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# Remote focusing spinning disk laser free confocal microscopy

## Application Note

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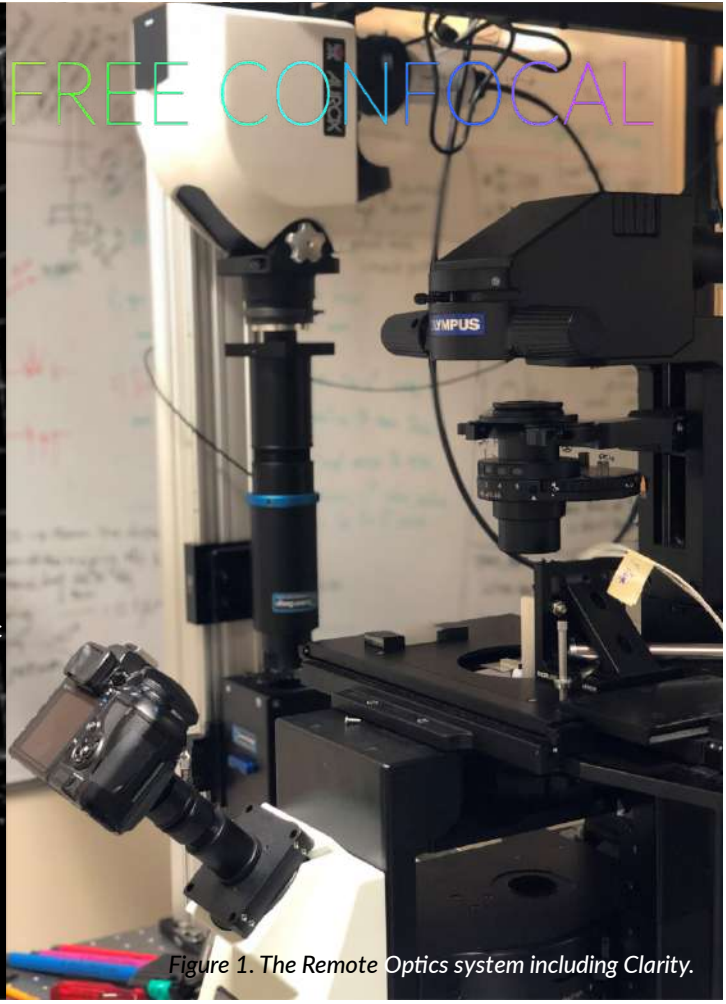


*Figure 1. The Remote Optics system including Clarity.*

### Introduction

A challenge of imaging live specimens in fluorescence microscopy is the ability to capture complex three-dimensional behaviour with high temporal and spatial resolution with minimal mechanical disturbance of the sample being studied. Remote focusing is a method that allows rapid refocusing without moving the sample or objective.

The authors introduce an optical design using the Aurox Clarity laser free confocal system to achieve fast optical sectioning in combination with a remote focusing system to rapidly acquire images of live samples at cellular resolution. A simplified optical scheme reproduced from the publication is shown in Figure 2.

Spinning disk confocal microscopes can acquire optical sections faster than traditional laser scanning systems for a small compromise in axial resolution. Traditionally, spinning disk confocal systems use lasers for illumination making them expensive, bulky and inflexible. The use of the small and compact Clarity laser free confocal spinning disk system with LED illumination in combination with the remote focusing system minimises complexity whilst retaining configurability and performance to cover most imaging tasks.

### Experimental

Structured excitation light from the Clarity spinning disk (SD) is imaged through the remote focusing unit and onto the sample.

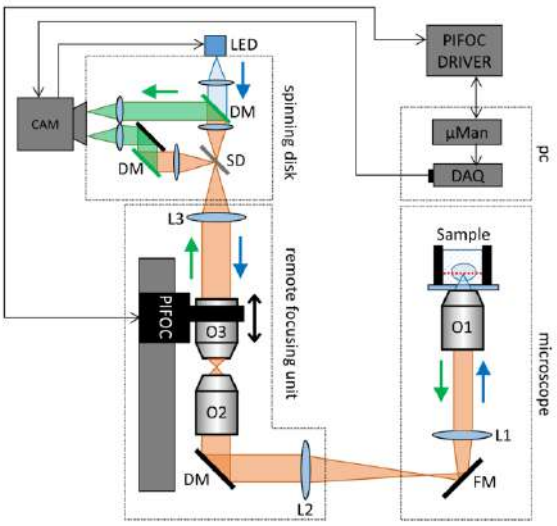


*Figure 2. Simplified optical scheme of the Clarity (SD) and Remote Focusing (RF) setup.*

Sample fluorescence is then collected by O1 (40X 0.8 NA, water) and the magnified image is demagnified by the refocusing lens, O2 (40X 0.95 NA air). A third reimaging objective O3, identical to O2 relays an image of the sample plane, back onto the spinning disk. In-focus fluorescence passes through the upper path onto one half of the sCMOS camera (CAM), with the out of focus component passing through the lower path onto the second half of the camera detector. On-the-fly processing of the two images then returns a confocal image of the sample. By scanning and de-scanning in Z, the position of O3 determines the axial location of the plane of interest in the sample. FM=fold mirror, DM2=dichroic mirror. Tube lenses L1 (f=180 mm) and L2 (f=140 mm).

