Imaging with the Super-resolution Microsphere Amplifying Lens (SMAL) Nanoscope

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Abstract. The basic principle of the SMAL (Super-resolution Microsphere Amplifying Lens) imaging technology that we invented has its roots in the SMON (Submerged Microsphere Optical Nanoscope) pioneering work. SMAL is a novel objective lens whose front lens assembly contains a microsphere and is replaceable. We built a nanoscope prototype with nano XYZ scanning capability, which integrates a SMAL objective lens, allowing us to achieve super resolution imaging (70 nm – 90 nm). We have resolved large area scans (200 µm x 200 µm) by contactless sample scanning.

1. Introduction and aim
The resolution limit for conventional optical microscopy is approximately 200 nm within the visible light spectrum due to the far-field diffraction limit. It follows that conventional techniques are not suitable for imaging objects with structures smaller than this limit, for example live viruses (typically 5–150 nm, with some up to 300 nm) [5]. Other techniques have been used to image this kind of samples, like Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), that are often used to observe specially prepared dead virus structures at very high resolutions (10 nm) in vacuum. These techniques require complex sample preparation, and are not suitable for in vivo imaging and measurements (the electron beam damages living cells, viruses, etc.). Atomic force microscopes (AFMs) offer good imaging of small features by using a contacting probe. However, samples may be easily damaged by the AFM tip. Moreover, this technique does not offer a real image, but a reconstructed image, and the configuration plus acquisition time is typically very long (up to hours). Stimulated Emission Depletion (STED) fluorescence optical microscopy is a recently established method for imaging cellular structures, bacteria and viruses beyond the diffraction limit, down to a resolution of 6 nm. STED offers a better resolution, but the sample also requires a complex preparation (fluorescent labelling), which may not always be suitable for imaging living organisms. The fluorescent imaging techniques give good results mainly for organic samples [4]. However, for high resolution, these techniques are confronted with the challenge of photo bleaching which limits the exposure time to tens of seconds. Microsphere super resolution imaging has been demonstrated using microspheres positioned between the objective lens and the sample using the SMON (Submerged
Microsphere Optical Nanoscope) technique [1]. The employed microspheres are typically in the order of 1-100 µm in diameter. The high spatial frequency information is carried by evanescent waves present at the boundary of two different media with different refractive indices. These waves decay exponentially within 1 µm, so they cannot be observed with conventional objective lenses. The use of microspheres allows to capture such waves and propagate them into the far-field. The basic principle of the SMAL (Super-resolution Microsphere Amplifying Lens) imaging technique that we invented has its roots in the SMON pioneering work. SMAL is a novel objective lens whose front lens assembly contains a microsphere and is replaceable. We built a prototype nanoscope with nano XYZ scanning capability which integrates a SMAL lens. The microsphere gives us the super resolution (down to 70 nm – 90 nm), while the piezo stage allows for autofocusing and arbitrarily large area scans (up to its travel range limit: 200 µm x 200 µm).

2. Experimental setup
We developed an original microscope comprising of: a regular optical microscope system from commercial available opto-mechanical components, a manual XYZ stage for coarse adjustment, a computer-controlled XYZ piezo stage, a SMAL objective lens, a metallic custom chassis and a protective enclosure. The schematic of the experimental setup is shown in Figure 3.

2.1. Objective lens
The setup consists of a regular 100x (NA = 1.24) oil immersion objective lens with a disk attachment on the top of its hemispherical front lens. We spincoated a uniform thin film of NOA®81 UV curable glue onto a glass disk, then we placed a BaTiO$_3$ microsphere (we tried different sizes in different setups) with a refractive index of 1.93 on the glue layer. Then we cured the attachment for 1 hour (Figure 1) with UV light. We calculated the total thickness of the attachment such that the microsphere is in the focal point of the objective lens. This thickness varies depending upon the focal distance of the objective lens, the diameter and refractive index of the microsphere, as well as the wavelength of the irradiating light. We confirmed the value of the thickness experimentally, by using a simple setup consisting of a microsphere positioned onto a sample and a micrometric Z adjustment to move the objective lens on top of it. By evaluating the sharpness of the image at different Z positions, it is easy to find the optimal distance between the lens and the sphere. Special care must be taken in aligning the microsphere with the optical axis before fixing it onto the glass disk. The position of the sphere can be evaluated in real time by observing the image acquired with the nanoscope. We designed SMAL with a replaceable front lens assembly to allow the replacement of the microsphere, in case it gets damaged by repeated contact with the sample.

