A comprehensive routine quality check protocol for facility microscopes using Argolight Calibration Slides.

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Abstract

Light microscopy allows quantitative analysis of whole biological systems in a minimally invasive way, but reliable quantification relies on reliable instrument performance, which needs to be monitored regularly by quantifiable quality checks (QC).

When establishing a routine QC protocol in the “MPI of Biochemistry Imaging Facility”, we were confronted with major challenges: (1) the large variety of microscope modalities and components (objectives, light sources, detectors, etc.), making it difficult to find common measurements reflecting the performance of all instruments; (2) different performance parameters requiring different samples types (small or large structures, or unstructured fluorescence, detectable across a large spectral range), leading to unmanageable numbers of permutations of components and samples, and of manual interventions (sample and objective changes).

To make the QC protocol routinely manageable, we set ourselves two goals: (1) Enough measurements need to be included to reliably monitor the performance of all microscopes; and (2) net staff time for measurements (excluding instrument warm-up, cleaning, trouble-shooting and data analysis) must not exceed 1 hour per instrument. To achieve both, we have built our routine QC protocol on Argolight™ Calibration Slides, which allow measurement of a large range of instrument parameters in three imaging dimensions across a large wavelength range with one single slide. The proprietary Daybook software is used to analyse the resulting image data and produces a range of QC measurements, from which we have chosen six key indicators for instrument performance. As additional parameter, excitation light intensity is measured with a power meter in the sample plane.

Our QC protocol allows tracing of instrument performance across a range of modalities (e.g. widefield or confocal, LED or laser excitation, camera, PMT, HyD or Airyscan detectors). We also show that our performance indicators are suitable to identify typical problems affecting data quality, like objective lens damage or condensation in cameras.