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ID-9-P-2524 Analysis of mitochondrial morphology and networks by Fiji

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MITOLAB does visual analyses of microscopic images of mitochondria of child patients with mitochondrial disorders. However, due to demands on statistical evaluation, semiautomatic analysis of the data became desirable. We propose a flow that provides main important parameters of mitochondria by using freeware software package **Fiji** (**fiji.sc**).

These parameters are of interest: 1. Number and density of mitochondria per one cell – disordered mitochondria tend to be degraded and sparsely distributed; 2. Number of mega-mitochondria, which are mitochondria collapsed into spheres instead of normal tubular shapes, which may appear in tissues of patients with disorders; 3. Directional distribution of mitochondria in cells – healthy cells have mitochondria forming a compact network of tubules in a few dominant directions distributing ATP throughout the whole cell. Disordered mitochondria networks tend to be fragmented, without dominant directions; 4. Branching of mitochondrial segments – mitochondria of healthy cells tend to form branching networks. Disordered cells contain high number of separated mitochondria, thus the branching is less observed.

The acquired images are generally of low quality, see **Fig. 1**, thus the preprocessing of data is essential. It includes: I. Conversion to greyscale images (Fiji menu item **Image→Color→Split Channels**); II. Removing inhomogeneous background (**Process→Subtract Background**); III. Removing noise (**Process→Noise→Despeckle**); IV. Improving contrast (**Image→Adjust→Brightness/Contrast**); VI. Selection of an individual cell by creation its convex hull (**Polygon selection**); V. Thresholding MITO (**Image→Adjust→Threshold**), **Fig. 2**.

Density and number of (mega-)mitochondria are obtained by **Analyze→Analyze Particles** and are expressed as Ratio AM/AC, Number of mitochondrial components and Number of mega-mitochondria, respectively, in **Fig. 3**. Directions of mitochondria were analyzed by generation of “directionality histogram” using **Analyze→Directionality** and by fitting a Gaussian function to the highest peak. Goodness of fitting directionality histogram in **Fig. 3** describes partially if predominant directions are present in this histogram (it goes to 1) or not (it goes to 0). Branching was found by creation of a skeleton using **Process→Binary→Skeletonize** and by skeleton analysis using **Plugins→Skeleton→Analyze Skeleton (2D/3D)**. Branchings is expressed by Ratio BP/EP in **Fig. 3**.

Fig. 3 shows an analysis of images of two healthy patients and four sick patients. Although analysis of medical image data is mostly difficult and ambiguous and we present results of a restricted number of patients, we conclude that Fiji can be used for successful analysis and statistical evaluation of such data.

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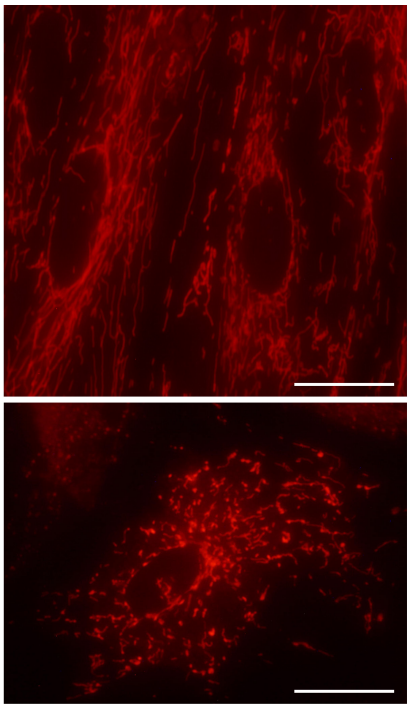

Fig. 1: Microscopic fluorescence images of mitochondria in fibroblasts of both healthy (upper; Healthy I, see Fig. 3) and sick patients (bottom; Sick III, see Fig. 3). Magnification 600×, stained by MitoTracker Red (Invitrogen), white bar is 40 µm.

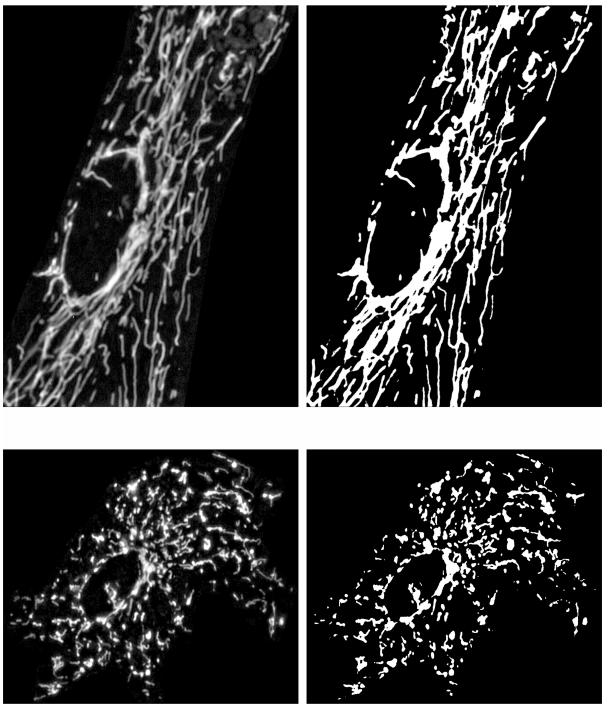

Fig. 2: Preprocessed greyscale 8 bit (left) and thresholded binary images (right) of mitochondria of cells from Fig. 1.

	Healthy I	Healthy II	Sick I	Sick II	Sick III	Sick IV
Area of the whole cell [nm^2] (AC)	5.6327+09	3.7856+09	9.2615+09	8.1979+09	8.8207+09	3.0236+09
Area of mitochondria [nm^2] (AM)	1.6734+09	1.3254+09	2.9397+09	1.6009+09	1.2464+09	0.5678+09
Ratio AM/AC	**0.2970**	**0.3501**	**0.3174**	**0.2248**	**0.1413**	**0.1878**
Number of mitochondria parts (size>25 pxl)	**72**	**75**	**326**	**174**	**229**	**69**
Number of mega-mitochondria (Circularity>0.8)	**2**	**6**	**51**	**24**	**37**	**9**
Goodness of fitting directionality histogram by a Gaussian function	**0.99**	**0.96**	**0.99**	**0.71**	**0.30**	**0.74**
Number of branching skeleton points (BP)	291	358	576	249	135	83
Number of ending skeleton points (EP)	338	444	709	506	655	264
Ratio BP/EP	**0.861**	**0.806**	**0.812**	**0.492**	**0.206**	**0.201**

Fig. 3: Results of image analysis of two healthy patients and four sick patients. Mitochondria of healthy cells tend to have higher Ratio AM/AC, smaller number of fragmented parts and mega-mitochondria, higher Goodness and Ratio BP/EP than mitochondria of disordered cells.