Applicability of non-invasive and live-cell holotomographic imaging on fungi[1]

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BACKGROUND

The ability to acquire three-dimensional (3D) information of cellular structures without the need for fluorescent tags or staining makes holotomographic imaging a powerful tool in cellular biology. The technique is based on quantitative phase imaging (QPI), an advancement of phase contrast microscopy (PCM), that utilizes the refractive index (RI) to visualize low-contrast specimens. Through illumination of the sample from all possible angles, as shown in Figure 1, a 3D RI map can be reconstructed[2]. In contrast to PCM, RI values can be assigned to each voxel, enabling quantification. Here, we demonstrate the applicability of holotomographic live-cell imaging on *Aspergillus niger* and study their subcellular structures.

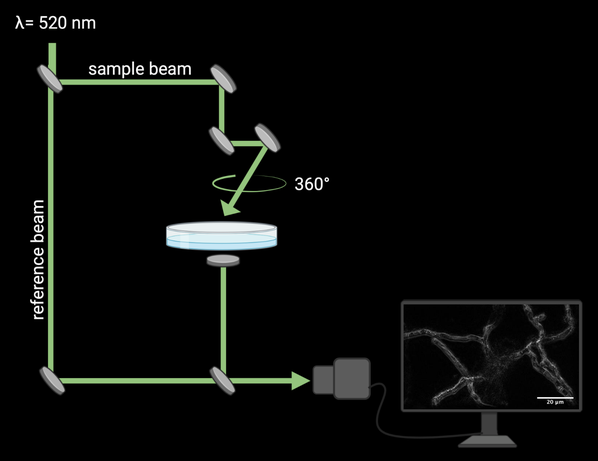

Figure 1: *Principle of holotomographic microscopy.*

RESULTS

The resulting data contains 96 individual 2D holotomograms. This data can be utilized to reconstruct a 3D RI map (Fig. 2A) or to generate a Z-projection (Fig. 2B). The Z-projection contains the most relevant information of the 2D holotomograms, depending on the projection type (i.e. maximum intensity, standard deviation, etc.).

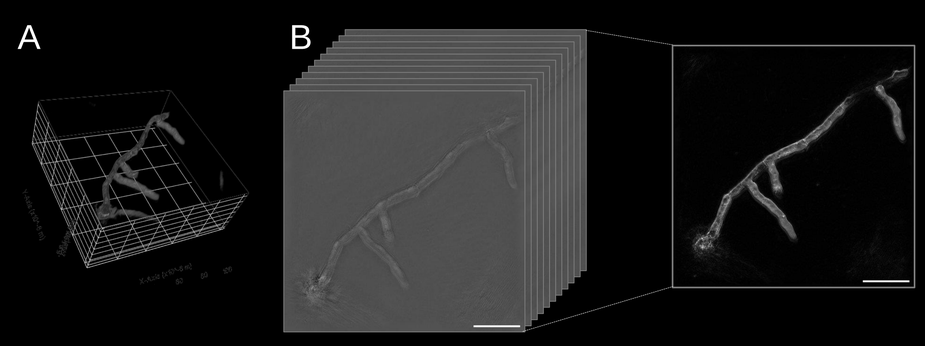

Figure 2: *(A) 3D RI map reconstruction by the software of the microscope. (B) Schematic view of the generation of a Z-projection from 2D holotomographic slices. Scalebar: 20 µm.*

spatio-temporal resolution:

- x,y: 180 nm; z: 360 nm
- t: 1.7 sec

Identification of subcellular structures by refractive index patterns in *Aspergillus niger*

