# Documentation of the TEM illumination correction plug-in for ImageJ

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### Abstract

Non-uniform illumination in TEM microscopy is a critical issue for image quantification. The TEM illumination correction plug-in for ImageJ is designed to remove such artefact and this document aims to be a guide for users.

# Contents

# 1   Introduction

Image acquisition within microscopes is often performed under non-uniform illumination, resulting in varying intensity bias across the image. While it is not disturbing for visual image inspection, it becomes critical for image quantification.

The best way to remove illumination bias is calibration, i.e. taking a picture without any sample (this image is called "flat field") during acquisition in order to capture only the illumination pattern. Then, the "flat field" is used to correct intensity bias by subtracting it from any acquired image. If no calibration is available, a retrospective correction method has to be used instead. There is a large literature of such restoration on MRI images (see Vovk et al. 2007) for a review). Usually intensity gradients or entropy are used within a model estimation framework (Likar et al. 2001; Samsonov et al. 2002). Some illumination correction are directly included in processing, such as for instance segmentation methods (Caldairou et al. 2011; Wells et al. 1996). About TEM images, Randall et al. (1998) have proposed a method for beam illumination removal under stationary assumption[1] of the true signal.

Unfortunately, this stationary assumption is in general not true, especially for images of mouse's embryos (see for instance figure 2(a)). The illumination correction plug-in implements the method described by Tasdizen et al. (2008), which is an estimation of a polynomial model of the illumination bias by a weighted least-squares fitting. Despite it can be applied to any image, the main purpose of this implementation was about TEM images of mouse's embryos. This document is meant to describe the use of the ImageJ plug-in for illumination-bias correction in transmission electron microscopy (TEM) images.

The rest of this document is subdivided into five sections. First, the illumination bias standard model is defined. Second, the bias correction method is explained. Then, the graphical user

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[1]i.e. the image contains large homogeneous area

interface and the implementations details are described. Finally, an example of use is given.

# 2   Illumination bias model

Non-uniform illumination in images can be due to the microscope (e.g. the lamp of a light microscope or the electron beam of an electron microscope create a non-uniform pattern on the field of view) or the sample (e.g. in fluorescence microscopy, the light emitted by fluophores is attenuated by the thickness of sample, resulting in a shading pattern on the axial view).

A standard model to describe illumination bias is a multiplicative model for a given pixel (Randall et al. 1998):

$$u = B \times I + \varepsilon \ , \tag{1}$$

where $u$ is the measured intensity, $B$ is the illumination bias factor, $I$ is the intensity without bias and noise and $\varepsilon \sim \mathcal{N}(\mu, \sigma^2)$ is the noise component. Note that within this model, we assume a Gaussian noise, which is relevant only for moderate to high signal-to-noise ratios[2].

# 3   Bias correction: removing low frequencies

Since the illumination bias is observed as a slow varying intensity shading across the image, it can be considered as a part of the low frequencies in image. Note that it is a strong assumptions for images with slow intensity variations that are not due to the illumination bias.

In TEM images (and especially in mouse's embryos images), hopefully there are not such slow variation patterns that could

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[2]see the NLM denoising plug-in manual for more details

interfere with the illumination bias. The components of interest are almost always in high-frequencies of the images. In such images, the main issue is the presence of abrupt intensity changes, for instance at edges of nuclear like bodies (see figure 2(a)). The correction method should not give the same weight for edge and non-edge pixels during the illumination model estimation.

Assuming a Gaussian noise component in image (see equation (1)), the noise impact can be attenuated by applying a Gaussian convolution with a bandwidth large enough (i.e. bandwidth should approximate the standard deviation of the noise). Then, assuming that the bias is a slow varying shading, it is approximately constant over the convolution kernel's domain and therefore the convolution only applies on the intensities:

$$
\begin{aligned}
u * K_\sigma &= (B \times I) * K_\sigma + \varepsilon * K_\sigma \\
&\approx (B \times I) * K_\sigma \\
&\approx B \times I * K_\sigma \ .
\end{aligned}
\tag{2}
$$

Since a multiplicative model is not easy to estimate, it can be rearranged into an additive model by using a logarithm:

$$
\begin{aligned}
u * K_\sigma &= B \times I * K_\sigma \\
\Leftrightarrow \quad \log\left(u * K_\sigma\right) &= \log\left(B\right) + \log\left(I * K_\sigma\right) \\
\Leftrightarrow \quad \nabla \log\left(u * K_\sigma\right) &= \nabla \log\left(B\right) + \nabla \log\left(I * K_\sigma\right) \ .
\end{aligned}
\tag{3}
$$

Finally, since TEM images do not have in general well separated object classes, gradient is used instead of intensity in equations.

