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Objective improvement of the visual quality of ion microscope images

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ABSTRACT

The need to operate with low ion beam fluences implies the images obtained using ion microscope (IM) are often grainy and have poor visual quality compared to what can be obtained using e.g. confocal microscopy. This results from the Poissonian distribution of counts in pixels. Here we report work on some different approaches for objectively improving the visual quality of IM images. In this work we present (i) dramatic improvement in the visual image quality of off-axis and direct-scanning transmission ion microscopy (STIM) images by suppression of zero-pixels; (ii) denoising of PIXE images using wavelet filtering and (iii) use of the feature preserving characteristics of wavelet filtering for co-localisation of weak trace elements.

1. Introduction

Speckle noise in ion microscope (IM) images can hide the information in the data because of the response of the human neural-visual system. This is because the perception of large scale information is perturbed by the present of high spatial frequencies. For example, localisation of fields of interest only requires information on a coarse-scale [1].

An IM produces a 2D pixelated map by probing the specimen with an ion beam and quantifying the information by counting detected particles to create a digital image. The image is often dominated by speckle noise [1]. The intensity is generally Poisson distributed [2] with probability, p of n counts in a pixel: $p(n) = \lambda^n \exp(-\lambda)/n!$. Here λ is the mean number of counts per pixel. Then for $\lambda > 0, p(0) \neq 0$, i.e. there is a finite probability that there are no counts recorded in a pixel. The significance of these zero-pixels is that they carry no information and we cannot differentiate between zero intensity in the image function, $f(x,y)$ and zero-counts from Poissonian noise. This noise gives rapid transitions from zero to a finite number of counts over a single pixel width, which implies the high spatial-frequency in these speckled IM images is significant. Generally, the visual quality of IM images is enhanced by subjectively tuning free-parameters (e.g. spatial filter kernel and function, pass-band of frequency domain filters, etc.). *Objective improvement of the visual quality of the image* was taken to mean that a standard procedure with no free-parameters was applied.

Conventional spatial and frequency domain approaches are poorly suited to denoising IM images. This is because they introduce smearing. Here we have investigated objective procedures for visually enhancing the image in IM. Two techniques are reported for objective visual enhancement: (i) Filtering by suppressing zero-pixels and (ii) denoising of PIXE images using wavelet filtering.

2. Zero pixel suppression filtering

Fig. 1 presents a direct-STIM image from a human breast cancer cell on Si_3N_4 using a 2 MeV H_2^+ beam measured at the National University of Singapore [3]. In the raw image (Fig. 1a), 13.3% of the pixels contain zero counts. The zero-pixels were replaced by a "best estimate" of their contents. The best estimate was taken to be a median filter with a 3×3 kernel. This was chosen because the median filters have the best edge preservation characteristics. Comparing the raw image and Fig. 1b shows the dramatic visual enhancement afforded by removal of the zero pixels. Details in the internal structure of the cell become visible that are masked in the raw data by speckle noise. This represents a very benign approach because it is only alters the small fraction of zero pixels. Clearly the method is therefore poorly suited when the fraction of zero pixels becomes large, which was verified by tests (not shown).

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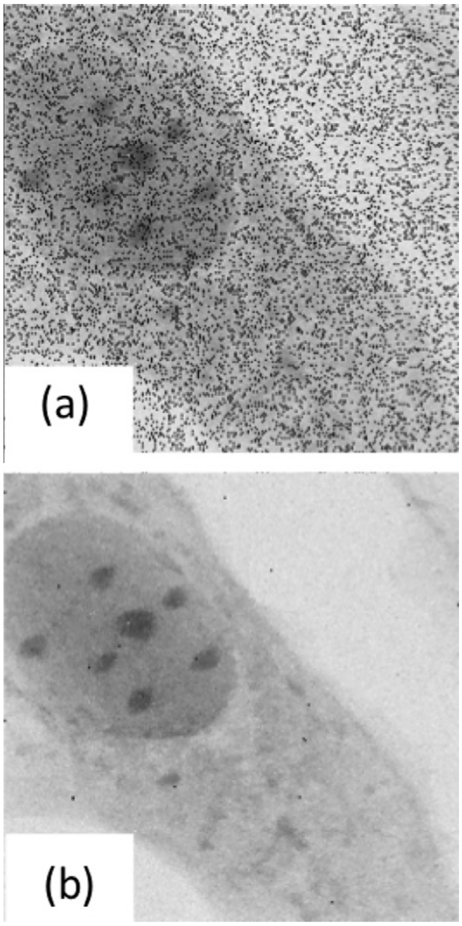

Fig. 1. A direct STIM image of a breast cancer cell on Si_3N_4 supporting film using a 2 MeV protons (a) raw and (b) zero pixel suppression filtering.

