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1. Introduction - Background and Aims

In 2 collections

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Disclaimer

This protocol collection was developed by members of **Working Group 2 "Detection System Performance"** of the international consortium **QUAREP-LiMi.**

The Consortium for Quality Assessment and Reproducibility for Instruments and Images in Light Microscopy (QUAREP-LiMi), formed by the global community of practitioners, researchers, developers, service providers, funders, publishers, policy makers and industry related to the use of light microscopy, is committed to democratizing access to quantitative and reproducible light microscopy and the data generated by it.

This protocol collection has undergone the internal approval process of QUAREP-LiMi.

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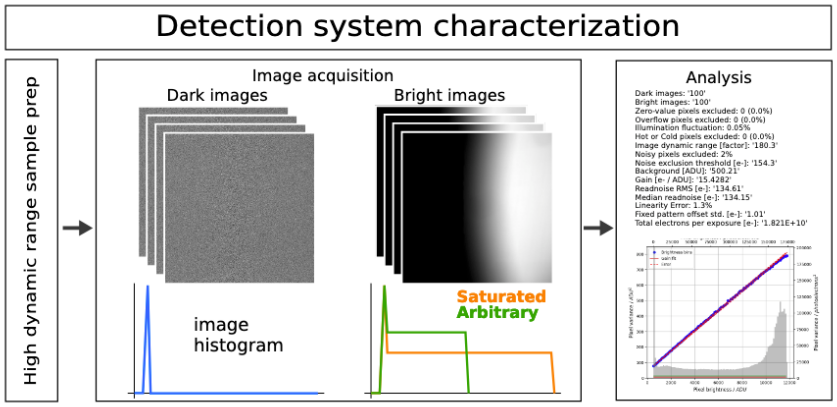
Abstract

This protocol is the introduction to the protocols collection "Characterization of the Photon Conversion Factor, Dark Noise, and Dynamic Range of Light Microscope Detection Systems". Here we describe in more detail the mission statement of QUAREP-LiMi Working Group 2, different aims of detection system characterization, a short guide to the protocols in the collection, and detection system basic theory underlying the protocols in this collection.

Guidelines

For sample preparation, measurement, and subsequent analysis, follow this workflow scheme:
- prepare sample
- identify detection system type (area or point)
- identify settings for "bright" images, measure
- measure "dark images" with same settings as for the "bright images"
- analyse



Safety warnings

Ensure you follow general lab safety guidelines for radiation sources and chemicals as outlined within your organisation.

Laser safety and regulations

- Please refer to the documentation provided by the manufacturer for additional warnings and preventive, protective equipment (PPE) requirements (e.g. laser safety goggles). Always consult your local Laser Safety Officer or Radiation Safety Officer and refer to your laboratory safety documentation for more information.
- You can also consult your Laser Safety Standards ANSI Z136 in North America, SUVA 66049.D in Europe, and BS EN 60825-1 in the UK. Additionally, laser safety standards and regulations are covered by IEC norm 60825-1, and LED eye safety standards and regulations are covered by IEC norm 62471 in Europe.

Safety information

Hazardous, visible, or invisible radiation from lasers, lamps, and other light sources used for microscopy **can cause permanent damage to the retina, skin burns, and fire**. Always follow proper laser safety protocols for your equipment and situation.

Mission statement

1 The Consortium for Quality Assessment and Reproducibility for Instruments and Images in Light Microscopy (**QUAREP-LiMi**) Working Group 2 (**WG2**) focuses on the "detection system", which acquires light intensity signal from the sample. Our goals are to standardize characterization of detection system performance and to establish guidelines for monitoring its performance over time.

2 To standardize characterization of detection system performance, we define universal, externally measurable parameters applicable to any type of detector, together with methods for retrieving these parameters from common detector types. These universal parameters are split out into the specific detector-type (cameras, photomultiplier tubes, ...) 'internal' parameters, which have already been defined by the community. These universal parameters allow to evaluate different types of detection systems and thus help with the choice of the most suitable technology for an application.

3 The measurement protocols will enable the monitoring of detection system performance over time, helping to identify potential faults or performance degradation. Combined with standards developed by
other QUAREP-LiMi working groups, this will contribute to improved data reproducibility, both within individual laboratories and between collaborating institutions.

Aims

4 Following our mission statement, characterization of the microscope detection system can follow different aims. In the following protocols, we will refer to three different contexts:

4.1 **Aim 1 - experiment quality control**. Characterize the microscope performance using detection settings that match the experiment.

Note

The goal is to calculate the photon conversion factor, the dark noise, and the dynamic range of a detector, as well as various other calibration and quality metrics for each individual experiment. This will enable the user to convert intensity values to number of photons for each individual experiment. In addition, the user obtains the offset and noise characteristics of the measurements.

The characterization results can help interpret the raw data correctly, yielding quantitative results. They can also help estimate noise and the statistical relevance of the experiment.

4.2 **Aim 2 - instrument quality control.** Monitor the microscope performance over time and identify possible service issues, to maintain a constant and high level of image quality.

Note

Microscope settings should be chosen such that changes in detection system performance can be observed for the full operation range used on the microscope system. These settings should therefore be most sensitive to changes in performance and do not need to reflect the conditions used during an experiment!

4.3 **Aim 3 - system characterization.** Fully characterize the detection path performance under the anticipated range of settings that users will apply.

