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Restoration of Scatter Blurred Video Fluorescence Micrography

by

Omar S. Sheshah, B.Sc.

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of
Master of Engineering

Ottawa-Carleton Institute for Electrical Engineering
Faculty of Engineering
Department of Systems and Computer Engineering
Carleton University,
Ottawa, Ontario, Canada, K1S 5B6

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**“Restoration of Scatter Blurred Video Fluorescence
Micrography”**

Submitted by

Omar S. Sheshah, B.Sc.

**in partial fulfillment of the requirements
for the degree of Master of Engineering.**

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November 7, 1994

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Abstract

Linear minimum mean-square error deconvolution filtering of biological images acquired through video microscopy has been investigated. The images are of cellular structures stained with fluorescent dyes causing a glowing aura which distorts the image. The major problem is that the true images are forever unknown, so that the distortion model can only be estimated. The glow is modelled from fluorescent plastic beads. Issues concerning the estimation of the model parameters and the consequence of using inaccurately-estimated parameters in optimally-derived Wiener filter restoration are investigated.

The derivation of the linear Wiener filter, implemented in the frequency domain, incorporating the presence of additive white noise is provided. Several examples of frequency domain Wiener filtering are examined, and significant improvements in image quality are demonstrated. Wiener filtering is also implemented in the spatial domain by convolving the image with a truncated Wiener kernel. However, very large kernels are required to compensate for the distortion introduced by the scatter blur, so that spatial domain restoration can be very impractical.

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To my beautiful loving mother

Table of Contents

Acceptance Sheet	ii
Abstract	iii
Acknowledgements	iv
List of Tables	viii
List of Figures	ix
Glossary	xi
1 Introduction	1
1.1 Background	1
1.2 Organization of the Thesis.	3
2 Image Characterization and Modeling	4
2.1 Introduction	4
2.2 Source of Images	4
2.2.1 Fluorescence Labeling of Cell Images	5
2.2.2 Fluorescence Microscope Imaging System.	7
2.2.3 Microbead Images.	10
2.2.4 Cell image	11
2.3 Blur Formation Model	12
2.4 Modelling of Distortion.	14
2.5 Observation Noise	20
3 Traditional Restoration Techniques	22
3.1 Introduction	22
3.2 Problems, Solutions, and Algorithms	22
3.3 Inverse Filter	23
3.4 Wiener Filter	27
4 Wiener Filtering In the Frequency Domain	30
4.1 Introduction	30

4.2 Implementation	31
4.3 Results and Analysis	37
4.3.1 Bead Experiment.	42
4.3.2 Cell Image Example	54
4.3.3 Microtubule Image Example.	59
5 Wiener Filtering In the Spatial Domain	62
5.1 Introduction	62
5.2 Implementation	63
5.3 Results and Analysis	68
6 Conclusions	76
6.1 Summary of Results	76
6.2 Proposals for Future Research.	78
Bibliography	82

List of Tables

TABLE 5.1 Computational load for various square convolution kernels	71
TABLE 5.2 Energy in truncated Wiener kernel and computation time	72

List of Figures

FIGURE 2.1 Original Cell Image.....	6
FIGURE 2.2 Fluorescence Microscope Imaging System. Methods in Cell Biology [19].	9
FIGURE 2.3 (a) Bead image (b) Cross section profile.....	10
FIGURE 2.4 (a) Cell image (b) Cross section profile	11
FIGURE 2.5 Linear Blurring Model	12
FIGURE 2.6 cross section profiles of different bead images	15
FIGURE 2.7 Curve Fitting of the two degradation PSF models.....	16
FIGURE 2.8 (a) Point Spread Function (PSF). (b) PSF transform.....	18
FIGURE 2.9 (a) Point Spread Function (PSF). (b) PSF transform	19
FIGURE 3.1 Linear Blurring Model.....	24
FIGURE 3.2 Linear Blurring Model with an additive noise term.....	25
FIGURE 4.1 Linear Blurring and Restoration Model	31
FIGURE 4.2 Implementation of the Wiener filter in the Fourier domain	36
FIGURE 4.3 Wiener TF plots. (a) Low Noise (b) Medium Noise (c) High Noise	39
FIGURE 4.4 Wiener TF plots. (a) Low Noise (b) Medium Noise (c) High Noise	40
FIGURE 4.5 Wiener TF plots.....	41
FIGURE 4.6 Filtered beads and profiles. Fixed $P_2 = 1.8$. Low Noise.....	44
FIGURE 4.7 Filtered beads and profiles. Fixed $P_1 = 0.8$. Low Noise.....	46
FIGURE 4.8 Filtered beads and profiles. Fixed $P_2 = 1.8$. Medium Noise	47
FIGURE 4.9 Filtered beads and profiles. Fixed $P_1 = 0.8$. Medium noise.....	48
FIGURE 4.10 Filtered beads and profiles. Fixed $P_2 = 1.8$. High Noise	51
FIGURE 4.11 Filtered beads and profiles. Fixed $P_1 = 0.8$. High Noise	52
FIGURE 4.12 Filtered beads and profiles. Medium noise	53
FIGURE 4.13 Filtered Cells and profiles. (a) Low noise (b) Medium noise (c) High noise	55
FIGURE 4.14 (a) Original cell image (b) Filtered cell image (optimum parameters) ..	57
FIGURE 4.15 Filtered Cells and profiles. Medium noise	58

FIGURE 4.16 Filtered Microtubule and profiles. Medium noise	60
FIGURE 5.1 Wiener filter spatial and frequency domain processing	63
FIGURE 5.2 Implementation of the Wiener filter in the spatial domain	67
FIGURE 5.3 Wiener IR plots. Medium noise	69
FIGURE 5.4 Wiener IR plots. Medium noise.	70
FIGURE 5.5 Filtered bead images and profiles. Medium noise.	74
FIGURE 5.6 Filtered bead images. Medium noise.	75

Glossary

LSI

Linear-Shift Invariant

LSV

Linear-Shift Variant

MSE

Mean- Square Error

PSF

Point Spread Function

DNA

Deoxyribonucleic Acid

SIT

Silicon Intensified Target

TF

Transfer Function

IR

Impulse Response

DFT

Discrete Fourier Transform

IDFT

Inverse Discrete Fourier Transform

FFT

Fast Fourier Transform

IFFT

Inverse Fast Fourier Transform

Chapter 1

Introduction

1.1 Background

Digital image restoration is a difficult task, one that challenges the most advanced computer systems. The problem is complex, since typical images contain a great deal of data, and many applications require real-time processing. A number of well-known digital image restoration techniques can effectively improve the fidelity of an image (e.g., the inverse filter [1], the Wiener filter [2][3][4], the constrained-least-squares filter [5]).

The goal of this thesis is to apply image restoration techniques to fluorescence microscope images to improve their appearance. The images investigated in this thesis have been acquired from a video camera attached to a fluorescence microscope. The images are of cellular structures stained with fluorescent dyes which emit light causing a glowing aura or what appears to be a “scatter blur”. Our objective is to reduce that distortion.

Restoration could be defined as the recovery of former position or condition. Restoration techniques are oriented towards modeling the degradation and applying inverse process in order to recover the original image from the distorted one. Thus, the goal of image restoration is to obtain an estimate of the original undistorted image from an observed degraded image.

The observed image may be degraded by a number of effects including lens distortion, media distortion, noise, and detector distortion. We assume that the degradation can be modeled as a linear system. The complexity of the blur identification problem suggests that the techniques be limited to linear systems with shift-invariant Point Spread Functions (PSF) [6]. The problem becomes much more difficult when the nature of the blur is allowed

to vary across the image plane. Thus, the problem of space-varying blur will not be discussed in this thesis, and we assume that the degradation may be modelled as a Linear Shift-Invariant systems (LSI).

In most real applications the cause of the blur is not known, hence the first step towards the restoration of a blurred image is to identify the kind of the blur that the image has suffered. This problem is complicated by the fact that no a priori image is available. This is the most difficult step in image restoration if there is no available a priori information about the source of the blur and the noise, which is the case in this thesis.

The problem of identifying the original image without a priori knowledge of the PSF has been termed blind deconvolution [7], blind deblurring [8], and blur identification [9]. The problem will generally be referred to as "deblurring" here.

Three basic elements for the design of a restoration filter [10] can be defined:

1. An image formation model: a mathematical model describing the image and how the observed image is related to the original image.
2. A restoration criteria: an objective stating conditions which the solution to the restoration problem must satisfy.
3. A computational algorithm: a practical filter specifying steps in which the solution defined by the restoration criterion is obtained.

In all but the most trivial cases the original image cannot be exactly determined from the degraded image, and the restored image can only approximate the original image. The restoration criterion therefore specifies the solution that represents an optimal restoration. The effectiveness of image restoration filtering depends on the extent and the accuracy of the knowledge of the degradation process as well as on the filter design criterion. Hence, in image restoration, it is important to develop realistic models for the image degradation process, the noise process, and the image data.

In this research, in order to describe the imaging process, from the original image to the observed image, we need a realistic model for the LSI blurring and for the noise. We also require a criterion by which to judge the quality of restoration. A frequently used

criterion is the minimum Mean-Square Error (MSE). Although, as a global measure of visual fidelity, its validity is questionable, it is a reasonable measure, and is mathematically tractable [11]. In this thesis, however, emphasis is placed on subjective criteria because of the lack of information about the original image.

Numerous optimal image restoration methods have been proposed for removing spatial degradation due to blur and noise; examples are the inverse filter [1], the Wiener filter [2][3][4], and constrained deconvolution [5]. In each case, a criterion to be optimized is chosen and particular assumptions and models are used to derive a practical restoration filter. Clearly the choice of restoration criterion will affect how the restored image relates to the original image.

The LSI Wiener filter is commonly used to restore linearly-degraded images. The restored image is a minimum MSE estimate of the original undistorted image. In this work an estimate of the distortion PSF was determined from measurements of the images of fluorescent beads, and it is utilized in the Wiener filter restoration algorithm, originally as a starting point for adaptive restoration.

1.2 Organization of the Thesis

The organization of this thesis is as follows: The mathematical modeling and characterization of the scatter blur distortion PSF measured from fluorescent plastic bead images is discussed in Chapter 2. Review of several traditional techniques for image restoration is the subject of Chapter 3. Different techniques use different models of the imaging process and criteria for image restoration. The most important of these techniques is the minimum MSE Wiener filter. In Chapter 4 the performance of the minimum MSE linear Wiener filter, implemented in the Fourier domain, is examined as a function of the PSF parameters. In Chapter 5, restoration is implemented by convolving the image with a truncated version of the Wiener filter, in the spatial domain, to reduce the number of calculations. We conclude the thesis with Chapter 6 where we summarize the results and discuss future work.

