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Automatic Correction of Non-uniform Illumination in Transmission Electron Microscopy Images

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Abstract:

Transmission electron microscopy (TEM) provides resolutions on the order of a nanometer. Hence, it is a critical imaging modality for biomedical analysis at the cellular level. One of the problems associated with TEM images is variations in brightness due to electron imaging defects or non-uniform support films and specimen staining. These variations render image processing operations such as segmentation more difficult. The correction requires estimation of the global illumination field. In this paper, we propose an automatic method for estimating the illumination field using only image intensity gradients. The closed-form solution is very fast to compute.

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Transmission electron microscopy (TEM) provides resolutions on the order of a nanometer. Hence, it is a critical imaging modality for biomedical analysis at the cellular level. One of the problems associated with TEM images is variations in brightness due to electron imaging defects or non-uniform support films and specimen staining. These variations render image processing operations such as segmentation more difficult. The correction requires estimation of the global illumination field. In this paper, we propose an automatic method for estimating the illumination field using only image intensity gradients. The closed-form solution is very fast to compute.

Chapter 1

Introduction

The field of image processing has made significant progress in the quantitative analysis of biomedical images over the last 20 years. In certain domains, such as brain imaging, scientific papers that test clinical hypotheses using sophisticated image filtering and segmentation algorithms are not uncommon. Compared to the vast amount of research in medical imaging modalities such as MRI and CT, the number of scientific papers on electron microscopy applications in the image processing community has been very limited.

In transmission electron microscopy (TEM), a thin specimen is cut and stained, then it is suspended in an electron beam. The staining agent, which blocks the electron beam, is selectively picked up by different structures such as membranes. As a result, stained structures appear darker which is the source of contrast in TEM images. One of the problems with TEM images is spatially varying contrast due to non-uniform illumination. Non-uniform illumination can have many sources: aging filaments, faulty reference voltages, contaminated apertures, or non-uniform support film fabrication [1]. Subtle electron illumination asymmetries are more evident at moderate-to-low magnifications and are often inadvertently enhanced by digital contrast adjustment. This effect is similar to the the intensity inhomogeneity problem observed in MRI. The MRI intensity inhomogeneity problem is manifested as a slowly varying multiplicative field in the acquired images. Similarly, the non-uniform illumination can be modeled as a multiplicative effect [2]. The observed image is given as

$$f(x, y) = s(x, y)I(x, y) + n(x, y), \tag{1.1}$$

where s is the true signal, I is the non-uniform illumination field and n is additive noise. The I field varies slowly over the image; in other words, it does not have any high frequency content.

Removal of non-uniform illumination effects is important for later processing stages such as image registration based on correlation metrics and segmentation based on intensity thresholding. An automatic correction for non-uniform illumination in TEM images captured by a CCD camera has been proposed [2]. This approach makes assumptions about the properties of the CCD camera and characteristics of the true signal. TEM acquired in this way are not as high resolution as TEM images captured directly

on film and scanned. Hence, the latter is the preferred method of acquisition in most applications. In this paper, we propose an approach that is applicable in general.

A larger amount of research effort has focused on the intensity inhomogeneity problem in MRI . Approaches using tissue class information [3] and combining the inhomogeneity correction with segmentation [4, 5, 6] have been proposed. Other methods perform inhomogeneity correction based on intensity gradients and entropy [7, 8, 9]. MRI intensity inhomogeneity correction approaches that rely on parametric class properties are not useful for TEM images because histograms of cellular TEM images do not have well separated classes. However, methods based on image gradients are suitable for adaptation to TEM images. In this paper, we propose an approach based on the MRI intensity inhomogeneity correction method of Samsonov *et al.* [9].

Chapter 2

Methods

Randall *et al.* [2] propose a radial model for the illumination. This is motivated by the observation that the electron beam has a radially symmetric nature. However, the estimation of a radial model requires knowing the precise position of the electron beam's center, which is not necessarily the center of the image (see Figure 3.1(c)). In [2], this is accomplished by the focus adjustment circle that is available on images captured with a CCD camera. Unfortunately, this focus adjustment circle is not present in TEM images captured on film and scanned, which is the typical acquisition method as discussed in Section 1. A more general model is the free-form, bivariate polynomial of degree N:

$$\hat{I}(x,y) = \sum_{i=0}^{i=N} \sum_{j=0}^{j=i} \alpha_{i-j,j} x^{i-j} y^{j}, \tag{2.1}$$

where α are the weights on the different monomial terms.

