Deconvolution algorithms of 2D Transmission
- Electron Microscopy images

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Deconvolution algorithms of 2D Transmission Electron Microscopy images

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Abstract

The purpose of this thesis is to develop a mathematical approach and associated software implementation for deconvolution of two-dimensional Transmission Electron Microscope (TEM) images. The focus is on TEM images of weakly scattering amorphous biological specimens that mainly produce phase contrast. The deconvolution is to remove the distortions introduced by the TEM detector that are modeled by the Modulation Transfer Function (MTF). The report tests deconvolution of the TEM detector MTF by Wiener filtering and Tikhonov regularization on a range of simulated TEM images with varying degree of noise. The performance of the two deconvolution methods are quantified by means of Figure of Merits (FOMs) and comparison in-between methods is based on statistical analysis of the FOMs.

Keywords: Deconvolution, Image restoration, Wiener filter, Wiener deconvolution, Tikhonov regularization, Transmission electron microscopy, Point Spread Function, Modulation Transfer Function
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Chapter 1

Introduction

1.1 The electron microscope

1.1.1 History

The Transmission Electron Microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. When using a light microscope, resolution is limited by the wavelength of light. TEMs use electrons as “light source” and their much lower wavelength make it possible to get a resolution a thousand times better than with a light microscope.

The first TEM was first built by Max Knoll and Ernst Ruska in 1931. The imaging properties of the TEM, particularly with regards to imaging of biological specimens, was improved at Siemens in 1936 [9] with the introduction of scanning transmission electron microscopy technique. An important development occurred with the invention in the 1970s of the field emission gun by Albert Crewe [4]. The TEM is by now an essential imaging tool in a wide range of fields, such as life sciences, material sciences, gemology, metallurgy and semiconductor industry.

1.1.2 Application to life sciences

One of the main goals for present-day electron microscopy is to look at the life processes within a cell at the molecular level. During recent years electron microscopy has developed into a most useful tool to study macromolecules, molecular complexes and supramolecular assemblies in three dimensions (3D electron microscopy). Computational methods for image restoration that seek to remove image artifacts introduced by the TEM are becoming increasingly important, especially for 3D electron microscopy (section 1.2.2).

1.1.3 Limitations

The inside of an electron microscope is under high vacuum as the molecules that make up air would otherwise scatter the electrons [3]. Hence, samples have to be imaged in a vacuum. Next, electrons are notoriously unable to penetrate through matter, therefore only the thinnest possible specimens are suitable for imaging with TEM [7]. Hence, sample preparation is required for producing a
sample thin enough to be transparent for electrons. This has to be done with minimal intervention to the structure of the sample. The sample preparation process for TEM is therefore relatively time consuming.

Another issue is that of image contrast and sample damage. When biological specimens are irradiated by the electron beam in the EM, the specimen structure is damaged as a result of ionization and subsequent chemical reactions. Ionization occurs when energy is deposited in the specimen as a result of inelastic scattering events. Hawkes [15] proposed that inelastic scattering can make the heating of the sample in the irradiated area, radiochemical decomposition of water, as well as other secondary chemical reactions. Combining more intense electron sources with more sensitive electron detectors allows one to minimize the total dose received by specimen can be used to get a high resolution image. But, the combinations of high keV beams with the intense electron sources also increase the risk of destroying the specimen.

1.2 Image processing in TEM

1.2.1 Image processing

Image processing is a physical process used to convert an image signal into a physical image. The image signal can be either digital or analog. The actual output itself can be an actual physical image or the characteristics of an image. Image processing techniques were first developed in 1960 through the collaboration of a wide range of scientists and academics. The main focus of their work was to develop medical imaging, character recognition and create high quality images at the microscopic level.

TEM gives high-resolution information of a sample, but the three-dimensional information is projected into a 2D image. When a specimen is sufficiently thin and the energy of the incident electrons is high enough, most of the incident electrons will pass through the specimen with less energy loss and can be ‘visualized’ on a fluorescent screen or registered on a photographic plate located below the specimen. The TEM image obtained in this way is called a conventional electron micrograph which is the result of the scattering, diffraction and absorption of the electrons as they pass through the specimen. Different regions of the specimen scatter the electrons by various degrees where the extent of the scattering depends on the local elemental composition and the local thickness and density of the specimen.

1.2.2 Three-dimensional imaging in TEM

Three main techniques have been applied for 3D electron microscopy: electron crystallography reviewed in [10, 6], single particle analysis reviewed in [5], and electron tomography reviewed in [12, 13, 11]. Electron crystallography permits structural analysis of macromolecules at or close to atomic resolution (0.4 nm or better). It relies on the availability of 2D crystals and has proven especially suited for membrane proteins. Larger biological complexes are preferably studied by single particle analysis, which in favorable cases allows the molecular objects to be examined at medium-resolution (1-2 nm). Notably, this resolution is good enough to permit high-resolution structures of subunits or domains.
(typically obtained by X-ray crystallography) to be fitted into the large structure at hand. This hybrid approach may reveal the entire complex at close to atomic resolution. Finally, electron tomography can provide structural information at the molecular level in the context of the cellular environment. Most importantly, small cells or sections through larger cells or tissues can be studied as cryo-specimens with a close-to-life preservation as shown by [1, 11]. The resolution is presently limited to 4-5 nm but it seems reasonable to reach higher resolution in a near future.

1.3 Summary of approach

The focus of this project is on deconvolution algorithms of 2D Transmission Electron Microscopy images, an important part in the study of signal processing fields. TEM and image reconstruction are core techniques to study complex biological three-dimensional structure of macromolecular. There are several types of deconvolution methods for image restoration. The report treats Wiener deconvolution and deconvolution by Tikhonov regularization. Chapter 2 explains the mathematically notion of an image. Here we also introduce the motivation for image restoration. Chapter 3 introduces the inverse problem associated with image restoration. The notions of well- and ill-posed problems are introduced and we show that deconvolution is an ill-posed problem. The concept of a regularization method is defined for solving ill-posed problems. Chapter 4 talks about Wiener deconvolution and Tikhonov regularization for deconvolving detector blurring in TEM images. Chapter 5 introduces the quantitative evaluation of deconvolution methods based on using Figure of Merits (FOMs). Chapter 6 introduces the TEM simulator used for generating the simulated data used in the report. Chapter 7 presents the training FOM and FOMs use in this project in detail. It also presents the optimal parameters for the two regularization methods. The FOMs are implemented in MATLAB and numerical experiments conducted using those functions and MATLAB image processing toolbox. Chapter 8 presents the results for both noise-free and noisy images. The regularizations are carried out by MATLAB. Chapter 9 investigates the sensitivity of deconvolution methods for misspecification of parameters in the TEM Modulation Transfer Function. We determine the parameter that is most significant and its acceptable intervals. In this chapter sensitivity test is carried out in both two deconvolution algorithms mentioned in Chapter 4. Chapter 10 gives some conclusions and a short discussion regarding further topics.
Chapter 2

Image restoration

2.1 Mathematics of imaging

We start out by formalizing the notion of an image. From a user’s viewpoint, an image is any quantity that is spatially distributed in 2D/3D that represents some physical property of an object. One can here also consider 4D images (movies) if temporal variation is involved. An image will always be supported in a finite region $\Omega$ (the region-of-interest). Mathematically, an image can therefore be represented by a function on $\Omega$ where grey-scale images are real valued functions $I: \Omega \rightarrow \mathbb{R}$. Color images are vector valued, but here we only consider grey-scale images.

2.1.1 Analog and digital images

The mathematical notion of an image as a real valued function represents a analogue image where greyscale values as well as resolution is infinitely fine. Since computers cannot handle this kind of information, images need to be digitized. This means that the function is sampled at finitely many points in the region-of-interest and its range is also digitized. Hence, a digital image is simply an array where each component represents the value of the function $I$ at a small region around a suitably chosen sample point in the region-of-interest $\Omega$.

