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Constrained Inverse Filtering Restoration of Scatter-Blurred Video Fluorescence Micrographs

by

Djamila Belhandouz, B.Sc., M.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,
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Scatter-Blurred Video Fluorescence
Micrographs”**

Submitted by

Djamila Belhandouz

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

Thesis Supervisor

Chair, Department of Systems
and Computer Engineering

Carleton University,
December 02, 1994

Abstract

Image restoration refers to the problem of removal or reduction of degradation in noisy blurred images. In this thesis we focus on the restoration of images produced by fluorescence microscopy. These images are blurred by a glowing aura that surrounds the fluorescently tagged structures and further degraded by an additive noise.

Parametrization of the distortion function and its use permits removal of image degradations by two-dimensional deconvolution techniques.

As a starting point Wiener filtering restoration is used. This restoration technique turned out to be not "optimum", contrary to what it is said in the literature, and is plagued with several problems that limit its effectiveness. First, Wiener filtering requires accurate knowledge of the covariances of the ideal image and the noise to obtain optimal results. Second, Wiener filtering restoration results in artifacts caused by excessive noise amplification at high frequency. Finally, the mean-square error (MSE) criterion of optimality is not particularly good if the image is being restored for the human eye. The MSE criterion weights all errors equally regardless of their location in the image.

An image restoration technique called Constrained Inverse Filtering is presented in this thesis. The system is developed by attempting to overcome subjectively some failures of the Wiener filtering restoration technique. The restoration system, which is a combination of inverse filtering and noise reduction, yields independent control of the distortion and the noise. Constrained Inverse Filtering is identified as an effective algorithm for image restoration. It successfully reduces the restoration artifacts, which manifest themselves as ringing and filtered noise artifacts, while reducing the blurring.

Constrained Inverse filtering restorations show significant improvement over the Wiener filtering restorations in reducing the restoration artifacts. Some examples are given to illustrate the performances of the Constrained Inverse and Wiener filtering image restoration systems.

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Table of Contents

Abstract	iii
Acknowledgements	vi
List of Figures	xi
Glossary	xiv
1 Introduction	1
2 Source image and model of distortion	5
2.1 Introduction.	5
2.2 Fluorescence microscopy.....	5
2.3 Model of a fluorescence imaging system	6
2.4 Origin and type of images in the restoration process	10
2.4.1 Beads	10
2.4.2 Cells	11
2.4.3 Labelling of the specific cell observed through the microscope	13
2.5 Source image and model of distortion.....	14
2.5.1 Modeling blurred images	16
3 Digital image restoration techniques	21
3.1 Introduction	21
3.2 Basic formulations of the restoration problem	21
3.2.1 Noise in restoration	24
3.3 Image restoration technique.....	25
3.3.1 Inverse filtering	26
3.3.2 Wiener filtering	28
3.3.3 Constrained Inverse filtering	29
4 Characterization and implementation of the restoration filter	31
4.1 Wiener filter.....	32
4.1.1 Wiener filter digital implementation	34
4.2 Constrained Inverse filter	39

5 Restoration of images distorted by fluorescence microscopy	48
5.1 Restoration procedure.....	49
5.2 Experimental measurements.....	51
5.3 Results and analysis.....	52
5.3.1 Wiener filtering restoration	54
5.3.2 Constrained Inverse filtering restoration	62
5.4 Quantitative fidelity measure.....	74
5.5 Examples: restoration of cell and microtubule images.....	82
5.6 Computational analysis of image filtering restoration.....	92
6 Concluding remarks	95
6.1 Summary of contributions	95
6.2 Conclusions.....	95
6.3 Recommendations for future research.....	97
Bibliography	100

List of Tables

TABLE 5.1	Execution time of the restoration algorithm for different PCs	94
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List of Figures

FIGURE 2.1 Model of a fluorescence imaging system [22]	7
FIGURE 2.2 The optical system of a modern fluorescence microscope [24]	9
FIGURE 2.3 (a) Microbead image and (b) a cross-section profile of the intensity.....	12
FIGURE 2.4 (a) Cell image and (b) a cross-section profile of the intensity.	12
FIGURE 2.5 Schematic representation of the labeling of the specific protein	14
FIGURE 2.6 PSF and Transfer function for $P_2=1.8$ and P_1 varying.	18
FIGURE 2.7 PSF and Transfer function for $P_1=1.0$ and P_2 varying.....	19
FIGURE 3.1 A model of the image degradation process.....	22
FIGURE 3.2 Reduction of blurring and additive noise.....	30
FIGURE 4.1 Digital implementation of the Wiener filter	35
FIGURE 4.2 Wiener filter transfer functions (a) P_1 varying and $P_2=1.8$ and (b) P_2 varying and $P_1=1.0$	37
FIGURE 4.3 Wiener filter transfer function for $P_1=0.8$, $P_2=1.8$ and variable noise N ..	38
FIGURE 4.4 Constrained Inverse filter	40
FIGURE 4.5 Constrained Inverse filter transfer functions for P_1 varying and P_2 fixed at 1.8	41
FIGURE 4.6 Constrained Inverse filter transfer functions for P_2 varying and P_1 fixed at 1.0	43
FIGURE 4.7 Constrained Inverse filter transfer functions for different roll off band- widths	44
FIGURE 4.8 Constrained Inverse and Wiener filters transfer functions	46
FIGURE 4.9 Amplitude spectrum of the acquired microbead image.	47
FIGURE 5.1 Schematic representation of the degradation and restoration processes of an image	49
FIGURE 5.2 Block diagram of the restoration procedure	52
FIGURE 5.3 The acquired original microbead image	53
FIGURE 5.4 Wiener filtering : original and restored images with respective profiles and amplitude spectra	55
FIGURE 5.5 Wiener filtering : original and restored images with respective profiles and amplitude spectra	56
FIGURE 5.6 (a) Original and Wiener filtered images and (b) respective cross-section brightness profiles.	58

FIGURE 5.7 (a) Cross-section brightness profiles and (b) Amplitude spectra of Wiener restored images and original image	60
FIGURE 5.8 (a) Cross-section brightness profiles and (b) Amplitude spectra of Wiener restored images and original image	61
FIGURE 5.9 Constrained Inverse filtering: original and restored images, cross-section profiles and amplitude spectra	63
FIGURE 5.10 Constrained Inverse filtering: original and restored images, cross-section profiles and amplitude spectra	64
FIGURE 5.11 (a) Original and optimum restored images and (b) respective cross-section profiles	67
FIGURE 5.12 (a) Multiple cross-section brightness profiles and (b) multiple amplitude spectra	68
FIGURE 5.13 (a) Multiple cross-section brightness profiles and (b) multiple amplitude spectra	69
FIGURE 5.14 Restored images for two extreme cases corresponding to low and high parameters	71
FIGURE 5.15 Restored images and respective cross-section profiles for $P_1=0.8$ and $P_2=1.8$	72
FIGURE 5.16 Restored images and respective cross-section profiles for $P_1=0.8$ and $P_2=1.8$	73
FIGURE 5.17 Sub-band energy plots of restored images by constrained inverse filter for different parameters	75
FIGURE 5.18 Amplitude spectra at fixed cycles for different values of the parameter P_2 and $P_1=1.0$	78
FIGURE 5.19 Sub-band energy plots of restored images by Wiener filter for different parameters	79
FIGURE 5.20 (a) Optimum restored images by Constrained Inverse and Wiener filtering and (b) cross-section profiles	80
FIGURE 5.21 Amplitude spectra of Constrained Inverse and Wiener filtered images ..	81
FIGURE 5.22 Constrained Inverse filtering of cell images, profiles and amplitude spectra	84
FIGURE 5.23 Constrained Inverse filtering of cell images, profiles and amplitude spectra	85
FIGURE 5.24 Constrained Inverse filtering of microtubule images, cross-section	

profiles and amplitude spectra	86
FIGURE 5.25 Constrained Inverse filtering of microtubule images, cross-section	
profiles and amplitude spectra	87
FIGURE 5.26 Wiener filtering of cell images, profiles and amplitude spectra	88
FIGURE 5.27 Wiener filtering of cell images, profiles and amplitude spectra	89
FIGURE 5.28 Wiener filtering of microtubule images, profiles and amplitude spectra .	90
FIGURE 5.29 Wiener filtering of microtubule images, cross-section brightness pro-	
files and amplitude spectra.	91

Glossary

CCD

Charged-Coupled Device.

DFT

Discrete Fourier Transform.

DMA

Direct Memory Acces.

DNA

Dioxynucleic acid

DSP

Digital Signal Processing.

FTIC

Fluorescein Isothfluoresiocyanate.

FFT

Fast Fourier Transform.

FLOP

Floating Point Operation.

IFFT

Inverse Fast Fourier Transform.

LSI

Linear Shift Invariant.

MA

Memory Acces.

MSE

Mean Square Error.

PSF

Point Spread Function.

SIT

Silicon Intensifier Target.

Chapter 1

Introduction

The field of image restoration in the modern sense of the term began in the early 1950's with the work of Mareshal and his co-workers[1]. Although the possibility of optical spatial filtering had been demonstrated by the experiments of Abbe and Porter some fifty years earlier, it was Mareshal who first recognized its potential for restoring blurred photographs. His success stimulated others to study image restoration from the point of view of compensation of the degradations.

In this thesis, we will be dealing with the restoration of images of biological structure produced by fluorescence microscopy. In cell biology, fluorescence microscopy is heavily used in imaging illuminated microscopic, basic structures within the cell. These structures are viewed through an optical microscope. It is usually hard to observe the microscopic cellular components directly, and one way to overcome this problem is to use fluorescent tagging of various biological structures. Fluorescence images observed through a microscope and recorded through a sensor contain a noticeable amount of distortion. The images acquired by fluorescence microscopy are distorted by the various optical components of the imaging system and blurred by the glowing aura that surrounds the fluorescently tagged structures within the cell. This scatter blur is essentially due to the interaction of the emitted light with the surrounding material which will scatter the light and produce a glowing aura. Blurring is a form of bandwidth reduction of the image owing to the image formation process.

The problem of image restoration has been extensively studied for its practical importance as well as its theoretical interest [2-14]. The ultimate goal of restoration techniques is to improve an image in some sense. We consider restoration to be a process

that attempts to reconstruct or recover an image that has been degraded by using some a priori knowledge of the degradation phenomenon. Evidently, any such estimation procedure must require some form of knowledge concerning the degradation function. In some cases, the physical phenomenon underlying the degradation can be used to determine the degradation function. In other situations, it may be possible to determine the degradation from the degraded picture itself, e.g., when it is known a priori that a certain portion of the degraded picture is the image of a point, line, or edge in the original picture. Image restoration when the point spread function, PSF, is unknown is a difficult nonlinear restoration problem. Restoration methods based on unknown PSF are called blind deconvolution methods [5], [44], [50].

In image restoration, the objective is to reduce or eliminate the degradation. Thus restoration techniques are oriented toward modeling the degradation and applying the inverse process in order to recover an approximation of the original image. This approach usually involves formulating a criterion of goodness that will yield some optimal estimate of the desired results. With many types of image restoration, it is difficult to determine the correct criteria to differentiate between wanted and unwanted information because the real image is usually not available. Judging the performance of any restoration process is then dependent on the comparison between the restored image and the expected "real image", otherwise expert judgement is necessary to evaluate the performance of the restoration filtering.

Restoration algorithms are typically mathematical and complex algorithms. In addition, they are designed to exploit detailed characteristics of the signal and degradation. The development of an image restoration system depends on the type of degradation. Algorithms that attempt to reduce additive random noise are different from those that attempt to reduce blurring [2], [3]. The removal of image blur by applying a deblurring function is considered as a restoration technique. The image deblurring problem is a classical example of an ill-conditioned problem because its solution is highly sensitive to measurement errors [2]. Accurate knowledge about the degradation is essential in developing successful restoration algorithms.

A common artifact associated with any of the restoration techniques is ringing, a

Gibbs-like oscillation introduced in the vicinity of abrupt changes in intensity. Methods for reducing noise amplification, such as regularization, tend to make this problem worse [41].

The following concepts are important to an understanding of both the capabilities and limitations of image processing [3].

- 1) Because of limitations on resolution and because of the presence of noise, any image contains only finite amount of information.
- 2) Image degradation always reduces the information content of an image.
- 3) In the presence of degradation, the human visual system is incapable of efficient extraction of that information which is contained in the image.
- 4) An image is processed in an attempt to present that information in such a way that it can be extracted visually.
- 5) Both the goal and the fundamental limit of image processing is achieved when the human visual system is able to extract from the processed image all of the information contained in the original degraded image.

The various restoration approaches that have appeared in the literature depend on the particular degradation model assumed. In this study, we assume that the degradation model is linear and space invariant with additive noise. As a starting point, the Wiener filter is used to restore images acquired by fluorescence microscopy. This filter is supposed to remove the blur caused by the excessive glow from fluorescent tags and suppress the noise due to quantization. When both noise and blur are present, the Wiener filter achieves a compromise between the low-pass noise smoothing filter and the high-pass inverse filter resulting in a band-pass filter. The blurring process is usually a low-pass filter, and the Wiener filter acts as a high-pass filter at low levels of noise [10]. We observe that the deblurring effect of the Wiener filter diminishes rapidly as the noise level increases.