Chapter 2

Image Characterization and Modeling

2.1 Introduction

Modeling plays an important role in blur identification and image restoration. The success of identification and restoration algorithms depends highly on how well the mathematical model fits the physical process of image formation and recording. In image restoration, it is important to develop realistic models for image degradations, especially for the blur formation. In this research work, we attempt to restore images produced by the fluorescence microscope imaging system. In most real applications the cause of the blur is not known, hence the first step towards the restoration of a blurred image is to identify the kind of blur that the image has suffered. This is the most difficult step in image restoration particularly if there is no available a priori information about the source of the blur and the noise [12]. In this chapter we assume linear distortion and we deal with the identification and modeling of the distortion PSF based on measurements of fluorescent bead images.

2.2 Source of Images

Fluorescence microscopy is a significant tool for the examination of cells and cellular constituents. Fluorescence microscopy is most often used to detect specific proteins or other molecules in cell and tissues. Fluorescent molecules absorb light at one wavelength and emit it at another, longer wavelength. If such a compound is illuminated at its absorbing wavelength and then viewed through a filter that allows only light of the emitted wavelength to pass, it is seen to glow against a dark background [13]. Because the background is dark, even a minute amount of the glowing fluorescent dye can be detected. The same number of molecules of an ordinary stain viewed conventionally would be

practically invisible because they would give only the faintest tinge of color to the light transmitted through this stained part of the specimen. Fluorescent probes and fluorescently marked immunological probes offer us the ability to visualize and quantify basic structures within the cell. The ability to perform quantitative measurements on fluorescence images, however, can be severely limited by the same instrument that provides us with the images, the microscope itself. When a fluorescence image is converted to a digital image for subsequent computer processing, the effect of the scanning instrument as well as the quantization process can further compound the problem [14][15]. The possibility that some components of the cell may be lost or distorted during specimen preparation has always worried microscopists. The only certain way to avoid the problem is to examine cells while they are alive, without fixing or freezing. For this purpose light microscopes with special optical systems are especially useful. Fluorescence microscopy is an important biophysical tool for studying living cells [16].

2.2.1 Fluorescence Labeling of Cell Images

In cell biology it is important to determine the quantities of specific molecules and to know where they are in the cell and how their level changes in response to extracellular signals. Sensitive methods have been developed for assaying these molecules, as well as for following the dynamic behavior of them in living cells. Antibodies are proteins produced by the immune system as a defense against infection. Antibodies are made most simply by injecting a sample of an antigen into an animal such as a rabbit or a goat then collecting the antibody-rich serum. The precise molecule (or antigen) specificity of antibodies makes them powerful tools for the cell biologist. Labeled with fluorescent dyes, and injected into the cell, or added to a tissue section, the position of the corresponding antigen can be determined [17]. The sensitivity of antibodies as probes for detecting and assaying specific molecules in cells and tissues is frequently by a signal-amplification method. For example, although a marker molecule such as a fluorescent dye can be linked directly to an antibody used for specific recognition (the primary antibody), a stronger signal is achieved by using an unlabeled primary antibody and then detecting it with a group of labeled secondary antibodies that bind to it [18][19].

The images produced by our imaging system described above, are distorted by the

glow that surrounds the fluorescently attached dyes [20]. The blur is caused by the interaction of the emitted light from the fluorescent dyes with the surrounding materials, causing what appears to be a scatter blur, or “glow” around the boundary of the acquired images, much like the appearance of a street lamp in the fog. An original cell image showing the blurring caused by the glow is depicted in Figure 2.1.

The images used in this thesis originated from a research program in molecular biology. The research is conducted in the department of Biology at Carleton University under the direction of Professor N. Chaly. The images were produced from a TV camera attached to the fluorescence microscope described in the next subsection. Two classes of images were acquired. The first were cell and nuclear images, and the second were images of fluorescent plastic microbeads.

FIGURE 2.1 Original Cell Image.

2.2.2 Fluorescence Microscope Imaging System

The images used in this thesis were produced by the fluorescence microscopy imaging system shown in Figure 2.2. The fluorescent dyes used for staining the cell images are detected with the help of the fluorescence microscope. The fluorescence microscope is a compound microscope which has been modified by incorporation of special filters. The optical paths for specimen image formation are similar to that of a standard bright field microscope. The light source is usually a high-intensity lamp which emits radiation in the blue-violet or ultra-violet region of the spectrum [21]. This radiation passes through an 'exciter' filter which allows only the required wavelength to pass. This light is capable of causing fluorescence in the specimen. The placing of a second filter beyond the objective lens removes any of the short exciting radiation while allowing the longer wavelength fluorescence to form an image. Since the image is formed entirely by light emanating from the specimen, fluorescing objects appear as intensely bright images in a uniformly dark background [22].

The video camera is a Dage-MTI Silicon Intensified Target (SIT) low-light-level, high sensitivity, TV camera. Low-light-level video cameras can substantially improve the visibility of the image and reduce the exposure needed to record picture photographically. They provide higher gain and the ability to detect very dim images with limited integration times. The other component of the TV camera based digitization system is normally not part of the camera itself [23].

The frame grabber (a Coreco Oculus 300) is mounted on an adapter card which is installed in a Intel 386-based personal computer. The frame grabber can manipulate large numerical arrays and perform limited types of arithmetic or logical operations on data in memory, but does not have the programing versatility or special functions found in a typical host computer. The host computer controls the operation of the frame grabber but does not manipulate image memory directly. The Oculus 300 can manipulate and process an image memory at video input rates of 30 frames/sec [24][25].

It is most desirable for the images acquired to have the same spacing of the pixel values in the horizontal and vertical directions(i.e., square pixels), as this simplifies many processing and measurement operations. The standard video image is not square, but has a

width-to-height ratio of 4:3, whereas the acquired image is: 512 by 512 pixels. The images used here were adjusted by applying linear interpolation, which matches the TV aspect ratio proportions while keeping the pixels square.

Once a processed image has been produced, it can be routed to several locations for display and storage [26]. The digital image can be reconverted to an analog video format by using a Digital-to-Analog Converter (DAC), and the video image can be displayed on a high resolution monitor or permanently stored on magnetic disks or other storage devices.

FIGURE 2.2 Fluorescence Microscope Imaging System. *Methods in Cell Biology* [19].

2.2.3 Microbead Images

The fluorescent beads used in this thesis have been provided by Polysciences Inc., and are made from a variety of polymers [19]. The beads used are about $5\ \mu\text{m}$ in diameter. Many kinds of fluorescently labeled beads have been synthesized and are used for system calibration and for setting system parameters. They can be obtained “plained”, i.e., with no reactive groups available on them, or may contain various reactive groups to which one can attach selected dyes or other molecules such as antigens or antibodies. They are available with fluorescent dyes bound to them spanning a range of excitation and emission wavelengths. In some cases the dye molecules are bound so that only the surface is fluorescent while in others, the dyes seem relatively homogeneously distributed throughout the bead [17]. In our case, the dye appears to be on the surface.

Beads are nominally homogeneous and spherical but this is not always the case. The images acquired are of 512×512 8-bit pixels. Figures 2.3 (a) and (b), show a typical 224×224 pixels bead image and a scan line profile across the diameter of the indicated bead. The glow around the bead, as well as the bright edges, are readily apparent.

The fluorescent bead images are used in this work as test measurements for the determination of the distortion PSF model.

(a)

(b)

FIGURE 2.3 (a) Bead image (b) Cross section profile

2.2.4 Cell image

Present-day living cells are classified as procaryotic (bacteria and their close relatives) or eucaryotic. Although they have a relatively simple structure, procaryotic cells are biochemically versatile and diverse. Eucaryotic cells are larger and more complex than procaryotic cells and contain more Deoxyribonucleic Acid (DNA), together with components that allow this DNA to be handled in elaborate ways [17]. Eucaryotic cells by definition have a nucleus which contains most of the cell's DNA, enclosed by a double layer of membrane. The DNA is thereby kept in a compartment separate from the rest of the contents of the cell, the cytoplasm, where most of the cell's metabolic reactions occur.

The nucleus is the most conspicuous organelle in the cell. It is separated from the cytoplasm by an envelope consisting of two membranes. In the microscope one sees that the nucleus is brightly fluorescent compared to the cytoplasm which also displays significant fluorescence. Total fluorescence of the cell, however, is relatively constant [27].

The cell images used in this research are of human cervical carcinoma called Hela cells. A 224×224 pixels cell image and a scan line profile across its diameter is shown in Figures 2. 4 (a) and (b).

(a)

(b)

FIGURE 2.4 (a) Cell image (b) Cross section profile

2.3 Blur Formation Model

Images recorded in electronic or photographic medium are often degraded due to recording system imperfections. These degradations may be caused by a variety of circumstances including diffraction effects, scattering, optical system aberrations, camera or object motion, and defocussed lens. In addition to these blurring effects, the recorded image is also corrupted by noise from the recording medium due to the randomness of the photoelectric effects [28]. Measurement errors due to the accuracy of the recording system, transmission errors, and digitization also degrade the image further.

In this thesis, the images are distorted primarily by what appears as a "scatter blur", as demonstrated later in this chapter.

In general, the goal of image restoration is to model these degradations, then estimate the original scene, given the degraded image. In practical situations little, if anything, is known about the characteristics of the recording system, and the degradations must be estimated from the degraded data itself. The linear blurring model with an additive noise process is shown in Figure 2.5. The degradations are classified into two categories: spatial degradations causing blur and random point degradations introducing noise.

FIGURE 2.5 Linear Blurring Model

The observed blurred image $g(x,y)$ in the exposure domain can be modeled by a linear system [29] that is described by

$$g(x,y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} h_1(x,y;\xi,\eta) f(\xi,\eta) d\xi d\eta + n(x,y) \quad 2.1$$

where $f(x,y)$ is the exposure distribution of the infinite extent original object, $h_1(x,y;\xi,\eta)$ is the PSF of the blurring system, and $n(x,y)$ is an additive white noise process. Exposure refers to the integral of light intensity over the time interval that the shutter is open. Due to the nature of image formation, both the observed and original images take nonnegative values only.

