

## Super-resolution with Clarity<sup>HS</sup>

### Application Note

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Figure 1. Super-resolution laser free confocal image of mouse fibroblast; Clarity<sup>HS</sup> (Inset)

#### Introduction

The Aurox Clarity<sup>HS</sup> was used to record and compare wide-field, confocal and super-resolution confocal (Figure 1) images of NIH3T3 mouse fibroblast.

#### Experimental

A multi-well slide of fixed NIH3T3 mouse fibroblast was prepared where the primary antibody was for  $\alpha$ -tubulin and the secondary was conjugated with Alexa Fluor 488.

The instrument set-up consisted of an Aurox laser free confocal Clarity<sup>HS</sup> device connected to a Nikon Ti-E inverted microscope, using a 63x 1.4 N.A oil immersion objective. The Clarity<sup>HS</sup> was fitted with a CoolLED pE300 Ultra light source, a Hamamatsu Orca Flash 4.0 V3 sCMOS camera and an Aurox GFP-LED filter cube (ex: 466 nm, em: 525 nm).

The wide-field image (Figure 2) was first recorded using the Clarity<sup>HS</sup> with an exposure time of 200 ms. The Aurox Visionary software and Clarity<sup>HS</sup> were then used to automatically record a single channel time series of 100 confocal images at 0.9  $\mu$ m sectioning and 200 ms exposure. The images were then automatically exported to ImageJ / Fiji using the Aurox Visionary one-click export function.<sup>1</sup> The first confocal frame of this time series is shown in Figure 3.

The freely available, open-source Super-Resolution Radial Fluctuations (SRRF) analytical approach, as implemented in ImageJ / Fiji was then applied to the 100 frame time series using default SRRF settings.<sup>2</sup> The resulting laser free super-resolution confocal image is shown in Figure 4.

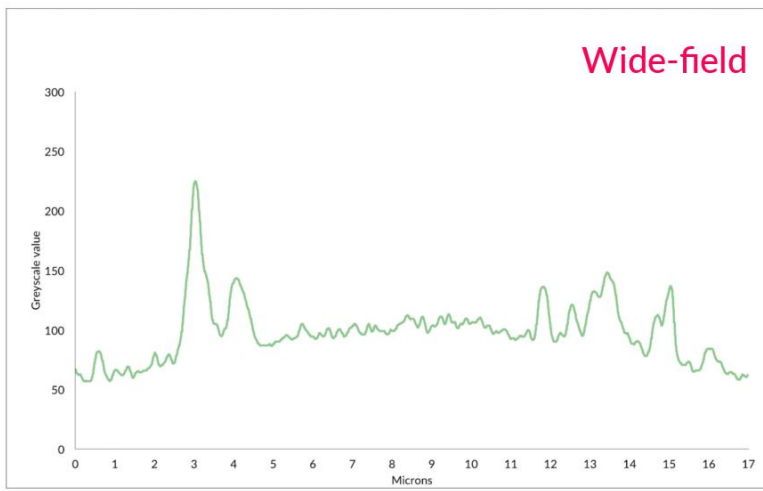
The wide-field, confocal and super-resolution confocal images were then visually compared and the section line profile plots 1-3 were generated across the regions indicated in Figures 2-4.

#### Results

As expected the laser free confocal image shows a significant improvement over the wide-field image with the removal of out-of-focus signal and much enhanced contrast, revealing better defined image structure detail as shown in Plot 2 and Figure 3. The application of the SRRF analysis to the laser free confocal imagery can provide further enhanced resolution and finer image details as shown in Plot 3 and Figure 4.

#### Conclusions

The Aurox Clarity<sup>HS</sup> laser free confocal device is an excellent tool for recording high quality confocal images in an easy to use and affordable way. Simply recording 100 frames of data over a few tens of seconds and using freely available post processing tools; such as SRRF, can provide super-resolution images without the need for expensive and difficult to use confocal microscopy equipment and software.



Plot 1. Wide-field image line profile plot

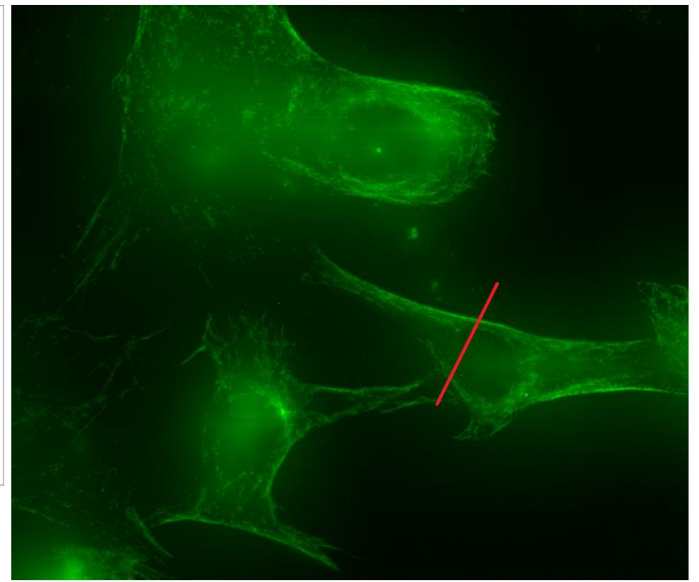
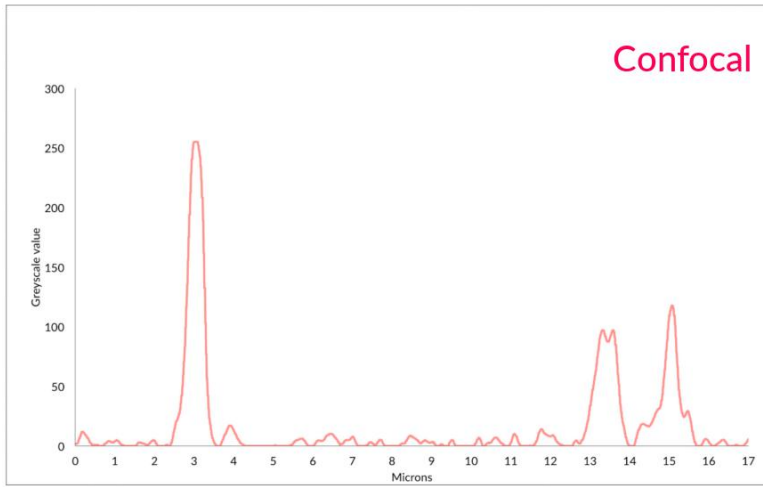


