Light sheet microscopy of cell sheet folding in *Volvox*

Half year report June 2017

Summary

Light sheet fluorescence microscopy (LSFM) is the state-of-the-art technique to study developmental processes *in vivo*. LSFM causes less photo-damage than confocal microscopy enabling longer time-lapse recordings. We had previously built a LSFM setup in the Goldstein group. The purpose of this project is to improve the quality of the generated LSFM data.

Optical sectioning is achieved by moving the sample through a light sheet and thereby creating z-stacks. In our previous setup images were recorded by a single camera. Due to light absorption and scattering the images of the sample half facing away from the camera showed a significant loss in image quality. In order to correct for this loss we have added a second camera and detection arm opposing the first one and covering the second half of the sample. This improved setup is doubling the thickness of a sample for which we can acquire useful fluorescence data. This significantly increases the variety of future applications including studies on the morphogenesis of entire embryos in the multicellular micro-alga *Volvox* and the development of feeding structures of the aquatic carnivorous plant Bladderwort.

Report and outcomes

Dr. Stephanie Höhn and Dr. Pierre Haas (DAMTP, Cambridge) are studying embryonic cell sheet folding events in *Volvox* that resemble the invagination of cell sheets in animals, e.g. during gastrulation **(Fig. 1A,B)** [1,2]. In collaboration with Dr. Karen Lee (John Innes Centre, Norwich) we are planning to study the development of the feeding bladders of the aquatic carnivorous plant *Utricularia* (Bladderwort). These bladders develop within spiral shaped structures. We are using LSFM to acquire long-term time-lapse recordings of these developmental processes (Fig. 1). Dr. Lee has visited the Goldstein lab in May 2017 to acquire preliminary data **(Fig. 1C)**.

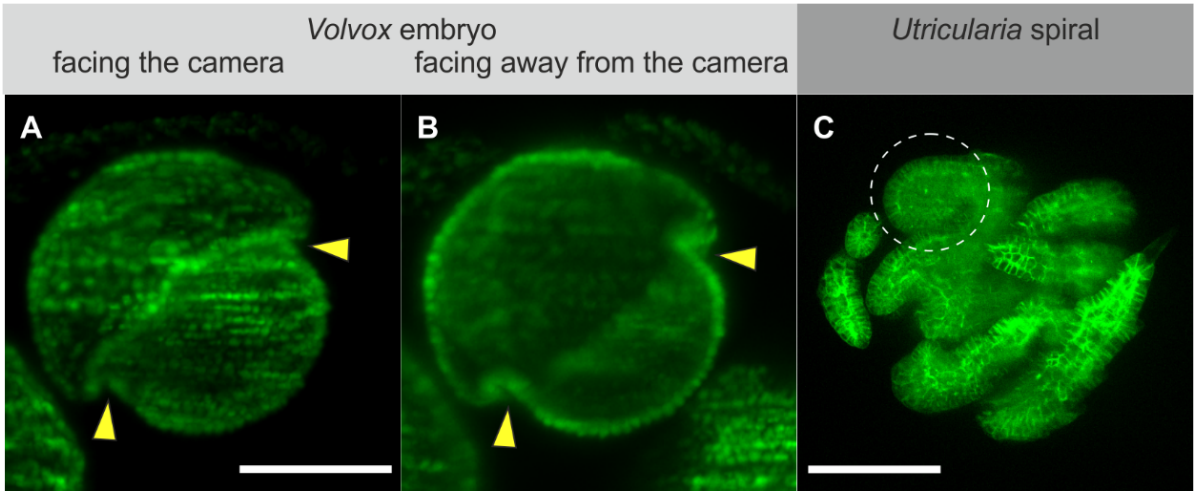

Figure 1 Light sheet fluorescence microscopy of *Volvox* and *Utricularia* (Bladderwort) with a custom-built setup. A, B: *Volvox* embryo undergoing invagination. The image quality is significantly lower in the sample half facing away from the camera (B). In this project we added a second detection arm to achieve a homogeneous image quality throughout the sample. Green: Chlorophyll-autofluorescence. **C**: *Utricularia* spiral (dotted line) from which a feeding bladder will develop. Green: GFP-tagged cell wall marker.

