Under the Microscope: Interview Series #4

Simultaneous *in vivo* fluorescence and DIC imaging of fast-moving samples

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Analysing dynamic gene expression patterns in fast moving model organisms like *C. elegans* can be challenging. At the Friedrich Miescher Institute in Basel, Switzerland, Platform Head at the Facility for Advanced Imaging and Microscopy, Laurent Gelman, explains a clever technique for acquiring fluorescence and DIC images simultaneously.

Using wide-field or spinning-disk confocal microscopy and a CoolLED pT-100 Illumination System for transmitted light, gene expression patterns can be automatically mapped in individual worms both spatially and temporally *in vivo* without movement artefacts, providing accurate insights into biological processes.

Tell us about your role in this project

As microscopy facility manager, I am responsible for purchasing or adapting the microscopes to enable scientists to answer their research questions. Lately we've had a couple of projects involving something more tricky than normal, and this is where our method comes in.

Why did you need a specialised technique?

The goal of this project was to monitor gene expression levels in different cells of individual worms over several days. Since fluorescence may oscillate differently in different cells over time, and since the worm population is not perfectly synchronised, each individual worm must be analysed separately. We cannot simply carry out ensemble approaches measuring fluorescence at once at the level of the whole population. Also, each individual worm must be imaged in detail, i.e. at a magnification allowing cell, tissue and organ identification.

The number of images acquired each time, over many worms and multiple days makes it impossible to manually quantify the images. We therefore needed an automatic approach. However, this requires the acquisition of two separate channels precisely overlaid: one for GFP quantification, and one to be used as a mask to delineate the worm body boundaries and substructures. To create such a mask, we used either brightfield or DIC images to reveal anatomical details.

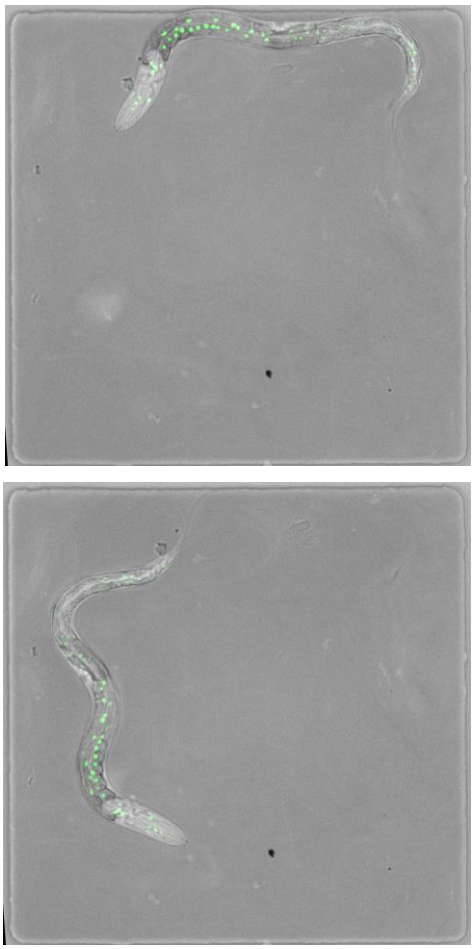

Figure 1: Simultaneous DIC and fluorescence (GFP, green) imaging enables *in vivo* analysis of gene expression patterns in *C. elegans*. Watch video here. Courtesy of Yannick Hauser and Helge Grosshans, FMI, Basel.

The problem when acquiring two channels sequentially is that any slight movement of the sample makes it impossible to perfectly overlay the two images and hinders automated segmentation and analysis. As worms are very mobile when exposed to light, we needed to find a way to take the two channels, brightfield/DIC and GFP, simultaneously.

How did you solve this challenge?

Firstly, we needed to isolate individual worms for analysis. In collaboration with the researchers working on this project, we adapted the method developed in the lab of Dr. Bringmann (Turek *et al.*, 2015): we made an agarose pad with a 2D-array of microwells, each of them populated with a single worm. The size of each microwell matches the field of view of our microscope, and we programme time-lapse experiments over multiple days and multiple positions (microwells) to monitor each individual worm.

We then fitted several spinning-disk or wide-field systems in the facility with a second camera and a 561 nm long-pass camera beam-splitter. The light path on the microscope was set the same way as for a dual-channel GFP and mCherry experiment with simultaneous acquisition. However, for the red channel we instead used transmitted light from a CoolLED pT-100 LED Illumination System, which emits a narrow bandwidth at 635 nm. In this configuration, one camera captures GFP emission and the second one the transmitted light, which follows the same path that fluorescence from a red-emitting fluorophore (e.g. mCherry) would follow. Overlaying both the fluorescence and brightfield/DIC images, we can then clearly see when a single worm starts to express a reporter gene, and in which region of the body.

Finally, we used an in-house designed machine learning workflow developed by our programmers Markus Rempfler and Jan Eglinger to automatically find the worms in the brightfield/DIC images and create a mask applied to the GFP channel for fluorescence intensity quantification.

Is this method useful for other studies?

Simultaneous acquisition of different channels is crucial when a Z-stack of a fast-moving object is required, or when the time resolution for a time-lapse experiment must be very high. The main issue when two channels are acquired simultaneously is usually crosstalk. This would be the case if attempting to use the white light sources usually present for transmitted light imaging on the microscopes together with GFP. However, thanks to the pT-100 635 nm Illumination System, we can record the GFP and transmitted light signals without crosstalk. I think few people realised this was possible and as easy as replacing the classical white LED with a red-shifted one (or blue if one wants to image mCherry!).

For more information on this facility and its research, visit www.fmi.ch

If you would like to know more about the benefits of LED Illumination Systems for transmitted light applications, visit www.CoolLED.com