After fixing the degree (N) of the polynomial model in equation 2.1, estimation of the non-uniform illumination field is reduced to the estimation of the α parameters. In [2], a direct estimation of parameters is proposed. This approach requires two assumptions: (i) I is constant over local neighborhoods, and (ii) the mean value of s in the same local neighborhoods is constant over the entire image. The first assumption is always valid owing to the physics of TEM imaging; however, the second assumption fails depending on the type of specimen being imaged. For instance, the large band structure at the upper left corner of Figure 3.1(a) is darker on average than the rest of the cells. Next, we describe an indirect method of parameter estimation based on the intensity gradients instead of intensity means. The advantage of this indirect method is that the second assumption about the means of s is replaced by a much weaker assumption on its gradients. The main idea behind this method is to choose the α parameters so that the spatial gradients of the illumination model in equation 2.1 fit the gradients of I in equation 1.1 as closely as possible. This idea was proposed by Samsonov *et al.* [9] for MRI intensity inhomogeneity correction. While our approach is similar, it differs in two important ways that will be discussed at the end of this section.

The gradient of I, which are needed to fit the model parameters, is not directly observable. The gradient of the observed signal is ∇f. It has three contributing com-

ponents:

1. Edges of distinct objects (cells): Large in magnitude; Spatially abrupt (high frequency), but organized geometrically.

2. Gradients due to noise: Varying magnitudes; Spatially abrupt (high frequency) and unorganized.

3. Gradients of I: Small magnitude and slowly varying (low frequency).

The goal is to eliminate the first two kinds of gradients, and fit the model only to gradients of I. We begin by convolving the image with a Gaussian kernel:

$$f_\sigma = (sI)_\sigma + n_\sigma \approx (sI)_\sigma . \tag{2.2}$$

If the standard deviation is chosen large enough, we can assume that the remaining contribution of n in the filtered signal is negligible. Furthermore, the convolution of the product of s and I with the Gaussian kernel K_σ (equation 2.3) can be rewritten in a simpler form. Since I is slowly varying, it is approximately constant in the Gaussian kernel's region of support. Therefore, it can be taken out of the integral yielding equation 2.4:

$$\begin{aligned} (sI)_\sigma (\mathbf{x}) &= \int_{\mathbf{u}} s(\mathbf{x}+\mathbf{u}) I(\mathbf{x}+\mathbf{u}) K_\sigma(\mathbf{u}) du && (2.3) \\ &\approx I(\mathbf{x}) \int_{\mathbf{u}} s(\mathbf{x}+\mathbf{u}) K_\sigma(\mathbf{u}) du && (2.4) \\ &= I(\mathbf{x}) s_\sigma(\mathbf{x}). \end{aligned}$$

In the above equation, we use $\mathbf{x}$ to denote the image coordinates (x, y). To transform the multiplicative nature of the illumination field into an additive one, we now take the logarithm of the filtered signal:

$$\log f_\sigma \quad = \log I + \log s_\sigma. \tag{2.5}$$

Taking the gradient of both sides, we get

$$\nabla \log f_\sigma \quad = \nabla \log I + \nabla \log s_\sigma. \tag{2.6}$$

The gradient of I can not be isolated exactly from the gradient of s_σ; however, we know that latter dominates the in pixels where an edge is present. To decrease the effect of such pixels in the illumination model, we define the weight at pixel i as

$$w_i = \exp\left(-\frac{|| \nabla f_{\sigma,i} ||}{\mu^2}\right). \tag{2.7}$$

This equation assigns monotonously decreasing weights to pixels with larger gradient magnitudes; the parameter μ controls the rate of decline. By an appropriate choice of

μ, edge pixels in s_σ can be assigned much smaller weights than non-edge pixels. Then, the model parameters are estimated by minimizing the following energy function:

$$E(\alpha) = \sum_i w_i \left(\left(\frac{\partial f_{\sigma,i}}{\partial x} - \frac{\partial I_i}{\partial x} \right)^2 + \left(\frac{\partial f_{\sigma,i}}{\partial y} - \frac{\partial I_i}{\partial y} \right)^2 \right), \tag{2.8}$$

where i enumerates all image pixels. The energy is written in terms of the model parameters α by substituting equation 2.1 for I. This is a least-squares fitting problem with a closed-form solution. The implementation of the least squares solution is beyond the scope of this paper. Finally, the corrected image can trivially be computed by dividing the original image by the estimated illumination field.

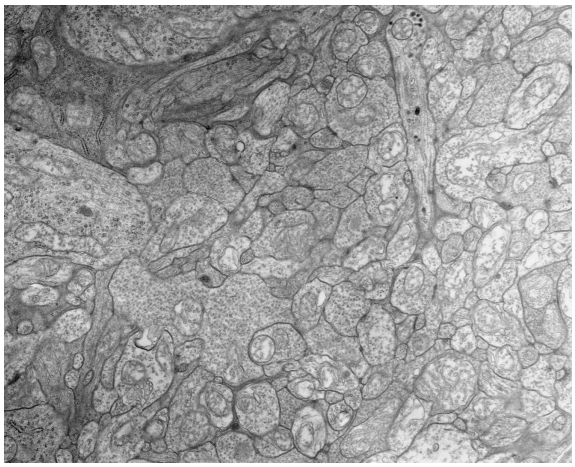
The first important difference of our method from the one proposed in [9] is the method of identifying $\nabla \log I$. In that work, Samsonov *et al.* use anisotropic diffusion [10] to filter the image. Then, the gradient magnitude image is thresholded to remove edge pixels. However, anisotropic diffusion is designed to filter piece-wise constant images. While MRI falls into this category, TEM images of cells (and textured images in general) violate this principle. Therefore, anisotropic diffusion filtering is not a viable option for our application. As discussed above, we use Gaussian filtering combined with an appropriate weighting in the energy equation (2.8) to eliminate gradients due to edges and noise from the polynomial fit.

