THREE DIMENSIONAL RECONSTRUCTION OF BIOLOGICAL SAMPLES

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We describe the use of spatially incoherent illumination to make tridimensional reconstruction of semi-transparent biological samples using a quantitative phase imaging technique [1]. The setup can be coupled with adaptive optics to enhance the contrast above several hundreds of µm imaging depth.

Quantitative phase imaging is commonly used with coherent illumination allowing relatively simple interpretation of the measurement. We propose to use spatially incoherent illumination which create lateral resolution increase and optical sectioning to image thick samples with intracellular resolution [2].

The 3D volume is imaged by axially scanning the sample with a quadri-wave lateral shearing interferometer while using spatially incoherent white-light illumination. This illumination configuration is known to lead to axial and lateral resolution improvement compared to classical coherent illumination.

We use a non-modified inverted microscope equipped with a Z-axis piezo stage. A z-stack is recorded by objective translation along the optical axis in order to reconstruct the 3D object structure. The native halogen source of the microscope is used with a 700 ± 30 nm band-pass filter to neglect the sample dispersion.

The main advantages of this approach are its easy implementation, compared to the other state-of-the-art diffraction tomographic setups, and its speed which makes even label-free 3D living sample imaging (such as cells) possible.

A deconvolution algorithm is used to compensate for the loss in contrast due to spatially incoherent illumination. This makes the tomographic volume phase values quantitative. Hence refractive index could be recovered from the optical slices.

We will present tomographic reconstruction of biological samples from individual COS-7 cell of about several micrometers thick to fixed brain tissue of 100 µm thick. We will also present the prospect to apply adaptive optics correction to compensate for spherical aberration due to thick imaging with large numerical aperture optics.