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Quantitative Analysis of Mitochondrial Tubulation Using 3D Imaging

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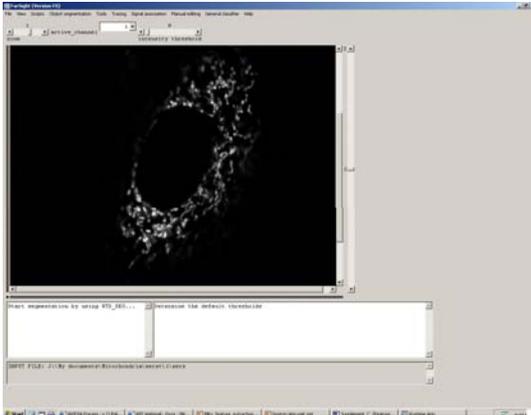


Abstract

Mitochondria are dynamic organelles capable of undergoing fission and fusion. Overexpression of a dominant negative mutant of a mitochondrial fission protein (DLP1-K38A) inhibits total mitochondrial fission resulting in tubular mitochondria. In this work, we present a quantitative analysis of mitochondrial tubulation using FARSIGHT, a multidimensional image analysis tool. It has been found that in a control cell, total mitochondrial volume is distributed into many isolated mitochondrial elements. In contrast, it is seen that tubular mitochondria caused by overexpression of DLP1-K38A contains 80% of the mitochondrial volume within a single mitochondrial element.

FARSIGHT Image Analysis

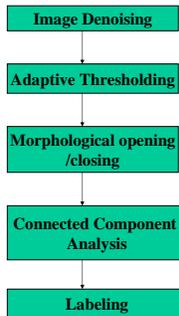
FARSIGHT Image Analysis Tool



Screenshot of FARSIGHT image analysis tool

Mitochondria Image Segmentation

Steps involved in image segmentation



- Input image is first **denoised** by **median filtering** with filter width 3.
- Adaptive thresholding** is applied to extract foreground
 - Binary image is generated with background zero.
 - Entire image is divided into 60×60 blocks
 - Intensity statistics such as mean μ and standard deviation σ is calculated for each block
 - Intensity threshold is assigned to the center of the block by $t = \mu + k \times \sigma$, where k is an adjustable coefficient.
 - Intensity threshold for each pixel of the entire image is obtained by interpolating the block thresholds by fitting a cubic spline.
- Morphological opening** is applied to above binary image.
 - Removes tiny island-like objects or artifacts.

- Morphological Opening**
 - Structuring element used - ellipsoid of width 3 in short axis.
 - Radius ratio between the short and long axis is determined by sample scaling of the image along x, y and z axis.
- Morphological Closing**
 - Removes small gap/hole inside objects due to non-uniform staining.
 - Structuring element used- ellipsoid of width 7 in short axis.
- Connected Component Analysis**
 - Identifies the connected pixel regions and labels them.

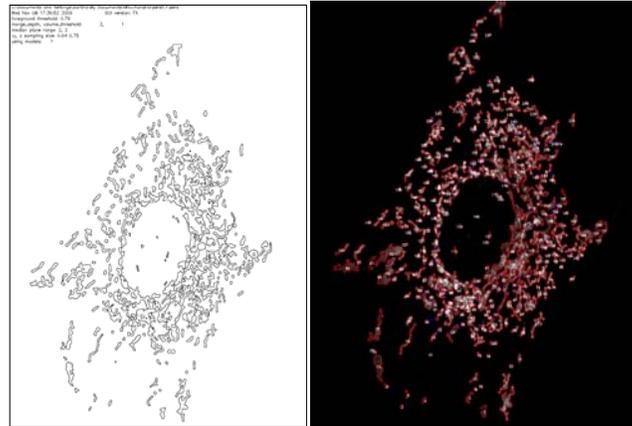


Image segmentation Output

Labelled Image with Links

Mitochondria Image Quantitation:

- After segmentation, **surface points** are extracted for each identified object in the image.
- Minimum distance between the surface points of the objects is calculated.
- To speed up this computation, we do not compute the distance between every pair of objects in our actual implementation, but only compute the distance between each object and some of its close neighbors, which can be searched quickly.
- A selective set of **quantitative features** like volume, surface area, shape, convexity, intensity, texture, can be computed for each object, which can be used for subsequent statistical analysis to get biological insights.
- Two fundamental types of measurements are computed – intrinsic measurements and associative measurements.
- The former are computed from individual image channels. The latter involve one or more image channels.

Volume $|\Omega|$: number of voxels in the object

Surface area: number of voxels on object surface

$|\Omega_s|$, where $\Omega_s = \{p | I_p = o; \exists N_p, I_{N_p} \neq o\}$ the set of surface voxels of the object

Shape: Compactness or thinness of the object

$$\frac{|\Omega_s|^3}{36\pi|\Omega|^2}$$

Convexity: ratio of the object volume and the volume of its convex hull

$$\frac{|\Omega|}{|convex_hull(\Omega)|}$$

Intensity: average intensity of the voxels in the

$$mean(I(\Omega))$$

Texture: average gradient of the normalized intensity

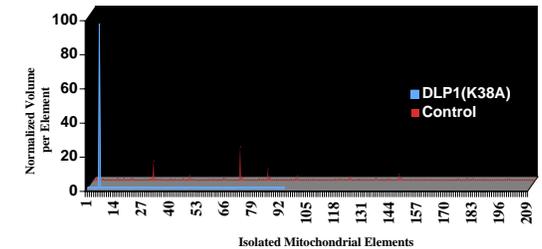
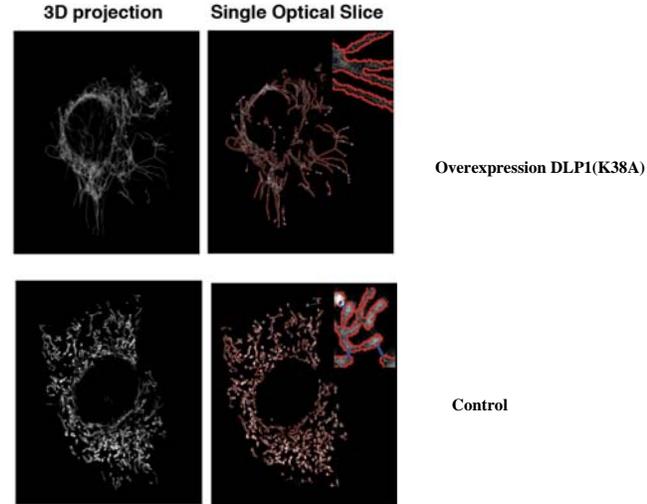
$$mean\left(G\left(\frac{c}{I} \times I(\Omega)\right)\right) \text{ Where } c \text{ is a constant}$$

$p = (x, y, z)$ - the coordinate of a voxel (three dimensional point in a volume image)
 N_p - a neighbor voxel of p

I_p - the segmentation label at p (usually $I_p = 1$ for foreground voxels and 0 for background voxels)

Experimental Results

Results: In a control cells total mitochondrial volume is distributed into many isolated mitochondrial elements. In contrast, tubular mitochondria caused by overexpression of DLP1-K38A appears to have more than 80% of the mitochondrial volume within a single mitochondrial element.



References

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Overview of the Strategic Research Plan

