Microsphere-assistance in microscopic and confocal imaging

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Abstract. Topographical as well as microscopic imaging of nanoscale surfaces plays a pivotal role across various disciplines. Nevertheless, achieving fast, label-free, and accurate characterization of laterally expanded structures below the diffraction limit remains challenging. Recent studies highlight the use of microsphere assistance for resolution improvement. Confocal microscopy, augmented by microspheres, enables the imaging of small structures that were previously inaccessible. This is experimentally compared with microsphere-assisted microscopy (MAM) to underline the decisive role of the confocal effect.

1 Introduction

In the characterization process of nanostructured surfaces, topographical measurements play a crucial role throughout different disciplines from quality control in fabrication processes to biological tissue observation. In order to further analyze surface features, the characterization process must be accurate, fast and, ideally, label-free. In microscopic and interferometric measurements, however, the system is fundamentally diffraction-limited in its lateral resolution capabilities.

With the help of microspheres, the lateral resolution of an imaging system can be extended so that surface measurements are even possible below the system's diffraction limit [1]. This is demonstrated in detail for both, microscopic and interferometric applications [2]. The theoretical background has also been highlighted in recent studies [3], including a complete simulation model that incorporates a rigorous simulation of the crucial near-field wave propagation involving the microsphere [4].

Confocal microscopy is a powerful tool that enhances the resolution capabilities of a conventional microscope significantly [5]. The additional confocal influence when imaging with microsphere assistance is discussed in [6–8] and also shown in [9]. Utilizing the unique optical properties of microspheres in combination with confocal microscopy for topographic measurements further increases resolving power while allowing fast processing and easy sample preparation. Measurement results show how the use of microspheres enables imaging topographical structures otherwise not accessible.

2 Methodology and instrumentation

The presented results are obtained using a commercial confocal microscope (Nanofocus µsurf custom). With a numerical aperture (NA) of 0.95 and a central illumination wavelength of $\lambda = 505\,\text{nm}$ a high resolution topographical measurement is already possible using the confocal effect provided by an integrated pinhole disc. The measurement capabilities are described by an extensive characterization in [10]. Figure 1 shows the schematic setup including a microsphere placed in the near-field of the specimen, a linewidth/pitch standard manufactured by Supracon, which is analyzed elsewhere [11].

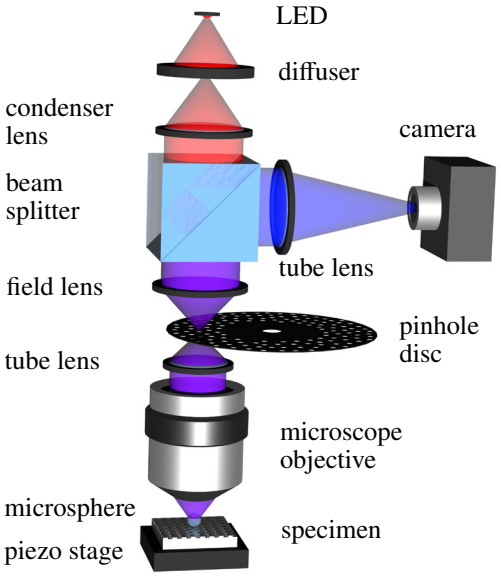

Figure 1: Schematical view of the experimental setup including a microsphere placed on the specimen enhancing the near-field wave propagation. The specimen is illuminated by an LED (central wavelength $\lambda = 505\,\text{nm}$, red beam path). The image stack is acquired by a CCD camera (imaging beam path in blue).

