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Mytoe: automatic analysis of mitochondrial dynamics

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ABSTRACT

Summary: We present Mytoe, a tool for analyzing mitochondrial morphology and dynamics from fluorescence microscope images. The tool provides automated quantitative analysis of mitochondrial motion by optical flow estimation and of morphology by segmentation of individual branches of the network-like structure of the organelles. Mytoe quantifies several features of individual branches, such as length, tortuosity and speed, and of the macroscopic structure, such as mitochondrial area and degree of clustering. We validate the methods and apply them to the analysis of sequences of images of U2OS human cells with fluorescently labeled mitochondria.

Availability: Source code, Windows software and Manual available at http://www.cs.tut.fi/%7Esanchesr/mito

Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Mitochondria play a key role in several cellular processes, from energy production to apoptosis and ageing. Malfunctioning in mitochondrial processes has been associated to several diseases (Westermann, 2010). Due to mitochondrial fusion and fission, the organelles form a highly dynamic structure that can change from fragmented to filamentous (Koopman *et al.*, 2005). A better understanding of the dynamics of this structure and its relationship with complex cellular processes may provide much insight on mitochondrial functioning and their role on the well-being of cells.

Recent works addressed the problem of segmenting mitochondria from fluorescence microscope images. (Koopman *et al.*, 2005) presented a segmentation method along with descriptors for mitochondrial properties. Another method uses 3D imaging (Song *et al.*, 2008). Mitochondrial motility has been assessed by the degree of colocalization between successive images and from the differences of subsequent image pairs (Koopman *et al.*, 2005; Yi *et al.*, 2004). These methods detect motion but do not yield data on directionality. Other methods to study motility include optical flow (OF) estimation (Gerencser *et al.*, 2008) and distance transform (Beraud *et al.*, 2009). Tracking methods for motility analysis exist, but rely heavily on human interaction (Saunter *et al.*, 2009; Silberberg *et al.*, 2008).

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We present an easy-to-use software, Mytoe, for automated study of mitochondrial structural dynamics from temporal confocal images. It has a simple graphical user interface and requires only a few parameters from the user. Mytoe includes a novel segmentation method which identifies individual branches of the organelles' structure by thresholding and by morphological image processing. In contrast to previous methods, this allows quantifying properties of both single branches and the macroscopic structure formed by the mitochondria. OF is used for motion analysis. The output can be examined in Mytoe with branch-level data being visualized by color-coding individual branches based on the data, and motion vectors being visualized as quiver plots. The results are saved as MATLAB MAT-files and comma-separated values. We first describe the methods in Mytoe and results of the validation procedure. Finally, we use Mytoe to analyze time-lapse sequences of U2OS human osteosarcoma cells.

2 METHODS, APPLICABILITY AND VALIDATION

Mytoe segments the nucleus and cell membrane from fluorescence microscope images. The outlines can be manually drawn if the automatic segmentation is unsatisfactory. The mitochondria are segmented in two steps. The first is similar to the method of (Koopman *et al.*, 2005) but uses morphological top-hat (TH) (Soille, 2003) instead of linear filtering. Each image of mitochondria is first denoised with a median filter, followed by TH, which enhances the separation of mitochondria from the background. The result is median filtered to remove noise enhanced by the TH. Finally, the denoised image is contrast stretched and binarized by Otsu's method (Otsu, 1979) to produce a mask. The next step, developed here for our aims, extracts individual branches of the network by applying to the mask a two-iteration thinning procedure (Guo *et al.*, 1989) and locating the branch points of the resulting skeleton.

Motion is analyzed by OF estimation, using a pyramidal implementation (Bouguet, 2000) of the method proposed in (Lucas *et al.*, 1981). OF yields the displacements of objects between each pair of subsequent frames in a movie, enabling the calculation of velocities. Results using this method are provided in the Supplementary Material. If desired, motion can also be analyzed by measuring the colocalization of mitochondria between successive images as proposed in (Koopman *et al.*, 2005).