Our previous custom-built LSFM setup comprised two illumination arms and a single detection arm **(Fig. 2A)**. A laser beam is split by a beam splitter and focused through cylindrical lenses to create two light sheets that illuminate the sample from two sides. The original detection arm is oriented perpendicularly to the light sheets. Due to light absorption and scattering the data quality for the sample half facing away from the camera was significantly lower **(Fig. 1B)**.

In this project a second detection arm was added opposing the first one **(Fig. 2B)** to enable sample imaging from two sides and thereby achieving a homogeneous image quality throughout the sample. The two detection arms each consist of a detection objective, a tube that encloses the light path and functions as filter holder, a tube lens and a camera with adapter-mount. The sample chamber had to be modified to add a fourth objective (the second detection objective) and a lamp had to be designed for bright field illumination **(Fig. 2C)**. All parts of the microscope are attached to an optical breadboard. To make space for the 2nd detection arm and camera, the setup had to be translated on the bread board. The breadboard was rotated by 90 degrees for better access. For safety reasons the laser path was changed, entailing a change in the position of the beam splitter **(Fig. 2B)**.

Details on our modified LSFM setup including photographs and drawings of the self-built parts have been made publicly accessible on the website of the OpenSPIM platform (see "Cambridge" on http://openspim.org/Who_has_an_OpenSPIM%3F).

Self-built parts:

All self-built parts were manufactured by the in-house machine shop at DAMTP.

- Sample chamber: We designed a sample chamber to hold fluid medium with inlets for the 4 water dipping objectives.
- Objective holder: we added a fourth objective holder to the metal case enclosing the sample chamber.
- Brightfield lamp: The original space for brightfield illumination was lost when adding the 4th objective (Fig. 1). We explored different options to keep illumination (other than laser) during positioning of the sample:
    - Shining light through the top of the sample chamber did not work.
    - The objective holder for the 2nd detection objective was designed to allow for darkfield images.
    - We designed a flat LED lamp to fit in the filter holder, determined the required voltage and adjusted the power supply accordingly.
- Tube to cover laser path and hold filters
- Clamp to hold the detection arm
- Filter cases
- Custom size mirror posts

Purchased parts:

Prices for the second camera, camera mount adaptor, tube lens, objective and multi-wavelength emission filter were negotiated before purchasing them.