For 2D images, the most common is to choose points $(x_1, y_1), \ldots, (x_n, y_m) \in \Omega$ that all lie on a regular lattice, i.e.,

$$x_k := x_0 + k \triangle x \quad \text{and} \quad y_l := y_0 + l \triangle y$$

for fixed $\triangle x, \triangle y \geq 0$ and $k = 0, \ldots, n - 1$ and $l = 0, \ldots, m - 1$. Then, the digital image corresponding to the 2D image $I: \Omega \rightarrow \mathbb{R}$ is given by the $(n \times m)$-matrix $I$ defined as

$$I := \begin{pmatrix}
I(x_1, y_1) & \cdots & I(x_1, y_m) \\
\vdots & \ddots & \vdots \\
I(x_n, y_1) & \cdots & I(x_n, y_m)
\end{pmatrix}$$
2.2 The Fourier transform and convolution

The Fourier transform is an important image processing tool which is used to decompose an image into its frequency components. The output of the transformation represents the image in the Fourier or frequency domain, while the input image is the spatial domain equivalent. In the Fourier domain image, each point represents a particular frequency contained in the spatial domain image. The Fourier transform is used in a wide range of applications, such as image analysis, image filtering, image reconstruction and image compression. Stated mathematically, the 2D Fourier transform takes a function $I$ (defined on $\mathbb{R}^2$ with suitable regularity properties) and produces another function $\mathcal{F}(I)$ in the following way:

$$\mathcal{F}(I)(\xi) := \int_{\mathbb{R}^2} I(x) e^{-2\pi i x \cdot \xi} \, dx.$$ 

One can then show that the inverse Fourier transform is given as

$$\mathcal{F}^{-1}(I) := \int_{\mathbb{R}^2} I(\xi) e^{2\pi i x \cdot \xi} \, d\xi,$$

i.e., if $\hat{I} = \mathcal{F}(I)$, then $\mathcal{F}^{-1}(\hat{I}) = I$ (the Fourier inversion theorem).

In image processing, many operations are based on modifying an image in a close neighbour of a point. This is e.g. the basis for image filtering when an images is to be smoothed, sharpened, or when edges are to be detected. These and many other image processing operations can be described as convolutions. Important for our purposes is that convolution is ideal for describing (non-random) blurring caused by an image acquisition device. Mathematically, the convolution between two functions $I$ and $J$ is a third function $I \ast J$ that is typically viewed as a modified version of one of the original functions, giving the area overlap between the two functions as a function of the amount that one of the original functions is translated. The formal definition is as follows:

$$(I \ast J)(x) := \int_{\Omega} I(x) J(x - y) \, dy \quad \text{for} \quad x \in \Omega.$$ 

There is now a relation between convolutions and Fourier transforms that is called the convolution theorem. Under suitable conditions, this theorem states that the Fourier transform of a convolution is the pointwise product of Fourier transforms. In other words, convolution in one domain (e.g., spatial domain) equals point-wise multiplication in the other domain (e.g., frequency domain). Stated mathematically,

$$\mathcal{F}(I \ast J)(\xi) = \mathcal{F}(I)(\xi) \cdot \mathcal{F}(J)(\xi).$$ 

2.2.1 Fourier transform and convolution of digital images

For digital images, the function representing the 3D images is replaced by a matrix as described in section 2.1.1. Hence, the Fourier transform and convolution operations defined above correspond to operations on these corresponding matrices.

The digitized version of the Fourier transform is called the Discrete Fourier Transform (DFT) and the corresponding inverse is the inverse DFT. The DFT
and its inverse operates on matrices representing digital 2D images and are defined as follows:

\[ \hat{I}_k = \sum_{l=0}^{m-1} I_l e^{-2\pi i k \frac{l}{m}} \]

and its inverse is

\[ I_l = \frac{1}{m} \sum_{k=0}^{m-1} \hat{I}_k e^{2\pi i k \frac{l}{m}} \]

Both the DFT and its inverse are efficiently computed by means of the Fast Fourier Transform (FFT) method.

The convolution is almost always calculated through the digital version of the convolution theorem. Hence, to calculate the digital convolution of two digital images, one first calculates their DFTs, then take the matrix product of the DFTs, and then calculate the inverse DFT of the resulting matrix product.

Now, when calculating the Fourier transform or convolution of a digital images, there can be ringing artifacts in the results. These are artifacts that appear as spurious bands or “ghosts” near edges. The term “ringing” is used because the output image oscillates at a fading rate around a sharp transition in the input image. These are probably due to spectral leakage and one way to handle that is through zero-padding.

Zero-padding consists of extending a digital image (or its spectrum) with zeros. In general, zero-padding a digital image prior to taking its Fourier transform DFT is equivalent to interpolation, or sampling more often, in the transformed domain. Here is a quick way of understanding this. If you sample a image in the spatial domain at higher rate, you get a more “squashed” spectrum, i.e., spectrum with more zeros at both ends. In other words, you can obtain more samples in the spatial domain by simply zero-padding in the frequency domain after DFT and then applying an inverse DFT on the result. The same effect holds in reverse when zero-padding occurs in the spatial domain. This is all because the perfect signal reconstruction is possible as long as a signal is bandlimited and sampled at least at the Nyquist rate. Now, often “resolution” means how well one can reliably (statistically) discriminate two adjacent points in the spatial or frequency domain. Then, the resolution actually depends on the size of the DFT matrix due to spectral leakage, i.e., smaller size, more blurry or smeared the transformed signal, and vice versa. Hence, zero-padding helps in clarifying the true peak of the sampled window transform. With enough zero-padding, even very simple interpolation methods, such as quadratic polynomial interpolation, will give accurate estimates of spectral peaks and thereby reduce ringing artifacts.

On a final note, when used in convolution, there are different ways to do the zero-padding. We always keep the Point Spread Function (PSF) in the upper left corner of the enlarged PSF, so that the small PSF and the big PSF have the same center. It is easy to do the computation, since the computation usually needs the information of the center of the PSF.

## 2.3 The Point Spread Function (PSF)

The Point Spread Function (PSF) describes the response of an imaging system to a point source or point object. A more general term for the PSF is a system’s
impulse response, the PSF being the impulse response of a focused optical system. The PSF in many contexts can be thought of as the extended point source in an image that represents an unresolved object, so for an imaging system, its quality can be measured in terms of the PSF as it encodes the degree of spreading of a point object. In functional terms it is the spatial domain version of the Modulation Transfer Function (MTF), i.e.,

$$\text{MTF}(\xi) = \mathcal{F}(\text{PSF})(\xi).$$

For a digital image, the PSF and MTF needs to be sampled. Since we can see both functions as 2D images, their digitized variants can be represented by matrices as described in section 2.1.1.

Blurring is often modeled by means of a convolution with a PSF, so deblurring the image corresponds to reversing the effects caused by convolution with the PSF. Now, no imaging systems can resolve all frequencies in an image. For those that are described in terms of convolutions, this translates into the fact that the PSF is bandlimited, or essentially bandlimited, so the corresponding MTF is zero, or decreases to zero, as frequencies increase. This can cause problems when one seeks to deblur the imaging data in order to recover the original, sharp image. This is discussed in more detail in section 3.1.1. When the PSF is unknown, the deblurring procedure is called \textit{blind deconvolution}. 

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Chapter 3

Inverse problems and imaging

Many Image restoration problems, such as deblurring, can be formulated as an inverse problem. The goal here is to obtain, given imaging data, the best possible approximation of the original image within the limits imposed by imaging conditions and noise level in the imaging data [14]. Stated formally, this is an inverse problem where we seek to recover the original image $I_{\text{true}} \in X$ from imaging data $I_{\text{data}} \in H$ where

$$I_{\text{data}} = T(I_{\text{true}}) + I_{\text{noise}}$$

with $T : X \to H$ a fixed given mapping. In the above, $X$ is the reconstruction space that contains the set of feasible images and $H$ is the data space containing all possible imaging data. The latter is always finite dimensional vector space of real numbers $\mathbb{R}$ since one can only measure finite number of data points. Furthermore, $T$ is the forward model that describes how the signal gives rise to imaging data in absence of noise. We assume it will always be linear. This also includes the actual finite sampling of the imaging data. Finally, $I_{\text{noise}}$ is the noise component in the imaging data, so it is a sample of a random variable.

3.1 Well- and ill-posed problems

The concept of a well-posed problem is proposed by J. Hadamard in 1923, who argued that every mathematical problem corresponding to some physical or technological problem must be well-posed.

According to Hadamard, the inverse problem of finding $I_{\text{true}}$ from the equation

$$I_{\text{data}} = T(I_{\text{true}}) + I_{\text{noise}}$$

in a metric space $X$ (with metric $\| \cdot \|_X$) from “initial data” $I_{\text{data}}$ in a metric space $H$ (with metric $\| \cdot \|_H$) is well-posed on the pair of spaces $(X, H)$ if each of the following holds:

(a) For every $I_{\text{data}} \in H$ there exists a solution $I \in X$ (existence).

(b) The solution is uniquely determined (uniqueness).
The problem is stable on the spaces \((\mathcal{X}, \mathcal{H})\), i.e., for every \(\epsilon > 0\) there is a \(\delta(\epsilon) > 0\), such that for any \(I_1, I_2 \in \mathcal{X}\) where
\[
\|I_1 - I_2\|_\mathcal{X} \leq \epsilon
\]
we have that
\[
\|T(I_1) - T(I_2)\|_{\mathcal{H}} \leq \delta(\epsilon).
\]
The problem is ill-posed if any of these criteria are not fulfilled.

