Fluorescent microscopy has turned into an invaluable tool for life sciences within the last 30 years. However, in order to achieve a variety of reasons: quantification of the obtained signal, providing a reliable relationship between the analyte (e.g., the protein of interest tagged with a fluorescent marker) and the detected signal is challenging. The concentration of a protein can vary substantially over time and/ or locally (different levels in different cellular compartments) leading to a heterogeneous signal with a fluorescent microscope over several orders of magnitude.

In order to enable quantitative analysis of microscopy images, or render restoration techniques (e.g., deconvolution) more reliable, it is necessary to acquire images with sufficient signal to noise and/or background noise. Therefore, the ability to detect and distinguish intensity levels over a large intensity range (without saturation of the detector) rely on the dynamic range capability of the detector as well as of the imaging modality.

**Workflows to evaluate the Dynamic Range of microscopes and facilitate High Dynamic Range imaging.**

**Workflow**

![Workflow Diagram](image)

**Dynamic Range**

Three images of each well were acquired with a widefield or confocal microscope. The images were then analyzed to extract intensity levels to create circular histograms of intensity (ODN) that were measured to each well based on its average intensity.

**InSpeck**

- The distribution of beads intensities is plotted in graphs A and C, respectively, for widefield and confocal images. InSpeck beads are expected to have a bi-normal intensity distribution. The average beads intensity of populations A1, A2, and B are respectively 600, 800, and 1200.

**Rainbow**

- The distribution of beads intensities is plotted in graphs A and C, respectively, for widefield and confocal images. Rainbow beads are expected to have a bi-normal intensity distribution. The average beads intensity of populations A1, A2, and B are respectively 600, 800, and 1200.

**Conclusion**

We are presenting a workflow to study and measure the dynamic range of different microscope systems. It is generic and can be applied to microscopes with area detectors as well as to detectors of point scanning confocal microscopes. By using the workflow we were able to confirm that:

- Photocounting detectors have a limited linear range (object brightness real vs. measured).
- 20 images of beads acquired with a confocal microscope lead to a considerable bias when analyzing the brightness.

The workflow can be used for instrument testing, compare different instruments, ensure quantitative imaging as well as provide an easy approach for High Dynamic Range (HDR) imaging.