2.2. The microscope
We mounted the SMAL objective lens (Figure 2) onto our custom made white light reflection microscope. The microscope includes two stages: a manual XYZ with micrometric stage to allow for coarse sample exploration and focusing, and a software-controlled XYZ piezo stage for controlling the sample scan and the autofocus. The best quality imaging was obtained with oil and water immersion.

2.3. The software
Typically, the field of view of SMAL is tiny when compared to a standard objective lens, and it is dependent upon the size of the microsphere. Moreover, the image can be affected by a slight pincushion distortion. In order to obtain an undistorted, large area image of the sample in super resolution, a scan process is necessary. This is achieved by using a piezo stage with nanometric accuracy and a custom software. The software controls the camera and the stage and automatically acquires several images in different positions of the sample, in a grid-like pattern. Each image is corrected for distortion and cut to preserve only the part with meaningful super resolution information (at the centre of the microsphere). Then, the software stitches all the images together to obtain a large
area mosaic. Sometimes, a grid-like pattern may appear in the final mosaic, depending on a number of factors, including the material of the sample and the immersion medium. So, as an optional final step is the removal of such pattern by using standard image processing algorithms. The scan is typically fast when compared to super resolution imaging techniques like AFM. When using SMAL, a typical 20 µm x 20 µm scan of a silicon microprocessor sample with a 15 µm diameter microsphere requires stitching 784 images and takes 2 minutes and 16 seconds.

![SEM imaging of our embedded microsphere in spin coated UV curable glue (20 µm diameter barium titanate microsphere)](image1)

![Front side rendering (not to scale) of the proposed objective lens with the replaceable front lens assembly](image2)

![Experimental Setup using SMAL](image3)

Our software suite allows the user to take large area scans up to 200 µm x 200 µm (the travel range of the stage) with a lateral resolution down to 70 nm, depending on the sample. Figure 4 (a) shows the scanning of a 80 µm x 80 µm of an Intel® Core i3-530 “Clarkdale” microprocessor. Figure 4 (b) highlights a zoomed in area of the same sample where it is possible to observe features with sizes between 70 nm and 90 nm, as well as different circuit copper layers in depth.
Figure 4 – (a) 80 μm x 120 μm scan of an Intel® Core i3-530 “Clarkdale” microprocessor using our SMAL based nanoscope; (b) zoomed in area of the same image

Figure 5 – (a) 100x, 1.24NA oil immersion objective lens imaging of a graphene sample; (b) the same sample scanned with our SMAL based nanoscope

3. Conclusion:
   • With SMAL, we resolved features down to 70 nm - 90 nm of an Intel® Core i3-530 microprocessor;
   • We could automatically scan arbitrarily large areas in super resolution;
   • The technique is non-contacting, non-invasive, and it works naturally in both wet and dry media;
   • This work opens new opportunities for the study of nanostructures, and is suitable for efficient quality control, as well as studying the interaction between cells/viruses/bacteria/drugs;
   • In comparison with expensive equipment like SEMs and AFMs, which can only image the sample’s surfaces, our method can see inner layers of materials with resolution beyond diffraction limit at a depth down to 3 μm (Figure 4);
   • Unlike other imaging technologies previously mentioned, colour imaging with super resolution can give an estimate of the thickness of graphene layers (Figure 5b).

References
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