Wiener filtering (minimizing mean square error) is commonly used to restore linearly degraded images. To obtain optimal results, there must be accurate knowledge of the covariance of the ideal image. A set of prototype images of the ideal signal is often suggested to be used for the estimation of the covariance. However, in practical situations,

it is unlikely that such a set of prototypes will be available, and consequently the degraded image is often used for the covariance estimation. This leads to undesirable restored results because of the lack of proper and sufficient images for the covariance estimate. From our observations, Wiener filter restoration appears to cause large artifacts such as considerable noise amplification in the high frequency range and ringing. Observation of the artifacts associated with Wiener filter restoration has led to the hypothesis of separating the restoration process into two steps: inverse filter restoration corresponding to the inverse of the degradation function followed by noise reduction. This technique is called Constrained Inverse filtering and provides the observer with independent control of the noise and the distortion [5].

A serious attempt has been made to compare the performance of Constrained Inverse filter restoration with other restoration techniques namely the Wiener filter restoration. The obtained results indicate that the performance of the restoration system developed using the Constrained Inverse filter restoration compares favorably with the Wiener filter restoration. The restored image appears relatively clear, sharp and free of artifacts such as high frequency noise. It is this blurring of the fine detail by the Wiener filter that has led, at least in part, to the constrained inverse filter.

The overall objectives of this research work are to develop a new image restoration system and illustrate its performance. In Chapter 2, we discuss the source image and develop the image degradation model. Various techniques related to digital image restoration, including the new image restoration system are described in general in Chapter 3. In Chapter 4, Wiener filter restoration and the new constrained inverse filter restoration are analyzed. Their performance is compared based on their transfer functions. In Chapter 5, we discuss and illustrate various examples in which the new restoration filter and the Wiener restoration filter are applied to restore blurry and noisy images. Conclusions and suggestions for further studies are discussed in Chapter 6.

Chapter 2

Source image and model of distortion

2.1 Introduction.

In this research work, the particular problem that we will be dealing with will be the restoration of distorted images produced by fluorescence microscopy. The microscope provides us with the fluorescence images on which we can perform quantitative measurements. However, various limitations and distortions are inherent and introduced in quantitative fluorescence microscopy [22], [23].

In order to design a digital image restoration system effectively it is necessary to characterize the image degradation effects of the physical imaging system quantitatively. Basically, the procedure is to model the image degradation effects and then perform operations to “undo” the degradation to obtain a restored image. It should be emphasized that accurate image modeling is often the key to effective image restoration. A mathematical model is described in this chapter for image degradation in our fluorescence microscopy imaging system [8], [16], [17]. The a posteriori modeling approach is used to develop the model for the image degradation effects based on measurements of a particular image to be restored. Also, in this chapter, some necessary information about the fluorescence microscopy imaging system is discussed in order to understand the nature and the effects of the degradation.

2.2 Fluorescence microscopy

Fluorescence microscopy has the ability to allow the examination of spatial distribution of specific proteins or other molecules in cells and tissues [22-35]. Fluorescence is a type of luminescence where light is emitted from molecules for a short period of time following the absorption of light [22], [23]. The emitted light interacts with matter and it may be

either scattered (diffracted) light or absorbed. Microscopy is concerned with the power of light per unit of cross-section since the light is spread out over some area of the specimen. Fluorescent probes and fluorescently marked immunological probes allow us to visualize and quantify basic structures within the cell [25]. A very powerful and widely used technique is to couple fluorescent dyes to antibody molecules, which then serve as highly specific and versatile staining reagents that bind selectively to the particular macromolecules they recognize in cells. One fluorescent dye that is commonly used for this purpose is fluorescein, which emits an intensive green fluorescence when excited with blue light. By coupling an antibody to fluorescein, specific molecules can be detected in the cell [24].

2.3 Model of a fluorescence imaging system

In recent years electronic imaging systems and the associated technology of image processing have had a major impact on light microscopy. They have enabled certain practical limitations of microscopes to be largely overcome [22], [23], [24]. Various components form a fluorescence imaging system which produces a digital image in a computer memory. The instrumentation required for fluorescence imaging microscopy involves the integration of fluorescence microscopy, electronic imaging, and digital image processing. The system includes a stable excitation light source, a shutter to control the duration of the excitation, a mechanism for changing excitation or emission wavelengths, an electromechanical stage, a low-light-level detector (camera), an image processor, and a host computer coordinated to control overall aspects of the system [22], [32], [34], as it is illustrated in Fig. 2.1. As stated earlier, images observed through an optical fluorescence microscope and recorded through a sensor contain a noticeable amount of distortion.

The fluorescent dyes used for staining cells are detected with the help of a fluorescence microscope. A fluorescence microscope is a compound microscope similar to an ordinary light microscope except that the illuminating light, from a very powerful source, is passed through two sets of filters. One to filter the light before it reaches the specimen and one to filter the light obtained from the specimen [23], [24].

FIGURE 2.1 Model of a fluorescence imaging system [22]

The first filter is selected so that it passes only the wavelengths that excite the particular fluorescent dye, while the second filter blocks out this light and passes only the wavelengths emitted when the dye fluoresces. Figure 2.2 illustrates the optical system of a modern fluorescence microscope [24]. The fundamental principle in the concept of a fluorescence microscope is to maximize the collection of fluorescent light while minimizing the collection of excitation light. This is achieved by optimizing the optical configuration and components of the fluorescence microscope [24]. In the real world the intensity of the fluorescence from the specimen is usually several orders of magnitude less intense than the intensity of illumination. This fact is a major constraint in the concept of a fluorescence microscope [23].

The desire for spatial information dictates the need for a two-dimensional detection system. Fluorescence images must be recorded using low-light-level cameras or cooled CCD imagers and digital image processing to minimize the damage that can be caused by a poor visibility of the image [23]. The video camera produces an output signal related to the brightness of the incident light. A Silicon Intensified Target (S.I.T) video camera has been used to produce the images that we are dealing with in this work. This camera is a high sensitivity TV camera. Low-light-level excitation is generally used with this type of camera which improves the image visibility [20], [27]. Because images produced by video cameras are in electronic form, they can be readily digitized, fed to a computer, and processed in various ways to extract latent information. Moreover, by using video systems linked to image processors, contrast can be greatly enhanced so that the eye's limitations in detecting small differences in light intensity are overcome. The video camera may contain from one to three stages of intensification.

The other components of a television camera based digitization system are normally not part of the camera itself. Often they will be mounted on an adaptor card which can be used in a personal computer or workstation. Such an adaptor is commonly known as a frame grabber. This must be a very high speed device to cope with the large data rate of the signal. The converter produces the digital values of the signal. A typical frame grabber will digitize each row of the incoming signal into 512 samples [37]. In our case, a Coreco Oculus 300 frame grabber has been used.

FIGURE 2.2 The optical system of a modern fluorescence microscope [24]

In quantitative fluorescence microscopy, the aim is to measure and interpret the light output from an excited fluorophore. Quantitative image analysis requires a storage medium that does not compromise the accuracy of the image. Digital devices provide faithful reproduction of all aspects of the images since the pixels are stored as numeric data. Most modern computer workstations have high-performance disks available that are capable of very fast transfer rates, adequate for most applications. Digital disks are ideally for quantitative imaging applications [23].

2.4 Origin and type of images in the restoration process

The images used in this thesis originated from a research program in molecular biology conducted in the Department of Biology at Carleton University under the direction of Dr. N. Chaly. The images were recorded from an S.I.T TV camera attached to the fluorescence microscope described earlier in section 2.3. Two classes of image were acquired. The first were images of fluorescent plastic microbeads used as test images, the second were cell and nuclear images.

2.4.1 Beads

Fluorescently tagged plastic beads were used to calibrate the imaging system. These beads, obtained from Polysciences Inc., are uniform spheres of known refractive index (polystyrene $n_D^{20}=1.60$) and are widely used as calibration standards. Such fluorescent functional latex spheres are used as highly sensitive fluorescent probes and quantitative reagents for biochemical and immunological studies. Polysciences uses proprietary dyes to enhance the detection of the beads. The beads available are made from a variety of polymers. The beads are available with diameters ranging from sub-micrometers to a millimeter or more. They can be obtained "plain", i.e., with no reactive group available on them, or they may contain various reactive groups to which one can attach selected dyes or other molecules such as antigens or antibodies. They are available with various 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 bead¹[23]. In general these beads are relatively constant with respect to their fluorescence intensities. Dye quality and levels are maintained to strict spectral specifications to insure the consistency and reproducibility of lots. Individual beads also show consistent levels of dye content. The protein binding activities of the particle are not adversely affected by the dye.

The fluorescent microbead consists of a disk of high luminosity with an aura of fluorescent decay. Figures 2.3(a) and (b) show an acquired bead image and a cross-section profile of the intensity, respectively. Beads in the images shown are nominally $5\mu m$ in diameter. The fluorescent microbead images are used to help in the evaluation of the distortion that the acquired images have suffered and as test images in the restoration process.

2.4.2 Cells

A typical animal cell is 10 to 20 μm in diameter, which is about five times smaller than the smallest particle visible to the naked eye [24]. Two major contents of the cell are the nucleus and the cytoplasm. The nucleus is the most conspicuous organelle in the cell. It is separated from the cytoplasm by an envelope consisting of two membranes. All of the chromosomal DNA is held in the nucleus. In the cytoplasm, most of the cell's metabolic reactions occur. In the microscope, labelled components of the nucleus display significant fluorescence. They are viewed as brightly fluorescent compared to the cytoplasm which also contributes to the total fluorescence of the cell, this low-level fluorescence however, is relatively constant.

The cell images used in the restoration process are images of a particular human cervical carcinoma cell called Hela. These images have only one particular protein defined at a time. Immunofluorescence microscopic labelling of a single type of protein is used to separate proteins from one to another. Figures 2.4(a) and (b) illustrate a cell image acquired by fluorescence microscopy and a corresponding brightness cross-section profile.

1. Our experiments would tend to indicate that the former is true in our case.

(a)

(b)

FIGURE 2.3 (a) Microbead image and (b) a cross-section profile of the intensity.

(a)

(b)

FIGURE 2.4 (a) Cell image and (b) a cross-section profile of the intensity.

2.4.3 Labelling of the specific cell observed through the microscope

Fluorescence microscopy is most often used to detect specific proteins or other molecules in cells and tissues. Cells that have been fixed and stained can be studied in a conventional light microscope, while antibodies coupled to fluorescent dyes can be used to locate specific molecules in cells in a fluorescence microscope. Cells have many different proteins, namely antibodies produced by the vertebrate animal immune system as a defence against infection[24]. These antibodies have a precise antigen specificity and when labeled with fluorescent dyes, they are invaluable for locating specific molecules in cells by fluorescence microscopy.

In the particular experiment from which we got our images, first, the cell is infected with a virus called adenovirus (5) which will grow inside the nucleus. Viral replication factories follow after infection, the cell will contain many types of protein, some coded by the germ of the virus and others by the germ of the cell itself. In this experiment, the type of protein labelled is the DBP protein. Second, the infected cells are then injected into animals, a mouse for example, which see them as foreign protein and start to generate antibodies against it. These antibodies, anti-DBP mouse immunoglobulin, are called primary. Finally, the complex formed by the protein and the primary antibody is incubated with a secondary antibody, for example a goat antibody, to obtain a goat anti-mouse antibody immunoglobulin. The secondary antibodies are chemically reacted with a molecule that is fluorescent, called fluorescein isothfluoresiocyanate, FITC. This process of labeling is called conjugation. A goat anti-mouse immunoglobulin fluorescein isothfluoresiocyanate, Ig FITC, is then obtained. A schematic representation of the described labeling process is given in Fig. 2.5. A group of labelled secondary antibodies with fluorescent dyes linked to them are used to detect and recognize the complex formed by the protein and the primary antibody. A fluorescence microscope is then used to look into the cell and detect the selected protein, DBP protein [23], [24]. Thus the protein produced by the animal immune system against that infection can be detected.

The acquired images are cell or bead images produced by the described fluorescence microscopy imaging system. The images acquired from the fluorescence microscope are blurred by the excessive glow that surrounds the fluorescent dyes linked to one of the

various biological structures. This glow appears to be due to the interaction of the emitted light with surrounding material scattering the light and producing a glowing aura.

FIGURE 2.5 Schematic representation of the labeling of the specific protein

2.5 Source image and model of distortion

The images that we will deal with in this thesis will be the digital images produced by fluorescence microscopy. A typical imaging system consists of an image formation system, a detector, and a recorder. A large number of imaging systems can be modeled as two-dimensional linear, shift invariant (LSI) systems. If this is true, all linear system theory is applicable. Although no imaging system is truly a linear, shift invariant system, most quality equipment represents a very close approximation [39], [40].

In our work, we assume that the fluorescence imaging system is a linear, shift invariant (LSI) system. This is a useful approximation because the captured image can be characterized and mathematical techniques associated with LSI systems can be applied.