Let $\Omega \subset \mathbb{R}^2$ an image domain and $p \in \Omega$ an image pixel with measured intensity $u$, true intensity $I$ and illumination bias factor $B$. The last can be modelled by polynomial with degree $D$ as:

$$
\hat{B}(p, \beta) = \exp\left(\sum_{i=0}^{D} \sum_{j=0}^{i} \beta_{i-j,j} \, p_x^{i-j} \, p_y^i\right) \ ,
\tag{4}
$$

where $\beta$ is the vector of model parameters. This polynomial model allows to constrain the illumination bias to a smooth and slow varying intensity shading.

The illumination model (4) can be estimated by a weighted least-square fitting from equations (3):

$$J(\beta) = \sum_{i=1}^{N} \omega(p_i) \left\| \nabla \log \left( u * K_\sigma(p_i) \right) - \nabla \log \left( \hat{B}(p_i, \beta) \right) \right\|_2^2 , \quad (5)$$

where $\omega$ is the weighting function defined as

$$\omega(p) = \exp \left( -\frac{\|\nabla u * K_\sigma(p_i)\|_2}{\mu^2} \right) , \quad (6)$$

where $\mu$ is a parameter controlling the decline rate of $\omega$. This weighting function allows to give less importance to strong edges and more importance to homogeneous areas, resulting in a better estimation of the illumination bias.

The decline rate parameter $\mu$ has been empirically set by default to 0.001. This value gives good results on TEM images of mouses' embryos. Do not hesitate to change it if needed.

# 4    Graphical user interface

Once installed, the plug-in can be found in menu Plugins > Restoration > TEM illumination correction. As shown by figure 1, its graphical user interface (GUI) accepts only two parameters:

- the *display illumination bias* option allows to see as an image the estimated illumination bias that has been removed from the input image (default is unchecked);

- the *weighting parameter* controls the decline rate of the weighting function (default is 0.001).

# 5    Implementation details

In implementation, the degree of the polynomial model is set to $D = 2$ (see equation 4). We assume that a quadratic model is enough to capture most of the observed illumination pattern.
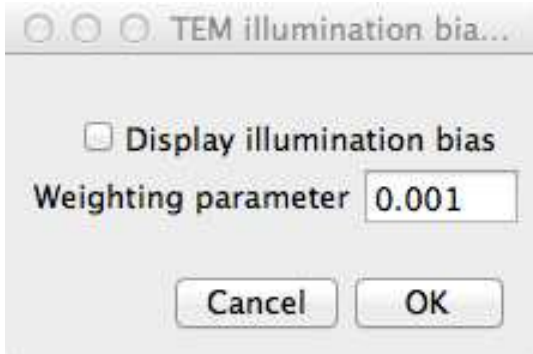
Figure 1: Graphical user interface (GUI) of the plug-in.

Using the weighted least-square fitting method, one can find a closed-form solution to equation (5). This cost function can be rewritten into a matrix form:

$$J(\beta) = \|WM\beta - WU\|_2^2 \quad , \tag{7}$$

where $U$ is the vector of all pixels intensity, $W$ is the diagonal matrix of the weights corresponding to pixels (see the weighting function (6)), $M$ is the matrix containing the model derivatives and $\beta$ is the model parameters to estimate. See Tasdizen et al. (2008) for a complete description of matrices. The closed-form solution to equation (7), in the sense of weighted least-squares, is

$$\hat{\beta} = (M^T W M)^{-1} M^T W U \quad . \tag{8}$$

This fitting is implemented in the plug-in using a modified version of the Jama[3] package.

