Image Processing Methods
in
Digital Autoradiography

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To

my parents and brother
and
Costas.

Αφιερωμένο με όλη μου την αγάπη
στους γονείς μου, στον αδερφό μου
και στον Κώστα.
Summary

Autoradiography is a common method in biomedical research for detecting and measuring biodistributions of labelled biomolecules within a specimen. The conventional method is based on using film or film-emulsions for the image acquisition. Although film autoradiography is still in widespread use, there are several disadvantages such as long exposure times, lack of sensitivity, non-linear response of the film and limited dynamic range that encouraged the development of digital autoradiographic systems. Most of the current digital imaging systems have demonstrated excellent performance as far as the above parameters are concerned but still cannot match the image resolution performance exhibited by film or film-emulsion.

This thesis is focused on developing image processing methods for improving the quality of digital autoradiography images corrupted with noise and blur obtained by a hybrid CCD autoradiography system at room temperature. Initially, a novel fixed pattern noise method was developed which takes into account the non-ergodic nature of the dark current noise and its dependence on ambient temperature. Empirical formulae were also deduced as a further improvement of the above method for adapting the parameters of the noise distribution for ambient temperature shifts. Image restoration approaches were developed using simulated annealing as a global optimisation technique appropriate for removing the noise and blur from high particle flux samples.

The performance of the proposed methods for low flux distributed sources (microscales and mouse brain sections) labelled with high energy beta emitters has also been demonstrated at different temperatures and integration times and compared with images acquired by the conventional film-based method.

Key words: Digital autoradiography, image restoration, simulated annealing, fixed pattern noise removal.
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Chapter 1

Introduction

Radioisotope imaging is used extensively in biological and clinical research either for disease diagnosis or further investigation of radiopharmaceuticals and imaging of specific biochemical processes. The method relies on the principle that radioisotopes (usually used in tracer\(^1\) quantities) used for imaging purposes behave in a similar way at a chemical and biological level as the natural elements in order to image the true biodistributions of the molecules under study. Thus, the images represent ‘snap shots’ of the bio-chemical process in a living organism.

Autoradiography is one of the oldest methods used in clinical and biological research for detecting and measuring the micro-distribution and quantity of tagged biomolecules within a specimen [6], [11]. The process is based on administering a labelled compound to a biological system [animal/human] and using thin slices of excised tissue held in intimate contact with an imaging sensor or imaging medium. Autoradiography techniques can be classified according to the type of radiation used (\(\alpha\), \(\beta\) or \(\gamma\) radiation autoradiography), the dimension of the sample investigated (macro- and micro-autoradiography) or the method of autoradiograph analysis (emulsion grain density or track autoradiography). Autoradiographic methods can also be classified into two main categories according to the detecting medium: conventional and digital autoradiography. Conventional autoradiography uses films and films-emulsions for detecting the particle emissions. However, there are several disadvantages associated with conventional methods

\(^1\)Concentration of a trace element is usually of the order of 0.01% [94] of the total weight.
such as long exposure times, non-linear response of the films, limited dynamic range and lack of sensitivity. These problems have motivated the development of dedicated digital imaging systems for use in autoradiography and dedicated image processing methods to improve image quality. The work of this thesis is in the area of digital autoradiography, specifically using a prototype CCD (Charge Coupled Device)-based imaging system and extending its use to room temperature by using image correction and image processing methods.

1.1 Motivation and aim

The most sensitive technique for monitoring biochemical processes within living tissue requires radioisotopes with the same chemical and biological properties as the natural elements to be introduced to the system [24], [81]. This requirement for the same properties is essential since the living system should not be significantly (or not at all) disturbed during experimentation. Most of the suitable radioisotopes are pure beta emitters and thus a large part of autoradiography is concerned with imaging beta emitters. The reason for labelling tissue with these beta ($\beta^-$ or $\beta^+$) emitters is because they are the most common natural elements found in glucose, proteins and amino acids and they have been widely used for labelling in biomedical research [37].

The system used in this work has previously produced good quality autoradiographs under cooled conditions, in order to limit dark current. However, cooling down the device or part of the imaging system is usually a time consuming process that can last up to two hours until it reaches thermal stability. Furthermore, imaging of short half-life isotopes is precluded if cooling is required. Therefore, this thesis is a contribution towards the development of an imaging system that can operate at room temperatures rather than under cooled conditions.

The flux of particles emitted from a radiolabelled tissue sample can vary dramatically. In some situations poor labelling efficiency in autoradiography can be a significant problem\(^2\). Even though labelling may be adequate, using a CCD system for autoradiographic imaging still requires significant correction for large inter-pixel variations. At

\(^2\)When a specific development programme is required for labelling new compounds, it is often a
room temperature, where the thermal noise increases significantly, the signal to noise ratio becomes even poorer. Therefore, it is relatively challenging to image low activity (low particle flux) samples at room temperature. In this work, image processing methods are developed for both low and high activity samples. The choice of the method used depends on the application and the specifications of the sample.

The ultimate aim of this project is to develop image processing methods for correcting dark current thermal effects for a CCD-based imaging system which would produce high resolution autoradiographs (images of radioactivity distributions) of varying activity distributed sources, at room and human body temperature. This thesis is one part of a larger project aiming to develop a system that would image live tissue cells. The two components of such a system are: the image sensing system and the tissue/cell preservation system. This work focuses on the development of the former component. Image processing methods would be developed in order to correct for the thermal effects of dark current in both low and high activity sources for operating the CCD at room and human body temperature. The latter component involves growing live cells on waterproof thin membranes - a technique being developed at the Cyclotron unit, Hammersmith Hospital, London, UK, which would be eventually integrated into the imaging system. The key parameters required from this project for such an imaging system are:

- operating in temperatures between 20° C and 30° C where cell/tissue sections can survive,

- having high spatial resolution in the order of few tens of μm,

- reducing background noise of the image using software-based corrections,

- demonstrating a linear response for a range of temperatures.

non-trivial problem because the labelling process can affect some metabolic processes. Additionally, the chemistry of the actual place that the tracer would bind should be investigated and the appropriate part of molecule structure should be fully understood. Therefore, when new compounds are imaged, the labelling efficiency is poor due to these difficulties and therefore the flux of particles emitted vary. A low flux of particles leads to poor signal to noise ratio because of either limited imaging time or radioisotope decay.
1.2 Outline of the thesis

In this work image processing methods have been developed for correcting/restoring autoradiographic images of low and high activity samples. The thesis consists of two main parts. The first part the characteristics of the hardware system are assessed and appropriate image processing methods developed and applied to image distributed sources of low activity. The second part is concerned with developing and evaluating image processing algorithms applied to simulated and real autoradiographic test images.

More specifically, chapter one provides an introduction to the field of autoradiography, explaining briefly the basic principles. The motivation of this work is discussed and the aim and objectives of the project are presented.

Chapter two includes a more extensive explanation about autoradiography and main application areas. Autoradiography is classified into two main types: conventional and digital. The basic principles and limitations of conventional autoradiography are presented and the reasons for developing digital autoradiographic systems are discussed. A brief overview of other digital imaging systems in autoradiography is presented. Since use of CCD technology has demonstrated promising results in this area, a brief description of CCD technology is included. An overview of the previous published performance of the CCD-based system used in this work is discussed here albeit under cooled conditions. However, since the main aim of this work is focused on software-based approaches rather than hardware development of the imaging system, a literature review of software-based approaches specifically used in autoradiography is presented in more detail and discussed. Finally, an introduction to the algorithms used for image restoration is presented.

In chapter three the experimental arrangement used throughout this work is presented in more detail. The chapter concludes with a discussion of the issues around room temperature image acquisition and the description of the test autoradiographic images used.

Chapter four contains experimental results using calibrated sources $^{14}$C microscale sources commonly used in biological research. The linearity of the system is investigated
1.3. Achievements of this thesis

At different temperature levels, including human body temperature (required for future imaging of live cells), and for different integration (exposure) times. Fixed pattern noise removal methods were developed for correcting small inter-frame temperature drifts and investigating the relationship between the dark current behaviour and temperature.

In chapter five several different software approaches to image restoration are developed. A fixed pattern noise method developed in the previous chapter was combined with the basic image restoration approach for correcting small temperature drifts and dark current offsets.

The methods described in chapter five are applied to simulated and real test data in chapter six. Simulated images are used in order to establish the sensitivity of each algorithm to its parameters and to define the optimal range of values for each of them and for each algorithm.

In chapter seven, a fixed pattern noise removal method developed in chapter four is applied to real distributed tissue samples. Images of three different mouse brain tissue sections labelled with $^{35}$S were obtained at two different temperatures and the results of the new method are presented and compared with images acquired using conventional film imaging.

Chapter eight concludes with an overview of the thesis and the final conclusions. Suggestions for further work are also included.

1.3 Achievements of this thesis

The achievements of this thesis are summarised to the following:

- A novel method for fixed pattern noise removal has been developed, taking into consideration the non-ergodic nature of the dark current noise and its dependence on ambient temperature (section 5.4.3).

- Empirical formulae have been derived for adapting the parameters of the noise distribution due to dark current for ambient temperatures in the range 5° C - 36° C (section 4.4).
• A framework of image restoration using a global optimisation technique as simulated annealing has been developed, appropriate for removing noise and blur from images of high flux samples (Chapters 4 and 5).

• The concept of autoradiography at room temperature has been clearly demonstrated with tissue samples imaged with a film-based method and with the proposed technique (Chapter 7).

The publications that have been produced so far from this thesis are listed at the end of the book.
Chapter 2

Literature Survey

Several methods have been developed in order to detect the emitted radiation of radioisotopes used as tracers. Autoradiography is an invasive method used in research to measure the quantity of tagged biomolecules within an *ex-vivo* specimen [11] and so infer the metabolised biodistribution. The tagged molecules labelled with a radioisotope (the most common used in autoradiography emit $\beta$ particles, fast electrons) have similar chemical and physiological processes as the stable molecular counterparts [93]. In order to detect the emitted radiation, modified photographic methods were used also to locate the radiotracers. The great advantage of autoradiography is that it integrates the photographic effect of very weak radiation intensities [6]. It is the most sensitive method of determining biodistributions because no other method can achieve the labelling efficiencies capable with autoradiography using radiotracers.

2.1 Conventional Autoradiography

Autoradiographic methods can be conveniently grouped into two main approaches: conventional (where film is used) and digital (where dedicated digital imaging systems are used). The conventional methods of autoradiography use various types of photographic layers as the detection medium for stopping the emitted $\beta$ radiation. The detection medium utilised by conventional autoradiography are films or film-emulsions. The principle in these methods is that the radioisotope decays within the specimen and
the particles from the emitted radiation, after suitable exposure time, cause changes in the silver halide photographic layer. These changes are then amplified in order to be visible with the naked eye. The developed image shows the distribution and, if imaged with a calibrated source and digitised, the quantity of the radioisotope present.

Although conventional imaging methods have demonstrated a superior spatial resolution (imaging in sub-cellular level) and low cost, there are several factors explained below that affect their overall performance. During a film exposure microscales are also required to be imaged in order to be able to calibrate the film image for further quantitative analysis. Furthermore, many factors exist during wet processing of the film that can affect or even destroy the image (such as the chemical composition of the developer or the temperature of the developing solution or other variables associated with wet film processing) [11]. For quantitative analysis, digitisation of the film images is possible by using CCD-cameras or micro-densitometers. Film autoradiography can also require long exposure times (over several days or weeks) due to lack of sensitivity. The sigmoid [11] shape of optical density versus the logarithm of exposure demonstrates a relatively small linear region and in general non-linear response of the film. Thus very low activities or very high activities cannot be (quantitatively) imaged simultaneously. In the former case there is not sufficient energy deposited to form a latent image and in the latter case saturation is almost immediately reached, demonstrating the low dynamic range (~ \(10^2\ [82]\)) of the films. However, despite their disadvantages, films and emulsions are still in widespread use because of the superior spatial resolution.

The specimen used in autoradiograms can be of different types according to the needs of the study. For example, the specimen used by biochemists could be chromatograms\(^1\) whereas biologists can use a whole organism or a slice of organism [22]. The thickness of the specimen also varies depending on the type of autoradiography (i.e. thin-tissue autoradiography).

The radioisotopes are chosen by applying criteria such as the type of emitted radiation,

---

\(^1\)Chromatograms are the products of chromatography. The official definition from the International Union of Pure and Applied Chemistry (1993) is that: "chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction."
2.1. Conventional Autoradiography

the biological relevance, the half-life and the energy of the emitted radiation [81], [24].

The most commonly used radioisotopes are presented in table 2.1.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life ($T_{1/2}$)</th>
<th>Useful decay</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tritium ($^3$H)</td>
<td>12.26 years</td>
<td>$\beta^-$</td>
<td>18.6 keV (max)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.72 keV (average)</td>
</tr>
<tr>
<td>Carbon-14 ($^{14}$C)</td>
<td>5570 years</td>
<td>$\beta^-$</td>
<td>155 keV (max)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>49.5 keV (average)</td>
</tr>
<tr>
<td>Sulphur-35 ($^{35}$S)</td>
<td>87.4 days</td>
<td>$\beta^-$</td>
<td>167 keV (max)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48.8 keV (average)</td>
</tr>
<tr>
<td>Phosphorus-32 ($^{32}$P)</td>
<td>14.4 days</td>
<td>$\beta^-$</td>
<td>1.71 MeV (max)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>695 keV (average)</td>
</tr>
<tr>
<td>Phosphorus-33 ($^{33}$P)</td>
<td>25.4 days</td>
<td>$\beta^-$</td>
<td>249 keV (max)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>76.9 keV (average)</td>
</tr>
</tbody>
</table>

Table 2.1: Table of the most commonly used radioisotopes in autoradiography [10], [21].

The energy of the $\beta$ particles varies from 0 to a maximum value $E_{max}$ (continuous spectrum) which characterises each radioisotope. When $\beta$ particles pass through matter, they deposit part of their energy and are deflected by orbital electrons, which is the main cause of energy losses. Generally, the rate of energy loss of $\beta$ particles is low and sometimes the $\beta$ particles pass through the silver halide crystals without imparting sufficient energy to form a latent image. Some crystals however can create a latent image if some minimum energy is given to them. Therefore the track of $\beta$ particles through an emulsion consists of silver grains, some small, some large and with gaps between these locations. The track is not a straight line either because of nuclear collisions or sometimes there are two branches (during formation of $\delta$ rays). Towards the end of the $\beta$ particle track the grains tend to be larger and the track more tortuous because as the energy decreases the rate of energy loss increases rapidly [93].

A photographic process follows in order to develop the film and make permanent changes of the silver bromide (or halide) grains a visible image. The efficiency of an autoradiographic system with films and emulsions is defined as "the proportion of
Table 2.2: Factors that affect the resolution and efficiency of a conventional autoradiographic system. The symbols ↑ and ↓ represent an increase or decrease of each factor respectively.([70],p.22)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Resolution</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy of β particles</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
<tr>
<td>Source-emulsion distance</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
<tr>
<td>Source thickness</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
<tr>
<td>Emulsion thickness</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
<tr>
<td>AgBr crystal size</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
<tr>
<td>Exposure length</td>
<td>↑ degrades</td>
<td>↓ improves</td>
</tr>
</tbody>
</table>

Table 2.2: Factors that affect the resolution and efficiency of a conventional autoradiographic system. The symbols ↑ and ↓ represent an increase or decrease of each factor respectively.([70],p.22)

Disintegrations occurring in the specimen which are recorded by the emulsion layer” [6]. Fewer than 5 % of the emitted beta particles from $^{32}$P can be detected by emulsion [45].

There are many definitions for the resolution of such system but the most commonly used defines as resolution “the distance which must separate two sources of equal strength if the grain density between them falls to half that seen over each source” [87]. The efficiency and the resolution of the autoradiographs are affected from various factors that mainly come from the source (choice of radioisotope, distance between the source and the emulsion, thickness of the source) or the emulsion (thickness of the emulsion, size of the silver bromide (or halide) crystals, length of exposure, sensitivity of emulsion. Table 2.2 shows how changes in the above factors affect the efficiency and the resolution.

Within the emulsion there are developed silver crystals present that were not created by the incident radiation. These crystals are defined as background and provide an
intrinsic lower limit on sensitivity. The main causes of background are: during development of the film (strength, temperature or duration), background due to exposure to light, background due to pressure, chemography (other chemical action produces latent image), contamination of the emulsion environmental radiation and spontaneous background. Some of the background causes cannot be eliminated and this is an additional disadvantage of conventional autoradiography.

There are many disadvantages in conventional autoradiography such as the limited dynamic range, the lack of sensitivity of films and the difficult quantitation of the image also because of the non-linear response. Additionally, the user has to compromise some factors in order to achieve good resolution as well as good efficiency. Lastly, conventional autoradiography is not a method for real-time imaging but is a long procedure until the image is finally visualised. All these disadvantages have long been noticed and many techniques have been developed in the area of digital autoradiography to address these problems.

2.2 Digital Autoradiographic Systems

The main objective in digital autoradiography is to achieve real time imaging with good spatial resolution (~ few \(\mu m\)) and efficiency. Generally, the digital methods that have been developed so far have good detection efficiency (superior to 60 % for some of the systems), linear response over wide dynamic range (almost six orders of magnitude) that allows simultaneous visualisation of the areas of low and high activities (i.e. linear response over a range such as 14-1295 kBq/g). Digitisation of the data within the imaging system makes quantitative analysis easier than using the conventional methods (photographic film)[81]. However, the spatial resolution of digital techniques is still inferior to that of films. Research is still on going in order to improve the spatial resolution of such systems.

Digital autoradiography imaging systems can be divided into three broad types: methods based on plate technology, gas detector-based systems and silicon imaging systems. The characteristics of each category are discussed below.
Plate Technology

There are three main systems belonging in this category which are the storage phosphor plates by Johnston [45], the microchannel plates by Lees et al [60], [61], [62], [63] and the Beta Camera by Ljunggren et al [65], [66], [68], [67]. The former system is based on exposing the sample against phosphor plates that are read out later by laser scanning. Although this system is still in use, it is the only digital imaging system that does not provide real-time imaging. Additionally the spatial resolution of approximately 100 $\mu$m has limited the number of applications. However, the plates are re-usable and have better efficiency and linearity compared to film. The system by Lees et al is based on direct detection of $\beta$ particles in a microchannel plate. Although, it was initially developed for astronomical purposes (X-ray photon counting) by the X-ray Astronomy Group at Leicester University, it has shown a very promising performance, especially for imaging low energy betas such as those emitted from $^3$H. The Beta camera utilises a combination of a scintillator and microchannel plates but still the spatial resolution is poor ($\sim 200 \mu$m) and it requires additional cooling of the system. However, being one of the first dedicated autoradiographic imaging system, further discussion is provided later on this chapter.

Gas detector-based systems

The gas-based detector systems such as those developed by Englert et al [28] and Petegnief et al [82] are based on utilising a multi-wire proportional chamber [8]. The main principle of the gas-based systems is that the beta particles which enter the detector can ionise gas molecules in a channel of a collimator. The produced electrons can result in further ionisation due to a strong electron field applied between the anode and the cathode. These systems have demonstrated accurate quantification of the activity and good linearity of up to 5 orders of magnitude but poor spatial resolution ($\sim 500 \mu$m) due to the wide angle of $\beta$ emission and the wide spread of the tracks, especially in the case of high energy beta particles. However, these systems are suitable for imaging of large sections ($\sim 480 \text{ cm}^2$).
2.2. Digital Autoradiographic Systems

Solid state or silicon-sensor based systems

Solid state or silicon-based imaging systems can be further classified into those systems that are based on CCD device and those based on silicon strip or silicon or gallium arsenide detectors. The CCD-based imaging systems utilise either just a CCD-device as the prototype system by MacDonald et al [70], [71], [79] or a CCD optically coupled to an intensifying screen by a lens system such as the one developed by Karellas et al [46], [47], [48] or the micro-imager by Charon et al [18], [19], [20], [56], [57] which is based on a combination of a scintillator, an image intensifier and a CCD. These systems have demonstrated good detection efficiency due to the low energy required (few eV) to generate the signal in silicon and small depletion layers which allows good spatial resolution (some systems better than 50 μm). However, they usually provide small imaging areas due to the read out process (large devices have long read out periods). Systems based on silicon strip detectors include examples such as the system by Bertolucci et al [12], [13] or silicon or gallium arsenide detectors [5], [2], [39], [75]. Two common systems with spatial resolution below 50 μm based on the same principles are the BIOSCOPE system by Overdick et al [80], [77] and the DEPFET Bioscope system using silicon strip detectors by Klein et al [53], [30]. Other systems of the same category include CCD arrays [91], [58], [59], [97] or the silicon pixel array by Puertolas et al [23]. The spatial resolution of all these systems is limited by the dimensions of the pixels. However, the silicon solid state systems have received the most attention because of the possibility of using small pixel dimensions.

Since the main concern of this thesis was not hardware development, only the most known systems will be briefly summarised and discussed.

Photostimulable storage phosphor materials were initially developed and used in medical radiography in order to reduce the exposure time required for X-ray film. This system is based on the result of incident radiation to the electrons of the material (excitation to the conduction band and then trapping in an ‘F-center’). The read-out procedure is performed by a laser beam scanning across the sample, which causes the return of the electrons to the conduction band by emitting photon radiation. The results have shown that the sensitivity of the phosphor plate is 60 times better than
conventional film autoradiography. Additionally, using samples labelled with $^{32}\text{P}$ has shown an increase in sensitivity up to 250 times compared with direct film autoradiography. However, the length of exposure would not necessarily give proportional detection by the phosphor plates since the background (cosmic and environmental radiation) increases over time as well. The response of the system was found to be linear within a range of activity 1:50000. The spatial resolution of the system was found 530 $\mu$m for $^{14}\text{C}$ and 670 $\mu$m for $^{32}\text{P}$, as measured by ink lines of 0.3 mm width separated by 0.15, 0.3, 0.5 or 1.0 mm [45]. Although phosphor plate technology is still used, there are several disadvantages like poor spatial resolution and that the optimal exposure time has to be known from experience. This system can be used satisfactorily in low resolution autoradiographic applications such as DNA sequencing [90] but still does not provide real-time imaging.

The Bioscope system, whose components are based on double-silicon strip detectors and self-triggering read-out chips, has shown a relatively good performance. The spatial resolution is below 50 $\mu$m when imaging samples labelled with $^{35}\text{S}$ (figure 2.1), which is considered to be state-of-the-art for digital autoradiography. The noise performance was also quite good ($60\,\text{e}^-\text{ENC}^2$) and therefore no background subtraction was necessary. The linear response (within the range of 30-250 keV) [80] of the system to the energy of different isotopes makes it a useful tool for multi-tracer studies [55].

The beta camera has many similar advantages such as the capability in performing dynamic studies. This detector consists of a scintillator, a fiber-optic window, a photocathode, two microchannel plates (MCP) and a resistive anode (see figure 2.2). When beta particles interact with the scintillator, photons are generated. These photons are then directed through a fiber-optic window to a photocathode. The produced photoelectrons then enter a microchannel plate, which work in a similar manner to a photomultiplier tube producing a cascade of electrons which interacts with a resistive anode producing pulses with different amplitudes depending on the position [65]. The detector was cooled down to a temperature of -20$^\circ$C to reduce dark current levels. The spatial resolution of the initial system was 500 $\mu$m and the sensitivity is $10^{-3}\text{sec}^{-1}\text{kBq}^{-1}\text{g}$. The beta camera demonstrated that real-time autoradiographic imaging can be possible.