The term image refers to a two-dimensional light-intensity function, denoted by $f(x,y)$, where the value or amplitude of f at spatial coordinates (x,y) gives the intensity (brightness) of the image at that point. As light is a form of energy, $f(x,y)$ must be nonzero and finite [21], [52-55], that is,

$$0 \leq f(x, y) < \infty \quad 2.1$$

The recorded digital image consists of a two-dimensional array of quantized samples, i.e., discrete values. Thus the image can be represented by a weighted sum of impulse functions. Any distribution of fluorescent light produced by illumination and/or fluorochromes can be represented by

$$image = f(i, j) = a(i, j) \cdot \delta(i, j) \quad 2.2$$

Where $a(i,j)$ is the distribution of the fluorescent light and $\delta(i, j)$ is the impulse function.

A linear, shift invariant system is characterized by its impulse response $h(i,j)$. Thus, the recorded digital image, $g(i,j)$, can be represented by the convolution of the impulse response of the fluorescence imaging system, $h(i,j)$, and the input digital image, $f(i,j)$, that is [52-55],

$$g(i, j) = h(i, j) \otimes f(i, j) + n(i, j) \quad 2.3$$

where $f(i, j)$ and $g(i, j)$ represent the input and output sequences, respectively, of a two-dimensional system.

In optics, the response $h(i,j)$ to a single input impulse $\delta(i, j)$, a single point of light, is called the Point Spread Function (PSF) of the imaging system. Equation (2.3) mathematically represents the two-dimensional (2D) convolution of the PSF, $h(i,j)$, with the undegraded image, $f(i,j)$, resulting in the detected image $g(i,j)$. In this equation, the symbol $\otimes$ represents the 2D convolution process and (i,j) the spatial coordinate system chosen to describe the process. The PSF, $h(x,y)$, is represented as a linear and rotationally symmetric function. Thus the higher order terms expressing the non-linear and

nonstationary system response are neglected, based on previous findings [16], [17]. The PSF, $h(i,j)$, has all the information necessary for the description of the imaging system and in the case of a noiseless imaging system, the original image can be recovered from the degraded one if the PSF is known.

The degradation process is modeled as an operator or system H , which together with an additive noise operates on an input image to produce a degraded output image [18]. Image restoration algorithms are designed to exploit characteristics of an image and its degradation function. For this reason, a precise knowledge of the degradation is essential to developing a successful image restoration algorithm [6], [7].

2.5.1 Modeling blurred images

Image restoration may be defined as applying the inverse of a mathematical model that describes the degradation to estimate the original image [38], [41-43]. 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. Hence, in image restoration, it is of utmost importance to develop realistic models for the image degradations. The degradations that we are concerned with in this thesis can be broadly classified as:

- (i) Spatial degradations (blur), and
- (ii) Random pointwise degradations (noise).

A realistic model of the blurred and noisy fluorescence images should include the blurring process during image formation, and the noise sources [5], [6], [7]. Next, we discuss modeling the blur during image formation.

In this thesis, we are dealing with a blind deconvolution problem since there is no a priori knowledge about the degradation phenomenon. One approach to obtaining information about degradation is to gather information from the degraded image itself. If an image is blurred and we can identify a region in the degraded image where the original undegraded image is known, we may be able to estimate the blurring function $h(i,j)$ which is typically of lowpass character [5]. Let us denote the known original undegraded signal in a particular region of the image as $f(i,j)$ and the degraded image in the same region as $g(i,j)$. Then $g(i,j)$ is approximately related to $f(i,j)$ by Eq. 2.3.

The blur identification problem reduces to first selecting the appropriate parametric form for the PSF, and then identifying the parameters characterizing it.

Looking at the cross-section profile of a microbead image illustrated in Fig. 2.3, it can be seen that the luminosity decays for a range of distances beyond the edges of the microbeads: the effect we have attributed to “scatter blur”. This observation yields a model for predicting the behavior of the fluorescent scatter blur and a PSF for deconvolution techniques that corrects for that fluorescent scatter blur [62]. For simplicity we model the scatter as being isotropic. The PSF of the fluorescent scatter blur can be reasonably well approximated by the radially symmetric function $h(r)$ given by [8], [15], [19], [42], [56]

$$h(r) = \frac{p_0}{1 + p_1 \cdot r_2^p} \quad 2.4$$

where r is the radial coordinate in the image plane, and P_0 , P_1 , and P_2 are respectively magnitude, magnification scaling factor, and decay that controls the roll-off of the PSF. For simplicity the parameter P_0 is kept constant and equal to unity. Parametrization of the system PSF for a given imaging situation requires the experimental determination of the constant P_1 and P_2 and, when substituted into Eq. 2.4, gives the system PSF as a function of radial distance. The selection of the PSF parameters is primarily empirical [62].

The PSF parameters P_1 and P_2 determine the low pass characteristics of the scatter blur degradation. Since the PSF has a radial symmetry, it is convenient and also consistent to study the PSF behavior in one dimension and particularly on the diagonal of the two dimensional PSF matrix. Illustrated in Fig. 2.6(a) and (b) is the effect on the PSF and its Fourier transform respectively of changing the P_1 parameter while keeping the parameter P_2 fixed. As P_1 increases, the PSF drops rapidly and becomes narrower, yielding less dispersion. On the other hand, the transfer function of the degradation system gets wider including more higher frequencies. There is less differential attenuation of the high frequencies as the parameters gets larger.

FIGURE 2.6 PSF and Transfer function for $P_2=1.8$ and P_1 varying

Figures 2.7(a) and (b) show the PSF and the transfer function pair as the P_2 parameter varies while P_1 is constant. In this case, we observe that the PSF varies in a similar way with P_1 , although we found out that the PSF is more sensitive to the variation of P_2 than to P_1 .

It should be noted that the system PSF is symmetrical and only half is shown in Fig. 2.6 and in Fig. 2.7.

The PSF parameters, P_1 and P_2 , may be combined to give the frequency attenuation / amplification required in the restoration filter to best match the frequency distortion occurring in the image as a result of the transfer process through the fluorescence microscopy system.

FIGURE 2.7 PSF and Transfer function for $P_1=1.0$ and P_2 varying

Chapter 3

Digital image restoration techniques

3.1 Introduction

Digital image restoration is a field that has seen a great amount of activity [1-14]. Images are recorded to portray useful information about a phenomenon of interest. Unfortunately, a recorded image will almost certainly be a degraded version of an original image or scene, due to imperfections of physical imaging systems and the particular physical limitations imposed in every application where image data are recorded. The situation becomes more complicated due to random noise, which is inevitably mixed with the data, and may originate from the image formation process, the transmission medium, the recording process, or any combination of these. Image restoration may be viewed as a process in which mathematical operations are performed on an observed or measured image to estimate the ideal image that would be observed if no degradations were present in an imaging system. The effectiveness of image restoration depends on the extent and the accuracy of the knowledge of the degradation process as well as on the restoration design criterion [9]. Once image restoration problems are quantified precisely and represented mathematically, digital restoration techniques [1-5] may be applied to achieve better looking images.

3.2 Basic formulations of the restoration problem

The image restoration problem can be described in terms of Fig. 3.1:

FIGURE 3.1 A model of the image degradation process

Take the recorded image, g , and process it in some fashion to obtain an estimate of the original image, f . The estimate, $\hat{f}$, of the original image is referred to as the restored image. In many practical situations, the image degradation can be adequately modeled by a linear blur and an additive white noise process. Then the degradation model is described by

$$g = f \otimes h + n \quad 3.1$$

With this model, the purpose of digital image restoration is to operate on the degraded image, g , in order to obtain an improved image which is as close to the original image, f , as possible, subject to a suitable optimality criterion. A frequently used criterion is the mean square error. Although, as a global measure of visual fidelity, its validity is questionable, it is a reasonable local measure and is mathematically tractable [11]. A number of techniques providing a solution to the image restoration problem have appeared in the literature [1-14].

Image restoration deals with images that have been recorded in the presence of one or more sources of degradation. There are many sources of degradation in imaging systems. Three major types of degradation can occur: blurring, pointwise nonlinearities, and noise. Blurring is a form of bandwidth reduction of the image owing to the image formation process. Some types of degradation affect only the gray levels of the individual picture points, without introducing spatial blur, these are sometimes called point degradations.

Other types which do involve blur are called spatial degradations. The second type of image degradation is a pointwise nonlinearity introduced by the non-linear response of the recording medium. The final source of degradation in recorded imagery is noise. This corrupts both the image formation and recording processes. It can be introduced by quantization of the data for original storage [10]. In this work, we will only be concerned with spatial degradation, blurring, and noise.

Given an ideal picture $f(x,y)$ and the corresponding degraded picture $g(x,y)$, we will assume that g and f are related by

$$g(x, y) = \iint h(x, y, x', y') \cdot f(x', y') dx' dy' + n(x, y) \quad 3.2$$

where $h(x,y,x',y')$ is the degradation function and $n(x,y)$ the random noise that may be present in the output picture. Evidently, in the absence of noise the degraded image of a point source described by $f(x', y') = \delta(x' - \alpha, y' - \beta)$ would be given by $h(x,y)$. Therefore, $h(x,y)$ is a point spread function which, in general, is dependent on the position (α, β) of the point in the ideal picture.

The assumption that the observed picture $g(x,y)$ is a linear function of the ideal picture $f(x,y)$ as in Eq. (3.2) is approximately correct only over a small dynamic range of gray levels. The assumption that the noise is additive is also subject to criticism. Many noise sources may be individually modeled as additive. In this section, we will further restrict ourselves to pictures that have suffered a shift invariant type of degradation; then the point spread function (PSF) takes the form $h(x-x', y-y')$ which leads to two-dimensional convolution and Eq. (3.2) becomes

$$g(x, y) = \iint h(x - x', y - y') \cdot f(x', y') dx' dy' + n(x, y) \quad 3.3$$

A discrete formulation of the equations (3.2) and (3.3) is given by the following

$$g_i(j\Delta x, k\Delta y) = \sum_{n=a_2}^{b_2} \sum_{m=a_1}^{b_1} h(j\Delta x, k\Delta y, m\Delta x_1, n\Delta y_1) f(m\Delta x_1, n\Delta y_1) \quad 3.4$$

And also,

$$g_i(j\Delta x, k\Delta y) = \sum_{n=a_2}^{b_2} \sum_{m=a_1}^{b_1} h((j-m)\Delta x, (k-n)\Delta y) f(j\Delta x, k\Delta y) \quad 3.5$$

Infinite images are not practical for digital restoration and we assume the images to be of finite extent.

An alternative point of view to the linear equations already described is in filtering. Equations (3.3) and (3.5) describe a linear spatial filter, and the image restoration problem can be described, in a formal fashion at least, as the choice of the filter inverse to the point spread function, so as to yield the original data when the degraded image is processed by the filter. The filtering viewpoint does contribute insight into the case where the simple inverse filter approach will work. If the original image blur is so slight that the bandwidth of the corresponding distorting filter remains appreciably greater than zero throughout the image bandwidth, and if the signal to noise ratio is very high over the image bandwidth, the inverse filter can be expected to achieve good results[13].

3.2.1 Noise in restoration

To restore a picture in the presence of noise, in addition to a knowledge of the PSF, one needs to know (at least in theory) both the statistical properties of the noise and how it is correlated with the picture. In this thesis, we assume that the noise is introduced by quantization of the original samples of the image, and is uncorrelated with the image.

The most basic requirement for computer processing of images is that the images be

available in digital form, that is, as arrays of finite length binary words. For digitization, the given image is sampled on a discrete grid and each sample or pixel is quantized using a finite number of bits. Uniform quantization of common images, where the pixels represent the luminance function, requires about 256 gray levels, or 8 bits [10].

Quantization necessarily results in loss of information since coded values are specified with less accuracy than in the original image. Replacing original pels by their quantized approximations causes errors in the reconstructed replica image that appear to the viewer as random noise or snow. For most applications involving photographic displays, this noise can be made practically invisible with 8-bit quantization, i.e., quantization with 256 judiciously chosen representation levels[20].

Visibility of quantization noise is strongly dependent on how much noise there is in the original image prior to quantization. It suffices to point out here that most applications require only enough bits of quantization to insure that quantization noise is small compared to the noise in the original samples. Seven bit quantization (or even six) is thus sufficient for some applications where the original image is noisy [20].

In practice the most common assumptions about noise are that it is white, i.e., its spectral density is constant, and that it is uncorrelated with the picture. Both these assumptions can be seriously questioned. The concept of white noise is a mathematical abstraction, but it is a convenient, though somewhat inaccurate, model provided the noise bandwidth is much larger than the image bandwidth.

Different restoration techniques require different amounts of a priori knowledge about the noise. The restoration filter based on Wiener's theory requires the characterization of the noise process in terms of its spectral density.

3.3 Image restoration technique

The FFT makes feasible the numerical solution of the large linear systems presented in the space invariant digital restoration problem. The ill-conditioned behavior of the systems is not affected by the use of the FFT for solutions. The ill-conditioned behavior is controlled from assumptions made to develop a particular restoration technique. Given the ill-conditioned behavior and the existence of noise, there is no unique solution. Instead, there

is an infinite number of solutions, and one must choose a criterion that determines the solution which is optimal in some sense [2], [58].

