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# 5. Analysis - Characterization of the Photon Conversion Factor, Noise, and Dynamic Range

In 1 collection

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## Abstract

The aim of this analysis is to obtain various calibration metrics from inhomogenous calibration images using the photon transfer method (**McFadden et al., 2022** and **Heintzmann et al., 2016**). The analysis is not completely automated, and relies on users to interpret the data, identify problems and scrutinize the results. It is generally unaware of the underlying detector technology, which can affect the results.

This guide therefore intends to provide an overview of the method, the choices that a user must make, how to interpret the data, and common pitfalls that should be avoided. Note that the method significantly differs from other calibration methods that use varying light levels over time (**Mullikin et al., 1994**, **van Vliet et al., 1998**, and **Murray et al., 2013**). It necessitates different definitions and the results will also differ. The algorithm is based on that which is described in the supplementary material of **McFadden et al., 2022**. A more detailed overview of the photon transfer method can be found in *Photon transfer* (**Janesick, 2007**).

## Guidelines

Please refer to protocol "**1. Introduction - Background and Aims**" of this collection for a more detailed description of a detection system, its parameters and various aims for performance monitoring.

This protocol is part of a collection of protocols developed by QUAREP-LiMi WG2 for characterising detection system performance.

## Materials

"gui_calibration_tool" (https://github.com/bionanoimaging/NanoImagingPack/releases, by D. McFadden)

## Before start

Acquire suitable inhomogenoeus calibration images following protocol "**3. Data Generation - Systems with an Area Detector**" or "**4. Data Generation - Systems with a Point Detector**" of this collection.

## Introduction

1 This protocol describes how to analyze a stack of inhomogeneous images using the python script **gui calibration tool from D. McFadden**. The analysis uses the photon transfer curve method (**Janesick, 2007**, **Mullikin et al., 1994**) to calculate the photon conversion factor (PCF), the readnoise, and the dynamic range of a detector, as well as various other calibration and quality metrics (**McFadden et al., 2022** and **Heintzmann et al., 2016**). Acquisition of suitable images is described in **protocol 3** and **protocol 4** of this collection. The analysis applies to images captured on both point detectors and area detectors. The protocol includes quality criteria for the results.

Note

The photon transfer method calculates the photon conversion factor (in units of electrons/ADU) from the photon shot noise.

With electron-multiplying detectors, e.g., photo-multiplying-tubes (PMTs, Art, 1990), electron-multiplying CCDs (EM-CCDs, **Ryan et al., 2021** and **Plakhotnik et al., 2006**), intensified CCDs (iCCDs), avalanche photodiodes (APDs), and single-photon avalanche diodes (SPADs), the photon transfer method does not yield the *physically* correct photon conversion factor. This is due to the "multiplication noise", which masks the photon shot noise (**Cho et al., 2006** and Art, 1990).

With an incorrect photon conversion factor, the total yield of photoelectrons will also be false, the method cannot yield a correct quantum efficiency.

Despite this, the photon-transfer method can be useful for electron-multiplying detectors, as it can quantify the noise in terms of "effective photoelectrons", or "noise-equivalent-photoelectrons", which can be useful for quantifying imaging quality.

Other caveats are discussed in **McFadden et al., 2022**, supplementary materials.

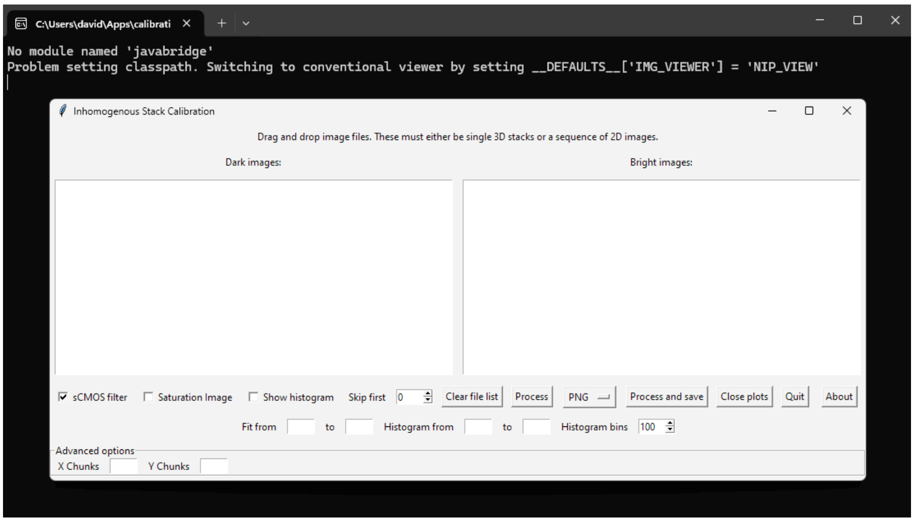
Note

In addition to the photon conversion factor, we also estimate the readnoise (mean and median) and calculate the dynamic range using the photon transfer method. An example is shown in the third note of step 7.

## Basic usage

2 Locate the file "gui_calibration_tool.exe" and run it in Windows. The default main window should appear.

Note




The GUI (foreground) and the command prompt window (background) of the analysis program.

Note

The software can be downloaded from Github, **gui calibration tool from D. McFadden**. Decompress the archive. The program "gui_calibration_tool.exe" is found in one of the sub-folders. Refer to the included "readme.txt" file for the most current documentation.

