Technical Note for 3rd party microscopes.

SMAL user guide.

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Introduction

Using the SMAL lens on a Nikon LV100 or a Leica DM2500.

This document details the method to achieve good results with the SMAL lens on these microscopes (other microscopes may also be suitable).

Although the Nanoro M microscope shows the highest quality results for Super Resolution imaging, the LV100 and DM2500 microscopes are known to be SMAL lens compatible and are able to achieve similar Super Resolution results albeit at reduced image quality.

For a detailed example of the benefits of the Nanoro M's image quality enhancements over the DM2500 and LV100 instruments for the SMAL lenses, please see our technical note here: https://bit.ly/36CNnCh

User Guide

Caution.

Please note: The SMAL lens is delicate and care should be taken throughout this procedure.

1. Microscope and Camera alignment

The microsphere optics are mounted in the exact centre of the SMAL objective lens. However, when mounted on 3rd party microscopes, the optical alignment may not place this in the same central position (as viewed through the camera). The first step is to mount the SMAL lens on the microscope away from the sample and ensure the epi-illumination light source is turned on and all irises are opened. Should the microsphere optics appear lopsided or not visible (centre of Figure 1), reposition of the camera until figure 1 is approximated.

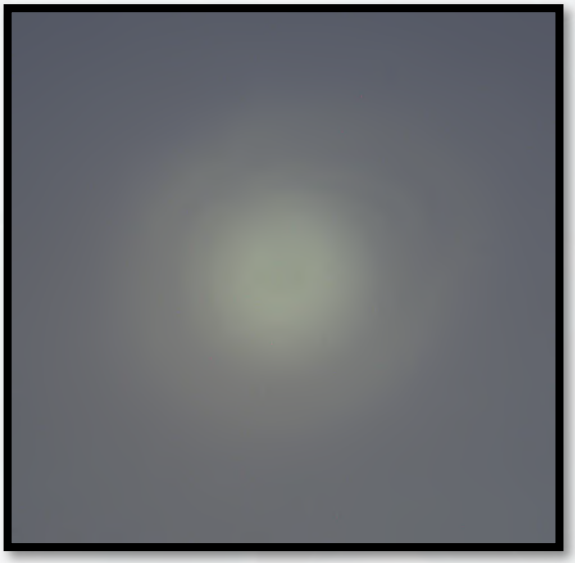

Figure 1: The microsphere must be visible on the monitor control

Figure 2: sample localisation

2. Region of Interest Location with lower magnification lenses

After initial alignment, it is recommended to switch to a low magnification objective lens to find the area of interest on the sample. Another higher magnification objective lens (e.g. x100) should then be used to refine the area further.

This step is useful in avoiding contact of the SMAL lens with the surface while searching for the area of interest and is also useful in avoiding low contrast areas of the sample which may be difficult to visualize during the approach.

3. Position the SMAL lens – turret rotation

When the region of interest is located, the lower magnification lens needs to be withdrawn from the surface in order to rotate the turret and select the SMAL lens. This withdrawal is required as 3rd party lenses cannot be guaranteed for parfocality with the SMAL lenses, and will help avoid a crash while rotating the turret.

Optional: For even higher accuracy in selection of the region of interest, the same turret lens position can be used for the SMAL lens as used by the lower magnification lens (e.g. where the x100 lens was located). This is because the tolerance of most turrets on rotation are within a few tens of microns, depending on quality. To proceed with this option, after finding the area of interest with the x100 objective lens, withdraw the lens from the surface and remove the lens from the turret and replace by the SMAL lens in the same slot.

4. Immersion

The use of oil immersion is recommended for SMAL immersion lenses, however, other immersion mediums are also suitable such as water, silicone and glycerol. If not already applied to the sample in step 2, please do so now.

5. Approaching the small lens toward the sample

When the SMAL lens is mounted on the turret, the microsphere should be visible on the screen. Approach the sample carefully using the finest height adjustment control, while always keeping an eye on the monitor or in the eye piece of the microscope. When the microsphere is entering the immersion medium, the image on the monitor will become suddenly darker; cf. Figures 3 and 4. After this point always use the fine adjustment knob for the z-axis adjustment.