This section looks at the problem of blur removal using a linear restoration filter, neglecting any pointwise nonlinearities that might be corrupting the image. In the space-invariant case the original and blurred images are related by

$$g(i, j) = \sum_{k, l} h(i - k, j - l) f(k, l) + n(i, j) \quad 3.6$$

3.3.1 Inverse filtering

The basic idea of inverse filtering is extremely simple. Let m and n represent the horizontal and vertical spatial frequencies. Let $f(i, j)$ represent the original undistorted image. We assume $g(i, j)$ to be the result of adding a noise intensity $n(i, j)$ to a blurred image $b(i, j)$ of $f(i, j)$. Thus,

$$g(i, j) = b(i, j) + n(i, j) \quad 3.7$$

where

$$b(i, j) = \sum_{k, l} h(i - k, j - l) f(k, l) \quad 3.8$$

Here $h(i, j)$, the point spread function, is the response of the blurring filter to a two-dimensional unit impulse $\delta(i, j)$.

An inverse filter is a linear filter whose point spread function $h_{inv}(i, j; k, l)$ is the inverse of the blurring function $h(i, j; k, l)$ in the sense that

$$\sum_{p, q} h_{inv}(i, j; p, q) h(p, q; k, l) = \delta(i - k, j - l) \quad 3.9$$

where

$$\delta(i, j) = \begin{cases} 1 & i = j = 0 \\ 0 & i \neq j \neq 0 \end{cases} \quad 3.10$$

Thus,

$$h_{inv}(i, j) * h(i, j) = \delta(i, j) \quad 3.11$$

which can be expressed in the discrete frequency domain as

$$H(m, n) \cdot H_{inv}(m, n) = 1 \quad 3.12$$

Then the Fourier transformation of equations (3.7) and (3.8) gives

$$G(m, n) = B(m, n) + N(m, n) \quad 3.13$$

$$B(m, n) = F(m, n) \cdot H(m, n) \quad 3.14$$

in the spatial frequency domain. Restoration by inverse filtering merely consists of dividing both sides of Eq.(3.13) by the transfer function H or of multiplying both sides by the inverse filter and taking the inverse transform. Thus, if the blurred image is passed through the inverse filter, the discrete Fourier transform of the output is given by

$$\hat{F}(m, n) = H_{inv}(m, n) \cdot G(m, n) \quad 3.15$$

Substituting for $G(m,n)$ using Eq. 3.13 and Eq. 3.14, Eq. 3.15 becomes

$$\hat{F}(m, n) = H_{inv}(m, m) \cdot (H(m, m) \cdot F(m, m) + N(m, n)) \quad 3.16$$

and finally,

$$\hat{F}(m, n) = F(m, n) + H_{inv}(m, n) \cdot N(m, n) \quad 3.17$$

provided H does not vanish at any point (m, n) . In the past, restorations obtained by this technique have been performed with minor modifications. The modifications are aimed at the fairly obvious flaw in this method. The transfer function (H) invariably decreases rapidly for large values of spatial frequencies (m, n) . Noise, in the other hand, has a fairly uniform spectral distribution. From Eq.(3.17) it is apparent, therefore, that the restoration enhances high frequency noise. This is because the power spectrum of the blurred image is typically highest at low frequencies and rolls off significantly for higher ones. In the presence of noise, any 'reasonable' set of sampling points will include many at which the reciprocal of H is very large. Thus the restoration will include a large amount of noise at spatial frequencies in the neighborhood of a zero of H [13].

To overcome the noise sensitivity of the inverse filter, a number of restoration filters have been developed. One way to avoid the arbitrariness of the inverse filtering approach is to minimize the discrepancy between the original and the restored images. The measure of this discrepancy, even if it were known in detail, would be too complex to use. As an alternative it is possible to find the optimum restoration for some simpler objectively defined criterion and see how much improvement this affords. The minimum mean-squared-error (MMSE) criterion is one of the few objective criteria for which the optimum restoration can be computed [13].

3.3.2 Wiener filtering

The Wiener filter is a linear space invariant filter which makes use of the power spectrum of both the image and the noise to prevent excessive noise amplification. The frequency response of this restoration filter $H_w(m, n)$ is chosen to minimize the mean squared restoration error ϵ given by [2-14], [60]

$$\begin{aligned} \epsilon &= E(|F(m, n) - \hat{F}(m, n)|^2) \\ &= E(|F(m, n) - G(m, n) H_w(m, n)|^2) \end{aligned} \quad 3.18$$

The solution to this minimization problem is given by

$$H_w(m, n) = \frac{H^*(m, n)}{|H(m, n)|^2 + \frac{S_{nn}(m, n)}{S_{ff}(m, n)}} \quad 3.19$$

The resulting restoration algorithm is a linear digital spatial filter which can be described in the frequency domain by $H_w(m, n)$ and the restored image Wiener estimate is described in the frequency domain as

$$\hat{F}(m, n) = H_w(m, n) G(m, n) \quad 3.20$$

The point spread function must be known so that its Fourier transform may be computed for the filter.

Wiener filter restorations possess the shortcoming of requiring extensive a priori information, namely, the point spread function and detailed knowledge of image and noise autocovariance functions. The excessive noise amplification as in the previous technique is no longer present because of the masking of the spectral zeros, but the filtered image is still somewhat blurred. The Wiener filters act mainly to suppress measurement noise, while performing only minor deblurring in the case of a large noise parameter [41].

The modifications suggested to overcome these drawbacks are intuitive. As an example we mention Harris's suggestion [3] of multiplying Eq. 3.13 by the transfer function of the noise reduction filter $H_0(m, n)$ before multiplying by H_{inv} . Also, in [5] a similar method for reduction of blurring and additive noise is presented. In the next section, we will present that method.

3.3.3 Constrained Inverse filtering

In practice, an image may be degraded by both blurring and additive noise:

$$g(i, j) = f(i, j) \otimes h(i, j) + n(i, j) \quad 3.21$$

From the previous equation, one reasonable approach to restoring $f(i, j)$ is to apply a noise reduction system to estimate $b(i, j)$ given by $b(i, j) = f(i, j) \otimes h(i, j)$ from $g(i, j)$ and then to apply a deblurring system to estimate $f(i, j)$ from the estimated $b(i, j)$ as shown in the next figure. This configuration is referred to as a Constrained Inverse filter [5].

FIGURE 3.2 Reduction of blurring and additive noise

By choosing the transfer function of the noise reduction system, H_0 , to be zero over the region of the spatial frequency plane where $B(m, n)$, which is the Fourier transform of $b(i, j)$, is dominated by the noise $N(m, n)$ we can reduce high frequency noise. The price we pay is that the estimate of the original image $\hat{f}$ is no longer a noisy version of f but a noisy version of f blurred through the filter H_0 . The requirements imposed on H_0 are intuitively reasonable. However, there is a noncountable infinity of functions that satisfy these requirements and we must choose one.

The approach of reducing one degradation at a time allows us to develop a restoration algorithm for each type of degradation and simply combine them when more than one type of degradations is present in the degraded image. In spite of all the limitations and arbitrariness Constrained Inverse filtering is capable of yielding reasonably good restorations in many situation.. where noise is somewhat quite important in the degradation. In addition, it is an optimal approach in some cases [5], [13], [45].

Chapter 4

Characterization and implementation of the restoration filter

In many cases restoration techniques are based on the assumption that an image can be modeled by a stationary random field, and restoration is achieved by filtering the degraded image with a linear space invariant restoration filter, the frequency response of which is a function of the Fourier transform of the degradation function. As a starting point, in this thesis we focus on the restoration approach that has the concept of seeking an estimate of the original image and minimizes a predefined criterion of performance. Normally one measure that can be extensively used on account of its mathematical simplicity is the mean squared error. Because of their simplicity, we start to focus our attention on least-squares criterion functions. The minimum-mean-squared-error (MSE) criterion is one of the few objective criteria for which the optimum restoration can be computed.

Here, we are dealing with blurred and noisy image restoration. Once the exact nature of the blur has been determined, many approaches can be taken to deconvolve it from the blurred image to produce a restoration [2], [3], [9] and [21]. A restoration filter that minimizes the mean squared error between the original image and its restoration is called the least squares filter or the Wiener filter as already formulated in the previous chapter. Wiener filtering is commonly used to restore linearly-degraded images. The frequency response of the Wiener filter is a function of the ideal image and noise power spectral densities. To obtain optimal results, there must be accurate knowledge of the covariance of the ideal image. In practical situations a single copy of the degraded image is often used for the covariance estimation. This leads to undesirable restored results because of the lack of proper and sufficient a priori information about the ideal image [47], [48].

Wiener filtering restoration yields poor restorations and introduces artifacts and excessive noise amplification at high frequencies.

In this thesis we develop an image restoration technique , the so-called Constrained

Inverse filter or CIF, by attempting to reduce the effects of these problems. We separate the restoration procedure into two phases: a noise reduction system accomplished by a low pass filter and a deblurring system using an inverse filter. This technique has superior performance, as it gives an independent control of the blur and the noise to the observer.

4.1 Wiener filter

As a starting point the Wiener filter is selected to be the restoration filter, the frequency response of which is a function of the ideal image and noise power spectral densities. There are at least two difficulties in this approach because there is no a priori knowledge about either the original image or the noise source. The covariances of the original image and noise are not known and have to be estimated from the available distorted image [45].

As already formulated in the previous chapter, the frequency response of the Wiener restoration filter is given by

$$H_w(u, v) = \frac{H^*(u, v)}{|H(u, v)|^2 + \frac{S_n(u, v)}{S_f(u, v)}} \quad 4.1$$

where $S_n(u, v)$ and $S_f(u, v)$ represent, respectively, the power spectral densities of the noise component and the original image, and (u, v) represent the spatial coordinates in the frequency domain.

The optimal solution of the Wiener filter for the particular image model $g = Hf + n$ is determined uniquely by the power spectra S_n and S_f , and the Fourier transform, $H(u, v)$, of the point spread function, PSF, $h(x, y)$. In order to implement the Wiener filter, we need to know the Fourier transform of the blurring operator H , and the power spectra S_n and S_f [46]. As H is assumed to be given, the Wiener filter can be implemented upon availability of S_n and S_f . Estimating the noise power spectrum is relatively simple, but estimating the original image power spectrum S_f is not. The noise process is assumed to be white, so that

$S_n(u, v)$ is a constant. This constant can be regarded as $S_n(0, 0)$ which is equal to σ_n^2 , the variance of the noise n .

In image restoration, a number of variations of the Wiener filter have been proposed either to improve its restoration performance or to replace lack of a priori knowledge [36], [49], [50]. One of the popular methods is to put

$$\frac{S_n}{S_f} = \frac{1}{SNR} \quad 4.2$$

for all spatial frequencies.

We assume that both terms of the previous ratio, S_n and S_f , vary in the same way and hence, they can be approximated by a constant or a noise constant N . Thus $H_w(u, v)$, the frequency response of the Wiener filter, reduces to

$$H_w(u, v) = \frac{H^*(u, v)}{|H(u, v)|^2 + N} \quad 4.3$$

Using Equation 4.3 as the Wiener filter frequency response and assuming the dispersion function $h(x, y)$ given by Equation 2.5, the restoration filter in this case is parameterized by the point spread function parameters, i. e., the magnification factor P_1 and the roll off factor P_2 , and by a noise parameter N . Ideally, N should be calculated from the statistical properties of the transforms. However, when this is impractical, it is common to approximate N by trial and error to find a satisfactorily restored image in which the sharpness of edges from high-frequency terms is balanced against the noise [36]. The noise parameter is adjusted to produce the restoration that most closely matches the ideal original image.

4.1.1 Wiener filter digital implementation

The impulse response of the Wiener filter is given by [10]

$$h_w(m, n) = \frac{1}{4\pi^2} \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} H_w(u, v) \times \exp \{j(mu + nv)\} du dv \quad 4.4$$

where $H_w(u, v)$ is the frequency response of the filter and (u, v) are the corresponding spatial coordinates to the frequency domain. This integral can be computed on a uniform $N \times N$ grid as

$$\tilde{h}_w(m, n) = \frac{1}{N^2} \sum_{(k, l) = -\frac{N}{2}}^{\frac{N}{2}-1} \sum \tilde{H}_w(k, l) W^{-(mk + nl)} \quad -\frac{N}{2} \leq m, n \leq \frac{N}{2} - 1 \quad 4.5$$

where

$$\tilde{H}_w(k, l) = H_w\left(\frac{2\pi k}{N}, \frac{2\pi l}{N}\right) \text{ and } W = \exp\left(-j2\frac{\pi}{N}\right)$$

The preceding series can be calculated via the two-dimensional DFT from which we can approximate $h(m, n)$ by $\tilde{h}(m, n)$ over the $N \times N$ grid defined above. Outside this grid $h(m, n)$ is assumed to be periodic.

