( \frac{1}{x-1} = 0.1 \). The system is defined by the function \( F = \frac{1}{x-1} \), and the output is 0.1. The inverse problem consists here in finding which input values of \( x \) provide 0.1 through the system defined by \( F \). In this case, there is only one value, which is 11: it is the solution of the inverse problem.

In real conditions, the data from an experiment contains some errors, so the equation should be written with the error bars. Let us imagine an experiment with the following error bars: \( \frac{1}{x-1} = 1 \pm 0.01 \). What would be now the accuracy on the result of the inverse problem? There are several ways to find this out. The most precise consists in finding the inverse function \( F^{-1} \) of the system, which, for a function \( F \), is defined in such a way that \( F(F^{-1}(x)) = F^{-1}(F(x)) = x \). In our example, the inverse function is \( F^{-1}(x) = 1 + \frac{1}{x} \). Then, \( F^{-1}(0.1 \pm 0.01) = 11 \pm 0.001 \), with 3 decimals precision.

However, a system does not always possess an inverse function. In such a situation, it is possible to use the Taylor series of the function, at the first order: \( \frac{dF}{dx} \approx \frac{\Delta F}{\Delta x} \), where \( \Delta F(x) \) is the error on the result (in our example: \( \pm 0.01 \)) and \( \Delta x \) is the error on the
Figure B.1: Ill-conditioned case of inverse problem for an arbitrary function.

initial variable. In the example above, this would give: \( \Delta x \approx \frac{\Delta F}{dF/dx} = -\Delta F(x - 1)^2 \), and hence, for \( x = 11 \), the error found is \( \Delta x = \pm 0.1 \). It can be seen that the result is only precise at 2 decimals now, so this method is less accurate than the previous one, because of the Taylor series approximation.

This last example shows that the precision on the initial variable depends on the derivative of the function considered, for a given value. Then, if the function has a segment of low gradient, such as shown in Figure B.1, the error bars on \( F \) are extended and so the precision on \( x \) is dramatically reduced. In some cases, the error on \( x \) can reach more than 100\%. Such cases are called ‘ill-conditioned’, or ‘badly conditioned’. An ill-conditioned problem multiplies the error and then needs a very accurate measure of the data in order to provide reliable solutions.

This notion can be extended to systems with multiple variables. In such cases, the problem can be ill-conditioned in regard to one or several of the variables, according to the gradient of the describing function in the corresponding dimension. Then, the inverse problem can recover the initial values corresponding to the low-gradient dimension, but the others are lost because of the error expansion.
Appendix C

User’s manual

In parallel with the hardware, a series of programs have been written to automatise the procedures of data acquisition, processing and visualisation. This appendix explains how to use these programs to control the DEP machine developed in this thesis.

The machine itself is composed by several devices, linked to the computer. The different devices should be checked before launching the first experiment, and after each time one of the devices has been removed or replaced from the machine. Let us start with a description of the different devices that form the DEP machine and their normal mode of functioning.

C.1 Devices

The DEP machine is composed by 6 devices:

1. The microscope
2. The pump
3. The signal generator
4. The oscilloscope
5. The microchip
6. The computer
Each device must be prepared before and after being powered. This preparation has to be performed once, before the measure of a sample and does not have to be renewed as long as the sample is not changed. If the sample is changed, some of the operations have to be re-performed: in that case the instruction to follow are listed again.

C.1.1 The microscope

The microscope used here is a Zeiss microscope, without light condenser. The objective has a 2x magnification but is modified with an extra lens. This lens has been fixed on the objective but a shock may make it move. If this happens, it can be re-arranged manually by twisting the Perspex box fixed at the end of the microscope objective. If the box falls, it can be fixed with a side screw (be careful though not to screw it too tight as this will damage the objective).

