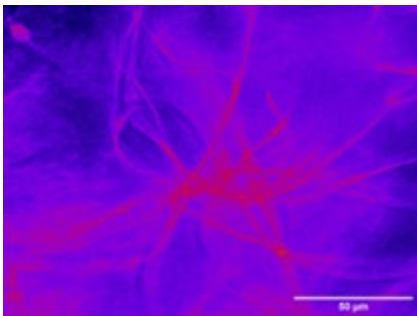


Image Cleanup and Signal Enhancement: A Fiji Workflow for HalolImages

HalolImages may include background gradients and illumination artifacts that can affect image quality. This workflow uses Fiji to efficiently subtract background and enhance contrast to improve visualization. While the steps are demonstrated on 2D projections of HalolImages, they can also be applied to individual channels prior to generating a composite.

Background Subtraction



Z-projection of 15 individual channels acquired from a fibre sample, displayed with the 'Fire' LUT. (Scale bar = 50 μm).

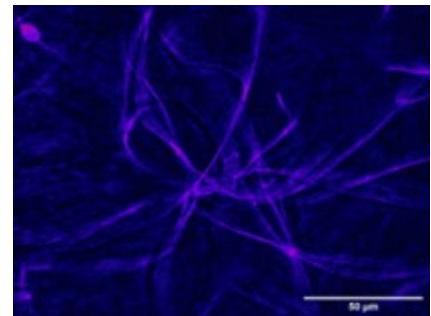
Subtract Background



Process > Subtract Background

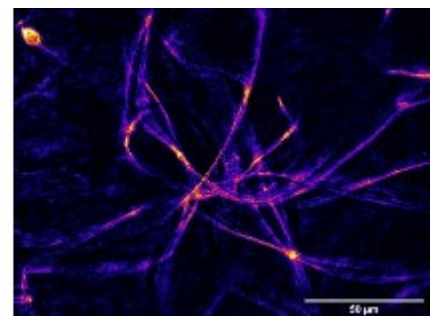
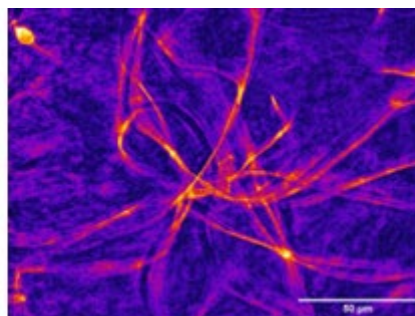
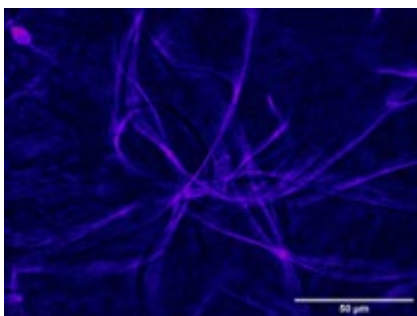
Set rolling ball radius: this value will depend on the size of the structure of interest. **Tip:** for small structures, a lower pixel value is recommended.

Workflow for Background Subtraction.



HalolImage processed using background subtraction with a rolling ball radius of 100 pixels. (Scale bar = 50 μm).

Enhance Contrast



After performing background subtraction (left), the signal can be further improved by enhancing contrast (*Process > Enhance Contrast*). Contrast enhancement can be applied either by normalizing the signal (centre), which performs a linear contrast stretch, or by equalizing the histogram (right), which redistributes pixel intensities non-linearly to achieve a more uniform histogram. For both images the saturated pixels were set at 0.01%. (Scale bar: 50 μm).