Fast And Reliable Workflow To Test The Performance Of Objectives In A Light Microscopy Facility
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INTRODUCTION
Microscopy Core Facility manages an increasingly large number of microscope users. Confocal Basis acquiring confocal microscopes, for example, are now more and more used in biomedical research. Only if these devices are correctly set, they can easily acquire images close to the distortion limit, given by the objective NA, entrance wavefront and reflective index (n) of the mounting medium.

The objective is the essential part which will contribute to the resolution of the image. In particular, macro lens cannot be corrected in context with the specimen suffers from damaged, or dirty, and decreases the performance of the entire setup.

Here we describe a simple and effective method to identify dirty or damaged objectives.

AIM
Objectives performing objectives are essential for successful light-microscopy. Several procedures are in place but they are often time-consuming making it cumbersome to prove that the lens is either not clean enough or even damaged.

The most obvious method is based on visual inspection. This requires to remove the objective and look at the front lens surface with a magnifying glass (e.g., or magnify) to observe the mount, dirt on the glass, and dust (if any). In the absence of the objective, you can easily identify damaged, worn, or dirty objectives. A blood-leaf open method is necessary to remove the lens. This is time-consuming and error-prone (e.g., damage/breaking) due to the mounting process.

A different, well accepted method to measure the performance of an objective is based on measuring the point spread function (PSF). This requires a capture of a set of images based on bright-field image and looking at the shape of the PSF to identify problems of the lens or spatial resolution (reflective index mismatch). The drawback of this method is that the acquisition of a PSF requires well-trained and experienced users, it is costly the PSF acquisition demands a lot of finances, expertise and time. Therefore, the performance of the PSF (even in advanced projection) cannot be reliably calculated to the damage of the objective.

We propose an easy and quick method that immediately provides a result on the quality of the lens, the idea is based on a resolution test target, whose stripes range from 2 mm to less than 0.1 mm; that works with any microscope and objectives. As long as the test has a resolution target of a continuous. This profile of the target, image can be used to indicate if the front lens of the objective is broken or dirty.

THE PTS

RESULTS

This table shows the differences (in PSF) between 5 different and 10 different microscopes, which have different conditions, NA, ratio NA and 0.9 NA (independent). The results are given for 4 different objectives: 40X1.3 NA 1.56 NA (uncoated), 40X1.3 NA (uncoated), 4X0.14 NA AlN-2.4. AlN improves the front lens is slightly damaged and at 10X0.8 NA AlN-2.4. AlN improves the front lens is clean.

Comparisons show:

- The obtained resolution is (as expected) influenced by the NA of the transmission conditions as well as by the wavelength of the used light.

- Profiles, damaged objectives in objectives used with an inadequate immersion oil can be easily identified (less than the 2 mm WT and plunger fails to calculate the value).

COMPARISON WITH PSF MEASUREMENTS

We additionally measure the PSFs with a protocol largely used in microscopy core facilities. To compare the results with our

We followed the protocol developed by K. Lienau (in [1]). This method proposes to acquire a stack of 1000 sections (as an

COMPARISON TO CLASSIC “SIEMENS STAR” TARGETS

We compared our PTS to already existing Siemens Stars like the “ Bayer Star Targets” in the LightPath and the “Star Targets” in the LightPath. This targets does not provide a short enough spacing at the center to assess the resolution (FWHM) of a typical imaging system with high NA and magnification.

Our PTS is constituted so that the point spread function is clear enough to get an answer to the FWHM (FWHM). It is then enough we can determine between a 2.5 mm and 1.0 mm objective, which is impossible to do with Siemens Stars we had from Tissue.