

Digital Holographic Microscopy Applied to Diffraction Tomography of a Cell Refractive Index

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Abstract: We present a approach of 3-dimensional refractive index tomography of biological specimen, based on the high accuracy phase measurement provided by Digital Holographic Microscopy, recorded for a complete rotation of the specimen in the setup

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1. Introduction

Digital holographic microscopy (DHM) provides quantitative measurement of the optical path length (OPL) distribution that enable to describe semi-transparent samples, such as living cells with a diffraction-limited transverse resolution and a sub-wavelength axial accuracy [1]. However, single images as presented in Ref. [1] do not reveal the three-dimensional (3D) internal distribution of cellular components, but a phase shift resulting from a mean refractive index (RI) accumulated over the cellular thickness. We show here that standard optical diffraction tomography (ODT) techniques can be efficiently applied to reveal internal structures and to measure 3D RI spatial distributions. Pioneer works [2, 3] have established the theoretical basis of reconstructing the 3D scattering potential of weakly scattering objects, by recording the waves scattered from the different directions of parallel illumination. The experimental setup used in the present work involves a fixed illumination beam and a rotating sample. The main advantages of DHM for complex diffracted wave retrieval is that only a single hologram is needed for each orientation of the specimen, leading to short acquisition time and low stability requirements for the system. The object field is recovered when the hologram is re-illuminated by a digitally computed replica of the reference wave, allowing quantitative measurement of both phase and amplitude [4, 1]. Amplitude measurements are equivalent in resolution and quality to classical optical microscopy. Phase measurements are performed with a precision of about 1 degree (corresponding to 2 nm of free wave propagation distance). The biological specimen observed is a yew pollen grain (30 μm diameter), having a 3D-structured nucleus, which makes it an ideal test specimen for the method.

2. Experimental Setup

Transmission DHM (Fig. 1) used for the present study is described in details in Ref. [5]. Results presented here have been obtained with a 63X 0.85 NA microscope objective (MO). The light source is a laser diode at 635 nm. The camera is a 512 x 512 pixels, 8 bits, black-and-white CCD, with square pixels of 6.7 μm , and a maximal frame rate up to 25Hz. The field of view is 80 μm x 80 μm . The transverse resolution (around 1 μm) and the transverse scale calibration are determined with a USAF 1951 resolution test target. The pollen cells are in a glass micropipette (MP) filled with a glycerol solution to prevent drying. The MP has an internal diameter of 100 μm , an external diameter of 510 μm , and is fixed on a motorized rotating stage mounted on a micrometric xyz-stage used to center the pollen cell in the field of view. A second xy-stage mounted on the rotating stage itself allows for centering the pollen cell under investigation on the rotation axis, to minimize lateral displacements of the specimen in the field of view during the

rotation. The rotation of the stage and the acquisition of the holograms are controlled with a PC. To minimize strong light refraction by the MP, which acts like a cylindrical lens regarding to the illuminating light, the volume between a glass coverslip and the MO is filled with an index matching fluid suppressing the air/glass interface.

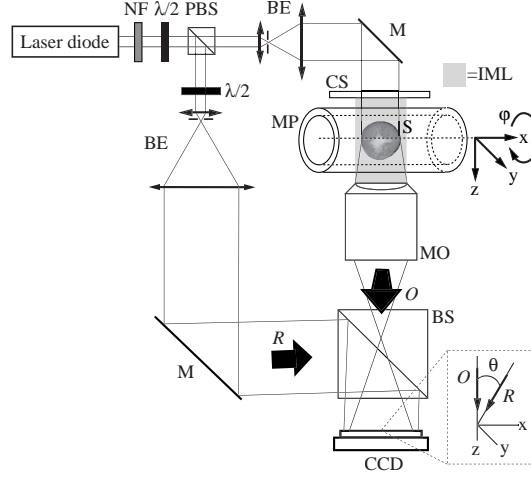


Fig. 1: Digital holographic microscope for transmission imaging: NF neutral density filter; PBS polarizing beam splitter; BE beam expander with spatial filter; $\lambda/2$ half-wave plate; MO microscope objective; M mirror; BS beam splitter; O object wave; R reference wave; MP micropipette; S specimen; IML index matching liquid. Inset: a detail showing the off-axis geometry at the incidence on the CCD