The PSF of the blur, $h_1(x,y;\xi,\eta)$ is defined as the image of a point object of unit brightness, $\zeta(x-\xi,y-\eta)$, located at (ξ,η) in the object plane. In its most general form, the PSF $h_1(x,y;\xi,\eta)$ depends on the position (ξ,η) of the point object in the object plane. If every spatial position in the image is blurred in the same way, we say that the blur is spatially invariant [30]. Then, we have

$$h_1(x,y;\xi,\eta) = h(x-\xi,y-\eta) \quad 2.2$$

where $h(x,y)$ denotes the image of a point object $\zeta(x,y)$ located at the origin of the object plane.

In this thesis, we consider only LSI blurs. In this case, the model of the blurred image (2.1) simplifies to the following convolution integral:

$$\begin{aligned} g(x,y) &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} h(x-\xi,y-\eta)f(\xi,\eta)d\xi d\eta + n(x,y) \\ &= \int_{(\xi,\eta) \in R} h(\xi,\eta)f(x-\xi,y-\eta)d\xi d\eta + n(x,y) \\ &= h(x,y) * f(x,y) + n(x,y) \end{aligned} \quad 2.3$$

In the blind deconvolution problem we are given $g(x,y)$ and must estimate $f(x,y)$ having incomplete knowledge of the blurring system $h(x,y)$. In this thesis $h(x,y)$ is found experimentally to be the PSF of a scatter blur. In equation (2.3), R denotes the support area

of the PSF which may be of infinite extent, and $*$ stands for 2-D convolution. Hence, according to equation (2.3), the observed blurred image $g(x,y)$ is modeled as the output of a linear system, with the impulse response $h(x,y)$, whose input $f(x,y)$ is the unknown original image to be estimated [31].

Fourier transforming both sides, and using the convolution theorem, we obtain

$$G(u, v) = H(u, v) F(u, v) + N(u, v) \quad 2.4$$

Where $G(u, v)$, $F(u, v)$, $H(u, v)$, and $N(u, v)$ are the Fourier transforms of $g(x,y)$, $f(x,y)$, $h(x,y)$, and $n(x,y)$, respectively [11]. The function $H(u, v)$ is the transfer function of the fluorescence microscope system that transforms the original image $f(x,y)$ into the degraded image $g(x,y)$.

2.4 Modelling of Distortion

Image restoration may be defined as applying the inverse of a mathematical model that describes the degradation in order to estimate the “original” image. The success of image restoration in a given application depends on how good the assumed mathematical model fits the real cause of the image degradation [32]. Before image restoration can be accomplished, the distortion PSF must be known. In some cases, this is known in advance, but here it must be determined experimentally from the blurred bead images. Our first motivation is to develop a model that predicts the behavior of the scatter given the fluorescence microscope imaging system. The second motivation is to develop a deconvolution technique for scatter correction. Given a PSF model, an inverse filter can be devised to correct for scatter in the blurred image.

Modelling our test bead image and taking cross-section profiles for many bead images as shown in Figure 2.6, leads to the observation that there is a scatter effect beyond the outside boundary of the beads and shows the exponential decay of the fluorescence intensity beyond the physical limits of the bead. As a result of this scatter, a degradation occurs in the acquired images.

These observation lead us to the assumption that the degradation PSF of the fluorescence imaging system could can be modelled by either of analytical forms:

(i) a simple exponential model

$$h(r) = \exp(-\alpha|r|) \text{ (Model 1)}$$

2.5

FIGURE 2.6 cross section profiles of different bead images

where α is the decaying constant of the scatter.

(ii) or by the radially symmetric function model suggested by [33][34][35], (Model 2)

$$h(r) = \frac{1}{1 + P_1|r|^{P_2}}$$

2.6

which is parameterized by two imaging parameters P_1 and P_2 . The parameter P_1 is the magnification scaling factor and P_2 is the scatter decay factor [36].

To test the soundness of our models and their ability to predict the spatial scatter behaviour of the test bead images, we took a finite sequence of data points along the scatter decay curve of different bead images and applied a curve fitting algorithm to the two analytical models based on the goodness-of-fit criterion [37]. Model 2 has a better fit to the data points taken from the bead images than model 1. Therefore, model 2 is chosen over model 1 as our scatter blur PSF.

Figure 2.7 shows the results of the curve fitting algorithm of the two above models and illustrates which of the two models have the best possible fit to the data. The average intensity curve was calculated for four different bead images and plotted against the two fitted curves. The standard error deviation measured between the empirical measured values of the beads and the calculated data for the second model was calculated to give an idea of how close the fit is [38][39].

FIGURE 2.7 Curve Fitting of the two degradation PSF models

The PSF is typically a small, radially symmetric function that is generally decreasing from the center out. The PSF of the fluorescence microscope system can be easily pictured and understood in one dimension. Figures 2.8 and 2.9 provide plots of the PSF for high and low values of P_1 and P_2 and show its Fourier transform. It should be noted that the scatter blur PSF is symmetrical and only half of it is shown here.

The PSF convolves with the image so that the wider it is, the more the dispersion. For a small increase in the values of P_1 and P_2 the effective width of the PSF narrows significantly causing less dispersion, therefore less filtering or less glow to subtract from the blurred image is required to correct for the blur. On the contrary, when P_1 and P_2 decrease, the PSF width becomes wider causing more dispersion, thus more filtering is required to compensate for the scatter blur.

The above experimental results and observations indicate that the analytical model provides a reasonably good model for the PSF, and may be considered as a predictor of the scatter's spatial-frequency behavior, and as a viable candidate for the PSF in the Wiener filtering implementation schemes that will correct for the scatter blur [34].

The value of this model ultimately lies in its ability to predict the behavior of the spatial distribution of the scatter blur, and its performance in the Wiener filtering algorithm for scatter correction. These will be discussed in Chapter 4.

FIGURE 2.8 (a) Point Spread Function (PSF). (b) PSF transform.

FIGURE 2.9 (a) Point Spread Function (PSF). (b) PSF transform.

2.5 Observation Noise

As the image is sampled, it must be quantized (converted to a finite, discrete scale with an analog-to-digital or A/D converter). A gray-scale employing eight-bit integers, allowing values 0 to 255, is a popular choice, and used in our experiments. Quantization introduces errors. These errors can be viewed as a random, additive noise process. The probability density function(pdf) for rounding noise is uniform in the range -0.5 to 0.5.

Image restoration algorithms are usually based on the assumption of additive, white noise, but imaging systems can introduce other types of noise. For example, noise can have a fixed pattern or it may be signal dependent; it can be additive, multiplicative, or effect the image in more complicated ways [40].

In this work, as successive images are integrated in the frame grabber to reduce the additive noise to a very low level, we assume that major source of noise in the images is quantization noise.

Chapter 3

Traditional Restoration Techniques

3.1 Introduction

This chapter describes several traditional image restoration techniques. In image restoration, an image has been degraded in some manner and the objective is to reduce or eliminate the degradation. Images are produced to record or display useful information, but the process of image formation and recording is imperfect. The recorded image invariably represents a degraded version of the original undistorted image. Because the available knowledge of the original image and the degradations is invariably incomplete, image restoration is an estimation problem. The effectiveness of the restoration procedure depends on the extent and the accuracy of the knowledge concerning the degradation process. From the digital degraded image and what is known about the scene and the imaging system, a restoration technique must estimate the original undistorted image [41]. The restored image should correspond closely to the original image, but it must be computed efficiently.

3.2 Problems, Solutions, and Algorithms

Practical methods of image restoration are conditioned on a model of the scene and the imaging process. The model characterizes the knowledge (or assumptions) that can be used to solve the problem. Different techniques may be conditioned on different models; it is important to consider the accuracy of a model for each particular problem. The common models are reasonable for most systems, but they are motivated by practical considerations as well-the model must not make the problem intractable. To be successful, a method must capture the essential aspects of the problem in a simple model. If the

technique is to be practical, the model must ignore some of the unpleasant complexities ever-present in real problems.

Practical techniques must yield an acceptable estimate. With incomplete knowledge, no method can always determine the correct solution. In many applications, a human observer is the best judge of the relative quality of possible solutions. Unfortunately, the criteria used by the human visual system are not well understood and even if human perception were fully modeled, it might be too complicated to be computed in a reasonable time with current technology [31]. A practical criterion must be simple to calculate.

The most popular image restoration techniques use a single LSI operator. The LSI restoration operator is conditioned on assumptions about the imaging process and on judgments about which estimate is best. These assumptions and judgments are influenced by practical considerations. Non-linear methods and shift-variant (adaptive) methods are usually more complicated and computationally expensive [42]. However, because even LSI operations on images require many calculations, computational complexity is often a critical concern.

Traditional restoration techniques are successful because they define the model of the problem and the nature of the solution so that an effective and efficient algorithm exists. The models capture the important aspects of the restoration problem without being too complex, the measures of quality for the possible solutions correlate well with the human perception, and the algorithm is tractable [43].

3.3 Inverse Filter

The first digital image restoration techniques focused on the problem of image blur (or high-frequency attenuation). One of the simplest techniques, the inverse filter [1], is based on the assumption that the imaging process is a non-singular, noise free, LSI operation:

$$g = f * h$$

FIGURE 3.1 Linear Blurring Model

where f is the original undistorted image of the scene, h is the system PSF, and g is the degraded image as shown in Figure 3.1. The original image of the scene f is unknown; the goal of restoration is to recover it from a digital distorted image g using knowledge of the PSF h . Assuming the model of equation 3.1, the original undistorted image can be recovered by applying the inverse of the system PSF:

$$\begin{aligned}
 \hat{f} &= g * s_I \\
 &= f * h * s_I \\
 &= f * \delta \\
 &= f
 \end{aligned}
 \tag{3.2}$$

Where $\hat{f}$ is the restored image, and s_I is the PSF of the inverse filter $h * s_I = \delta$

The inverse filter assumes knowledge of the system PSF h . Previous techniques for characterizing acquisition devices were designed for analog systems (e.g. film cameras) and rely on oversampling the analog output. The question of the existence of the inverse of the PSF is considered later in this section.

The inverse filter can also be applied in the frequency domain [4][44]:

$$\begin{aligned}
 \hat{F} &= GS_I \\
 &= FHS_I \\
 &= F
 \end{aligned}
 \tag{3.3}$$

Note that the system transfer function H may be complex, so the inverse filter transfer function is defined as:

$$\begin{aligned} S_I &= H^{-1} \\ &= \frac{H^*}{|H|^2} \end{aligned} \quad 3.4$$

Before the filter S_I can be applied in the frequency domain, the image must be transformed. After the filter is applied, the resulting product must be inverse transformed. Except for rounding error differences, the spatial domain and frequency domain processing yield identical results.

