\textbf{Abstract:} In this paper, we demonstrate simultaneous AFM/NSOM using a dual-tip normal tuning-fork based scanning probe microscope. By scanning two SPM probes simultaneously, one dedicated for AFM with a standard tip diameter of 20 nm, and the second having a 150 nm aperture NSOM fiber with 200 nm thick gold coating, we combine the benefits of $\sim 20$ nm spatial resolution from the AFM tip with the spectral information of a near-field optical probe. The combination of simultaneous dual-tip scanning enables us to decouple the requirements for high resolution topography and probe functionality. Our method represents a marked shift from previous applications of multi-probe SPM where essentially a pump-probe methodology is implemented in which one tip scans the area around the second. As a model system, we apply dual-tip AFM/NSOM scanning to a sample of spin-cast nano-clustered Lumogen dyes, which show remarkable brightness and photochemical stability. We observe morphology features with a resolution of 20 nm, and a near-field optical resolution of 150 nm, validating our approach.

\textbf{Keywords:} multiprobe; NSOM; SPM; lumogen; FRET; tuning fork probe.

\section{Introduction}

Atomic force microscopy (AFM) since its introduction in 1986 [1] has been limited in its technology to single probes investigating a sample surface. Nonetheless, the goal of achieving multiprobe AFM operation was a most desirable objective which was difficult to achieve. This article describes the application of a highly developed multiprobe platform and one of its applications to near-field scanning optical microscopy (NSOM). In this particular application, we demonstrate that such a platform can readily address the dichotomy between achieving the best NSOM resolution while not sacrificing on the on-line AFM resolution of the structure.

The development of multiprobe scanning probe microscopy (SPM) technology based on AFM was quite complex. This complexity arose from a complicated interaction between probe technology, feedback technology, and the geometry of the construction of the SPM.

The history of such multiprobe technology was therefore initially based on scanning tunneling microscopy and the guiding of the probes to relatively close proximity was defined by an on-line scanning electron microscope. There are several reports on the development of multiprobe STM equipped with up to four probes [2–9]. The first ambient example of a two probe STM however did not occur until 2007 [10].

 Nonetheless, such technological advances are less than general due to the fact that STM could not be used on non-conducting surfaces and without some alternate methodology, such as SEM or actually performing a scan with each probe, one could not rapidly place two probes together. Both of these issues were addressed in a paper published in 2009 [11].

To put this report in perspective, it is appropriate to note that Roco et al. [12] in their report in nanotechnology developments in 2020 emphasized multiprobe SPM as an important technological advancement placed along with aberration correction transmission electron microscopy, electro holography, energy dispersive X-ray, etc. This perspective also noted that novel methods to measure electrical, optical, and magnetic properties at the nanoscale (including multi-probe SPMs) were needed to cause a paradigm shift that would bring invaluable advancement in a wide variety of technological fields.

The 2009 paper noted above was the first paper based on a patent originally filed in 2001 [13]. This technology
addressed all of the outstanding aspects of how one would build such a multiprobe SPM based on AFM technology. Furthermore, the platform could be used not only for AFM imaging but for the broad spectrum of multifunctional imaging methodologies that include AFM feedback. It is for such functional imaging that multiprobe technology has its greatest potential.

In fact in this first paper by Dallapiccola et al. [11], the developed multiprobe platform was applied for pump-probe near-field optical imaging of a plasmonic waveguide in which the propagation of a plasmonic wave was measured. In this particular experiment, one NSOM probe was used as a point source of illumination that provided all “k” vectors to effectively excite plasmons at any point in the waveguide and the second NSOM probe was used for collecting the light from the propagating plasmons in the near-field. A 1/e decay length of 340 nm was measured.

It was already seen in this 2009 study that in a multiprobe system such as this, one probe could image another probe. This has since been supported by the work of Klein et al. [14] who has demonstrated with the same platform model, MultiView 4000 Multiprobe SPM (Nanonics Imaging Ltd, Jerusalem, Israel), that one probe’s feedback is extremely sensitive to an additional probe’s nanometric presence. This makes it possible to bring multiple probes rapidly together and even into contact.