3 Locate the image data.

Note

The image data should consist of two time series of 2D images:

- bright image series of a non-fluctuating calibration image (see the acquisition protocols for details).
- dark images series, with all light sources switched off and environmental light eliminated

Note

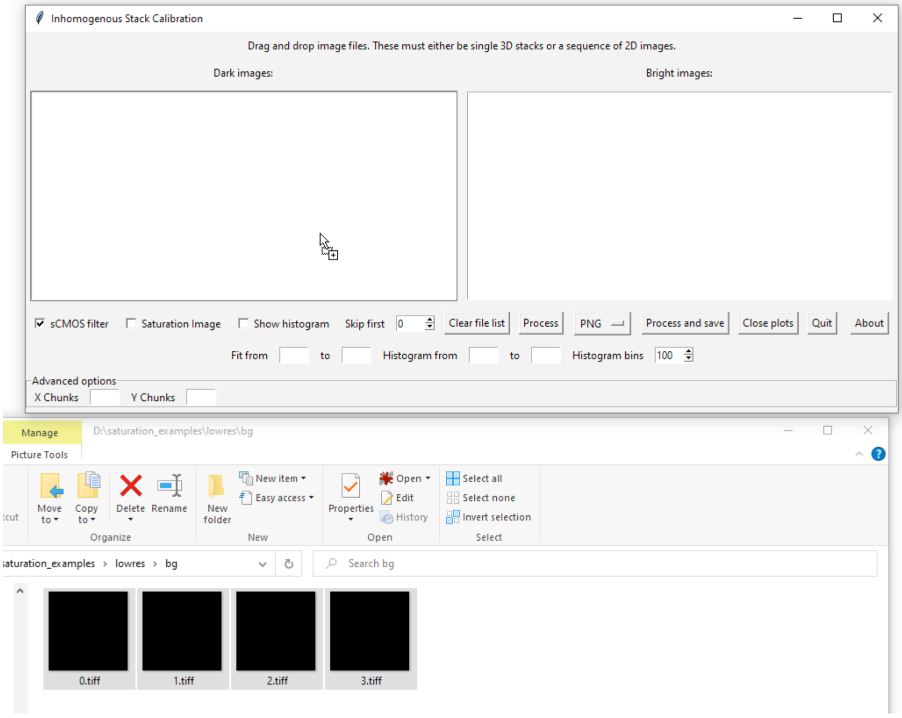
At present, the gui_calibration_tool can read TIFF and .npy files. Other formats, such as manufacturer specific file formats, should be first converted to (multi-page) TIFF files.

Note

The data must be in a lossless raw image format for the calibration to be successful. In particular, the common viewable image formats JPEG (due to lossy encoding) and PNG (RGB encoding rather than raw pixels) are not suitable. If a file format (such as TIFF) supports both lossy and lossless compression, then only lossless compression (such as LZW) should be used.

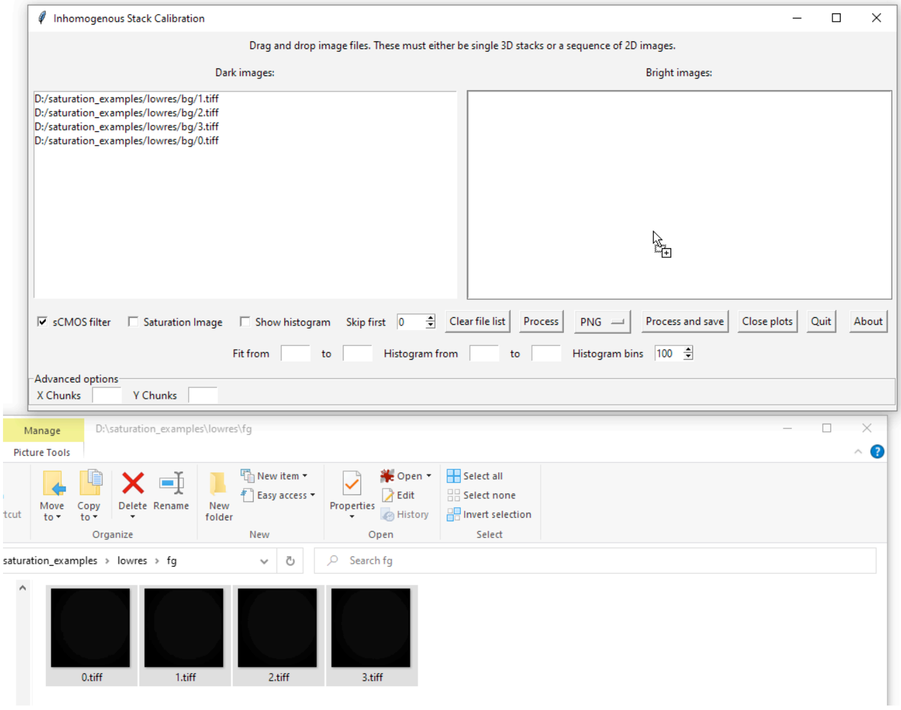
4 Drag and drop the file(s) for the dark series into the left box, and the bright series file(s) into the right box.

Note




Drag and drop the dark image data into the analysis program.

Note




Drag and drop the bright image data into the analysis program.

Note

The dark and bright series can consist of either multiple 2D files or a single 3D (multi-page) file. Mixing 2D and 3D files will likely result in an error.

5 Edit the options in the main window according to your preference. This may necessitate some adjustments, as outlined in the following steps.

Note

If the bright images have saturated pixels, check the **"Saturation Image" option**. The analysis will calculate the linear range and detector capacity. Note, data acquired using protocol *4. Data Generation - Systems with a Point Detector* generally produces saturated images.

Do not use the "Saturation Image" option for images without saturated pixels, since the linear range and detector capacity analysis will be invalid.

Please refer to step 8 for image saturation evaluation. go to step #8

Note

The **"sCMOS" option** improves the analysis results for CMOS-type image sensors.
CMOS sensors typically suffer from "popcorn noise", which results in a small fraction of excessively noisy pixels. This option attempts to identify and exclude excessively noisy pixels from the data.
For other detector types or if the image histogram exhibits very low values, this option should not be used since it may affect the analysis results negatively.

6 Process the data.

Note

Processing large datasets takes a long time, in which the program is unresponsive. The available RAM must be many times larger than the dataset that is analyzed.