The second difference is in the use of global histogram information. Samsonov *et al.* minimize a weighted combination of the gradient fitting and the energy of the histogram power spectrum [9]. The weight for the histogram energy term is negative; therefore, it is being maximized while the gradient fitting energy is being minimized. Due to the presence of this non-linear term in the energy, an iterative solution is required to estimate α. By dropping this term, we obtained an energy (equation 2.8) that could be solved very fast in a non-iterative (closed-form) manner.

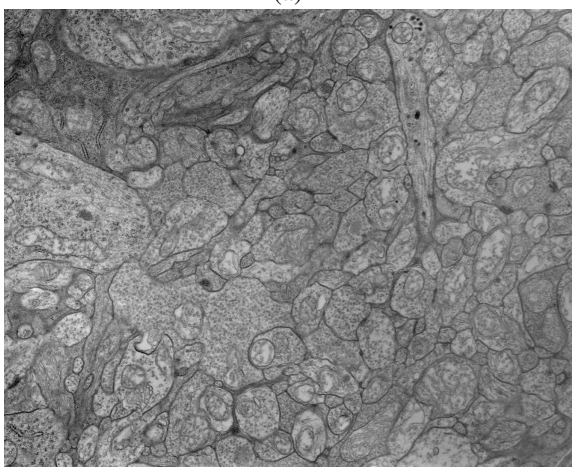
Chapter 3

Results

Figure 3.1(a) shows a TEM image of a portion of the rabbit retina. Notice that structures in the right side of the image appear brighter than those on the left. This is due to non-uniform electron illumination. However, also notice there is a darker band of cells in the upper-left corner of the image. Since, these cells are sharply darker than their immediate surroundings, this is not a case of non-uniform illumination. The illumination corrected image for this 1328×1069 example takes approximately 10 seconds to compute on a high-end PC. The corrected image and the estimated illumination field are shown in Figures 3.1(b) and (c), respectively. Recall that stained cell membranes appear darker in TEM images than their surroundings. Figure 3.2 shows the results of a simple thresholding experiment to identify cell membranes. The results with the illumination corrected image are spatially more consistent.



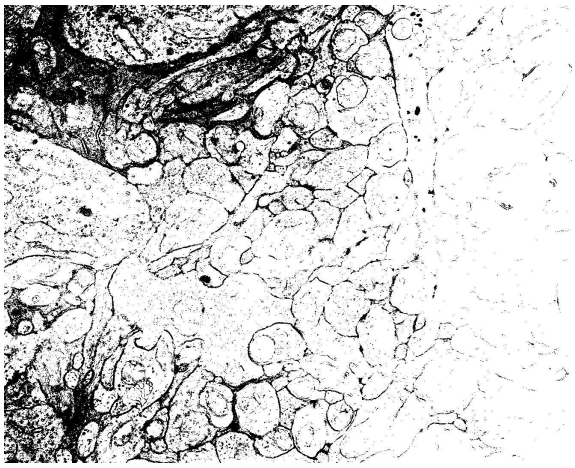
(a)



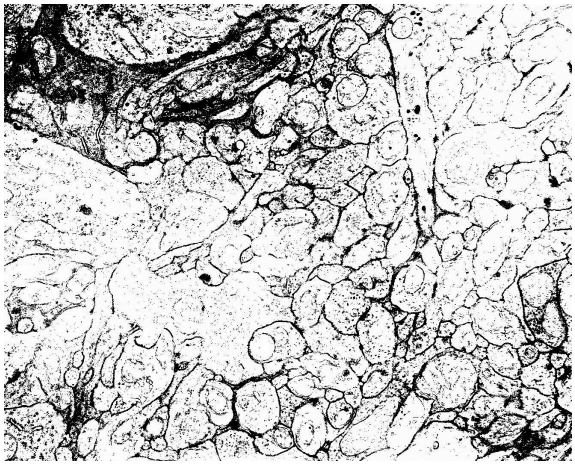
(b)

(c)

Figure 3.1: (a) Original TEM image, (b) same image after illumination correction, (c) estimated illumination field.



(a)



(b)

Figure 3.2: (a) Result of thresholding (a) original image, (b) illumination corrected image.

Chapter 4

Conclusion

In this paper, we proposed an automatic illumination correction for TEM images that does not rely on strong assumptions about the true signal. The method draws on ideas from the MRI intensity inhomogeneity correction method introduced in [9]; however, it uses a different strategy for identifying image gradients due to non-uniform illumination that is more suitable for TEM images. Furthermore, we proposed an energy function with a closed-form solution that can be computed very fast.

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