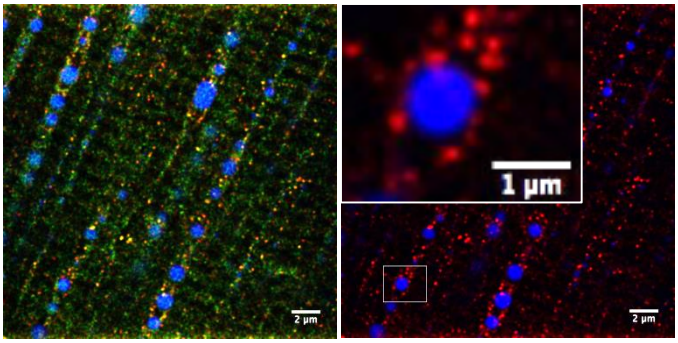
STED-imaging of lipid droplets, mitochondria and PLIN5 in human skeletal muscle biopsies with a novel lipid dye

Anne Gemmink[1], Sabine Daemen[1], Helma JH Kuijpers[2], Gert Schaart[1], Marc AMJ van Zandvoort[2], Matthijs KC Hesselink[1]
Dept. of [1]Human Biology and Movement Sciences, and [2] Genetics and Cell Biology
Maastricht University Universiteitssingel 50, 6229 ER Maastricht, The Netherlands
E-mail: anne.gemmink@maastrichtuniversity.nl

High intramyocellular lipid (IMCL) content associates with insulin resistance. However, endurance trained athletes also have high levels of IMCL whilst being very insulin sensitive; the so-called athlete's paradox. Thus, IMCL content is not causally related to insulin resistance. Other factors, like lipid droplet (LD) coating proteins and LD-mitochondria interaction, may help to understand this paradox. To study these factors at the nanoscale level, and the involvement of PLIN5 (LD coat protein) herein, super-resolution microscopy (like STED) is warranted. Here we aim to simultaneously image LDs and PLIN5 along with mitochondria using STED and confocal microscopy in human skeletal muscle sections. To avoid undue overlap in emission and excitation of currently available STED probes, we explored monodansylpentane (MDH) as a novel LD dye to combine with staining of other proteins.

Currently available LD dyes emit green or red light and are excited by 488 nm or 592 nm, hence interfering with STED probes in one- depletion laser (592 nm) based STED systems. Due to their relatively large size, LDs do not require imaging by STED, but can theoretically be imaged in blue by confocal microscopy, permitting simultaneous imaging of PLIN5 and mitochondria with STED. MDH is a commercially available probe to image LDs in blue which thus far has only been used in cultured cells rather than tissue sections.

Five µm thick muscle sections were stained for TOMM20 (mitochondrial protein), PLIN5, and MDH (LDs) with Abberior Star 440 (AS440) and AlexaFluor 488 (AF488) as secondary antibodies, respectively. A 100x 1.4 N.A. objective and a 5x optical zoom was used, resulting in a pixel size of 23 nm. MDH was excited at 405 nm, while TOMM20-AS440 and PLIN5-AF488 were excited at 470 nm and 514 nm, respectively. Emission was collected at 475-500 nm and 535-550 nm, respectively. The STED depletion laser was at 30% at 592 nm.



LDs were stained in blue (see figure, left and right panel) with TOMM20 (green, left panel) and PLIN5 (red, left and right panel) imaged by STED. The zoomed image of LDs (insert in right panel) revealed that PLIN5 presents as dots decorating LDs rather than in a rim-like structure coating LDs as reported by confocal microscop.

In conclusion, we successfully combined imaging of LDs with mitochondria and LD coat proteins in sections using MDH to stain LDs combined with STED imaging of green-light emitting dyes like AF488 and AS440. This novel staining and imaging procedure revealed that PLIN5 decorates the LD surface in a dot-like fashion rather than a rim-like fashion, as was conventionally believed to be the case based upon confocal imaging.