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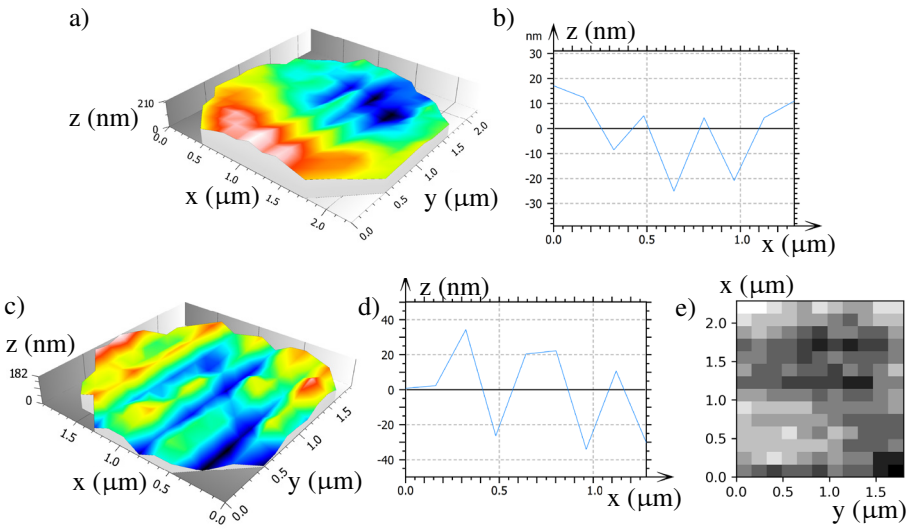

Figure 2: Confocal microsphere-assisted topography measurements of grating structures on the linewidth/pitch standard. Reconstructed surface data is shown as well as a profile cross section of the grating structure for a period length of a), b) $\Lambda = 260\,\mathrm{nm}$; and c), d) $\Lambda = 300\,\mathrm{nm}$. The limited field of view induced by the microspheres (SiO_2, diameter d = 5 µm) is visible as a circular cut-out of the measurement area. e) shows a MAM image of the same field as c).

Besides the optical limitations, the system is also limited by the pixel pitch of 7.4 µm leading to a lateral sampling interval on the specimen of $\Delta x = 163.4\,\mathrm{nm}$. Using Nyquist's sampling theorem this leads to a limited resolvable period length of $\Lambda_{Ny} = 326.8\,\mathrm{nm}$. As it is shown in [10], in practise the lateral resolution of the confocal microscope corresponds to $\Lambda_{min} = 0.565\,\lambda/\mathrm{NA}$ leading to $\Lambda_{min} \approx 300\,\mathrm{nm}$

Taking advantage of the microsphere-induced additional magnification, the overall resolution of the optical system can be enhanced, which is shown in the following section.

3 Results

After acquiring the image stack, signal analysis provides reconstructed topography data. 3D representations of measured surfaces for different grating periods ($\Lambda = 260\,\mathrm{nm}$ and $\Lambda = 300\,\mathrm{nm}$) as well as profile cross sections are shown in Fig. 2a)-d). Neither structure is resolvable with conventional or confocal microscopy due to the large pixel pitch of the camera. The additional magnification induced by the microspheres is M ≈ 1.3. The limited camera resolution of the commercially available confocal microscope becomes relevant when measuring small structures. Nevertheless, the resolution of the system is improved overall by the use of microspheres, as the topographic measurement of $\Lambda = 260\,\mathrm{nm}$ shows.

When comparing the confocal microsphere-assisted result with the MAM image of the same field of view in Fig. 2e), it becomes clear that the confocal technique affects the imaging process. The microscopic imaging does not resolve the $\Lambda = 300\,\mathrm{nm}$ structure, whereas the topographic evaluation of the confocal imaging even improves the effective resolution to smaller lateral structures.

4 Conclusion

Confocal microscopy is a powerful tool that enhances the lateral resolution capabilities of a conventional microscope. When coupled with microsphere assistance, this technique achieves even higher resolution. Like it is discussed in [7], the confocal effect on microscopic imaging plays a pivotal role for the capability of the system to resolve structures below the diffraction limit. Furthermore, for commercially available systems the pixel resolution can be improved due to the additional magnification by the microspheres.

Future investigations considering rigorous simulations should meticulously analyze the transfer behavior of a confocal microscope considering microspheres, enabling a comprehensive comparison with MAM and interferometry.

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