Figure 2. Wide-field image of mouse fibroblast



Plot 2. Laser free confocal image line profile plot

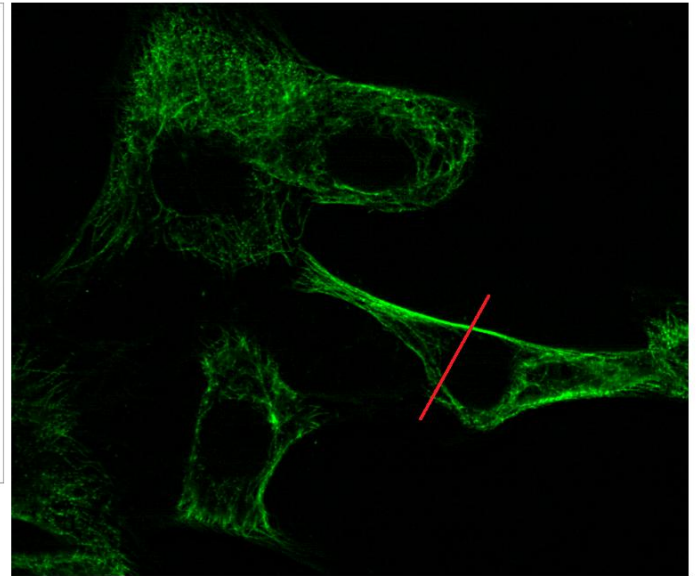
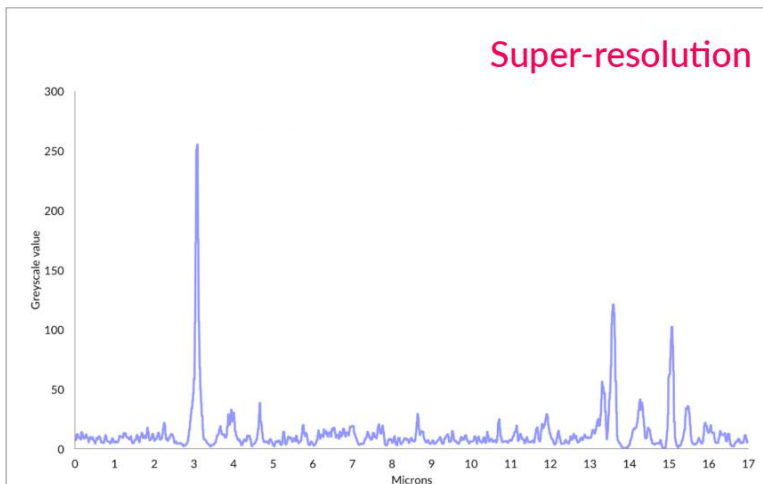


Figure 3. Laser free confocal image of mouse fibroblast



Plot 3. Super-resolution laser free confocal image profile plot

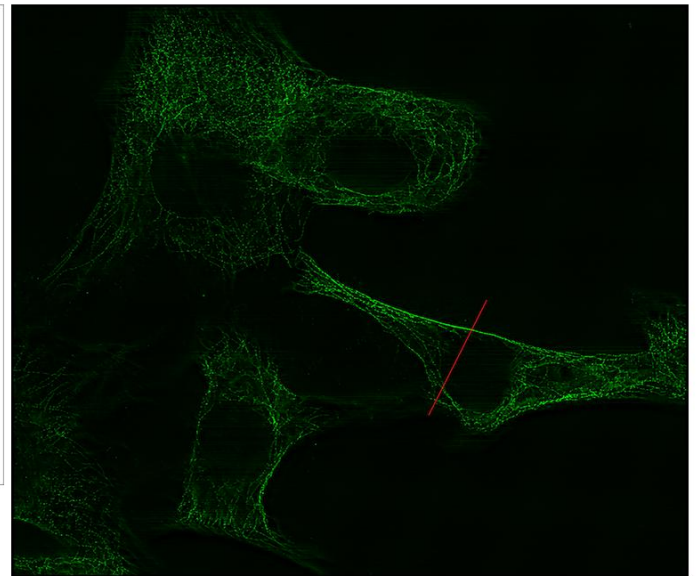


Figure 4. Super-resolution laser free confocal image of mouse fibroblast



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