6.1 Click "Process" and wait for the plots to appear.

6.2 Inspect the results, adjust the options as needed as explained in Steps go to step #8 go to step #9 go to step #10 go to step #11 , and press "Process" again to repeat the calculations

7 To save the results, click "Process and save".

Note

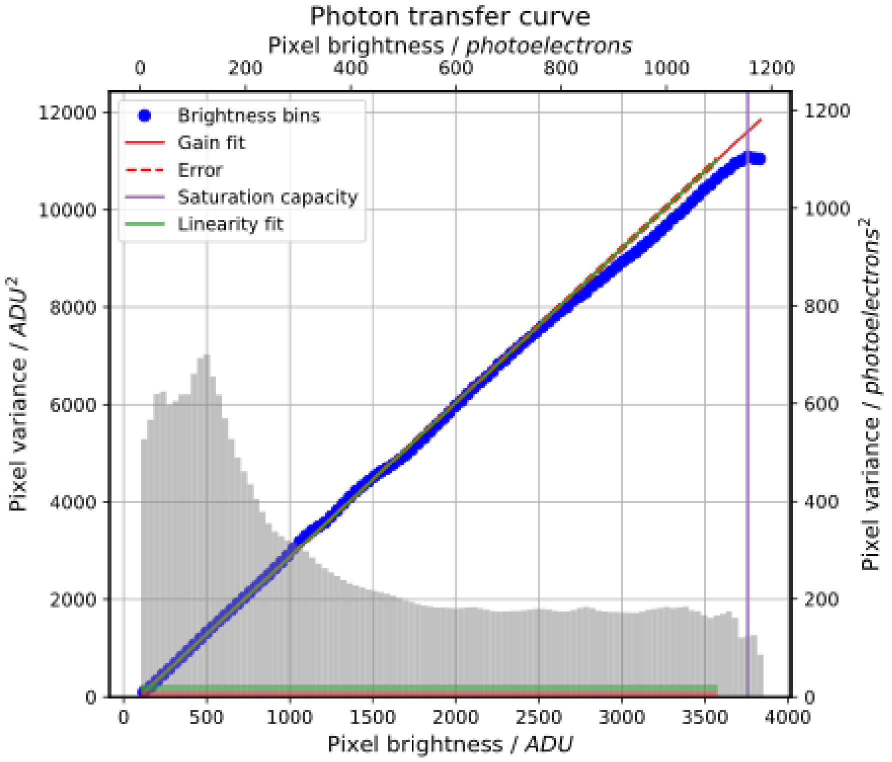
The script will show the "Save As" dialog in which you can specify the folder where to store the analysis results. The saved data consists of images of the graphs, a YAML text file with the analysis results, and the file 'definitions.txt' with more detailed explanations of the metrics.

Note

The saved result files have a fixed name and the script will overwrite existing files without notice. Specify a dedicated folder for each data-set and confirm that the folder is empty or that it is OK to overwrite the existing files.

Note

This is an example of how the results look like, see the image of the photon transfer curve and the results below.




Example of the Photon_Calibration.png file with the Photon Transfer Curve (blue), Linear Fit (green) and Saturation capacity indicator (purple).

```
# Results from calibration tool for inhomogenous image stacks.
# See definitions.txt for extended definitions.
# The results are in the YAML format, readbale by humans and machines
# Written by David McFadden, FSU Jena
# NanoImagingPack library: https://gitlab.com/bionanoimaging/nanoimagingpack/
# Questions, bugs and requests to: david.mcfadden777@gmail.com
# heintzmann@gmail.com

Dark images: '60'
Bright images: '60'
Illumination fluctuation: 0.08%
Hot or Cold pixels excluded: 0 (0.0%)
Bright image dynamic range [factor]: '153.7'
Noisy pixels excluded: 2%
Noise exclusion threshold [e-]: '3.7'
Background [ADU]: '100.88'
Gain [e- / ADU]: '0.3157'
Readnoise, RMS [e-]: '1.89'
Readnoise, median  [e-]: '1.65'
Detector dynamic range [factor]: 578, 27.6
Detector dynamic range [power dB]: '27.6'
Detector dynamic range [root-power dB]: '55.2'
Saturation value [ADU]: '3758'
Saturation capacity [e-]: '1154'
Linearity Error: 2.0%
Fixed pattern offset std. [e-]: '1.16'
```

Example of the image_calibration.txt file with the analysis results.

```
# Extended definitions for calibration results.
# Written in the YAML format, readbale by humans and machines
# Written by David McFadden, FSU Jena
# NanoImagingPack library: https://gitlab.com/bionanoimaging/nanoimagingpack/
# Questions, bugs and requests to: david.mcfadden777@gmail.com
# heintzmann@gmail.com

Dark images: Number of dark images used for calibration
Bright images: Number of bright images used for calibration
Illumination fluctuation: "Illumination fluctuation for bright images. The fluctuation
  in the total amount of light between the bright frames. If this value is high, it
  suggests an unstable light source or possibly problems with the detector's acquisition. "
Hot or Cold pixels excluded: Total number of excluded hot or cold pixels. Pixels are
  considered hot or cold if their mean value in the background image is more than
  4 standard deviations removed from the background values of all background image
  pixels.
Bright image dynamic range [factor]: Bright image dynamic range, expressed as a factor.
  It represents the difference between the darkest and brightest parts of the bright
  calibration images. Above 20 is good.
Noisy pixels excluded: Percentage of pixels which were excluded. By default, 2% of
  the noisiest background pixels are excluded from the analysis. This deals with RTS
  noise in CMOS cameras. CCD and scanning imagers should be unaffected.
Noise exclusion threshold [e-]: Threshold for exclusion for noisy pixels. Units are
  electrons.
Background [ADU]: The background, or black level of the signal, obtained by the mean
  pixel value of the dark exposures. Units are ADU.
Gain [e- / ADU]: Conversion factor (Gain) determined by fit to photon transfer curve.
Readnoise, RMS [e-]: The mean readnoise, calculated by multiplying the mean of the
  individual pixel standard deviations with the gain. Units are photoelectrons.
Readnoise, median  [e-]: The median readnoise, which is a commonly quoted metric for
  CMOS cameras. Units are electrons.
Detector dynamic range [factor]: Electron dynamic range of the detector (Jannesick,
  Photon transfer, p. 50). Expressed as a dimensionless factor.
Detector dynamic range [power dB]: Electron dynamic range of the detector. Expressed
  power dB
Detector dynamic range [root-power dB]: Electron dynamic range of the detector. Expressed
  as root-power dB, which is twice the power dB value. Commonly used for video cameras.
Saturation value [ADU]: Value at peak of photon transfer curve. Units are ADU
Saturation capacity [e-]: Saturation capacity in units of electrons
Linearity Error: Mean absolute deviation from fit within linearity fit range.
Fixed pattern offset std. [e-]: Gain multiplied with standard deviation of the background
  mean projection. Units are electrons.
```