Note

The characterization can be used to compare microscope systems with each other and detectors within a microscope system. Furthermore, it can be used to predict optimal detection system settings for a specific type of measurement to be performed.

Protocols Collection guide

5 The presented collection of protocols is built around the photon transfer curve method (**Janesick, 2007**, **Mullikin et al., 1994**). This method consists in capturing and analysing of images with an inhomogenous, high-dynamic range distribution of signal intensities over an image area (**McFadden et al., 2022** and **Heintzmann et al., 2016**).
Thus, the collection contains protocols for the preparation of a high dynamic range fluorescent sample, the detector characterization measurements, and an analysis protocol.
For measurements, the protocols distinguish between **point** and **area detectors**. Choose the respective protocol according to your detection system.

5.1 **Point detectors** belong to detection systems that can detect one pixel (picture element) information at a given time. To form an image, pixel information is collected in a series, one after another. Generally, point detectors consist of one dexel (detector element). The pixel size and number of pixels in the image depend on the optical settings (scan area, scan speed, pixel dwell time...) of the point scanning detection system and is freely selectable.

5.2 **Area detectors** belong to detection systems that can detect more than one pixel (picture element) information at a given time. They detect information for several pixels simultaneously, in parallel.
Area detectors consist of an array of dexels (detector elements). The size of the array area and number of dexels is detector specific. In the final image, pixel size depends on the dexel size of the area detector, binning, and the overall magnification of the area detection system.

Detection system - basics

6 The microscope's detection system can be subdivided in two parts, the **optical and the electronic part.** The optical part collects photons emitted by the sample, with an efficiency that in general depends on the instrument and the configuration. Note, that the rate of the collected photons is not constant, but follows a stochastic noise distribution, called **shot noise**. Measuring the shot noise (signal variation) allows for estimation of the absolute number of detected photons.
After travelling through the **optical path,** emission photons arrive at the surface of the detector. A fraction of these photons is converted into photoelectrons. The ratio of the number of electrically measurable photoelectrons (Npe) to the number of photons arriving at the surface of the detector (Np) is referred to as the detector **quantum efficiency (QE)** (see Figure 1). The method used for this protocol collection does not allow for determining QE since the incident light intensity is not known.

In the **electronic part** the number of photoelectrons (corresponding to the physical charge) are read out by an electrical circuit and converted to digital values (referred to as analog-digital-units or ADUs) by an analog-to-digital converter. This readout has an uncertainty, the **read noise,** which is why the precise number of photoelectrons is also uncertain (**Janesick, 2007**, Vliet et al., 1998, **Deagle et al., 2017** and **Murray et al., 2013**). The number of photoelectrons corresponding to one ADU is termed the **photon conversion factor (PCF**, also referred to as the electron conversion factor, or photoelectron conversion).

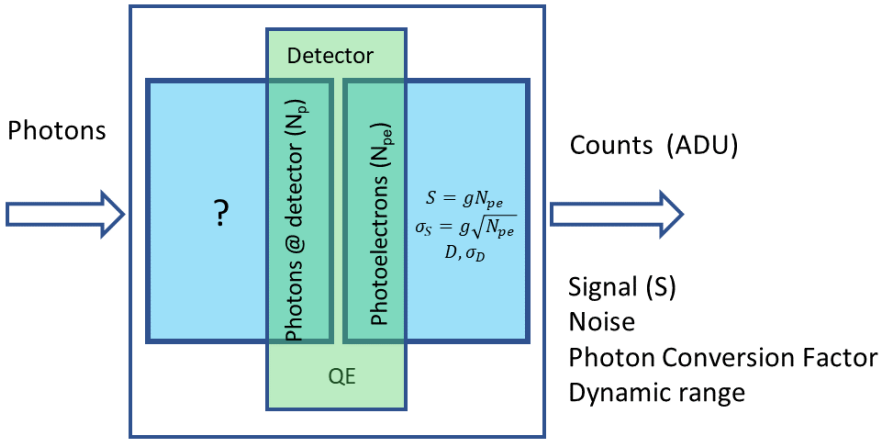


Figure 1: schematic representing the detection system. Adapted from **EMVA1288**.

By acquiring and analyzing data according to the workflow described in protocols 3,4, and 5 of this collection, we can obtain a number of useful characteristics of the electronic part. This characterization yields, among others, the photon conversion factor, electron-equivalent readout noise, as well as the detector dynamic range.

Note

The model described above **does not apply to electron-multiplying detectors,** which rely on impact ionization to multiply the photoelectrons. Electron-multiplying detectors include in particular 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). These can improve the signal, but also introduce additional noise, the "multiplication noise", which masks the photon shot noise (**Cho et al., 2006** and Art, 1990). It is important to note that in this case the protocol does not yield the physically correct photon conversion factor.

The protocol can be useful despite this, as it can quantify the noise in terms of "effective photoelectrons", or "noise-equivalent-photoelectrons". Other caveats are discussed in **McFadden et al., 2022**, supplementary materials.

Note

Our considerations are limited to linear detection systems and a spectral range that generates at maximum one charge carrier per photon.

Note

The **optical part** of the detection system is more difficult to characterize quantitatively. Microscopes are usually much more configurable on this end, but at the same time a standardized configuration is needed to achieve aims 2 and 3. Additionally, characterizing the collection efficiency and optical quantum efficiency requires a well calibrated light source or detector, in order to control the amount of light going into the detection system. Further tools may need to be developed.

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