3. Wavelet denoising

Wavelet transformation methods are a powerful tool for image processing because they represent images analogously to the human neural-visual system [4]. Wavelet transformations of a image function, $f(x,y)$ yield a position and scale (i.e. frequency) representation. This facilitates their use for de-noising, image compression [4,5] and multi-resolution representation of images [1]. Wavelets, $\psi(x,y)$ are oscillatory functions that are non-zero over a restricted region of space and frequency and have no DC component. The basis function of the continuous wavelet transform is a mother wavelet $\psi(x,y)$ with 2D shift, (ν,τ) and dilation, α according to the wavelet coefficient, $C(\nu,\tau,\alpha)$ [4]:

$$C(\nu,\tau,\alpha) = \frac{1}{\sqrt{\alpha}}\int^{+\infty}\int_{-\infty} f(x,y)\psi\left(\frac{x-\nu}{\alpha},\frac{y-\tau}{\alpha}\right)\mathrm{d}x\mathrm{d}y \qquad (1)$$

The idea behind wavelet denoising is that the Poissonian noise in the IM image gives large difference in intensity between adjacent pixels which contributes mainly to the small-scale coefficients after wavelet transformation [5].

Natural images, on the other hand have their information concentrated in the coarse-scale global coefficient components. Thus "shrinking" the wavelet coefficients by applying soft and hard threshold functions to the coefficients, $C(\nu,\tau,\alpha)$ followed by an inverse wavelet transform [4,5], allows the noise to be removed from the image while preserving the natural image information.

The problem then reduces to objectively selecting threshold form and level. We have tested three schemes that have no-free parameters, but are based on using the variance of the noise in $f(x,y)$ to select the thresholds. Using the wavedec2 function in the Matlab wavelet toolbox, we obtained the wavelet transform of the image using the Daubechies-4 wavelet [5,6]. This was chosen because it is relatively smooth and approximately symmetric. However, tests showed the choice of wavelet had little effect on the outcome. The thresholds were then applied to the coefficients using wdencmp function to denoise the image. To prevent smearing due to filters built into image handling software, the images were handled using an uncompressed bitmap format.[1]

The simplest scheme is *VisuShrink* [4,5] which uses a soft threshold in combination with a single universal threshold calculated from the noise variance. The second was a variant of VisuShrink where the coefficients are *penalised* depending on the sparsity [7]. The third was the *LevelShrink* scheme that gives thresholds that are adapted to the different decomposition levels [4]. Fig. 2 presents raw and denoised data from a human blastomer cell on Si_3N_4 supporting film [8]. (a) presents a direct-STIM image taken with 2 MeV $^4He^+$ ions, (b) shows the off-axis STIM image taken with protons and (c) the denoised image using the optimal denoising threshold selection (LevelShrink with soft thresholds and three levels of decomposition).

It was found that all three thresholding procedures gave significant visual improvement of the image by removing the noise, while retaining the integrity of features such as edges. Visushrink gave somewhat greater smearing, while penalisation of the coefficients gave more ringing. In all cases the ringing was more significant if hard thresholds were used. Increasing the decomposition level gives more smearing of the image, but decreases the noise contribution.

4. Co-localisation using wavelets

The use of wavelet smoothing where only the highest order (coarse scale) coefficients are preserved has been investigated for study of co-localisation of a low-concentration trace element (Fe) with a minor elements (P and Ca) that is otherwise difficult to observe. Fig. 3 presents colour-channel co-localised [9] PIXE images of a rabbit aorta. The co-localisation of the low-concentration trace-element Fe and the minor element, P can be clearly seen in Fig. 3b, whereas it is not visible in the raw colour- channel co-localised data (Fig. 3a). It should be noted that the intensities were modified by the wavelet processing hence visual enhancement introduced some perturbation into the image.

5. Conclusions

The zero-pixel suppression filter approach gives a very small alteration to the image but for not too large image zero-pixel fractions gives a dramatic improvement in visual quality.

Wavelet denoising has been tested for removing the noise from indirect-STIM images. It was found that VisuShrink, penalised threshold and LevelShrink thresholding procedures gave a clear improvement in the visual quality by removing the noise. The LevelShrink gave somewhat less smearing and smaller ringing artifact than the other threshold selection methods tested. Wavelet smoothing based on the highest order coefficients could be used to colocalise weak trace element and minor elements in PIXE maps.