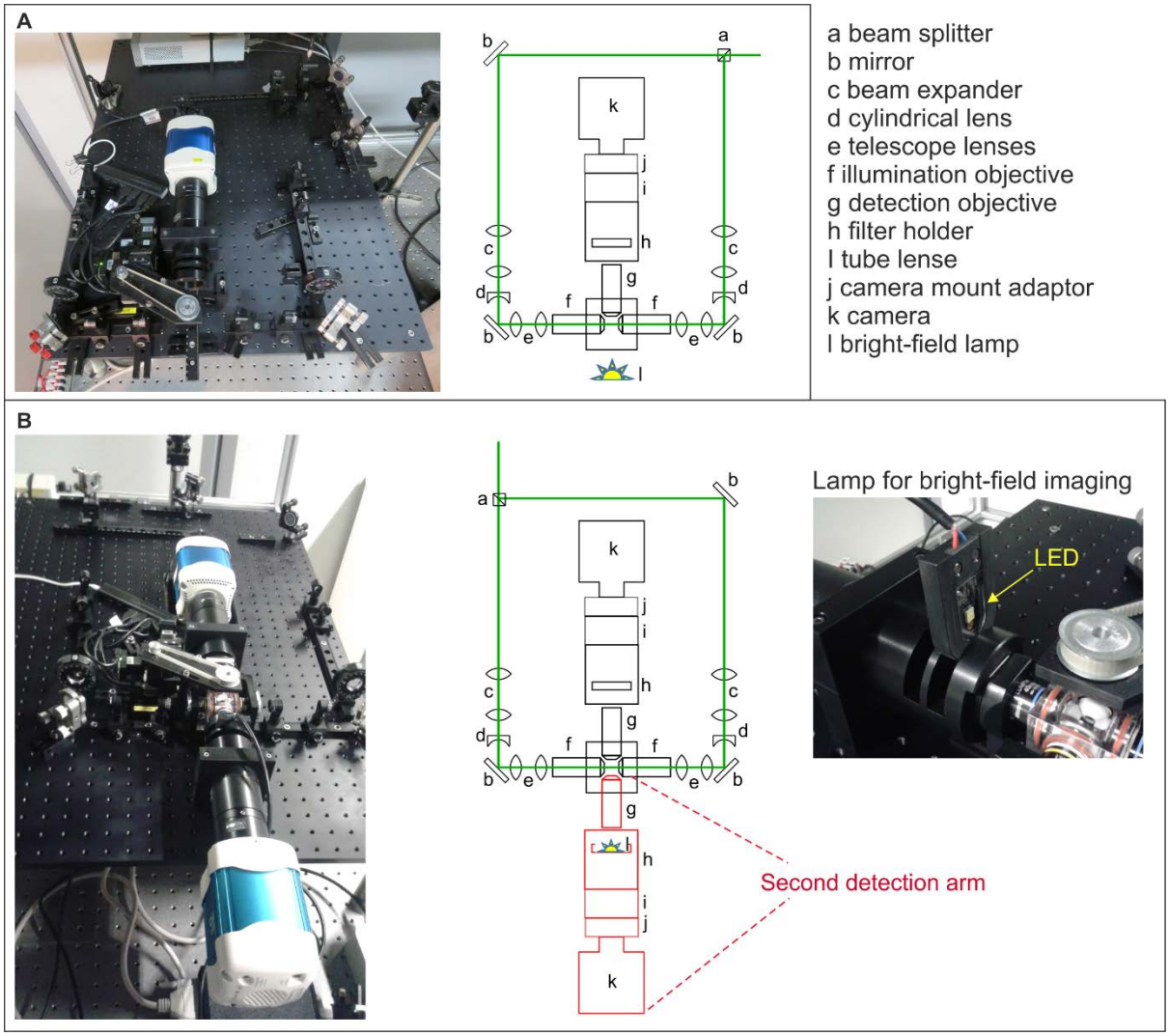

Figure 2 Adding a second camera and detection arm to a custom-built light sheet microscope. A: Previous LSFM setup. The laser beam is split by a beam splitter and focused through cylindrical lenses to create two light sheets that illuminate the sample from two sides. The original detection arm is oriented perpendicularly to the light sheets. **B:** The second detection arm increases the quality of 3-dimensional datasets by imaging samples from two sides. An LED lamp was designed to be temporarily inserted into the filter holder during sample positioning.

References

[1] Höhn S and Hallmann A. There is more than one way to turn a spherical cellular monolayer inside out: type B embryo inversion in *Volvox*. BMC Biology **9**, 89 (2011). **[2]** Höhn S, Honerkamp-Smith AR, Haas PA, Khuc Trong P, and Goldstein RE. Dynamics of a *Volvox* embryo turning itself inside out. Phys. Rev. Lett. **114**, 178101 (2015).

Expenditure

The £4,000 granted by the OpenPlant Fund were used to buy the second camera, a CoolSnap Myo camera (Roper Industries, UK) for £4,125. The exceeding £125 were paid from internal funds.

In addition, we purchased the following items to complete the second detection arm of the microscope (paid from internal funds):

Tube lens U-TLU-1-2 (Olympus, UK):	£350.15
0.5x C-mount-adapter U-TV0.5XC-3-7 (Olympus, UK):	£502.56
Emission filter, 405/488/561/635 nm StopLine quad-notch filter:	£1, 071.00

Follow on Plans

We have previously been using 20x detection objectives with an N.A. of 0.5. In order to increase the resolution of our data for cell tracking we are planning to purchase a 40x, N.A. 0.5, water dipping objective LUMPLFLN40XW (Olympus, UK), priced £1,969.96. We are requesting the additional £1000 from the OpenPlant Fund to be allocated towards this 40x objective as soon as possible. The remaining £969.96 would be paid from internal funds.

We are currently integrating the second camera into the acquisition software. Both cameras will be controlled by the Multi-Camera adapter within MicroManager (https://micro-manager.org/).

We are very grateful that the OpenPlant Fund is enabling us to improve our LSFM setup and to expand our developmental studies in *Volvox*, *Utricularia* and other future model systems.