Reconstruction of mitochondria 3D images using image processing techniques

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Mitochondrial dysfunctions are associated with number of cardiac disorders including ventricular hypertrophy. Gradual alternations in the mitochondrial functions already in the early phase of hypertrophy lead to a diminished energy production, a loss of cardiomyocyte contractility and changes of cell's electrical properties. Recent experimental studies indicate that the spatial organization of mitochondria in cells is one of the factors playing an important role in mitochondria bioenergetics [1]. A question arises if there is any association between spatial mitochondrial organization and the stage of hypertrophy.

Electron microscopy provides detailed information about spatial organization of mitochondrial networks, however only in fixed tissue specimens. On the other hand, confocal microscopy with optical sectioning capability allows monitoring the spatial distribution of fluorescence labeled specimens in the living cells, what can be effectively utilized for study of spatial distributions and properties of labeled mitochondria.

An average size of mitochondria is reaching the range where the resolution limit of optical microscopy starts to take effect. Determination of correct size and location of mitochondria in 3D space is therefore often hampered by out-of-focus light and the noise (see Figure 1). The degree of data distortion is dependent mostly on the optical system and can differ between channels used for signal detections. All these factors affect the results of spatial analysis and can lead to misinterpretations of the recorded information. One way of how to minimize the influence of imaging conditions on the reliability of confocal data analysis is to employ digital image restoration techniques [2].

This study introduces data processing protocol that should overcome or at least minimizes the above mentioned problems with denoising, blur removal and especially enhancement of z-direction resolution which is several times worse than lateral resolution. The protocol is based on suitable application of reconstruction technique known as deconvolution on the measured 3D stacks of mitochondrial images by confocal microscope. The stacks are post-processed using various deconvolution algorithms based on well-known imaging model [3]:

$$g = N(h \otimes f + b)$$

where g, h, f, b denote blurred image, PSF function of imaging device, original image and background, respectively. N stands for general noise function and operation $\otimes$ denotes convolution. In this case of confocal microscope, due to low light intensities and photon counts, Poisson noise distribution of registered photons is assumed. The algorithms being used are described in detail in [4] and [5]. For the purpose of sub-resolution image reconstruction, performance evaluation of un-regularized Richardson-Lucy algorithm (RLA) for Poisson noise distribution as well as its modified versions with Tikhonov-Miller regularization and Total Variation Regularization is carried out. Reconstruction regularization can be easily incorporated into multiplicative iterative formula for Richardson-Lucy algorithm in this way:

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$$f^{n+1} = \left[h^T \otimes \left(\frac{g}{h \otimes f^n} \right) \right] \frac{f^n}{1 + \lambda \frac{\partial r}{\partial f^n}}$$

where f^n is n-th estimate of undistorted image, h^T is the transpose of h and r is selected regularization function. Such modification has enabled testing custom regularization functions as well, which might show better results in case of mitochondria images reconstruction. Influence of mathematically modeled and experimentally measured PSF functions, effect of data preprocessing (e.g. denoising) and various parameters of algorithms implementation on restoration performance were statistically evaluated and taken into account to obtain optimal quality of reconstruction process.

Using proposed image processing protocol we can significantly improve visual quality of experimentally recorded mitochondria spatial data, as well as to preprocess data for next analysis or visualizations, etc. volume rendering or creation of image based models of mitochondria.

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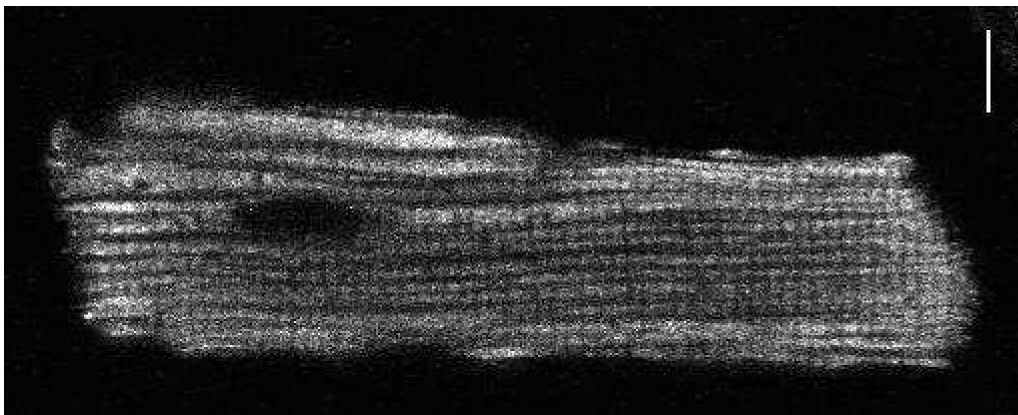

Figure 1. Isolated rat cardiomyocyte stained using JC1 marker. Excited at 488 nm. Imaged using Zeiss LSM 510 Meta with 40x/1.2 objective. Upper right line is 10 µm scale.