When one is dealing with an ill-posed problem, one needs to address each of the issues of existence, uniqueness and stability. First, existence can be commonly enforced by considering maximum likelihood (least squares) solutions to (3.1), i.e., (3.1) is replaced with
\[
\min_{I \in \mathcal{X}} \|T(I) - I_{\text{data}}\|_{\mathcal{H}}
\]
for a suitable “notion of distance” \(\| \cdot \|_{\mathcal{H}}\) in data space \(\mathcal{H}\). However, two serious problems remain:

- **Non-uniqueness**: Infinitely many maximum likelihood solutions.
- **Instability**: Maximum likelihood solutions do not depend continuously on the data \(I_{\text{data}}\).

For handling uniqueness and stability, the recovery method must involve some stabilization (regularization method).

### 3.1.1 Deconvolution is ill-posed

Consider now the problem of deconvolution of 2D images, i.e., we seek the 2D image \(I_{\text{true}}\) from the equation
\[
I_{\text{data}} = \{\text{PSF} \circledast I_{\text{true}}\} + I_{\text{noise}}. \tag{3.2}
\]
Note here that \(I_{\text{true}}\) and \(I_{\text{data}}\) are real-valued functions defined on some 2D region-of-interest \(\Omega\) that represent grey scale images. The same applies to \(I_{\text{noise}}\) which is the 2D noise component of the image \(I_{\text{data}}\) and PSF is the PSF of the imaging system which we seek to deconvolve. Hence, this problem corresponds to (3.1) with
\[
T(I) := \text{PSF} \circledast I.
\]

One attempt at solving this problem is by inverse filtering. This procedure works as follows: Begin by take the Fourier transform of data \(I_{\text{data}}\) in (3.2). Then, we get
\[
\mathcal{F}(I_{\text{data}}) = \mathcal{F}\left(\{\text{PSF} \circledast I_{\text{true}}\} + I_{\text{noise}}\right).
\]
Next, the Fourier transform is linear, so
\[
\mathcal{F}(I_{\text{data}}) = \mathcal{F}(\text{PSF} \circledast I_{\text{true}}) + \mathcal{F}(I_{\text{noise}}).
\]
Now, the convolution theorem stated in section 2.2 gives us that
\[
\mathcal{F}(I_{\text{data}}) = \mathcal{F}(\text{PSF}) \cdot \mathcal{F}(I_{\text{true}}) + \mathcal{F}(I_{\text{noise}}).
\]
Hence, dividing with $\mathcal{F}(\text{PSF})$ gives us

$$\frac{\mathcal{F}(I_{\text{data}})}{\mathcal{F}(\text{PSF})} = \frac{\mathcal{F}(I_{\text{true}})}{\mathcal{F}(\text{PSF})} + \frac{\mathcal{F}(I_{\text{noise}})}{\mathcal{F}(\text{PSF})}.$$ 

Now, it is tempting to define the solution to (3.2) as the image $I^*$ where

$$\mathcal{F}(I^*) := \frac{\mathcal{F}(I_{\text{data}})}{\mathcal{F}(\text{PSF})} = \frac{\mathcal{F}(I_{\text{data}})}{\text{MTF}}.$$ 

This is precisely what inverse filtering suggests us to do. Then we get that

$$\mathcal{F}(I^*) = \mathcal{F}(I_{\text{true}}) + \frac{\mathcal{F}(I_{\text{noise}})}{\text{MTF}}.$$ 

When $I_{\text{noise}} = 0$ and $\text{MTF}(\xi) \neq 0$, then we see that $\mathcal{F}(I^*)(\xi) = \mathcal{F}(I_{\text{true}})(\xi)$, so for all such points $I^* = I_{\text{true}}$. But what happens when $I_{\text{noise}} \neq 0$ and/or $\mathcal{F}(\text{PSF})(\xi) = 0$?

First, note that when $\mathcal{F}(\text{PSF})(\xi) = 0$ we run into trouble since the spectral noise becomes infinitely large at that point unless $\mathcal{F}(I_{\text{noise}})(\xi) = 0$. However, there are problems even when $\mathcal{F}(\text{PSF})(\xi) \neq 0$. Most imaging systems, including detectors in TEM, are bandlimited. This means that the imaging system cannot resolve all frequency information in the original image. For the case of detectors, this is manifested by the decline of the MTF towards zero as the frequency $|\xi|$ increases. Hence, the PSFs that are of relevance, we know that their Fourier transform $\mathcal{F}(\text{PSF})$ will decline. The noise $I_{\text{noise}}$ is independent of the signal, so one cannot expect the noise to cancel out the $\mathcal{F}(\text{PSF})$. Hence, for large values of the frequency, the spectral noise will grow. This causes instability, so the deconvolution problem is ill-posed.

### 3.2 Regularization

Regularization is an important tool for solving ill-posed problems of the type in (3.1), such as deblurring in image restoration. The main idea in regularization is to replace the original ill-posed inverse problem by a parameterized family of well-posed inverse problems that converge to a least-squares solution as the data error goes to zero.

For an ill-posed inverse problem, as already mentioned, it does not make sense to try to approximate $T^{-1}$. This was e.g. clear with the example of noise amplification when using inverse filtering for deconvolution.

One option is to regularize by calculating a stable approximation to $T^{-1}$. This is e.g. the approach taken in Wiener deconvolution for deconvolution. Another more generic approach is Tikhonov regularization which is widely used in image restoration applications.

#### 3.2.1 Tikhonov regularization

Consider the general inverse problem of recover the 2D image $I_{\text{true}} \in \mathcal{X}$ from data $g \in \mathcal{H}$ assuming $I_{\text{data}} = T(I_{\text{true}}) + I_{\text{noise}}$ where $T$ is the linear forward
model. The Tikhonov regularization method is defined as the solution to

\[
\min_{I \in X} \lambda S(I) + \|T(I) - I_{data}\|^2_{\mathcal{H}}
\]  

(3.3)

where

- \(S : X \rightarrow \mathbb{R}^+\) is the regularization functional which enforces uniqueness and also acts as a stabilizer by incorporating a priori knowledge about the unknown true image \(I_{true}\),
- \(\|T(I) - I_{data}\|_{\mathcal{H}}\) quantifies the dissimilarity in data space \(\mathcal{H}\) between the measured data and the data generated by the image \(I\), and
- \(\lambda > 0\) is the regularization parameter that quantifies the compromise between accuracy (resolution) and stability.

The most common choice of regularization functional in imaging is

\[
S(I) := \sqrt{\int_X |\nabla I(x)|^2 dx}
\]

which corresponds to the assumption that the true image \(I_{true}\) is smooth.

The choice of dissimilarity measure in data space should be based on knowledge of the probability model for the noise component \(I_{noise}\) of data \(I_{data}\). We will consider additive independent Gaussian noise with zero mean so \(\|\cdot\|_{\mathcal{H}}\) is the regular 2-norm.

Finally, there is the choice of regularization parameter \(\lambda\). Its choice should be based on the model for the noise component \(I_{noise}\) of data and the particular form of the dissimilarity measure \(\|\cdot\|_{\mathcal{H}}\).

**Digital images** When discretized, such as when we consider digital images, the linear forward model \(T\) is simply multiplication with a matrix \(T\), i.e,

\[
T(I) := T \cdot I.
\]

We can then formulate the Tikhonov regularization problem as finding the 2D digital image \(I\) from 2D imaging data \(I_{data}\) by

\[
\min_{I \in \mathbb{R}^{n \times m}} \lambda \|L \cdot I\|^2_2 + \|T \cdot I - I_{data}\|^2_{\mathcal{H}}
\]

In the above, \(L\) is the matrix that represents the discrete version of calculating the image gradient, so \(L \cdot I\) is the discrete version of \(S(I)\). The minimizer of the above optimization problem can be expressed as a solution to the normal equations

\[
(T^T \cdot T + \lambda L^T \cdot L) \cdot I = T^T \cdot I_{data}.
\]

The above can be solved by singular value decomposition or by an iterative method [8, 18].
Chapter 4

Deconvolution of TEM images

A TEM is a rather complex image formation device and image restoration can seek to reverse degradation introduced during different parts of the image formation in the TEM. Here we consider only the detector part in the TEM. The detector introduces correlations in-between neighboring pixels which in turn blurs the image. This is commonly modeled through a convolution. For slow-scan CCD camera based detectors used in modern TEMs, the PSF is common to be expressed in terms of its MTFs. The latter is a rotationally symmetric function with a closed form parametrization \[20\]:

\[
\text{MTF}(\xi) := \frac{a}{1 + \alpha |\xi|^2} + \frac{b}{1 + \beta |\xi|^2} + c. \tag{4.1}
\]

Note that the MTF parameters \(a\), \(b\), \(c\), \(\alpha\), and \(\beta\) are detector specific and typical values are \(a = 0.7\), \(b = 0.2\), \(c = 0.1\), \(\alpha = 10\) and \(\beta = 40\). Figure 4.1 shows a plot of a typical MTF.