$^{2}\text{ENC stands for Equivalent Noise Charge.}$
Figure 2.1: Autoradiograph of two thin hairs labelled with $^{35}$S obtained by the Bioscope system [80].

ble even for dynamic or multi-nuclide studies [67]. A modified beta camera was found to demonstrate the spatial resolution at FWHM varied between 100 - 240 $\mu$m. However, there are many factors that affect the spatial resolution such as multiple scattering in the detector and the scintillating light distributions of the photocathode [23]. The main contribution to the field from this work was that it represented the first serious development of a digital autoradiography system and demonstrated the real-time imaging potential of using dedicated imaging technology.

Figure 2.2: A schematic drawing of the beta camera. [65]
Direct detection using microchannel plates improved the resolution of another type of beta camera to 400 μm and increased the efficiency in imaging tritium because of the high electron detection efficiency of the plates [62] (figure 2.3).

![Image of tritium labelled whole body thin tissue slice of rat](image-url)  

**Figure 2.3:** Image of tritium labelled whole body thin tissue slice of rat [62], page 638.

The now commercial micro-imager system, previously developed under the name High Resolution RadioImager (HRRRI) [57], took the basic detection principles seen in the beta camera and scaled these down to improve the imaging performance. Spatial resolution of 15 μm was achieved on a 1.2 cm² area (23 × 23 μm², 576 × 384 pixels). However, the signal to noise ratio of the system was poor as in this case the photocathode was not cooled. Another limitation of the system is the small active area, which can make the system unsuitable for some applications [56]. In figure 2.4 the micro-imager is presented with three images of rat kidney section labelled with tritium. This improvement compared to the beta camera was achieved principally by using patented methods of producing a very thin scintillator, and the use of a CCD rather than resistive anode for event localisation.

During this section several digital autoradiographic systems that aim to replace con-
Figure 2.4: The micro-imager system. A 5 μm thick rat kidney section (A) standard radioactive image after 60 hours exposure, (B) optimal density image (20 minutes) (C) superimposed images. The distribution shown in the images is a protein tagged with tritium ([56], page 3).

2.2. Digital Autoradiographic Systems

Conventional systems were discussed. However, the spatial resolution of most systems still remains modest compared to film-emulsion. Of these systems, those using CCD technology appear to show the most promising performance. In the next section a brief overview of CCD imaging technology is therefore presented.
2.3 General principles of CCD imaging

2.3.1 CCD Technology

The charge-coupled device (CCD) was first developed in 1969 although it was actually a new way of using an older, more familiar device, the MOS transistor. CCDs were initially utilised for optical imaging and for subsequently X-ray astronomy [69], [40] and high energy particle physics applications. The CCD device is structurally an array of MOS capacitors held in close proximity to each other. The recorded signal represents the integrated charge in each of these MOS-capacitors. The signal/charge generation and the read out process are described briefly in the following two sections.

Signal generation within the CCD volume

The signal generation in the CCD can be caused by interaction with visible light, X-ray or charged particles such as $\beta$ particles. Photons deposit their energy within the CCD volume using photoelectric effect [29], whilst the $\beta$ particles continuously depositing charge (loss of energy) by ionisation and excitation along their path within the device. The charge that is generated in the sensitive volume of the CCD is stored under the electrodes that define the device’s pixels.

A cross section of a CCD device is displayed in figure 2.5. Apart from the electrodes in the upper part, the CCD device consists of two major areas: the depletion layer and the field-free region or substrate. The location in which the signal charge is generated is defined by the characteristics of the particles: the type of the particle (positron, electron, photons) and the energy (low, medium, high). The charge that is generated in the depletion layer is drifted towards the electrodes and usually there is minimum spread between adjacent pixels (unless charge is generated exactly between two pixels). The charge generated in the field-free region either moves by diffusion [41] or recombines or reaches the edge of the depletion layer [69].

---

3MOS transistor is an n-channel Metal-Oxide Semiconductor (MOS) capacitor composed of a p-type silicon layer, a silicon dioxide layer and a metal plate. [42]
2.3. General principles of CCD imaging

Figure 2.5: Cross section of a CCD device. Signal charge is generated in the depletion layer and rapidly collected by the pixel electrodes or it diffuses in the field-free region to adjacent pixels ([69], page 1379).

Read out process in a CCD

The simplest way of arranging the MOS capacitors to form the CCD device is the three-phase device [43]. An example of a three-phase CCD device is shown in figure 2.6.

Each of the electrodes presented in figure 2.6 forms the gate of a MOS transistor. The pixel electrodes are separated in the perpendicular direction (perpendicular to the page in figure 2.6) by regions which are called channel-stops. Each of the gates is connected to each of the three phases: phase-1, phase-2 and phase-3 composing a pixel register. The generated charge is confined within the area defined by the three electrodes at potential 0 V, + V and 0 V, separated by the channel-stops in the opposite direction. This type of device based on the three electrodes is known as three-phase device and it requires three sequential voltage pulses to operate the device. Charge is serially transferred along the device by pulsing the detectors as shown in figure 2.6. At the end of each pixel column in the device is an element of the read-out register, which collects the shunted charge. Once a line of charge has been transferred to the read-out register, the charge is then serially shunted towards the output amplifier by three additional
Chapter 2. Literature Survey

Figure 2.6: (a) Charge accumulates under the positive (+V) electrodes, caused by thermal dark current, lattice defects and due to ionisation caused by light or particulate radiation entering under the positive electrode. Statically accumulating charge in this way occurs during exposure often referred to as the integration time, prior to read out. (b) Read out of the deposited charge via charge-coupling. The electrodes are pulsed sequentially so the charge is moved along the CCD [26].

Radiation damage in the CCD

There are references in literature mentioning the radiation damage caused on the CCD devices from exposure to ionising radiation [44], [88]. There are two main categories of radiation damage caused by high flux of particles: bulk or displacement damage and ionisation-induced damage. Bulk damage occurs when high energy particles displace silicon atoms from their original place in the lattices resulting in silicon vacancies. The other type of damage is ionisation-induced where an increase is observed in the amount of generated electron-hole pairs within the gate insulator, resulting in an increase in the
2.3. General principles of CCD imaging

![BASIC CCD ARRAY](image)

Figure 2.7: CCD Array: the arrows represent the direction of the charge transfer during read out. During read out the charge of one potential well moves to its adjacent and is added to the existing charge, then the total moves to the next potential well and so on until the charge moves along to the horizontal read out register. [26]

dark current level. The first problem from the ionisation induced damage is that holes generated in the epitaxial layer are trapped generating a flat potential that effectively shifts the bias voltage of the clock and the output amplifier. The second problem is that the dielectric of the Si-SiO₂ interface changes causing an increase in the surface dark current level [43]. On the manufacturer’s data sheet it is mentioned that “device parameters may begin to change if subjected to greater than $10^4$ rads” [27]. This is equivalent of $3.7 \times 10^{11}$ ionising particles. The estimated dose used during this work is much less than this amount, so there was not expected to be any significant radiation
damage while this work was carried out.

2.4 Use of direct irradiation for CCD Autoradiography

Although a number of systems have used CCD technology for autoradiography, the work contained in this thesis uses a prototype CCD-based imaging system based on direct irradiation. This has demonstrated good performance under cooled conditions, as described in the literature [79], [71], [70]. In this section a short overview of its performance is presented.

Ott et al [79] tested the system using an X-ray $^{241}$Am source (11.9, 13.9, 15.88, 17.5 and 22keV and $\gamma$-rays of 26, 33 and 59.5 keV) and $\beta$ emitting sources of $^{35}$S, $^{14}$C, $^{18}$F and $^{32}$P. It was found by using calibrated low (0-102.57 nCi/g$^{-1}$) and high (31-883 nCi/g$^{-1}$) activity microscales of $^{14}$C that the system has a linear response up to three orders of magnitude (see figure 2.8) and the minimum detectable level of activity was $\sim$ 25 mBq [79].

![Figure 2.8](image)

**Figure 2.8:** The linearity of the system was checked using the low and high activity microscales of $^{14}$C. The total integration time was 3 hours and 20 minutes. The linearity of the system is extended over three orders of magnitude [70], page 161.

The spatial resolution of the system was measured using a profile through a line source labelled with $^{125}$I and found to be approximately 35 $\mu$m at FWHM. Similar results
were obtained for other $\beta$ sources.

The method used for summing the images is explained in figure 2.9. A threshold was set at 2 or 3 standard deviations away from the mean/mode according to the confidence level that is required. This threshold value was subtracted from all pixels, thus removing background noise. The frames were then binarised and summed in order to form a composite image (figure 2.9).

**Figure 2.9:** The method by MacDonald ([70], page 132) for thresholding: from the histogram of each frame the threshold was set equal to mode $\pm 2\sigma$ or $\pm 3\sigma$. Each frame was binarised using this global threshold. The composite image consisted of the sum of the binarised frames.

An additional investigation was made by MacDonald [70] involving the distribution of the beta tracks recorded for different sources and energies. The size of each beta event could be determined by performing a background subtraction first and then applying 8-connectivity connected component analysis. This allowed small isolated groups of pixels to be associated with individual beta events, assuming that pixel noise was negligible and that the probability of two events clusters overlapping was insignificant. The size distribution of these individual beta events or tracks were expressed as a frequency distribution as shown in figure 2.10. The histograms of the three different $\beta$ sources used are shown in figure 2.10. It was calculated that the mean event size for the cases of $^{35}\text{S}$, $^{18}\text{F}$ and $^{32}\text{P}$ was 3.8, 2.7 and 2.8 pixels respectively [79]. Generally the lower energy isotopes produced larger events. High energy sources such as $^{32}\text{P}$ generated...
Landau-like distributions due to the fact that the high energy particles tend to travel straight through the device, thus leaving a smaller signature, while the lower energy ones follow a more erratic path.

Figure 2.10: Histograms of the recorded event size from a variety of common radioisotopes used in autoradiography. The majority of the events seemed to be concentrated at the 1-2 pixel level ([70], page 141) but with a rapid drop off up to 10 pixels long.

Finally the ultimate test was performed using a $^{14}$C labelled slice of mouse brain tissue. Sections of the same brain tissue were exposed to film for three weeks and then the image was digitised at 25 μm pixel resolution. An adjacent section was imaged by the CCD imaging system and, although the main tissue features were visible after a few hours, the image shown in figure 2.11(b) was acquired after 24 hours exposure. This total exposure time comprised of the sum of 1440 binarised frames of sixty seconds.
2.4. **Use of direct irradiation for CCD Autoradiography**

integration time. Additionally, saturated areas in the film image that are absent in the CCD image indicate the wider dynamic range of the system.

![Image of mouse brain tissue](image)

**Figure 2.11:** Mouse brain tissue of 10 µm thick labelled with $^{14}$C. The left image (a) shows a digitised image after film exposure for 3 weeks. The right image (b) demonstrates that the CCD imaging system can acquire comparable image data in only a fraction of time: 24 hours of exposure, consisting of 1440 frames of 60 seconds [79], [70].

Although direct contact of the sample with the CCD surface could potentially lead to surface contamination, post-acquisition tests showed no significant residual effect.

Furthermore, one of the major attractions of using direct irradiation of the CCD for autoradiography is that the technology is relatively low cost; the hardware cost approximately $£\ 2,000$ compared to $£\ 20,000$ for other digital autoradiographic systems. The ability to operate without cooling would make the system far more user-friendly and allow development of specialist applications such as imaging of radiolabelled live tissue or cell colonies.

In order to achieve this aim, image processing methods will be developed to minimise the effects of thermal noise. To place this approach in perspective, an overview of published work and covering of image processing as applied to autoradiography is presented in
the following section.

2.5 Image Processing in Autoradiography

An entirely separate community of researchers have, over the last decade, been developing machine-vision or image processing methods aimed at improving the quality of autoradiographic data. However, the data used has primarily been obtained by digitisation of conventional film/emulsion based samples. Nevertheless, impressive results have been forthcoming. In the following section some of the notable achievements are received.

At the beginning of the last decade, Yee [104] suggested several image processing methods for better display of total nucleic acids labelled with $^{32}$P and exposed for two weeks. The images used in the work were digitised by using a scanning densitometer rather than a CCD camera. The basic routines that Yee [104] suggested included local and global contrast enhancement with background subtraction, digital smoothing, deblurring, edge sharpening and image averaging. Yee comments that use of contrast enhancement helped to visualise faint areas but also during this process dark outlier specks and heavy mottling were exaggerated. This problem was solved by using median smoothing (figure 2.12). During median smoothing the outlier specks were removed without significantly distorting the image. For better display of weak signals against high background, contrast boosting following by median or mean averaging was proposed. Additionally, sharpening techniques were developed to correct for intrinsic blurring caused by the dimensions of the digitising laser spot in the microdensitometer. Although additional features were recovered in the corrected images, it was mentioned that image enhancement methods are very subjective and therefore easily misused.

Adryan et al also used image processing for analysis of autoradiographic gene expressions profiles [4]. In this case the exposed autoradiographs were scanned at 100 $\mu$m pixel size before processing and digitised. This group used background subtraction by removing the average optical density of a defined blank area. They used sixteen autoradiographic images combined with averaging of reference points considering the local background in order to equalise inconsistent exposure. Then the signal was equalised
Figure 2.12: Electrophoretic separation of nucleic acids labelled with $^{32}$P. (a) original autoradiograph (b) contrast enhancement and (c) five-point median smoothing. ([104], page 787)

to the full dynamic range and background subtraction followed. Although this method has shown increased sensitivity compared with the conventional approach (background subtraction), it causes loss of image information. Additionally, it is probable that this method can create image artefacts.

Marshall & Pickering [73] were concerned with the case of cross-fire in emulsion autoradiography. Cross-fire refers to the phenomenon where activated film grains are displaced from the true source of origin of the electrons. Marshall & Pickering used a simulated autoradiograph of a uniform disc source in order to demonstrate the performance of their method. They produced a mathematical model of the image spread based on calculating the number of grains exposed per unit area. They convolved the image with the proposed model of image spread and then used an inverse Fourier transform method to restore the original image. During the restoration process various cut-off frequencies were used, but the user has to compromise between image sharpness and noise. Comparisons between initial and restored image density versus the radial distance demonstrates good performance of the algorithm. However, the method was found to cause ringing effects [73] and in order to correct this effect image enhance-
ment was used based on a spatially moving average smoothing filter. Despite showing promising results with simulated data, it is worth stating that the method has not been tested on real autoradiographic data.

All the previous methods suggested approaches based on background subtraction, smoothing or image enhancement techniques. However, image enhancement is not an objective tool and so smoothing or background subtraction can result in either image distortion or net information loss. To address this shortcoming new image restoration approaches have been developed. The following reviewed methods are thus based on restoring autoradiographs using objective criteria, applied on digitised images obtained using conventional methods and not having a direct digital acquisition.

A significant contribution from Goyette et al [36], [35] concerning image restoration techniques in autoradiography has been reported, motivated by the need to measure and quantitatively analyse drug delivery in the extracellular space between capillaries. Goyette et al referred to the analysis of the point spread function and its mathematical description. This group identified the difficulties in describing the point spread function due to the random process of emitted radiation (radioactive decay) and its interactions (reflection, absorption) in tissue. Additionally they explained the noise present due to the size and distribution of the grains of the film. The approach developed was initially using an adaptive noise smoothing filter that uses variant mean and variance based on local spatial statistics in order to correct for film grain noise. In regularisation techniques there is always the task of preserving the fidelity of the data by also achieving smoothing or other desirable properties of the degraded image. A parameter is usually included in the expressions of the cost function that controls the strength of its part. Goyette et al in [36] suggested an adaptive iterative regularisation algorithm, where this parameter was estimated for each iteration, at each step and therefore it was not necessary to define it in advance. They checked their approach using $^{51}$Cr microspheres and found a relative improvement in the spatial resolution at FWHM of 27 % in comparison with the standard approach (non-adaptive iterative algorithm) that showed an improvement of 23.58 %. The noise variance of the background became half, which proves the level of smoothing. Because in the previous work they did not consider the remaining additive noise as signal-dependent, their work continued and in
2.5. *Image Processing in Autoradiography*

[35] another approach was presented. They modelled the point spread function with an inverse square relationship and the film grain noise with an additive signal-dependent noise source. Another addition to the previously developed adaptive iterative technique was a weighting matrix that represented the inverse of the local variance at different location. The results have shown an impressive relative improvement of 43 % in spatial resolution at FWHM, measured using radiolabelled spheres acting as idealised point sources. Therefore, the spatially adaptive (weighted) iterative restoration algorithm have presented excellent performance. However, several assumptions were made such as that the images were in the linear part of the D-logE curve $^4$ [100].

During the last few years there have been several methods of implementing image processing and pattern recognition algorithms for restoring severely blurred autoradiographic images or towards a more accurate quantitative analysis. For example, Zhao *et al* [105] have used a contour-tracing tool to extract film background in combination with a Bayes classifier in order to determine more accurately an optimal threshold between active and non-active pixels having minimised the risk of misclassification. Similar problems such as impulse noise and blurring can be found in the literature. For example, Bedini *et al* [9] have developed edge-preserving restoration algorithms for low intensity fluorescence images obtained with wide-field microscopy. They have compared results in both simulated and real images using a standard regularisation algorithm with simulated annealing and maximum entropy and using edge-preserving stabilisers. Their proposed method of edge-preserving performed very well in microscopy images by showing a good noise suppression and defining correctly the cell edges present. The moderate amount of blurring introduced by the microscope has been partially removed by the proposed algorithm even without introducing the point spread function.

Similar approaches have been developed for astrophysical images where the same problems as seen as with autoradiographic images: edge preserving [14] and spatially adaptive iterative algorithms for restoration [51] have been utilised in order to ameliorate the quality of the image. Several groups have worked in restoring images contaminated with various types of noise (Gaussian, impulse, etc.). The presence of outliers in the noise distribution of real images is unavoidable and therefore most techniques

$^4$Optical density $D$ versus the log of exposure $E$. 
Chapter 2. Literature Survey

Figure 2.13: Results from the weighted regularised iterative algorithm applied to a digitised autoradiograph ([35], page 819).

(a) Original (I) and restored (II) image after applying regularised weighted iterative algorithm.

(b) Profiles of a line passing through three spheres.

have utilised an outlier detector before applying any algorithm to the data. Xu & Lai [103] followed this approach creating an outlier detector, where the intensity value of its pixel was compared with each of the neighbouring pixels. Then they used a "minimum-maximum estimator" [103], in which the intensity value of the outlier was replaced by the mean between the remaining maximum and minimum intensity values. Of course in this method there is the empirical problem of choosing the threshold that defines
the outlier value. Results were presented concerning different thresholds and also comparing this method with median filtering (different sizes). Using mean square error as an assessment criterion demonstrated improved performance of the suggested method compared with median filtering. This approach could be used as a pre-processing technique combined with image restoration algorithms but only in the case where the outlier intensity values are not likely to be confused with real data. A similar approach was presented by Han & Lin [38] in order to remove impulse noise from highly corrupted images.

One can conclude thus, that over the last decade there has been noticeable step towards techniques that use objective criteria for image improvement due to an increase in the demand for more accurate quantitative analysis of the images. Regularisation techniques have been preferred and specifically image restoration methods using either simulated annealing or maximum entropy for optimisation. It is therefore proposed that using image restoration based on simulated annealing would be an appropriate strategy for examining noise corruption in CCD autoradiography imaging. Because this work will address standard image restoration with simulated annealing, a review of image restoration techniques is included in the next section.

2.6 Image restoration approaches.

Image restoration is an area of engineering where the original image/scene is recovered from the degraded, observed one. The methods used to solve this problem are focused on modelling the degradation processes, usually blurring and noise, and application of inverse procedures to recover the original image. Areas of application of digital image restoration techniques are found in astronomical photography, where pictures are degraded due to refraction in the atmosphere or motion blur, in autoradiography, in image and video coding, in restoring aging and deteriorated films and several inspection procedures such as blurry X-ray images of aircraft wings [7].

In mathematical terms, the problem of restoration can be expressed as [3]:

\[ g = D \ast f + n \]  

(2.1)
where $g$ is an $M \times N$ matrix representing the observed image, $f$ is the original image of the same dimensions, $D$ is a spatially invariant blurring kernel and $n$ represents a signal independent additive noise. In the above expression it was assumed a linear invariant point spread function but there are problems where the point spread function can be spatially varying.

The result of an image restoration technique depends on the prior knowledge of the identification, estimation and restoration processes. These processes are concerned with the knowledge of the degradations occurred in the image, the original image and type of noise [7], [34]. The different approaches for solving the restoration problem can be classified according to the image model or the way the problem is approached.

A well defined classification between different image restoration approaches is between deterministic and stochastic algorithms. Deterministic algorithms have good knowledge of the degradation processes and the original image is usually obtained by applying transformations that are inverse to the degradation [92]. Stochastic algorithms have partial knowledge of the degradation such as the point spread function or noise and the best quality of restoration is obtained by applying some stochastic criteria such as the least squares method [92], Metropolis dynamics [74] or the Gibbs sampler [33]. For example, during the least squares solution the norm-square of the residual $J(f)$ is minimised as shown in equation (2.2) [72]:

$$J(f) = \| g - Df \|^2$$

(2.2)

where $g$ and $f$ are the observed and the original image respectively and $D$ is the blur operator.

According to the type of algorithm the different methods can be classified as iterative or recursive. During the iterative methods there is no explicit need for having inverse transformations of the degradation and the restoration progress can be monitored and stopped when necessary [7]. One benefit of iterative algorithms is also that they can restore images suffering from different types of degradation such as linear, non-linear, spatial invariant, spatial variant etc. Iterative algorithms can be represented as:

$$f_{k+1} = f_k + \beta(Df_{k+1} - Df_k)$$

(2.3)
2.6. Image restoration approaches.

where $\beta$ is just a varying parameter and $k$ is an indicator of the iteration number [49]. Recursive algorithms permit spatial adaptivity and require less storage space than iterative techniques. The other advantage of recursive methods is that they do not require inversion of a matrix such as the inverse of $D$. Most variations of recursive methods based on Kalman filtering [85], [101].

Additionally algorithms can be classified as linear and non-linear. When the degradation can be modelled by a linear operator then the solution, which will be computationally less expensive than in the non-linear case, can be the inverse of the matrix representing the degradation (inverse filter or Wiener filter). For the case of inverse filtering the solution is described by:

$$f = D^{-1}g - D^{-1}n$$

where $D^{-1}$ is the inverse matrix of $D$. However, this approach has the disadvantage of being sensitive to noise and that it is often computationally difficult to compute the matrix $D^{-1}$ because of its large size [83].

In the case of non-linear models, the solution is computationally expensive for more complicated algorithms such as simulated annealing and maximum entropy algorithms [72]. There are several definitions for entropy but the most commonly used is by Frieden [32], which defines the entropy $S_f$ as $S_f = -\sum_{\text{pixels}} O \ln O$, where $O$ is the original image. For example, in the maximum entropy algorithm the effort is focused in maximising the entropy that enforces the smoothness of the restored image. A constant-value image has maximum entropy. Expressing this principle with mathematical terms the sum of the image and noise entropy that should be maximised is given by:

$$J_{ME}(f) = -\sum_{i} f(i) \ln f(i) - \sum_{i} n(i) \ln n(i)$$

Both maximum entropy and simulated annealing are known in literature as being computationally complex and slow respectively.