Wiener filtering can be implemented in the Discrete Fourier Transform domain, DFT, as shown in Fig. 4.1. First, we generate a 256×256 Point Spread Function (PSF) $h(m, n)$ and we multiply it by a raised-cosine window function $w(m, n)$ that reduces the edge value of the impulse response to zero, because the impulse response at the edge of the filtering block is significant enough to introduce edge effects. Then, we convert the windowed PSF function into the frequency domain by using the FFT algorithm and compute the Wiener filter's coefficients according to Eq.4.3 with a given PSF and noise estimation N . Finally, we normalize the Wiener filter's coefficients to unity DC gain and save those acquired 256×256 coefficients for later use in the image restoration process.

FIGURE 4.1 Digital implementation of the Wiener filter

The Wiener filter is parameterized by the parameters of the scatter blur P_1 , P_2 , and by the additive noise factor N . Values for P_1 , P_2 and N are experimentally tested and, when substituted into Eq. 4.6 give the system PSF as a function of radial distance and will leading to the Wiener restoration filter by substituting for $H(k, l)$ and N in Eq. 4.3.

$$h(r) = \frac{1}{1 + p_1 \cdot r_2^p} \quad 4.6$$

The Wiener filter restoration performance depends on the filter characteristics as the parameters P_1 , P_2 and N change. Illustrated in Fig. 4.2(a) is the effect of changing the parameter P_1 while keeping the parameters P_2 fixed to 1.8 and N fixed to the energy density of the dispersion transfer function, the Fourier transform of the PSF. As P_1 increases the filter gain decreases, the bandwidth gets wider, and the high frequency noise components are more amplified in this case, because the Wiener filter magnitude gets larger at high frequency range as P_1 increases. Low frequency content is less amplified when P_1 becomes larger. This parameter controls the Wiener filter gain which decreases as P_1 increases. Also as P_1 varies, we observe some changes in the filter decay. Experimental values for P_1 range from 0.1 to 3.0. The range of optimal behaviour of the Wiener filter for P_1 is 0.8 to 1.0.

Figure 4.2(b) shows the effect of changing the parameter P_2 while keeping P_1 constant ($P_1=1.0$) and N fixed to the energy density of the dispersion transfer function. As P_2 increases, the Wiener filter transfer function decays slowly to a certain value in the high frequency range, and the high frequency components get more attenuated because the Wiener filter magnitude becomes smaller when P_2 gets larger. For low values of P_2 the low frequency magnitudes of the Wiener filter are larger than for high values. Thus, the P_2 parameter controls the roll off of the filter. The typical range for P_2 is from 0.8 to 3.0. The range of optimal behaviour of the Wiener filter for P_2 is 1.8 to 2.0.

As seen in Fig. 4.3(a) and (b), the noise parameter N controls substantially the roll-off of the filter at high frequencies. As the noise increases, the Wiener filter magnitude gets flattened and attenuated, also high frequency components are more attenuated. When the noise is equal to zero, the Wiener filter is, in this case, a high pass inverse filter as observed

FIGURE 4.2 Wiener filter transfer functions (a) P_1 varying and $P_2 = 1.8$ and (b) P_2 varying and $P_1 = 1.0$

FIGURE 4.3 Wiener filter transfer function for $P_1=0.8$, $P_2=1.8$ and variable noise N

in Fig. 4.3(a).

As a result from what we stated earlier, the parameters P_1 , P_2 and N act as a selective attenuator of the low frequency components and as an amplifiers of high frequencies relatively to the low frequencies dependent on the values of those parameters. We can interpret the Wiener filter frequency response as follows: when both blur and noise are present, the Wiener filter achieves a compromise between the low pass noise smoothing filter and the high pass inverse filter resulting in a band pass filter. The blurring process is usually a low pass filter [5], [6] and the Wiener filter acts as a high pass filter at low levels of noise [10]. We observe that the deblurring effect of the Wiener filter diminishes rapidly as the noise level increases, as observed in Fig. 4.3. The Wiener filter acts mainly to perform minor deblurring, while enhancing high frequency noise components in the restored image.

4.2 Constrained Inverse filter

To overcome Wiener filtering image restoration difficulties a pragmatic approach to restore the given images has been suggested as indicated in the previous chapter and experimentally tested as we will see in the next chapter. A Constrained Inverse filter is implemented and tested in the same way as with Wiener filter. In the new filter digital implementation, we use the same block diagram structure given in Fig.4.1 where we replace the Wiener restoration filter block with new blocks composed of noise reduction and deblurring systems as illustrated in Fig. 4.4. The new overall restoration filter obtained is called the "Constrained Inverse Filter". As described in Chapter 3, this filter is a combination of two filters, a noise reduction filter associated with a deblurring filter that is the inverse filter of the degradation function. The Constrained Inverse Filter allows a user to vary the restoration filter gain and its roll off bandwidth independently from each other and, in effect, implements the filter described in Equation 4.2.

FIGURE 4.4 Constrained Inverse filter

As a noise reduction filter we use a low-pass raised-cosine filter whose transfer function $H_{RC}(r)$ is given by the following closed form formula [57], [63]:

$$H_{RC}(r) = \begin{cases} \frac{1}{2} (1 + \cos(\pi \frac{r-a}{b-a})) & a < r < b \\ 0 & r > b \\ 1 & r < a \end{cases} \quad 4.7$$

where, $r = \sqrt{u^2 + v^2}$, is the radial distance in the frequency domain and a and b are the lower and upper passband bounds. This filter is used because of its excellent frequency and spatial domain responses, its impulse response is simple and non-oscillatory.

The point spread function empirically derived in the second chapter permits the derivation of an inverse filter used in the deblurring process of the degraded image. With this approach, there is no need for a priori knowledge about power spectra of the original and noise sources in order to implement the restoration filter, as it is the case in the Wiener filter. Since the noise reduction and the deblurring systems do not depend on the space-variant image power spectra because of the design decision, and blurring is assumed to be invariant, there is no nonstationarity problem as in the case of Wiener filtering restoration. Consequently, the overall restoration system can be applied to the entire image. The noise reduction filter is parameterized by two parameters, a and b , which define the roll-off pass-

band of the filter. The deblurring filter which is the inverse filter (inverse of the degradation system) is parameterized and controlled by two parameters, P_1 and P_2 as it is the case with the PSF. The parameters P_1 , P_2 , a , and b determine the bandpass characteristics of the Constrained Inverse filter. This filter enhances the mid-frequency range relatively to the low frequency content while eliminating the high frequency noise.

In Fig.4.5(a) and Fig.4.5(b), we illustrate the effect of changing the P_1 parameter while keeping P_2 , a , and b fixed. As P_1 increases, the filter gain decreases, and the filter bandwidth gets broader. There is not much change in the low frequency range content as P_1 varies, however, the mid frequency range is very affected by the variation of the parameter P_1 . The high frequency noise is completely attenuated by the noise reduction filter. The restoration filter has higher gain for lower values of P_1 , and thus the mid frequency content is more enhanced. When P_1 is low and equal to 0.1 the filter gain is maximal and, as P_1 increases, the filter magnitude drops dramatically and flattens yielding less filtering.

In the other case where P_2 varies while P_1 and the roll-off bandwidth B are constant, we observe that this parameter controls the roll-off of the Constrained Inverse restoration filter. We notice almost the same effects as in the previous case where P_1 was varying, the main difference is that the overall filter is more sensitive to the variation of the parameter P_2 than to P_1 as seen in Fig.4.6(a) and Fig.4.6(b). For low values of P_2 , for example 0.8, the filter has higher gain resulting in more filtering i.e. higher mid-frequency gain. When P_2 is high and equal to 3.0, the Constrained Inverse filter transfer function is almost flat and there is no filtering in this case. As P_1 and P_2 decrease, the filter gain increases, and there is amplification of mid frequency content relative to the lower frequency content.

Figures 4.7(a) and 4.7(b) show the effects of the variation of the roll-off bandwidth on the restoration filter for a fixed value of the parameter P_1 and P_2 . This roll-off bandwidth is found to control the decay of the filter and the high frequency noise content. By moving the upper bound of the bandwidth to the right in the frequency axis while keeping the lower bound fixed, we include more high frequency components in the filter bandwidth resulting in an enhancement of the noise during restoration process, in this case the filter gain gets larger and the roll off of the filter becomes faster. On the other hand, if the

(a) Filter roll off is between 10 and 40 cycles.

(a) Filter roll off is between 0 and 50 cycles.

FIGURE 4.5 Constrained Inverse filter transfer functions for P_1 varying and P_2 fixed at 1.8

(a) Filter roll off is between 10 and 40 cycles.

(b) Filter roll off is between 0 and 50 cycles.

FIGURE 4.6 Constrained Inverse filter transfer functions for P_2 varying and P_1 fixed at 1.0

(a)

(b)

FIGURE 4.7 Constrained Inverse filter transfer functions for different roll off bandwidths

lower bound of the roll off bandwidth is near zero, the filter gain gets smaller and the low frequency content is distorted during filtering.

Hence, when both noise and blur are present in the acquired image, the Constrained Inverse filter achieves a compromise between a low pass noise reduction filter and the high pass inverse filter resulting in a band pass filter. The high frequency noise components are completely removed which is not the case with the Wiener filter whose transfer function does not actually go to zero as illustrated in Fig. 4.2 and Fig. 4.3. Also we observe that the deblurring effect of the Wiener filter diminishes rapidly as the noise level increases. The blurring process is usually of low pass character, and the Wiener filter acts as a high pass filter at low levels of noise, as seen in Fig. 4.3. Figs. 4.8(a), (b), and (c) illustrate that both Constrained Inverse and Wiener restoration filters act like high pass filters in the low frequency range that is the one corresponding to the range 0-10 cycles in the spatial frequency domain.

Most of our images have their frequency content concentrated at very low frequencies as illustrated in Fig. 4.9 which shows the power spectrum of the acquired microbeads image given in Fig. 2.3(a). Thus most of the performance of both restoration filters is localised in that frequency range. The signal-to-noise ratio of the low-frequency components, associated with coarse structures, is often much greater than that of the high-frequency components, so that restoration of that part of the image is simpler or completely unnecessary.

Figures 4.8(a), (b), and (c) show that the Wiener filter enhances high frequency noise considerably resulting in noticeable restoration artifacts while the Constrained Inverse filter suppresses completely that noise.

FIGURE 4.8 Constrained Inverse and Wiener filters transfer functions

FIGURE 4.9 Amplitude spectrum of the acquired microbead image

Chapter 5

Restoration of images distorted by fluorescence microscopy

Images acquired by fluorescence microscopy systems are degraded by a scatter blur. The excessive glow caused by fluorescence of tagged components causes a blur in the images obtained using such a device. Removal of this degradation is accomplished by knowledge of the image point spread function, PSF, and the application of mathematical deconvolution techniques. In this chapter, the two methods for deblurring, the Wiener filter and the Constrained Inverse filter, are compared.

The latter system is developed by attempting to estimate more accurately the frequency response of an image restoration filter according to subjective criteria. It is well known that restorations which are produced using the convolutional inverse of the blur are usually of very poor quality. This is the case because the inverse filter attempts to restore information lying in bands of frequency that may be dominated by noise [13]. An attempt must therefore be made to limit the gain of the restoration filter, $H_R(u,v)$, in those areas of the image spectrum that have a low signal-to-noise ratio. The following constraint has been found [5], [13] to be consistently useful: a noise reduction system followed by a deblurring system permits the estimation of the original image from the distorted one. In some cases, this approach, which we have termed a constrained inverse filter [5], is found to be very effective. The resulting system provides the observer with independent control of the blur and noise. The computations required by the preceding described system for restoring an image are not any greater than typical restoration techniques such as Wiener filtering. And also, the new system appears to compare favorably in performance with other restoration techniques.

Some examples are given to illustrate the performance of the CIF image restoration system. Experimental results are demonstrated on images blurred by fluorescence microscopy and are presented to illustrate the proposed algorithm.

5.1 Restoration procedure

As stated in the previous chapters, it will be assumed that the cause of the blurring can be modelled as a spatially invariant linear system. The blurred image $b(x,y)$ is then equal to the convolution of the object intensity function $f(x,y)$ and the Point-Spread-Function (PSF) $h(x,y)$ of the blurring system. All noise $n(x,y)$ is modeled as additive, although this may not always reflect reality. The image formation process is illustrated in Fig. 5.1.

FIGURE 5.1 Schematic representation of the degradation and restoration processes of an image

Once the exact nature of the degradation has been determined, the appropriate approach can be taken to deconvolve it from the distorted image to produce a restored image. The PSF, analytically derived in section 2.5.1 and experimentally confirmed in section 5.3.1, permits the derivation of an inverse filter and a Wiener filter. The form and parameters of the point spread function are determined from a least-squares fit to the scatter blur decay curve of cross-section profiles of the bead images [62]. These values are unique to each image acquired by the fluorescence microscopy system and thus affect the characteristics of the specific restoration filter used in the restoration process.

In this chapter, two-dimensional Wiener filtering and Constrained Inverse filtering are applied to restore the qualitative integrity of the spatially encoded information that was degraded by the scatter blur PSF and additive noise, as demonstrated by blurred images of specific spatial extent.

A fluorescence microscope coupled to a digital camera video system is the acquisition apparatus. Bead, cell and microtubule images are imaged, digitally acquired, and deconvolved with the Wiener and constrained inverse restoration filters.