The definitions.txt file with the explanation of the calculated metrics (adapted from **EMVA1288** and Janesick, 2007).

## Interpretation of the Photon Transfer Curve

8 **Photon Transfer Curve**

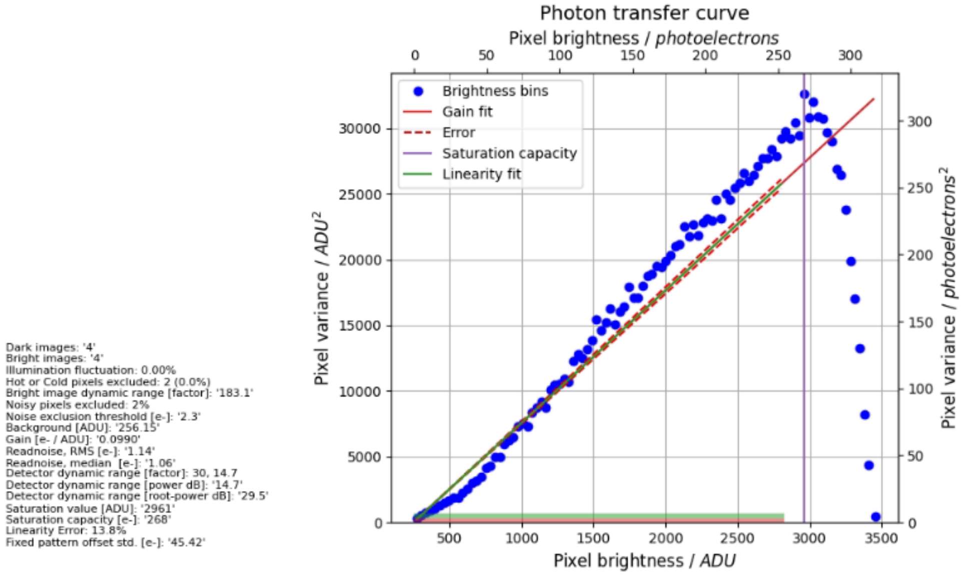
The Photon_Calibration.png image shows the mean-variance photon transfer curve: the Pixel Variance (noise) of the pixels against their mean Pixel Brightness. The slope of the curve is the Photon Conversion Factor.

What additional features the analysis can calculate depends on whether the captured bright image series has saturated pixels or not. A Photon Transfer Curve graph of images with saturation shows a peak followed by a drop-off. In that case you can select the 'Saturation Image' analysis option and the results and graph look like as described in step 8.1.

If the images do NOT have saturated pixels, the 'Saturation Image' option should not be selected. The analysis graphs for such images is described in step 8.2.

8.1 **Saturation Level Image Series**

The photon transfer curve of saturation-level image series initially resembles a line which reaches a peak (the saturation level) and quickly drops off. The purple line shows the saturation capacity.  If the purple line is not present, select the "Saturation Image" checkbox and re-process the data.




The purple vertical line shows the peak of the curve, which is the estimated saturation capacity. Unless manually specified otherwise, the fit range is selected relative to this value.

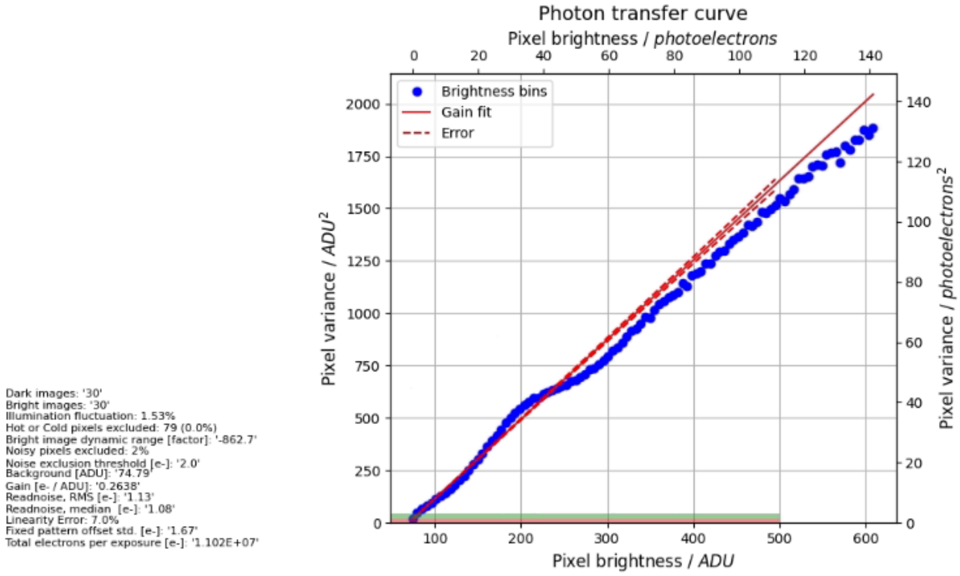
> Note
>
> With a saturation level image, the algorithm will calculate a linear fit for the data between the origin and 95% of the curve peak location. This produces information on the dynamic range of the detector. But the saturation causes loss of photometric information, which is why the total number of photo-electrons (which would enable calculating the quantum efficiency) cannot be shown.