The light source of the microscope is linked to a stable power supply (EA-PS 3016 10B). This power supply has been specially selected for best performance so it should not be changed. It is linked directly to the light bulb, so the maximum admissible voltage is 12V DC. Most of the time, the power does not have to be that high and 8V DC is enough in most cases. Should there be a problem the user will be prompted by the software before the experiment starts.

The camera connected on top of the microscope is a Dolphin F145B (Allied Vision) and is controlled by the computer via a FireWire connection.

C.1.2 The syringe pump

The pump is sitting next to the microscope. It is composed of a syringe, a valve and an electronic board. It is completely driven by the computer via an RS-232 connection. The RS-232 connexion cable can be plugged on any of the COM ports of the computer; the software should detect it automatically.

The electronic board is connected to a separate power supply (WEIR 413D). It needs a voltage of 24V DC ±5%. If the power supply has been moved or changed, these settings should be set before connecting the power cables. Special care should be taken about the polarity as inverting the cable may damage the board and the pump.
The syringe used has a volume of 1mL (BD Plastipak) and can be changed by removing the four holding screws noted on Figure 1, loosing the axial screw and unplugging the tubing connector. The new syringe must be placed in such a way that its body sits in the base and that the body holder touches the base face. The plunger holder should be placed in the moving unit and secured with the axial screw, and then the cover and the four holding screws can be placed again. The holding screws should not be too tight or too loose, the correct tension is when the syringe cannot move axially but is not compressed.

C.1.3 The signal generator

The signal generator (FG100, Digimess) is remotely controlled by the computer via an RS-232 connection. The cable can be plugged to any serial port of the computer; the detection is made by the software. This device powers the microchip via a peak detector. The peak detector is a small electronic unit that connects the signal generator, the microchip and the oscilloscope together; the signal generator is plugged into the BNC connector.
C.1.4 The oscilloscope

The oscilloscope is driven by computer via an RS-232 connection. The cable can be plugged to any serial port of the computer; the detection is made by the software. It is connected to the two wires that goes from the peak detector unit.

C.1.5 The microchip

The microchip is held in a Perspex capsule placed under the microscope. It is fixed to the microscope plate so that the user can move it with the side wheels of the microscope. It is connected to the peak detector via the crocodile clips, and to the sample via the Teflon tubing. The measure of DEP is very light-sensitive, so this unit should remain covered with an absorbent material in order to protect it from external light sources.

C.2 Data Acquisition

The machine is controlled almost entirely by computer, with a Matlab script. First, start Matlab (r2007a). If this is the first use, make sure that the directory ‘DEP project’ is placed into the Matlab path list, as well as all its sub-directories. When ready, enter the command ‘DEP’ in the command line. This starts the main GUI panel that controls the machine. This may take a little while to appear because of the serial ports check. Eventually GUI appears; it should look like Figure C.2.

1. Directory and description

   This panel is at the top left corner and contains the directory browser and the tag editor. The browser allows the user to choose or create the directory where the data will be saved. For this, click on ‘Browse’. The tag editor is a short description of the experiment performed; it is saved in the data and can appear in the figures as a legend, after data processing.

2. Controls

   This is the top-right panel. It is the panel that contains all the links to actions that are not directly related to the experiment, i.e. data processing, device control,
device checking and signal calibration. It contains 4 push buttons with different effects.

- **Device control**
  Clicking on this item opens a secondary GUI that contains different options for a direct control upon the devices. See the ‘device control’ section for more information.

- **Calibration**
  Clicking on this item launches the signal generator calibration procedure. This allows the program to detect any frequency effect of the microchip that could damage the AC signal during the experiment and to compensate it. Before running the calibration, the user must fill the system with the conductivity medium he will use for the experiment, then select the frequency range and points per decade. Then the calibration is optimised. Ideally, the calibration should be repeated every time the conductivity is changed.
Appendix C. User’s manual

3. Procedures

The procedure panel lists all the different procedures that appear in the experimental protocol. It is where the different actions are selected by the user. Usually, a DEP experiment uses the first 4 buttons in the order, from top to bottom.