As already described in sections 2.5 and 3.2 the relationship between the undegraded image $f(x,y)$ and the actual detected image $g(x,y)$, as illustrated in Fig. 5.1, is given by

$$g(x,y) = f(x,y) \otimes h(x,y) + n(x,y) \quad 5.1$$

The 2D convolution in the spatial domain, given in Eq.(5.1), can be represented in the frequency domain by

$$F_2[g] = F_2[f] F_2[h] + F_2[n] \quad 5.2$$

where $F_2[]$ is a 2D Fast Fourier Transform (FFT) operation. The right hand side of Eq.(5.2) is a multiplication of the frequency domain representation of the undegraded image with the PSF on a point-by-point basis and the acquired result is added to a noise factor. Computing the restoration filter $F_2[h_R]$ and taking the inverse FFT yields

$$f(x,y) = g(x,y) \otimes F_2^{-1} \{ F_2[h_R] \} \quad 5.3$$

where $F_2^{-1}[]$ is the inverse 2D FFT operation and h_R is the restoration filter impulse response.

Fig. 5.1 represents the degradation process of a particular image during its formation and the image restoration process. The undegraded image f can be obtained by direct 2D deconvolution on the acquired spatial domain image with the selected restoration filter, if the PSF, $h(x,y)$, is known. The efficiency and speed of the Fast Fourier Transform permits rapid deconvolution to be carried out in the frequency domain by solving for $F_2[f]$ in

Eq.(5.2) and inverse transforming the result, the estimate of the original image is then given by

$$f = F_2^{-1} \{ F_2[g] F_2[h_R] \} \quad 5.4$$

Since we assumed a closed form for the PSF, $h(x,y)$, the Wiener and the Constrained Inverse restoration filter transfer functions, denoted by H_R , can be calculated, with the tremendous benefit of avoiding singularities and oscillatory behavior, present in a digital calculation caused by finite sampling width, truncation errors, and problems associated with noise.

5.2 Experimental measurements

Video images were acquired with a standard fluorescence imaging system with a fluorescence microscope coupled to a video image processor¹. The images were linearly acquired with a 10-Mhz 8-bit analog-to-digital converter and stored as 512×512 pixel files. The 512×512 matrix was reduced to 256×256 by windowing to minimize computation time. To avoid edge effects a 224×224 window has been extracted from the acquired 256×256 image and the resulting image is padded with zeros to obtain a 256×256 image [63]. Standard 2D FFT techniques were used to convert the images into the frequency domain. Point-by-point multiplication with the precalculated transfer function of the restoration filter followed. Retransformation of the product to the spatial domain was accomplished by inverse 2D FFT techniques.

One dimensional profiles taken along the diagonal of the two-dimensional power spectra were computed for each of the restored images to evaluate the performance of the restoration. Also respective cross-section brightness profiles are illustrated for each case of restoration. A block diagram summarizing the restoration process is given in Fig. 5.2.

1. The images were acquired during research conducted in the Carleton University Department of Biology under the direction of Professor N. Chaly.

FIGURE 5.2 Block diagram of the restoration procedure

In the next part of this chapter, we illustrate and discuss some examples of bead and cell images where the constrained inverse and Wiener filter techniques developed in Chapters 3 and 4 are applied to restore blurred and noisy images. As a starting point, we first consider the test case of bead images degraded by the excess glow caused by fluorescence and additive quantization noise. A noisy and blurred image of 256 x 256 pixels, with each pixel represented by 8 bits, is shown in Fig. 5.3.

5.3 Results and analysis

Experiments were performed to evaluate 1) the constrained inverse filter, and 2) the Wiener filter. Test image g is available and is degraded by a linear shift invariant blurring system H and an additive independent white noise n . The two filters were applied to restore the test image g . The distorted image g is illustrated in Fig. 5.3, its cross-section brightness profile and its amplitude spectrum are shown in Fig. 5.4(a) and in Fig. 5.5(a). The original image looks blurry, a considerable glow is detected apparently emanating

FIGURE 5.3 The acquired original microbead image

from areas of the image that extend beyond the physical boundaries of the beads and edges are not well defined as seen in Fig. 5.3. The image depicts the extent of scatter blur degradation illustrated clearly by the cross-section brightness profile, Fig. 5.4(a), showing a blur at the edges of the beads. The amplitude spectrum in Fig. 5.4(a) shows that the [one dimensional, taken along the diagonal, Fourier-transformed (magnitude)] blurred and noisy image has most of its energy concentrated at very low frequencies, between 0 and 10 cycles in the spatial frequency domain. For most images the amplitude spectrum at low spatial frequencies is relatively larger than those at high spatial frequencies. The amplitude spectrum decays as the frequency increases to become more-or-less constant. Since the image content and the blur are of low-pass character, we can state that the high frequency components correspond to the additive white noise. The noise level is approximately four orders of magnitude lower than the signal in the acquired image. Thus the objective for the restoration filters is to eliminate completely the noise level and to reduce the excessive glow in the acquired image.

The selection of the restoration filter parameters is primarily empirical. The purpose of varying these parameters is to demonstrate that a) an operator can achieve good results; and, b) to provide data that might be used to formulate a quantitative fidelity measure.

5.3.1 Wiener filtering restoration

In this section, we will look at and analyze images restored by Wiener filtering. Also we attempt to compare the performance of the Wiener filtering technique to the Constrained Inverse filtering technique. In this image restoration, we use the same blurry and noisy acquired image as in the Constrained Inverse restoration shown in Fig. 5.4(a) and in Fig. 5.5(a).

Figures 5.4 and 5.5 contain the results of Wiener filtering image restoration and restored images are given for different values of the parameters P_1 and P_2 in Eq. 2.5. In all cases the noise parameter N is kept fixed, equal to the average energy density of the dispersion transfer function. First, P_2 is kept fixed at 1.8 and $P_1 = 0.1, 0.8, 3.0$, and the results are shown in Fig. 5.4. Next, P_1 is kept fixed at 1.0 and $P_2 = 1.0, 1.8, 3.0$, and the results are shown in Fig. 5.5. This range of values for P_1 and P_2 are similar to the ones

FIGURE 5.4 Wiener filtering : original and restored images with respective profiles and amplitude spectra

FIGURE 5.5 Wiener filtering : original and restored images with respective profiles and amplitude spectra

corresponding to the constrained inverse image restoration, presented in section 5.3.2. Cross-section brightness profiles and one-dimensional amplitude spectra computed along the diagonal and using log scale are illustrated for each of the two cases indicated above in Fig. 5.4 and in Fig. 5.5 respectively next to the restored images.

By looking at the restored images and their respective cross-section brightness profiles and amplitude spectra for different parameters P_1 and P_2 , we notice in general that the Wiener filter performs in three different ways. First case is the overfiltering or overcompensation of the degradation when the parameters as well as the dispersion are underestimated, that is for $P_1=0.1$ and $P_2=1.8$ or $P_1=1.0$ and $P_2=1.0$. In this case the restored image shows a lot of artifacts namely ringing, edge effects and noise enhancement, as seen in Fig. 5.4(d) and in Fig. 5.5(d). Artifacts created by the edges of the image can be considerable in restoration. They are created because the edges, which do not correspond to scene properties in any way, contain high-frequency spatial Fourier components that may be orders of magnitude higher than those due to the scene itself. The images show evidence of undershoots and overshoots due to the Wiener restoration artifacts.

Second case corresponds to compensation of the degradation for a "good" selection of the parameters, that is for $P_1=0.8$ and $P_2=1.8$ or $P_1=1.0$ and $P_2=1.8$. The restored image shows a noticeable reduction of the glow caused by the scatter blur. Note, however, that the restored image looks noisy because of some graininess shown on the image illustrated in Fig. 5.4(c), and in Fig. 5.5(c). The background part of the microbead image especially has a granular appearance that is characteristic of Wiener filtering. This occurs because the signal-to-noise ratio is overestimated in this region. If the noise parameter is larger, this effect is less prominent, but edges and other features are blurred. The cross-section brightness profile and the amplitude spectrum shown in Fig. 5.4(c) and in Fig. 5.5(c) exhibit significant noise fluctuations due to Wiener filtering restoration. The edge-enhancing effect of the restoring method is seen in the cross-section brightness profile as shown in Fig. 5.6(b). The amplitude spectrum plot, illustrated in Fig. 5.7(b) and Fig. 5.8(b), indicates that the mid-frequency range components are enhanced relatively to the lower range, also high-frequency noise amplification is evidenced in these figures.

Finally, we have the case of overestimation of the parameters, that is for $P_1=3.0$ and

Original image

Restored image for $P_1 = 1.0$ and $P_2 = 1.8$
(Optimum case)

(a)

(b)

FIGURE 5.6 (a) Original and Wiener filtered images and (b) respective cross-section brightness profiles

Finally, we have the case of overestimation of the parameters, that is for $P_1=3.0$ and $P_2=1.8$ or $P_1=1.0$ and $P_2=3.0$ corresponding to an undercompensation for the degradation. In this case Wiener filtering restoration reflects a poor reduction of glow and poor resolution at the edges of the microbead image leading to no filtering and the restored image is similar to the original one, as illustrated in Fig. 5.4(b) and in Fig. 5.5(b).

The cross-section brightness profiles shown in Fig. 5.7(a) and in Fig. 5.8(a) illustrate the filtering artifacts phenomenon and show clearly the three different cases stated above. The cross-section brightness profiles corresponding to the images restored by Wiener filtering as indicated above show some periodic oscillations due to the noise enhancement by the Wiener restoration filter. The amplitude spectra plots show an increase in amplitude in the mid-frequency range as the parameters vary, this is illustrated in Fig. 5.7(b) and in Fig. 5.8(b).

(a) P_1 varying and P_2 fixed at 1.8

(b) P_1 varying and P_2 fixed at 1.8

FIGURE 5.7 (a) Cross-section brightness profiles and (b) Amplitude spectra of Wiener restored images and original image

(a) P_2 varying and P_1 fixed at 1.0

(b) P_2 varying and P_1 fixed at 1.0

FIGURE 5.8 (a) Cross-section brightness profiles and (b) Amplitude spectra of Wiener restored images and original image

5.3.2 Constrained Inverse filtering restoration

Figures 5.9 and 5.10 show the result of Constrained Inverse filtering on the noisy and blurred observed image, shown in Fig. 5.3, with varying parameters. The restored images obtained by applying the Constrained Inverse filtering technique to the image in Fig. 5.3 while altering the parameters P_1 , P_2 of the assumed distortion filter and the roll-off bandwidth of the noise reduction filter B are analyzed in this section.

Figures 5.9(b), (c), and (d), show restored images corresponding respectively to a constant $P_2 = 1.8$, a fixed roll-off between 10 and 40 cycles, and P_1 varying, and equal to 0.1, 0.8 and 3.0. Fig. 5.10(b), (c), (d) shows restored images corresponding to a fixed P_1 and equal 0.1, same roll-off parameter as in the previous case, and P_2 varying, and equal to 0.8, 1.8, and 3.0. Respective cross-section brightness profiles and power spectra (magnitude), computed along the diagonal of the spectrum of the two dimensional restored image, are shown next to each image.

Figures 5.9 (d) and 5.10 (d) illustrate that for low parameters P_1 and P_2 , (this is the case of overestimation of the dispersion), we have considerable artifacts that are ringing and extreme edge effects due to the overfiltering. The images show evidence of an undershoot artifact characterized by the thin black ring around the bead edge. The cross-section brightness profile shows edges and not much information content of the image as seen in those two figures, the profile shows also undershoots and overshoots. Undershoots around bright areas cause the background brightness, that is usually dark, to be bright. Undershoots are negative brightness levels that appear as very bright pixels because small negative numbers in the 2's complement number system are large unsigned binary numbers. This could be overcome by truncating all negative values to zero. Undershoots are obvious artifacts, since there are negative brightness levels that have no physical meaning. We observe some overshoots which are somewhat more difficult to interpret as obvious artifacts as they could represent bright areas.

The "bright" edges to the beads are an indication that the fluorescent material is concentrated on the surface of the beads and this is illustrated in the cross-section brightness profiles in Fig. 5.9 and in Fig. 5.10.

FIGURE 5.9 Constrained Inverse filtering: original and restored images, cross-section profiles and amplitude spectra

FIGURE 5.10 Constrained Inverse filtering: original and restored images, cross-section profiles and amplitude spectra

Undershoots and overshoots, created from the filtering process, reduce the image's dynamic range as it is illustrated by the cross-section brightness profiles in Fig. 5.9 and in Fig. 5.10. The amplitude spectra plots shown in Fig. 5.9 and Fig. 5.10 illustrate that when the parameters are low, underestimated, the corresponding restoration filter has a higher gain in the mid-frequency range, that is between 5 and 35 cycles in the spatial frequency domain, and thus enhances the energy in that particular range of frequencies. This is illustrated in the amplitude spectrum by an increase in its magnitude in the mid-frequency range. The high frequency noise has been substantially reduced but not to zero as expected from the restoration filter, and the reason can be explained by the effects of the Discrete Fourier Transform analysis of finite length sequences and of edges in the signals. Abrupt cuts at the edges of an image are traduced in the frequency domain by periodic oscillations. These periodic oscillations are due to the Gibbs phenomenon arising from the finite two-dimensional signal bandwidth [64].