![Figure 4.1: 2D plot of a typical MTF of the form (4.1). The value is equal to 1 when \(\xi = 0\) and then it monotonically decreases and approaches 0 as \(|\xi|\) increases.](image)

Figure 4.1: 2D plot of a typical MTF of the form (4.1). The value is equal to 1 when \(\xi = 0\) and then it monotonically decreases and approaches 0 as \(|\xi|\) increases.
To deconvolve the detector PSF in 2D TEM images can then be stated as the problem of recovering the true image $I_{\text{true}}$ from TEM imaging data $I_{\text{data}}$ where

$$I_{\text{data}}(i,j) = C_{i,j} \{\text{PSF} \ast I_{\text{true}}\}(x_{i,j}) + I_{\text{noise}}(i,j)$$

(4.2)

for a pixel $(i,j)$ in the detector. In the above, $C_{i,j}$ is a known scaling term (partly detector dependent), $x_{i,j}$ is typically the mid-point of the $(i,j)$:th pixel in the imaging data $I_{\text{data}}$, and PSF is the detector PSF given by its MTF.

Note that we can always divide imaging data $I_{\text{data}}(i,j)$ with $C_{i,j}$, so without loss of generality, we can set $C_{i,j} = 1$. Furthermore, stated in the Fourier domain, the problem is to recover the Fourier transform $\hat{I}_{\text{true}}$ of the true image from the Fourier transform of TEM imaging data where

$$\hat{I}_{\text{data}}(i,j) = MTF(\xi_{i,j}) \cdot \hat{I}_{\text{true}}(\xi_{i,j}) + \hat{I}_{\text{noise}}(i,j).$$

Since PSF is a filter that suppresses high frequency components of the input image, the recorded image $I_{\text{data}}(i,j)$ has loss of information even when data is noise-free, i.e., $I_{\text{noise}}(i,j) = 0$.

### 4.1 Inverse filtering

The basis for inverse filtering was already outlined in section 3.1.1. It is an attempt at directly inverting $T$. The inverse filtering solution to the deconvolution problem is defined as

$$I^* := \mathcal{F}^{-1}\left(\frac{\mathcal{F}(I_{\text{data}})}{\mathcal{F}(\text{PSF})}\right) = \mathcal{F}^{-1}\left(\frac{\mathcal{F}(I_{\text{data}})}{MTF}\right).$$

We have used MATLAB to calculate the inverse filtering solution to the above stated deconvolution problem and the result is shown in figure 4.2. Even when data is noise-free, i.e., $I_{\text{noise}} = 0$, the inverse filtering solution is useless due to severe instability. This further supports our claim in section 3.1.1 that deconvolution is ill-posed.

### 4.2 Wiener deconvolution

Wiener deconvolution is a deconvolution method that deconvolves an image by convolving it with a cleverly chosen filter (Wiener filter):

$$I^* := I_{\text{data}} \ast W.$$ 

The Wiener filter $W$ is most easily described in the frequency domain:

$$\tilde{W}(\xi) := \frac{|MTF(\xi)|}{|MTF(\xi)|^2 + \frac{\sigma_{\text{noise}}(\xi)}{\sigma_{\text{data}}(\xi)}}$$

where $\sigma_{\text{noise}}(\xi)$ is the mean spectral density of the noise component $I_{\text{noise}}$ of data and $\sigma_{\text{data}}$ is the mean spectral density of the imaging data $I_{\text{data}}$ itself. The ration

$$\text{NSR}(\xi) = \frac{\sigma_{\text{noise}}(\xi)}{\sigma_{\text{data}}(\xi)}$$
is called the Noise-power-to-Signal-power Ratio (NSR). For noise-free data, we have that $\sigma_{\text{noise}}(\xi) = 0$, so the NSR is zero as well. The NSR acts as a regularization parameter and properly estimating it from data is important for obtaining the best performance with Wiener deconvolution.

We have used MATLAB Image Processing Toolbox implementation of Wiener deconvolution. Wiener deconvolution is then performed by the command `deconvwnr`. Note however that zero-padding (see section 2.2.1) is needed for increased accuracy as shown by figure 4.3.

Figure 4.3: Left figure shows an intensity profile along a line after Wiener deconvolution without zero-padding. Right image shows the same intensity profile when Wiener deconvolution is used with zero-padding.
4.3 Tikhonov deconvolution

This is simply to solve our deconvolution problem using Tikhonov regularization (section 3.2.1), i.e.,

\[
\min_I \lambda \int_\Omega \left\| \nabla I(x) \right\|^2 dx + \left\| I \ast \text{PSF} - I_{\text{data}} \right\|^2
\]

(4.3)

or, stated equivalently in Fourier space,

\[
\min_i \lambda \int_\Omega \left\| \nabla (\mathcal{F}^{-1}(\hat{I}))(x) \right\|^2 dx + \left\| \hat{I} \cdot \text{MTF} - \hat{I}_{\text{data}} \right\|^2
\]

(4.4)

where \( \hat{I}_{\text{data}} \) is the Fourier transform of \( I_{\text{data}} \). For digital image, we can formulate the Tikhonov regularization problem as finding the 2D digital image \( I \) from 2D imaging data \( I_{\text{data}} \) by

\[
\min_I \lambda \| L \cdot I \|^2 + \left\| I \ast \text{PSF} - I_{\text{data}} \right\|^2
\]

where \( I \) is the matrix representing the digital image and \( L \) the discrete version of calculating the image gradient:

\[
L_x = \begin{pmatrix} -1 & 0 & +1 \\ -2 & 0 & +2 \\ -1 & 0 & +1 \end{pmatrix} \ast I
\]

\[
L_y = \begin{pmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ +1 & +2 & +1 \end{pmatrix} \ast I
\]

In the above, \( L_x \) and \( L_y \) are two digital images which are the horizontal and vertical derivative approximation of each point. The gradient magnitude \( L \) is then

\[
L = \sqrt{L_x^2 + L_y^2}
\]
Chapter 5
Quantitative evaluation of deconvolution methods

In order to make a quantitative evaluation of deconvolution methods, we need to be able to compare a deconvolved image $I$ against the original uncorrupted image $I_{\text{true}}$, henceforth called the phantom.

One issue is how to make this comparison. The comparison between the deconvolved image $I$ and the phantom $I_{\text{true}}$ is quantified by means of a Figure of Merit (FOM). Another issue is on choosing the appropriate settings for the deconvolution method. There will be different results from the deconvolution method depending on its settings, the most important one being the choice of regularization parameter(s). Hence, before the deconvolution method is tested, it needs to be calibrated where the regularization parameter(s) are selected as to minimize a training FOM.

The quantitative evaluation of a fixed deconvolution method can now be summarized as follows:

1. Training data: Select a fixed set of phantoms $I_{\text{true}}^1, \ldots, I_{\text{true}}^n$ which represent the true uncorrupted images. For each phantom $I_{\text{true}}^k$, generate a set of TEM imaging data:
   $$I_{\text{train}}^{k,1}, \ldots, I_{\text{train}}^{k,m}.$$  
   Hence, note that there can be several data related to one phantom. This training data should have signal-to-noise and distortion properties that resemble real TEM images of biological specimens.

2. Define a single FOM, a training FOM denoted by $t_{\text{FOM}}$, that will be used for the calibration.

3. Calibration: Choose the regularization parameter $\lambda^*$ for the deconvolution method that provides the best match across all training data and all phantoms in the training set. The matching is to compare, using $t_{\text{FOM}},$
the deconvolved image $I_{k,l}^{(\lambda)}$ (obtained from training data $I_{\text{train}}^{k,l}$) against the corresponding phantom $I_{\text{true}}^{k}$. Mathematically, we determine $\lambda^*$ as

$$
\lambda^* := \arg \max_{\lambda} \left\{ \max_{k,l} \text{tFOM}\left(I_{k,l}^{(\lambda)}, I_{\text{true}}^{k}\right) \right\}.
$$

4. Test data: Select a fixed set of phantoms

$$J_{\text{true}}^{1}, \ldots, J_{\text{true}}^{n'}$$

which represent the true uncorrupted images. For each phantom $J_{\text{true}}^{k}$, generate a set of TEM imaging data:

$$J_{\text{test}}^{k,1}, \ldots, J_{\text{test}}^{k,m'}.$$

This test data should have signal-to-noise and distortion properties that resemble real TEM images of biological specimens.

