BRIGHTFIELD MULTIVIEW RECONSTRUCTION IN A LIGHT SHEET MICROSCOPE

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Light Sheet Fluorescence Microscopy (LSFM) is a powerful tool for live imaging of developing organisms as well as imaging of large, chemically cleared samples. Optical Projection Tomography (OPT) has shown to complement the fluorescence reconstruction obtained with LSFM. In particular, OPT with bright-field contrast is used to correct absorption artifacts in LSFM [1] and to reconstruct the whole anatomy of the specimen [2].
Here we show that the Bright-Field (BF) contrast can be efficiently reconstructed in 3D by combining stacks of images acquired at multiple angles.

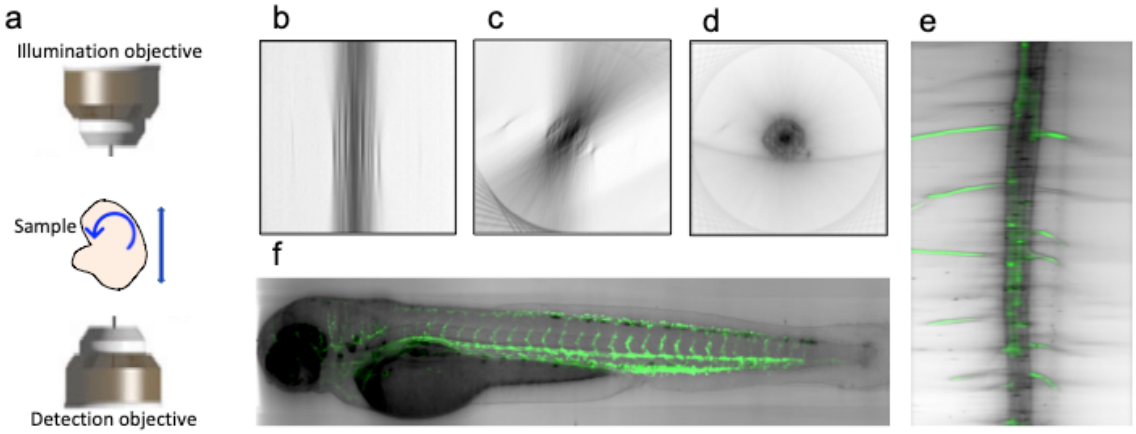

Figure 1: Schematic of the setup, consisting in a BF microscope equipped with a stage for sample translation and rotation (a). Transverse section of the sample obtained at a single angle view (b), combining 10 views (c) and combining 45 views taken around 360° (d). Lateral section of an Arabidopsis thaliana, overlapped with LSFM (e). BF reconstruction (intensity projection), reconstruction of a 4 days post fertilization zebrafish, kdrl:GFP (f). Images are obtained with a 4X, NA 0.13 detection objective.

We describe the reconstruction method, based on multiview deconvolution of the BF data (Fig 1a-d) and we assess resolution and contrast as a function of the spatial and angular sampling. The BF multiview reconstruction, compared to OPT, reduces the number of acquired angles by an order of magnitude, eliminates diffraction artifacts and can be readily combined with LSFM. We then discuss an efficient approach to identify the position of the rotational axis of the system, and we show results obtained in developing Arabidopsis thaliana (Fig. 1e) and zebrafish embryos (Fig 1f).

[1] Mayer, J. et al. "OPTiSPIM: integrating optical projection tomography in light sheet microscopy extends specimen characterization to nonfluorescent contrasts" Optics Letters, 39, 1053-1066 (2014).
[2] Bassi, A., Schmid, B. and Huisken, J. "Optical tomography complements light sheet microscopy for in toto imaging of zebrafish development" Development 142, 1016-1020 (2015).