RESOLUTION ENHANCEMENT FOR LOW-TEMPERATURE SCANNING MICROSCOPY BY CRYOSTAT IMMERSION IMAGING

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The diffraction limit, hampering the microscopy of nanoscopic particles or structures, can be overcome by various super-resolution techniques. One convenient way to increase the resolution of e.g. fluorescence images can be done by confocal microscopy combined with an objective of high numerical aperture and immersion oil with refractive index close to glass. The usage of immersion fluids and high-performance objectives is however exceedingly problematic under low temperature conditions [1].

Several ways have been devised in order to avoid the emerging issues, e.g. by placing the objective outside the cryogenic system which causes other problems like the requirement for large working distances and mechanical instabilities.

A new construction of a scanning stage enables us to immerse an objective together with the sample positioned inside a cryostat [2]. The combination with a transfer system allows the loading of frozen or vitrified samples with immersion fluids attached on top of them into the cold cryostat.

Heating the cryogenic chamber over the melting point of an appropriate chosen immersion fluid leads to the possibility to move the objective into the melted immersion droplet and to thereby increase the refractive index between the objective lens and sample.

We recorded confocal images of fluorescence beads at 150 K for the case with and without immersion fluid. By determining the point spread function of imaged single fluorescence beads the effective numerical aperture was appointed to be around unity for an objective with NA = 0.75 (as specified by the manufacturer).

Increasing the resolution for low temperature microscopes provides new opportunities e.g. for studies on biological systems like vitrified cells, which can only be imaged by label-free auto-fluorescence. The here presented sample transfer system together with the demonstrated practicability to use immersion objectives at low temperature would be also of relevance for correlative light and electron cryo microscopy (cryoCLEM)[3].