PSF acquisition and analysis (QUAREP-LiMi WG 5) + FIELD HOMOGENEITY (QUAREP-LiMi WG 3)

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Abstract

These two workshops will be held in parallel; you can attend only one at a time!

PSF acquisition and analysis (QUAREP-LiMi WG 5)

Presenters (in alphabetical order)**: Ioannis Alexopoulos, Nadia Halidi, Laure Plantard, Glyn Nelson**

Authors: Laure Plantard, Nadia Halidi, Laurent Gelman, Glyn Nelson, and WG 5

A microscope with stable performance over time is necessary to ensure quantifiable and reproducible results. Measuring the Point Spread Function (PSF) as a proxy for the optical lateral and axial resolution is a first step in monitoring performance stability. The size, shape, and symmetry of the PSF, as compared to the theoretical ideal resolution, characterize the entire optical setup, including the objective and affect the image quality and any subsequent experimental quantification analysis, especially for advanced microscopy techniques.

This workshop would be composed of two sub-parts: PSF acquisition and PSF analysis. The PSF acquisition will be done on a microscope with a discussion on some essential parameters, such as Nyquist sampling. Image analysis would be performed with an open-source plugin (MetroloJ_QC) to measure the quality control metrics values. Analysis of PSF from other microscopes will also be done to illustrate different cases and highlight how to identify potential aberrations.

FIELD HOMOGENEITY (QUAREP-LiMi WG 3)

Presenters (in alphabetical order)**: Kees van der Oord, Sabine Reither, Sandra Ritz, Martin Spitaler, Tomasz Wegierski**

Authors: Sandra Ritz, Martin Spitaler and WG 3

Quantitative light microscopy generally depends on the direct correlation between the measured fluorescence intensity and the number of labeled molecules in the sample. This correlation depends on many factors (most of which are covered by QUAREP-LiMi working groups), but a key factor is the field homogeneity of the fluorescence excitation and emission light. Only if the amount of excitation light is equal for all fluorophores in the sample, and if the efficiency of detection is homogeneous across the field of view, the image data can be used for quantitative analysis of the sample.

To analyze the field homogeneity of fluorescent microscopes, we have developed a simple protocol suitable for most fluorescent microscope modalities. Starting from affordable samples (solutions of FITC and DAPI, or alternatively fluorescent plastic slides), fluorescence images are acquired and corrected for background signal with "dark images" (no excitation light). To extract quantitative measurements for field homogeneity, we have collaborated with the developers of the MetroloJ_QC plugin for ImageJ to optimize the existing tool for our needs. We propose CENTRICITY, POLYNOMIAL LINE FIT, and COEFFICIENT OF VARIATION as quantitative readouts, which in our tests, best reflect the quality of the field homogeneity across a large variety of microscopy modalities and objective lenses. These measurements will allow routine tracking of microscope field homogeneity, and in case of aberrations, will allow the user to make informed decisions on further actions like further detailed system checks, alignment, or flat-field correction of data.

- https://quarep.org/working-groups/wg-3-uniformity-of-field-flatness
- https://www.protocols.io/file-manager/A6200C449FCF11EC85CC0A58A9FEAC02