In regularized image restoration, the initial assumption is that the true image is reasonably smooth, therefore a function representing extra 'roughness' is imposed in the
expression that is minimised. A regularized solution is then found by minimising an expression such as:

\[ \phi(\hat{f}) = \| g - D\hat{f} \| + \lambda \| L\hat{f} \|^2 \]  

(2.6)

where \( \hat{f} \) is the estimated true image, \( L \) is the regularization operator and \( \lambda \) is a scalar that controls between the fidelity of the data and the degree of smoothness [85]. The regularization algorithms can be space-variant or space-invariant. A usual approach for the latter case is by minimising the sample mean-square error that can be computed as:

\[ T_s(\lambda) = \frac{1}{N} \| g - Df(\lambda) \|^2 \]  

(2.7)

As mentioned before, the blurring process of the image might be mathematically and physically modelled correctly and details of this process may be known a priori. Usual cases of known blurring models are:

- **Motion Blur**: common problem during to movement of the camera, usually modelled by averaging the neighbouring pixels [85].

  \[
  d(x, y, L, \phi) = \begin{cases} 
  \frac{1}{L} & \text{if } \sqrt{x^2 + y^2} \text{ and } y/x = -\tan \phi, \\
  0 & \text{elsewhere}
  \end{cases}
  \]  

(2.8)

where \( L = uT \) is the length of motion during exposure if \( u \) is the velocity and \( T \) the time of exposure and \( \phi \) is the angle with the horizontal axis.

- **Atmospheric Turbulence Blur**: problem often appears in remote sensing and aerial imaging. The blur can be modelled by a Gaussian point spread function [7].

  \[
  d(x, y) = Ke^{-\frac{x^2 + y^2}{2\sigma^2}}
  \]  

(2.9)

where \( K \) is a normalising constant and \( \sigma^2 \) is the variance of the distribution representing the severity of the blurring.

- **Uniform out-of Focus Blur**: this type of blurring can be found in many imaging systems due to de-focusing. This process can be modelled by a uniform intensity
distribution within a circular disk \[7\].

\[
d(x, y) = \begin{cases} 
\frac{1}{\pi R^2} & : \text{if } \sqrt{x^2 + y^2} \leq R \\
0 & : \text{otherwise}
\end{cases}
\] (2.10)

where \( R \) is the radius of the disk.

- **Uniform 2D Blur**: the de-focusing in this case is more severe and it is commonly used in research simulations \[7\].

\[
d(x, y, L) = \begin{cases} 
\frac{1}{(L)^2} & : \text{if } -\frac{L}{2} \leq x, y \leq \frac{L}{2} \\
0 & : \text{otherwise}
\end{cases}
\] (2.11)

The image restoration problem in biomedical imaging and especially in autoradiography does not include a well-defined modelling of the blurring as in the above cases. Approaches to the solution based on Bayes formulation and theory \[64\] use stochastic algorithms such as simulated annealing.

### 2.7 Discussion

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**Table 2.3**: Comparison between the basic characteristic of conventional autoradiography (AR) and digital systems.

In summary, in this chapter the main disadvantages of the conventional autoradiographic methods have been discussed and the motivation for moving to digital imaging systems were presented. Most of the digital systems have fulfilled the requirements (of real-time imaging, linear response over wide dynamic range and good detection
efficiency), nevertheless, the spatial resolution of dedicated digital imaging technology remains modest compared to film autoradiographs.

Table 2.3 summarises the basic characteristics of these two types of autoradiography. Recent approaches have focused in applying image processing methods in digitised autoradiographs acquired by conventional systems. The results have shown significant improvements in image quality. In particularly those techniques using image restoration algorithms can provide useful information for quantitative imaging. CCDs are among the most promising technology for use in autoradiography. A summary of the performance, under cooled conditions, of one such system has been presented. In the following chapter, the specific problems associated with using this system at room temperature are presented.
Chapter 3

Image Acquisition

The performance of direct irradiation CCD imaging in autoradiography was described in the previous chapter. The motivation for moving to room temperature is presented, along with the problems incumbent with such operating conditions. The CCD-based imaging system used for data acquisition is presented in more detail in this chapter. Additionally, an investigation is included concerning the issues from moving from cooled to room temperature conditions. Finally, an exemplar set of experimental test data acquired by the imaging system at room temperature are presented in section 3.3.

3.1 Experimental Arrangement.

The experimental arrangement used in this work is essentially the same as described by MacDonald et al [71].

3.1.1 CCD sensor

The CCD device is a large area inverted-mode EEV 05-20 CCD mounted on a copper cold finger. It is enclosed in an aluminium cryostat, evacuated when required, to prevent condensation when operating under cooled conditions. The active area of the CCD is 17.3 mm × 25.0 mm representing 1152 × 826 pixels of 22.5 μm × 22.5 μm. In figure 3.1 the CCD sensor inside the enclosure well (cryostat) is displayed.
The cryostat or the cryogenic chamber provides a light-tight enclosure to house the CCD and a buffer board used to amplify the small analogue input voltage signals from the CCD. The CCD is mounted on an insulated cold finger which can be inserted in liquid nitrogen or subjected to thermostatically controlled coolant (see schematic diagram in Appendix A).

### 3.1.2 Read out electronics

Apart from the CCD sensor, the cold finger and the enclosure well, the hardware is composed of electronics that are responsible for the data acquisition: a timing box, a readout box and a PC (figure 3.2 shows the experimental arrangement in the laboratory).

During integration time the read out clocks are halted. Once an integration period has elapsed, the grab signal becomes low and the pixel clocks restart. The grab line is used to initiate acquisition in the data acquisition card, so data is continuously acquired while the grab line remains low. The sampling of the video data is externally synchronised by using a pixel clock control line taken from the electronics read out box. This process continues until the grab line goes high, initiating a new integration period. During readout period, pixel data is clocked out at a rate of one pixel per microsecond.
3.2. Data acquisition issues at room temperature

Having summarised the performance of the system under cooled conditions, it is pertinent to discuss the potential problem and issues involved in moving to room temperature operation. The major issue to be addressed is dark current correction. This is the major source of image corruption when using a CCD for long integration times. There are many different types of dark current such as depletion dark current, surface dark

Figure 3.2: The whole experimental arrangement of the CCD prototype imaging system in the laboratory. The equipment consists of the CCD sensor, cryostat, cold finger, read out electronics, PC, vacuum pump and a bath circulator.

(see figure 3.3 for a schematic diagram). Read out of each frame takes approximately 1 second ($\sim 10^6$ pixels $\times$ 1 $\mu$sec for each is $\sim$ 1 sec). The CCD is serially read out at a clock speed of 1 MHz, in full-frame mode, using an EEV-GEC CDB01-1 driver assembly. Full frame mode means that the whole area of the device is sensitive to radiation exposure [69]. The characteristics of the system are summarised in Appendix B.

3.2 Data acquisition issues at room temperature

Having summarised the performance of the system under cooled conditions, it is pertinent to discuss the potential problem and issues involved in moving to room temperature operation. The major issue to be addressed is dark current correction. This is the major source of image corruption when using a CCD for long integration times. There are many different types of dark current such as depletion dark current, surface dark
Chapter 3. Image Acquisition

Figure 3.3: Schematic representation of the imaging system. The CCD sends the signal to the read out box. The integration time is set in the timing box. The pixel clock pulses constantly data to the acquisition card. The video signal contains the data from the CCD. Grab shows the voltage change during integration and read out. Read out at a clock speed of 1 MHz takes approximately 1 second per frame until the read out is completed.

current, diffusion dark current or substrate dark current, originating at the corresponding levels of the CCD device. The main noise source for room temperature acquisition is the surface dark current with smaller contribution of the other types.

Room temperature CCD beta imaging presents a number of problems due to dark current, which is the principal noise source [43] in slow scan mode at 295K: small (few °C) drifts in ambient conditions can produce significant changes (up to 10 %) in pixel dark current because of the exponential dependence on thermal bond-breaking in silicon (equation 3.1). Step changes in dark current/temperature can be easily corrected, but pixel dark current variability across the device (arising from discrepancies in pixel dimensions and impurity levels introduced in manufacture) are exacerbated by long
3.2. Data acquisition issues at room temperature

(~10 seconds) exposure at 295 K: complete lines and groups of pixels may reach saturation early during an exposure or give a highly irregular background upon which beta particle signatures are superimposed.

The number of available charge carriers, \( n \), in a semiconductor varies as

\[
n \propto e^{-(E_g/(2kT))}
\]

where \( E_g \) is the energy gap (1.1 eV in silicon), and \( kT \) represents the thermal energy of the charge carriers. Hence for ultimate noise reduction, cooling is often used which reduces dark current by about a factor of ten \([102]\) between liquid nitrogen and room temperature.

![Figure 3.4: Mean pixel intensity as a function of integration time. At room temperature, pixels reach saturation in a few minutes (~ 5 min), whilst at cooled conditions even after 45 min exposure, saturation was not reached.](image)

For room temperature operation, inverted-mode structures can be used to successfully reduce the dominant source of dark noise arising from Si-SiO\(_2\) surface states by around two orders of magnitude \([79]\), \([70]\), albeit with some reduction in full well capacity\(^1\). Nonetheless, continuous dark current accumulation using such a device at room temperature can cause pixel saturation in just a few minutes (figure 3.4, \([102]\)). Given that the typical full well capacity of an inverted-mode device is \(\sim 10^5\) electrons and given

\(^1\)Well capacity is a measure of how many electrons a pixel can hold \([43]\)
that it is expected typically $\sim 10^3 - \sim 10^4$ electrons are generated per detected beta [79],
then clearly the device must be used with an integration time significantly shorter than
the average pixel saturation point. Additionally, because there is no shutter to prevent
exposure during readout, the CCD is still sensitive to the photons/particles resulting
in an effect known as image smearing. In order to reduce the effects of smearing the
integration time should be much longer than readout time. As mentioned above longer
integration times can lead to pixel saturation, so a solution would be to increase the
readout rate. However, an increase of the readout rate can scale the noise by a factor
approximately equal to the root of the readout frequency [69].

Figure 3.5 shows accumulated dark current histograms of an entire image frame at
both room temperature ($\sim 25 \, ^\circ C$) and under cooled conditions ($\sim -55 \, ^\circ C$) when in-
tegrated for 10 seconds and read out at a clock speed of 1 MHz. Clearly, when the
device is cooled, the histogram exhibits a sharp normal-like distribution originated by
the Poisson random process underlying dark current generation [43]. However, at room

(a) Cooled conditions.  
(b) Room temperature.

Figure 3.5: Histograms of pixel intensity values of a single blank frame using an integration time
of 10 seconds. Under cooled conditions (left), the distribution is dominated by electronic readout
noise, whereas the distorted distribution at room temperature (right) shows the effects of pixel
variations in accumulated dark current over the entire device. Note that this is aggravated by using
long integration times, and by using a device with large numbers of defects.
temperature, and with longer integration times, the distribution is significantly distorted. This is attributed to enhanced inter-pixel variations in the accumulated dark current.

### 3.3 Description of Real Test Data and Image Acquisition

Test data for use during the methodological development (see chapter 4) were obtained as follows while evaluating the different algorithms: eighteen consecutive image frames (12-bit format) were acquired with the prototype CCD-based imaging system [70] at room temperature ($\approx 22^\circ C$) with an integration time of 10 seconds per frame. The integration time was chosen to be 10 seconds considering the plot of figure 3.4 and the level of activity of the samples, which was quite high at approximately 162 nCi ($\sim 6kBq$). The sample to be imaged consisted of two microfibres of 20 $\mu$m diameter held in a ‘T’ shape, and sealed in approximately 100 $\mu$m thick cling film. The fibres were labelled with $^{18}$F positron emitter ($T_{1/2} = 109.7$ minutes, $E_{\text{max}} = 1.65$ MeV [99]) and placed in direct contact with the CCD sensor's surface. Therefore, no collimation or focusing was used. For computational purposes, the images were cropped around the area of interest so each image consisted of $501 \times 251$ pixels. Figure 3.6 shows a raw single image frame and 3.7 includes the sum image of the eighteen frames. The images were converted from 12-bit to 8-bit for display purposes using:

$$X_{8\text{bit}} = \frac{X_{12\text{bit}} - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}} \times 255 \quad (3.2)$$

where $X_{12\text{bit}}$ is the matrix with the 12-bit data, $X_{\text{min}}$ and $X_{\text{max}}$ are the minimum and the maximum intensity values of each frame respectively and $X_{8\text{bit}}$ is the image frame with the 8-bit data.

As can be observed comparing the images in figure 3.6a and 3.7a, a single image represent a very low statistics image, whilst the sum image has better signal to noise ratio. These experimental data were used for the subsequent development of the image correction and image restoration methods described in chapters 4 and 5.
Figure 3.6: A single 8-bit image of one of the 18 consecutive test images and its profile along column 130.

Figure 3.7: A sum initial image of the 18 frames and the corresponding profile of column 130.
Chapter 4

Assessing the thermal characteristics of the system

As mentioned in chapter 2, the conventional systems (films or emulsions) have demonstrated non-linear response and they have limited dynamic range [100]. Therefore, the developed digital imaging systems should demonstrate, apart from good spatial resolution, a linear response and the ability to image over a wide dynamic range. Most of the digital systems presented in the literature [80], [48] have always been assessed in terms of good performance in the following three areas: linearity, dynamic range and spatial resolution. The spatial resolution of the system and ways to improve it for high flux samples will be discussed later on this thesis. In this chapter the remaining two criteria will be assessed and results will be presented.

In the first part of this chapter the sensitivity and linearity of the system will be investigated over a range of temperatures between 5 °C and 36 °C and different integration times. The same system had shown good performance concerning the above two factors when operating under cooled conditions [70], [71], which was also confirmed during this work. Thus, similar experiments should be performed in order to assess these characteristics of the system at elevated temperature. The sample used for assessing the hardware performance was 14C calibrated microscales. A fixed pattern noise removal method was developed and used for correcting these images.

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Chapter 4. Assessing the thermal characteristics of the system

The fixed pattern noise removal method requires a library of blank frames for every temperature and integration time, which has to be obtained after each image acquisition. However, this process, apart from being time consuming, can not always guarantee that the blank scan acquisition is repeated under the same conditions as the image acquisition. For this reason, an attempt to overcome these problems and create a model that can be used to correct the dark current offsets (mode values). The thermally induced changes in standard deviation of the normal-like distributions describing the noise of each pixel are discussed in the second part of this chapter.

4.1 Description of the microscales and image acquisition

The linearity of the system was investigated using calibrated $^{14}$C microscales. These microscales consisted of a labelled polymer arranged in order of increasing activity and separated by inactive areas. The specific activity of the microscales varied between 31 nCi/g and 883 nCi/g (the whole range is shown in figure 4.1). The microscales were made from tissue equivalent material of 20 $\mu$m sections of rat brain grey matter impregnated in vivo. The actual activity can be found by multiplying specific activity of each band with its mass. The microscales were weighted on a set of Sartorius Basic BA61 scales and the average mass was $6.45 \times 10^{-4}$ g. Therefore the range of activity is between 0.019 nCi and 0.57 nCi. One strip of the microscales was attached to a cut microscopic slide as shown in figure 4.1 using the instructions given by the manufacturer. The microscopic slide was then placed directly onto the CCD surface without any other intermediate covering material. Figure 4.2 shows the exact arrangement of the microscales on the CCD surface.

After placing the microscales onto the CCD surface, the cryostat was sealed and evacuated down to a vacuum of $10^{-1}$ torr. Vacuum was applied in order to prevent water condensation when operating under cooled conditions. Before image acquisition the cryostat system was set to the required temperature and left for one hour to ensure thermal equilibrium had been achieved. During this, the CCD read out electronics were switched on and left for two hours [78] to reach its normal quiescent operating temperature. When operating under cooled conditions, liquid nitrogen was added every
4.1. Description of the microscales and image acquisition

Figure 4.1: The microscales as delivered (left), the strip that was attached on the microscopic slide (middle) and a 10p coin (right) for comparison of the size. Tape was attached on the back of the slide in order to be able to handle the slide on the CCD device without contaminating its surface.

Figure 4.2: The microscopic slide was placed directly on the CCD surface without using any extra collimation or focusing. The whole system was under vacuum in order to prevent water condensation.
thirty minutes in order to maintain stable temperature.

Experimental frames were acquired using the microscales at different integration times: 5 s, 10 s, 15 s and 20 s and for six temperature values, creating 24 different data sets. Using the same combination of temperature and integration time, 500 blank frames were also acquired in order to yield information on the individual pixel behaviour.

The temperature was controlled by a water bath circulator (except for the investigation using liquid nitrogen described in the following section). The recorded temperature values: 5 °C, 10 °C, 16 °C, 22 °C, 28 °C and 36 °C represent the temperature of the water coolant circulated around the cold finger. The circulator and the arrangement of the pipes are shown in figure 3.2 in chapter 3. There was no measurement of the actual temperature of the CCD device, but since there was a relative study on the temperature change this was not a problem. However, it is reasonable to assume that the true CCD temperature was within a few degrees of the coolant temperature [70].

4.2 Image acquisition of the microscales at liquid nitrogen temperature

In order to confirm the behaviour of the system as reported by MacDonald [70], an investigation concerning the linearity of the system was repeated. The integration time was set to 15 seconds and 608 frames were acquired. The dimensions of each image were cropped 328 x 1152. Each image was corrected (see figure 4.3) according to the method referred in [79] by Ott et al: the threshold of each frame was set at the intensity value found from the mode pixel intensity value of the background peak of the frame plus two standard deviations. The value of each pixel above the threshold was then set to one, with all other pixels set to zero. The resulting binary images were finally summed to create a composite image.

Regions of interest were placed over each band position to determine the average ADC value per pixel at each band. This was plotted against specific activity, so the investigation would be representative of the results obtained using thin (~ 20 μm) tissue. The plot displayed in figure 4.4 demonstrates that there was linearity of the system over the
4.2. Image acquisition of the microscales at liquid nitrogen temperature

Figure 4.3: The corrected image of the microscales obtained at liquid nitrogen temperature. The initial composite image consisted of 608 frames of 15 seconds integration time each. The image is displayed in 8-bit format.

whole dynamic range. The fitted line was found using the least squares method [95], where a simple linear equation of the form:

\[ y = ax + b \]  

(4.1)

where \( a \) was the slope and \( b \) was the offset was fitted. The \( x \) and \( y \) variables in this case were the activity (nCi/g) and the ADC/pixel, respectively. The value of \( R^2 \) displayed on the plot represents the quality of the fit. The parameter values were calculated so that the vertical distances of the data points from the fitted line were minimum.

Although a threshold of \( 2\sigma \) was selected, repeating the method in [79], it was also important to examine the effect of varying this threshold. This investigation was performed concerning the effect of different threshold levels in the number of events recorded. The number of events were calculated using connected component analysis (8-connectivity) [92] to determine the number of beta events per frame (as described in section 2.4). A single frame was selected and the threshold was systematically increased and the number of events were determined at each threshold. The two plots are shown in figure 4.5. When the threshold was set very low, then it would appear that pixels with just dark current were classified as data pixels and therefore there was an artificial increase in the number of events recorded. Applying a very high threshold means that many
Figure 4.4: Linearity plot obtained by using $^{14}$C microscales of different specific activity levels: 31 nCi/g, 60 nCi/g, 122 nCi/g, 235 nCi/g, 356 nCi/g, 530 nCi/g, 708 nCi/g and 888 nCi/g distributed at 8 different bands. Estimates of absolute activity for 20 $\mu$m tissue equivalent thickness can be obtained by multiplying by $6.45 \times 10^{-4}$ g. The abscissa is the specific activity in (nCi/g) and the ordinate represents the ADC level per pixel at each band. A linear fitting was performed using least squares and the $R^2$ represents the quality of fit. The dots represent the experimental data and the solid line the fitting. The error in each measurement for each pixel was ±0.5 ADC units, therefore following error propagation analysis [54] the error in each of the displayed points is too small for display.

valid beta events were classified as noise and therefore the actual number of events shown was artificially reduced.

4.3 Thermal behaviour of the CCD imaging system.

Previously (section 3.2) it has been shown that the system was very sensitive to small drifts in ambient temperature. In order to investigate these effects further, and as part of an attempt to correct for these changes, a series of experiments was conducted aimed at systematically examining the effects of temperature change in the range 5 °C - 36 °C. In order to investigate also the effects of the integration time, four different settings were
4.3. Thermal behaviour of the CCD imaging system.

Figure 4.5: The two plots show the number of events detected for one frame while varying the threshold. The number of events was calculated performing connected component analysis with connectivity 8. The right diagram shows the lower part of the left plot better by cropping the large values.

investigated between 5 seconds, representing the lower limit (below which image smear starts to become noticeable) and 20 seconds representing the corresponding higher limit (above which large numbers of pixels become saturated).

Five hundred blank frames were additionally acquired for each set of parameters and the mean and the standard deviations of each individual pixel were determined in order to make a fixed pattern noise correction. Fixed pattern noise refers to the inter-pixel variability in sensitivity or non-uniformity [43]. This is a standard correction technique for CCD imaging. However, in this application (using long integration times to image rare or sparse events) there is significant fluctuation in interframe pixel dark current bias, attributed to small changes in temperature. These fluctuations were experimentally observed to be of similar magnitude to the size of the signals induced by beta ionisation in the device. A fixed pattern noise correction method was therefore developed where the mean value of each pixel $\mu_{ij}$ is scaled to account for these changes. It was also observed that the ensemble pixel intensity distribution scales with temperature. Using the ratio of the mode values as a measure of relative temperature change,
between the image \((\hat{G})\) and the blank scan acquisition \((\hat{B})\) applied to each frame then the thermally corrected pixel bias is estimated to be:

\[
\mu_{ij} = \hat{G} - \hat{B}
\]

This method is based on the assumption of linear relationship between the two acquisitions for small temperature drifts. The mode instead of the mean was used for each pixel due to the presence of saturated pixels that tend to move the mean towards higher intensity values. Additionally an extra post-processing step was followed, as described below.

The post-processing step involved binarisation of data corrected for fixed pattern noise. A composite image was formed by adding all corrected binary frames. Because broken or saturated pixels that have not been corrected with the fixed pattern noise method would always be classified as data, their intensity value was always set to one. Using the measurements obtained at liquid nitrogen temperature as 'ground truth', it was observed that the maximum number of events associated with pixel in the composite image was equal to 10 implying that most pixels only rarely contained data. Thus, all composite image pixels that contained greater than 10 ADC units were set to zero because from the data collected at liquid nitrogen temperature, such pixel data has been adequately corrected. The number of frames acquired for each integration time are shown in table 4.1. The total exposure time remained the same (9110 seconds) for all integration times and temperatures also it was the same total exposure time as when imaging the microscales under cooled conditions (previous section).

For displaying purposes, the initial frame and the corrected frame of the 15 seconds integration time obtained at 10 °C are shown in figure 4.6. In the initial composite image the regions of activity were completely hidden due to high level of dark current. While performing fixed pattern noise removal the main dark current offsets were removed and using the post-processing procedure, the remaining saturated uncorrected pixels were also corrected, so the result was to observe clearly six out of eight bands.

The region of each band was defined from the corrected composite image acquired at 10 °C with 15 seconds integration time, as this represents the highest quality image over all data sets. Then the total sum of ADC units was found for each band and the
4.3. Thermal behaviour of the CCD imaging system.

<table>
<thead>
<tr>
<th>Integration time (seconds)</th>
<th>Number of acquired frames</th>
</tr>
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<tr>
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<td>1822</td>
</tr>
<tr>
<td>10</td>
<td>911</td>
</tr>
<tr>
<td>15</td>
<td>608</td>
</tr>
<tr>
<td>20</td>
<td>456</td>
</tr>
</tbody>
</table>

Table 4.1: The total exposure time remained the same for all combinations of integration time and temperatures. This table shows the number of frames for each integration time that forms a total exposure of 9110 seconds.

