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## 🌐 EMCCD Gain and Offset Calibration

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### ABSTRACT

EMCCD Gain and Offset Calibration

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**Protocol status:** Working

We use this protocol and it's working

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## 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.**