Feb 01, 2024

EMCCD Gain and Offset Calibration

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx3dpdg8j/v1

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Protocol Citation: Joseph Beckwith 2024. EMCCD Gain and Offset Calibration . **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx3dpdg8j/v1

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

Created: February 01, 2024

Last Modified: May 31, 2024

Protocol Integer ID: 94556

Keywords: ASAPCRN, offset calibration, emccd gain, gain

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000509

Abstract

EMCCD Gain and Offset Calibration

EMGain 0 Camera gain and offset measurement protocol

1 Place a lens tissue on the sample stage above the objective lens to serve as a diffuser.

2 Acquire brightfield mode images from the microscope with a 100ms exposure time, capturing at least 500 frames consecutively under no light. Ensure EM Gain is off.

3 Repeat step 2 with increasing exposure times (at least 5 times, including step 2).

4 Save the images taken under different exposure times in the 'camera_calibration' folder in .tif format and prefix the file names with 'exposure_'. For instance, the first image captured under no light should be named '0_filename', and the second image taken with slightly increasing exposure should be named '1_filename'.

5

EMGain on Camera gain and offset measurement protocol

6 Acquire brightfield mode images from the microscope with a 100ms exposure time, capturing at least 500 frames consecutively under no light. Ensure EM Gain is set to the setting of interest (*e.g.* 250).

7 Repeat step 2 with increasing exposure times (at least 5 times, including step 2). Ensure no pixels are above/at saturation.

8 Save the images taken under different exposure times in the 'camera_calibration_EMGain_N' folder in .tif format and prefix the file names with 'exposure_'. For instance, the first image captured under no light should be named '0_filename', and the second image taken with slightly increasing exposure should be named '1_filename'.

Gain calculation

9 **Use the GDSC-SMLM plugin for imageJ to calculate gain at no gain, ("Mean-Variance Test") to calculate camera gain.**

Then, using this gain parameter, use the "Mean-Variance Test (EM-CCD)" function to calculate the overall game and offset.