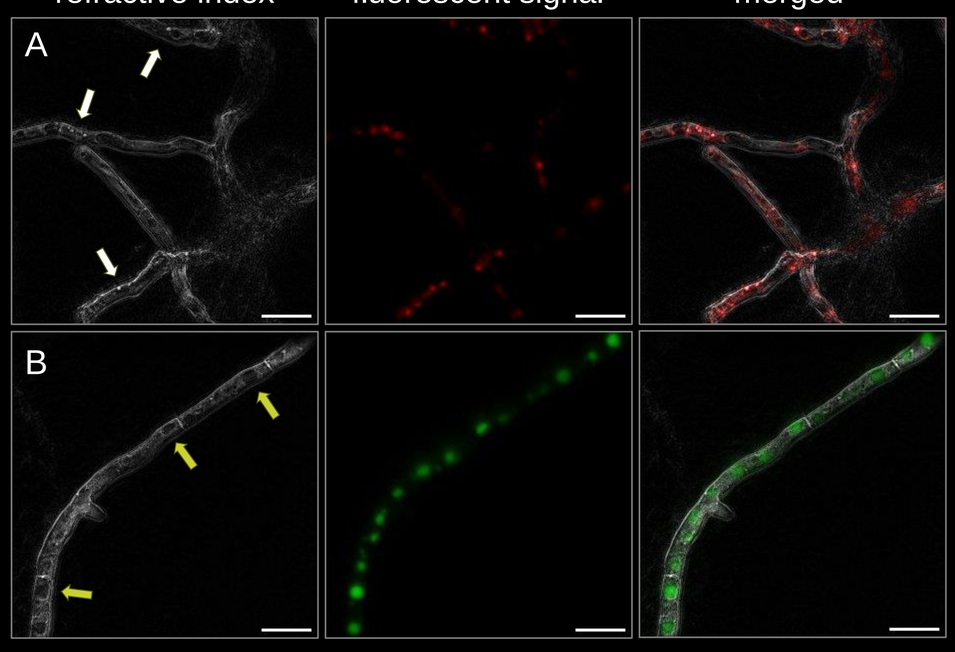

Figure 3: *(A) Lipid droplets (LDs) in A. niger were stained with Nile Red. (B) Vacuoles of A. niger were stained with CDCFDA. Scalebar: 10 µm.*

LDs appear as bright dots (white arrows) and vacuoles as darker areas (green arrows) in the RI signal.

Degradation of the fungal cell wall enables non-invasive localization of nuclei in conidia and hyphae

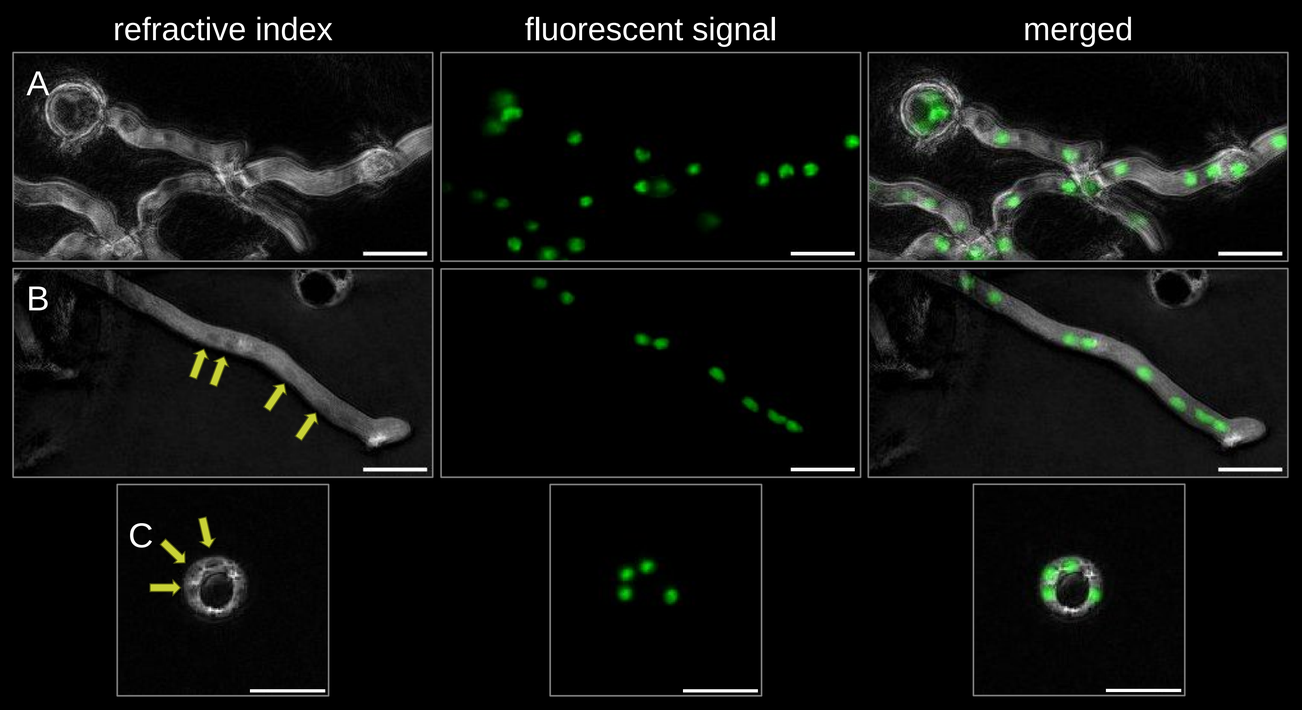

Figure 4: *Images of an engineered A. niger strain expressing the histone H2A tagged with sGFP. (A) shows the strain with an intact cell wall. (B) shows a hypha after cell wall digest and (C) shows a protoplast. Scalebar: 10 µm.*

