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Characterization of the Photon Conversion Factor, Noise, and Dynamic Range of Light Microscope Detection Systems

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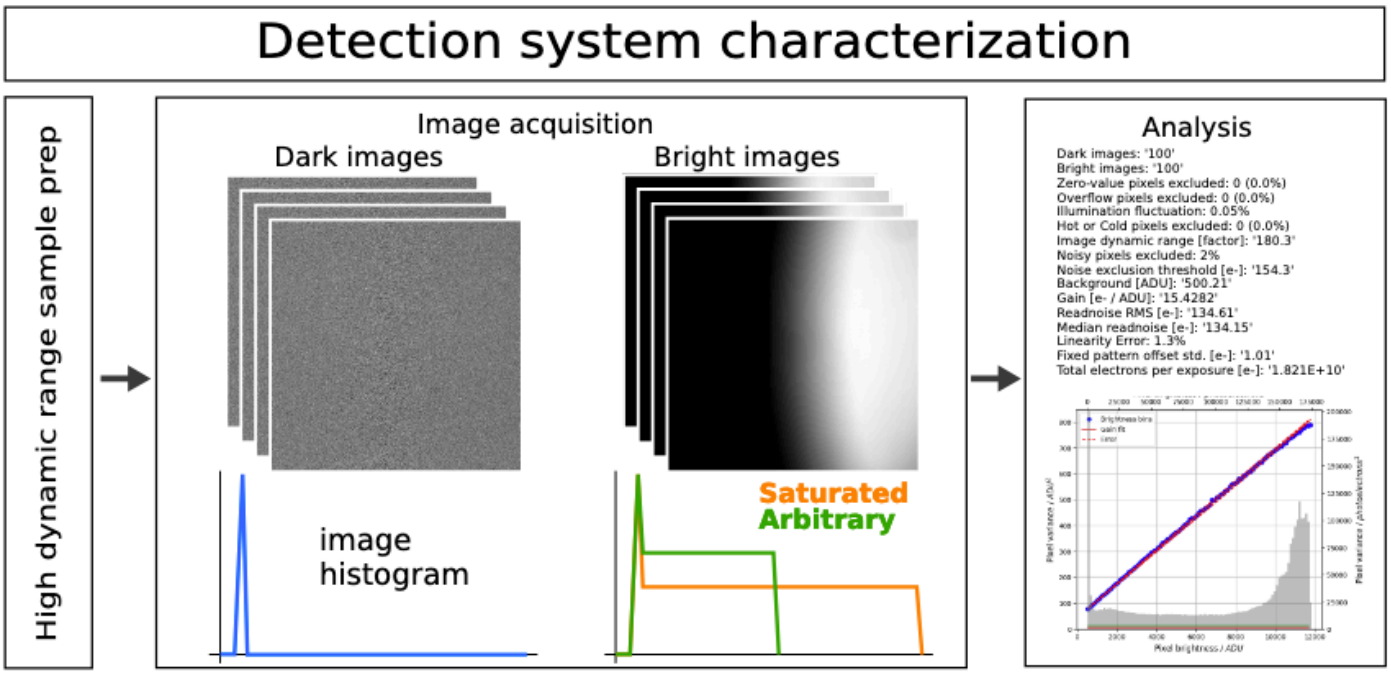
Abstract

The protocols in this collection describe how to measure and analyze the photon conversation factor (PCF photo-electrons/count), readnoise, and dynamic range of a light microscopy detection system; which can either be a point detector or an area detector, using an in-homogeneous detector illumination scheme (as opposed to uniform illumination). The collection includes protocols on how to prepare a suitable sample for the acquisition, how to acquire data, as well as a respective analysis protocol.

There are three aims a detection system can be characterized for, briefly:
Aim 1 - experiment QC: Characterize the microscope performance using detection settings that match the experiment.
Aim 2 - instrument QC.: Monitoring of microscope performance over time for service purposes, to maintain image quality constant and at high level.
Aim 3 - system characterization: Full characterization of detection path performance under the range of settings applied by users.

For a more detailed description refer to protocol 1. Introduction - Background and Aims.



Workflow of detection system characterization using the Light Microscopy Detection System Characterization toolset.

This protocol collection represents the collective experience of over 100 imaging scientists and industry experts. Measurements made by our working group with these protocols will be available in a public database.

Please note, that this is an evolving document, to be versioned and updated, based on community feedback and new data.

Materials

For materials please refer to the repsective protocol.

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 guideline: 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.

Before start

For a detailed introduction and description, please read **protocol 1**.
For characterization of an area detector (e.g., sCMOS camera), please follow **protocol 2** , **protocol 3**, and **protocol 5**.
For characterization of a point detector (e.g., PMT or HyD detector), please follow **protocol 2**, **protocol 4** and **protocol 5**.

Make sure the microscope is turned on at least one hour prior to the measurements to allow the system to stabilize.
The test sample can be prepared well in advance.

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Files

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Protocol

NAME

1. Introduction - Background and Aims

VERSION 1

CREATED BY

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Protocol

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2. Sample Preparation - An Easy-to-Prepare Sample Slide

VERSION 1

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3. Data Generation - Systems with an Area Detector

VERSION 1

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4. Data Generation - Systems with a Point Detector

VERSION 1

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Protocol

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

VERSION 1

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