Materials & methods: Fibroblasts were settled on Y-shaped concave micropatterned coverslips (Cytoo SA) to adopt standardized triangular shapes. The cells were examined under an inverted, wide-field Leica microscope equipped with a high-sensitivity CCD Roper CoolSnap HQ2 camera using Metamorph 7.6® software. Huygens and Imaris 7.3® software were used for 3D processing.

Control fibroblast Our approach was validated by using fibroblasts with mutations in the OPA1 and MFN2 genes, known to be involved in mitochondrial fusion, as well as with drugs inducing mitochondrial oxidative phosphorylation inhibition which elicited structural mitochondrial defects such as changes in total mitochondrial volume, connectivity of the network, number of mitochondrial branch points, and mitochondrial organization in general (E). Our results showed that mitochondria emerging from the microtubule organizing centre may be crucial to mitochondrial biogenesis since it appeared to be protected against mitochondrial fragmentation induced by OPA1 mutations (E, F).

Mitochondria are dynamic organelles that adapt to cellular requirements by changing their shape through processes of fission and fusion to form tubular networks (A). Defective mitochondrial metabolism is associated with a wide range of human diseases covering most medical specialties. The increasing importance of defective mitochondrial dynamics in several diseases including common neurodegenerative disorders calls for the development of reference imaging tools for medical investigation and drug screening. Current approaches to study mitochondrial morphology are limited by low data sampling coupled with manual identification and classification of complex morphological phenotypes (B).

We have therefore normalized the quantification of mitochondrial dynamics by means of micropatterned (from CYTOO SA) designed to standardize the size and shape of cultured fibroblasts (C,D). In addition, we present a novel method of imaging mitochondrial networks in 3D, in models of live or fixed cells.

Fig. C: Cytoo micropattern array : MitoTracker Green signal : Mitochondrial network, branch point (red). The color codes show the tubules length between branch points. Control fibroblast

Fig. E: Mitochondrial network fragmentation induced by mitochondrial complex III inhibitor Antimycin, 10µM, 4h or by an OPA1 S545R mutation (Dominant Optic Atrophy patients’ fibroblasts). Increased mitochondrial network connectivity by MFN2 R94Q. Corresponding quantifications are shown.

Fig. D: Reproducibility of mitochondrial network distribution in control fibroblasts : mitochondrial volume/ cellular volume (convex Hull algorithm).


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Mitochondrial fusion and fission play critical roles in maintaining functional mitochondrial network when cells experience metabolic or environmental stresses. Fusion helps maintain stress by mixing the contents of partially damaged mitochondria as a form of complementation (A). Fission is needed to create new mitochondria, but it also contributes to quality control by enabling the removal of damaged mitochondria and can facilitate apoptosis during high levels of cellular stress.

The challenge is to design therapeutic drugs that promote mitochondrial fusion but also allow fission events.