These advances arose from three inter-related developments. The first is the development of a series of functional probes that have probe geometries that permit probe tips to be in touching distance while keeping the optical axis completely clear from above and below the platform. A video of such an operation can be seen on the internet where a gold nanoparticle probe comes within the near-field of an NSOM probe and changes the boundary conditions of the emission from the NSOM probe as the gold nanoparticle approaches (http://www.youtube.com/watch?v=xnFAW8AXn6Y). The second is the development of ultrathin scanners that can achieve large ranges in X, Y and Z up to 100 microns and permits new open architecture geometries of scanned probe microscopes [15]. The third is the development of normal force tuning-fork feedback which shows that the ultimate in force sensitivity with glass based multiprobes enabled probes for NSOM, electrical, thermal, plasmonic, ion conductance, fountain-pen nanolithography, and scanning electrochemical microscopy applications (http://www.nanonics.co.il/products/spm-probes-and-nanotools.html). For example it has been shown that with such probes and the platform used in the present study, a force sensitivity of 1.6 pN could be achieved, which is more sensitive than any other AFM based feedback technique developed thus far [16].

A major challenge that is pervasive in nearly all functional applications that include SPM is that the functional probes associated with these applications do not always achieve the ultimate in AFM topographic resolution. This is due to the additional dimensions inherent in some functional probes that are based on AFM feedback. The example that we focus on in this paper is nano-optical measurements with NSOM. Since the inception of NSOM, this has been a problem that has limited the AFM resolution of an NSOM probe due to the coating around the active area that defines the near-field optical element.

In this paper we concentrate on fluorescence imaging with NSOM. For such imaging, the best choice is aperture based NSOM. This is due to the fact that fluorescence is very sensitive to bleaching and thus, in aperture based NSOM only the pixel that is being interrogated is bleached. Fluorescence however requires maximal photon flux from the nano-aperture of an NSOM probe. For such maximal photon flux, the cone angle of the probe has to be large and such cone angles compromise the AFM resolution which is defined by the outer diameter of the probe and not the aperture which defines the NSOM resolution. Thus, multiprobe technology permits one the potential to achieve maximal AFM and NSOM resolution on line for excellent structural and optical nano-characterization.

In addition, without the best AFM resolution one often requires the NSOM probe itself to be used to search for potential fluorescence structures. However, this in itself compromises one of the advantages of fluorescence NSOM where a virgin pixel not exposed to light can be interrogated with this singular nano-optical technique which confines light nanometrically in X, Y and Z unlike any other optical technique known.

All of the above problems, which have been associated with NSOM fluorescence since its inception [17], can be overcome by using the multiprobe technology that has been applied to fluorescence NSOM imaging for the first time in this paper. Specifically, the main concept introduced here is the use of a dual probe. The first probe can image the surface at the highest AFM resolution to locate the position of particles with potential fluorescent spectral properties. The second is chosen to give the highest NSOM signal. Thus, this can involve making a relatively large cone angle probe which reduces the length of the transport of light in the tapered region of the NSOM fiber probe which has a diameter that is below the cut-off frequency of the optical fiber where light cannot propagate without loss in this metal coated region. Furthermore, such a probe which generally has a large force constant in the 1–10 N/m range can readily be used to scan soft surfaces such as the materials studied in this paper since
the probe is complexed to a tuning fork for normal force tuning fork force sensitivity.

As a model system, we investigate dual tip AFM/NSOM scanning on thin films of Lumogen dyes. Lumogen dyes, manufactured by BASF, are good initial examples since they exhibit high fluorescence quantum yield and relative photochemical stability even in air. This allowed in this initial study multiple scans with the NSOM probe to verify our conclusions.

The dye Lumogen Red that was used is reported to have a quantum yield exceeding 90%, and the dye Lumogen Orange is characterized by similar performance. Thin films of Lumogen doped into polymeric host-matrices are being implemented as solar concentrator layers due to their ability to withstand high intensity solar excitation for very long periods of time and are also used in thin film organic waveguide laser architectures [18, 19].

Thus, these dyes permit scans of long duration for the highest signal to noise in scanning optical measurements. Therefore, Lumogen dye is an attractive candidate material to investigate.