Mytoe extracts various statistics. From individual branches it computes properties such as thickness, length and orientation. From the OF, the average speed and the average direction of each branch are calculated. In addition, it extracts cell-level quantities such as number of branches and total mitochondrial area. The list of extracted quantities is shown in Table 1. The methods of computation of each feature and their practical implementation are described in Supplementary Material.

We apply Mytoe to confocal images of U2OS cells. Cells were transfected with a vector expressing mitoDsRED2, an red fluorescent protein targeting the mitochondrial matrix. The nuclei and cell membranes are visualized by

Table 1. Quantitative features from Mytoe

Length	Distance to cell membrane
Thickness	Orientation relative to *x*-axis
Tortuosity	Orientation relative to centroid
Intensity	Number of branches (C)
Speed	Number of connected components (C)
Direction	Total mitochondrial area (C)
Wiggle ratio (Gerencser *et al.*, 2008)	Degree of clustering (C)
Distance to centroid	Colocalization (C)
Distance to nucleus membrane	

Cell-level features marked with (C).

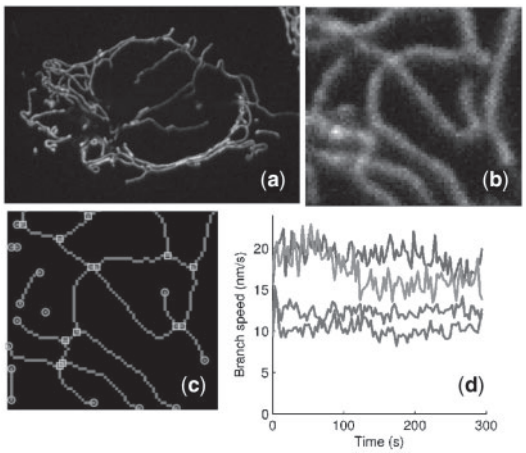

Fig. 1. Original image (**a**) and cropped region (**b**). (**c**) The skeleton (grey), branch points (squares) and end points (circles) found from the mask. (**d**) Time series of mean mitochondrial speed in four cells.

Hoechst 33342 and WGA647 fluorescent dyes, respectively. The images were acquired with a Nikon Eclipse Ti with 100× Apo, a Wallac-Perkin Elmer Ultraview spinning-disk confocal system, Andor EMCCD camera and an autofocus system. Each cell was imaged every 3 s for 10 min in 2D. An example image is shown in Figure 1a. In Figures 1b and 1c, we show a region of the same image, and the structure segmented from that region, respectively.

The quality of microscope imaging is degraded by noise and saturation, thus we added noise and increasingly saturated the images (Supplementary Material). We applied Mytoe and observed how the features changed with increasing levels of degradation. Visual inspection showed that the methods are robust to levels of degradation much higher than in real images. In Supplementary Material we present the results of the analysis of structure and motion and of the features in Table 1 for a set of images. As an example, Figure 1d shows time series of mean branch speeds of mitochondria of four cells, revealing that they differ widely, likely due to differing cell cycle stage.

3 DISCUSSION

Mytoe provides an easy means to study mitochondrial structure and kinetics by automated image analysis of temporal images of fluorescently labeled mitochondria. The structure analysis was found robust to noise and insensitive to saturation. Thus, the method can be used to obtain reliable measurements from saturated images, provided that the mitochondrial branches are separable.

So far, we tested Mytoe on one cell type. Its efficiency may depend on the shapes of cell type and mitochondria. Here we showed that it is accurate enough to, for example, distinguish the kinetics of the mitochondria structure from one cell to another.

In the future, this framework can be extended by including additional descriptors and adding other shape analysis techniques that can be chosen as a function of the cell type. Additionally, we aim to further develop Mytoe so as to automatically detect abnormalities in the mitochondria structure, due to chemicals (such as in drug screening) or disease.

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Conflict of Interest: none declared.

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