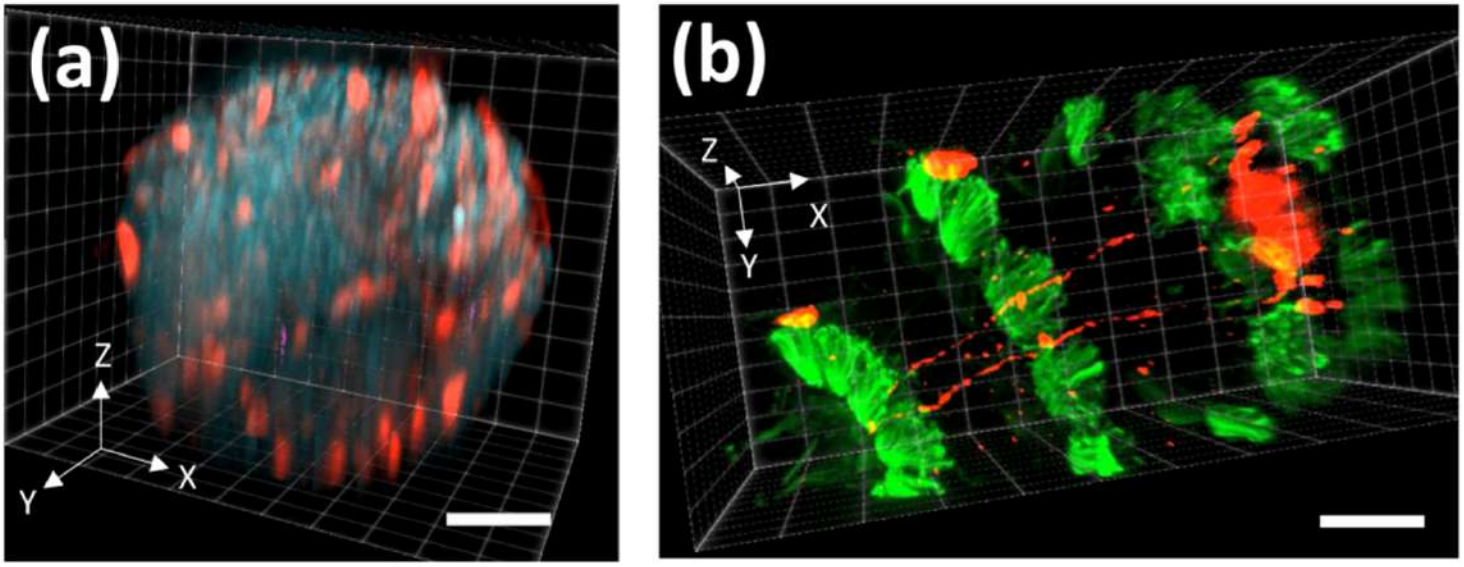


*Figure 4. Multi-channel images of fixed Platynereis dumerilii larvae after (a) 2 days post-fertilisation and (b) 3 days post-fertilisation. Labels: (a) blue (DAPI) = nuclei; red (DsRed) = pERK immunostaining, (b) red = THDa immunostaining, green = cilia bands (acetylated tubulin immunostaining). Scale bars (a) 30 µm (b) 50 µm.*

**Calcium imaging**

Axial and lateral full width half maxima less than 5 µm and 490 nm respectively were demonstrated over 130 µm axial range with a 256 × 128 µm field of view. A water-index calibration slide was used to achieve an alignment minimising image volume distortion.

The high temporal resolution of the system then enabled imaging of changes in electrical activity through Ca2+ binding to GCaMP in the anterior nervous system region of *Platynereis dumerilii*. A sampling rate of 1Hz was observed for an entire 24 µm thick image volume with good signal to noise ratio. The system was also used to record multi-channel images of fixed samples as shown in Figure 4.

**Conclusions**

The flexibility of the Clarity laser free confocal system and the new Micro-Manager plug-in from Aurox allowed the set up of a unique optical arrangement for fast confocal imaging with remote focusing. Even when imaging through the fast Z-scan module, the Clarity was able to provide plenty of signal for fine sectioning.

*Figure 5. (L-R) Michele Gintoli, Sharika Mohanan and Alex Corbett demonstrate remote focusing with Clarity.*

**References**

*M. Gintoli, S. Mohanan, P. Salter, E. Williams, J. Beard, G. Jekely, A. Corbett. (2020). "Spinning Disk -- Remote Focusing Microscopy."*