Prior to our work, to the best of our knowledge, these dyes have not been reported in the NSOM/SNOM literature. Perhaps the reason is that their fluorescence is severely quenched when a neat film is prepared as occurs for perylene and other laser dyes [20]. Other forms of perylene diimide have been studied by NSOM [21] but not for near-field fluorescence [22], likely due to pronounced “concentration quenching”.

In order to obtain the high brightness of the Lumogen dyes even in solid state, we prepared composite films of Lumogen Red doped into a Lumogen Orange host matrix. In the doped layer, the spacing between Lumogen Red molecules is sufficient to prevent concentration quenching of the fluorescence. Using Lumogen Orange as the host introduces the possibility of FRET to occur between the host and dopant dye molecules, due to the good spectral overlap between Lumogen Orange’s fluorescence and Lumogen Red’s absorption. FRET sensitizes the fluorescence of the acceptor molecule and thus enables a much larger fraction of laser light to be absorbed and transferred to a smaller fraction of Lumogen Red molecules. Lumogen Orange also has stronger absorption of the 532 nm light used for the near-field excitation. On glass and silicon substrates, for specific preparation conditions, the dye molecules can pool into sub-micron scale islands that are suitable for dual-tip AFM/NSOM SPM characterization.

2 Experimental section

Samples of nano-clustered Lumogen dyes were probed using AFM, NSOM, and simultaneous dual-tip AFM/NSOM following the schematic of Figure 1A,B. All measurements were performed using a dual microscope from Nanonics Imaging which was free-space coupled to a LabRam HR Micro-Raman microscope from Horiba Jobin Yvon as shown in Figure 1B.

Upon initial examination with white-light far-field illumination, samples were scanned using standard AFM tuning fork probes. Gain and feedback parameters were adjusted to obtain high resolution images. Fluorescence of the sample was mapped via far-field excitation and

![Figure 1](image-url) Figure 1 Schematic of dual-tip SPM system and the experiment. (A) Microscope image of approaching AFM and NSOM tuning fork probes. (B) Experimental set-up consisting near-field NSOM probe with 150 nm aperture illuminated with 532 nm fiber coupled laser, AFM probe with 20 nm diameter, and bottom microscope with 50× objective and aligned APD for light collection.
emission using the LabRam coupled to the multiprobe SPM system in order to obtain on-line micro-PL spectra. Then, NSOM was performed using near-field excitation and a photon-counting avalanche photo-diode (APD) to collect the near-field excited fluorescence through the objective of the microscope. From the NSOM scan, we obtained the near-field mapping of fluorescence and information about topography. Finally, the AFM tip was brought into feedback with the sample and then into soft-contact with the NSOM probe (See Figure 1A). The details of achieving soft-contact are described below. With both probes in feedback and soft-contact with each other, the sample was scanned to obtain dual-tip AFM/NSOM SPM images. A closed loop scanner from Physik Instruments (PI) was used to perform substrate-scanning of the samples.

2.1 System design

The experimental setup consists of a commercial scanning probe microscope (SPM) system Hydra Multi View 4000 (Nanonics Imaging Ltd, Jerusalem, Israel). The Multi View 4000 consists of a dual microscope to provide optics for both upright and inverted microscopy based upon two Olympus BXFM microscopes. This configuration allows for exciting the sample using transmission mode far-field and near-field optics and also reflection mode if so desired. For these initial steps in multiprobe fluorescence NSOM described in this paper, the sample was excited with a 532 nm laser in the near-field through an NSOM fiber probe with 150 nm aperture. The fluorescence was collected in transmission mode through a long working distance 50× objective (N.A.=0.45, W.D.=17 mm) of the inverted microscope from below. The fluorescence intensity was detected in transmission mode with an APD from Excelitas (SPCM-AQRH-14) after filtering out the laser excitation using a Semrock 532 nm Razor Edge long pass filter (LP03-532RE-25).