## 8.2 Arbitrary Brightness Image Series

If the data was acquired well below the saturation level of the camera, then there will be no obvious peak in the curve. The curve will typically start off as a line, and then either end abruptly or diverge. In this case, there is no obvious reference for the fitting range. By default, the tool selects a range that depends on the structure and statistics of the images.

Ensure that the fit range only contains "good" (non-diverging) data. If necessary, choose a range to fit for manually and enter the values into the corresponding fields in the main window (remember to re-process the data by clicking "Process").

A manual range selection is also useful for repeatability and monitoring (**Aim 2**).




Except for the small "bump" at a value of 200 ADUs, the plot appears to be fairly linear. A manual fit range between 0 and 500 or 600 ADUs therefore seems sensible.

Note

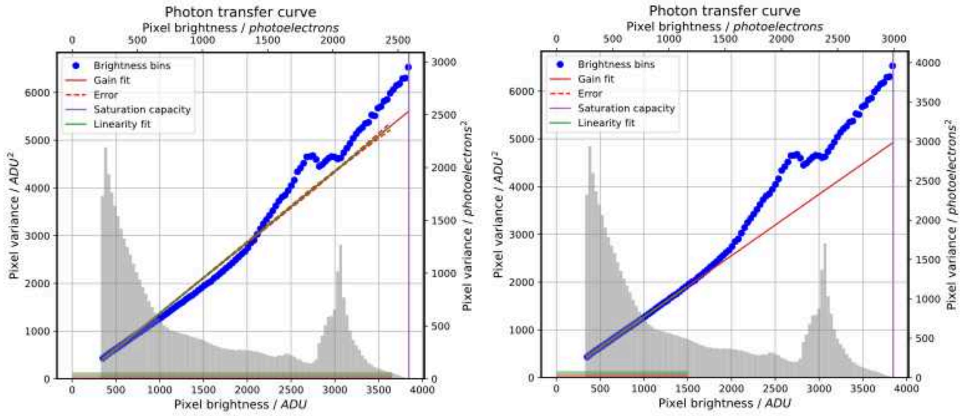
If the curve reaches a peak on the right side of the plot and quickly drops off, this indicates that the detector was partially saturated. (c.f. saturation-level analysis go to step #8.1). In this case, the radiometric data ("electrons per exposure") will be false. Use either the saturation-level analysis, or re-acquire the data with a lower exposure.

Note

If the illumination was below the saturation level, then it is possible to specify the total number of photo-electrons in the frames. If the photometric flux of the light incident on the detector is also known, then it is possible to calculate the quantum efficiency of the detector.

9 **Detector with correction for non-linearity**

Some detectors implement a correction for pile-up effects or other non-linear responses of the detector hardware. The correction will affect the noise characteristics of the data, which interferes with the estimation of the photon conversion factor (PCF) from the noise. For an optimal PCF calculation, the fit range should be limited to the brightness range that is not affected by the correction.




The photon transfer curve of a detector that implements pile-up correction over 1500 ADU. As a result of the correction, the graph shows a higher noise level in the data for intensities over 1500. The PCF should be determined from the intensity range that is not affected by the corrections. The left-hand figure shows the linear fit over the full range. In the right-hand figure, the fit range is limited to the ADU range 0, 1500.

## 10 Deviation from Linearity and Noise Level

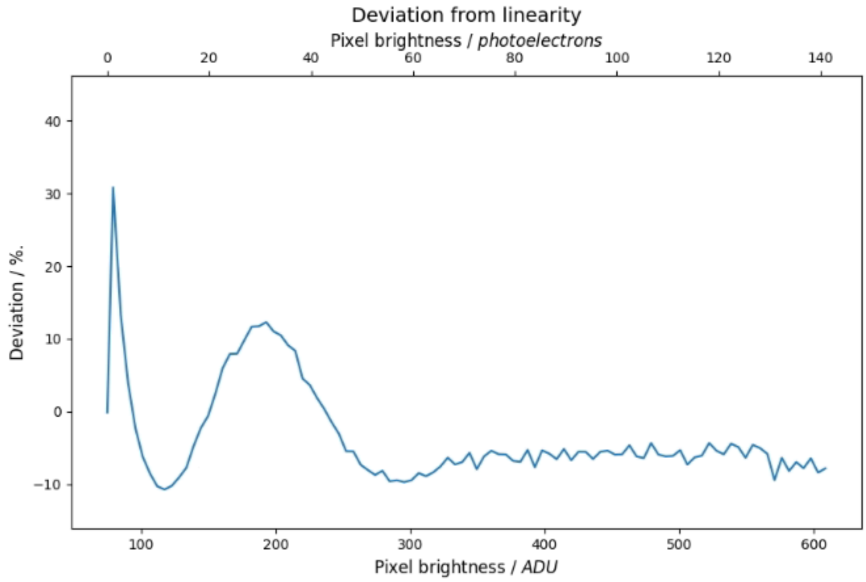
The slope of the fit of the photon transfer curve (PTC) corresponds to the photon conversion factor (PCF). The PCF is used to calculate the electron-equivalent noise level. It is therefore crucial for the noise level calculation to find a satisfactory curve and fit. When the fit does not seem to match well with the linear range in the PTC plot, specify the linear range as fit range and restart the calculations. This will change the PCF and the noise level results.

