AUROX LASER FREE CONFOCAL

Bring Clarity to Mycology

Application Note

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Figure 1. Macrophage and Candida; Clarity (Inset)

Introduction

Confocal microscopy is an essential tool in mycology research, producing images free of out-of-focus blur with high contrast and high spatial resolution. Fully in focus projections through a living or fixed sample can be obtained allowing the researcher to observe features which would have been hidden by out-of-focus information. The principal uses of confocal microscopy in mycology research include detection and identification of fungi in clinical specimens and the identification of fungi based on their morphology. Confocal laser scanning microscopy is a useful tool. However, spinning disc confocal microscopy is often preferred due to the reduced photo-toxicity and photo-bleaching.

In this application note, the Aurox Clarity, structured illumination spinning disc device was used to record confocal images of mycology specimens.

Experimental

Experiment 1: Comparison of wide-field & confocal imaging

The mould specimen 1, *Rhizopus Sporangia* (bread mould) and specimen 2, *Penicillium*, were stained in Harris hematoxylin and counterstained with Phloxine B. Eukitt, a clear, fast drying mountant was used. For both specimen 1 & 2 a z-stack was recorded in both the wide-field and the confocal mode using the Aurox Clarity and a 10x, 0.45 NA objective.

For specimen 1, the channel settings were FITC: Exposure time 40 ms; MCherry: Exposure time 40 ms. The confocal z resolution was 14 µm and the total stack size was 196 µm. For specimen 2, the channel settings were FITC: Exposure time 40 ms; MCherry: Exposure time 60 ms. The confocal z resolution was 14 µm and the total stack size was 168 µm.

Experiment 2: Multi-colour acquisition of a fixed medical mycology sample.

A *Candida albicans* strain SC5314, was combined with murine bone marrow-derived macrophages, fixed with 2% Formaldehyde and stained with: Rhodamine Phalloidin to visualise macrophage Actin (blue); Calcofluor White to visualise fungal Chitin (red); and Fc-Dectin-1 probe labelled with FAb-Alexafluor488 to visualise exposed fungal β-Glucan (green). Cells were grown and imaged on Ibidi 8-well µ slides with PBS. A 3 colour confocal z-stack was recorded for the sample using a 40x, 0.95 NA objective. Channel settings were DAPI: Exposure time 20 ms; GFP: Exposure time 700 ms; DsRed: Exposure time 120 ms; The confocal z resolution was 1.2 µm and the total stack size was 10 µm.

All images were collected using an Aurox Clarity and the Aurox Visionary software. The Clarity was mounted on a Nikon Ti-E microscope. The light source used was a CoolLED pE300 Ultra and the detector was a sCMOS Hamamatsu Orca Flash 4.0 V3.

Image data were exported to ImageJ/Fiji using the one-click export function of the Aurox Visionary software.[1] Maximum projection images were then created using ImageJ/Fiji.

Results

The laser free confocal images (Figure 1: *Rhizopus*, and Figure 2: *Penicillium*) obtained from Experiment 1 show a significant improvement in resolution and clarity when compared to the wide-field images (Figure 3: *Rhizopus*, and Figure 4: Penicillium), which were collected under the same data collection parameters.