Noise is a serious problem for the inverse filter. Restoration is an ill-conditioned problem-small errors in the image can result in large errors in the restored image. In equation 3.2, the restored image is identical to original image of the scene. This equality is consistent with the model of the imaging system in equation 3.1, but this model is unrealistic-some noise is invariably present [30]. Figure 3.2, shows a more accurate model of the imaging process that includes an additive noise term n :

$$g = f * h + n \quad 3.5$$

FIGURE 3.2 Linear Blurring Model with an additive noise term

Using this model, the inverse filter restoration contains an error term:

$$\begin{aligned}
 \hat{f} &= g * s_I \\
 &= (f * h + n) * s_I \\
 &= f + n * s_I
 \end{aligned}
 \tag{3.6}$$

The frequency domain equations provide clearer insight into the nature of the ill-conditioning:

$$\begin{aligned}
 \hat{F} &= GS_I \\
 &= (FH + N) S_I \\
 &= F + NS_I
 \end{aligned}
 \tag{3.7}$$

That is, the restored image spectrum $\hat{F}$ differs from the scene spectrum F by the error term NS_I . Each component of the restored image spectrum is

$$\hat{F}(u, v) = F(u, v) + \frac{N(u, v)}{H(u, v)}
 \tag{3.8}$$

If a system transfer function value $H(u, v)$ is very small at some frequency, then even a small amount of image noise at this frequency can result in a large error. In the worst case, when $H(u, v)$ is zero, the system is singular (i.e. cannot be inverted) and the image cannot be fully restored even if there is no noise [6][44].

Because we are dividing by the Fourier transform of the filter, any very small values of coefficients can have disproportionately large effects on the results. These small values, which can arise as a result of the necessary approximate definition of the filter, can cause large, spurious terms to appear in the transform of the restored image, and lead to artifacts

in the final results. A simple modification to the filter is to locate any values of 0 and to replace them by 1. This effectively ignores these values in the calculation. The resulting filter is known as the pseudoinverse filter. Unfortunately, even this does not overcome all of the difficulties inherent in the technique.

The difficulty with the above formulation, Equation 3.8, is that it is very rarely that the form of the noise component is known in sufficient detail to allow its Fourier transform to be derived. Consequently, a technique has been devised which can cope with noise, without needing to know its form in such detail. This technique is known as the Wiener filter.

3.4 Wiener Filter

The Wiener filter takes its name from an analogous one-dimensional result first established by Norbert Wiener in the 1940's [45]. The Wiener filter is the linear filter that minimizes the expected MSE between the original and the restored image:

$$e_w^2 = \frac{1}{MN} \sum_{m=1}^M \sum_{n=1}^N (f(m, n) - \hat{f}(m, n))^2 \quad 3.9$$

The Wiener filter is also called the least-squares filter and the minimum MSE filter [2][3]. The method is premised on the model of Equation 3.5.

Most derivations of the filter treat the scene and the noise as stochastic processes and assume that the noise and the scene are independent, that both processes are stationary, and their power spectra are known. The power spectra of the scene and the noise are seldom known, but approximations are usually adequate. The Wiener filter is conditioned on the desirability of minimizing the minimum MSE. The minimum MSE measure does not correspond exactly to human perception of image quality, but it is generally satisfactory and it is easily computed [6][46]. The popularity of the Wiener filter attests to its utility.

The filter is typically defined in the Fourier frequency domain as

$$F(u, v) = \left[\frac{1}{H(u, v)} \frac{|H(u, v)|^2}{|H(u, v)|^2 + \frac{S_n(u, v)}{S_f(u, v)}} \right] G(u, v) \quad 3.10$$

Where S_f is the scene power spectrum and S_n is the noise power spectrum. $F(u, v)$ is the restored image and $H(u, v)$ is the model of degradation PSF. $G(u, v)$ is the degraded image. At frequencies where the scene power spectrum is zero ($S_f(u, v) = 0$), the transfer function of the filter should be zero.

At frequencies where the noise power spectrum is zero ($S_n(u, v) = 0$), the Wiener filter is identical to the inverse filter. As the ratio of the energy of the noise to that of the scene increases, the value of the Wiener filter transfer function is attenuated. The Wiener filter uses the ratio of the noise energy to the scene to attenuate the restoration filter. As has already been mentioned, it is unusual for the necessary amount of information to be available about the noise component to allow its Fourier transform, and hence its spectral density function, to be computed [11][47]. However, an approximation is possible, on the assumption that these functions vary in similar way to one another, as u and v vary. If this is true, we can approximate the ratio of the two functions by a constant value N . Equation 3.10, then reduces to

$$F(u, v) = \left[\frac{1}{H(u, v)} \frac{|H(u, v)|^2}{|H(u, v)|^2 + N} \right] G(u, v) \quad 3.11$$

Where the noise energy is low compared to the signal energy, the Wiener filter becomes the inverse filter, thus we have

$$W(u, v) = \begin{cases} \frac{1}{H(u, v)} & H(u, v) \gg N \\ 0 & H(u, v) \ll N \end{cases} \quad 3.12$$

Thus no ill-conditions arise in Wiener filtering. This approximation will tend to work best in those cases where the noise varies with the signal. The noise power spectrum will then tend to resemble the image power spectrum and their ratio will tend to be reasonably constant¹.

One advantage of the simplicity of the form of equation 3.11, is that it possible to try a range values of N to see which has the best effect in noise removal. This can be done even when nothing is known about the actual form of the noise component. The Wiener filter algorithm implementation that is used in this thesis is presented in Chapter 4.

The expected mean-square error of the Wiener filter restoration is

$$e_w^2 = \sum_u \sum_v \frac{S_f(u, v) S_n(u, v)}{|H(u, v)|^2 S_f(u, v) + S_n(u, v)} \quad 3.13$$

If the noise is negligible, e_w^2 approaches zero. If noise dominates the signal, e_f^2 is bounded by the energy of the signal [48][49]. In comparison, the expected mean-square error of the inverse filter (using the assumptions about the signal and noise used to derive the Wiener filter) is

$$e_i^2 = \sum_u \sum_v \frac{S_n(u, v)}{|H(u, v)|^2} \quad 3.14$$

The expected mean-square error of the inverse filter is an unbounded function of the noise. At each frequency, the expected square-error of the inverse filter is at least as large as that of the Wiener restoration filter.

1. This is seldom as the case in cellular images, where the noise is constant while the signal is very localized spatially.

Chapter 4

Wiener Filtering In the Frequency Domain

4.1 Introduction

Linear filters have been used successfully and made great contributions in image restoration of the conventional class because of their simple formulation and economical computational efforts. As described in Chapter 3, the Wiener filter is one of the earliest methods that was applied to image restoration. The Wiener filter also finds extensive applications in control, signal processing, communication, and many other science and engineering fields. The Wiener filter incorporates a statistical knowledge of the image and the noise into the restoration scheme. The Wiener filter gives the linear least mean square estimate of the object image from the observations and have been used extensively for the restoration of noisy and blurred images [50].

In this work, the essential idea behind the Wiener filter implementation is to make use of the distortion PSF estimated from the fluorescent bead measurements, assuming additive white noise. The minimum MSE measure does not correspond exactly to human perception of image quality, but it is generally satisfactory and it is easily computed. The popularity of the Wiener filter attests to its utility. The reason for the popularity of the Wiener filter is due to its simple but powerful criterion and also because its solution has closed form [51]. This chapter describes Wiener filter restoration of blurred cell images in the Fourier domain. Experimental results are included to illustrate the proposed Wiener algorithm.

4.2 Implementation

The Wiener filter is a LSI filter which makes use of the power spectrum of both the image and the noise to prevent excessive noise amplification. The frequency response of the Wiener filter $W(u, v)$ is chosen to minimize the minimum MSE, e_w , given by:

$$e_w^2 = E(|f(x, y) - \hat{f}(x, y)|^2) \quad 4.1$$

Where $\hat{f}(x, y)$ is usually called the least square estimate of $f(x, y)$ with a given observed blurred image $g(x, y)$ [52]. As discussed in section 2.3, $g(x, y)$ can be modeled by a LSI system shown in Figure 4.1, and can be represented by the convolution operation as

$$g(x, y) = \sum_{\alpha=0}^{N-1} \sum_{\beta=0}^{N-1} h(\alpha - x; \beta - y) f(\alpha, \beta) + n(x, y) \quad 4.2$$

or

$$g(x, y) = f(x, y) * h(x, y) + n(x, y) \quad 4.3$$

FIGURE 4.1 Linear Blurring and Restoration Model

The restoration system consists of the LSI Wiener filter defined by the impulse response $w(x, y)$. After restoration with the filter, the restored image becomes

$$\hat{f}(x, y) = \sum_{\alpha=0}^{N-1} \sum_{\beta=0}^{N-1} w(\alpha - x, \beta - y) g(x, y) \quad 4.4$$

or

$$\hat{f}(x, y) = g(x, y) * w(x, y) \quad 4.5$$

as illustrated in Figure 4.1.

It is analytically convenient to consider the restored image in the Fourier transform domain. By the Fourier transform convolution theorem

$$\hat{F}(u, v) = G(u, v) W(u, v) \quad 4.6$$

Where $\hat{F}(u, v)$, $G(u, v)$, and $W(u, v)$ are respectively, the 2D-DFT's of $\hat{f}(x, y)$, $g(x, y)$, and $w(x, y)$. This implies that once the transfer function of the Wiener filter $W(u, v)$ is calculated, the restored image $\hat{f}(x, y)$ is computed by the IDFT of Equation 4.6, as

$$\hat{f}(x, y) = IDFT[G(u, v) W(u, v)] \quad 4.7$$

Complete descriptions of both the degradation PSF and the noise are required to specify $W(u, v)$ [53]. The full formulation is

$$W(u, v) = \frac{H^*(u, v) S_f(u, v)}{|H(u, v)|^2 S_f(u, v) + S_n(u, v)} \quad 4.8$$

Which may also be written as

$$W(u, v) = \frac{H^*(u, v)}{|H(u, v)|^2 + S_n(u, v) / S_f(u, v)} \quad 4.9$$

The above equation is in the Fourier domain. $W(u, v)$ is the Wiener filter and $H(u, v)$ is the model of the degradation PSF. The term $|H(u, v)|^2$ is defined as

$$|H(u, v)|^2 = H^*(u, v) H(u, v) \quad 4.10$$

It is the product of the complex conjugate of the degradation PSF model with itself. $|H(u, v)|^2$ is known as the spectral density function or the power spectrum of the degradation model. In equation 4.9, the terms $S_n(u, v)$ and $S_f(u, v)$ are also spectral density functions [54].