- **Rinse the system**
  This is the first step of an experiment. This procedure allows the user to rinse the system with a solution. Usually, the system is filled with a detergent when the system is powered so it has to be washed with a low-conductive solution. Ideally, the conductive medium prepared for the experiment should be used. The procedure uses about 500µL of solution, and should be repeated three times. Place the solution into an Eppendorf tube and then place the Eppendorf on the sample holder. Then place the tubing end in the Eppendorf and start the procedure. A message will appear to make sure the sample is in place, just click ‘yes’. Once the system is rinsed, it is a good practice to change the syringe if the previous solution was too different. For that, see the ‘Pump’ section.

- **Load the sample**
  This procedure rinses the system with the conductive media and places the sample into the microwell so the machine is ready for data acquisition. First place an Eppendorf on the sample holder with 1.5mL of rincing medium, and then place the tubing end inside. Make sure the tubing reaches the bottom of the Eppendorf. Then start the procedure: a first message will appear to ask if the solution is into place. Click ‘Yes’ and the loading starts. Once the system
is clean, a second message appears that asks the user to place the sample. The minimum amount of sample usable by the machine is 300 µL, less than that generates some bubbles in the microwell that damages the signal. Remove the previous Eppendorf and replace it with the sample. Make sure that the tubing reaches the bottom of the tube again, and click ‘Ok’. After that, the loading sequence finishes and the system is ready for data acquisition.

- Launch the acquisition

Clicking on this button starts the data acquisition. Before doing that, the user must make sure that the system is clean and filled with the sample, and that all the experimental parameters are set on the ‘Experimental parameters’ panel (see the ‘Experimental parameters panel’ section for more details). The light source should be switched on now if it has not been done yet. When launching an acquisition, the user is asked how many replicates he wishes to obtain. These are independent replicates of the experiment that will be performed successively by the machine. Once the number of replicates is set, the experiments begin.

If the level of light is too bright or too dark, the program detects it and asks the user to modify it before it starts. A progress bar appears on the screen that shows the time left before the completion of the current replicate. Closing this progress bar stops the data acquisition.

The data collected by this procedure are saved in the folder selected in the ‘Directory and description’ panel on the top-left corner. Each replicate generates a dedicated folder (i.e. ‘Replicate1’, ‘Replicate2’, and so on) in which a folder ‘Data’ is created. This is where is stored the raw data collected during that phase; it consists in sequences of images saved into mat-files. This ‘Data’ folder can use quite a lot of memory depending on the parameters set for the acquisition, but once the data processing has been performed it is safe to delete it if necessary.

At the end of each replicate, the experimental conditions are stored in the file ‘Experimental_conditions.mat’ in the folder corresponding to that replicate. This file can be opened in Matlab later and contains all the information present on the GUI, plus the order in which the frequency have been measured (variable ‘permutation’), the date of the beginning and end of the measure,
the date when each image was taken and the voltage peak to peak measured experimentally.

- Wash and finish

When all the experiments are done, it is necessary to wash the system with a detergent so that it remains clean. The detergent used is a solution of Decon 5%. 1.5mL of this solution must be placed in a tube on the sample holder, with the tubing in it, before starting this procedure. Clicking on ‘Wash and finish’ displays a message that checks if the system is ready. Clicking ‘ok’ starts the procedure; it washes the system and fills it with the solution so that the devices can be switched off and the system can wait several days before another experiment.

- Edit a text

Clicking on this button opens a text file and save it on the path selected in the ‘Directory and description’ panel. It is a model text file where the user can detail the experiment he is to perform. The model file can be changed in the DEP folder where the program is stored.

- Check image

Clicking on this button opens a preview of the camera and a histogram. This is useful if one wants to check the alignment of the microwell in the system, or the saturation of the camera. However, the machine contains an algorithm for the detection of the level of light so it should not be too sensitive to the illumination.

