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3D Volume rendering of invertebrates using Light-Sheet Fluorescence Microscopy

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Light-Sheet Fluorescence Microscopy (LSFM) has recently emerged as the technique of choice for obtaining high quality 3D images of whole organisms with low photo-damage and fast acquisition rates. Unlike conventional optical microscopy or scanning electron microscopy systems it offers the possibility of obtaining multi-views of the sample by rotating it. Here we show that the use of light sheet microscopy for the analysis of invertebrates provides a fair compromise compared to scanning electron microscopy (SEM) in terms of resolution but avoiding some its drawbacks such as sample preparation or limited three-dimensional perspectives.

We have developed a scanning light sheet microscope based on open hardware and software [1], suited for multicolor fluorescent imaging with sample rotation. All the details for the replication of the system as well as the source code for the microscope control are accessible through our webpage (https://sites.google.com/site/openspinmicroscopy/). The scheme of the setup is shown in Fig. 1. We have tested the imaging capabilities of our system in different organisms (*Drosophila melanogaster*, zebrafish, ants, spiders, moths, daphnia) obtaining high quality relevant morphological information. Fig. 2 shows a collection of images obtained with the LSFM system for different samples and conditions illuminating the sample with the 488 nm laser line. Those samples did not require any preparation at all, being just hold on the designed sample holder. In Fig. 2 (a) we can observe different morphological details of a moth head and wings. The main advantage of LSFM microscopy, compared with confocal or SEM is the ability of sample rotation. Fig. 2 (b) shows the maximum projection of a spider imaged for 4 different view angles, 90 degrees apart. Different magnifications are possible by replacing the detection objective, allowing modifying the field of view from 6.6x6.6 mm^2 to 133x133 μm^2. Fig. 2 (c) shows the maximum projection of an ant obtained with 2x, 4x, 10x and 60x, respectively. The last one corresponds to the tip of the ant antenna. Fig. 3 (a) shows an example of different views of an *Oecophylla smaragdina* ant's head, obtained using 488, 568 and 647 nm excitation laser. Different sample features show autofluorescence depending on the excitation wavelength, providing multicolor images. Due to its specific configuration, with illumination and image collection at perpendicular axis, images suffer from shadowing on the side of the sample furthest from the laser. In addition, for thicker samples, images suffer strong quality degradation as imaging deeper, due to scattering. These problems can be minimized by rotating the samples and acquiring images from multiple angles, and then computationally merging them into a single 3D dataset (Fig. 3 (b)) using a freeware solution for multi-view fusion [2]. Fig. 3 (c) shows the inner brain structures inside the ant's head. In this talk we will show how LSFM techniques could provide a cheap, high-qualitiy, multicolor, 3D alternative to scanning electron microscopes for the study of the morphological structure of insects and invertebrates in morphogenetic studies of the whole animal.

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2. Preibisch, S. *et al.*, *Nature Methods*, **7**, 418-419, 2010.

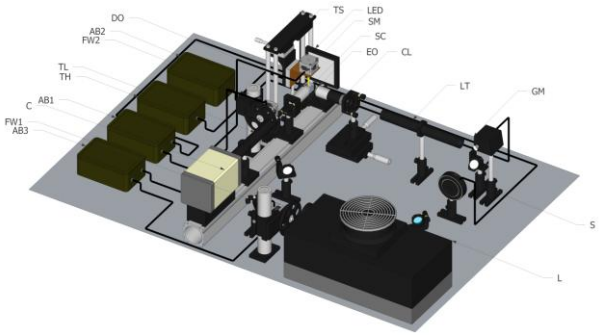

Figure 1. Schematic of the Light Sheet Fluorescence Microscope at IGC.

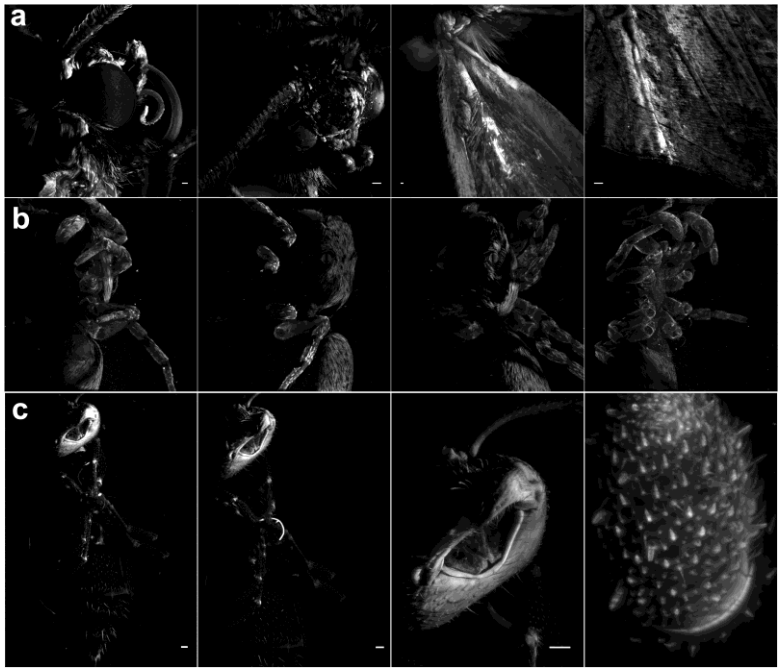

Figure 2. Different samples imaged with LSFM: (a) moth head and wings, (b) four views of a spider and (c) ant with different magnifications.

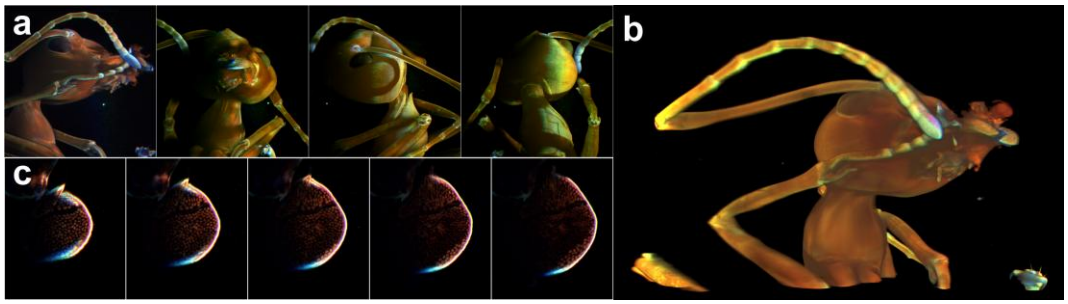

Figure 3. (a) Different views of an *Oecophylla Smaragdina* ant's head (b) multi-view fusion of 8 views and (c) brain structure at different depths.

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