It is unusual for the necessary amount of information to be available about the noise component to allow its Fourier transform, and hence its spectral density function, to be computed. However, an approximation is possible, on the assumption that these functions vary in a similar way to one another as u and v vary. If this is true, we can approximate the ratio of the two functions by a constant scalar value [55].

Equation 4.9, then reduces to

$$W(u, v) = \frac{H^*(u, v)}{|H(u, v)|^2 + N} \quad 4.11$$

which is called the Wiener filter transfer function in its simple form.

This function contains the transform of the estimated PSF of the scatter blur as calculated in Section 2.4 and shown by the following equation

$$h(r) = \frac{1}{1 + P_1 |r|^{P_2}} \quad 4.12$$

The PSF of the scatter blur is parameterized by the parameters P_1 and P_2 . The Wiener filter restoration performance depends on the filter characteristics as the parameters P_1 , P_2 , and N change. One advantage of the simplicity of the form of Equation 4.11, is that the evaluation may be performed incrementally while changing the noise constant N until a satisfactory result is obtained. This can be done even when nothing is known about the actual form of the noise component [56].

The Wiener filter restoration implementation in the frequency domain is shown in Figure 4.2. First, the scatter blur PSF estimated in Equation 2.6 with the two variable input parameters P_1 and P_2 is generated in size 256×256 . The PSF is multiplied by a raised-cosine weighted rolloff window to drop the PSF values at the edges to zero and make it decay smoothly [57]. These edge values when transformed into the frequency domain are significant, thus introducing artifacts in the filtered image that reduce the visual and measurable quality of the restored images [58].

The PSF is converted into the frequency domain via the 2-D Fast Fourier Transform (FFT). The Wiener filter transfer function in Equation 4.11 is calculated with an input for the experimental noise parameter N , thus providing the Wiener filter coefficients. The Wiener filter is normalized to unity AC gain so that the output energy is equal to the input energy when the input is white noise.

The second phase of the Wiener filtering process starts with loading the input distorted image of size 256×256 . The actual size of the input image is 224×224 pixels, but padded with zeros to 256×256 pixels. The input image is scaled to cover the range of 0 to 255 of gray levels for display purposes [59]. The input image is transformed into the

frequency domain by applying a 2D-FFT to the image. Next, the image is multiplied by the Wiener filter coefficients and then inverse transformed via a 2-D Inverse Fast Fourier Transform (IFFT) [60] producing the restored filtered image $\hat{f}(x, y)$ which is an estimate of the original unknown image $f(x, y)$.

The Wiener filtering in the Fourier domain can be summarized as:

Phase 1:

- Generate the scatter blur PSF.
- Multiply the PSF by a raised-cosine rolloff window.
- Transform the PSF into the frequency domain.
- Calculate the Wiener filter transfer function.
- Normalize the Wiener filter to unity AC gain.
- Save Wiener filter coefficients.

Phase 2.

- Load Wiener filter coefficients.
- Load the 256×256 input image.
- Scale input image to 256 gray levels.
- Transform input image into the frequency domain.
- Multiply input image by filter coefficients.
- Scale filtered image to 256 gray levels.
- Perform IFFT on filtered image.
- Output and save the restored image.

FIGURE 4.2 Implementation of the Wiener filter in the Fourier domain

The Wiener filtering software programs were written with the Borland C++ 3.1 compiler. The experiment was implemented on a 33 MHz 80486-based PC compatible microcomputer, with 8MB of RAM. The Phar Lab DOS-Extender was used to overcome the constraint imposed by the 640KB memory limitation and the real mode addressing used by DOS [61].

4.3 Results and Analysis

To demonstrate the Wiener filter transfer function characteristics when varying the function parameters P_1 , P_2 , and the noise N , illustrative plots of the Wiener transfer function are shown in Figures 4.3-4.5. The range of values tested for parameters P_1 and P_2 , respectively, were in the range of 0.1-1.5 for P_1 , and 1.0-2.5 for P_2 . This range of values covers the final fit parameters resulting from the curve fitting computations described in Section 2.4, which determined the estimated scatter blur PSF parameterized by the two parameters P_1 and P_2 .

Figures 4.3-4.5, show the effect of varying the parameters P_1 , and P_2 on the filter response for:

- a) Low noise ($N = 0.2 * \text{average signal energy}$)
- b) Medium noise ($N = \text{average signal energy}$)
- c) High noise ($N = 5 * \text{average signal energy}$).

Increasing P_2 while keeping P_1 and N constant, as in the case of Figure 4.3(b), for medium noise, will decrease the Wiener filter gain and tend to reduce the frequency response of the filter in the high frequency range. Thus, the high frequency components get more attenuated because the Wiener filter magnitude becomes smaller when P_2 gets larger. Increasing the noise constant N shown in Figure 4.3(c), will suppress and flatten the magnitude of the Wiener filter transfer function, thus decreasing remarkably the filter gain. On the contrary, making the noise very small as in Figure 4.3(a) will boost the filter gain to higher values. This results in amplifying the high frequency components, especially when

both P_1 and P_2 are increasing simultaneously or separately, resulting in a Wiener filter similar to a high pass inverse filter.

Keeping P_2 and N constant and increasing P_1 results in a slight decrease in the low frequency filter gain and broader bandwidth as shown in Figure 4.4(a) for low noise level. As P_1 increases we observe a shift in the filter response, thus P_1 acts as a magnification factor. For low values of P_1 , some low frequency components are attenuated. Experimentation in varying the filter parameters, demonstrates that when P_1 and P_2 are very low a wide impulse response of the PSF results causing more dispersion, thus more blur to filter or subtract from the distorted image. On the contrary, when P_1 and P_2 are very high, a narrow impulse response of the scatter PSF results causing less dispersion, thus little filtering is needed to compensate for the scatter blur.

Figure 4.5 illustrates the clear effect of the different noise levels on the Wiener frequency response for specified values for P_1 and P_2 . For example, Figure 4.5(b) shows three curves for low, medium, and high noise levels with respect to $P_1 = 0.8$ and $P_2 = 1.8$. Clearly from the figure, it shows the obvious effect of the noise on the Wiener filter gain. As the noise increases, the filter gain decreases rapidly to some low magnitude values. On the other hand, making the noise very low tends to increase the filter gain to higher values causing it to act almost as an inverse filter, especially when the noise is at very low values.

As a result, the parameters P_1 , P_2 , and N act together as a selective amplifier of the low frequency components and as an attenuator of high frequencies relative to the low frequencies dependent on the values of these parameters. Experimentation and observation of the Wiener filter transfer function behaviour when both blur and noise are present leads to a compromise between a low pass smoothing filter and a high pass inverse filter resulting in a band pass filter [46].

FIGURE 4.3 Wiener TF plots. (a) Low Noise (b) Medium Noise (c) High Noise

FIGURE 4.4 Wiener TF plots. (a) Low Noise (b) Medium Noise (c) High Noise

FIGURE 4.5 Wiener TF plots.

4.3.1 Bead Experiment

As a starting point, 256×256 8-bit pixels images of the fluorescent manufactured-beads were used as test case images in experimenting and determining the best combination of the filter parameters P_1 , P_2 , and the noise N . Once the optimum parameters were found, they were exploited in filtering cell and microtubule images to compensate for the fluorescence decay of luminous bodies. The tests determined the effect of varying the parameters P_1 , P_2 , and N on the Wiener filter, with respect to its performance and its ability to provide the best restored images. The filtered images were examined for obvious artifacts that tend to reduce the visual quality of the restored images [62]. In addition, the filtered images were tested for the success of removing the scatter blur and the excessive glow

Visual comparison is a subjective process. The ultimate criterion is a visually assessed appearance, the judge is the “expert eye” in the absence of quantitative fidelity criterion. Sometimes, it is difficult to conclude from visual inspection of two filtered images which one is the best. The restored image quality is defined in subjective terms, and can only be measured in terms of an observer’s response. There is no reason to suppose that subjective quality is a one dimensional quality, and if it is multidimensional, it can’t be ranked [63]. In practice it is treated as a scalar, and is defined in terms of the protocol used to measure it. An attractive scale is graded in just-noticeable difference in image quality, so that subjective measurement of relevant system parameters is quite a difficult and tedious procedure. Measurement of subjective image quality is very difficult [64][65].

Series of restoration tests were performed for different range of values for P_1 , P_2 , and the noise N . The tests and observation results were subjectively evaluated and scaled as “*undercompensated*”, “*good*”, and “*overcompensated*”. Throughout this analysis, we will rely on a subjective comparison between the original image, attached in the special reference page (page no. 78) and the restored images by human observer to illustrate the performance of the Wiener filter.

Figures 4.6 and 4.7 show the results for fixed $P_2 = 1.8$ and varying $P_1 = 0.2, 1.0, 2.5$, and for fixed $P_1 = 0.8$ and varying $P_2 = 1.0, 1.8, 2.5$ respectively, for low noise level. The

same tests for the same range of P_1 and P_2 values are done respectively, in Figures 4.8 and 4.9 for medium noise level and in Figures 4.10 and 4.11 for high noise level.

Figure 4.6(a) shows a bead image when $P_1 = 0.2$ and $P_2 = 1.8$, for low noise level. The restored image has overshoots and undershoots from the bright areas around the bead edges, and in addition is noisy. The result is a grainy image with exaggerated details and sharpened edges around the bead. This is due to the filter gain at high frequencies as shown in Figure 4.4, that led to overfiltering the image¹.

When P_1 increases for the same P_2 value as in Figure 4.6(b), a clear decrease in the size of the ringing artifacts around the edges of the beads when is apparent. The Wiener filter successfully restored the image better than the one in Figure 4.6(a), but enhanced the noise components. Because edges or fine details are the primary contributors to the high frequency components of an image, therefore, the Wiener filter performed as a high pass filter which increased the local contrast and sharpened the bead image. The inevitable noise fluctuations are significantly more, due to the amplification of the noise component in the high frequency region [66].