4. Experimental parameters

This panel contains all the data related to the experimental conditions. It is split into two areas, the main parameters and the advanced parameters. The advanced parameters are explained later.

- Lowest frequency

This is the value of the lowest frequency in the spectrum, in Hz. When setting this parameter, one should consider that too low a frequency can lead to hydrolysis in the microwell, which create bubbles and increases the toxicity of the medium. 1KHz is the default value because it is safe, but according to the suspension medium one can go lower.
• Highest frequency
  This is the value of the highest frequency in the spectrum, in Hz. The maximum frequency is 20MHz.
• Points/decade
  This is the number of points per frequency decade in the spectrum.
• Voltage
  This is the amplitude of the signal provided by the signal generator during the experiment. The maximum is 20Vpp but too high a voltage can be a problem, especially with microflows. The ideal voltage depends on the cell line, medium conductivity and frequency band, and has to be found experimentally.
• Medium conductivity
  This is the conductivity of the suspension medium, in µS/cm. It is necessary for the signal processing and should be provided if the user needs to measure the permittivity and conductivity of the sample.
• Cell concentration
  This is the concentration of the sample, in cells per mL. This parameter is not necessary for the data processing but is stored with the experimental conditions for ease of use.

5. Advanced parameters
  This panel contains all the parameters that affect the behaviour of the machine during an experiment.

• Syringe volume
  This is the volume of the syringe in mL; it is used by the machine for setting the motor speed.
• Syringe length
  This is the length of the syringe in mm; it is used by the machine for setting the motor speed.
• Resuspended volume
  This is the amount of sample which passes through the microwell each time the sample needs to be refreshed, in mL. It corresponds to the volume of the tubing between the sample holder and the microwell.
• Images/points
  This is the number of images taken by the camera for the measure of each point of the DEP spectrum. It influences the precision of the measure.

• Blanks/points
  Before starting the measure of DEP, a number of images are taken in order to be used as a reference during the data processing. This sets their number.

• Time/points
  This is the amount of time spent for the measure of each point of the DEP spectrum, in seconds.

• Motor speed
  This sets the speed of the pump, in % of the full scale.

• Dead volume
  This corresponds to the volume between the microwell and the syringe, in mL.

• Well diameter
  This is the diameter of the microwell, in µm.

6. Messages
  This panel displays the information about the processing. This can be the beginning and end of an experiment, the list of devices detected, the start and end of the rinsing procedure, and so on.

C.3 Data processing

The data processing is performed by a dedicated GUI. This appears when pressing the ‘Data processing’ button on the top-right corner of the Data Acquisition GUI.

The data processing GUI contains three panels:

• File description
  This panel presents some information about the state of the processing.

• Data acquired
  This is the panel on the left-hand side. It lists all the experiments that have
been performed, allow retrieving previous experiments and pre-process the data acquired to obtain the DEP spectrum. All the data listed in the box is affected by the buttons.

• **Refresh**
  Pressing ‘Refresh’ refreshes the list of experiments. This is useful every time a new experiment has been performed.

• **Add...**
  Pressing ‘Add...’ launches a file browser so the user can add an experiment done previously; for that, select the file named ‘experiment.mat’ in the corresponding folder.

• **Remove selection**
  Pressing ‘Remove selection’ removes the selected item from the list.

• **Launch**
  Pressing ‘Launch’ starts the pre-processing of all the data listed. If the user selected
a set of experiments that has already been pre-processed, a question dialogue opens
to ask if the data needs to be re-processed or if the data processing is skipped. Once
this first choice is made, a second question dialogue opens to check if the decision
applies to all the pre-processed files. When finished, the data pre-processed appears
on the left panel (see Figure 4) and several files are created: a file ‘Results.mat’
and a file ‘Spectrum.mat’ appear in the folder corresponding to each replicate.
The first one contains the result of the pre-processing: the numerical results (vari-
able ‘results’) with the error bar (variable ‘errbar’), the list of frequency (variable
‘freqlist’), the position of the rings (variable ‘position’), the radius of the microwell
in pixels (variable ‘radius’), the date when the data has been acquired since the
application of the electric field (variable ‘deltat’) and the tag set by the user in the
‘Directory and description’ panel, previous to experiment.
The second contains information about the DEP spectrum measured. This com-
prises the values of the frequency (variable ‘Frequency’), the values of the spectrum
(variable ‘K’), the error bars (variable ‘dK’) and, after the curve fitting, it can also
contain the fitted curve (variable ‘Kfit’) and the fit object (object ‘fitobj’).

