EXCITATION-SCANNED TRANSMISSION OPTICAL PROJECTION TOMOGRAPHY

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In optical projection tomography (OPT, [1]), a 3D attenuation map of the sample is reconstructed from angular projections using a filtered backprojection. OPT is easily integrated into a light sheet fluorescence microscope to provide an additional contrast [2,3]. To date, transmission OPT has only been acquired with "white" light sources or single illumination wavelengths, even though a specimen's transmission spectrum may contain valuable information, e.g. chemical composition, potentially useful to infer sample health.

We present a spectrally resolved transmission OPT with excitation scanning. A supercontinuum source with an AOTF for wavelength selection was used for illumination at 520-820 nm. The laser power was adjusted to compensate for the spectral power density of the laser and the spectrally varying AOTF efficiency, thus ensuring constant illumination intensities across the whole spectrum. Both, dynamic and static approaches to reduce speckle were evaluated. OPT needs a large depth of focus (DOF) to encompass the whole sample in each projection, but may suffer from reduced resolution when using low NA lenses. The spiral acquisition presented in [2] extended DOF for high NA detection objectives. Thirty frames were typically acquired for each of the 720 angles. We optimized the detection NA and the parameters of the spiral to maximize acquisition speeds.

We acquired spectrally resolved projections in living zebrafish embryos and reconstructed the 4D (x, y, z, λ) or 5D (x, y, z, t, λ) data with an inverse Radon transform. Spectral phasors [4] were used to reduce the dimensionality of the data and visualize all spectra present in the sample in a 2D polar plot.

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