EXPANSION AND STED NANOSCOPY A NEW TOOL FOR PUSHING THE RESULUTION AT THE LIMIT, THE FLUORESCENT LABEL.

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Expansion microscopy (ExM) is a novel method that allows super-resolution imaging with conventional microscopes (1, 2). It consists in soaking the cells with a polymer, inducing the polymerization to form a dense meshwork throughout the cell, cross-linking the fluorophores to the polymer and, after digestion of cellular protein, rehydrating of the sample. The swelling of the polymer gel led to a fourfold isotropic stretching of the sample. Therefore, it increases the distance between two objects that otherwise couldn't be seen as two different things with an ordinary microscope. One of the drawback of such a technique is the long preparation made of several stages, i.e. immunostaining, gelation, digestion and expansion. They are really crucial steps for a good imaging post-expansion.

In our work we present a comparison between ExM and stimulated emission depletion (STED) nanoscopy (3). Our aim is to study the e possible combination of STED and ExM as a method to further enhance the final resolution achievable. We will in particularly take advantage of the use of separation of photons by lifetime tuning (SPLIT) STED (4).

We show application of these methods from single fixed cells (Figure 1) to slices of fixed mouse retina tissue.

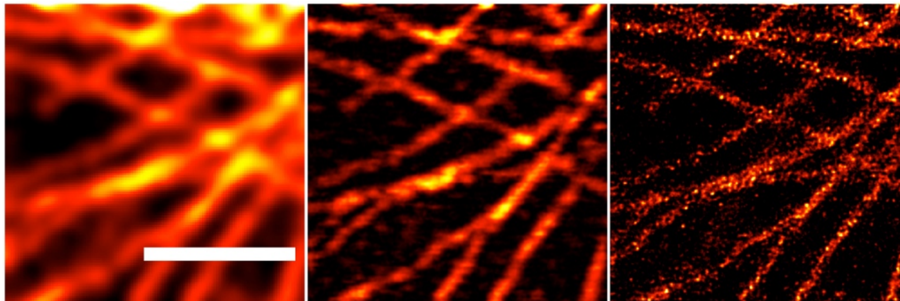

Figure 1: from left to right: simulated confocal image pre-expansion, confocal image post-expansion, STED image post- expansion. Scale bar 10 μm

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