Figure 4.6: The initial sum image of 608 frames acquired with 15 seconds integration time at temperature 10 °C in contrast with the composite image of individually corrected frames using fixed pattern noise removal and post-processing. The results are shown in negative polarity for better visualisation.

average ADC level per pixel was plotted against activity. Four integration times of six temperatures resulted in 24 different combinations and therefore 24 data sets. The plots that demonstrate the linearity of the imaging system at those high temperatures are shown in figures 4.7, 4.8, 4.9 and 4.10 for the four integration times respectively. The parameter values of the fitted line are shown in table 4.2, including the $R^2$ value.

Conclusions can be deduced from the values of the slope $(a)$ and the offset $(b)$ of the
Chapter 4. Assessing the thermal characteristics of the system

Figure 4.7: Linearity plots for integration time of 5 sec for 1822 frames and six different temperatures. The error for each plotted point was estimated as too small to be plotted.
Figure 4.8: Linearity plots for integration time of 10 sec for 911 frames and six different temperatures. The error for each plotted point was estimated as too small to be plotted.
Chapter 4. Assessing the thermal characteristics of the system

Figure 4.9: Linearity plots for integration time of 15 sec for 608 frames and five different temperatures. The error for each plotted point was estimated as too small to be plotted.
4.3. Thermal behaviour of the CCD imaging system.

Figure 4.10: Linearity plots for integration time of 20 sec for 456 frames and six different temperatures. The error for each plotted point was estimated as too small to be plotted.
Chapter 4. Assessing the thermal characteristics of the system

<table>
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<tr>
<td>T ${}^\circ C$</td>
<td>ADC/pixel nCi/g</td>
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Table 4.2: The parameters $\alpha$ and $b$ of the least squares fitting ($y = \alpha x + b$) for the different data sets. The $R^2$ value represent the quality of the fitting.

fitted lines. From the plots shown in figures 4.7, 4.8, 4.9 and 4.10 it was found that the imaging system demonstrated linear behaviour for the different temperature values and the different integration times as indicated by the good levels of the $R^2$ values, all of them being higher than 0.98. The physical meaning of slope $\alpha$ indicates the sensitivity of the system. When the slope is large, then the same relative change in activity will result in a greater change in the ADC level per pixel and therefore the change in activity is detected more easily. Therefore, it was reasonable to observe an increase in the slope between 5 seconds and 20 seconds integration time since the exposure per frame was greater and thus the recorded ADC level was higher and the effects of image smear, caused during read out have been reduced.
Additionally, it was also expected to observe that at lower temperature levels the slope would be greater than at higher temperatures due to the fact that at high temperatures the level of noise is high and therefore the signal to noise ratio is poor. This indeed was observed. However, at high temperatures many pixels are close to saturation and therefore the extra charge that can be deposited in the pixel is limited. When the pixel reaches saturation, no extra charge and no extra information can be stored, whilst other problems—phenomena like over-flowing of charge to neighbouring pixels starting to occur.

Parameter $b$ represents an intrinsic lower limit in terms of sensitivity, estimated to be the predicted ADC level recorded if there was no sample present and thus no activity. Therefore, high values of $b$ could be interpreted as high noise levels. The expected performance was that $b$ would be increasing with temperature, which can be shown in table 4.2. It was also demonstrated that for short integration times, eg observed in the values of 5 seconds, the value for $b$ was greater than for the other integration times, which can be explained by the image smearing during readout of the device. The smearing occurs because the device is still sensitive to radiation whilst being read out [43]. This could be reduced by increasing read out speed [69]. However, increasing the readout frequency results in changes in the noise level proportional to the square root of the readout frequency [69].

### 4.4 A simple empirical model to correct for thermal dark current fluctuations.

#### 4.4.1 Thermal variation of $Mode_{ij}$

The correction method of fixed pattern noise removal requires a reference set of blank frames. The reason for this extra set is because the behaviour and therefore the distribution of noise values for each pixel has to be estimated. Ideally this set must be acquired under the same conditions (integration time and temperature) as the real data set. Unfortunately, changes in ambient temperature can mean this is not always possible. Therefore, it is necessary to make an attempt to create a model that will correct
Figure 4.11: The microscales images corrected with fixed pattern noise removal. It is also confirmed by visual inspection that the image acquired at 36 °C is more noisy than the one at 5 °C. The results are shown in negative polarity for better visualisation.
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Figure 4.12: Corrected microscales frames obtained with 10 seconds integration. Each image consists of 911 individually corrected frames. The results are shown in negative polarity for better visualisation.
Chapter 4. Assessing the thermal characteristics of the system

Figure 4.13: The 15 seconds integration time is one of the suggested imaging times because it is much greater than the read out time but still not long enough for the majority of pixels to reach saturation. The results are shown in negative polarity for better visualisation.
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Figure 4.14: The images of the $^{14}$C microscales corrected with fixed pattern noise removal. The threshold during post-processing remained the same. The increase in the dark current level is indicated by a rise in the number of pixels with high intensity values. The results are shown in negative polarity for better visualisation.
the dark current offsets for different temperatures.

For this purpose, 500 frames of different temperatures were obtained using 15 seconds integration time for each frame. The reason for choosing 15 seconds integration time for this investigation was that the system had always demonstrated a stable and reliable behaviour for such an integration time. Longer integration times resulted in a large number of saturated pixels, whilst shorter integration times resulted in an increase of the image smear. Additionally, ten randomly chosen pixels, including one that demonstrated a saturated performance, were followed during the different data sets. The mode intensity value of each frame was plotted versus the number of frame for each temperature and is shown in figure 4.15.

![Figure 4.15: The mode dark current level (ADC units) calculated for each frame is plotted against the frame number for 15 seconds integration time. The different colours indicate different temperatures.](image)

The demand for creating a theoretical model to define the dark current level in each pixel became more obvious observing the variation among frames with time. Initially, the relative change of the average mode dark current level over the 500 frames had to be investigated for certain temperatures. The mode was chosen instead of the mean in order to have a more robust estimator because of the long tails of the dark current distribution due to the irregular high intensity or broken pixels.
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Temperature measurements, where quoted, refer to the thermostatically controlled temperature of the circulating water. This means there will be a temperature difference, \( \Delta T \), between the CCD sensor and that of the water circulating around the cold finger due to imperfect thermal coupling across different surfaces. Thus an empirically derived formula for dark current variation with temperature was found to give a better fit, compared to the exponential behaviour previously cited in equation 3.1. The deviation from pure exponential behaviour is attributed to the change in heat load, and therefore changes in \( \Delta T \), at different circulating water temperatures.

An empirically derived polynomial was fitted to the measured mode values for various temperatures:

\[
\text{Modeframe}(T) = aT^2 + \beta T + \gamma
\]

where \( \text{Modeframe}(T) \) is the mode value of the frame at temperature \( T \) and \( a, \beta \) and \( \gamma \) the coefficients of the polynomial. The plan for designing the theoretical model of the dark current level was to model the relative change in the dark current level during acquisition at temperature \( T \) compared with a reference temperature \( T_0 \). Thus, there would be no need for any absolute temperature measurements. The reason for producing a relative model based on a reference set of frames was in order to be able to correct for any deterioration or any change of the equipment as for example an increase in the number of broken pixels or in those that demonstrated an irregular high level of dark current.

Basically, the reference set of frames would be used as a ‘calibration’ set for the system. Therefore, if \( T_0 \) was the reference temperature and \( \text{Modeframe}(T_0) \) the mode value, then equation 4.3 becomes:

\[
\text{Modeframe}(T_0) = aT_0^2 + \beta T_0 + \gamma
\]

Subtracting equations 4.3 and 4.4, expression 4.5 is derived that calculates the relative change of the mode dark current level:

\[
\text{Modeframe}(T) - \text{Modeframe}(T_0) = \alpha(T^2 - T_0^2) + \beta(T - T_0)
\]

Using the 5 °C data set as the reference ‘\( T_0 \)’ data set and the corresponding set of blank frames, the parameters \( \alpha, \beta \) and \( \gamma \) were found in a least square error sense equal
to 0.099, 3.2 and -260 respectively. The $R^2$ value was found equal to 0.989. Figure 4.16 shows the plot of the average mode level per set for each temperature (marked as points) and the fitting curve marked as solid line.

![Figure 4.16: The average mode dark current level per set of 500 blank frames for each temperature versus the temperature and the fitted quadratic curve, using as reference the 5° C blank data set.](image)

Equation 4.5 expresses the relative change in the mode dark current level of the frame as a function of change in temperature. Since it was shown in chapter 4 that the noise was not ergodic and that a local noise model should be built for each pixel, the individual pixel behaviour should also be studied and modelled. Similar plots as the one shown in figure 4.16 were made for ten randomly selected pixels mentioned before. For displaying purposes, four of them are presented in figure 4.17. The remaining six appear in Appendix H for completeness.

The fitted polynomial for each individual pixel also follows the expression:

$$Mode_{ij}(T) = \alpha T^2 + \beta T + \gamma$$  \hspace{1cm} (4.6)
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Figure 4.17: The average mode of the distributions of 4 different pixels versus temperature. Poor fit in the last example is because the pixel exhibits erratic saturated behaviour. Additionally, the fitted curves described by equation 4.4 are plotted.

as:

$$Mode_{ij}(T) - Mode_{ij}(T_0) = \alpha(T^2 - T_0^2) + \beta(T - T_0)$$

(4.7)

This expression is similar with equation 4.5, apart from the coefficients $\alpha$ and $\beta$. Table 4.3 includes the polynomial coefficients of the fitted curves to the data points and the corresponding $R^2$. The saturated pixel was not included, since these saturated pixels do not follow the model and are therefore uncorrectable.

From table 4.3, the average values were calculated for each coefficient and found: $\alpha = 0.108 \pm 0.039$, $\beta = 3.23 \pm 0.51$ and for $\gamma = -258.8 \pm 9.28$. Comparing these values with...
Chapter 4. Assessing the thermal characteristics of the system

### Table 4.3: The coefficients of the fitted polynomial for 9 randomly chosen pixels. The broken pixel is not included here.

<table>
<thead>
<tr>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
<th>Pixel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.082</td>
<td>0.098</td>
<td>0.18</td>
<td>0.091</td>
<td>0.080</td>
<td>0.1</td>
<td>0.17</td>
<td>0.088</td>
<td>0.085</td>
</tr>
<tr>
<td>$\beta$</td>
<td>3.6</td>
<td>3.4</td>
<td>2.4</td>
<td>3.4</td>
<td>3.7</td>
<td>3.1</td>
<td>2.4</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>-260</td>
<td>-260</td>
<td>-250</td>
<td>-270</td>
<td>-260</td>
<td>-260</td>
<td>-260</td>
<td>-270</td>
<td>-260</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.987</td>
<td>0.990</td>
<td>0.988</td>
<td>0.989</td>
<td>0.990</td>
<td>0.991</td>
<td>0.987</td>
<td>0.993</td>
<td>0.989</td>
</tr>
</tbody>
</table>

those found for the relative change of the frame, $\alpha$, $\beta$ and $\gamma$, it was observed that for the 9 pixels randomly selected these coefficients were very similar. Thus, assuming that this sample is representative of the entire pixel ensemble, then:

$$\alpha = \alpha, \quad \beta = \beta, \quad \gamma = \gamma$$ \hfill (4.8)

The validity of this assumption will be reflected in the quality of the correction. Combining then equations 4.5, 4.7 and 4.8, it was found that:

$$\text{Mode}_{ij}(T) - \text{Mode}_{ij}(T_0) = \text{Mode}_{\text{frame}}(T) - \text{Mode}_{\text{frame}}(T_0) \quad (4.9)$$

The above equation demonstrates that the relative change of the mode value of the dark current distribution of each pixel is the same as the corresponding change of the mode value of the frame. Re-arranging equation 4.9, the expected mode value of each pixel at any temperature $T$ can be deduced from:

$$\text{Mode}_{ij}(T) = \text{Mode}_{ij}(T_0) + \text{Mode}_{\text{frame}}(T) - \text{Mode}_{\text{frame}}(T_0) \quad (4.10)$$

In equation 4.10:

- $\text{Mode}_{ij}(T_0)$: is known from the reference set of frames.
- $\text{Mode}_{\text{frame}}(T_0)$: is also known from the reference set.
- $\text{Mode}_{\text{frame}}(T)$: can be measured from the image frame at temperature $T$. 
4.4. A simple empirical model to correct for thermal dark current fluctuations.

- $Mode_{ij}(T)$: can be calculated from the above three known values and equation 4.10.

Thus a predicted mode value of the dark current for each pixel can be calculated having only one reference set of blank frames known. The current process has the advantage that the knowledge of the absolute temperature of the CCD device was not a necessity for calculating dark current values and that only one set of blank frames is required that can be updated regularly. The fitted curves in figure 4.17 were deduced by using the same polynomial coefficients as for the average mode of the frame. It was shown that the model did not apply for broken or saturated pixels.

Figure 4.18 demonstrates the linear relationship between the changes in the mode values of each frame and the changes to the mode value of each pixel with temperature.

![Graph showing linear relationship between mode values](image)

**Figure 4.18:** The two experimental plots show that the change in the mode of each pixel is linearly related to the relative difference of the average mode value of the frame between different temperatures. The direction of either of the axes represent also the relative drift in temperature. The lines are just to guide the eye.

4.4.2 Thermal variation of $\sigma_{ij}$

Within the expression for the cost function, the factor $S_{ij}$ includes information on the standard deviation of the dark current distribution of each pixel (equation 5.25), where
Chapter 4. Assessing the thermal characteristics of the system

\( S_{ij} = \frac{1}{2\sigma_{ij}^2} \) for a valid pixel. Plotting the distributions of the 10 random pixels over the 500 blank frames, it was observed that the standard deviation for each pixel is different and varied slightly between different temperatures. It is therefore important to consider whether the thermally modulated variation in dark current, \( \sigma_{ij} \) should be accounted within the cost function.

In figure 4.19 the distributions of the pixels are displayed for two different temperatures. At this point, a theoretical model of the value of the standard deviation between different temperature levels was investigated.

From a theoretical point, the number of thermionic electrons produced in a silicon semiconductor is given by the Richardson-Dushman equation as [86], [52]:

\[
\begin{align*}
    n_i &= AT^{3/2} e^{-\frac{E_g}{2k_B T}} \\
    \text{where } A \text{ is a constant depending on the material. Therefore, for a temperature change between } T_o \text{ and } T: \\
    \frac{n_i(T)}{n_i(T_o)} &= \left( \frac{T}{T_o} \right)^{3/2} e^{-\frac{E_g}{2k_B} \left( \frac{1}{T} - \frac{1}{T_o} \right)} \\
    \text{The exponential factor is the dominant factor in the above expression [52], [86]. For fixed temperature } T, \text{ if we measure many values } n_i, \text{ they exhibit Poisson-like behaviour [52], and therefore the natural variation on the data is given by the square root of the measurement } \left( \sigma = \sqrt{n} [54] \right). \text{ Therefore, the relative change in the standard deviation with temperature, from equation 4.12 is given as:} \\
    \frac{\sigma_{ij}(T)}{\sigma_{ij}(T_o)} &= \sqrt{\left( \frac{T}{T_o} \right)^{3/2} e^{-\frac{E_g}{2k_B} \left( \frac{1}{T} - \frac{1}{T_o} \right)}} = \left( \frac{T}{T_o} \right)^{3/4} \sqrt{e^{-\frac{E_g}{2k_B} \left( \frac{1}{T} - \frac{1}{T_o} \right)}} \\
    \text{Since the exponential part is dominant [52], therefore:} \\
    \frac{\sigma_{ij}(T)}{\sigma_{ij}(T_o)} &\approx \sqrt{e^{-\frac{E_g}{2k_B} \left( \frac{1}{T} - \frac{1}{T_o} \right)}} \approx e^{-\frac{E_g}{2k_B} \left( \frac{1}{T} - \frac{1}{T_o} \right)} \\
    \text{Replacing the physical constants in the above expression with another lumped constant } C, \text{ where } C = e^{E_g/(4k_B)} \approx 1.8 \text{ for room temperature } (T \approx 300 \text{ K}) \text{ and } \Delta T = T - T_o, \text{ equation 4.14 is re-written as:} \\
    \frac{\sigma_{ij}(T)}{\sigma_{ij}(T_o)} &\approx Ce^{\Delta T/T_o} \propto e^{\Delta T/T_o} \\
\end{align*}
\]

\( AT^{3/2} e^{-\frac{E_g}{2k_B T}} \)
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Figure 4.19: The dark current distribution of 10 randomly chosen pixels over 500 frames acquired for 15 seconds each for the temperatures of 5 °C (top two rows) and 28 °C (bottom two rows). The last graph corresponds to a saturated pixel.
For a temperature change between 5 °C and 28 °C, the ratio of the standard deviations of the two temperatures was 1.17, representing a predicted 17% change in the standard deviation over the extremes of temperature investigated. This can be seen by reference to figure 4.20 which plots the standard deviation for each pixel versus temperature. Apart from the saturated pixel, the average standard deviation was approximately 4 ADC units for temperatures between 5 °C and 28 °C. However, the 17% change represents 0.68 - 1.2 ADC units. Given that the ADC digitisation error of ± 0.5 LSB\(^1\) then this change may not always be significant. The measurement of the standard deviation at 36 °C observed to be high, although this can be largely attributed to variations of temperature during acquisition as it was difficult to maintain constant T. Therefore 36 °C data should be viewed with caution. However, for the temperature range 5 °C-28 °C, this demonstrates that the expected thermal changes in \(\sigma\) for different temperatures are expected to be negligible and therefore such effects have been ignored in any subsequent correction/restoration method.

4.4.3 Evaluation of the new method on the microscale images.

The fixed pattern noise removal method developed in section 4.4.1 was applied to the microscales images obtained using 15 seconds integration time. A further comparison was also made with the method developed in section 4.3 (equation 4.2). In order to avoid confusion the term fixed pattern noise 1 is used to refer to the method from section 4.3 and the term fixed pattern noise 2 refers to the method described in section 4.4.1. If \(g_{ij}\) are the initial experimental pixel intensity values from the microscale data, then the expected pixel values \(\tilde{g}_{ij}\) were estimated by simple subtraction:

\[
\tilde{g}_{ij} = g_{ij} - Mode_{ij}(T)
\]  

(4.16)

An analysis concerning the linearity and sensitivity of the system was performed as before by plotting the average ADC units per pixel versus the activity level and calculating the slope and the offset of each fitted line for the different temperatures. The corrected microscales images are shown in figure 4.21 and the corresponding linearity

\(^1\) LSB stands for Least Significant Bit.
4.4. A simple empirical model to correct for thermal dark current fluctuations.

Figure 4.20: Each plot demonstrates the change of standard deviation with temperature. The abscissa is the temperature $T$ and the ordinate the standard deviations of the dark current distribution of each pixel over 500 blank frames acquired using 15 seconds integration time. Note that pixel 10 represents a saturated pixel.

plots in figure 4.22. In table 4.4 the coefficients of the fitting ($y = ax + b$) were compared between the two methods. The objective was to increase the slope, since the sensitivity increases proportionally, and to reduce the offset, which was the recorded level of dark current with no activity present.

As shown from the table there was an improvement in both parameters for all temperatures, since the parameter $\alpha$ for the results of the new method was greater than the previous values. The relative improvement in the slope and therefore in the sensitivity was between 3 to 15 %. Parameter $b$ had also been reduced comparing with the old values and this reduction was within the range of 35 to 55 %. Additionally, the slight
### Table 4.4: The parameters $a$ and $b$ of the least squares fitting ($y = ax + b$) after correcting with fixed pattern noise method 1 and 2. The slope $a$ has been increased and the offset $b$ has been reduced, while the linearity ($R^2$) has also been improved. The relative improvement of each measurement is shown inside the brackets.

<table>
<thead>
<tr>
<th>T °C</th>
<th>f.p.n. method 1</th>
<th>f.p.n. method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha \times 10^{-4}$</td>
<td>$b \times 10^{-2}$</td>
</tr>
<tr>
<td>5</td>
<td>8.7</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>7.9</td>
<td>2.7</td>
</tr>
<tr>
<td>16</td>
<td>8.3</td>
<td>3.3</td>
</tr>
<tr>
<td>22</td>
<td>8.6</td>
<td>2.7</td>
</tr>
<tr>
<td>28</td>
<td>7.8</td>
<td>1.9</td>
</tr>
</tbody>
</table>

but consistent improved $R^2$ values suggest there may be a small improvement in the linearity response of the system compared with the old method.

### 4.5 Conclusions

Two main issues were investigated in this chapter. The first was concerned with the linearity and the sensitivity of the system at different temperatures and integration times using calibrated microscales. A fixed pattern noise removal method was developed initially and it was applied to correct for interpixel and interframe dark current variations. The results demonstrated reproducible linearity of the system for all integration times and temperatures, even with those obtained under cooled conditions. It was also observed that the system can successfully image activities of at least two orders of magnitude, showing satisfactory results for this dynamic range. The same dynamic range was found also by using the image frames acquired under cooled conditions, thus a similar performance of the imaging system was observed between cooled conditions and room temperature.

The initial fixed pattern noise method (f.p.n. 1) although it corrected efficiently the
Figure 4.21: The corrected images of the $^{14}$C microscales using the model in order to predict the mode values of each pixel in any temperature. The reference set of blank frames were at 5 °C. The results are shown in negative polarity for better visualisation.
Figure 4.22: The linearity plots for the microscales images for 15 seconds integration time corrected using the model that described the dark current behaviour.
4.5. Conclusions

Microscale frames and it was convenient when there was a small number of frames due to be corrected. However, it had also some disadvantages: because this method required an extra set of blank frames for the different temperatures and integration times, it was necessary to repeat a blank scan acquisition under similar conditions with only small variations in temperature permitted every time an image acquisition was performed. Therefore, a method was developed that would correct the dark current offsets taking into consideration significant different temperature variations. Observing the thermal behaviour of the dark current, an empirical model was developed for the mode values of the dark current distribution of each pixel that would predict the mode dark current level having only one set of blank reference frames. The advantages of this method were that knowledge of the absolute temperature or any relative drift of temperature was not necessary since each frame was corrected separately as well as each pixel. An advantage of having a single set of reference frame data is that these can be updated at regular intervals and used in conjunction with the empirical model to correct each pixel. This latest fixed pattern noise estimation method (f.p.n. 2) was tested on the same microscale data as the previous fixed pattern noise estimation method (f.p.n. 1). The results showed that the new correction method improved the sensitivity and the linearity of the system as expressed by parameters $\alpha$ and $b$, where the former parameter was increased by up to 15% and the latter was decreased by up to 55%.
Chapter 4. Assessing the thermal characteristics of the system
Chapter 5

Methodology

5.1 MAP Image Restoration

The problem that image restoration techniques have to solve is to recover the original image, given the degraded image, by modelling the degradation and applying inverse procedures [50], [83]. A global optimisation technique for image restoration was used in this work. The restoration problem is formulated in terms of a cost function that has to be minimised with respect to the true (unknown) values at all pixel positions. The optimisation problem was solved using simulated annealing.