Overestimating P_1 as in the case of Figure 4.6(c), results in little and poor restoration of the image and undercompensation. This agrees with the explanation of the Wiener transfer function behaviour shown in Figure 4.4. When P_1 increases for fixed P_2 , the Wiener filter gain reduces to low magnitude values, resulting in a little reduction of the glow.

As already discussed for the Wiener frequency response plots in Figure 4.4, the noise parameter N has a vital effect on the Wiener filter performance. Assuming that a little noise will increase the filter gain more precisely in the high frequency areas, thus accentuating and enhancing the noise frequency components. Overestimating the noise will taper-off and flatten the frequency response of the Wiener filter, therefore dropping the filter gain to low magnitude values.

1. The white areas surrounding some of the beads represent negative values, i.e. extreme undershoots. They are up as white because small negative two's complement numbers are interpreted as large unsigned binary numbers in the DAC converter driving the display.

Overcompensated

Good

Undercompensated

FIGURE 4.6 Filtered beads and profiles. Fixed $P_2 = 1.8$. Low Noise

Keeping P_1 and N fixed and varying P_2 for low noise as in the case of Figure 4.7, led to more noticeable effect on the Wiener filter performance and comparatively on the restored images. Underestimating P_2 for $P_1 = 0.8$ as in Figure 4.7(a), led to slightly larger undershoots and overshoots and edge effects represented in thin dark rings around the bead edges, when compared to underestimation of P_1 . The Wiener filter is overcompensated. The background noise is due to the high frequency component emphasis by the Wiener filter acting like high pass filter when P_2 is underestimated for very low N . The noise actually involves high rates of change of gray level, and it actually becomes stronger than the image signal at high frequencies, thus high pass filtering tends to increase the background noise power [67].

Increasing P_2 to a higher value as in the case of Figure 4.7(b), resulted in obvious reduction of the undershoots and the dark rings around the edges that was observed when P_2 is underestimated. The Wiener filter smoothed the image and showed a good improvement in the image resolution and more defined image details in the bead image.

Collectively, overestimating P_2 resulted in undercompensated bead images as depicted in Figure 4.7(c). The Wiener filter seemed to have no filtering effect on the bead image, as it looks almost like the distorted original image shown in the attached reference page (page no. 78) for visual comparison. This is more interesting to visualize when comparing Figure 4.9(c) and Figure 4.11(c), respectively, for medium noise and high noise level. This comparison is just to show the clear effect of noise on the filtered images for the same P_1 and P_2 . In justification with the scatter blur PSF tested in Section 2 4, P_2 has more effect than P_1 on the rolloff decay of the scatter blur. Less dispersion resulted from an increase in P_2 value, thus less glow to filter from the blurred bead image.

The Wiener filter performed best in the medium noise level mode. Figures 4.8(b) and 4.9(b), demonstrate good examples of Wiener filtered images. For these optimum parameters, there is a clear evidence of the Wiener filter success in eliminating the scatter blur and considerably diminishing the excessive glow contaminating the bead image, besides the ability to resolve fine features.

Overcompensated

Good

Undercompensated

(c) $P_1 = 0.8$
 $P_2 = 2.5$

FIGURE 4.7 Filtered beads and profiles. Fixed $P_1 = 0.8$. Low Noise

Overcompensated

(a) $P_1 = 0.2$
 $P_2 = 1.8$

Good

(b) $P_1 = 1.0$
 $P_2 = 1.8$

Undercompensated

(c) $P_1 = 2.5$
 $P_2 = 1.8$

FIGURE 4.8 Filtered beads and profiles. Fixed $P_2 = 1.8$. Medium Noise

Overcompensated

(a) $P_1 = 0.8$
 $P_2 = 1.0$

Good

(b) $P_1 = 0.8$
 $P_2 = 1.8$

Undercompensated

(c) $P_1 = 0.8$
 $P_2 = 2.5$

FIGURE 4.9 Filtered beads and profiles. Fixed $P_1 = 0.8$. Medium noise.

Overestimation of the noise is illustrated clearly in Figures 4.10 and 4.11 for the same range of values for P_1 and P_2 as demonstrated earlier in this text. For the optimum case as in Figures 4.10(a) and 4.11(a), one can see the dominant effect of noise on the filtered beads when compared to the medium noise mode as in Figures 4.8(b) and 4.9(b). It is evident from observation that overestimation the noise does reblur the filtered images, thus obscuring the finer details in the bead images.

The Wiener filter performance seems to be very insensitive to the change of the wide range of parameters P_1 and P_2 , while its obvious sensitivity to the noise is inevitable. When either P_1 or P_2 are underestimated the filter gain becomes very high in magnitude, especially in the P_2 case, thus amplifying the image signal at the low frequency region causing undesirable artifacts. The Wiener filter demonstrated its effectiveness for the range of values for $P_1 = 0.8-1.0$ and $P_2 = 1.8-2.0$, for medium noise mode. From a series of tests and observation results, parameter P_2 slightly showed more impact than P_1 on the Wiener filter performance and the filtered beads.

Overestimating P_1 and P_2 leads to undercompensation of the distorted image, hence no noticeable effect on filtering the bead image nor any indication of eliminating the unwanted glow. Therefore, the Wiener filter performed ineffectively in this case. When both P_1 and P_2 are underestimated the images become reblurred due to overfiltering of the Wiener filter.

Figure 4.12(a), illustrates one of the extreme cases where both P_1 and P_2 are underestimated for medium noise level. Figure 4.12(c) shows the second extreme case when both P_1 and P_2 are overestimated. The filtered image in Figure 4.12(b) for the optimum parameters $P_1 = 0.8$ and $P_2 = 1.8$ is given to provide a closer look and more definite conclusion of the pronounced difference between the three filtered images.

In Figure 4.12(a), P_1 and P_2 are underestimated, it looks like the filtered image has been reblurred due to overcompensation of the Wiener filter. The image is obscured and has obvious ringing artifacts. As discussed in Section 2.4, when P_1 and P_2 are low a broad impulse response creates more dispersion, thus more low frequency amplification is required to compensate for the scatter blur.

When overestimating P_1 and P_2 as in Figure 4.12(c), the Wiener filter has done little or no filtering at all to the bead images. The filtered beads look almost the same as the original blurred bead shown in the reference page enclosed (page no. 78). It seems that the Wiener filter has no effect beyond this range of values for P_1 and P_2 .

Overcompensated

(a) $P_1 = 0.2$
 $P_2 = 1.8$

Good

(b) $P_1 = 1.0$
 $P_2 = 1.8$

Undercompensated

(c) $P_1 = 2.5$
 $P_2 = 1.8$

FIGURE 4.10 Filtered beads and profiles. Fixed $P_2 = 1.8$. High Noise

Overcompensated **Good** **Undercompensated****(c) $P_1 = 0.8$
 $P_2 = 2.5$** **FIGURE 4.11 Filtered beads and profiles. Fixed $P_1 = 0.8$. High Noise**

Overcompensated

Good

Undercompensated

(a) $P1 = 0.2$
 $P2 = 1.0$

(b) $P1 = 0.8$
 $P2 = 1.8$

(b) $P1 = 1.5$
 $P2 = 2.5$

FIGURE 4.12 Filtered beads and profiles. Medium noise

4.3.2 Cell Image Example

The cell image is a biological digital image acquired through the fluorescence microscope imaging system depicted in Figure 2.2. The motivation behind of Wiener filtering is to remove the fluorescent decay of the luminous bodies. So far, the Wiener filter demonstrated its ability in reducing the scatter blur and removing the glow obscuring the test bead images under optimal conditions. In this example, the Wiener filter is applied to the cell image under optimum and incorrect settings. Figure 4.13 shows the cell image filtered for the optimum parameters $P_1 = 0.8$ and $P_2 = 1.8$, in low, medium, and high noise levels. For a better visual comparison, the original cell image is shown in Figure 4.14(a) along with the filtered cell image for the optimum parameters in medium noise level, in Figure 4.14(b). Moreover, Figure 4.15 shows the optimum case as in Figure 4.15(b), along with the underestimated and overestimated extreme cases, respectively, as in Figures 4.15(a) and 4.15(c).

Figure 4.13(a) shows the cell image result for low noise mode. The Wiener filter succeeded in removing the glow from the bright circular structures. As well, the edges are more defined and slightly sharpened. Comparatively, the image looks grainy and differentiated with respect to Figures 4.13(b) and 4.13(c). This is due to the amplification of the noise components in the high frequency region, as has been tested in the bead experiment, and shown in Figure 4.4(b). In the original image some structures are obscured by the glow, and in the filtered image some of these bright structures have dark spots, thus it is difficult to tell if a hole exists or just a filtering artifact.

The image in Figure 4.13(b) displays a good restoration result indicating the Wiener filter success in retrieving more features that have been suppressed by the glow distortion. There is a clear reduction of glow, moreover the dark rings (undershoots) are quietly reduced, but it is very hard to analyze due the tedious complex structure of the cell. Though, by looking at the profile one can see a reduction in the undershoots and in the noise oscillation, compared to Figure 4.13(a). The indicated circle shown in the profile of Figure 4.13(b), illustrates the capability of the Wiener filter in resolving the specified circled peak into two or more distinct peaks, thus providing a clear definition of the structure and also increasing its contrast. This is the objective of this work.

Overcompensated

Good

Undercompensated

(a) $P1 = 0.8$
 $P2 = 1.8$ (b) $P1 = 0.8$
 $P2 = 1.8$ (c) $P1 = 0.8$
 $P2 = 1.8$ **FIGURE 4.13** Filtered Cells and profiles. (a) Low noise (b) Medium noise (c) High noise

The effect of overestimating the noise is shown in Figure 4.13(c). The cell image is never resolved because the inverse filtering is suppressed by the effects of the high value of the noise parameter N .

The filtered cell image for the optimum parameters shown in Figure 4.13(b) is enlarged in Figure 4.14(b) along with the original distorted cell image depicted in Figure 4.14(a). The success of the Wiener filter in reducing the glow obscuring details in the original blurred image in Figure 4.14(a) and providing a clear, resolved and enhanced image is clear in Figure 4.14(b).

Figure 4.15(a) illustrates the overcompensated case when both P_1 and P_2 are very low. As has been shown from the bead experiment, blurring occurs when both P_1 and P_2 are underestimated. The cell image in Figure 4.15(a) is obscured by the glow and the bright structures are more indistinct and vague. The filtered cell image in Figure 4.15(b) shows a reduction of glow and more clearly defined edges, as previously explained in Figure 4.13, for the three noise modes. Figure 4.15(b) is shown here to show the clear distinct difference between the optimum, the overcompensated, and the undercompensated cases.