In this section it is shown that the filter performs well under optimal conditions. That means correct selection of parameters P_1 , P_2 and the roll-off bandwidth B . In this case, the "good" values for the parameters P_1 , P_2 and B are respectively 0.8, 1.8 and [0- 40] cycles, the corresponding restored image looks clear, sharp and noiseless. The corresponding restored images are given in Fig. 5.9(c) and Fig. 5.10(c). We notice a substantial reduction of blur if we compare the restored image with the acquired one. The cross-section brightness profiles of the restored images as illustrated next to the restored images indicate a good reduction of the glow due to scatter blur and a good edge resolution, edges are well defined and seem to be sharper in this case.

By looking at the plot of the energy distribution in the frequency domain illustrated in the two previously indicated figures, the energy in the mid-frequency range is enhanced but not as much as it is in the overcompensation case and this can be explained by the variation of the filter gain as the parameters vary. The low frequency content of the image has not been distorted meaning that the main information content of the image is saved. The fluorescence scatter blur has been considerably attenuated. We notice also from the plot that the noise level has been substantially decreased by the restoration filter.

In the extreme case of undercompensation of the distortion due to the under-estimation of the dispersion, that is when the parameters P_1 and P_2 are high, around 3.0, the restored image does not show much difference in brightness compared to the acquired image. The restored image is still blurry because of the poor reduction of the scatter blur, however, the background noise is attenuated. The restored images corresponding to this case are shown in Fig. 5.9(b) and Fig. 5.10(b). The cross-section brightness profiles illustrated next to the images in the same figures show no resolution at all at the edges and this is indicated by the observed effect of the scatter blur at those edges of the profile, the bead width in this case is larger than the nominal one. From the amplitude spectrum plots given by Fig. 5.9(b) and Fig. 5.10(b), we can notice the similarity between the amplitude spectra of the acquired and restored images in the low and mid- frequency ranges, however, the high frequency noise has been considerably reduced.

When the filter is operating under optimal conditions, the restored image demonstrates the effectiveness of the Constrained Inverse Filter to remove the high frequency noise and selective low frequency blur without distorting the image. Figure 5.11 illustrates the qualitative performance of the Constrained Inverse filtering restoration. Use of the constrained inverse filter tends to increase the resolution in the degraded image and to define edges more sharply as seen in Fig. 5.12(a) and in Fig. 5.13(a). The edge-enhancing effect of this restoring method is seen in Fig. 5.11(b). The corresponding deconvolved image demonstrates also an improvement in the energy distribution in the mid-frequency range relatively to lower frequency range, high frequency noise is considerably reduced and these facts are observed in Fig. 5.12(b) and in Fig. 5.13(b). The ability of the constrained inverse filter to remove the blur and the noise is depicted by the cross-section brightness profile and the power spectrum, and qualitatively shows that the basic spatial information remains similar. These restorations illustrate the trade-off between the level of artifacts and the degree of deblurring, as seen in the cross-section brightness profiles of the restored image in Fig. 5.12(a) and in Fig. 5.13(a).

There are two extreme cases of restoration: undercompensation and overcompensation for the degradation in the restoration process. The overestimation of the parameters P_1 and P_2 , and that happens when both of them are high, e.g. equal to 3.0, is illustrated in Fig.

Original image

Bright edge
Restored image
 $P_1=0.8, P_2=1.8$ (optimum case)

(a)

(b)

FIGURE 5.11 (a) Original and optimum restored images and (b) respective cross-section profiles

(a)

(b)

FIGURE 5.12 (a) Multiple cross-section brightness profiles and (b) multiple amplitude spectra

(a)

(b)

FIGURE 5.13 (a) Multiple cross-section brightness profiles and (b) multiple amplitude spectra

5.14(d), (e), (f). The restored image shown in Fig. 5.14(d) demonstrates no filtering at all because of the poor removal of the image glow and the beads edges are not well defined leading to a poor resolution, and this is illustrated by the cross-section brightness profile in Fig. 5.14(e). The amplitude spectra of the original and restored images do not exhibit a noticeable difference in the frequency range between 10 and 40 cycles, in fact they look similar. In this case, the dispersion is under-estimated and the distortion is under-compensated.

The other extreme case corresponds to the underestimation of the parameters P_1 and P_2 when both of them low, and equal to 0.1 for P_1 and 0.1 for P_2 . The restored image given in Fig. 5.14(a) shows extreme edge effects, it actually looks very differentiated. The cross-section brightness profile in Fig. 5.14(b) shows overshoots and undershoots at the bead edges resulting in severe restoration artifacts. The power spectrum shows overfiltering of the degradation resulting in a considerable enhancement of the energy in the mid-frequency range, as seen in Fig. 5.14(c). In this case, the dispersion is over-estimated and the distortion is over-compensated.

The range of the roll-off bandwidth B in the restoration system is chosen as a compromise between noise reduction and signal distortion. The roll-off bandwidth B controls the reduction of high frequency noise in the image during the restoration process. If B is chosen in the low or mid-frequency range, then large noise reduction can be achieved, but this can happen at the expense of signal distortion. When the roll-off bandwidth B is selected carefully, the restoration filter demonstrates an excellent ability to attenuate high frequency noise. Various restored images for optimum selection of the parameters, that is when $P_1 = 0.8$ and $P_2 = 1.8$, and different roll-off bandwidths B are shown in Fig. 5.15 and in Fig. 5.16. When the roll-off of the filter starts from zero, the content of the restored image is slightly distorted at low frequencies and this is observed in the restored image itself by a loss in sharpness if we compare it to the one obtained by a restoration filter with a roll-off starting from 5 or 10 cycles in the spatial frequency domain, as seen in Fig. 5.15. If the roll-off bandwidth B includes more high frequencies, then the corresponding power spectrum contains some high frequency components and the noise is not considerably attenuated in the image but it is appreciable on the restored image which

FIGURE 5.14 Restored images for two extreme cases corresponding to low and high parameters

Roll off between 0-40

Roll off between 5-40

Roll off between 10-40

Roll off between 20-40

FIGURE 5.15 Restored images and respective cross-section profiles for $P_1=0.8$ and $P_2=1.8$

Roll off between 50-60

Roll off between 40-60

Roll off between 30-60

Roll off between 10-60

FIGURE 5.16 Restored images and respective cross-section profiles for $P_1=0.8$ and $P_2=1.8$

shows in this case some graininess. As there is more noise compensation, the image becomes less grainy, as seen in Fig. 5.16.

Based on the previous examples that we discussed already, we have found that a reasonable choice of the roll-off bandwidth B at which substantial noise reduction is achieved without noticeable signal distortion is approximately between 10 and 40 cycles in the spatial frequency domain.

The Constrained Inverse filtering restoration technique performs well in the presence of noise and produces meaningful restorations. Moreover, the granularity created by the noise is substantially reduced. This technique has improved the overall appearance of scenes greatly without creating significant losses of information, because noise seems to be objectionable in regions where there is little scene detail.

5.4 Quantitative fidelity measure

In this section we attempt to provide data that might be useful in formulating a quantitative fidelity measure. For this purpose, energies in different sub-bands of spatial frequencies have been computed. For each subsequent eight cycles in spatial frequency domain we look at the variation of the fraction of energy relative to the total as the filter parameters vary. We consider two experiments. First P_2 is fixed at 1.8 and P_1 is varied 0.1, 0.4, 1.0, 3.0. Figure 5.17(a) illustrates the energy in the sub-bands as P_1 varies. In the second experiment we fix P_1 at 1.0 and P_2 takes the values 1.0, 1.2, 1.8, 2.5. In both cases the restoration filter is rolled off between 10 and 40 cycles. Figure 5.17(b) shows the variation of the energy in the sub-bands as P_2 varies.

From the previously indicated figures we notice that most of the signal energy is concentrated in the first sub-band of frequency. The plots show more variation in energy when P_2 varies than in the case where P_1 is varying. From the energy plots we notice that the constrained inverse restoration filter is more sensitive to P_2 than to P_1 and this can be explained by looking at the variation of the filter transfer function as the parameters vary in Chapter 4.

Figures 5.17(a) and (b) exhibit a considerable enhancement in energy for the mid-

(a) Roll off bandwidth between 10-40

(b) Roll off bandwidth between 10-40

FIGURE 5.17 Sub-band energy plots of restored images by constrained inverse filter for different parameters

frequency range, that is between 10 and 30 cycles, relative to the other ranges and specially for the extreme overcompensation case illustrated in Fig. 5.17(b) where most of the energy is contained in the band between 16 and 24 cycles in the frequency domain. The restoration filter is supposed to achieve an optimum restoration when the parameters are correctly selected, and this means that the filter gain is properly selected to enhance enough the energy in the band of frequency of interest. However Figs 5.17(a) and (b) do not show much difference in energy between the optimum restoration and the undercompensation case.

To illustrate the effect another way, the magnitude of the power spectrum at fixed frequencies in the low frequency range, as seen in Fig. 5.18(a), and in the mid frequency range, as seen in Fig. 5.18(b), is computed for P_1 fixed at 1.0, the roll off bandwidth between 10 and 40 cycles, and P_2 varying. As P_2 increases first the magnitude of the amplitude spectra increases for P_2 between 0.8 and 1.2, then it starts to decrease slowly around $P_2=1.5$ for low cycles, 3-5 cycles, and around $P_2=1.2$ for mid cycles, 10-20 cycles, and finally it keeps on decreasing to attain the no-filtering restoration that is for P_2 in the region 2.5-3.0. We observe that the energy peaks at P_2 around 1.5 for low cycles resulting in an optimum restoration in this region. Also same energy behaviour is noticed for P_2 around 1.2 in the mid-cycle range. This result illustrates quantitatively the difference in amplitude spectra between the over-filtering, optimum, and under-filtering restorations.

Wiener filtering restoration does not show a significant variation in energy in the low and mid-frequency range relative to the case with the constrained inverse filter that demonstrated an enhancement of the amplitude spectrum in the mid-frequency range components over the low and high-frequency range components of the amplitude spectrum, as seen in Fig. 5.19(a) and Fig. 5.19(b).

Also Wiener filtering enhances considerably the high frequency component which appears in the restored image as graininess because the Wiener frequency response does not actually go to zero where there is no signal. We know that for most images the amplitude spectrum at low spatial frequencies is typically highest and rolls off significantly for higher spatial frequencies, in this case $p_f(u,v) \gg p_n(u,v)$ and the Wiener estimate

(a) Low frequency range from 3 to 8 cycles

(b) Mid frequency range from 10 to 20 cycles

FIGURE 5.18 Amplitude spectra at fixed cycles for different values of the parameter P_2 and $P_1=1.0$

(a) Noise is fixed at the energy density of the dispersion transfer function

(b) Noise is fixed at the energy density of the dispersion transfer function

FIGURE 5.19 Sub-band energy plots of restored images by Wiener filter for different parameters

$H_w(u,v)$ becomes nearly equal to the inverse of the degradation $H(u,v)$ meaning that the Wiener filter acts like an inverse at those frequencies. Figures 5.20 and 5.21 illustrate noise enhancement in Wiener filtering restoration over Constrained Inverse filtering restoration. Figure 5.20 compares the performances of the constrained inverse filter and the conventional Wiener filter. The conditions are otherwise the same as for the images shown in Fig. 5.5(c) and in Fig. 5.10(c). It can be observed that the constrained inverse filter provides appreciably sharper and clearer images, that are free of noise, than does the Wiener filter.

Also we notice that Wiener filter restoration appears to be “grainier” than for the constrained inverse filter restoration as it is illustrated in Fig. 5.20(a) and (b). As expected, the high frequency noise fluctuations in the Constrained Inverse filtering restoration are significantly less than in the Wiener filtering restoration as shown in Fig. 5.21.

It has been widely observed that linear, shift-invariant restoration algorithms, such as the ones described in Chapter 3, often introduce ringing artifacts (superwhites, superblacks, overshoots, and undershoots) near sharp-intensity transitions. This ringing seriously reduces both the visual and measurable quality of the restoration.

Both images, restored by Wiener filter and constrained inverse filter operating under optimum conditions, exhibit some improvement. However, the improvement obtained by using our procedure over the conventional Wiener filter is noteworthy!

Constrained inverse filtered image
 $P_1=1.0$ and $P_2=1.8$

roll off between 10-40 cycles

Wiener filtered image
 $P_1=1.0$ and $P_2=1.8$

noise=energy density of
 the dispersion transfer function

(a)

FIGURE 5.20 (a) Optimum restored images by Constrained Inverse and Wiener filtering and (b) cross-section profiles

FIGURE 5.21 Amplitude spectra of Constrained Inverse and Wiener filtered images

5.5 Examples: restoration of cell and microtubule images

In this section we illustrate and discuss some examples where the Constrained Inverse and Wiener filtering restoration techniques are applied to restore cell images degraded by blurring, from the excessive glow of fluorescently stained structures in the cells, and additive noise. Artifacts were observed from filtering that are excessive noise, undershoots around bright areas and poor edge resolution around bright areas.