2.2 Sample preparation

Lumogen Red (BASF Lumogen F Red 305) and Lumogen Orange (BASF Lumogen F Orange 240) were obtained from BASF free of charge and used without further purification. Chemical structures of the two lumophores are shown in Figure 2A,B respectively. Solutions for doped layers of Lumogen consisted of 95% Lumogen Orange and 5% Lumogen Red. First, 9.5 mg of Lumogen Orange and 0.5 mg of Lumogen Red were combined and then dissolved in 10 ml of acetone to obtain a solution with net concentration of 1.0 mg/ml. Then the solutions were kept stirring for 3 h in order to dissolve the dye completely in the solvent.

![Figure 2](image-url)  
**Figure 2**  Chemical structure and corresponding fluorescence spectra of Lumogen dyes under study. (A) Molecular structure of Lumogen Orange, (B) Lumogen Red, and (C) fluorescence spectra of Lumogen Orange (open circles), Lumogen Red (thick line) and the doped film of Lumogen Orange and Red, 19:1 (w/w) shown in (filled squares).
Lumogen films were prepared on a glass substrate of sizes 25×25 mm² using spin coating method. The substrates are cleaned via sonication in Micro-90 semiconductor grade detergent, de-ionized water, and acetone, and then rinsed in boiling isopropyl alcohol and dried under a stream of nitrogen. Immediately following cleaning, substrates were exposed to oxygen plasma for 120 s. Lumogen layers were prepared by adding sufficient quantity of solution to cover the substrate surface. Then the substrate was rotated at 1000 rpm for 90 s with an acceleration of 1000 rpm/s, using a Headway Research PWM32 spin-coater.

3 Results

Figure 2C shows the fluorescence spectra of Lumogen Orange, Lumogen Red, and the doped film, from upon excitation with a laser at 532 nm. The Lumogen Orange sample has a photoluminescence peak (PL) at a wavelength of 622 nm, and the PL peak for the Lumogen Red sample is at 609 nm. The doped film shows two peaks; the major peak corresponds to Lumogen Red (628 nm), and the small satellite peak is from Lumogen Orange (542 nm). The intensity of the doped film compared to the neat film of Lumogen Red shows a dramatic increase in PL intensity. This is suggestive of FRET sensitized fluorescence of the acceptor fluorophore. The acceptor Lumogen Red is only 5% of the film concentration, and yet it shows 2.3 times more fluorescence at the PL peak compared to the neat Lumogen Red sample. At the same time, since in the doped film, the Lumogen red molecules are further apart, the quantum yield is expected to be higher than in a neat film even without FRET being operative. While the mechanism of fluorescence enhancement still needs to be investigated, the doped film clearly provides greater PL signal than either of the neat films, and therefore it was chosen for the investigations of dual-tip AFM/NSOM scanning.

Figure 3A shows the microscope image of the sample using far-field white-light illumination. The Lumogen molecules cluster into sub-micron sized islands. Far-field micro-PL mapping of the fluorescence is shown in Figure 3B. The integration time was set to 250 ms per point, and a 100×0.9 NA objective was used, with a 532 nm wavelength laser excitation. Based on these images, the spatial profile of a typical island is on the order of 1 μm. AFM scans of the sample show a much higher resolution image of the morphology, indicating the actual profile of such features is 600 nm in lateral extent or less depending on the island chosen.

After performing baseline AFM-only and NSOM-only measurements, dual-tip AFM/NSOM simultaneous scanning was implemented. Figure 4A–C show the topography obtained from the NSOM probe and AFM probe, and the simultaneously obtained near-field fluorescence image. Figure 4F is the height profile from the NSOM probe, which shows good correlation between maximum height and maximum fluorescence, but poor lateral topographical resolution. For the same features, the simultaneous AFM scan shows much better lateral resolution (See Figure 4D) revealing sub-140 nm diameter islands on the film. The near-field fluorescence of the same trace is

Figure 3  Far-Field images of doped Lumogen sample. (A) White-light image from reflection of the sample. (B) Micro-PL mapping using a 532 nm laser as the source of excitation.
shown in Figure 4E. The spatial extent of the nano-islands obtained from the optical signal is comparable to the AFM topography with the AFM probe, indicating the complementarity between AFM with an AFM probe and the associated NSOM probe fluorescence images. This correlation is far superior to anything that could be discerned with the AFM resulting from the convolution of the NSOM probe tip diameter (550 nm) with the sample. This is even though the NSOM tip by itself has a core diameter of 150 nm.