5. Define a set of FOMs that will be used for evaluation.

6. For each test data $J_{\text{test}}^{k,l}$, calculate the deconvolved image $I_{\text{test}}^{k,s}(\lambda^*)$ using the regularization parameter $\lambda^*$ from the calibration step. Also, calculate the FOMs defined earlier for evaluation by comparing deconvolved image $I_{\text{test}}^{k,s}(\lambda^*)$ against the corresponding phantom $J_{\text{true}}^{k}$.

7. Perform a statistical analysis on the FOMs values.

In our case, we test two deconvolution methods, Wiener deconvolution and Tikhonov regularization. In the former, the regularization parameter is the NSR value, in the latter it is $\lambda$.

5.1 Figure of Merits (FOMs)

In imaging, different Figure of Merits (FOMs) have been proposed depending on what aspect one seeks to emphasize.

In this project we mainly focus on structural consistency measures. These FOMs can be measured using simple magnitudes such as dynamic range, absolute errors, squared errors, differences in the means, and differences in standard deviations [17]. We also include another FOM for image quality, the Structural Similarity Index (SSIM) [19]. The FOMs are defined so that they are in-between 0 and 1, with 1 when $I = J$ and smaller values correspond to worse agreement.

To describe the FOMs, we need to introduce some notation. First, given an image $I$, define $m(I)$ and $M(I)$ as its relative (after normalization to $[0, 1]$) minimum and maximum intensity values. Also, let $\mu(I)$ denote the mean and $\sigma(I)$ the standard deviation, obtained after images has been normalized to $[0, 1]$. Finally, we have $\rho(I, J)$ that denotes the covariance between $I$ and $J$.

Mean Square Error (MSE) The MSE is a FOM that measures the average of the squared errors. Give two images $I$ and $J$ on a region-of-interest $\Omega$, it is defined as

$$
\text{MSE}(I, J) := 1 - \frac{1}{|\Omega|} \int_{\Omega} \left( \frac{I(x) - J(x)}{2} \right)^2 dx.
$$
Mean Absolute Error (MAE) The MAE is a FOM that measures the average of the absolute errors. Given two images $I$ and $J$ on a region-of-interest $\Omega$, it is defined as

$$\text{MAE}(I, J) := 1 - \frac{1}{|\Omega|} \int_{\Omega} \frac{|I(x) - J(x)|}{2} \, dx.$$ 

Mean Density Value (MDV) The MDV is a FOM that measures the correspondence between the density averages over the whole region. Given two images $I$ and $J$ on a region-of-interest $\Omega$, it is defined as

$$\text{MDV}(I, J) := 1 - \frac{1}{2} |\mu(I) - \mu(J)|.$$ 

Density Standard Deviation (DSD) The DSD is a FOM that measures how well the standard deviations agree. Given two images $I$ and $J$ on a region-of-interest $\Omega$, it is defined as

$$\text{DSD}(I, J) := 1 - \frac{1}{2} |\sigma(I) - \sigma(J)|.$$ 

Range The range is a FOM that measures how well the dynamic range agrees. Given two images $I$ and $J$ on a region-of-interest $\Omega$, it is defined as

$$\text{Range}(I, J) := 1 - \frac{1}{2} \left( |M(I) - M(J)| + |m(I) - m(J)| \right).$$ 

Structural Similarity Index (SSIM) The SSIM is a FOM that measures the similarity between two images. It is designed to improve on traditional methods like peak signal-to-noise ratio and MSE, which have proved to be inconsistent with human eye perception. The difference with respect these other FOMs is that these approaches estimate perceived errors on the other hand SSIM considers image degradation as perceived change in structural information. Structural information is the idea that the pixels have strong inter-dependencies especially when they are spatially close. These dependencies carry important information about the structure of the objects in the visual scene. Given two images $I$ and $J$ on a region-of-interest $\Omega$, the SSIM is defined as

$$\text{SSIM}(I, J) := \ell_{\text{mean}}(I, J)^\alpha \ell_{\text{var}}(I, J)^\beta \ell_{\text{cov}}(I, J)^\gamma$$

where $\alpha, \beta$ and $\gamma$ are parameters used to adjust the relative importance of the three components

$$\ell_{\text{mean}}(I, J) := \frac{2\mu(I)\mu(J) + C}{\mu(I)^2 + \mu(J)^2 + C}$$

$$\ell_{\text{var}}(I, J) := \frac{2\sigma(I)\sigma(J) + C}{\sigma(I)^2 + \sigma(J)^2 + C}$$

$$\ell_{\text{cov}}(I, J) := \frac{\rho(I, J) + C}{\sigma(I)\sigma(J) + C}$$

In the above, $C$ is a constant used for adjusting the values so that the FOM is in $[0, 1]$. 

Chapter 6
The simulated TEM data

The simulated data is obtained using the TEM simulator described in [16] (its source code is available from http://tem-simulator.sourceforge.net/).

The specimens (phantom) is a single RNA Polymerase II molecule embedded in aqueous buffer. The aqueous buffer is “meniscus” shaped and 150 nm thick at the edges and 50 nm thick at the center. The molecule in question is given by a real-valued function sampled on a grid with cubic voxels, each with size 0.1 nm. Simulated 2D TEM images are given on a detector that is 175 × 200 pixels in size.

6.1 Common imaging parameters

The images are all simulations of 300 keV at 25 000× magnification and a defocus of 3 µm. The physical values related to the electron and the imaging parameters relevant for TEM imaging are listed in table 6.1.

Detector parameters are the gain (which is 80), quantum efficiency DQE (which is 0.4), and pixel size, which is 16 µm. Hence, the pixel area $\Delta_{\text{pixel}}$ after magnification (25 000×) becomes 0.9216 nm$^2$. This gives the value

$$C_{i,j} := \Delta_{\text{pixel}} \cdot \text{gain} \cdot \text{DQE} \cdot \text{dose} \approx 29.5 \cdot \text{dose} \quad \text{for all } i, j$$

for $C_{i,j}$ in (4.2) where the dose is given in e$^-$/nm$^2$ and varies between images, see table 6.2. The detector MTF parameters $a$, $b$, $c$, $\alpha$, and $\beta$ in (4.1) are $a = 0.7$, $b = 0.2$, $c = 0.1$, $\alpha = 10$ and $\beta = 40$ for all simulations.

6.2 Varying imaging parameters

The imaging parameter that varies between simulated images is the dose (directly related to the noise level in the images) and whether noise and detector and/or optics PSFs is activated or not. The variations are summarised in table 6.2 on page 25. In a low-dose image, the noise one would expect corresponds to a dose of 5–50 electrons per pixel. Hence, the simulations with a dose of 50 or more correspond to images with little noise compared to real images.
### Universal physical constants

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest mass of electron</td>
<td>$m_0 = 9.10938 \cdot 10^{-31}$ kg</td>
</tr>
<tr>
<td>Velocity of light in vacuum</td>
<td>$c = 2.9979245 \cdot 10^8$ m/s</td>
</tr>
<tr>
<td>Planck’s constant</td>
<td>$h = 6.6256 \cdot 10^{-34}$ Js</td>
</tr>
<tr>
<td>Rest energy of the electron</td>
<td>$E_0 = 8.1871 \cdot 10^{-14}$ J $\approx 511$ keV</td>
</tr>
</tbody>
</table>

### Imaging dependent physical constants (at 300 keV)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic energy of incident electron</td>
<td>$E_{\text{acc}} = 4.80653 \cdot 10^{-14}$ J</td>
</tr>
<tr>
<td>Wavelength of incident electron</td>
<td>$\lambda = 1.96875 \cdot 10^{-12}$ m</td>
</tr>
<tr>
<td>Wave number of incident electron</td>
<td>$k = 3.19146 \cdot 10^{12}$ m$^{-1}$</td>
</tr>
</tbody>
</table>

### TEM illumination parameters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean energy spread</td>
<td>$\Delta E = 0.7$ eV $\approx 1.12152 \cdot 10^{-19}$ J</td>
</tr>
<tr>
<td>Illumination angle</td>
<td>$\alpha_c = 0.1$ mrad</td>
</tr>
</tbody>
</table>

### TEM optics parameters for the primary lens

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal length</td>
<td>$f_p = 2.7 \cdot 10^{-3}$ m</td>
</tr>
<tr>
<td>Defocus</td>
<td>$\Delta z$ $\in [0, 1.2 \cdot 10^{-5}$ m</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>$C_s = 2.0 \cdot 10^{-3}$ m</td>
</tr>
<tr>
<td>Chromatic aberration</td>
<td>$C_c = 2.0 \cdot 10^{-3}$ m</td>
</tr>
<tr>
<td>Objective aperture diameter</td>
<td>$D = 4.0 \cdot 10^{-5}$ m</td>
</tr>
</tbody>
</table>

Table 6.1: The values are for imaging using 300 keV. The defocus value is given as a range and a common value for defocus when imaging unstained biological phase contrast objects is $\Delta z = 5$ µm $= 5.0 \cdot 10^{-6}$ m.
Table 6.2: Summary of simulated data. All other parameters are the same across simulations and are given in section 6.1.
Chapter 7

Training

7.1 The training data and the training FOM

As training FOM we choose the MDV. The training data 50 are randomly choose sets of data from TEM simulations described in section 6.