In general, if $g_{ij}$ is the degraded value at pixel position $(i,j)$ and $x_{ij}$ is the true value at this position, then the problem is to estimate $x_{ij}$, given the degraded image and the degradation model. If $X$ is the configuration of all undegraded values about to be estimated, then the estimate $\hat{X}$ is:

$$\hat{X} = \text{arg}(\max_X P(X|\text{data, model}))$$  \hspace{1cm} (5.1)

where $P(X|\text{data, model})$ is the posterior probability density function for some configuration of pixel values $X$ to exist, given the actual data and the corrupting model that applies to them. This is the so called "Maximum A Posteriori" (MAP) solution to the problem. From Bayes theorem [92]:

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Chapter 5. Methodology

\[ P(X|G, \text{model}) = \frac{P(G|X, \text{model})P(X)}{P(G)} \quad (5.2) \]

where \( P(X) \) is the prior probability of the particular combination of pixel values to appear, \( P(G|X, \text{model}) \) is the posterior probability of the data configuration, \( G \), to arise, given the combination of the true grey level values \( X \) and the model of degradation, and \( P(G) \) is the prior probability of the data configuration to arise. As the data exist, the latter is constant and the problem of estimation is altered to:

\[ \hat{X} = \text{arg} \left( \max_X P(G|X, \text{model})P(X) \right) \quad (5.3) \]

Each of these probabilities is usually expressed as \( \text{Probability} \propto e^{-\text{function}} \) where the function is called cost function or Hamiltonian. The Hamiltonian representing the posterior probability density function of the data is denoted by \( H_d(G, X, \text{model}) \) and the Hamiltonian representing the prior model is denoted by \( H_p(X) \). Then (5.3) can be written as:

\[ \hat{X} = \text{arg} \left( \max_X e^{-[H_d(G, X, \text{model}) + H_p(X)]} \right) \quad (5.4) \]

Maximising the expression on the right hand side of equation (5.4) is equivalent to minimising its exponent. So the estimation problem may be expressed as the minimisation of the following cost function:

\[ H(G, X, \text{model}) = H_d(G, X, \text{model}) + H_p(X) \quad (5.5) \]

This theory would be applied in order to restore autoradiography images. The two parts of the cost function will be determined in sections 5.3 and 5.4.

5.2 Optimisation tool

The problem of global optimisation is often encountered in the literature. The main objective is to find the global optimum by escaping efficiently from any local optima. A method that satisfies the above requirement is the algorithm of simulated annealing. Many approaches have been presented in the literature of different methods of simulated
Annealing, such as the classical simulated annealing or Boltzmann machine presented by Geman and Geman [33], the fast simulated annealing or Cauchy machine explained by Szu and Hartley [96], the generalised simulated annealing by Tsallis and Stariolo [98] and the threshold accepting algorithm by Dueck [25] and Moscato and Fontanari [76].

The method of annealing is known from condensed matter physics. It is the physical process of heating up a solid until its melting point, which is followed by cooling it down until it crystallises into the ground state with a perfect lattice. From practice, it is known that if the cooling process is not done carefully, then it is very likely for the system to get trapped in locally optimal lattice structures with crystal imperfections [1].

The general steps of the simulated annealing algorithm, in this case for minimising the cost function $H(G, X, \text{model})$, are:

1. Choose at random an initial starting configuration $X^0$, which means an initial value for each pixel of the image. In this case we chose $X^0 = G$. We chose $X^0 = 0.5$ only during attempts to estimate the optimal parameter values.

2. Compute the value of the cost function $H(G, X, \text{model})$ for this configuration, $H(X^0)$.

3. Set $H^{old} = H(X^0)$

4. Set $T_k = f(T_0, k)$, where $f(T_0, k)$ is a function called the 'cooling schedule' that depends on an initial parameter value $T_0$ and the counter $k$. We define the 'decrement function' as $T_k = aT_{k-1}$, where $a$ is a constant between 0.8 and 0.99 [1] and $T_0 \sim 10$.

5. Pick one of the pixels in the configuration. Let us say that its value is $x^n_{ij}$.

6. Choose at random a new value for this pixel from the range of possible values. Let us say that the chosen value is $x^n_{ij}$.
7. Calculate the value of the cost function for the new configuration which is identical to the previous one, except for the value of the picked pixel which has been replaced by \( x_{ij}^{new} \), and call this \( H^{new} \).

8. Calculate \( \Delta H = H^{old} - H^{new} \)

9. • If \( \Delta H > 0 \), we replace the value of the pixel by \( x_{ij}^{new} \), set \( H^{old} = H^{new} \) and proceed to the next step (10).
   • if \( \Delta H < 0 \) we draw a random number \( q \) from a uniform distribution between 0 and 1.
     - if \( q > e^{\Delta H/T_k} \), we leave the value of the pixel unaltered and proceed to the next step (10).
     - if \( q < e^{\Delta H/T_k} \), we change the value of the pixel to \( x_{ij}^{new} \), set \( H^{old} = H^{new} \), and proceed to the next step (10).

10. If a certain termination criterion is fulfilled, we exit the program, else we proceed to the next step.

11. If we have not visited all pixels in the image for the particular value of \( k \), we go to step 5. If we have visited all pixels for the particular value of \( k \), we set \( k = k + 1 \) and go to step 4.

The criterion presented in step 9 is known as the Metropolis criterion [74]. This criterion corresponds to the physical situation when a system with energy \( H \) and temperature \( T \) is perturbed, and its energy changes by \( dH \). If the change in energy between the new and old state of the system is negative, then the new configuration is accepted, otherwise (if \( dH > 0 \)) it is accepted with a probability given by a Boltzmann factor \( \exp(-dH/T) \) [74]. This procedure is repeated until the temperature is close to zero or is interrupted by a termination criterion.

Examples of termination criteria are: if there is no significant change between two consecutive iterations e.g. the relative difference between the old and new value of the cost function is less than 0.01 %, then the process can be stopped. Another example of termination criterion is the acceptance ratio, which is defined as the number of
accepted transitions over the number of proposed transitions \([1]\) or for a certain number of consecutive updates the pixel values to be altered only when the number \(q\) is drawn.

Simulated annealing is a slow process that, if accelerated in an ‘improper’ way (fast solution), then the global optimum is not guaranteed. The solution usually found is near-optimal \([92]\). The speed of simulated annealing may be improved by designing fast sequential or parallel algorithms.

5.3 Prior Model

If there is no noise in the image, then all pixel values will be exactly the same (provided dark current biases have been removed), unless a particle has travelled through and deposited part of its energy in the pixels. In this case, there will be a peak in the pixels with the extra charge.

Therefore, a desirable prior model is one that favours those pixel configurations which encourage neighbouring pixels to have the same value (similar charge). The model that is adopted is the so called “membrane model”:

\[
P(X) = \frac{1}{Z} e^{-\sum_{i=1}^{M} \sum_{j=1}^{N} \lambda ((x_{ij} - x_{i,j+1})^2 + (x_{ij} - x_{i+1,j})^2)}
\]

(5.6)

where \(Z\) is a normalising constant, \(M\) is the number of rows and \(N\) is the number of columns of the image and \(\lambda\) is a parameter that represents a scaling factor of the \(H_p\) contribution to the cost function. Therefore, in the cost function of equation (5.5) the contribution of the prior probability for this data configuration is:

\[
H_p(X) = \lambda \sum_{i=1}^{M} \sum_{j=1}^{N} (x_{ij} - x_{i+1,j})^2 + (x_{ij} - x_{i,j+1})^2
\]

(5.7)

This cost function, however, tends to oversmooth the sudden jumps in pixel values caused by real recorded events. To allow sudden changes in values to exist, this quadratic term is multiplied with a function that switches off the smoothing process when the difference in values between neighbouring pixels is above a certain threshold,
\[ h_{ij} = \frac{1}{1 + e^{\frac{(x_{i,j} - x_{i,j+1})^2}{\mu}}} \quad u_{ij} = \frac{1}{1 + e^{\frac{(x_{i,j} - x_{i,j+1})^2}{\mu}}} \quad (5.8) \]

The above expression means that when the difference between adjacent pixels tends to zero, \( h_{ij} \) and \( u_{ij} \) tend to 0.5, and the corresponding regularization term of equation (5.7) is allowed to influence the outcome. If this difference tends to infinity, \( h_{ij} \) and \( u_{ij} \) tend to zero, and the effect of the corresponding regularization term is switched off. The behaviour of the function between these two extreme values (0 and 0.5) is controlled by parameter \( \mu \), which plays the role of a soft threshold.

After this modification, the prior model contribution to the cost function is:

\[ H_p(X) = \lambda \sum_{i=1}^{M} \sum_{j=1}^{N} (x_{i,j} - x_{i+1,j})^2 h_{ij} + (x_{i,j} - x_{i,j+1})^2 u_{ij} \quad (5.9) \]

### 5.4 Degradation Model

The Hamiltonian \( H_d(G, X, \text{model}) \) is determined by modelling the degradation processes. Initially, the degradation process was modelled as additive noise, homogeneous over the whole frame. However, experiments showed that the noise was not ergodic i.e. different noise models applied to different CCD elements. To deal with the problem of inhomogeneous noise, the noise process was modelled individually for each pixel. This approach allows us to deal with different bias at each pixel location, different noise processes if necessary, and even with damaged individual sensors of the CCD surface. In addition, the point spread function was modelled and it was taken into consideration when formulating the restoration problem. These different approaches of degradation modelling are described in the following sections.

#### 5.4.1 Global Noise Model

The histogram of the pixels with noise that were believed to be away from the radiolabelled fibres in figure 3.7a is shown in figure 5.1. The ordinate represents the pixel value and the abscissa shows the frequency with which each value appears in the image. The
negative values of the pixels simply reflect the negative voltage offset of the imaging system.

![Image of histogram](image.png)

**Figure 5.1:** The histogram of pixel values with accumulated dark current. The ordinate represents the pixel intensity values and the abscissa the frequency of each pixel value. The negative values are due to negative voltage offset of the imaging system.

A number of candidate functions were examined as potential models for the noise probability density functions. The best was empirically found to be an exponential function of the form [84]:

$$f(x) = \begin{cases} 
    Ke^{-(x-a)c/b} & : x \geq \alpha \\
    0 & : x < \alpha 
\end{cases}$$  \hspace{1cm} (5.10)

This empirical noise model is a parametric function that depends on three positive parameters: $\alpha$, $b$ and $c$. $K$ is a normalising constant. The values of these parameters can be adjusted so that the model fits the data.

As shown in figure 5.2, parameter $\alpha$ defines from which bin the exponential decay commences; below $\alpha$ all values are set to zero. Parameter $K$ is a normalising constant that is a function of $b$ and $c$. Parameter $b$ is strongly related with the maximum value of the function. Finally, parameter $c$ controls the rate of exponential decay. The best values of the parameters for the average histogram of all eighteen frames were found empirically, to be:

$$\alpha = -130 \hspace{1cm} b = 11.5 \hspace{1cm} c = 1.00$$  \hspace{1cm} (5.11)
The probability density function of equation 5.10 with the above values of the parameters is shown in figure 5.3 (solid line) superimposed on the histogram of figure 5.1 (dots).

If the range of values for \( x \) is \([\alpha, x_{\text{max}}]\), then the normalisation constant \( K \) is calculated to be:

\[
K = \frac{1}{\int_{\alpha}^{x_{\text{max}}} e^{-\frac{|x-\alpha|}{b}} \, dx} = \frac{1}{\left(1 - e^{-\frac{x_{\text{max}}-\alpha}{b}}\right) b} \quad (5.12)
\]

If \( g_{ij} \) is a degraded measured pixel value, and \( x_{ij} \) is the true uncorrupted value, then

\[
g_{ij} = x_{ij} + n_{ij} \quad (5.13)
\]
where \( n_{ij} \) is the value of the noise process at pixel \((i,j)\). This value is drawn from a distribution of the form shown in equation (5.10). Therefore \( g_{ij} - x_{ij} \) is distributed according to equation (5.10). If it is assumed that the noise is white, i.e. uncorrelated, then the joint probability density function of the noise values in all pixels is equal to the product of the probabilities of the individual pixel values to arise:

\[
P(n) = \prod_{i,j} Ke^{-\frac{[n_{ij} - \alpha]^c}{b}}
\]  
(5.14)

where \( n \) is the configuration of all noise values \( n_{ij} \). This, however, is the joint probability of the data values to arise, given the true pixel values and the noise model:

\[
P(G|X, model) = \prod_{i,j} Ke^{-\frac{[n_{ij} - \alpha]^c}{b}}
\]  
(5.15)

\[
P(G|X, model) = \prod_{i,j} e^{-\frac{[n_{ij} - \alpha]^c}{b} - ln K}
\]  
(5.16)

\[
P(G|X, model) = e^{-\sum_{ij} \left(\frac{[n_{ij} - \alpha]^c}{b} - ln K\right)}
\]  
(5.17)

It is obvious then that

\[
H_d(G, X, model) = \sum_{ij} \left(\frac{[n_{ij} - \alpha]^c}{b} - ln K\right)
\]  
(5.18)

As \( K \) is a constant it may be omitted from this expression without affecting the minimisation of the cost function. Finally, by replacing \( n_{ij} \) by \( g_{ij} - x_{ij} \) (equation (5.13)) the following expression of the \( H_d(G, X, model) \) is obtained:

\[
H_d(G, X, model) = \sum_{i=1}^{M} \sum_{j=1}^{N} |g_{ij} - x_{ij} - \alpha|^c
\]  
(5.19)

where \( M \) and \( N \) is the total number of rows and columns respectively of the corrupted image, and \( b \) is a constant that can be accounted for by the \( \lambda \)-factor of the prior model.
The final expression of the cost function that is minimised is:

\[
H(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} |g_{ij} - x_{ij} - \alpha|^c + \lambda \sum_{i=1}^{M} \sum_{j=1}^{N} (x_{ij} - x_{i+1,j})^2 h_{ij} + (x_{ij} - x_{i,j+1})^2 u_{ij}
\]  

(5.20)

5.4.2 The Ergodicity Assumption

In the previous section a noise model was derived valid at every pixel of the frame that is going to be restored. This is the standard approach to image restoration problems. However, it is based on the tacit assumption that the noise value \( n_{ij} \) is drawn from the same distribution in all pixel positions, so that computing the noise statistics over a whole frame is equivalent to computing the ensemble statistics at any pixel position. In this section this assumption is questioned by examining the noise statistics at each pixel position over 500 frames of dark current.

The above property is known as ergodicity and its physical meaning is that the natural variability among \( N \) images is statistically the same as the natural variability of each image separately [83]. Therefore, for a set of \( N \) images of similar type, for example the mean can be calculated from a single image of the data set, producing the same result.

The 500 consecutive frames of dark current were acquired at room temperature with 10 seconds integration time. Random pixels were chosen and the distribution of the pixel dark current values were plotted over the 500 frames and displayed in figure 5.4. It can be seen from figure 5.4 that the distribution consists of two peaks close to each other and that each histogram is not centered around the same pixel intensity value. In order to investigate any temporal variation, the mean dark current intensity level was plotted against the frame number and it is presented in figure 5.5.

From figure 5.5 it can be observed that the mean intensity level per frame increases across the number of frames (towards more positive values). The mean value of frame number 500 is 11 % higher than frame number 1. There is a noticeable step in frame number 350 in the dark current level. This drift in the mean value results to a shift in the individual pixels. Therefore, the second peak of the histograms in figure 5.4 is
5.4. Degradation Model

Figure 5.4: Histogram of dark current values over 500 frames at room temperature of four random pixels of the image. The pixels are located by the coordinates shown in the brackets.

attributed to the shift in the mean dark current level. Figure 5.6 shows the histogram distribution of the pixel values over the first 350 frames.

The histogram distributions of the dark current level of the different pixels suggests that the noise of the imaging system is assumed to be modelled by a normal-like distribution with different mean dark current and variance for each pixel. Looking at the values of individual pixels, it was concluded that there is a fluctuation on the pixel value (different mean values and variances) and therefore the noise of the system is not ergodic. Additionally, a shift in the mean dark current level was observed. This shift might be due to non-complete read-out of the pixel values, resulting in an increase of the remaining charge of the pixels. Normalising the pixel values with respect to the mean dark current level over the entire frame may allow us to overcome the problem of
5.4.3 Local Noise Model

As shown in the previous section, the ergodicity assumption is not valid and therefore it is necessary to model the noise process at each pixel individually. It was found by trial and error that the Gaussian model fits well the distribution of the dark current values at each pixel when considering the first 350 frames of the blank frame data, but with parameters that are different at each locality (5.21):

\[
P(n) = \prod_{i,j} \frac{1}{\sqrt{2\pi \sigma_{ij}^2}} e^{-\frac{(n_{ij} - \mu_{ij})^2}{2\sigma_{ij}^2}}
\]

(5.21)

where \(n_{ij}\) is the value of dark current in each pixel, \(\mu_{ij}\) is the mean value of each pixel over the 350 frames (figure 5.6) and \(\sigma_{ij}^2\) its variance.

The measured data is the sum of the dark current and the actual data, as described by
5.4. Degradation Model

Figure 5.6: Histogram of dark current values over the first 350 frames at room temperature of four random pixels of the image. The coordinates of each pixel are shown in the brackets.

Combining equations (5.13) and (5.21), we obtain:

$$P(G|X, model) = \prod_{i,j} \frac{1}{\sqrt{2\pi\sigma_{ij}^2}} e^{-\frac{(g_{ij} - x_{ij} - \bar{m}_{ij})^2}{2\sigma_{ij}^2}}$$ \hspace{1cm} (5.22)

and for the cost function

$$H_d(G, X, model) = \sum_{i=1}^{M} \sum_{y=1}^{N} \left( \frac{(g_{ij} - x_{ij} - \bar{m}_{ij})^2}{2\sigma_{ij}^2} - \frac{1}{2} \ln (2\pi\sigma_{ij}^2) \right)$$ \hspace{1cm} (5.23)

The second term inside the sum is constant and it cannot influence the optimum of the cost function. Therefore, it can be omitted to yield:

$$H_d(G, X, model) = \sum_{i=1}^{M} \sum_{y=1}^{N} \left( \frac{(g_{ij} - x_{ij} - \bar{m}_{ij})^2}{2\sigma_{ij}^2} \right)$$ \hspace{1cm} (5.24)
To deal with damaged cells of the sensor which obtain saturation value in all cases, the value of $\frac{1}{2^2}$ is set to zero, so that the pixels may acquire restored values from their neighbouring pixels, but their own totally corrupted datum value is entirely ignored.

$$S_{ij} = \begin{cases} \frac{1}{2^2} & \text{if pixel is valid.} \\ 0 & \text{if pixel is damaged.} \end{cases}$$ \hspace{1cm} (5.25)

Considering the above expression in equation (5.24) and including the Hamiltonian of the prior model (equation (5.9)), the cost function becomes:

$$H(G, X; \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} (g_{ij} - \mu_{ij})^2 S_{ij} + \lambda \left( \frac{(x_{i,j} - x_{i,j+1})^2 h_{ij} + (x_{i,j} - x_{i,j+2})^2 u_{ij}}{2} \right)$$

(5.26)

where $h_{ij}$ and $u_{ij}$ are defined by equations (5.8).

The mean value $\mu_{ij}$ for each pixel is a constant, and so that we do not have to subtract it every time from $g_{ij}$ when we run the simulated annealing algorithm, we can subtract it once at the beginning.

However, the noise model was computed from 350 blank frames obtained separately from the frames with the real data. It is expected that there may have been some drift in the ambient temperature between blank frame acquisition and experimental data acquisition. For this reason, before we subtract $\mu_{ij}$ from $g_{ij}$ we adjust it so that we refer it to the same ambient temperature as $g_{ij}$. The mode $\bar{G}$ of the frame was used as an indicator of the ambient temperature based on the relationship found in section 4.4.1 that there is a linear relationship between the value of the ambient temperature and the most frequent value recorded during a single frame grabbing process. The mean of the modes $\bar{B}$ of the 350 frames used to compute the noise model is taken to be an indicator of the temperature to which the extracted noise model refers. Therefore, the mean noise value $\mu_{ij}$ we subtract from each pixel is modified to be:

$$\mu_{ij}^* = \frac{\bar{G}}{\bar{B}} \hspace{1cm} (5.27)$$

This represents the same correction previously described as f.p.n. 1 in section 4.3. Of course the ambient temperature may even affect the standard deviation of the noise model. However, this is a secondary effect and as shown previously in section 4.4.2
and it is much less significant than the shifting of the mode. This effect was therefore ignored. The assumed linear relation between dark current and ambient temperature is approximately true for small thermal shifts (see section 4.4.1). Taking the mode as opposed to the mean of each frame we safeguard robustness against real data values, which are much fewer than the background dark current values which we wish to calibrate for.

Therefore, the new expression of the $H_d(G, X, \text{model})$ Hamiltonian becomes:

$$H_d(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} (b_{ij} - x_{ij})^2 S_{ij}$$

(5.28)

and the cost function to be minimised is:

$$H(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} (b_{ij} - x_{ij})^2 S_{ij} + \lambda [(x_{i,j} - x_{i+1,j})^2 h_{ij} + (x_{i,j} - x_{i,j+1})^2 u_{ij}]$$

(5.29)

where $b_{ij}$ is the measured data after the subtraction.

$$b_{ij} = g_{ij} - p_{ij}$$

(5.30)

5.4.4 Image blurring

If the point spread function of the imaging system is $R_{kl}$ the signal value recorded at pixel position $i,j$ will be given by the convolution of $R_{kl}$ with $x_{ij}$ plus the accumulated dark current $n_{ij}$:

$$g_{ij} = \sum_{k} \sum_{l} x_{i-k,j-l} R_{kl} + n_{ij}$$

(5.31)

If the blurring process can be modelled by a $D_1 \times D_2$ matrix with elements $R_{kl}$ where $k$ takes integer values in the range of $[-\frac{D_1}{2}, \frac{D_1}{2}]$ and $l$ takes its values in $[-\frac{D_2}{2}, \frac{D_2}{2}]$, then

$$g_{ij} = \sum_{k=-\frac{D_1}{2}}^{\frac{D_1}{2}} \sum_{l=-\frac{D_2}{2}}^{\frac{D_2}{2}} x_{i-k,j-l} R_{kl} + n_{ij} = x_{ij} \otimes R_{kl} + n_{ij}$$

(5.32)

where $\otimes$ represents the convolution between the true pixel values $x_{ij}$ with the point spread function, $R_{kl}$. 
Figure 5.7: Schematical model showing the parameters involved in determining the point spread function: $\phi = 161.57^\circ$, $\theta = 18.43^\circ$ and $d$ is the distance of a pixel from pixel $(i_0, j_0)$ that belongs to the wire.

To model the point spread function the procedure described in [83] was followed. First it was necessary to establish whether the point spread function is isotropic or not. To do that the profiles across the images of either two thin lines or two ideal step edges orthogonal to each other have to be examined. The data depict two orthogonal wires, so they are ideal for working out the point spread function. It was found that the angle between the horizontal axis and the wire was $\theta = 18.43^\circ$ and therefore $\phi = 161.57^\circ$ as it is shown in figure 5.7. In the absence of any blurring, these profiles of the cross-sections of the two wires would have been two pulses.

