Live-cell microscopy of mitochondrial (dys)function

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Mitochondria play a central role in cellular (patho)physiology and display a highly variable morphology that is probably coupled to their functional state. My research aims to quantitatively understand the molecular connection between mitochondrial metabolism and (ultra)structure with particular attention to redox signaling and biomolecule diffusion. To this end we study various cell models and a mouse model of mitochondrial complex I (CI) deficiency. As a key technology, protein-based and chemical fluorescent reporter molecules are introduced in the cells and their signals are quantified using life cell microscopy, image processing/quantification and data mining. Protein diffusion is studied by combining photobleaching strategies, single-molecule spectroscopy and *in silico* techniques. In primary fibroblasts from Leigh Syndrome (LS) patients, isolated CI deficiency is associated with mitochondrial morphological and functional changes and increased reactive oxygen species (ROS) levels. We developed a strategy allowing unbiased and automated quantification of mitochondrial "morphofunction" (*i.e.* morphology and membrane potential), cellular parameters (size, confluence) and nuclear parameters (number, morphology) in intact living Primary Human Skin Fibroblasts (PHSFs). Cells are cultured in 96-well plates and stained with tetramethyl rhodamine methyl ester (TMRM), Calcein-AM and Hoechst 33258 (**Fig. 1**). Next, multispectral fluorescence images are acquired using automated microscopy and processed to extract 44 descriptors. Subsequently, the descriptor data is subjected to a quality control (QC) algorithm based upon Principal Component Analysis (PCA) and interpreted using univariate, bivariate and multivariate analysis. Although specifically developed for PHSFs that are widely used in preclinical research, the protocol is portable to other cell types and can be up-scaled for implementation in High-Content Screening (HCS). Using a machine learning strategy, we discriminated between fibroblasts of a healthy individual and an LS patient based upon their mitochondrial morpho-functional phenotype. This allowed evaluating the therapeutic potential of newly developed small molecules.

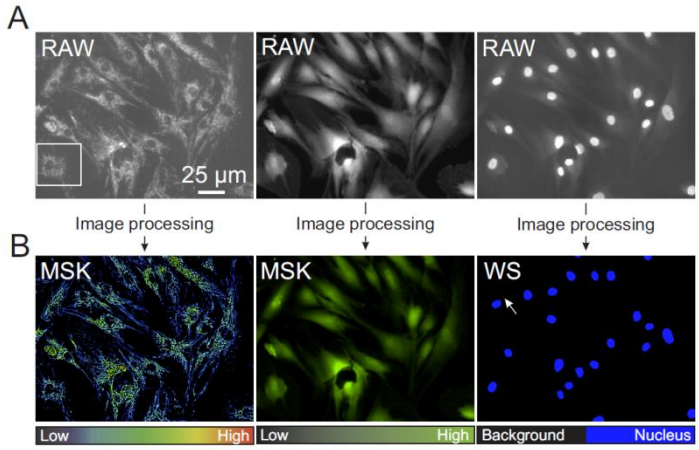

Fig.1: Overall image quantification strategy. *(**A**) Typical example of a TMRM, Calcein and Hoechst image (from left to right) obtained from the same well (RAW images). (**B**) Images obtained after image processing (MSK=masked image, WS=after watershed operation).*

References

1. Basit, F., van Oppen, L.M.P.E., Schöckel, L., Bossenbroek, H.M., van Emst-de Vries, S.E., Hermeling, J.C.W., Grefte, S., Kopitz, C., Heroult, M., Willems, P.H.G.M., Koopman, W.J.H. (**2017**) Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells. *Cell Death Dis.* 8:e2716.
2. Iannetti EF, Smeitink JAM, Beyrath J, Willems PHGM, Koopman WJH. (**2016**) Multiplexed high-content analysis of mitochondrial morphofunction using live-cell microscopy. *Nature Protoc.* 11:1693-1710.
3. Koopman WJH, Beyrath J, Fung W, Koene S, Rodenburg RR, Willems PHGM, Smeitink JAM. (**2016**) Mitochondrial disorders in children: toward development of small-molecule treatment strategies. *EMBO Mol. Med.* 8:311-27.
4. Liemburg-Apers, D.C., Wagenaars, J.A.L., Willems, P.H.G.M., Koopman, W.J.H. (**2016**) Acute inhibition of mitochondrial complex I and III triggers LKB1-, AMPK-, and SIRT2-dependent stimulation of GLUT1 activity. *J. Cell Sci.* 129:4411-4423.
5. Blanchet L, Smeitink JAM, Breuer ME, van Emst - de Vries SE, Vogels C, Pellegrini M, Jonckheere AI, Rodenburg RJT, Buydens LMC, Beyrath J, Willems PHGM, Koopman WJH. (**2015**) Analysis of small molecule phenotypic effects using combined mitochondrial morpho-functional fingerprinting and machine learning. *Sci. Rep.* 5:8035.
6. Willems PHGM, Rossignol R, Dieteren CEJ, Murphy MP, Koopman WJH (**2015**) Redox homeostasis and mitochondrial dynamics. *Cell Metab.* 22:207-18.
7. Koopman, W.J.H., Distelmaier, F., Smeitink, J.A.M., Willems, P.H.G.M. (**2013**) OXPHOS mutations and neurodegeneration. *EMBO J.* 32:9-29.
8. Dieteren, C.E.J., Gielen, S.C.A.M., Nijtmans, L.G.J., Smeitink, J.A.M., Swarts H.G., Brock, R., Willems, P.H.G.M., Koopman, W.J.H. (**2011**) Solute diffusion is hindered in the mitochondrial matrix. *Proc. Natl. Acad. Sci. USA* 108:8657-8662.