Localizing ER/Mitochondria contact sites using a Structured Illuminated Microscope

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The mitochondria and the endoplasmic reticulum (ER) have, amongst others, a common function in calcium (Ca^{2+}) homeostasis. Direct Ca^{2+} transfer from the ER to mitochondria occurs at their physical interaction site: the Mitochondria Associated ER-membrane (MAM) [1]. This specialization of the ER is emerging as a signaling hotspot, bypassing known cytoplasmic signaling cascades. Even though both the MAM and its composition are being reported as dynamic, most of our knowledge about MAMs is either from biochemistry or else from still images taken with electron microscopy [2]. Due to the MAM's sub-diffraction size, constant remodeling and poor definition, MAM imaging in live cells is a challenge. For this reason we have developed a fast, dual color, full field total internal reflection-structured illumination microscope which allows 50 x 50 µm field of view at 3.3 Hz and 100 nm of isotropic spatial resolution, **fig. 1a** [3]. This tool has permitted us to take dual-color images of the ER (labeled with EGFP) and the mitochondria (labeled with MitoTracker Deep Red), **fig. 1b**, and, via an analysis of colocalization of both images, to determine the position of the contact sites.

Furthermore, we developed a detailed study of how common descriptors of colocalization between the ER and mitochondria vary as a function of the images spatial resolution **(fig. 1c)**, the temporal resolution and the size and position of the region of interest (ROI) chosen. Our study calls for prudence when interpreting co-localization data and suggests that cell and organelle motility, the choice of the ROI analyzed, the effective spatiotemporal resolution all impact on the result and hence should systematically be stated, particularly when co-localization arguments are used to assess the effect of drug application on cellular signaling pathways [4].

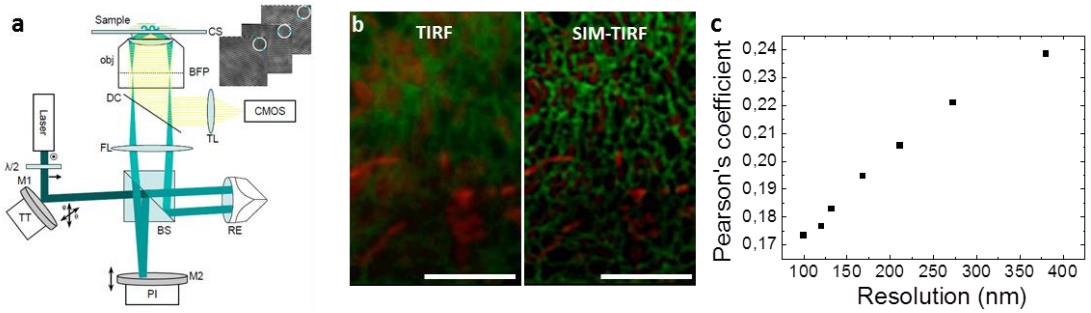

Fig. 1: (a) High resolution TIRF-SIM microscope schema. (b) Dual color images of ER (in green) and mitochondria (in red) taken in TIRF (left) and in TIRF-SIM (right). Scale bar: 1 µm. (c) Pearson's colocalization coefficient as a function of the images spatial resolution.

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