7.1.1 Training for Wiener deconvolution

The regularization parameter in Wiener deconvolution is the NSR. The optimal NSR value is taken as the one that gives the maximum MDV value. Computational evaluation of different values of the NSR in MATLAB shows the following results:

<table>
<thead>
<tr>
<th>Dose of TEM data</th>
<th>Optimal value of NSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10000 \text{ e}^{-}/\text{nm}^2$</td>
<td>NSR = 0.21</td>
</tr>
<tr>
<td>$5000 \text{ e}^{-}/\text{nm}^2$</td>
<td>NSR = 0.23</td>
</tr>
<tr>
<td>$1000 \text{ e}^{-}/\text{nm}^2$</td>
<td>NSR = 0.27</td>
</tr>
</tbody>
</table>

See also figure 7.1 that shows plots of the training FOM (the MDV) as well as the plots of MDV ± one Standard Deviation (STD). The associated figures 8.7 show the actual images.

7.1.2 Training for Tikhonov deconvolution

The regularization parameter in Tikhonov deconvolution is the $\lambda$ in (3.3). The optimal $\lambda$ value is taken as the one that gives the maximum MDV value. Computational evaluation of different values of the $\lambda$ in MATLAB shows the following results:

<table>
<thead>
<tr>
<th>Dose of TEM data</th>
<th>Optimal value of $\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10000 \text{ e}^{-}/\text{nm}^2$</td>
<td>$\lambda = 0.27$</td>
</tr>
<tr>
<td>$5000 \text{ e}^{-}/\text{nm}^2$</td>
<td>$\lambda = 0.97$</td>
</tr>
<tr>
<td>$1000 \text{ e}^{-}/\text{nm}^2$</td>
<td>$\lambda = 3.6$</td>
</tr>
</tbody>
</table>

See also figure 7.2 that shows plots of the training FOM (the MDV) as well as the plots of MDV ± one STD. The associated figures 8.12 show the actual images.
Figure 7.1: Plots showing the training FOM (the MDV) and its STD for Wiener deconvolution with different NSR. Each of the plots show results for three different training sets of TEM simulations, each with different doses: 10000 e<sup>−</sup>/nm<sup>2</sup> (top left), 5000 e<sup>−</sup>/nm<sup>2</sup> (top right), and 1000 e<sup>−</sup>/nm<sup>2</sup> (bottom). The maximal value for the training FOM is when NSR = 0.21 (dose=10000 e<sup>−</sup>/nm<sup>2</sup>), NSR = 0.23 (dose=5000 e<sup>−</sup>/nm<sup>2</sup>), and NSR = 0.27 (dose=1000 e<sup>−</sup>/nm<sup>2</sup>).
Figure 7.2: Plots showing the training FOM (the MDV) and its STD for Tikhonov deconvolutions with different $\lambda$. Each of the plots show results for three different training sets of TEM simulations, each with different doses: 10000 $e^{-}/\text{nm}^2$ (top left), 5000 $e^{-}/\text{nm}^2$ (top right), and 1000 $e^{-}/\text{nm}^2$ (low middle). The maximal value for the training FOM is when $\lambda = 0.27$ (dose=10000 $e^{-}/\text{nm}^2$), $\lambda = 0.97$ (dose=5000 $e^{-}/\text{nm}^2$), and $\lambda = 3.6$ (dose=1000 $e^{-}/\text{nm}^2$).
Chapter 8

Testing

In all test we assume perfect information about the MTF, i.e., the same MTF is used for both simulations and deconvolution. Testing against misspecification in MTF parameters is done in section 9.

8.1 The test data sets

We start out by testing the deconvolution methods on noise-free test data. Here, the phantom is the same noise-free image simulated assuming a perfect detector, so MTF is 1. The data is shown in figure 8.1.

![Figure 8.1: Noise-free image data: A simulated noise-free TEM image. The molecule is clearly visible but imaged is distorted due to the MTF.](image)

We also have three sets of 100 TEM images. All TEM images are of the same molecule acquired using the same imaging settings apart from the dose. The first set corresponds to TEM images acquired using a dose of 10000 e\(^{-}/\text{nm}^{2}\),
the second set to a dose of 5000 e$^{-}/\text{nm}^2$, and finally the third set to a dose of 1000 e$^{-}/\text{nm}^2$. Hence, apart from noise, all TEM images within a set are identical. Examples of images from these three sets of data are shown in figure 8.2.

8.2 Wiener deconvolution

8.2.1 Results for noise-free data

The results of Wiener deconvolution on noise-free data (section 8.1) are summarized in figure 8.3. Since data is noise-free, the NSR in Wiener deconvolution is set to 0 during deconvolution.
Figure 8.3: Wiener deconvolution of noise-free images: In the left column, (a) and (c) shows the phantom and a zoom-in, i.e., the noise-free image without any MTF distortion. In the right column, (b) and (d) show corresponding images obtained from Wiener deconvolution on noise-free image data shown in figure 8.1.

A close investigation shows that the original image is sharper than the phantom. This is perhaps easier to see if one considers intensity variation (contrast) along a line that passes through the molecule used in the simulations. In figure 8.4 we plot this intensity variation along the horizontal center line for each of the three images, the phantom, the data, and the image obtained after Wiener deconvolution. There, it is clear that the deconvolved image is closer to the phantom than the data.
Figure 8.4: Intensity variations in Wiener deconvolution: Here we plot intensity variations along the horizontal center line. The blue line shows the result in the phantom and represents the true intensity variations. The red line shows the case for the data and the green line is the case for Wiener deconvolution. It is clear that the result for the deconvolved image is closer to the phantom than the data.

Furthermore, we also consider the variance of the images which shows how much the values are spread around the mean. This is done both on the entire image as well as on the center part, i.e., the molecule (an RNA Polymerase II molecule). The latter is simply extracted from the whole image. Results are summarized in table 8.1.

<table>
<thead>
<tr>
<th></th>
<th>Entire image</th>
<th>Center part only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phantom</td>
<td>$3.104273825752572 \cdot 10^{-6}$</td>
<td>$3.913716138041773 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Data</td>
<td>$3.097199519455397 \cdot 10^{-6}$</td>
<td>$3.122020887131801 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Deconvolved</td>
<td>$3.097199519455397 \cdot 10^{-6}$</td>
<td>$3.122020887131801 \cdot 10^{-6}$</td>
</tr>
</tbody>
</table>

Table 8.1: Wiener deconvolution: Comparison of images variances, the phantom, data and Wiener deconvolution.

See also figure 8.5 where it is clear that Wiener deconvolution is always closer to the original image than data, this no matter if one considers the whole image or only its central part containing the molecule.
8.2.2 Results for noisy data

Here we consider the noisy data shown in figure 8.2. The results for deconvolution are shown in figure 8.6. The NSR used in the Wiener deconvolution is chosen according to the training, see section 7.1.1.

As for the noise-free case, we also investigate the intensity variation (contrast) along the horizontal central line that passes through the molecule. Since all images are noisy, including the phantom, we need to form an average contrast variation to discriminate random contrast variations from the one that comes from the molecule. Hence, we pick at random 50 simulated images of the same molecule and their associated phantoms (which are noisy but MTF-free images). Then, choose the horizontal center line for each image and calculate the contrast along that line. Next, form an average over all 50 images. The maximum and minimum variation in intensities that the phantoms manifest provide a band of acceptance for what the contrast should be in the deconvolution. Results are shown in figure 8.7 where we see that Wiener deconvolution for noisy images works well for all dose levels. The fluctuation in the background is reasonable and the contrast for the central molecule is in the acceptance region.
Figure 8.6: Wiener deconvolution of noisy images: Top row shows the deconvolution (a) and the phantom (b) when dose is 10000 e\(^{-}\)/nm\(^2\). Middle row shows the deconvolution (c) and the phantom (d) when dose is 5000 e\(^{-}\)/nm\(^2\). Finally, bottom row shows the deconvolution (d) and the phantom (e) when dose is 1000 e\(^{-}\)/nm\(^2\). As the dose decreases, the noise level increases. For the bottom row, the central molecule is hardly visible due to the noise.
Figure 8.7: Contrast variation for Wiener deconvolution of noisy images: The three figures show average contrast variation (green) along the horizontal central line for imaging data at three different noise levels corresponding to dose of $10000 \text{ e}^{-}/\text{nm}^2$ (top), $5000 \text{ e}^{-}/\text{nm}^2$ (middle), and $1000 \text{ e}^{-}/\text{nm}^2$ (bottom). The dotted upper and lower plots in each image show the acceptance region for the contrast at that noise level. This region is obtained by considering the maximum and minimum variation in intensities for the phantoms. In the central part for all three cases, which is the most important information, the contrast makes variations at the same position.
8.3 Tikhonov deconvolution

8.3.1 Noise-free data

The results of Tikhonov deconvolution on noise-free data (section 8.1) are summarized in figure 8.3. Since data is noise-free, the $\lambda$ in Tikhonov deconvolution is set to 0 during deconvolution.