Note

Photon transfer curves can (and typically will) deviate from the ideal of a straight line. This may be the expected behavior for the detector, or indicate a problem with the acquisition or a bug in the processing.

The photon transfer method can be used on a broad range of detectors, but the algorithm cannot guarantee "correctness". The method ultimately relies on the user to interpret the quality of the results.

It is beyond the scope of this protocol to provide a complete discussion of the photon transfer method. But at least within the fit range, it is possible to plot the deviation from the fit, shown in the "deviation from linearity plot".

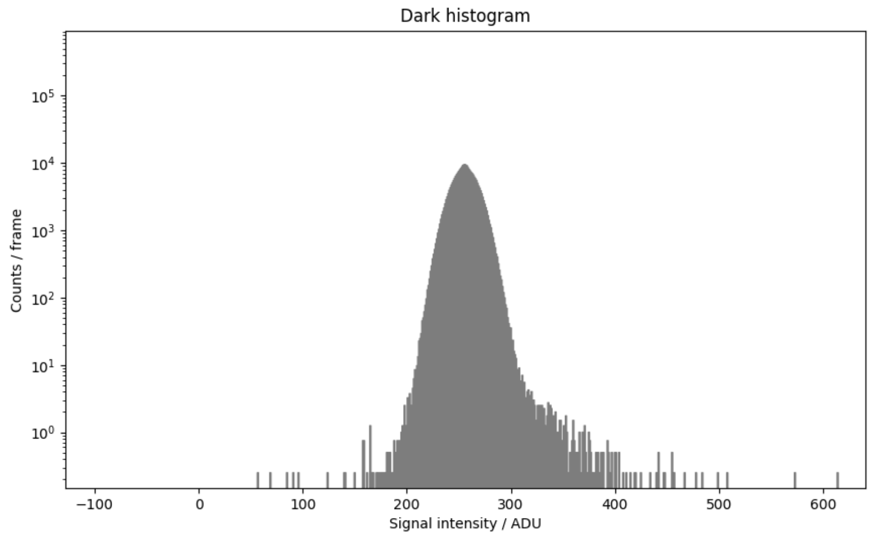



The linearity deviation plot from the above photon transfer curve. At the "bump", the linearity fluctuates by as much as 20%.

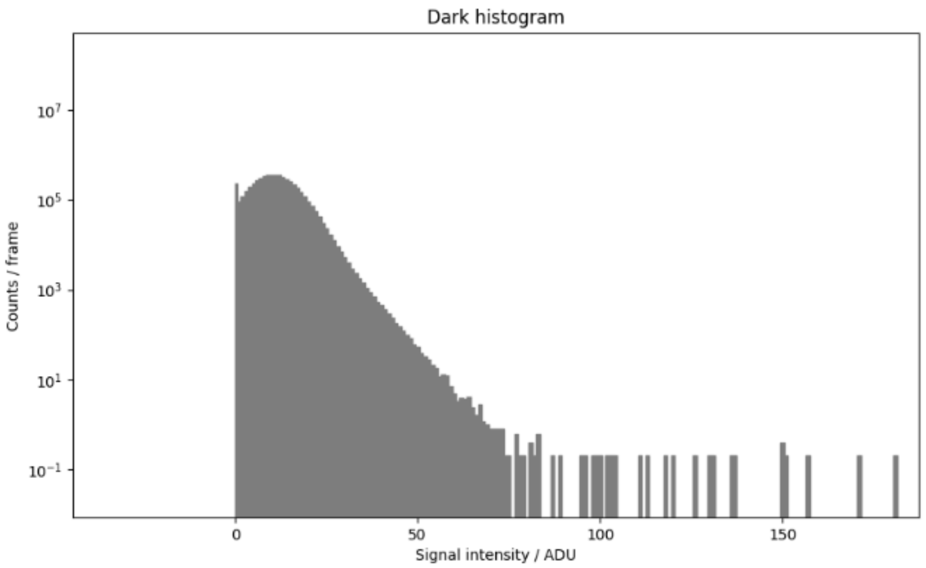
## Detecting problems in image acquisition settings

11 The generated graphs allow for checking if the dark and bright image series were captured with correct conditions and settings. If one of the checks below fails you will need to record new images with improved acquisition settings.

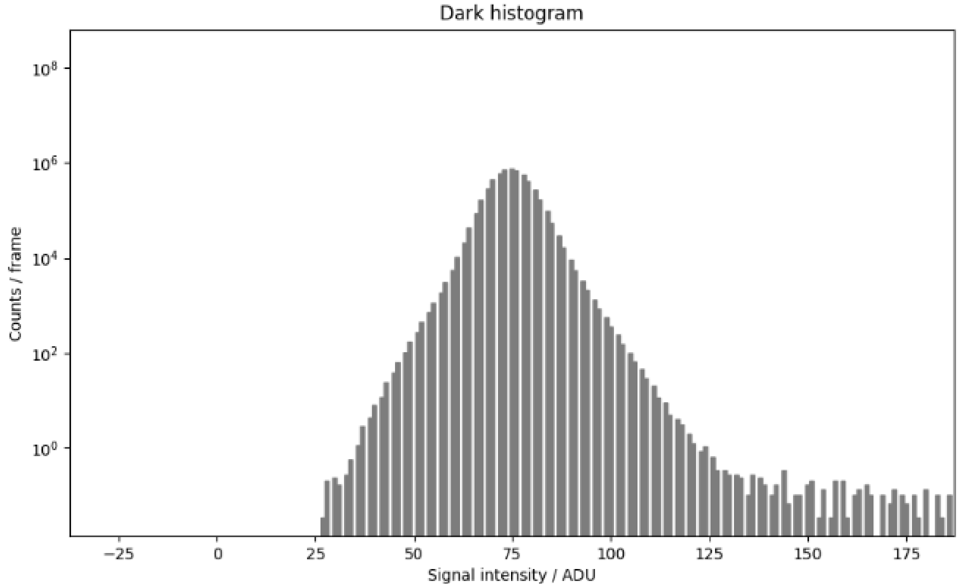
11.1 **Dark level clipping, missing grey-values**
The dark_histogram.png file shows the histogram of the dark image series. Ensure that there is no clipping of the background signal by looking at the dark histogram. The graph should resemble a bell-shaped curve with no zero values. The graph should be continuous with no missing intermediate grey values in the center.