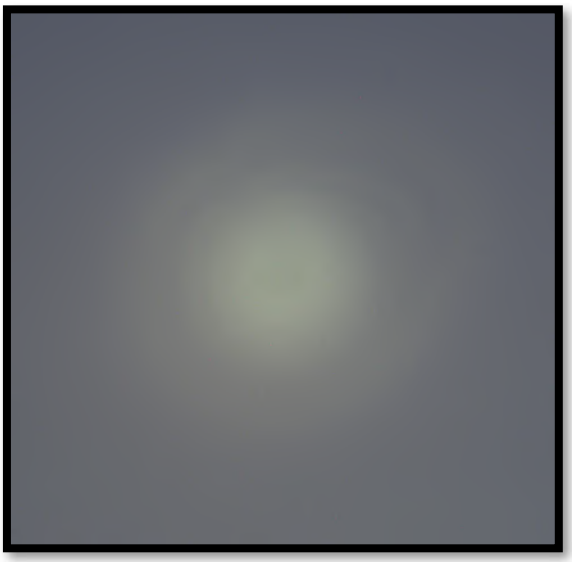

Figure 3: Microsphere appearance before entering the oil

Figure 4: Microsphere appearance after entering the oil

6. Focusing with the SMAL lens

It is important to ensure that the epi-illumination light intensity is high enough to ensure sample visibility. The light source should be increased to a reasonable level and adjusted to avoid any saturation of the camera.

The SMAL lens is composed of a number of optics that allow Super Resolution to be achieved. Part of the optical setup is such that a x100 magnification can be seen around the microsphere optics when the correct lens-to-sample distance is achieved (the outside of the red circle in Figure 5). On approach to the sample, this x100 magnification occurs first, providing a safe way to check the progress towards Super Resolution imaging with the microsphere optics.

Approach towards the surface to achieve the result of Figure 5 should be performed using the fine focus wheel of the microscope.

Figure 5 below shows the desired end result for this point in the approach. Here, we can use this area external to the red circle to fine-tune the area we wish to approach, and to a micron-level accuracy.

Nb. 1. The centre of the image of Figure 5 remains blurry due to the microsphere optics located in this region.

Nb. 2. When moving the sample in the (x, y) directions, the user must be careful of the sample roughness and to avoid collisions of the SMAL lens with the sample.

Figure 5: Focusing through the x100 of the SMAL lens (external part of red circle). The red circle indicates a region of slight blurring and also the location of the microsphere optics.

7. Imaging through the microsphere

After imaging the sample at standard optical resolution with the outside of the SMAL lens' field of view, keep approaching the sample using the finest adjustment knob. The image through the x100 magnification part of the SMAL lens will disappear and the microsphere optics will become more and more visible. Initially, it will be possible to see a halo around the microsophere optics, and then finally, thorough the optics to the sample beneath as shown in Figures 6 through 8 below.

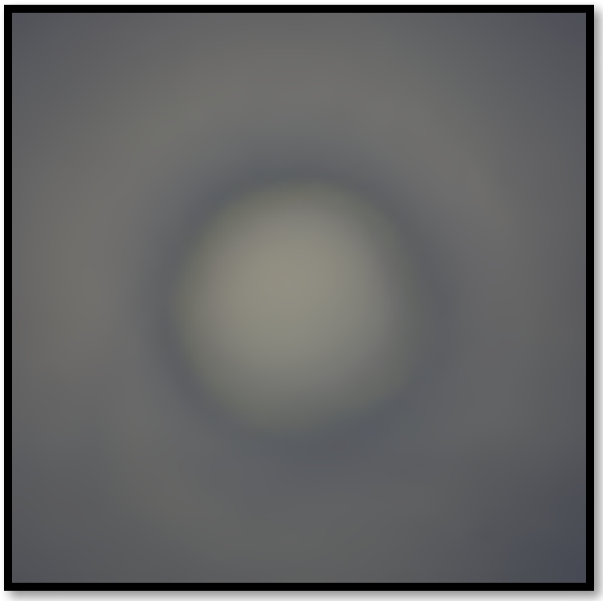

Figure 6: The microsphere is becoming more and more visible as the SMAL lens get close to the sample.