![Image showing Tikhonov deconvolution of noise-free images](image.png)

Figure 8.8: Tikhonov deconvolution of noise-free images: In the left column, (a) and (c) shows the phantom and a zoom-in, i.e., the noise-free image without any MTF distortion. In the right column, (b) and (d) show corresponding images obtained from Tikhonov deconvolution on noise-free image data shown in figure 8.1.

A close investigation shows that the original image is sharper than the phantom. This is perhaps easier to see if one considers intensity variation (contrast) along a line that passes through the molecule used in the simulations. In figure 8.9 we plot this intensity variation along the horizontal center line for each of the three images, the phantom, the data, and the image obtained after Tikhonov deconvolution. There, it is clear that the deconvolved image is closer to the phantom than the data. Furthermore, we also consider the variance of the images which shows how much the values are spread around the mean. This is done both on the entire image as well as on the center part, i.e., the molecule (an RNA Polymerase II molecule). The latter is simply extracted from the whole
Figure 8.9: Intensity variations in Tikhonov deconvolution: Here we plot intensity variations along the horizontal center line. The blue line shows the result in the phantom and represents the true intensity variations. The red line shows the case for the data and the green line is the case for Tikhonov deconvolution. It is clear that the result for the deconvolved image is closer to the phantom than the data.

Images. Results are summarized in Table 8.2.

<table>
<thead>
<tr>
<th></th>
<th>Entire image</th>
<th>Center part only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phantom</td>
<td>1.12976521374108 \cdot 10^{-5}</td>
<td>7.042525758510638 \cdot 10^{-4}</td>
</tr>
<tr>
<td>Data</td>
<td>8.601335157742903 \cdot 10^{-6}</td>
<td>5.10564542982219 \cdot 10^{-4}</td>
</tr>
<tr>
<td>Deconvolved</td>
<td>1.060077956060319 \cdot 10^{-5}</td>
<td>6.506293541279284 \cdot 10^{-4}</td>
</tr>
</tbody>
</table>

Table 8.2: Tikhonov deconvolution: Comparison of images variances, the phantom, data and Tikhonov deconvolution.

See also Figure 8.10 where it is clear that Tikhonov deconvolution is always closer to the original image than data, this no matter if one considers the whole image or only its central part containing the molecule.
8.3.2 Results for noisy data

Here we consider the noisy data shown in figure 8.2. The results for deconvolution are shown in figure 8.11. The $\lambda$ used in the Tikhonov deconvolution is chosen according to the training, see section 7.1.2.

As for the noise-free case, we also investigate the intensity variation (contrast) along the horizontal central line that passes through the molecule. Since all images are noisy, including the phantom, we need to form an average contrast variation to discriminate random contrast variations from the one that comes from the molecule. Hence, we pick at random 50 simulated images of the same molecule and their associated phantoms (which are noisy but MTF-free images). Then, choose the horizontal center line for each image and calculate the contrast along that line. Next, form an average over all 50 images. The maximum and minimum variation in intensities that the phantoms manifest provide a band of acceptance for what the contrast should be in the deconvolution. Results are shown in figure 8.12 where we see that Tikhonov deconvolution for noisy images works well for all dose levels. The fluctuation in the background is reasonable and the contrast for the central molecule is in the acceptance region.
Figure 8.11: Tikhonov deconvolution of noisy images: Top row shows the deconvolution (a) and the phantom (b) when dose is 10000 e⁻/nm². Middle row shows the deconvolution (c) and the phantom (d) when dose is 5000 e⁻/nm². Finally, bottom row shows the deconvolution (d) and the phantom (e) when dose is 1000 e⁻/nm². As the dose decreases, the noise level increases. For the bottom row, the central molecule is hardly visible due to the noise.
Figure 8.12: Contrast variation for Tikhonov deconvolution of noisy images: The three figures show average contrast variation (green) along the horizontal central line for imaging data at three different noise levels corresponding to dose of $10000 \, e^-/nm^2$ (top), $5000 \, e^-/nm^2$ (middle), and $1000 \, e^-/nm^2$ (bottom). The dotted upper and lower plots in each image show the acceptance region for the contrast at that noise level. This region is obtained by considering the maximum and minimum variation in intensities for the phantoms. In the central part for all three cases, which is the most important information, the contrast makes variations at the same position.
8.3.3 Comparison for two deconvolution methods

We use FOMs discussed in section 5.1 to compare Wiener and Tikhonov deconvolutions. The following table shows the FOMs for each of the three different data sets.

<table>
<thead>
<tr>
<th>FOM</th>
<th>Wiener deconvolution</th>
<th>Tikhonov deconvolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSIM</td>
<td>0.97395 ± 0.0054</td>
<td>0.94061 ± 0.0016</td>
</tr>
<tr>
<td>MAE</td>
<td>0.98032 ± 0.0025</td>
<td>0.96999 ± 0.00038</td>
</tr>
<tr>
<td>MDV</td>
<td>0.99711 ± 0.00054</td>
<td>0.99961 ± 0.00038</td>
</tr>
<tr>
<td>DSD</td>
<td>0.98491 ± 0.0055</td>
<td>0.97906 ± 0.0011</td>
</tr>
<tr>
<td>MSE</td>
<td>0.99775 ± 0.00063</td>
<td>0.99862 ± 0.00038</td>
</tr>
<tr>
<td>Range</td>
<td>0.91973 ± 0.014</td>
<td>0.91686 ± 0.046</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test data with dose = 1000 e⁻/nm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSIM</td>
</tr>
<tr>
<td>MAE</td>
</tr>
<tr>
<td>MDV</td>
</tr>
<tr>
<td>DSD</td>
</tr>
<tr>
<td>MSE</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test data with dose = 5000 e⁻/nm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSIM</td>
</tr>
<tr>
<td>MAE</td>
</tr>
<tr>
<td>MDV</td>
</tr>
<tr>
<td>DSD</td>
</tr>
<tr>
<td>MSE</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test data with dose = 10000 e⁻/nm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSIM</td>
</tr>
<tr>
<td>MAE</td>
</tr>
<tr>
<td>MDV</td>
</tr>
<tr>
<td>DSD</td>
</tr>
<tr>
<td>MSE</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

Table 8.3: FOM values for Wiener and Tikhonov deconvolutions for each of the three data sets acquired using a dose of 10000 e⁻/nm², 5000 e⁻/nm², and 1000 e⁻/nm². Regularization parameters in the deconvolutions, NSR for Wiener deconvolution and λ for Tikhonov deconvolution, are chosen during the training phase as in sections 7.1.1 and 7.1.2.

It can be seen that for both two deconvolution algorithms, the result is best when the dose is high, i.e., noise in data is low. This is to be expected. Next, Tikhonov deconvolution has slightly better performance for most of the FOMs, e.g., the MSE, MAE, MDV, and DSD. From this, we conclude that Tikhonov deconvolution is slightly better than Wiener deconvolution. On the other hand, Wiener deconvolution obtains satisfactory results when image quality is quantified by the SSIM, so Wiener deconvolution is helpful to retain the structure.
Chapter 9

Sensitivity analysis for MTF parameters

In all test we assume perfect information about the MTF, i.e., the same MTF is used for both simulations and deconvolution. In reality this is of course never the case. Here we test the performance of both deconvolution methods against misspecification in MTF parameters.

In Chapter 4, the MTF for a detector in a TEM is parametrized as

\[ \text{MTF}(\xi) := \frac{a}{1 + \alpha |\xi|^2} + \frac{b}{1 + \beta |\xi|^2} + c. \]

The MTF parameters \( a, b, c, \alpha, \) and \( \beta \) are detector specific and values used in all simulations are \( a = 0.7, b = 0.2, c = 0.1, \alpha = 10 \) and \( \beta = 40 \).