The choice of an implementation favouring speediness instead of memory consumption have been made for the plug-in. Assuming an input image of $N$ pixels, the memory consumption is asymptotic in $\mathcal{O}(N)$, with at most $29 * N$ data storage. For instance, if you have an image of size $3000 \times 2000$, the plug-in will need

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[3]http://math.nist.gov/javanumerics/jama/

approximately 1.5 Go for processing (without the memory needed for ImageJ).

The plug-in is implemented in Java with the plug-in interface of ImageJ and its source code is available on demand. The plug-in is working fine with the version 1.49r of ImageJ and Fiji.

# 6    Use case

The use of the plug-in to correct for non-uniform illumination is really simple. There is only one parameter controlling the pixel weighting function and that can be chosen empirically. It should be the same for a common batch of images acquired in a similar way.

An example is given in figure 2 on a TEM image of a mouse's embryo with the parameter set to 0.001 (the default value). It is obvious that for a threshold-based quantification (or any quantification), the illumination correction is critical.

# 7    References

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Likar, B., M. A. Viergever, and F. Pernus (2001). "Retrospective correction of MR intensity inhomogeneity by information minimization". In: *Medical Imaging, IEEE Transactions on* 20.12, pp. 1398–1410.

Randall, G., A. Fernandez, O. Trujillo-Cenoz, G. Apelbaum, M. Bertalmio, L. A. V. Rueda, and P. Morelli (1998). "Image enhancement for a low-cost TEM acquisition system". In: *BiOS'98 International Biomedical Optics Symposium*, pp. 140–148.

(a) Original image      (b) Estimated illumination

(c) Image before      (d) Image after

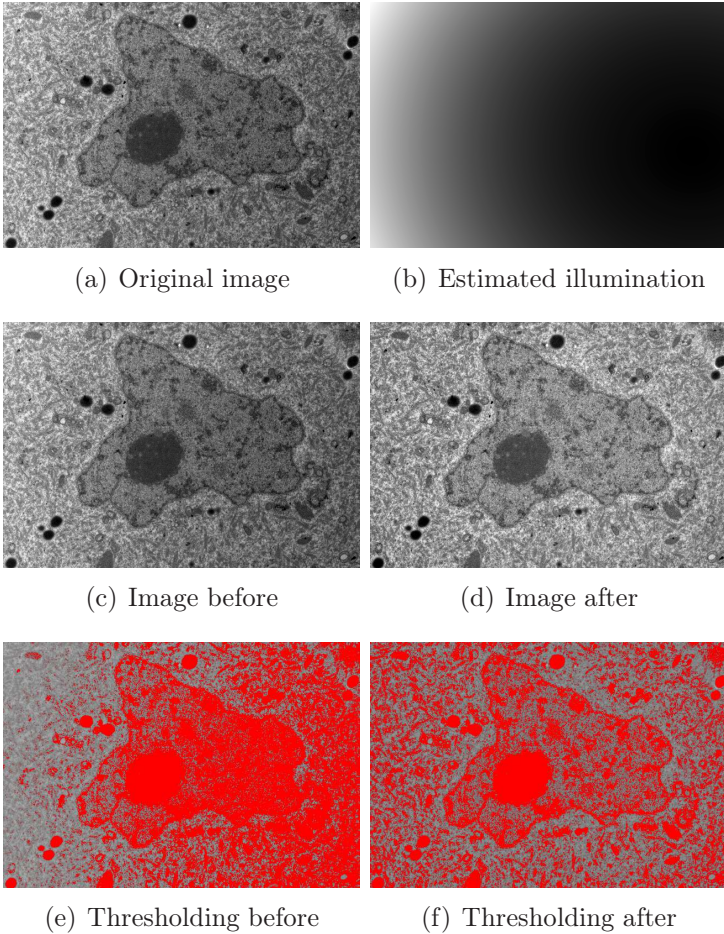(e) Thresholding before      (f) Thresholding after

Figure 2: Example of plug-in use on a TEM image of mouse's embryo (a). While the illumination bias (b) is not disturbing when visual inspection of structures (c-d), its estimation is crucial for any quantification, as thresholding of electron-dense regions for instance (e-f)

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