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[1] Images in the PDF open standard format for documents may be internally stored using wavelet compression algorithms, which give rise to a considerable degree of denoising.

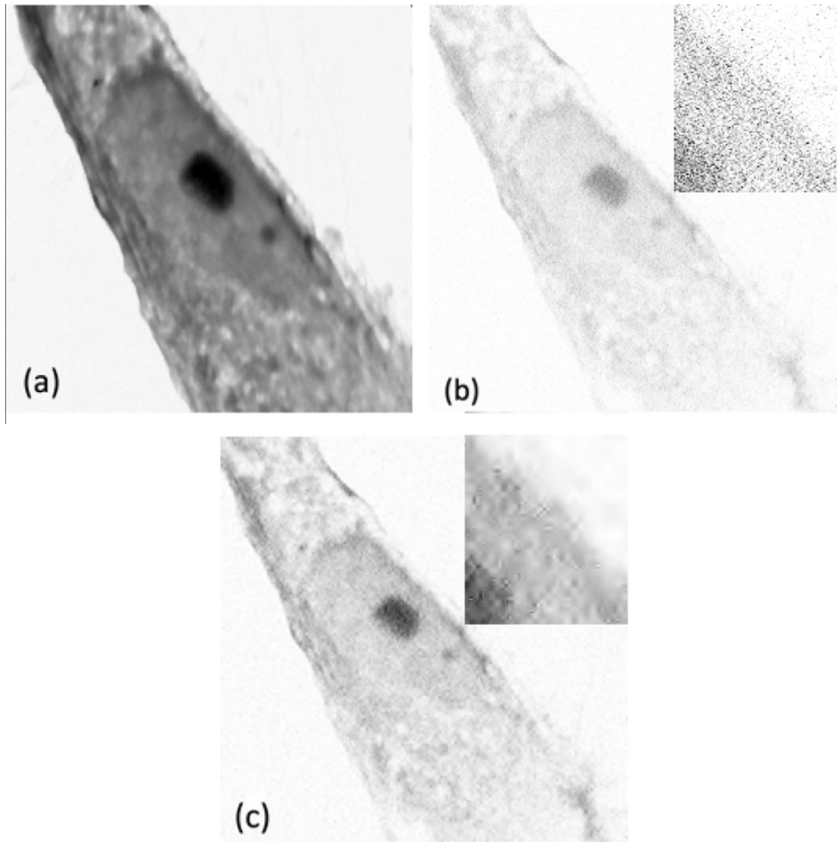

Fig. 2. (a) 2 MeV $^4He^+$Direct-STIM [3] image of a fibroblast cell. 50 μm × 50 μm scan. (b) Contrast expanded and inverted 1.5 MeV proton off-axis STIM image [8]. (c) LevelShrink soft-threshold denoised image of (b). Details of the image are shown in the insets in (b) and (c).

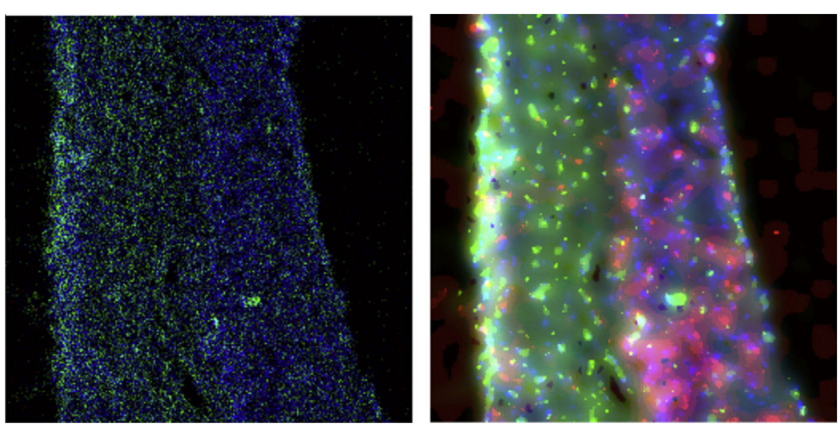

Fig. 3. Colour channel co-localised PIXE maps of a rabbit aorta. Red: Fe, Green: Ca, Blue: P. (a) Without wavelet smoothing. (b) After wavelet smoothing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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