Good example: This plot shows a good distribution for the dark histogram.



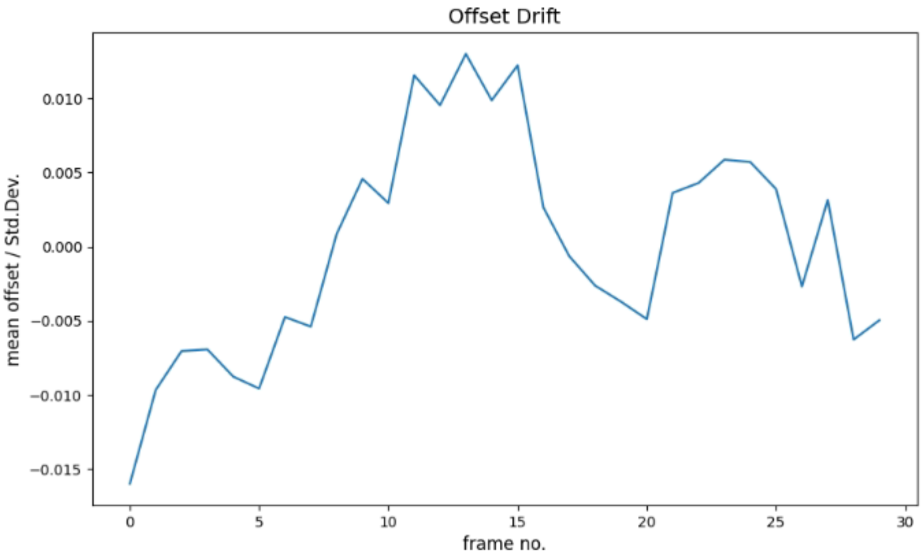

Bad example: It is apparent in this plot that the dark signal is being clipped at the value zero. This biases the results and falsifies the noise estimation. To resolve this issue, change the setting for the detector dark level.




Gaps in the histogram may indicate that a multiplication ("digital gain") was applied during acquisition. Change the detector digital gain setting to 1x to resolve this problem.

## 11.2 Offset Drift

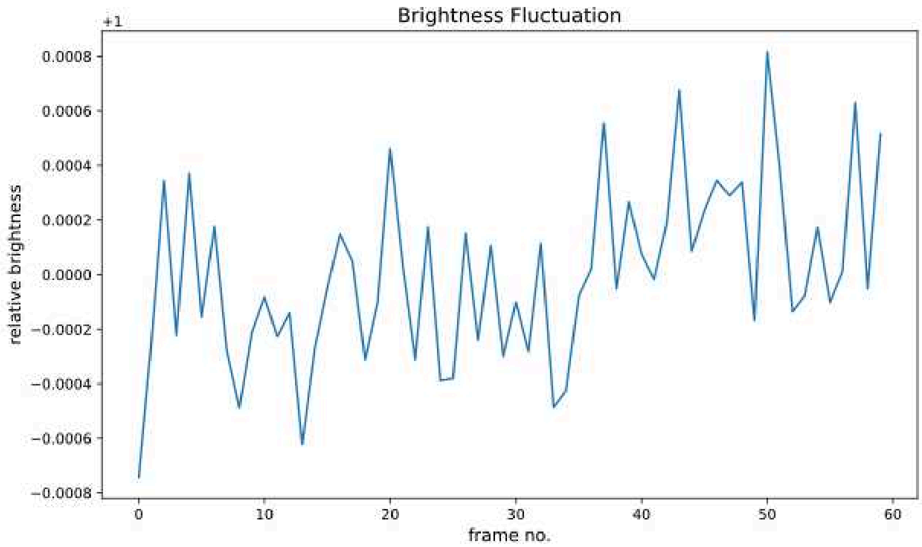
The correctOffsetDrift.png plot shows the average offset of the dark series over the time series. This should be flat. Noticeable drift or fluctuations compromise the data quality. A common cause of noticeable drift is sensor cooling issues. If the graph shows a significant trend or fluctuations, wait until the detector internal temperature has stabilized and recapture the data.




This offset graph shows that the offset was very stable.

## 11.3 Brightness Fluctuation

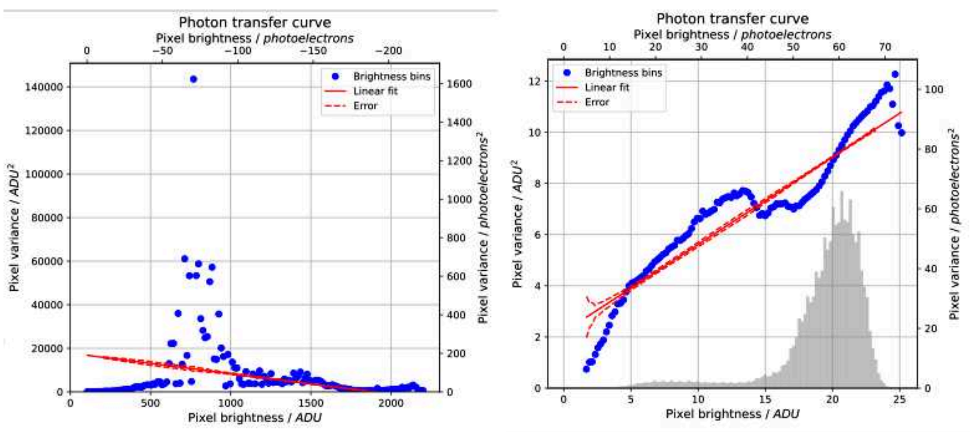
The Brightness_Fluctuation.png graph (below) shows the fluctuation in the total light of the bright image series. The fluctuations should be below 0.01. Bleaching of the sample as well as instability of the light source or sensor cooling are common causes of drift and fluctuations. When the brightness fluctuations graph shows a significant trend or fluctuations, try to identify the origin of this problem and recapture the data with more stable conditions.