Due to the robustness of the Lumogen dyes, the sample showed negligible photo-bleaching during the measurement. Thus, for these initial steps in multiprobe NSOM fluorescence, it allowed for multiple measurements to verify fully this new direction in fluorescence NSOM. In particular, we were able to scan the same location more than 10 times without any observable decrease in PL signal.

4 Discussion

From these first demonstrations, we can surmise that simultaneous dual-tip AFM/NSOM SPM appears to be a general method for obtaining high-spatial resolution AFM topography combined with the spectral functionality of an NSOM probe. There seems to be every indication that as a methodology, dual-scanning can be applied to many other nano-optical modalities including AFM/TERS and, in addition, a wide variety of hyphenated AFM/SPM techniques in order to decouple the requirements of obtaining high-resolution AFM together with additional SPM functionalities. Although these conclusions were a result of our use of the Nanonics MultiView 4000 Multiprobe SPM platform, we believe these conclusions should generally apply to any future multiprobe system based on the principles of the platform we have used in this paper.

Figure 5 shows the three-dimensional image of the AFM topographical map overlapped with the NSOM fluorescence, and the good correlation between the two simultaneously obtained scans. In performing the dual-tip SPM scanning, we noticed that there is an offset between the two images of about 0.5 μm. This offset means that as the fluorescent material is scanned below the dual-tips, each tip senses the same morphology at a slightly different sample scanner position. This is entirely reasonable because the two tips have some finite lateral extent and
the points of contact with the sample for each tip are not necessarily the points of the tips that are in contact with one another.

On a technical note to carry out such dual-tip SPM measurements, it is necessary to achieve “soft-contact” between the AFM tip and the NSOM probe. First, each tip is brought into feedback with the sample. Then, while both tips are still in feedback, they are brought together into soft-contact using piezo-positioners. The Nanonics Multi-View 4000 is especially suited for landing both tips together because the system allows for off-setting, i.e., positioning, one of or more tips using piezo-positioners, and has a tip-scanning mode of operation which can readily be switched to sample scanning. Furthermore, the system can be configured so that feedback is maintained via the tip-scanner, even though the substrate is being rastered during the measurement. From the piezo-controller, one observes the onset of soft-contact by noticing an alteration in the z-channel piezo-voltage, at which point the dual-tip scan can be initiated. Once soft contact was achieved, any offset in relative tip positions of the two probes could be readily adjusted since the probes were continually maintaining feedback. This is especially due to the fact that the controller offsets permit lateral adjustments even while maintaining tapping mode feedback without lateral force. Finally, based on the sensitivity of the probes to each other’s presence, it is feasible to expect that future developments will include automatic algorithms for closely fixing the relative probe positions.

In summary, the data shown in Figure 4 clearly show that the NSOM resolution closely follows the AFM resolution obtained with the AFM probe. Using this comparison, the interpretation of the NSOM relative to the structural characteristics of the sample are readily delineated while a similar delineation of even these fairly large structures is difficult to unravel with the AFM from the NSOM probe. Thus, this indicates the potential of enhancing fluorescence NSOM to a level that adds excellence of structural correlation that is critical in a wide variety of fields from fluorescent nanoparticles to biological membranes. This level of structural correlation has the potential to lead to new horizons in such imaging.

5 Conclusion

We have shown that simultaneous dual-tip AFM/NSOM scanning combines the benefits of high spatial resolution morphological imaging with near field spectral information. As a model system, we chose to implement dual-tip simultaneous SPM on spin-cast Lumogen dye nano-clusters. The clusters were composed of donor and acceptor molecules, Lumogen Orange and Lumogen Red respectively, to achieve high absorption of the NSOM excitation and yet high brightness. This work is the first report of NSOM on Lumogen dyes, and the first demonstration of simultaneous dual-tip scanning, fluorescent NSOM. We are confident that the results of this study should lead to many exciting future applications.

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