Figure 4.15(c) shows the undercompensation of the Wiener filter. It is clear from the filtered cell image that internal structures in the cell are not resolved. The image looks the same as the original. Therefore, the Wiener filter performance in this range is ineffective.

(a)

(b)

FIGURE 4.14 (a) Original cell image (b) Filtered cell image (optimum parameters)

Overcompensated

(a) $P1 = 0.2$
 $P2 = 1.0$

Good

(b) $P1 = 0.8$
 $P2 = 1.8$

Undercompensated

(b) $P1 = 1.5$
 $P2 = 2.5$

FIGURE 4.15 Filtered Cells and profiles. Medium noise

4.3.3 Microtubule Image Example

The microtubule image is a good example due to its unique complex, maze-like structure, which demonstrates the Wiener filter performance and its ability of reducing the glow, resolving features, and enhancing fine details

The microtubule image in Figure 4.16, is filtered for the two extreme cases when both P_1 and P_2 are underestimated and overestimated, respectively, as in Figures 4.16(a) and 4.16(c). Figure 4.16(b) shows the microtubule filtered image for the optimum parameters $P_1 = 0.8$ and $P_2 = 1.8$, for medium noise level. The effect of low and high levels of the noise was demonstrated in filtering the test bead image and which applied to the cell image experiment. Increasing the noise caused reblurring to the image and poor reduction of the glow, while reducing the noise resulted in overcompensating the glow and providing differentiated filtered images.

Figure 4.16(a) shows the reblurred resulted image of the Wiener filter when both P_1 and P_2 are underestimated. Details in the filtered image are obscured due to insufficient bandwidth of the Wiener filter as shown in Figure 4.16(a). Figure 4.16(b) illustrates the Wiener filter capability of resolving fine details in the microtubule image under correct filter settings. There is a considerable enhancement of the bright white cell in the image and resolving of the many string-like structures in the image. The filtered image show more resolved details when compared to the original in the attached reference page (page no. 78).

Obviously, the filtered image in Figure 4.16(c) is not far different from the original shown across in the reference page (page no. 78). The image has been undercompensated, thus no clear evidence of any glow reduction or resolved features, this is due to the overestimation of P_1 and P_2 .

Overcompensated

(a) $P1 = 0.2$
 $P2 = 1.0$

Good

(b) $P1 = 0.8$
 $P2 = 1.8$

Undercompensated

(c) $P1 = 1.5$
 $P2 = 2.5$

FIGURE 4.16 Filtered Microtubule and profiles. Medium noise

Chapter 5

Wiener Filtering In the Spatial Domain

5.1 Introduction

Many imaging systems must operate within stringent time constraints, in real time. Because of the large number of pixels in typical images and the computational complexity of restoration, real-time image restoration is a challenging goal. The traditional techniques described in Chapter 2 are global operations—each element of the output is a function of every element in the input. Global operations are practical only when implemented in the frequency domain, but the hardware and highly customized systems required for high-speed frequency-domain processing is prohibitively expensive for many applications [68]. The existence of advanced Digital Signal Processors (DSPs) such as the TMS320 and the use of spatial parallelism provide less expensive ways to speed up image filtering with substantial flexibility [69]. The work in this chapter illustrates a Wiener filtering algorithm that restores an image by convolving it with a truncated Wiener kernel. The challenge is to design a small Wiener kernel that yields good results. Convolving an image with a kernel that has only a few values is an efficient approach that facilitates fast implementation and possible adaptive processing.

Many of the common image restoration techniques (e.g., the inverse filter, the Wiener filter, and the constrained least-squares filter) are traditionally derived and implemented in the frequency domain. The restoration IR associated with these methods is as large as the image, $M \times N$ elements, so spatial convolution is $O(M^2N^2)$. For typical image sizes (e.g., 256×256 or 512×512), spatial convolution with a kernel as large as the image requires far too much computation and is impractical [70]. Fast transforms allow the equivalent computation to be performed in the frequency domain with significantly fewer

calculations. Frequency domain filtering requires only pointwise multiplication of the image spectrum $G(u, v)$ and the transfer function $W(u, v)$ and is therefore $O(MN)$. In most cases, this requires a Fourier transform to compute the image spectrum and an inverse Fourier transform to compute the resulting restored image.

Fast Fourier transforms are $O(MN \log(MN))$, thus the need for more efficient processing has motivated consideration of local algorithms as restoration implemented by convolution kernels that have only few elements. If the kernel has very few elements, spatial processing would require fewer operations than frequency domain processing. In restoration, as in most filtering operations, each output value is principally determined by the values in a relatively small region around the corresponding point in the input image. Therefore, local operations can be nearly as effective as global operations [72]. Spatial convolution is computationally practical only if the Wiener kernel is small. Spatial convolution of an $M \times N$ image with a K -element kernel is $O(KMN)$.

5.2 Implementation

LSI Wiener restoration techniques can be applied equivalently in either the spatial domain (with convolution) or in the spatial-frequency domain (with pointwise multiplication), as shown in Figure 5.1.

FIGURE 5.1 Wiener filter spatial and frequency domain processing

The response of the Wiener restoration filter derived in the frequency domain as in the previous Chapter

$$W(u, v) = \frac{H^*(u, v)}{|H(u, v)|^2 + N} \quad 5.1$$

where $W(u, v)$ is parametrized by the scatter blur PSF parameters P_1, P_2 , and the noise N . In this Chapter the Wiener filter is implemented in the spatial domain. The Wiener filter IR can then be obtained by inverse transforming $W(u, v)$ as

$$w(m, n) = \sum_{u=0}^{M-1} \sum_{v=0}^{N-1} W(u, v) \exp[un + vm] \quad 5.2$$

where $w(m, n)$ is the IR of the Wiener filter defined over the grid $M \times N$ which is 256×256 in our implementation [46]. The above formulation is implemented by means of the IDFT with the help of the FFT algorithm.

The windowed truncated kernel $w_k(m, n)$ of the Wiener IR is defined over the grid $K \times K$ where $K = 2L + 1$, [53]. The restored filtered image is then given by

$$\hat{f}(x, y) = \sum_{m=-L}^L \sum_{n=-L}^L g(x-m, y-n) w_k(m, n) \quad 5.3$$

where

- g is the 256×256 blurred image.
- w_k is the truncated Wiener $K \times K$ IR kernel.
- $\hat{f}$ is the 256×256 restored image.

To minimize the computation time, the truncated Wiener kernel IR must be designed, in

consideration of the following constraints:

1. In order to reduce the number of multiplications per pixel, in performing the convolution, maximum use of the symmetry must be made.
2. The extension of $w_k(m, n)$ must be limited to be less than 11×11 [73].
3. The final restoration quality of $w_k(m, n)$ has to remain comparable with that of the Wiener restoration filter.

The Wiener filter implementation in the spatial domain is illustrated in Figure 5.2. The Wiener filtering algorithm in the spatial domain is almost the same implementation as in the frequency domain. The only difference is that the blurred input image is convolved with the Wiener truncated kernel instead of multiplying both Fourier transforms, then inverse transforming as in the frequency domain.

For spatial restoration, the Wiener filter $W(u, v)$ must be transformed into the spatial domain using the IDFT. However, the resulting spatial Wiener kernel $w(m, n)$ is as large as the image $G(u, v)$, so the computational cost of the convolution is prohibitive. The computational cost of convolution is proportional to the number of nonzero elements in the kernel. Because of this, spatial kernel are not practical unless they have only a few nonzero elements [74][75]. If the spatial kernel is restricted to a few nonzero values, spatial convolution requires significantly less computation than frequency domain restoration [73].

The algorithm in this work constrains the Wiener truncated kernel $w_k(m, n)$ to a few specific locations. The unconstrained Wiener filter is mean-square-optimal, so the spatially constrained kernel cannot do any better. However, it may perform nearly as well. In practice, the most significant elements of restoration kernels are located near the kernel center and elements far from the center are small. Therefore, it is reasonable to expect that a small spatial kernel with a few well-chosen values near the kernel center can be nearly as effective as the Wiener filter in the frequency domain.

The Wiener filter can be implemented simply in the spatial domain as summarized in the following steps:

- Generate the 256×256 scatter blur PSF.
- Multiply the PSF by a raised-cosine rolloff window.
- Transform the PSF into the frequency domain.
- Calculate the Wiener filter transfer function.
- Normalize the Wiener filter to unity AC gain.
- Inverse transform the Wiener filter transfer function
- Truncate the filter IR to $K \times K$ Wiener kernel.
- Load the 256×256 input blurred image.
- Scale input image to 256 gray levels.
- Convolve input image with the truncated Wiener IR kernel.
- Scale filtered image to 256 gray levels.
- Output and save the restored image.

FIGURE 5.2 Implementation of the Wiener filter in the spatial domain

5.3 Results and Analysis

Small Wiener restoration kernels defined for efficient processing can yield excellent results. However, if the resulting restoration kernel is very large it is impractical. In evaluating the effect of the Wiener finite kernel size, comparisons were made with restoration of the same images that were restored using frequency domain filtering.

The Wiener IR is plotted for fixed $P_1 = 1.0$ and varying $P_2 = 1.0, 1.8, 2.5$ in Figure 5.3, and for fixed $P_2 = 1.0$ and varying $P_1 = 0.1, 0.8, 1.5$ as in Figure 5.4, for medium noise level.

The Wiener IR kernel elements are symmetrical in magnitude and have positive and negative values. The Wiener restoration IR has a peak at the origin and ripples outward. Generally, the width of the central lobe is inversely related to resolution. A PSF with a narrow central lobe has a wide frequency passband while an IR with a wide central lobe attenuates high frequencies. Large sidelobes will cause ringing adjacent to sharp edges [75]. The most significant values of the restoration kernel are located near the origin and the magnitude of the ripples decreases with greater distance from the origin. The existence of one large side lobe and many small ones is very undesirable [76][77] as illustrated in Figures 5.3(a) and 5.4(a). This is the case where P_1 and P_2 are over and underestimated as demonstrated in the results of Chapter 4.

It is interesting to compare the results of this spatial domain Wiener filtering to that accomplished in the frequency domain. As discussed in Chapter 4, multiplying the image transform by the filter transform kernel function is equivalent to convolving a kernel with the image in the spatial domain.