- Data processed

This panel lists all the data that has been processed by the GUI during the session.
Only the data selected is affected by the buttons in this panel.

- Plot raw data

Pressing ‘Plot raw data’ opens a figure and displays the DEP spectra of the files
selected. This allows a visualisation of the pre-processed data.

- Fit the data

Pressing this button opens a GUI dedicated to the curve fitting of the data. This
uses the multi-shell model, and allows the selection of the thin-membrane approx-
imation. When the fit is performed, this create the variable ‘Measure.mat’ in each
replicate directory. This file contains information about the values found from
the fit (variable ‘values’), their respective names (variable ‘names’), the initial
guess proposed by the user (variable ‘guess’), the goodness of fit (object ‘gof’) and
the tag that describes the experiment as previously selected by the user. More
information can be found in the ‘Curve fitting’ section.
• Plot the results

This button launches the plotting procedure. This asks the user which data are plotted on the X-axis and if the data should be averaged or not. Then it plots the result of the fit in different windows.

• Cytogram

This button launches the cytogram view. This asks the user which data correspond to the X and Y-axes and plots the data accordingly.

C.4  Device control

This GUI appears when the button ‘Device control’ is hit, on the top-right corner of the main acquisition GUI. It allows the direct access and basic control to some of the devices.

figure5 : device control

C.4.1 Signal generator panel

This panel proposes three options to control the signal generator: voltage, which set the output voltage peak to peak, signal frequency, which set the frequency of the sine wave, and a push button which switches the generator on and off.

C.4.2 Pump panel

This panel can control the pump and valve system. The upper button switches the valve to in order to connect the syringe to the sample, or to the bin. The lower button is used to calibrate the position of the syringe: this empties the syringe completely. The upper selector, together with the value of the injection speed, regulates the speed of the stepper motor. The lower selector, together with the value of the syringe position, moves the syringe to the desired value. Be careful with this last value, as it updates the syringe position instantly and it is easy to inject some air in the system if the value is too high.
C.4.3 Camera panel

This panel can access the camera in order to provide a visualisation of the microwell.

C.5 Curve fitting

The curve fitting panel works on the data selected in the ‘Processed data’ box of the data processing GUI. It fits the data automatically with the provided guesses and model.

The model can be selected in the top left corner dropdown menu. Up to six shells can be added to the model, but the more shells are modelled, the more difficult it is to find a good fit. The values of the guesses corresponding to the properties of each shell can be set in the ‘Permittivity’ and ‘Conductivity’ panels. The small drawing on the left reminds that $\varepsilon_1$ and $\sigma_1$ stand for the inner part, $\varepsilon_2$ and $\sigma_2$ for the 1st shell, and so on. The last values of $\varepsilon$ and $\sigma$ correspond to the properties of the medium. Finally, the ‘Geometry’ panel contains information about the dimensions of each layer.

It is also possible to select the band of frequency on which to perform the fitting. Because of EHD effects, the default value cuts the data corresponding to a frequency lower than 5kHz. Putting 0 can cancel the effect of filtering.

Pushing the ‘Close button’ closes the fitting GUI; pushing ‘Fit the data’ starts the automatic fitting of each data selected, with the initial guess provided. If the data is not fitted correctly, you can try another guess and re-try.
Appendix D

Electronic data

The CD contains all the electronic data developed during this thesis, including the programs and the present thesis.