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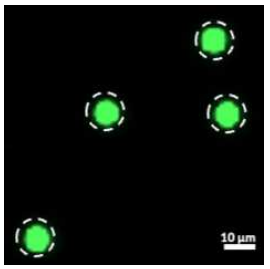
Autofluorescence Microscopy QC for Multimodal Molecular Imaging



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Protocol status: Working
We use this protocol and it's working

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Abstract

Autofluorescence QC is performed prior to collecting on-tissue AF. Fluorescence intensity can be tracked for each fluorescence channel (DAPI, EGFP, and DsRed), allowing the microscope performance and the LED light source to be benchmarked and tracked over time.

Materials

StarLight Calibration Slides (Bang Laboratories) - Glacial Blue (SL1GB), Dragon Green (SL1DG), Envy Green (SL1EG)
Zeiss AxioScan Z1 Microscope

Troubleshooting

Autofluorescence Microscopy QC

1 Fluorescence QC is collected prior to any on-tissue AF collection.

Use fluorescence calibration slides - StarLight Calibration Slides - Bang Laboratories DAPI (excitation-360nm, emission-450nm), Dragon green (excitation-480nm, emission-520nm), and Envy green (excitation-525nm, emission-565nm)

2 Load calibration slides into Zeiss AxioScan.Z1 slide scanner

3 Select the same ~ 25 × 25 mm square region in each calibration slide

Note

To reproducibly sample the exact region each time, the square region can be saved as a "region of interest", which can be imported for each slide.

4 Acquire fluorescence microscopy data for each calibration slide.

Note

Example set-up and relative ranges:
DAPI QC: DAPI (ex. 360 nm, em. 450 nm) Calibration Slide
LED Light: 385 nm
Light Source Intensity: 90%
Exposure time: 20 ms

eGFP QC: Dragon Green (ex. 480 nm, em. 520 nm) Calibration Slide
LED Light: 475 nm
Light Source Intensity: 90%
Exposure time: 60 ms

DsRed QC: Envy Green (ex. 525 nm, em. 565 nm) Calibration Slide
LED Light: 567 nm
Light Source Intensity: 90%
Exposure time: 250 ms

5 Data Analysis:
Once data are collected, background pixels are subtracted, and the average intensity of each channel is calculated and monitored over time.