Image Quality Enhancement in X-Ray Microscopy

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Abstract: The goal of this work is to develop digital image processing tools for improving the quality of the images provided by the Göttingen transmission X-ray microscope at BESSY \cite{1, 2}. We first present a method for correcting the inhomogeneous illumination. Then, we present results of image restoration techniques applied to X-ray microscopy images of test objects and of biological specimens containing specific gold probes. Since this restoration process requires knowledge of the point spread function (PSF), we propose a handy method for the measurement of the line spread function (LSF) of the optical system, which can be used as an estimate of the point spread function. Finally, we show the results of applying noise reduction procedures on images of biological specimens.

1 PSF Estimation

The knowledge of the PSF is needed for the restoration process. We developed a method for PSF-estimation based on the measurement of the ESF (Edge Spread Function). A gold edge obtained by cutting a gold covered polyimide foil is used as a test-object. To avoid problems with noise, we fit an empirically found function which models the ESF with sufficient precision. The model function we propose is the following:

\[
\text{ESF}(x) = a_x + \frac{a_x}{1 + \exp(-a_x(x-a_x))} + (a_x(x-a_x)\exp(-a_x(x-a_x)^2)) = A(x) + B(x) \quad (1)
\]

The first part of this function $A(x)$ is well suited to describe an image of a perfectly focused edge as well as an out of focus image of an edge. The second part $B(x)$ can be added to give a better model of the image of an edge in the case of partially coherent illumination. Fig. 1 shows an example of a fitted profile and the corresponding line spread function.

For an optical system with rotational symmetry, the LSF can be used as an estimate for a cross-section of the PSF, since a section of the theoretical PSF for incoherent illumination has approximately the same shape as the corresponding LSF.

This model has the great advantage to be quite robust to noise. Moreover, once the fitting is achieved, we obtain an analytical formulation of the ESF. It is now easy to calculate the LSF and the MTF (Modulation Transfer Function) in one or two dimensions.
2 Background Correction

At the Göttingen X-ray microscope at BESSY, the source is imaged into the object-field to illuminate the specimen. The spatial inhomogeneity of the synchrotron radiation source leads to an inhomogeneous illumination of the objects. Since it is not always possible to correct this background using a flat-field image, other methods that do not need accurate knowledge of the illumination-function have to be applied. We use an interactive method for correcting the inhomogeneous background by interpolating the illumination field with the following function [4]:

$$Z(x, y) = \exp(\sum_{l=0}^{2} \sum_{m=0}^{2} k_{lm} x^l y^m)$$

(2)

This function is fitted to a set of N values which are derived from the average of square regions that are interactively placed on the background of the image (Fig. 2).

Fig. 1. ESF determination for a microzoneplate with an outermost zonewidth of 39 nm [3]: example of a fitted function using the empirical model (left). The corresponding LSF is obtained by derivating the ESF. The model-LSF fits with a section of the theoretical PSF for incoherent illumination (right).

Fig. 2. Background correction by fitting a model-function to the illumination-field. Initial image with the set of points used for the interpolation step (left) and background-corrected image (right).
3 Image Restoration and Noise Reduction

The image restoration process is used to suppress the blur and decrease the contribution of the noise in the image. In the case of imaging biological specimen, which is one of the major applications of soft X-ray microscopy, we are under certain circumstances constrained to work with low exposure times in order to keep the radiation dose low. Consequently, the noise present in the image can be an important source of image degradation and has to be taken into account during the image restoration process. This is one of the reasons why we have decided to use the Richardson-Lucy algorithm [5] for the restoration step. This class of iterative algorithms requires the knowledge of the optical system's PSF. Fig. 3 presents the result of image restoration using the empirical model, applied to a germanium test object.

The restored image displays a better contrast, and the smallest structures of the test object are more clearly visible than in the original image.

![Fig. 3. Results of restoration using an empirical model of the PSF, applied to an image of a test object. The inner structures of the star pattern have a width of 24.7 nm. Original image (left). Result of restoration (right). A microzoneplate with 29 nm outermost zonewidth was used for imaging.](image)

One biological application of this technique is the use of the TXM in combination with specific staining with gold probes. Fig. 4 shows the result of the restoration process followed by a background correction for an image from in situ hybridisation on Xist RNA using biotinilated DNA probe. Signal revelation was done with gold-labelled streptavidin enhanced with silver. Some methods for quantitative characterisation of images, like statistical evaluation of the position of the gold probes, require a segmentation process. Fig. 4 demonstrates that image segmentation by thresholding leads to better results after restoration and background correction.

Another way to use the Richardson-Lucy algorithm is to suppress noise without loosing resolution as described in [4] and [6]. The major application of this work is the treatment of images with low signal to noise ratio, which is the case when imaging biological specimens that are very sensitive to soft X-rays. Fig. 5 demonstrates the result of noise reduction applied to an image of a chromosome of Chironomus Thummi larvae. The dosage applied to the object in the image (c) is about 100 times...
higher than in the image (a), and we can notice some loss of material and shrinkage
due to radiation effects. This observation is in good agreement with the theoretical
calculations [7]. Fig. 5-(b) shows the result of noise suppression applied to the image
with low signal to noise ratio. The decreasing of the noise is evident and we can see
the enhancement of the image quality approaching that of the image obtained after a
long exposure time (Fig 5(c)).
4 Conclusion

In this work we have presented results of image restoration and noise suppression applied to images of biological specimen. We showed that digital image processing can be applied to enhance quality of images with different signal to noise ratio. We demonstrated the capabilities of image restoration to enhance contrast of structures near the resolution limit of the X-ray microscope. As a prerequisite for image restoration we proposed a fast, easy to implement and noise-robust model for the characterisation of the optical system.

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