A Label-free Multicolor Optical Surface Tomography (ALMOST) imaging method for nontransparent 3D samples

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The recent advent of 3D imaging techniques, like light sheet microscopy in all its variations, has shown the need for 3D imaging specifically on the mesoscale. However, the main disadvantage that remains is that the samples need to be transparent to be imaged. However, many biological samples are of opaque nature, be it in the form of adult tissue in vertebrates or exoskeletons of insects, crustaceans, and mollusks, so samples need to be biochemically rendered transparent in a process called clearing. Although this is effective, it is not applicable to all samples. For imaging samples of solid nature typically micro-CT or soft x-ray imaging are applied with the downside that the actual appearance of the sample such as color or reflectivity is lost. Also, micro-CT and cleared samples are typically dead. This is a severe limitation for many research areas, as the 3D color surface morphology of opaque samples like intact adult *Drosophila*, *Xenopus* embryos, and other non-transparent samples cannot be assessed.

Hence, we have developed "ALMOST", a novel optical method for 3D surface imaging of reflective opaque objects utilizing an optical projection tomography device in combination with oblique illumination and optical filters. We demonstrate the image formation, give background information and explain the reconstruction and consequent rendering using a standard filtered back projection algorithm and 3D software. We expanded our approach to fluorescence and multi-channel spectral imaging and validated our results with micro-computed tomography. We highlight the versatility of our approach with different biological and inorganic test samples, including, resistors, and *Drosophila*. We also visualized the dynamic closure of neural folds during neurulation of live *Xenopus* embryos and show the complementarity of ALMSOT by imaging a *Xenopus* tadpole with transmitted light, fluorescence OPT, and ALMOST [1].

Our technique complements other valuable approaches, such as OPT, micro-CT, X-ray, or light sheet microscope, for 3D representation of the sample's surface morphology thereby adding complete preservation of the actual characteristic color without the need to use contrast agents, sample preprocessing, or digital post-processing to reintroduce the colors. Overall, we believe that our new modality for spectral/color, macro/mesoscopic 3D imaging is applicable to a variety of model organisms and enables to reveal longitudinal surface dynamics during development. Given that the device is cheap and online resources are available for building OPTs, we expect quick acceptance and implementation of this novel imaging application.

References;

[1] A. Kerstens; N. Corthout; B. Pavie; Z. Huang; F. Vernaillen; G. Vande Velde; and S. Munck, “A Label-free Multicolor Optical Surface Tomography (ALMOST) imaging method for nontransparent 3D samples,” *BMC Biology* **17**:1(2019).