The distance of two pixels $(i, j)$ and $(i_0, j_0)$ is given by:

$$d^2 = (i - i_0)^2 + (j - j_0)^2$$  \hspace{1cm} (5.33)

if $(i_0, j_0)$ is a pixel of the fibre, and $(i, j)$ is a pixel at a distance $d$ away from pixel $(i_0, j_0)$ and along a direction perpendicular to the fibre, we have:

$$j - j_0 = \tan(\phi + 90^\circ)(i - i_0)$$  \hspace{1cm} (5.34)

Combining equations 5.33 and 5.34, it is deduced that:

$$d^2 = (i - i_0)^2 + \tan^2(\phi + 90^\circ)(i - i_0)^2$$  \hspace{1cm} (5.35)
and therefore

\[ t - t_0 = \frac{\pm d}{\sqrt{1 + \tan(\phi + 90^\circ)}} \] (5.36)

The coordinates of a pixel that is at distance \( d \) from pixel \((t_0, j_0)\) are calculated from the above equations but they might not be integer numbers. Therefore, in order to find the pixel intensity value at that point, bilinear interpolation was performed as described in [83]. This procedure was followed for both wires. Plotting the pixel intensity values, \( g_{t_j} \), over distance \( d \) for many different \((t_0, j_0)\) along the wires, the point spread function of the imaging apparatus was deduced for two image directions, namely the directions perpendicular to the two wires. It was found that both profiles of the point spread function could be modelled by a Gaussian distribution (see figure 5.8) given by

\[ R_{kl} = \frac{1}{Q} e^{-\left(\frac{k^2}{2\sigma^2}\right) - \left(\frac{l^2}{2\sigma^2}\right)} \] (5.37)

with standard deviation \( \sigma = 6.5 \). Where \( Q \) is a normalising constant so that \( \sum_{k,l} R_{kl} = 1 \). The size of discontinuity of the point spread function was chosen to be such that the discontinuity of the truncated Gaussian was 0.02.

![Figure 5.8: The profiles as plotted along the two directions. The fitted Gaussians are shown as red solid lines. The x-axis represents the distance \( d \) from pixel \((t_0, j_0)\) and the y-axis is the pixel value in the normalised frame.](image)

Including the blurring model to the faithfulness to the data part of the cost function
Figure 5.9: Two dimensional, normalised point spread function. Its standard deviation is $\sigma = 6.5$. Its dimensions are $27 \times 27$.

(equation 5.28), $H_d(G, X, \text{model})$ becomes:

$$H_d(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} \left( b_{ij} - \sum_{k=-\frac{D-1}{2}}^{\frac{D-1}{2}} \sum_{l=-\frac{D-1}{2}}^{\frac{D-1}{2}} x_{i-k,j-l} R_{kl} \right)^2 S_{ij}$$

(5.38)

where $D=27$ is the size of the mask. A more compact way of the above expression is:

$$H_d(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} (b_{ij} - x_{ij} \otimes R_{kl})^2 S_{ij}$$

(5.39)

where $S_{ij}$ is defined by equation 5.25. Combining equations (5.9) and (5.39), the cost function that is going to be minimised has the following form:

$$H(G, X, \text{model}) = \sum_{i=1}^{M} \sum_{j=1}^{N} (b_{ij} - x_{ij} \otimes R_{kl})^2 S_{ij} + \lambda [(x_{i,j} - x_{i,j+1})^2 h_{ij} + (x_{i,j} - x_{i,j+1})^2 u_{ij}]$$

(5.40)

5.5 Relevance to fixed pattern noise removal methods

The term fixed pattern noise is used in conjunction with CCD technology to indicate different sensitivity to noise between pixels. Pixel non-uniformity is attributed to processing variations and alignment errors during the CCD fabrication, so the generated
pixels demonstrate different responsivities [43]. There is an additional difference between the levels of charge deposited to each pixel that is due to the process of read out as explained in section 2.3.1. There is a gradient in the level of dark current between the pixels of the same row and there is an additional gradient between the rows. On both sides of the CCD device there is a light-proof overlying layer applied during manufacture. The standard method for correcting for this gradient is to subtract the average dark current value of the first and the last four pixels of each row [43] which are under the light-proof layer. When this method is applied the same dark current level is subtracted from all pixels of the each row.

However, instead of using the standard fixed pattern noise removal method, it is possible to use the method described in section 5.4.3, according to which the mean level of dark current for each pixel adjusted for small drifts in ambient temperature is subtracted from the recorded value. Therefore, one can use this approached as an improved fixed pattern noise removal method for removing the dark current offsets more efficiently than using the standard method. In the next chapters the term fixed pattern noise removal refers to this subtraction of the individual, thermally adjusted levels of dark current, rather than to the standard method.
Chapter 6

Experimental Results

6.1 Introduction

The experimental data files are in 12-bit format, which means that they may include values within a range of $[-2047, 2047]$. Because it is computationally expensive to work with such large values, the measured data are normalised as shown below:

$$g_{ij}^{new} = \frac{g_{ij}^{old} - minimum}{maximum - minimum}$$

where $minimum$ and $maximum$ are the minimum and maximum value for each frame, respectively. The maximum value is always equal to 2047, which is equivalent to the maximum value for 12-bit signed integers. The minimum value differs for each frame. Each pixel behaves like a capacitor that charges up and discharges. All pixels do not charge up and discharge the same way each time, depending on the initial charge and the physical characteristics of the pixel. Thus, the minimum value for each frame varies within $\pm 5\%$ of the average level. The restored values are within the range $[0,1]$. It was found that the normalisation has accelerated the execution time of the experiments described in this chapter by approximately 75\% of the initial execution time.

To be able to assess the validity of each method real and simulated data were used. Simulated images were created and degraded realistically so that the data bear all the characteristics of the real data.
The simulated data generated were images of the same dimensions as the experimental data (501 x 251). The procedure used to create a simulated frame was as follows: all pixel values were set to zero apart from the pixels supposedly presenting the imprint of the active T-shaped wires (the image of the wires was single pixel wide). The produced image represents the ideal simulated image, shown in figure 6.1 (8-bit display).

![Ideal simulated image and its profile along column 130.](image)

**Figure 6.1:** Ideal simulated image and its profile along column 130. Apart from the wire pixels all other pixel values are equal to zero.

A convolution of this image with the point spread function of the device, defined in section 5.4.4, was the next step. From the real data the variance and the mean of the noise distribution at each pixel position were known and so a random number generator was used to draw for each pixel a value from a normal distribution with the same parameters. This value was then added to the original value of the pixel.

Because the experimental data were stored in 12-bit frames, the possible pixel values were within the range of [-2047,2047]. Simulated pixel values outside this range were changed into the corresponding extreme value of the range. Additionally, the saturated damaged pixels in the real data were also assigned saturated values in the simulated data. The final result was a simulated image with inhomogeneous additive noise, including degradation due to the point spread function of the imaging system, and saturated, damaged pixels. Figure 6.2 shows the degraded simulated image and its profile along column 130.
6.1. Introduction

Figure 6.2: Initial degraded simulated image and its profile along column 130. The random white dots in the image represent saturated pixels or pixels with high level of noise.

Restoring such images has a twofold advantage: a) the optimal parameters of the algorithm can be chosen so that the estimated image is as close as possible to the known perfect image. b) The prior knowledge of the ground truth allows an investigation of the sensitivity of the algorithm with respect to its parameters and thus establish its breaking points.

The criterion used in order to assess how close to the truth are the corrected simulated images is the Mean Square Error ($MSE$). The mean square error is defined as:

$$MSE = \frac{1}{MN} \sum_{i=1}^{M} \sum_{j=1}^{N} (f_{ij,restored} - f_{ij,ideal})^2$$  \hspace{1cm} (6.2)$$

where $f_{ij,ideal}$ and $f_{ij,restored}$ are the pixel values in the ideal and the restored image. When $MSE$ is minimum, the mean difference between the two images is minimum. Therefore, the optimal value of the parameter is defined at the minimum point of $MSE$.

The parameters of each method are either parameters of the cost function or of the algorithm of simulated annealing. Each of these parameter affects differently the performance of each method.

The parameters are listed below according to the influence they have on the outcome,
from the highest to the lowest as was empirically observed from many experiments. However, even the parameter with the lowest influence plays an important role in the result.

\[ \lambda \rightarrow \mu \rightarrow \alpha \rightarrow k_{\text{max}} \rightarrow T_0 \]

As also mentioned before, the above parameters have the following physical meaning:

- \( \lambda \): a weighting factor between the two parts of the cost function.
- \( \mu \): the soft threshold used in the peak-functions of the prior model.
- \( \alpha \): the parameter of the cooling schedule in simulated annealing.
- \( k_{\text{max}} \): the maximum number of iterations, when the process of simulated annealing is terminated.
- \( T_0 \): the initial temperature in the algorithm of simulated annealing.

The order of optimising the algorithm with respect to each parameter follows the order of the influence it has to the results. Whilst the algorithm was optimised with respect to one parameter, the other parameter values were fixed. The decision for the most suitable value was based on the values of \( \text{MSE} \). Once a parameter value was optimised, it was fixed for all subsequent experiments.

For the assessment of the real images the following four measures of the quality of restoration are used:

1.) The spatial resolution at Full Width at Half Maximum (FWHM) [54]. The FWHM is calculated as follows:

\[ \text{FWHM} = \sqrt{(\text{FWHM}_{\text{measured}} \cos \theta)^2 - 20^2} \]  \hspace{1cm} (6.3)

where \( \text{FWHM}_{\text{measured}} \) is the measured FWHM in \( \mu \text{m} \) from the profiles, \( \theta \) is the angle between the wire and the horizontal axis (shown in figure 5.7) and 20 \( \mu \text{m} \) is the thickness of the wire.

2.) The spatial resolution at Full Width at Tenth Maximum (FWTM) [54]. The FWTM is calculated similarly to the FWHM by equation:

\[ \text{FWTM} = \sqrt{(\text{FWTM}_{\text{measured}} \cos \theta)^2 - 20^2} \]  \hspace{1cm} (6.4)

where \( \text{FWTM}_{\text{measured}} \) and \( \theta \) are defined as above.
3.) Background uniformity. This is checked in a region away from the activity area (between rows 50 and 150 and columns 150 and 220) located in the upper left corner of the image frame. The standard deviation of the pixel intensity values of dark current located in this specified region was used as a uniformity measurement.

4.) Overall reduction of the background dark current. The noise suppression is measured by comparing the average (mean) intensity values in the specified area mentioned above between the initial and restored image.

For all the following methods, the parameters of the algorithms were chosen by experimenting with the simulated data. For each approach, each of the eighteen frames was restored individually and the results were summed to produce the final outcome. The restored version of a single frame and the final sum frame are presented and the spatial resolution at FWHM and FWTM were measured from 10 different profiles between columns 120 and 143 (columns used: 120, 123, 125, 128, 130, 133, 135, 138, 140, 143) of the restored sum frame.

The following five series of experiments were performed:

1.) Fixed pattern noise removal only using equations (5.27) and (5.30).

2.) Simulated annealing restoration using as cost function the one given by equation (5.20) in order to check the validity of the global noise model.

3.) Simulated annealing restoration using as cost function the expression of equation (5.26) using a local noise model.

4.) Simulated annealing restoration using a local noise model, with the mean value of the noise removed from each pixel adjusted for ambient temperature i.e. minimizing the cost function expressed by equation (5.29).

5.) Simulated annealing restoration using a local noise model with the mean value of the noise removed from each pixel adjusted for ambient temperature and taking into consideration the point spread function of the system. The expression of the cost function is given by equation (5.40).
6.2 Fixed Pattern Noise Removal

6.2.1 Simulated Data

The fixed pattern noise removal method developed in section 5.4.3 was investigated independently from the image restoration algorithm in order to assess the validity of the method when it is used separately. However, since the method does not involve any of the parameters mentioned before, the degraded initial simulated image was corrected and it is displayed in figure 6.3 with its profile along column 130.

Comparing the initial simulated image in figure 6.2 (repeated here for convenience) with the corrected image, it can be seen that many pixels with high intensity values have been corrected. This can also be observed by comparing the corresponding profiles. There is a noticeable reduction in the dark current offset but no smoothing. However, there are still some ‘hot’ pixels with only dark current that have not been corrected. This is because approximately 1 % of the total number of pixels do not follow a normal-like distribution but they have demonstrated a non-normal distribution, with a character which varies considerable for different pixels.

6.2.2 Real Test Data

The experimental frames have also been corrected in a similar way to the simulated image and added in order to form the summed corrected frame. A single corrected real image and its profile along column 130 are shown in figure 6.4. The corrected summed image and the corresponding profile 130 are shown in figure 6.5. The remaining profiles of the corrected sum frame are shown in comparison with the initial ones in Appendix C. The spatial resolution at FWHM and at FWTM of each of the profiles are given in table 6.1.

The average level of the spatial resolution almost remained the same (from 189 \(\mu m\) to 183 \(\mu m\)), whereas the mean spatial resolution at FWTM was reduced by approximately 7 % compared to the initial (from 638 \(\mu m\) to 589 \(\mu m\)). The standard deviation of the spatial resolution values were reduced by approximately 34 % and 33 % for the
6.2. Fixed Pattern Noise Removal

Figure 6.3: The original simulated image and its corrected version with fixed pattern noise removal, as well as its profile along column 130 before and after correction. From the profile it is shown that there is a reduction in the dark current offset, compared with the initial profile but no smoothing.

FWHM and FWTM respectively which was attributed to the small reduction of the spikes. The error of each experimental measurement was estimated to be ±7 μm, so the spatial resolution at FWHM might not have been improved since the new mean value was within the range of measurement error. In some cases also the spatial resolution became worse which demonstrates a non-stable behaviour of the algorithm to improve the spatial resolution.
The main achievement of this method though was that it could correct for the dark current offset of each pixel by also adjusting the mean intensity values for small temperature drifts. There was a 30 times reduction (the average level was reduced to 3% of the initial value) in the mean dark current level of the specified background region. However, it did not affect the inter-pixel fluctuations, so there was still a remaining component of noise. As it could be observed from the profiles in figure 6.5 and those
6.2. Fixed Pattern Noise Removal

Figure 6.5: The original and the corrected sum real image using fixed pattern noise removal, as well as its profile along column 130 before and after the correction. Approximately 10% of the pixels were overcorrected as showing by the downward spikes in the profile.

shown in Appendix C the spikes of high intensity pixels have been corrected in almost all cases.

Additionally, it was found that this method tends to overcorrect approximately 10% of all pixels and to undercorrect 1-5%. This was due to the fact that the method only corrects for the mean value. The asymmetry in the percentage of overcorrected and undercorrected pixels is probably due to the fact that the noise distribution is
Table 6.1: Comparison between the initial and corrected sum frame using only fixed pattern noise removal. There is a significant reduction in the mean dark current level by a factor of 30. However, the smoothing is only due to the reduction of the spikes (high intensity pixels). Positive values of "improvement" indicate actually worsening of the indicator value. The measurements have measurement accuracy of ±7 μm leading to 1-3 % uncertainty in the values of improvement.

not a symmetric function. This method is also valid under the assumption that the temperature change between the acquisition of the blank frames and the image frames is small, so the shift in the dark current, as represented by the mode value, is linear.

6.2.3 Conclusions

The fixed pattern noise removal is a method based on subtracting dark current offsets, different for each pixel, but also correcting for small drifts in ambient temperature
between blank scan and image acquisition. The results have confirmed the reduction in the dark current level but the spatial resolution of the system was almost at the same level as in the initial measurements. However, there was an improvement in the spatial resolution at FWTM due to the removal of the spikes of the high intensity pixels that tend to deteriorate the spatial resolution at that level.

In conclusion, fixed pattern noise removal deals only with mean dark current offsets, and to deal with statistical fluctuations it is necessary to use more sophisticated noise reduction method, like simulated annealing.

### 6.3 Using a Global Noise Model

A homogeneous noise model was used to build the faithfulness to the data part of the cost function. Although, this assumption was shown to be incorrect, results are presented in order to serve as the minimum benchmark of achieved improvement by the simulated annealing approach.

#### 6.3.1 Simulated Data

The first parameter altered was $\lambda$ within the range of $[1, 300]$. The pre-selected values of the other parameters were: $\mu=0.04$, $\alpha=0.99$, $k_{max}=1700$ and $T_0=10$. These pre-selected parameter values were close to optimal, as found by trial and error, but not the optimal values. The mean square error (MSE) was calculated and plotted against $\lambda$ values. The plot is displayed in figure 6.6.

From figure 6.6, the optimal range of $\lambda$ values, where $MSE$ was small, was from $\lambda=70$ to $\lambda=110$. However a clear optimal was shown at the point where $\lambda=90$. For all subsequent experiments, $\lambda$ was set to this value.

The next parameter was the soft threshold $\mu$. Its values were varied between 0.01 and 0.2. The actual value of the threshold with which the difference between two adjacent pixels was compared, according to the membrane model, was $\sqrt{\bar{\mu}}$. The calculated values of $MSE$ were plotted against $\mu$ and shown in figure 6.7. The suggested range of
Figure 6.6: The calculated values for $MSE$ for different values of $\lambda$ in the range of $[1, 300]$, using a global noise model. The optimal value for $\lambda$ was defined where $MSE$ was minimum.

The operation from this plot is for $\mu$ between $\mu=0.04$ and $\mu=0.09$. However, a high threshold may lead to oversmoothing, so the chosen value for the subsequent experiments was set to 0.04.

Figure 6.7: The calculated values for $MSE$ for different values of $\mu$ in the range of $[0.01, 0.2]$, using a global noise model. The parameter values used were: $\lambda=90$, $\alpha=0.99$, $k_{\max}=1700$ and $T_0=10$.

The above two parameters control the cost function and therefore their influence was direct. The next three parameters are characteristics of the simulated annealing process.
The parameter with the next highest influence was that of the cooling schedule, $\alpha$. It is known from the literature that simulated annealing is an optimisation method that can guarantee a global optimum [33]. However, in order to achieve this, a slow 'cooling' process is required for sufficient number of iterations. The values of $\alpha$ varied from 0.1 to 0.99. The results of a fast or slower cooling process for a fixed number of iterations at $k_{\text{max}}=1700$ are shown in the plot of $MSE$ versus $\alpha$ in figure 6.8. The other parameter values were set at $\lambda=90$, $\mu=0.04$ and $T_0=10$. From the plot a suggested range of $\alpha$ is above 0.90. From the $MSE$ plot it is clear that the slower the process, the closer to the ideal the restored image becomes. Another value of $\alpha$ that was tried was that of 0.999. In that case, $MSE$ was maximum ($MSE=0.308$) because the process was very slow and 1700 iterations was not sufficient to allow the corrupted image to reach an acceptable level of restoration. One has to compromise between a slow cooling process and an acceptable number of iterations (execution time). The optimal value for $\alpha$ was then set equal to 0.99.

![Figure 6.8: The calculated values for $MSE$ for different values of $\alpha$ in the range of [0.1, 0.99], using a global noise model. The global optimum is guaranteed only when the 'cooling' process is slow.](image)

The maximum number of iterations, $k_{\text{max}}$, was the next parameter that was investigated, within the range 200 to 3000. $k_{\text{max}}$ was one of the termination criteria used by the algorithm of simulated annealing. The other termination criterion was that when
for 50 consecutive times, the only acceptable pixel values were through drawing a $q$
value (see section 5.2). However, this criterion was strong and it was never reached.
The algorithm was always terminated by the maximum number of iterations ($k_{\text{max}}$).
$MSE$ was calculated for each $k_{\text{max}}$ and is shown in figure 6.9. It can be observed that
after a certain number of iterations (1000) the quality of restoration did not undergo
further improvement.

![Figure 6.9: The calculated values for $MSE$ for different values of $k_{\text{max}}$ in the range of [200, 3000], using a global noise model. The restoration process starts to converge after 1000 iterations.](image)

For better judgement of the maximum number of iterations used, the acceptance ratio
$\chi(k)$ was calculated for every experiment. The acceptance ratio is defined as the number
of random accepted transitions over the number of proposed random transitions [1]:

$$\chi(k) = \frac{\text{number of accepted transitions}}{\text{number of proposed transitions}}$$

Some examples of $\chi(k)$ versus $k_{\text{max}}$ are shown in figure 6.10.

For a small number of iterations, most proposed transitions were accepted, therefore
$\chi(k)$ was close to 1. As the number of iterations increases, fewer transitions were
accepted and for very large numbers of iterations even fewer transitions were accepted.
Therefore, when $\chi(k)$ tends to zero for many iterations, it shows a good point to set
the maximum number of iterations and terminate the algorithm. Combining the plots
6.3. Using a Global Noise Model

Figure 6.10: The calculated acceptance ratio, $\chi(k)$ for different number of iterations. It can be seen that for a small number of iterations the accepted ratio of transitions of new configuration is higher than for a larger number of iterations.

of $MSE$ and $\chi(k)$, it was concluded that 1400 iterations was an acceptable number of iterations at which to terminate the algorithm.

Figure 6.11: The calculated values for $MSE$ for different values of $T_0$ in the range of [1, 100], using a global noise model. The fact that $T_0$ has a small effect on the restoration quality is shown from the small variation of $MSE$ values.

The parameter with the lowest influence is the initial temperature, $T_0$. The meaning
of the initial temperature, and generally the meaning of temperature is equivalent to
the probability with which a new configuration is accepted, defined by the Boltzmann
factor (section 5.2). The range of values investigated was between 1 and 100. The
Corresponding \( MSE \) values are displayed in figure 6.11. The differences in the cal­
culated \( MSE \) values were located in the fourth decimal place, which confirms the initial
assumption that this parameter has a small influence on the outcome of restoration.
Therefore, concerning \( MSE \) all the values in the range of \([1, 100]\) can be used as \( T_0 \).
The optimal value was defined at minimum, which was at \( T_0 \) equal to 10.
The parameter values used to restore the experimental frames are listed below. The
results are presented in the next section.

\[
\lambda=90, \mu=0.04, \alpha=0.99, k_{\text{max}}=1400 \text{ and } T_0=10
\]

6.3.2 Real Test Data

Each of the eighteen experimental frames were processed using the global noise model
and the optimal parameter values. A restored single frame and its profile along column
130 are shown in comparison with the initial in figure 6.12. The restored sum image and
its profile along the same column are displayed in figure 6.13. The remaining profiles
of the restored sum image are given in Appendix D for further comparison.

Table 6.2 shows the measurements of each of the assessing criteria compared with the
initial values. The assessment was performed between the initial and the restored sum
frame. The mean spatial resolution at FWHM in the initial sum image as calculated
by the 10 profiles was 189 \( \mu m \pm 7\mu m \) with a standard deviation of 59 \( \mu m \). The cor­
responding values for the restored sum image were found to be 172 \( \mu m \pm 7\mu m \) and 96
\( \mu m \) showing an improvement in mean spatial resolution at FWHM of approximately
10\%. The spatial resolution at FWTM in the initial image was 638 \( \mu m \pm 7\mu m \) with a
standard deviation of 146 \( \mu m \). The mean spatial resolution in the restored image was
583 \( \mu m \pm 7\mu m \), demonstrating an improvement of 2\%. The standard deviation was
also reduced from 146 to 96 \( \mu m \), showing that maybe there was not significant smooth­
ing. The modest improvements in the spatial resolution were expected using the global
6.3. Using a Global Noise Model

Figure 6.12: An initial and restored single frame and their corresponding profiles along column 130. The remaining white lines represent saturated pixels that have not been corrected.

noise model since it was shown that the noise was not ergodic. The uniformity of the background as measured by the standard deviation has increased by 11 %, where the dark current offset has been suppressed by 15 %. This is a relatively small improvement compared with the previous method.
Figure 6.13: Initial and restored sum image of 18 individually restored real frames and their profiles along column 130. The white dots are pixels with high intensity values that were preserved. The parameter values used were: $\lambda=90$, $\mu=0.04$, $\alpha=0.99$, $k_{max}=1400$ and $T_o=10$.