We will now do sensitivity analysis where we change one variable at a time. Each parameters is changed linearly \( \pm 10\% \) from its nominal value. The, deconvolution is performed with the misspecified parameter and the result is compared against the original image. As a FOM, we use the MDV to quantify how well the deconvolved image fits the original one.

### 9.1 Sensitivity in Wiener deconvolution

We use simulated noise-free image data which is degraded by the MTF. Image data is then deconvolved by Wiener deconvolution. Table 9.1 and figure 9.1 summarizes how the MDV changes as the MTF parameters are changed.
Table 9.1: Summary of the sensitivity in parameter variation. Sensitivity of MDV is most for parameter $a$ followed by parameters $\alpha$ and $c$. The remaining ones do not effect the deconvolution that much.

<table>
<thead>
<tr>
<th>MTF parameter</th>
<th>Change in parameter</th>
<th>Change in MDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>$+10%$</td>
<td>$-5.6%$</td>
</tr>
<tr>
<td></td>
<td>$-10%$</td>
<td>$+6.3%$</td>
</tr>
<tr>
<td>$b$</td>
<td>$+10%$</td>
<td>$-0.51%$</td>
</tr>
<tr>
<td></td>
<td>$-10%$</td>
<td>$+0.52%$</td>
</tr>
<tr>
<td>$c$</td>
<td>$+10%$</td>
<td>$-3.43%$</td>
</tr>
<tr>
<td></td>
<td>$-10%$</td>
<td>$+3.69%$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$+10%$</td>
<td>$+4.38%$</td>
</tr>
<tr>
<td></td>
<td>$-10%$</td>
<td>$-4.66%$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$+10%$</td>
<td>$+0.44%$</td>
</tr>
<tr>
<td></td>
<td>$-10%$</td>
<td>$-0.52%$</td>
</tr>
</tbody>
</table>

Figure 9.1: Sensitivity of MDV in Wiener deconvolution: Change of MDV for Wiener deconvolution image when MTF parameters are misspecified. The horizontal axis describes the relative parameter change from its nominal true value, so $0.1$ means an increase of $10\%$.

Next we’ll study how these changes affect the intensity in the image. As the MDV changed linearly, we pick three situations for comparison. First, is when parameters are increased $10\%$. Second, is when parameters have the true nominal value. Finally, third is when parameters are decreased $10\%$. Wiener deconvolution is applied to data with these parameter settings. Next, we consider the intensity variation along three lines in the deconvolved images. One line is from the top, the other from the middle, and the final one is from the
Figure 9.2 shows the resulting intensity profiles. Here we see that, even though the effect of $a$, $c$, and $\alpha$ is noticeable, the shape of the intensity profile is retained. Hence, the image is not distorted. Furthermore, parameters $a$, $b$, and $c$ change reversely against the mean intensity. Changes in parameters $b$ and $\beta$ hardly effect the intensity and $\alpha$ and $\beta$ change in the same direction with the mean intensity.

Figure 9.2: Sensitivity of intensity in Wiener deconvolution: The red lines show the intensity when parameters are decreased by 10%, the blue lines show the intensity when parameters have their correct values, and the green lines show the intensity when parameters are increased by 10%.

9.2 Sensitivity in Tikhonov deconvolution

We use simulated noise-free image data which is degraded by the MTF. Image data is then deconvolved by Tikhonov deconvolution. Table 9.2 and figure 9.3 summarizes how the MDV changes as the MTF parameters are changed.
Table 9.2: Summary of the sensitivity in parameter variation. Sensitivity of MDV is most for parameter a followed by parameter b. The remaining ones do not effect the deconvolution that much.

<table>
<thead>
<tr>
<th>MTF parameter</th>
<th>Change in parameter</th>
<th>Change in MDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>+10%</td>
<td>−6.03%</td>
</tr>
<tr>
<td></td>
<td>−10%</td>
<td>+6.79%</td>
</tr>
<tr>
<td>b</td>
<td>+10%</td>
<td>−1.71%</td>
</tr>
<tr>
<td></td>
<td>−10%</td>
<td>+1.77%</td>
</tr>
<tr>
<td>c</td>
<td>+10%</td>
<td>−0.87%</td>
</tr>
<tr>
<td></td>
<td>−10%</td>
<td>0.88%</td>
</tr>
<tr>
<td>α</td>
<td>+10%</td>
<td>+0.15%</td>
</tr>
<tr>
<td></td>
<td>−10%</td>
<td>−0.15%</td>
</tr>
<tr>
<td>β</td>
<td>+10%</td>
<td>+0.080%</td>
</tr>
<tr>
<td></td>
<td>−10%</td>
<td>−0.085%</td>
</tr>
</tbody>
</table>

Figure 9.3: Sensitivity of MDV in Tikhonov deconvolution: Change of MDV for Tikhonov deconvolution image when MTF parameters are misspecified. The horizontal axis describes the relative parameter change from its nominal true value, so 0.1 means an increase of 10%.

Next we’ll study how these changes affect the intensity in the image. As the MDV changed linearly, we pick three situations for comparison. First, is when parameters are increased 10%. Second, is when parameters have the true nominal value. Finally, third is when parameters are decreased 10%. Tikhonov deconvolution is applied to data with these parameter settings. Next, we consider the intensity variation along three lines in the deconvolved images. One
line is from the top, the other from the middle, and the final one is from the bottom. Figure 9.2 shows the resulting intensity profiles. Here we see that, even though the effect of $a$, $c$, and $\alpha$ is noticeable, the shape of the intensity profile is retained. Hence, the image is not distorted. Furthermore, parameters $a$, $b$, and $c$ change reversely against the mean intensity. Changes in parameters $b$ and $\beta$ hardly affects the intensity and $\alpha$ and $\beta$ change in the same direction with the mean intensity.

![Intensity Profiles](image)

Figure 9.4: Sensitivity of intensity in Tikhonov deconvolution: The red lines show the intensity when parameters are decreased by 10%, the blue lines show the intensity when parameters have their correct values, and the green lines show the intensity when parameters are increased by 10%.

9.3 Conclusion

The comparison suggests that Wiener deconvolution is more sensitive than Tikhonov deconvolution to parameter misspecification. It also shows that Tikhonov deconvolution is a better algorithm.
Chapter 10

Conclusion and further work

Wiener and Tikhonov deconvolutions, two kinds of 2D deconvolution algorithm for TEM image processing are described and discussed in this project. The algorithms are assessed by simulated images generated by simulator introduced in [2]. In order to compare noisy images, we also talk about several FOMs such as SSIM, MSE and MDV. These algorithms could successfully deconvolve 2D simulated data in both the presence and absence of noise.

Chapter 8 presents results of the MATLAB experiments for Wiener and Tikhonov deconvolution to restore blurred images with simulated data. They all work well for simulated noise-free images. When applied to simulated noisy images, after experimentally choosing the “optimal setting” for deconvolution, both methods work well even when the dose decreases, i.e., noise increases.

According to the results of the MATLAB experiments presented in Chapter 8, Tikhonov deconvolution gives smaller variances of the errors than Wiener deconvolution. This is because Wiener filters are linear filters operating in the Fourier domain. These estimators assume stationary properties for the unknown MTF. Though Wiener deconvolution has slightly larger errors, it is still comparable to Tikhonov deconvolution, as it can successfully keep the image of the molecule, which is the most important information. The chief advantage of Wiener deconvolution is that it is fast and easy to implement. Tikhonov deconvolution effectively removes estimation noise and preserves the sharp features in the original image.

It is also a good comparison for these two methods when analyzing sensitivity for parameters in the typical MTF function in Chapter 9. Parameters are obviously stable in Tikhonov deconvolution than them in Wiener deconvolution. This result shows that Tikhonov deconvolution is more applicable in image restoration.

Both Wiener and Tikhonov deconvolution can be applied to restore TEM images. Usually Tikhonov deconvolution is more suitable than Wiener deconvolution as the estimator is nearly minimal over the whole domain.

While these two deconvolution methods both need training to select an “optimal” parameter, actually, the deconvolution with parameter estimation will decrease the accuracy since an imaginary noise is added to the model replaced
the real noise. The parameter is not a real optimal one. Taking Wiener de-
convolution as example, when NSR is small, the high frequency information
appears sharp but there is a lot of noise in deconvolution image; when NSR is
large, the noise is diminished but the object edges appear blurred. Another lim-
itation about deconvolution algorithm is PSF estimation. In fact, PSF is often
not symmetric and can’t be estimated exactly, so algorithm maybe cannot work
well for all kinds of TEM images.

Algorithms discussed in the thesis need to be tested for more TEM samples,
to determine the restoration performance. And corrections can be done for
denoising. As all of these regularization methods rely on information about PSF,
which isn’t known all the time, blind deconvolution method is an interesting
research field.
Bibliography