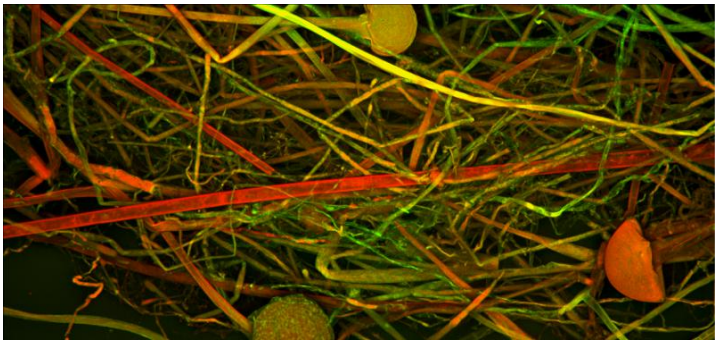

Figure 1. Confocal max projection image of *Rhizopus Sporangia*

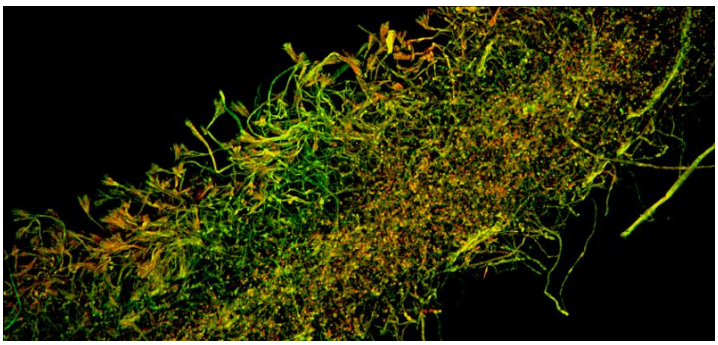

Figure 2. Confocal max projection image of *Pencillium*

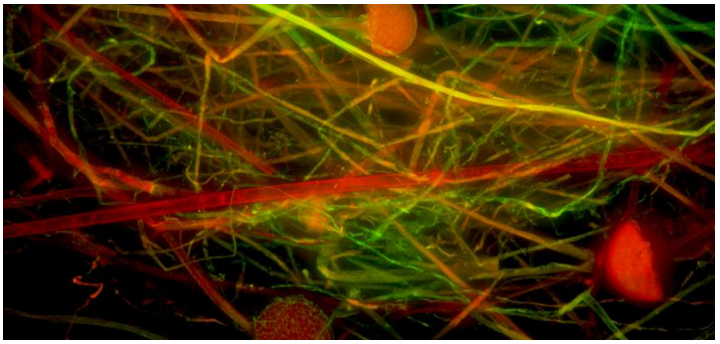

Figure 3. Wide-field max projection image of *Rhizopus Sporangia*

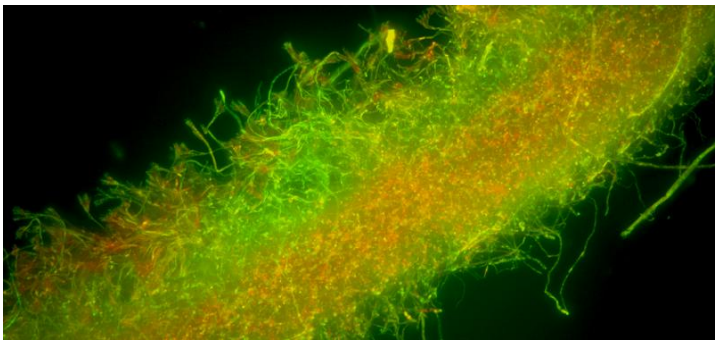

Figure 4. Wide-field max projection image of *Penicillium*

Experiment 2, provided clear and crisp three colour images of the sample (Figure 5 and main front image). These images allowed the easy identification of the species present in the sample and their interactions. The same method of data collection could be easily replicated using a living specimen.

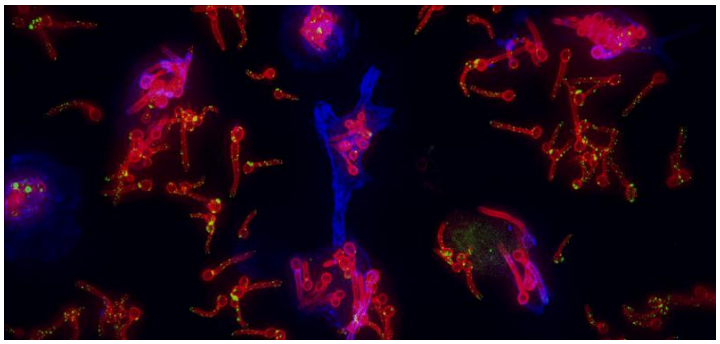

Figure 5. Three channel confocal maximum projection image of macrophage and Candida sample

References

1. Schindelin, J. et al. Nat. Methods 9, 676–682 (2012).

Conclusions

The Aurox Clarity laser free confocal device is an excellent tool for recording high quality 5D (x,y,z,t,λ) confocal images of mycology samples, achieving great optical sectioning, in an easy to use and affordable way.