While the nuclei are not visible in the RI signal of image A, they can be seen as darker spots in images B and C, marked with green arrows.

GFP-tagged histone H2A enables visualization of nuclear migration and division during hyphal growth and branching

Between 11:08 h and 13:20 h, lateral branching events occur. At 13:44 h, all visible nuclei in the depicted hypha have divided. The image at 14:00 h shows the formation of two septa, arresting one nucleus in a subapical compartment (green arrow). In subsequent images, nuclei in the adjacent apical compartments show simultaneous nuclear division, while the marked nucleus in the subapical compartment does not undergo nuclear division or migration.

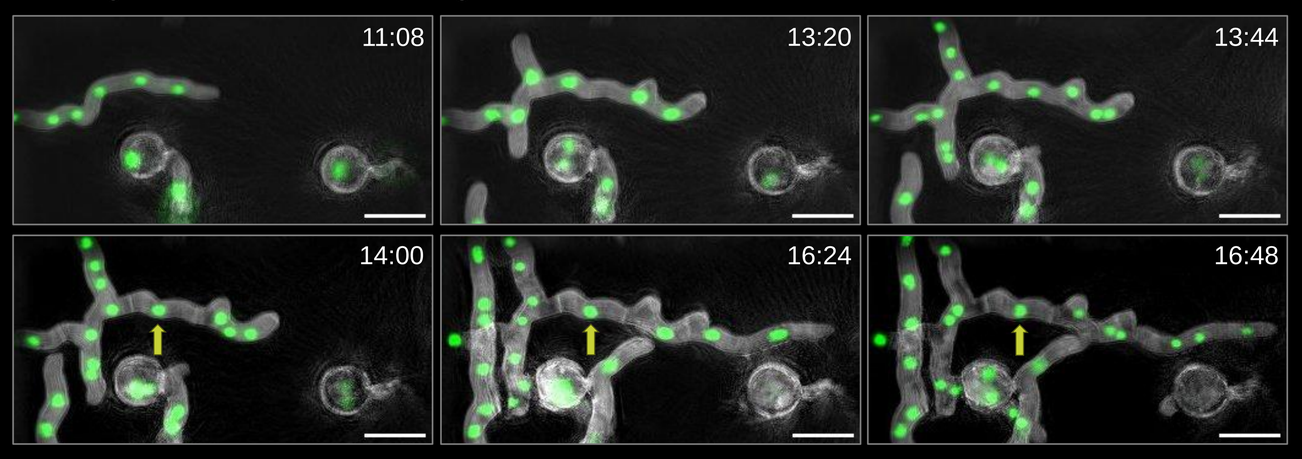

Figure 5*: Time series of an engineered A. niger strain expressing the histone H2A tagged with sGFP. The image of the RI signal is merged with the image of the fluorescent signal. Scalebar: 10 µm.*

CONCLUSION

Live-cell holotomographic imaging of fungal organisms offers advantages over conventional high-resolution live-cell imaging techniques. The absence of fluorescent labels and the low light intensity make it an excellent choice for long-term 4D imaging. In addition, quantitative 3D information can be obtained. Here, we show that distinct subcellular structures, such as vacuoles and lipid droplets, are visible in the RI map. We have also identified the fungal cell wall as a major limitation of this microscopy technique, as it interferes with the illumination laser. However, in combination with fluorescence microscopy, it is possible to track tagged proteins inside the fungal cell.

[1] Fritsche, S., Fronek F., Mach R., Steiger M. Applicability of non-invasive and live-cell holotomographic imaging on fungi. *Journal of Microbiological Methods*, (2024), 106983, 224. https://doi.org/10.1016/j.mimet.2024.106983
[2] Cotte Y., Toy F., Jourdain P. *et al.* Marker-free phase nanoscopy. *Nature Photon* **7,** 113–117 (2013). https://doi.org/10.1038/nphoton.2012.329

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