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## 🌐 Global Field Intensity Quantification in ImageJ (Fiji)

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ImageJ\_fiji



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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

Global Field Intensity Quantification in ImageJ (Fiji)

This protocol describes how to quantify global field fluorescence intensity from confocal microscopy images using ImageJ (Fiji).

It is intended for analyses where the entire image's fluorescence level is meaningful, rather than segmentation of specific structures

## Troubleshooting

## Global Field Intensity Quantification in ImageJ (Fiji)

- 1 This protocol describes how to quantify global field fluorescence intensity from confocal microscopy images using ImageJ (Fiji).  
It is intended for analyses where the entire image's fluorescence level is meaningful, rather than segmentation of specific structures.  
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**## 1. Open Your Image**
  - Launch Fiji/ImageJ.
  - Go to File **Ⓜ** Open.
  - Load your confocal image (.tif, .czi, .lif, .nd2, etc.).
  - Fiji will usually use Bio-Formats to import multi-channel files.
  
- 2 **## 2. Convert to 8-bit (Optional but Recommended)**  
This standardizes dynamic range across images.  
Steps:
  1. Go to Image **Ⓜ** Type **Ⓜ** 8-bit.
  2. Verify the image values are now 0–255.---
  
- 3 **## 3. Subtract Background**  
Rolling ball background subtraction reduces uneven illumination.  
Steps:
  1. Go to Process **Ⓜ** Subtract Background.
  2. Recommended rolling ball radius: 50–100 pixels.
  3. Ensure "Light Background" is unchecked for typical fluorescence images.
  4. Click OK.---
  
- 4 **## 4. Normalize or Enhance Contrast (Optional)**  
Useful if images were acquired under slightly different brightness conditions.  
Steps:
  1. Go to Process **Ⓜ** Enhance Contrast.
  2. Check "Normalize".
  3. Optionally check "Saturated pixels = 0.1%".
  4. Click OK.---
  
- 5 **## 5. Measure Global Field Intensity**  
Instead of thresholding or masking, here the entire image is measured.  
Steps:
  1. Press Ctrl+A (Select All).

2. Go to Analyze  Measure.

3. In the Results window, note:

- Mean Intensity (most commonly used)
- Integrated Density
- Min and Max Intensity

The mean intensity is the primary metric for global field comparison.

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## 6 ## 6. Export Results

- In the Results window, choose File  Save As... to export in CSV format.

- Repeat the process for all images and compare values in statistical software.

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## ## Notes and Tips

- Ensure identical imaging settings (laser power, gain, pinhole, exposure) for all compared samples.

- Avoid saturated pixels (255 in 8-bit), as they distort intensity measurements.

- Do not threshold or mask unless you intend to exclude background regions.

- If background varies significantly between images, consider subtracting a uniform background

(Process  Math  Subtract).

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This protocol provides a reproducible workflow for full-image intensity measurement in ImageJ/Fiji, suitable for global brightness comparison, imaging quality assessment, or experiments where overall fluorescence reflects biological differences.