6.3.3 Conclusions

In this section, it was demonstrated that using a homogeneous noise model in the expression of $H_d(G, X, model)$ resulted in a relative improvement in the spatial resolution of the images of 10% and 2% as measured at FWHM and FWTM respectively. Additionally, the pixel intensity values of a region of background has not been sufficiently
6.3. Using a Global Noise Model

<table>
<thead>
<tr>
<th>Profile</th>
<th>FWHM Initial</th>
<th>FWHM Restored</th>
<th>Improvement (%)</th>
<th>FWTM Initial</th>
<th>FWTM Restored</th>
<th>Improvement (%)</th>
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<td>224μm</td>
<td>-3</td>
<td>646μm</td>
<td>675μm</td>
<td>+4</td>
</tr>
</tbody>
</table>

| Mean noise | 335 ADC | 283 ADC | -15 |
| St.dev. of noise | 924 ADC | 818 ADC | -11 |

Table 6.2: Comparison between the initial and restored sum frame using global noise model. The assessment criteria were the spatial resolution at FWHM and at FWTM, deduced from 10 different profiles, the background uniformity and noise reduction as defined by the standard deviation and the mean intensity value from the pixels in a region of interest away from the sample area. The error in each measurement of the spatial resolution was estimated to be ±7μm, resulting in 1-3 % uncertainty in the values of improvement. Positive values of “improvement” indicate actually worsening of the indicator value.

smoothed (improved only by 11 %) and dark current offsets have not been significantly suppressed (15 % improvement) compared to the previous method. However, it was shown that small improvements can happen. Additionally, pixels with high intensity values attributed to a high level of noise have been preserved. It was demonstrated in section 5.4.2 that the noise of the system due to dark current generation at room temperature was not ergodic so modest results were expected. In the next section the
homogeneous noise assumption will be dropped and a per pixel noise model will be used without and with correction for changes in ambient temperature.

6.4 Local Noise Model

6.4.1 Simulated Data

For the choice of optimal parameter values of this algorithm, the same procedure was followed as in section 6.3. The initial parameter values were: \( \mu = 0.05, \alpha = 0.99, k_{\text{max}} = 1700 \) and \( T_0 = 10 \).

Parameter \( \lambda \) was varied in the range 0.0001 to 10. The plot of \( MSE \) values of the restored frame versus \( \lambda \) is shown in figure 6.14. The suggested optimal range of values of \( \lambda \) then is from 0.3 to 0.7. The optimal value was set at the middle of the optimal range, which was at \( \lambda = 0.5 \).

![Figure 6.14: The calculated values for MSE for different values of \( \lambda \) in the range of [0.0001, 10], using an inhomogeneous noise model. An operating range was identified between the values of \( \lambda = 0.3 \) and 0.7 with the adopted value at \( \lambda = 0.5 \) for all subsequent experiments.](image)

The values tried for the soft threshold \( \mu \) ranged between 0.001 and 1. The calculated values of \( MSE \) are shown in figure 6.15. After the value of \( \mu = 0.4 \) the plot starts to converge to a certain value of \( MSE \), until the threshold becomes high enough so
that pixels which belong to the image of the wire are also smoothed. A range of values with acceptable level of restoration was found to be between $\mu=0.06$ and $\mu=0.1$. The optimal value was set as previously at the middle of the optimal range, which was at $\mu=0.08$.

![Figure 6.15: The calculated values for $MSE$ for different values of $\mu$ in the range of [0.001, 1], using an inhomogeneous noise model. A very low or very high threshold can lead to a non-sufficient restoration or disregard of real signal respectively. The other parameter values were: $\lambda=0.5$, $\alpha=0.99$, $k_{max}=1700$ and $T_0=10$.](image)

Having determined the parameters of the cost function, this evaluation continued to the next parameter, which was the cooling schedule, $\alpha$. The range of values tried was from 0.1 to 0.99 as well as the value of 0.999. The level of restoration for each corrected image was shown in figure 6.16 by plotting the $MSE$ for each $\alpha$. It can be observed that since the parameters $\lambda$ and $\mu$ were fixed to their optimal values, the changes of the $MSE$ were smaller indicating that parameter $\alpha$ had smaller influence in the result of restoration. However, it was clear that when $\alpha$ was increasing, $MSE$ was decreasing. When $\alpha$ was equal to 0.999 though $MSE$ was found to be 0.326, since the process was really slow. Suggested values of $\alpha$ can be either 0.98 or 0.99, but with a clear optimal at $\alpha=0.99$.

The maximum number of iterations is closely related to the cooling schedule. Having chosen a slow cooling process, one needs to adjust the maximum number of iterations,
Figure 6.16: The calculated values for $MSE$ for different values of $\alpha$ in the range of $[0.1, 0.99]$, using an inhomogeneous noise model. The different cooling schedules had smaller influence on the level of restoration compared with the previous two parameters $\lambda$ and $\mu$.

$k_{max}$ so that the algorithm would be terminated when the quality of the restored image cannot undergo further improvement. The algorithm was terminated at different levels, at various numbers of iterations between 100 and 3000. The calculated $MSE$ for each $k_{max}$ are shown in figure 6.17. As shown from the plot, $MSE$ started to converge after 1400 iterations. The relative change in $MSE$ between 1400 and 3000 iterations was only 1%. In order to judge better the optimal value for the number of iterations, the acceptance ratio $\chi(k)$ was used. Figure 6.18 includes the three plots of $\chi(k)$ versus $k_{max}$ for values within the suggested range of 1400-3000. Judging from the plots of the acceptance ratio, the optimal value for $k_{max}$ was determined to be 1700, where there was a good compromise between the quality of restoration and execution time.

The last parameter altered was the initial temperature, $T_0$ in the range of $[0.05, 100]$. The plot of $MSE$ versus $T_0$ is displayed in figure 6.19. As mentioned in the previous section, $T_0$ had the smallest influence on the results of restoration, which was also confirmed in this case. Although the relative change between the smallest and the greatest value of $MSE$ was less than 1%, there was a suggested operating range between the values of $T_0 = 0.5$ to $T_0 = 4$. The optimal value was defined at the minimum $MSE$, which was found at $T_0$ equal to 1.
6.4. Local Noise Model

Figure 6.17: The calculated values for \(MSE\) for different values of \(k_{\text{max}}\) in the range of \([100, 3000]\), using an inhomogeneous noise model. There was no significant improvement in the quality of restoration after 1400 iterations.

![Graph showing MSE vs k_max](image)

(a) \(\chi(k)\) when \(k_{\text{max}}=1400\)  
(b) \(\chi(k)\) when \(k_{\text{max}}=1700\)  
(c) \(\chi(k)\) when \(k_{\text{max}}=3000\)

Figure 6.18: The acceptance ratio \(\chi(k)\) for different \(k_{\text{max}}\) values: (a) 1400, (b) 1700 and (c) 3000 iterations. 1400 iterations might be a small number in order to terminate the algorithm, whilst 3000 iterations can guarantee convergence but the execution time was greater. A good compromise between the two was found at 1700 iterations.

A different local noise model for each CCD element was used during this set of experiments. The optimal value for each parameter was determined and they were fixed for restoring the real frames:
Chapter 6. Experimental Results

Figure 6.19: The calculated values for $MSE$ for different values of $T_o$ in the range of $[0.05, 100]$, using an inhomogeneous noise model. The suggested values can be anything between 0.5 and 4, with an optimal found at $T_o=1$.

$$\lambda=0.5, \mu=0.08, \alpha=0.99, k_{max}=1700 \text{ and } T_o=1$$

The expected performance of this algorithm when applied to the real frames is a significant reduction in the dark current offsets and good smoothing of the background fluctuation.

6.4.2 Real Test Data

As mentioned in the introduction of this chapter, each experimental frame was normalised before entering the simulated annealing algorithm in order to shorten the execution time. The mean and variance of the noise model for each pixel were also normalised by the same factor of each real frame. The optimal values of the parameters were used. The results for a single image frame are shown in figure 6.20.

Each of the restored frames was re-scaled to the 12-bit format and summed in order to form the summed restored frame. For displaying purposes, the summed image was then converted to 8-bit and displayed. The restored sum frame and its profile are shown in figure 6.21. The remaining profiles of the restored sum frame compared with the profiles from the initial sum image are shown in Appendix E.
6.4. Local Noise Model

Figure 6.20: The initial and restored single frame (No 18) and their profiles along column 130. The reduction in the mean dark current level was significant due to the individual noise model applied by the algorithm.

For assessing the quality of restoration in the real images the spatial resolution was calculated at FWHM and FWTM from 10 different profiles. The results are given in table 6.3. The mean spatial resolution at FWHM was found equal to 208 μm showing a deterioration, compared with the initial, of approximately 15 %. This might be explained due to the fact that single pixels with high intensity values due to dark current have been corrected and smoothed. Additionally, mean intensity values have been
Figure 6.21: The initial and restored sum images and their profiles along column 130. Background uniformity was dramatically improved due to the smoothing process after correcting the dark current offset.

...subtracted without any further adjustment to temperature changes, which as shown in section 3.2 can be significant. Therefore, there might be a case of overcorrecting pixels so real information was also removed alongside the noise. The spatial resolution at FWTM has been improved by approximately 10 % (from 638 \( \mu m \) to 548 \( \mu m \)) with an also significant reduction in the standard deviation of the values to 50 \( \mu m \) instead of 146 \( \mu m \). The mean dark current level has been reduced by almost 88 % and the...
6.4. Local Noise Model

background uniformity has been improved almost 9 times due to smoothing.

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<thead>
<tr>
<th>Profile</th>
<th>FWHM Initial</th>
<th>FWHM Restored</th>
<th>Improvement (%)</th>
<th>FWTM Initial</th>
<th>FWTM Restored</th>
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<td>106 ADC</td>
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Table 6.3: Comparison between the initial and restored sum frame using a local noise model. Noticeable is the significant improvement in the background uniformity, as shown by the standard deviation compared with the previous method. The error in each measurement of the spatial resolution was estimated to be ± 7μm. Positive values of "improvement" indicate actually worsening of the indicator value. The improvement values are within 1-3 % of accuracy.

6.4.3 Conclusions

Using a different noise model for each pixel requires a library of mean and variance factors for each integration time and each temperature. However, it was demonstrated that the results of restoration were better than those using a homogeneous noise model as far as the background uniformity and noise reduction were concerned. However, the
significant noise reduction indicates that some pixels with real information might have been overcorrected, due to non-adjustment of temperature drifts, so intensity values attributed to real signal may have been removed. This explains the deterioration in the spatial resolution at FWHM and the moderate improvement of the FWTM. Therefore, it was important, as mentioned in section 5.4.3, that the mean dark current offsets would be adjusted to correct for small temperature drifts.

6.5 Local noise model and mean noise values adjusted for ambient temperature.

6.5.1 Simulated Data

In this section the input frame to the algorithm of image restoration is the corrected with fixed pattern noise removal frame presented in figure 6.3c. The order of the experiments was the same as in the previous two sections. Therefore, parameter \( \lambda \) was changed first, taking values in the range of \([0.0001, 30]\). The other parameter values were: \( \mu = 0.04 \), \( \alpha = 0.99 \), \( k_{\max} = 1700 \) and \( T_o = 10 \). The plot of the MSE values is shown in figure 6.22. The optimal range for \( \lambda \) was found to be between 0.3 and 0.6. The optimal value was fixed then at \( \lambda = 0.5 \) as in the previous method, which was expected since the initial values were similar.

The range of soft threshold, \( \mu \) was between \([0.001, 1]\) as in the previous algorithm. The fact that the acceptable range of values is getting smaller than for the previous two methods, as shown in figure 6.23, shows that the method becomes more sensitive to its parameters values. The acceptable range of values for \( \mu \) suggested by the plot of MSE is from 0.07 to 0.1. Since this threshold represents intensity change between neighbouring pixels and a fixed pattern noise removal has already been applied, it is prudent to apply a small soft threshold within the optimal range in order to avoid disregarding data with real information. Therefore, the optimal value for \( \mu \) was set equal to 0.08.

The cooling schedule \( \alpha \) was changed in the range of \([0.1, 0.999]\). It can be observed that for values up to 0.80, the MSE values were very similar, which means that the
6.5. *Local noise model and mean noise values adjusted for ambient temperature.*

**Figure 6.22:** The calculated values for \(MSE\) for different values of \(\lambda\) in the range of \([0.001, 30]\), using a local noise model with the mean noise adjusted for ambient temperature.

**Figure 6.23:** The calculated values for \(MSE\) for different values of \(\mu\) in the range of \([0.0001, 1]\), using a local noise model with the mean noise adjusted for ambient temperature. A very tight range of optimal values was defined from \(MSE\) values, showing an increase in the sensitivity of the method to the values of its parameters.

Estimated pixel values may have been trapped in similar minima. Of course this can be obvious only when a slower cooling schedule is chosen, as shown by the gradual reduction of \(MSE\) after the value of 0.90. The \(MSE\) value for the very slow cooling with \(\alpha = 0.999\) was found equal to 0.327, which was 30 times worse than for \(\alpha = 0.99\).
Therefore the optimal value for $\alpha$ was set again to 0.99.

![Graph showing MSE vs alpha](image)

**Figure 6.24:** The calculated values for $MSE$ for different values of $\alpha$ in the range of $[0.1, 0.99]$, using a local noise model with the mean noise adjusted for ambient temperature. A slow cooling process was desirable in order to have guaranteed a global minimum.

The maximum number of iterations $k_{\text{max}}$ is directly related to the cooling schedule. The only reason for not having the maximum possible number of iterations was of course the long execution times. The execution time of this algorithm was approximately the same as for the other approaches with simulated annealing. The plot of $MSE$ in figure 6.25 suggests that 1000 iterations could be the minimum number of iterations for terminating the algorithm. However the acceptance ratio was checked for the different $k_{\text{max}}$ values within the optimal range (figure 6.26) in order to judge and define the optimal value, which was finally set to 1500 iterations.

The last parameter altered was the initial temperature $T_0$ and the range was from 0.05 to 100. Figure 6.27 shows the plot of $MSE$ against various values of $T_0$. The suggested operating range could be within this range, since the fluctuation of the $MSE$ values was small with the greatest deviation only 0.6% of the average level. However, a clear minimum was found at $T_0=1$.

This method has been more sensitive to the parameter values than the other approaches, as demonstrated by the tighter optimal range suggested by the plots of $MSE$. The optimal values for each parameter found and applied to the real frames are shown
6.5. Local noise model and mean noise values adjusted for ambient temperature.

Figure 6.25: The calculated values for $MSE$ for different values of $k_{max}$ in the range of [200, 3000], using a local noise model with the mean noise adjusted for ambient temperature. Convergence was reached faster, so the total execution time is shorter than for the other methods.

Figure 6.26: The acceptance ratio $\chi(k)$ for different $k_{max}$ values: (a) 1000, (b) 1500 and (c) 3000 iterations. 3000 iterations required long execution time and 1000 iterations was the minimum number of iterations with an acceptable restoration. Therefore, the maximum number of iterations was fixed at 1500.

below:

$$\lambda=0.5, \mu=0.08, \alpha=0.99, k_{max}=1500 \text{ and } T_0=1$$

The expected performance of this algorithm applied to the real data is a reduction in
Figure 6.27: The calculated values for $MSE$ for different values of $T_0$ in the range of $[0.05, 100]$, using a local noise model with the mean noise adjusted for ambient temperature. The greatest fluctuation from the average $MSE$ value was only by 0.6%, so the whole range can be an operating range. However, there was a minimum value at $T_0 = 1$ and therefore the optimal value was set to 1.

the dark current offsets and a smoothing in the fluctuation of the pixel intensity values.

6.5.2 Real Test Data

There was a lower limit fixed for the intensity values after the fixed pattern noise removal in order to prevent overcorrection of pixels. In figures 6.28 and 6.29 the restored single and summed images are shown in addition to their profiles along column 130. The plots of the 10 remaining profiles along the restored sum image are given in Appendix F.

The measurements of the assessment criteria concerning spatial resolution, uniformity and background reduction are given in table 6.4. As it was expected there was a significant reduction in the mean dark current offset of 86%. Comparing the remaining dark current between the local noise method and this method, it can be observed that in the latter case it was slightly greater by 5 ADC units, representing an increase of 12.5% which demonstrates the result of the temperature drift between the image and the blank scan acquisition. The effect of this adjustment were also reflected in the improved values of the spatial resolution at FWHM and FWTM.
6.5. Local noise model and mean noise values adjusted for ambient temperature.

Figure 6.28: The initial and restored single images and their profiles along column 130. From the profiles it is shown that there is a reduction in the dark current offset with an additional smoothing, compared with the initial profile.

By visual inspection we can see that the image is much sharper, with more data shown in the 'T-shape' area. The mean spatial resolution of the 10 profiles at FWHM was improved to 124 μm, a reduction of 33 % with the standard deviation of the measurement down to 44 μm, although this was improved to 27 μm, when an outlier measurement was excluded. Similarly, in the mean spatial resolution measured at FWTM there was a reduction of 37 %, demonstrating a mean spatial resolution of 391 μm. This significant
Figure 6.29: The initial and restored sum images using a local noise model with the noise mean adjusted for ambient temperature. A relative improvement of 33% was found for the spatial resolution at FWHM.

improvement in the spatial resolution both at FWHM and FWTM is an indicator that thermal adjustments are important in the mean dark current value of each pixel.

The standard deviation demonstrated a smoothing of approximately one order of magnitude (90% improvement), which was similar with the uniformity shown in the previous method using the local noise model only. Therefore, a good adjustment of the mean dark current values of each pixel and simulated annealing may result in an im-
6.5. Local noise model and mean noise values adjusted for ambient temperature.

age with improved spatial resolution, increased background uniformity and significant suppression of the background dark current.

<table>
<thead>
<tr>
<th>Profile</th>
<th>FWHM Initial</th>
<th>FWHM Restored</th>
<th>Improvement (%)</th>
<th>FWTM Initial</th>
<th>FWTM Restored</th>
<th>Improvement (%)</th>
</tr>
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<tr>
<td>120</td>
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<td>103 μm</td>
<td>-52</td>
<td>787 μm</td>
<td>400 μm</td>
<td>-49</td>
</tr>
<tr>
<td>123</td>
<td>175 μm</td>
<td>160 μm</td>
<td>-8</td>
<td>576 μm</td>
<td>457 μm</td>
<td>-20</td>
</tr>
<tr>
<td>125</td>
<td>153 μm</td>
<td>110 μm</td>
<td>-28</td>
<td>668 μm</td>
<td>323 μm</td>
<td>-51</td>
</tr>
<tr>
<td>128</td>
<td>153 μm</td>
<td>146 μm</td>
<td>-4</td>
<td>520 μm</td>
<td>449 μm</td>
<td>-13</td>
</tr>
<tr>
<td>130</td>
<td>103 μm</td>
<td>20 μm</td>
<td>-80</td>
<td>365 μm</td>
<td>153 μm</td>
<td>-58</td>
</tr>
<tr>
<td>133</td>
<td>224 μm</td>
<td>160 μm</td>
<td>-28</td>
<td>843 μm</td>
<td>400 μm</td>
<td>-52</td>
</tr>
<tr>
<td>135</td>
<td>298 μm</td>
<td>132 μm</td>
<td>-55</td>
<td>647 μm</td>
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<td>-40</td>
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<td>138</td>
<td>224 μm</td>
<td>125 μm</td>
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<td>794 μm</td>
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<td>-49</td>
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<tr>
<td>140</td>
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<td>110 μm</td>
<td>-6</td>
<td>534 μm</td>
<td>428 μm</td>
<td>-19</td>
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<tr>
<td>143</td>
<td>231 μm</td>
<td>182 μm</td>
<td>-21</td>
<td>646 μm</td>
<td>513 μm</td>
<td>-20</td>
</tr>
</tbody>
</table>

Table 6.4: Comparison between the initial and restored sum frame using fixed pattern noise removal and local noise model. The spatial resolution is shown at FWHM and at FWTM and measurement in background uniformity and noise reduction such as the standard deviation (St.dev.) and mean in a region of interest away from the active area. The improvement in all assessment criteria was significant. The error in each measurement of the spatial resolution was estimated to be ± 7 μm, resulting in 1-3 % uncertainty in the values of improvement. Positive values of “improvement” indicate actually worsening of the indicator value.

6.5.3 Conclusions

This approach had combined the advantages of two methods: the fixed pattern noise removal where the dark current offsets were adjusted for ambient temperature, and im-
age restoration using a local noise model, where the smoothing of pixel intensity values below a soft threshold reduced the extra fluctuations in the generated dark current. The combined approach demonstrated an excellent performance as far as background uniformity was concerned (the standard deviation decreased by approximately a factor of 10). The spatial resolution at FWHM and FWTM were also significantly improved since the dark current offsets more effectively compensated than when using the local noise model only. However, this method was more sensitive to the parameter values than the previous ones. In order to achieve good image quality the parameter values used have to be the optimal. It is easier for the user to set the cooling schedule, the maximum number of iterations and the initial temperature, since a slow cooling process can be set ($\alpha=0.99$), a sufficient number of iterations (i.e. $k_{\text{max}}=2000$) and an initial temperature between 1 and 15. However, the weighting factor $\lambda$ and the soft threshold $\mu$ are more difficult to be determined. One can decide about the threshold level $\mu$ and then the weighting factor $\lambda$ can be more easily determined by plotting the two components of the cost function for each iteration and choosing $\lambda$ by trial and error so that the two components of the cost function remain roughly equal for all iterations.

6.6 Local Noise Model, with temperature adjustment for the mean dark current and modelling of blurring effects.

6.6.1 Simulated Data

A more sophisticated approach for restoring images may model the blurring effects of the system. In this case the point spread function was defined using the experimental data. Because this method was computationally very expensive compared to the previous algorithms due to the convolution with a large size mask, for the evaluation the simulated image was cropped around a region of interest that included the horizontal wire with dimensions $46 \times 46$. This did not affect the results of the evaluation or the accuracy
6.6. Local Noise Model, with temperature adjustment for the mean dark current and modelling of blurring effects.

in determining the optimal values. The starting parameter values were: $\mu = 0.05$, $\alpha = 0.99$, $k_{\text{max}} = 1200$ and $T_0 = 10$.

![Graph](image.png)

**Figure 6.30:** The calculated values for $MSE$ for different values of $\lambda$ in the range of $[0.001, 16]$, using noise mean adjusted for ambient temperature and image restoration taking into consideration the point spread function of the system. A single optimal value was clearly found at $\lambda = 0.8$.

The range of values under investigation for parameter $\lambda$ was from 0.001 to 16. From the plot of $MSE$ values shown in figure 6.30, the suggested optimal range for $\lambda$ is between 0.6 to 1, when a small deviation from the minimum is allowed. However, a very clear single minimum exists at point $\lambda = 0.8$ and thus the optimal value was set at $\lambda = 0.8$. This algorithm has shown an increased sensitivity to parameter $\lambda$ compared with the previous algorithms.

Having determined the optimal value for $\lambda$ the evaluation continued to the next parameter, $\mu$. The range of investigated values was $[0.001, 0.3]$ and the plot of the calculated $MSE$ values is displayed in figure 6.31. The suggested range as observed from the graph is from 0.01 to 0.06. However, a single minimum exists at $\mu = 0.05$ and therefore the optimal value was fixed at this value.

The cooling schedule $\alpha$ was altered within the same range of $[0.1, 0.99]$ in order to investigate the sensitivity of the algorithm and observe its performance. The big fluctuation in the values of $MSE$ confirmed for once more the fact that during a fast cooling process, simulated annealing can be trapped in local optima. However, since
Chapter 6. Experimental Results

Figure 6.31: The calculated values for $MSE$ for different values of $\mu$ in the range $[0.001, 0.3]$, using a local noise model with the noise mean adjusted for ambient temperature and accounting for the point spread function. A single minimum of $MSE$ exists at $\mu=0.05$.