This Brightness Fluctuation graph shows that the images were taken under very stable conditions.

## 11.4 Low histogram counts

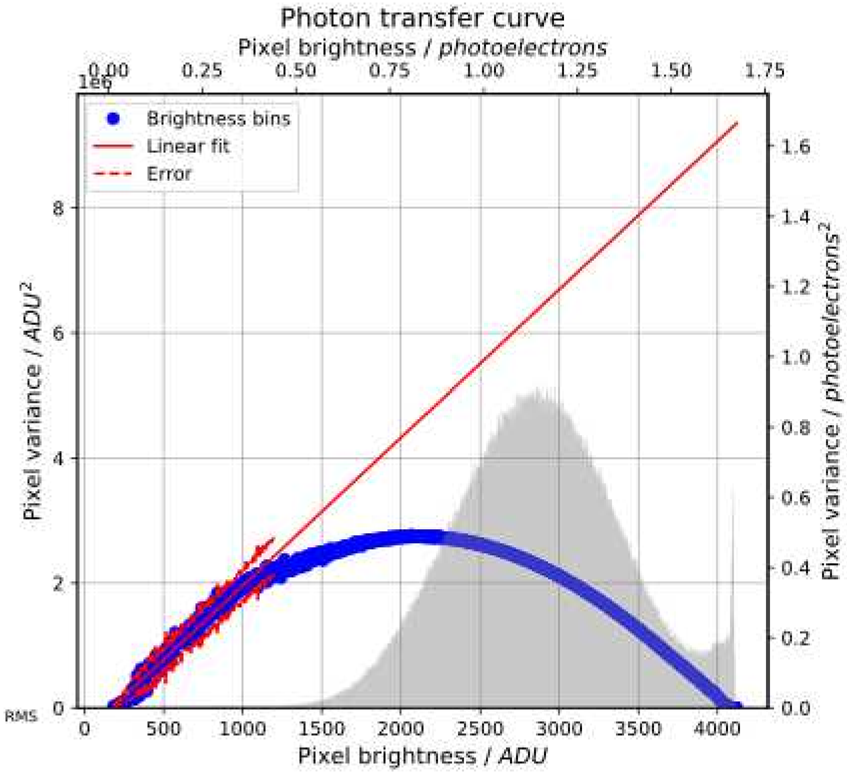
The analysis requires many pixel brightnesses within the histogram bins to have good statistics for the curve. When this is not the case, the photon transfer curve might diverge or exhibit other strange behaviours. This can be alleviated by changing the sample (e.g. defocussing the edge and creating a smoother transition), acquiring a  longer image series in order to improve the statistics, or ignoring the affected part of the curve by specifying a manual fit range.




These photon transfer curves do not show a straight line.

## 11.5 Early Saturation

Most point detectors will feature a non-linear response when the gain is set too high. This will be reflected in a curved PTC graph.

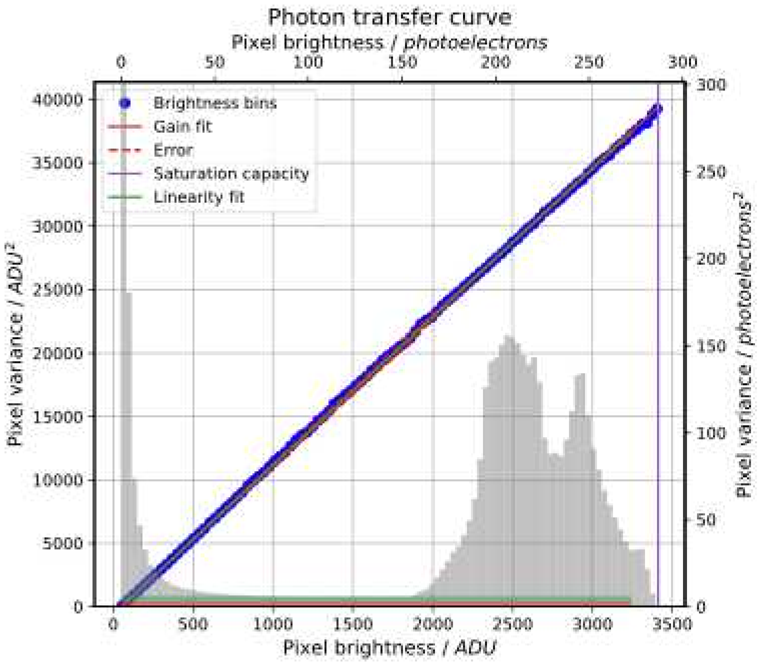



This photon transfer curve is from an acquisition where the photomultiplier gain was set too high, limiting the linear range to the first 1000 grey values. A more moderate gain setting was able to alleviate the issue.

## 11.6 **No Saturation, despite the option being selected**

With a saturation level image series, it is possible to calculate the dynamic range and full well capacity of the detector. However, if this option was selected in the tool and the images are not exposed at the saturation level, then the results will be falsified.

Saturation level curves are characterized by a clear peak in the curve at the right of the graph, after which the curve drops off rapidly. If this is not the case, re-acquire the images at a higher exposure level




The blue curve does not drop off after the peak, indicating that the saturation capacity and dynamic ranges values will be wrong.

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