PYCALIBRATE: FULLY AUTOMATED PSF ANALYSIS

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STANDARDISING IMAGE ANALYSIS: One of the key barriers to data reproducibility is the lack of standardization in microscope quality control (QC). Microscope QC requires standardization of both the sample used to measure microscope performance and the software used to analyse the images acquired. Despite there being several commercial (e.g. SVI Huygens) and freely available (PSFJ, MetroloJ) software solutions available there is currently no single package that is widely used by the microscopy community. Moreover, the solutions that do exist are semi-automated, requiring user input to define acquisition parameters and providing a window for error. This lack of automation and standardization makes performance comparisons between different microscopes unreliable.

FULLY AUTOMATED ANALYSIS: In response to the above problems, PyCalibrate was developed. PyCalibrate is able to provide fully automated analysis of images of point-like objects (e.g. beads or PSFcheck slide features) that describe the microscope point spread function (PSF). Using a "scale-space" approach to the image analysis, the PyCalibrate algorithm is able to automatically determine the feature size of the PSF features in a 3D data set (Figure 1). Once the individual features have been identified, the lateral and axial full width at half maxima (FWHM) values are determined. By applying a 2D Gaussian fit to the XY plane, major and minor widths, as well as orientation of the major axis can be quantified.
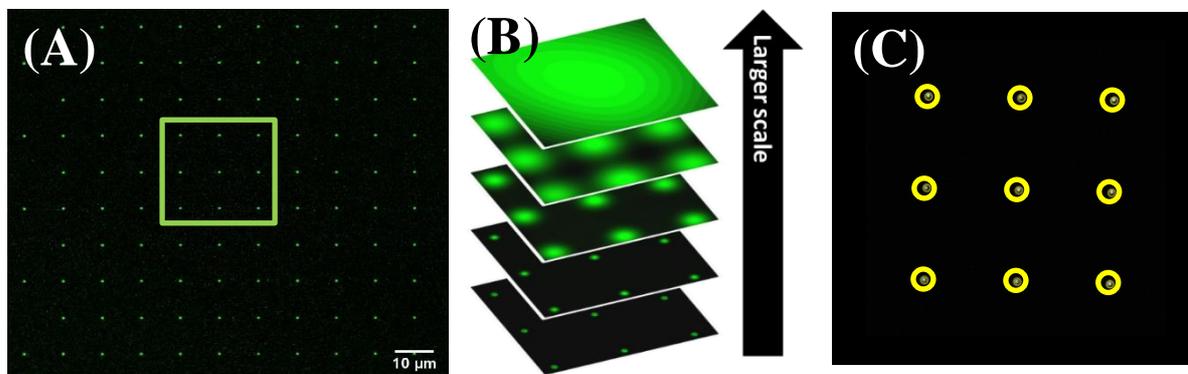

Figure 1 (A) A confocal stack of a 2D array of features (PSFcheck slide). (B) Scale-space representation of the highlighted region in (A). (C) Feature detection result: each feature is identified through its centre of mass coordinates and an ROI (yellow circle) of a fixed width.

GLOBALLY ACCESSIBLE HISTORY: To avoid problems associated with platform-dependent performance and maintaining the most recent software version, PyCalibrate has been developed as a web app. This requires only an internet connection to upload raw data to the web app and then download the analysis as a PDF or CSV file. As previous records are maintained in the cloud, this allows the full history of your microscope to be recorded to track sudden changes or slow drifts in performance. Using a cloud-based solution, data can be uploaded, processed and the results retrieved from anywhere in the world.

REFERENCES:
[1] www.psfcheck.com