The global minimum was desired, the value of $\alpha$ was set equal to 0.99.

Figure 6.32: The calculated values for $MSE$ for different values of $\alpha$ in the range of $[0.1, 0.99]$, using a local noise model with the noise mean adjusted for ambient temperature and accounting for the point spread function of the apparatus. An optimal value was set to $\alpha=0.99$.

The maximum number of iterations $k_{\text{max}}$ that the algorithm would be terminated at was important to be selected in the optimal point. The reason was that this algorithm was at least 40 times slower than the previous approaches when executed under the
6.6. **Local Noise Model, with temperature adjustment for the mean dark current and modelling of blurring effects.**

same computer specifications (6 hours for this approach per image, where the previous approaches took only for 9 minutes for 1200 iterations). A very slow algorithm cannot be realistically applicable but at the same time, convergence was necessary. $k_{\text{max}}$ was altered from 100 to 1200 iterations with a step of 100. The calculated $MSE$ values are shown in figure 6.33. From the plot of $MSE$ it was found that 800 iterations was the minimum numbers of iterations to terminate the algorithm. The acceptance ratio was calculated again as shown in figure 6.34. The optimal value for $k_{\text{max}}$ was then set to 1200 iterations so there was compromise between execution time and quality of restoration.

![Graph showing Mean Square Error (MSE) vs. Parameter $k_{\text{max}}$](image)

**Figure 6.33:** The calculated values for $MSE$ for different values of $k_{\text{max}}$ in the range of [100, 1200], using a local noise model with the noise mean adjusted for ambient temperature and accounting for the point spread function of the imaging system. Convergence was reached after 800 iterations.

The last parameter altered was $T_0$ in the range from 0.05 to 100. It was mentioned previously that the parameter with the lowest influence should not be considered as negligible. This concept was demonstrated better here where, as shown in figure 6.35, the fluctuation between the two extreme values of $MSE$ was 5.54 %, which can be considered as significant for this parameter. The suggested values for $T_0$ was between 10 and 15, with a clear minimum $MSE$ at $T_0=10$.

The optimal values for each parameter were determined to be:
Figure 6.34: The acceptance ratio $\chi(k)$ for different $k_{\text{max}}$. The optimal value was set at 1200 iterations because from the $\chi(k)$ values it was found that less than 1200 iterations were not adequate.

Figure 6.35: The calculated values for $MSE$ for different values of $T_0$ in the range of $[0.05, 100]$, using noise mean value adjusted for ambient temperature and image restoration accounting for the point spread function of the system. The clear optimal was found at $T_0=10$.

$\lambda = 0.8$, $\mu = 0.04$, $\alpha = 0.99$, $k_{\text{max}}= 1200$ and $T_0= 10$.

For further comparison, the initial cropped simulated image and the restored are presented in figure 6.36. Comparing the two images it is noticeable that the pixels with intensity values lower than the threshold have been smoothed. Additionally, there were
6.6. **Local Noise Model, with temperature adjustment for the mean dark current and modelling of blurring effects.**

fewer pixels representing the wire in the restored image than in the initial, which means an improvement in spatial resolution. In the next section, the results of the algorithm on the real frames are presented.

![Initial simulated image](a) Initial simulated image ![Restored simulated image](b) Restored simulated image

**Figure 6.36:** The initial cropped simulated image in comparison with the restored simulated image using the optimal values for each parameter. A background smoothing is noticeable as well as an improvement in spatial resolution.

### 6.6.2 Real Test Data

Each of the experimental frames corrected with fixed pattern noise removal was cropped in order to have the same dimensions as the previous simulated image, so the effects of the method would be demonstrated in a reasonable execution time. The restored single image is shown in comparison with the initial single image in figure 6.37. Because the image was cropped new profiles of the initial cropped sum image were taken from column 14 to 32, with a step of 2 which are given in Appendix G. The profile shown in figures 6.37 and 6.38 is along column 20. The remaining profiles of the restored sum image are given in Appendix G.

What is noticeable from the images in figure 6.37 is a decrease in the length of the wire. This effect is due to border effects of the algorithm. Additionally, the two blots-like formation is due to the fact that the pixel values of the wire were not necessarily high
Figure 6.37: The initial cropped single image in comparison with the restored single image. The non-uniformity of the activity along the wire is shown clearly in the left image. The pixel values which were restored are within the white box.

Figure 6.38: The profiles along column 20 of the initial cropped single image and from the restored single image.

intensity values (the activity was not necessarily uniform as shown in figure 6.37). A suppression of the dark current offsets and a smoothing are also demonstrated by the
6.6. Local Noise Model, with temperature adjustment for the mean dark current and modelling of blurring effects.

profiles in figure 6.38. The initial and restored sum image are shown in figure 6.39 and the corresponding profiles along column 20 in figure 6.40.

![Initial sum image](image1)
![Restored sum image](image2)

**Figure 6.39:** The initial cropped sum image in comparison with the restored sum image. There is a noticeable reduction in the wire dimensions that is due to the border effects of the deblurring mask. The pixel values which were restored are located within the white box.

![Initial sum profile](image3)
![Restored sum profile](image4)

**Figure 6.40:** The profile of the initial sum image in contrast with the corresponding one of the restored sum image.
The spatial resolution was measured at FWHM and FWTM at both the initial and restored cropped sum images along column 20 and the results are given in table 6.5. The mean spatial resolution at FWHM was improved by 6% (from 194 \( \mu m \) to 172 \( \mu m \)) and at FWTM was improved by 50% (from 539 \( \mu m \) to 264 \( \mu m \)). However, in many profiles there was a deterioration in the spatial resolution at FWHM and additionally, it was observed that the pixel intensity values remained at the same level of intensity, although the fixed pattern noise removal method applied, so dark current offsets were already subtracted. These results indicate may that the restoration process was not complete and the algorithm was trapped in a local minimum. However, if the number of iterations was extended the execution time would be very long and therefore not realistically applicable.

<table>
<thead>
<tr>
<th>Profile</th>
<th>FWHM Initial</th>
<th>FWHM Restored</th>
<th>Improvement (%)</th>
<th>FWTM Initial</th>
<th>FWTM Restored</th>
<th>Improvement (%)</th>
</tr>
</thead>
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<tr>
<td>14</td>
<td>178( \mu m )</td>
<td>262( \mu m )</td>
<td>+47</td>
<td>594( \mu m )</td>
<td>305( \mu m )</td>
<td>-48</td>
</tr>
<tr>
<td>16</td>
<td>170( \mu m )</td>
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<td>600( \mu m )</td>
<td>321( \mu m )</td>
<td>-46</td>
</tr>
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<td>321( \mu m )</td>
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<tr>
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<td>551( \mu m )</td>
<td>256( \mu m )</td>
<td>-53</td>
</tr>
<tr>
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<td>546( \mu m )</td>
<td>278( \mu m )</td>
<td>-49</td>
</tr>
<tr>
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<td>-65</td>
<td>514( \mu m )</td>
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</tr>
<tr>
<td>26</td>
<td>197( \mu m )</td>
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</tr>
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<td>+2</td>
<td>509( \mu m )</td>
<td>235( \mu m )</td>
<td>-53</td>
</tr>
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Table 6.5: Comparison between the initial and restored cropped sum frames. The spatial resolution was measured at FWHM and at FWTM. Measurement of the background uniformity was not possible because the region of interest (cropped image) was within the active area of the wire. The error in each measurement of the spatial resolution was estimated to be \( \pm 7\mu m \). Positive values of “improvement” indicate actually worsening of the indicator value.

The background uniformity was not measured because the cropped image was mostly
within the active area of the wire. However, there was a visual improvement in the background pixels.

6.6.3 Conclusions

This approach included the point spread function in the expression of the cost function. Because the blurring mask used was a two-dimensional mask of size $27 \times 27$ the execution time increased significantly in comparison with the previous algorithms (40 times slower per iteration). There was a general improvement in the spatial resolution both at FWHM and FWTM although in the first case, some measurements showed the opposite. This was attributed to the fact that the restoration process might have terminated early but also it could be an error due an inaccurate definition of the point spread function. Therefore, including the point spread function in the cost function may improve the spatial resolution and, judging by visual inspection, the uniformity of the background. However, there are limitations such as the long execution time of the algorithm that do not allow practical use of the algorithm in the case of digital autoradiography, where the number of frames might be up to 2000 or more.

6.7 Conclusions

The purpose of this chapter was to investigate different approaches in reducing the noise of simulated and real autoradiographic images of high particle flux. There were five methods developed in the previous chapter and an evaluation of each of them was performed in this chapter in order to show the validity of each method and to determine the breaking points and the optimal values of each parameter.

The approach of using a homogeneous noise model was less sensitive to the parameter values than any other method, but at the same time there was not significant improvement in the spatial resolution or sufficient smoothing in the fluctuation of the background intensity. Having confirmed that the noise was not ergodic, different noise models were applied to each CCD element. With this approach the uniformity of the background was improved by a factor of 9. However, broken pixels that do not follow
The defined normal-like distribution remained in the restored image because they were misclassified as pixels with useful information and therefore preserved.

The fixed pattern noise removal demonstrated that it can suppress the noise levels efficiently but without achieving any smoothing. A reasonable approach was to combine the two methods of fixed pattern noise removal and image restoration using a local noise model. The former method can reduce the dark current offsets, correcting also for small shifts in ambient temperature between blank scan and image acquisition, whilst the latter can effectively smooth the residual fluctuation. With this approach, a relative improvement of up to 33 % was achieved in the spatial resolution (at FWHM). Additionally, the fluctuation of the background pixels was reduced by a factor of ten. The execution time of this method was shorter than in the previous approaches because the desired image quality was reached faster due to pre-processing.

Finally, the point spread function of the system was included in the expression of the cost function. During this evaluation, it was demonstrated that the deblurring process may improve the spatial resolution of the image. However, this algorithm was computationally very expensive and may not be suitable for applications that require the restored images in almost real time. Lastly, determination of the point spread function should be as accurate as possible since the results can be significantly affected.

For further comparison between the methods, the mean spatial resolution at FWHM and FWTM of the four first methods are presented in table 6.6. The standard deviation of the mean spatial resolution is also included.
6.7. Conclusions

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Initial</th>
<th>FPN</th>
<th>SA Global</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean FWHM</td>
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<td>183 ± 7μm (0 %)</td>
<td>172 ± 7μm (-10 %)</td>
</tr>
<tr>
<td>Mean FWTM</td>
<td>638 ± 7μm</td>
<td>589 ± 7μm (-7 %)</td>
<td>583 ± 7μm (-2 %)</td>
</tr>
<tr>
<td>σ of FWHM</td>
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<td>39μm (-34 %)</td>
<td>67μm (+14 %)</td>
</tr>
<tr>
<td>σ of FW TM</td>
<td>146μm</td>
<td>98μm (-33 %)</td>
<td>96μm (-34 %)</td>
</tr>
</tbody>
</table>

Table 6.6: Comparison between the initial and restored sum frame. The relative improvement between the restored sum image and the initial is included inside the brackets. The abbreviations are: FPN for fixed pattern noise removal, SA Global for simulated annealing using a global noise model, SA Local for simulated annealing using a local noise model and FPN+SA Local for fixed pattern noise removal with simulated annealing using the local noise model. Clearly, the last method demonstrated the best performance compared with the former ones presented in this table.
Chapter 7

Tissue Imaging

The ultimate test for any method developed in the area of digital autoradiography imaging is to examine the results of imaging a distributed source. In this chapter the image processing method suggested in section 4.4 for low particle flux images, such as real tissue images is tested on a set of three mouse brain sections labelled with $^{35}$S.

7.1 Description of the images

The samples represent three mouse brain sections from the level of the caudate (bregma 1.34 mm [31]). The three coronal sections approximately 10 $\mu$m thick were incubated in a buffer containing 1 mMol$^1$ GDP (guanosine-dephosphate [37]) and 0.04 nMol $[^{35}\text{S}]$GTP$\gamma$S (guanosine-5'-0-(3-$[^{35}\text{S}]$thio) triphosphate. Using the radioisotope $^{35}$S for labelling the G-protein allows detection of its distribution and quantification of the binding concentration in different cerebral regions in a mouse model. The three different mouse brain sections were initially held in direct exposure with film (Hyperfilm-$\beta$max provided by Amersham, UK) for 4 days. This film has an increased sensitivity and resolution compared to standard X-ray film and is designed for direct autoradiography of most $\beta$-emitting radioisotopes. Then the images were digitised into 8 bit format using a Nixon XC-77CE CCD camera and MCID image analysis software by Imaging Research, Canada. The three different film images are shown in figure 7.1. The activity

$^1$I Mol=1 mole per litre.
of the samples was initially approximately 81 nCi (~3 kBq) per section but the exact activity contained in the tissue was not known due to the procedure of washing out and drying. The relative loss in activity due to radioactive decay between the end of exposure of the film images\textsuperscript{2} and acquisition of the CCD-based images was calculated to be 7.4 \%, corresponding to 10 days interval between the two imaging sessions.

7.2 Results and Discussion

The three mouse brain sections were attached on one microscopic slide and placed directly on the CCD surface. The integration time used was 15 seconds per frame. Composite images were obtained from data sets containing 650 frames, which represents a total exposure time of 2 hours and 42 minutes. Frames were acquired at two different temperatures: 10 °C and 22 °C for comparison. The method used for correction is the fixed pattern noise method presented in the previous chapter (equations 4.10 and 4.16). The post processing procedure remained the same as described in section 4.3. However, this case despite similar activities with the microscales, the activity spread out over many more pixels. Therefore, the average number of events per pixel remained very low. Each frame was corrected separately. The results are shown in figures 7.2 and 7.3 for the two different temperatures. A different colormap is used for better visualisation and contrast between the hot (red) and cold (blue) areas.

This study represents a particularly challenging imaging problem: the biological section of interest is to determine whether G-protein binds or not to structures in and around the caudate. This is because this particular problem is used for indicating the penetration of new drugs into the caudate area of the brain. From an imaging perspective, the challenging aspect of this study arises because G-protein tends to bind throughout the mouse brain section albeit at slightly different concentrations in different structures. Therefore, to determine binding in the caudate, the observer is looking for either higher or low activities compared to the surrounding structures. Thus, the result may be an

\textsuperscript{2} The preparation of the tissue samples, film acquisition and digitisation were performed by Alexis Bailey from the School of Biomedical and Life Sciences and Chemistry and they were made available prior to publication.
7.2. Results and Discussion

Figure 7.1: Computer enhanced autoradiographs of coronal brain sections from mouse labelled with $^{35}$S. The yellow areas in the tissue demonstrate low binding of the G-protein. Total exposure time: 4 days.

From a radioisotope imaging perspective, whilst selecting 'hot spot' imaging is a standard practice, detecting 'hot spot' against high background is often challenging, and detecting cold spots in a hot background is of-
Figure 7.2: Images of mouse brain sections labelled with $^{35}$S corrected with the fixed pattern noise removal method 2. The structures of interest are the 'cold' areas such as the two spots on the top of each tissue section and in the periphery. Each composite image was composed of 650 frames of 15 seconds exposure acquired at 10 °C. Red areas represent high concentration whereas dark areas represent low or no binding of the G-protein. The initial composite images are shown on the left and the corrected images on the right.

ten very difficult. The cold structures are the most difficult to image by the CCD-based imaging system due to the high level of dark current at room temperature. As it can
7.2. Results and Discussion

Figure 7.3: Images of mouse brain sections labelled with $^{35}$S corrected with the fixed pattern noise removal method 2. Each initial composite image was composed of 650 frames of 15 seconds exposure acquired at 22 °C. The total exposure time was 2 hours and 42 minutes. The initial composite images are shown on the left and the corrected images on the right.

be observed from figures 7.2 and 7.3 the raw composite images were totally dominated by thermal dark current effects. Although the correction method was checked in low flux emission images with poor signal to noise ratio, it has showed good performance and recovered the binding and non-binding areas of the G-protein in only a fraction of
the time (36 times faster) compared to conventional exposure with film imaging, albeit not unexpectedly, with some loss of contrast.

### 7.3 Conclusions

In this chapter the fixed pattern noise removal method was applied to three real mouse brain sections labelled with $^{35}$S. Initial images obtained by the CCD-based imaging system demonstrated that dark current effects dominate the data when samples of approximately few kBq or less are imaged. This can be understood from the fact that any given pixel can be expected to contain data only in a fraction of all image frames. Data sets were obtained at two different temperatures of $\approx 10 \, ^\circ \text{C}$ and $\approx 22 \, ^\circ \text{C}$. In both cases, the structures were not visible in the initial composite images. Applying the fixed pattern noise removal method 2 (section 4.4), which was developed for correcting images with very poor signal to noise ratio, the three different functional images were visible and the 'cold spots' could be recognised. Additionally, the CCD-image acquisition lasted only a small fraction of time (36 times faster) compared with the exposure time of the film images (2 hours and 42 minutes compared with 96 hours). Therefore, it has demonstrated that the CCD-based imaging system combined with the fixed pattern noise removal method 2 can image low particle flux images of distributed samples and it can result in a faster qualitative analysis about the penetration of the radiolabelled molecule into the target region (caudate in this case) and a relative quantitative analysis about the different penetration levels (concentration).
Chapter 8

Conclusions and Future Work

8.1 Overview and general discussion

Conventional autoradiography has many disadvantages such as lack of sensitivity, limited dynamic range and long exposure times. However, film autoradiographs demonstrate the best spatial resolution for imaging at the cellular level. A wide variety of technological developments can now produce systems capable of producing fast, real-time, high dynamic range digital autoradiograms, though none of these is able to replicate the exquisite sub-cellular resolution achievable with film emulsion techniques. Independently of this work, other researchers have undertaken derivation of image enhancement and image restoration methods for use in autoradiography. The novel step in this thesis is to bring together techniques from both areas in order to allow elevated temperature operation of a hybrid CCD autoradiography imaging system.

The literature review presented in chapter 2 includes two approaches to autoradiography: either improving the hardware characteristics of the current systems or performing image processing methods such as image enhancement and image restoration to digitised film images. This thesis is concerned with the problem of improving the quality of images obtained directly by a CCD-based digital autoradiography imaging system by applying image processing techniques. The aim of the work was to operate the CCD-based imaging system by MacDonald [70] at room and human body temperature and to develop image correction methods for the thermal effects of dark current. A brief
description of the CCD technology and operation was included in chapter also in chapter 2. The prototype imaging CCD system was described further and its performance was presented under cooled conditions.

In Chapter 3 the issues concerning room temperature image acquisition were then explained in more detail and the main problems were stated. At room temperature the level of dark current increases significantly and the signal to noise ratio becomes very poor. Improvement of the signal to noise ratio can be achieved by exposing the image for longer times. However, with a CCD system, the exposure time is limited due to the fact that the pixels reach saturation very quickly when operating at room temperature. In chapter 3 it was shown that the optimal exposure time per frame is between 10 and 20 seconds maximum.

Calibrated microscales labelled with \textsuperscript{14}C used for imaging showed the linearity of the system over two orders of magnitude in chapter four. There are various approaches which can be used for correcting for pixel non-uniformity including blank frame subtraction, individual line correction and flat field corrections [43]. However, the major problem in this application is caused by thermal noise, which previous methods do not need to address (as integration times are shorter and signal to noise ratio higher). Thus, a fixed pattern noise removal was performed here by modelling the noise distribution of each pixel individually, and correcting for changes in ambient temperature. Empirical formulae developed for adjusting the fixed pattern noise parameters for ambient temperature changes showed that the sensitivity of the system can be improved by up to 15 \%, with a further reduction in background noise of up to 55 \%.

Chapters 5 and 6 presented the methodology developed in an attempt to improve the quality of the images by applying image processing techniques. It was shown that the dark current noise in an image frame is not ergodic. The behaviour of dark current of individual pixels was examined over many blank frames and it was shown that the dark current of each pixel follows a normal-like distribution but with different mean and variance for each pixel. A global optimisation approach taking into consideration the non-ergodic nature of noise, the smoothness constraint and the blurring function of the imaging device was presented. The inclusion of the model of the blurring function
8.2. Further work

proved to be too expensive computationally, and it had to be abandoned. The reason being the type of image from which it was modelled: the wire frame that was imaged did not touch firmly on the device, so its activity was spread in a way that is not necessarily repeatable. Soft tissue that sits better on the device is bound to produce much less spread and therefore require a model blurring mask of much smaller dimensions. A smaller blurring mask may not slow the program too much and it may prove a very useful part of the methodology. (The results on the small images used to evaluate the method were good.)

In chapter 6 extensive evaluation of the methods developed showed that an improvement of 90% can be achieved in background noise suppression, while the spatial resolution at FWHM was improved by 33% and at FHTM by 37% using a local noise model with the noise adjusted for ambient temperature.

The cold structures of three brain sections studied in chapter 7, which are the most difficult to image by digital systems, were clearly shown in the corrected images, demonstrating the fact that room temperature image acquisition of low activity distributed sources can be performed by the CCD-based imaging system applying image processing methods.

8.2 Further work

Further work can be carried out towards the acceleration of the algorithm that takes into consideration the blurring function in the expression of the cost function using simulated annealing, which was proved to be computationally very expensive, although it provided good results.

Future work can be developed in dual-isotope imaging at room temperature by performing linear unmixing of the two spectra. Similar work has been carried out by Kvinnsland and Skretting [55], where they tried to separate the contributions of two linearly mixed radioisotopes. The task of linear unmixing is common in remote sensing [16], [17] but it has not been introduced yet in digital autoradiography. An exhaustive search and a gradient descent method could be implemented in order to separate counts
or events generated from each radioisotope so dual-imaging quantitative analysis could be possible.

Another area where this research can be extended to is for imaging line structures in tissue images. It is known that the structure of an image can be locally approximated by a Taylor expansion. The first order coefficients of this expansion constitute the structure tensor and the second order coefficients make up the Hessian matrix. Use of the structure tensor or the Hessian matrix and their eigenvalues to determine the local image structure are found in many application areas such as vessel enhancement of 3D medical digital images [89] or in identifying horizons in seismic data [15]. Similarly, a model based on the eigenvalues of the Hessian matrix can be developed for line preserving structures and used in comparison to the membrane model.
Appendix A

A.1 Schematic diagram of the cryostat

Figure A.1: Schematic diagram of the cryostat.
Appendix B

B.1 Specifications of the CCD imaging system

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<th>Specification</th>
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</tr>
</thead>
<tbody>
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<td>$17.3 \text{ mm} \times 25.9 \text{ mm}$</td>
</tr>
<tr>
<td>Pixel format</td>
<td>$826 \times 1152$</td>
</tr>
<tr>
<td>Pixel pitch</td>
<td>$22.5 \ \mu\text{m} \times 22.5 \ \mu\text{m}$</td>
</tr>
<tr>
<td>Total sensitive thickness</td>
<td>$20 \ \mu\text{m}$</td>
</tr>
<tr>
<td>Depletion region thickness</td>
<td>$7 \ \mu\text{m}$</td>
</tr>
<tr>
<td>Peak signal</td>
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</tr>
<tr>
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Appendix C

C.1 The 10 profiles of the corrected sum image using only fixed pattern noise removal method

Figure C.1: Profiles 120 and 123 of the initial (left) and the corrected (right) sum image using fixed pattern noise removal.
Figure C.2: Profiles 125, 128, 130 and 133 of the initial (left) and corrected (right) sum image.
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