A series of experiments have been carried out for varying P_1 , P_2 , and N in process of restoring the 256×256 bead image with the truncated Wiener IR kernel. It was found that large size kernels are required to achieve the identical restored bead images resulting from the frequency domain for the same settings of Wiener filter parameters P_1 , P_2 , and N . This is disappointing for two reasons:

- The first is that applying a large kernel takes time, even with very fast computers

FIGURE 5.3 Wiener IR plots. Medium noise

FIGURE 5.4 Wiener IR plots. Medium noise.

and with careful programming to achieve the most efficient method for carrying out additions and multiplications in optimum order.

- The second is that the elements of a small kernel can be adaptively altered to achieve good performance, whereas this becomes impractical with large kernel.

Table 5.1 shows the number of operations involved for a number of different kernel sizes and an input bead image of size 256×256 . The first column shows the size of the convolution kernel. The second gives the number of weights in the kernel. The third column shows the number of multiplications required. An equal number of additions is also needed. The fourth column gives the number of divisions required if the convolution result needs to be normalized. The final column shows the sum of all numeric operations needed to perform the convolution. These kernels are relatively modest in size when compared with the 256 pixels square bead image, yet the number of computations rapidly becomes enormous. It must be noted that the table shows only the number of computations. It does not take into account other operations which must be performed. These include extracting the correct pixels from the image and the kernel, writing the result pixel to the output image and maintaining the various indexes and counts which control the whole operation [78].

TABLE 5.1 Computational load for various square convolution kernels

Kernel size K	Kernel elements	Adds and Multiplies	Division	Total
3	9	589824	65536	1 245 184
5	25	1638400	65536	3 342 336
7	49	3211264	65536	6 429 664
9	81	5308416	65536	10 682 368
11	121	7929856	65536	15 925 248
15	225	14745600	65536	29 556 736
25	625	40960000	65536	81 985 536

Convolution is an expensive operation in terms of the amount of computation involved. Sometimes it is useful to normalize the result of the convolution computation. Since it is a sum of products, it is quite possible for the results to be too large to store in the same type of data as the original bead image. This is particularly true of 8-bit unsigned integer images. Naturally, during the computation for an individual pixel, larger data types are used to maintain precision [79][80]. Conversion to the output data type is made as the pixel is written used to maintain precision.

As emphasized earlier, employing large kernels in the spatial domain restoration is undesirable. The objective of the work reported here is to determine the kernel size that compares subjectively to the Wiener restoration results in the frequency domain for the same filter settings. A series of experiments were conducted for the same range of Wiener filter parameters P_1 , P_2 , and N that were investigated in the frequency domain. Table 5.2 presents the results of applying the optimum parameters $P_1 = 0.8$ and $P_2 = 1.8$ for medium noise level. Column 1 shows the kernel sizes. The second column gives the energy in the kernel size as a percentage of the total energy in the Wiener IR. The Kernel size 27 contains the whole energy of the Wiener IR. The need for large kernels to perform Wiener filtering in the spatial domain is due to the wide spread of the scatter blur PSF estimated from the test bead images. The time it took for Wiener spatial convolution for these kernel sizes is shown in column 4.

TABLE 5.2 Energy in truncated Wiener kernel and computation time

Kernel size K	Energy in truncated IR %	Time (min:sec)
3	85.9874%	1:35
7	86.5818%	1:50
11	86.5826%	2:14
23	99.9980%	4:15
27	100%	5:14

Performing the convolution with a 11×11 kernel on the 256×256 pixels bead image required about 2 minutes and 14 seconds on a 33 MHz 80486-based PC compatible computer. The same process took about 1 minute and 52 seconds in the frequency domain. It took less time for kernel sizes 3×3 and 7×7 as shown in Table 5.2.

Figure 5.5 shows an example of the filtering results for different kernel sizes. For kernel size 3×3 as shown in Figure 5.5(a), there is no visual indication that the bead image has been restored. The filtered image looks similar to the original bead image shown in the attached reference page (page no. 78). The profiles show the bead image profile filtered in the frequency domain superimposed on the spatial domain filtered bead image profile.

Subjectively, the filtered bead images get closer in appearance to those filtered in the frequency domain when the kernel size increases, as shown in Figures 5.5 and 5.6. This is simpler to judge when looking at the image profiles. They tend to overlap as illustrated in Figure 5.5(c) for kernel size 27.

It is clear from the results that unless a very powerful machine is available or special hardware assistance is provided, Wiener spatial convolution rapidly becomes prohibitive in terms of computational cost [81]. But, there are some important factors which may prove helpful in solving the above problem:

- Image filtering can be speeded up somewhat by the use of special hardware, such as a TMS320 DSP processor board. This provides relatively low cost means of solving certain problems as image filtering applications [69][82].
- Because of the localized nature of image processing. This suggests the use of spatial parallelism--processing different areas of an image simultaneously with different computers [73].

$K = 3 \times 3$

$K = 11 \times 11$

$K = 27 \times 27$

FIGURE 5.5 Filtered bead images and profiles. Medium noise.

frequency domain filtered bead

$K = 3 \times 3$

$K = 11 \times 11$

$K = 27 \times 27$

spatial domain filtered beads

$P1 = 0.8$
 $P2 = 1.8$

$K = 35 \times 35$

FIGURE 5.6 Filtered bead images. Medium noise

Chapter 6

Conclusions

6.1 Summary of Results

The image restoration problem deals with the estimation of an original image from its noisy and blurred observation. Since the blur and noise may mask important information in the image, it becomes necessary to restore the observed distorted images to recover the information. We have shown that the linear minimum MSE Wiener filter is an effective scatter blur filtering algorithm.

A number of assumptions were made in this research to simplify the image restoration problem. The distortion model was estimated from the blurred test bead images. The procedures developed in this thesis apply to the identification and restoration of LSI blurs only. All the parameters needed were estimated from the blurred test bead images. These assumptions may not always be entirely valid. Various assumptions may be imposed on the restoration in order to compensate for the loss of information incurred in the degradation process. Oftentimes, however, these assumptions are nothing more than educated guesses made to facilitate the restoration process. In our work, the parameters of the model were varied to achieve “optimal” results. Thus, the image restoration field is clearly in need of a method for testing assumptions imposed on the restoration process. Therefore, an important goal for solving the general image restoration problem is the development of techniques that relax assumptions made in this research.

The scatter blur PSF analytical model was chosen over the exponential model as our candidate distortion PSF, because it gave a better fit to the empirical data measured from the test bead image using the curve fitting algorithm based on goodness-of-fit criterion.

The LSI scatter blur PSF was modelled from the fluorescent plastic bead images. The PSF model is parametrized by two parameters P_1 , and P_2 . The value of this model ultimately lies in [33];

- its ability to predict the behavior of the spatial distribution of the scatter blur.
- its performance in the Wiener deblurring algorithm to compensate the scatter blur.

We have demonstrated the Wiener filter performance as a function of the scatter blur PSF parameters P_1 , P_2 , and the additive noise parameter N . The Wiener filter is a very effective filtering algorithm, demonstrated by its ability to remove the glow distortion and resolve fine details from bead and the cell images.

In order to evaluate the performance of the Wiener filter and the comparison of filtered images, we need some quality measurements. Unfortunately, quantitative measurements are very difficult to define and standardize, therefore subjective qualitative evaluation is the best choice, which is the approach in this thesis.

In applications where processing speed is important, the most significant problem with the Wiener filter, and other Fourier transform based techniques is computational cost. High-speed implementation of these global algorithms requires expensive, special-purpose hardware. Restoration implemented by convolution with truncated kernels requires significantly less computation than the frequency domain processing provided that the kernel size does not exceed 11×11 [73]. However, for any computer-based system, increasing the kernel size eventually reaches a point at which it is more efficient to perform the operation in the Fourier domain. The time needed to perform the FFT transformation from the spatial domain to the frequency domain and back is more than balanced by the speed with which the convolution can be carried out. The tests and restoration results presented in Chapter 5 indicate that the truncated Wiener kernel restoration is not as effective as the Wiener restoration in the frequency domain.

6.2 Proposals for Future Research

These assumptions are of much interest for future research:

- One such assumption is that the blur process is linear. This assumption is not always valid. Methods of incorporating sensor nonlinearities into the restoration process are an important open area of research. However, no automatic technique has yet been perfected for this problem.
- Another assumption is the LSI blur. Linear Shift-Variant (LSV) identification problem requires some rather restrictive assumptions. The proper choice and imposition of assumptions for space varying blur identification are important areas for future research in image restoration.
- The implementation of the TMS320 DSP board would provide less expensive means to speed up image filtering and achieve faster processing. More importantly, DSP boards and similar other image graphic processors will become more and more an integral part of the future workstations.

The results from Chapter 5 should spur research into related problems:

- The results of Chapter 5 suggests that truncated Wiener kernels are ineffective when compared to the robust performance of the Wiener filter performance in the frequency domain. Robustness is a significant advantage in real applications. This hypothesis should be thoroughly tested.
- Knowledge of the relationship between restoration kernel size and error would be very helpful. Undoubtedly, this relation is a complicated function of all of the components of the imaging system, but a parametric expression for predicting error would be useful in setting the kernel size [83].
- Cascade convolution is a technique for efficient spatial processing. A cascade is a series of convolutions. Certain types of large kernels can be decomposed into a series of small kernels and applied much more efficiently. Cascading small

spatial kernels is an area of active research [84][85].

- One might wish to impose other constraints on the restoration. For example, the kernel values might be limited to integers so that the implementation can be restricted to integer arithmetic. Constraints also can be placed on the restoration result (e.g., as in the constrained-least-squares filter). These restrictions and others have received widespread consideration, but should be integrated with constraints on restoration kernel size.
- Lahart [76] noted that truncated kernels can be varied adaptively to deal with spatially variant PSF's, noise, and autocorrelation. In an example, he classed pixels as lying either in regions with high or low autocorrelation and applied one of two restoration kernels according to the pixels class. He noted that this binary division could be expanded to a range of classes. Because the assumption of a stationary process is seldom realistic, space-invariant methods merit investigation. Because adaptive processing requires more computation, efficient algorithms are particularly important.

The Wiener filter restoration is based on a rigorous derivation, but restoration research should not stray from practical solutions. The Wiener filter restoration is a practical technique. It is simple, yet efficient and effective.

folded page

Microbead Image

Cell Image

Microtubule Image

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PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
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PRECISIONSM RESOLUTION TARGETS

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