3. Results: Pollen Grain Tomography with DHM

In the case of a weakly diffracting object such as a single biological cell, the optical path length of the collimated illuminating photons across the specimen is parallel to the optical axis [6]. The planar phase distribution $\varphi(x, y)$ provided by DHM is directly proportional to this optical path length. In our experimental setup, the rotation axis is parallel to the x -axis, while the optical axis is the z -axis. $\varphi(x, y)$ can then be expressed as

$$\varphi(x, y) = \int \frac{2\pi}{\lambda} \Delta n(x, y, z) dz, \quad (1)$$

where λ is the wavelength of the light source and $\Delta n(x, y, z)$ is the 3D RI spatial distribution difference between the pollen cell and its surrounding medium. $\varphi(x, y)$ is thus only proportional to the integration of $\Delta n(x, y, z)$ along the z -axis. To proceed to a standard tomographic reconstruction, one must record such 2-dimensional (2D) planar phase distribution for different sample orientations covering an angle of 180 degrees. In our study, 90 images were acquired with a 2 degrees step at a rate of 1 Hz. The representation of the data as a function of the angle is known as a sinogram. The 3D signal $\Delta n(x, y, z)$ can be reconstructed from the sinograms by a filtered backprojection algorithm (see for ex. Ref. [7]). For this purpose, the standard inverse radon transform *iradon* from the Matlab programming environment was used in a slice-by-slice implementation. The use of filtered backprojection algorithm instead of backpropagation [8] algorithm usually recommended in ODT is consistent with the assumption of a phase proportional to the optical path length across the specimen. The maximal spatial resolution of $\Delta n(x, y, z)$ depends on the sampling step used to cover the 180 degrees during the rotation of the specimen and on the spatial resolution of $\varphi(x, y)$. The 2 degree step used in this study is sufficient for the maximal spatial resolution to be reached.

The reconstruction is summarized on Fig. 2. Fig. 2.1 illustrates a cut in the 3D function $\Delta n(x, y, z)$ along the xy -plane in the middle of the pollen cell, while Fig. 2.2 and 2.3 show cuts at different positions in the cell along the yz -plane and the xz -plane respectively. On this figure, one can appreciate the 3D structure of the nucleus of the pollen grain. Knowing that the RI of the glycerol surrounding the pollen is 1.473, a $\Delta n = 0.06 \pm 0.01$ is measured in the nucleus, leading to a measured value of 1.53. The RI of the pollen wall, around 1 μm thick, is not clearly measurable in the present study. As the wall thickness is comparable to the lateral resolution of the system, difficulties appear during the unwrapping procedure involved in the reconstruction process to avoid 2π -jumps in the phase signal, leading to some discrepancies in the phase measurement from an image to another for this critical part of the cell. An increase of the lateral resolution of the system or an adaptation of the embedding medium RI should improve the reliability of the reconstructed phase images. Secondly, the rotation system induces small movements (few microns

range). Even if a numerical procedure based on the center of mass determination was used to re-center the cell on its rotation axis, the accuracy of this procedure was also limited to about $1\ \mu\text{m}$, making the tomographic reconstruction of this around $1\ \mu\text{m}$ -thick wall difficult. The slowly varying RI of the $12\ \mu\text{m}$ nucleus is not affected by the two artifacts described above.

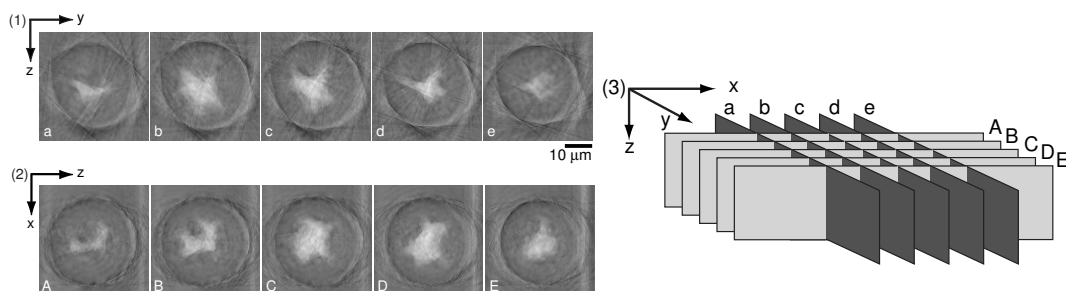


Fig. 2: Tomography of a pollen cell refractive index: cuts at different positions in the cell resp. along the yz -plane (1) and the xz -plane (2), schematic of the presented cuts (3). The cuts are distant of $2.5\ \mu\text{m}$ from each other.

The knowledge of the 3D RI spatial distribution of a cell leads to invaluable information concerning the distribution and the optical properties of the intracellular organelles. In spite of this major issue, ODT applied successfully to cell imaging has to our knowledge not provided quantitative results till today [9, 10].

4. Conclusion

In conclusion, we have shown for the first time to our knowledge the 3D distribution of RI of a semi-transparent object, in our case a pollen grain, provided by backprojecting OPL values collected with DHM on a series of projections of the preparation taken at various incidence angles. The accuracy of the RI determination is better than 0.01 and the 3D spatial resolution is better than $1\ \mu\text{m}$ in all 3D. This approach could find interesting application as a reference measuring technique in material and life sciences.

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