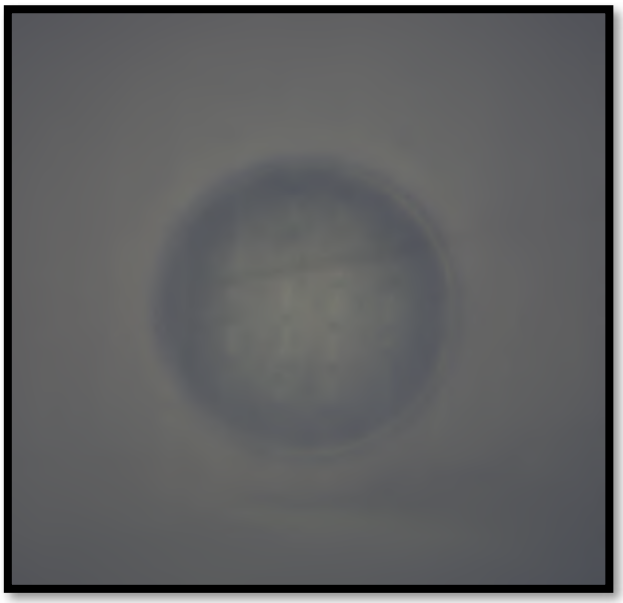

Figure 7: The sample appears within the SMAL microsphere optics.

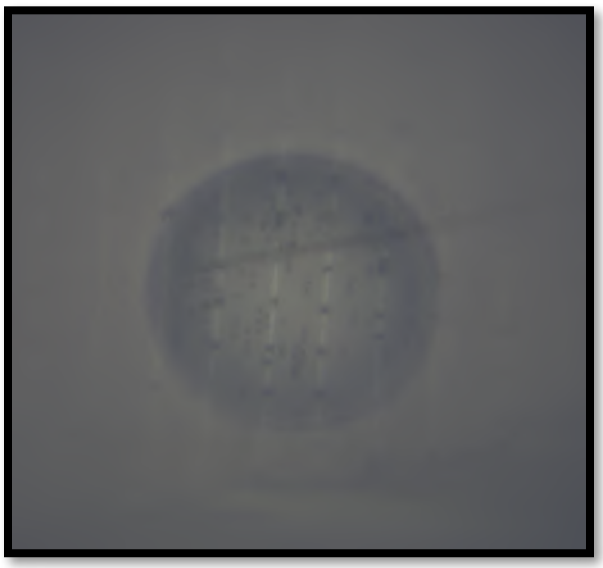

Figure 8: Fine focus inside the microsphere

Note:

If the image is not clear then it is likely the microsphere is not in precise focus:

- The image is blurry:
  Sample is too far.
- Doubling of sample features:
  Sample is too close.

8. Sample contrast

Typically, 3rd party microscopes are not suitable for low contrast samples in this setup. However, increase in contrast can be obtained by camera and lighting adjustments. These including light intensity, light alignment, camera exposure and use of aperture and field stops. Adjustments in these areas can help increased contrast and clarity considerably, but the exact setup will need investigating for each microscope/sample combination.

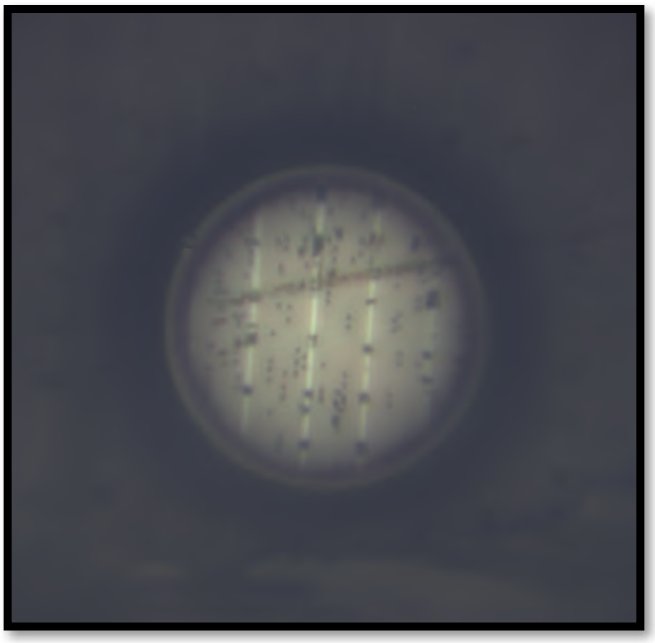

Figure 9: The image of Figure 8 obtained by adjusting the illumination conditions.

9. Observation of the sample

If the sample is smooth and flat, it can be investigated in the (x, y) direction. However, as the working distance is a few microns, sample investigation should proceed with caution and always with the finest-adjustment available on the microscope used.

If any assistance is required, we will be pleased to walk you through the procedure or offer our advice. Please contact your local distributor or LIG on our website: www.lig-nanowise.com

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