We first consider the case of cell image shown in Fig. 5.22(a) and in Fig. 5.23(a). The images restored by applying the Constrained Inverse filter to the distorted image in Fig. 5.22(a), for different values of the parameters, are shown in Fig. 5.22(b), (c) and (d) and in Fig. 5.23(b), (c) and (d). In the original image the boundaries are obscured by the glow. Overestimation of the parameters yields reblurring of the structure resulting in an undercompensation of the distortion as seen in Fig. 5.22(b) and in Fig. 5.23(b). When the restoration filter is operating under optimum conditions, that is for a “good” selection of the parameters, the filtered image, illustrated in Fig. 5.22(c) and in Fig. 5.23(c), shows a reduction of glow. The bright circular structures have more clearly defined edges. This result is a good indication that the filter is actually restoring previously indiscernible information. For “good” parameters the cell structures are well resolved. For low parameters the restored image shows clear dark rings around the circular glowing structures, as seen in Fig. 5.22(d) and in Fig. 5.23(d). These ringing artifacts result from the overcompensation of the dispersion.

Filtering of the cell does provide some image improvement, by more clearly defining its internal structures.

Next we consider the case where a microtubule image is filtered. The acquired image is shown in Figs. 5.24(a) and 5.25(a), the original image shows a glowing mass. In Fig. 5.24(b) and in Fig. 5.25(b) we observe that the restored image is similar to the original one because of under-estimation of the dispersion resulting in undercompensation of the distortion. When the filter operates under optimum conditions a dramatic reduction of glow is observed in the filtered image. The restored image shows many string structures, as seen in Fig. 5.24(c) and Fig. 5.25(c). The string-like structures are definitely present in the real image and are enhanced in the restored image. In the case of overcompensation of the

FIGURE 5.22 Constrained Inverse filtering of cell images, profiles and amplitude spectra

PRECISIONSM RESOLUTION TARGETS

FIGURE 5.23 Constrained Inverse filtering of cell images, profiles and amplitude spectra

FIGURE 5.24 Constrained inverse filtering of microtubule images, cross-section profiles and amplitude spectra

FIGURE 2.25 Constrained Inverse filtering of microtubule images, cross-section profiles and amplitude spectra

degradation, which corresponds to low parameters, the restored image shows some evidence of undershoots indicated by dark edges around the structure as illustrated in Fig. 5.24(d) and in Fig. 5.25(d).

Wiener filtering restoration of cell and microtubule images are illustrated in Fig. 5.26, Fig. 5.27, Fig. 5.28 and Fig. 5.29. The filtering conditions are the same as with the beads image. The graininess of both the structure and the cell make the boundaries of the structure hard to determine, as seen in Fig. 5.26(c) and in Fig. 5.27(c).

FIGURE 5.26 Wiener filtering of cell images, profiles and amplitude spectra

FIGURE 5.27 Wiener filtering of cell images, profiles and amplitude spectra

FIGURE 5.28 Wiener filtering of microtubule images, profiles and amplitude spectra

FIGURE 5.29 Wiener filtering of microtubule images, cross-section brightness profiles and amplitude spectra

5.6 Computational analysis of image filtering restoration

A digital image restoration filter is fully characterized by its impulse response ($h_R(i,j)$) in the spatial domain and by its frequency response in the frequency domain. The relationship between the undegraded image $f(i,j)$ and the actual detected image $g(i,j)$ in the frequency domain is given by

$$G(u, v) = f(u, v) \cdot H_R(u, v) \quad 5.21$$

Where $G(u,v)$, $F(u,v)$, and $H_R(u,v)$ are the discrete Fourier transform (DFT) of $g(i,j)$, $f(i,j)$, and $h_R(i,j)$.

The computational cost is measured in terms of the number of floating point operations (FLOPs) and the number of memory accesses (MAs) [59]. These values give an approximate indication of the processing time required to complete a task. The total number of FLOPs will provide a sufficient indication of processing time.

The acquired image and the filter frequency response are of size 256x256. A 256x256 2-D DFT has the following definition [5]

$$X(k, l) = \sum_{n=0}^{255} \sum_{m=0}^{255} x(n, m) \exp \left[\frac{-j2\pi}{N} (kn + lm) \right] \quad 5.22$$

$$0 \leq k, m \leq 255$$

The computation of a direct 2-D DFT can be very expensive and requires a great amount of time to complete a job. The 2-D DFT is computed using the row-column decomposition method based on Fast Fourier Transform (FFT) principles. This method is easy to implement, first the transforms of all the rows are computed and then the transforms of all the resulting columns. This allows direct use of the 1-D FFT algorithm.

The cost of this method is 2x256 multiplied by the cost of a 1-D FFT of size 256. The 1-D FFT consists of a sequence of two element, in place computations called butterflies [5].

We consider the radix-2, decimation in frequency (DIF) butterfly:

$$A \text{-----} A+B$$

$$B \text{-----} (A-B)W_N^k$$

Where $W_N^k = e^{-j\frac{2\pi k}{N}}$

For a single butterfly calculation, the number of floating point operations (FLOPS) is:

2 complex additions: 4FLOPs

1 complex multiplication: 6FLOPS

Total : 10 FLOPS

The number of memory accesses (MAs) necessary for the calculation of a single butterfly is:

2 complex data reads: 4 MAs

2 complex data writes: 4 MAs

complex exponential value: 2MAs

Total: 10 MAs

The 2-D DFT requires 512 computations of a 1-D FFT which itself requires $N/2\log_2 N$ butterfly calculations. Thus, the total cost for a 256×256 2-D DFT using radix-2 1-D FFTs is

$$\text{Number of FLOPs} = 2N \times N/2\log_2 N \times 10$$

$$\text{Number of MAs} = 2N \times N/2\log_2 N \times 10 \quad \text{where } N=256$$

The transform filtering restoration method requires the use of three such transforms: transform of the original image, transform of the filter impulse response and inverse transform of the result to yield the restored image. The restored image is obtained by multiplying the transform of the degraded image by the filter frequency response on the element by element basis. Hence 256×256 complex multiplications are necessary to complete the filtering process. For each calculation, four memory reads, a complex

multiplication and two memory write are performed. Thus the filtering process by itself requires:

$$\text{Number of FLOPs} = 6N^2$$

$$\text{Number of MAs} = 6N^2$$

For the entire restoration process, consisting of three 2-D Fourier transform and the complex 2-D multiplication, the number of FLOPs and MAs are given by the following:

$$\text{Number of FLOPs} = 30N^2 \log_2 N + 6N^2$$

$$\text{Number of MAs} = 30N^2 \log_2 N + 6N^2$$

For $N=256$:

$$\text{Number of FLOPs} = 16,121,856 \sim 16\text{MFLOPs}$$

$$\text{Number of MAs} = 16,121,856$$

The number of FLOPs and MAs are the same in this situation. The execution time of the restoration algorithm for different PC's is given in the following table.

PC Type	Execution Time of the Restoration Algorithm
386, 33 MHz	98.4 sec
486, 33 MHz	36.9 sec
486, 66 MHz	21.5 sec

TABLE 5.1 Execution time of the restoration algorithm for different PCs

Chapter 6

Concluding remarks

6.1 Summary of contributions

In this study we have developed a new image restoration system, termed Constrained Inverse filtering restoration, that is applicable to the problem of restoring an image degraded by blurring and additive noise. The restoration system consists of a noise reduction system and a deblurring system. The main feature of the noise reduction system is to attenuate substantially the high frequency noise in the available degraded image. The deblurring system corresponds to inverse filtering the blurring distortion.

Restoration examples were shown to illustrate the performance of this new image restoration system.

6.2 Conclusions

1. The Constrained Inverse filtering restoration provides independent control of the noise and the distortion to the observer.
2. The approach of reducing one degradation at a time allows us to develop a restoration algorithm for each type of degradation and simply combine them when more than one type of degradation is present in the degraded image.
3. The high frequency noise is completely attenuated which is not the case in Wiener filtering restoration where the high frequency noise may be considerably enhanced.
4. The Constrained Inverse filter performs well in the presence of noise and produces meaningful restorations: it resolves fine details, and removes enough glow distortion to show hidden features. The restored image appears relatively

sharp and free of artifacts.

5. Because of the design decision discussed in Chapters 3 and 4, the Constrained Inverse filtering restoration does not depend on the space-variant image and noise power spectr. There is no necessity for a priori knowledge about the ideal image, however, a significant amount of prior knowledge is required for the Wiener restoration filter, including knowledge of the degradation function and the covariance of the original image noise [11]. Although the Wiener filter is optimally derived, its true success in restoring real-world images depends on accurate estimation of the image and noise covariances [47]. To obtain an accurate estimate of the covariances, an ensemble of many samples of the ideal image (as random variables) is required. However, in practical applications it is unlikely that there will be an ensemble of the ideal image samples (that is, a set of prototype images) available for the estimation; therefore spatial averages are often used in place of ensemble averages in implementation. To make the situation worse, in most applications only the image to be restored is available and all prior knowledge about the ideal image signal has to be estimated from it. Hence, the covariances estimated from this single copy of the degraded image are far from the true covariances of the ideal image which is required by the minimum mean-square error estimate. For these reasons, it is expected that the Wiener restoration filter is no longer optimal because of the lack of accurate prior information [48].
6. For the noisy observation, the restoration artifacts are more noticeable in the Wiener filtering restoration than for a Constrained Inverse filter. The Wiener filter accentuates the background noise and is useful only when the background noise level is very low. Hence, Wiener filter restorations appear to be “grainier” than for the Constrained Inverse filter.
7. The optimality of the Wiener filter is derived on the basis of linear theory, however, the human visual system perceives images with a number of known

nonlinear effects, and probably a number of others that are unknown.

8. The Constrained Inverse filter has the advantage of being simple and yet preserves most of the information associated with the signals at regions of high signal-to-noise ratios (SNR's) in the frequency domain.

6.3 Recommendations for future research

1. The restoration methods presented in this thesis have all used a linear image formation and recording model in which the effects of any pointwise nonlinearities have been ignored. Thus one of the particular points to look at would be the restoration of blurred and noisy images described by the nonlinear model which incorporates the nonlinearity due to video cameras for example. Video cameras have an inherent nonlinear relationship between signal voltage and light intensity. The values of the digitized two-dimensional signal samples are related to the actual light intensity by a power law called "gamma". The nonlinear relationship between signal voltage and light intensity makes the image processing nonlinear and results in image distortion if linearity is assumed. For this reason digitized samples should be "gamma" corrected prior to any linear processing.
2. The restoration technique developed in this study is based on the assumption that the acquisition system can be approximately modeled by a stationary field. For a typical image, each part of an image generally differs sufficiently from other parts so that the stationarity assumption is not generally valid. To reduce the effect of nonstationarity problem, the restoration technique can be then implemented on a restricted space basis in which an image is segmented into many subimages and each subimage is restored separately and then combined. This short space implementation, when performed properly, may achieve a better system performance than the approach to process an entire image at one time [2], [64].

3. An open area in image restoration is the identification of space-varying blurs. The Constrained Inverse and Wiener filtering restorations used in this research assumed that the blur is space-invariant; a frequency-domain expression could then be used to evaluate the blur identification algorithm error. If the space-invariance assumption is not valid anymore, then the blur identification algorithm becomes more difficult to evaluate. The proper choice and imposition of assumptions for space-varying blur identification are important areas for future research in image restoration [2], [64].
4. The Constrained Inverse and Wiener filtering are one-step techniques that provide a solution to the image restoration problem. It would be interesting to try the iterative techniques for restoring noisy-blurred images. The iterative structure of the restoration algorithm offers a number of advantages over noniterative techniques. Among the advantages of iterative approaches are the following: i) there is no need to determine or to implement the inverse of an operator; ii) knowledge about the solution can be incorporated into the restoration process; iii) the solution process can be monitored as it progresses; iv) constraints can be used to control the effect of noise [41], [48]. They are restricted, however, by the lack of any quantitative quality measure.
5. The traditional development of image-restoration filters, including the Wiener and Constraint Inverse filters, is based on the assumption that the images are the result of a process that can be mathematically expressed as the convolution of the radiance field with the point-spread function of the image-gathering system plus the noise generated by the detection mechanism. The sampling required to convert the continuous-input radiance field into a digital signal is tacitly assumed to be sufficient so that sampling artifacts can be disregarded as a source of degradation. However, insufficient sampling is common in image gathering, and its neglect is a fundamental error in current formulations of image-restoration filters. This neglect to account properly for the conversion of continuous-input

radiance field into a digital signal extends also to the conversion of the digitally processed signal into the continuous image produced by the image-display system. Therefore image-restoration filters should account for the insufficient sampling that causes aliasing as well as for the blurring and noise [61].

6. Another possible suggestion for future study would be the implementation of the Constrained Inverse filter developed in this thesis. This restoration filter can be implemented either using single chip digital signal processors (DSP boards), which provide a relatively low cost means of image filtering with great speed, or using spatial parallelism, that is, processing different areas of an image simultaneously with different computers. Because image processing is often very localized in nature, processing of one area of an image is often independent of the processing of other areas. Hence, several DSPs operating together could provide very efficient image filtering.

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