VERY HIGH RESOLUTION CHEMICAL IMAGING WITH INFRARED SCANNING NEAR-FIELD OPTICAL MICROSCOPY (IR-SNOM)

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ABSTRACT

In this paper we present chemically highly resolved images obtained with Scanning Near-field Optical Microscopy (SNOM) coupled with an Infrared (IR) Free Electron Laser (FEL) at Vanderbilt University, Nashville, USA.
Main principles governing SNOM imaging as well as essential components of the experimental setup are described. Chemically resolved images showing the distribution of different phases within the boron-nitride films are presented. Universal character of the experiment and its huge potential applications in biophysics and medical sciences domain are illustrated with highly resolved SNOM images of pancreatic cells.

INTRODUCTION

The ability to see very small features remains fundamental in the continuous effort of understanding the Universe. Thus, first microscopes appeared already in the fifteenth century. Their improvement in resolution, i.e. in the ability to see smaller and smaller features, followed closely the general technology progress until today. However, both theoretical predictions (mathematically demonstrated by Ernst Abbe in 1870) and the experimental evidence point out that all the classic lens-based microscopes are limited in their resolution. This fundamental limitation of the resolution is usually called "diffraction limit" since it is a direct consequence of the diffraction phenomenon.
The smallest object that can be distinctly seen, with the best possible lens based optical microscope, must be bigger than $\lambda/2$ (λ being the wavelength of the light used to illuminate the object) (1,2,3). According to this, the best resolution achievable with visible light is around 250 nm. Several microscopy techniques were developed in order to get higher resolution: Scanning Tunneling Microscopes (STM), Atomic Force Microscopes (AFM), Scanning Electron Microscopes (SEM) … Still none of these microscopy techniques is really "*optical*". They allow "*sensing the shape*" of the studied object in different ways, but none of them is capable of "*seeing*" the object, i.e. of performing spectroscopic measurements. That was the main motivation for the development of Scanning Near-field Optical Microscopy (SNOM). SNOM is an optical technique allowing us to see much smaller objects than those allowed by the diffraction limit. The SNOM surpasses this limit by far. With SNOM we already achieved resolutions of the order of $\lambda/70$ working with the infrared light and attained resolutions of the order of 50 nm, independently of the wavelength of the light used to illuminate the sample. These were the record resolution values, but we routinely get optical resolutions of the order of 100 nm, much better than the conventional microscopy whose maximal resolution is around 500 nm.

WORKING PRINCIPLES OF SNOM

With a classic microscope we are always looking at a large zone of the sample (Figure 1). That is where the problem comes from: we are always seeing a superposition of light coming from every part of the sample under the objective, which means a superposition of diffraction patterns of every shape on the sample.
In 1870 E. Abbe calculated that this allows us to see distinctly only details that are set apart by more than $\lambda/2$, and also bigger than $\lambda/2$, and this with the best possible lens-based oil immersion optical microscope.

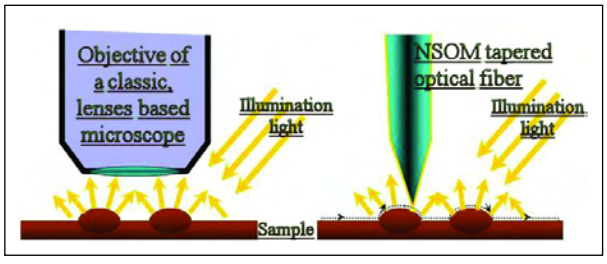

Figure 1: Conventional optical microscope (left) and SNOM (right) at work

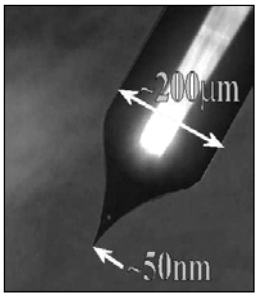

Figure 2: Photo of the tapered end of our Chalcogenide optical fiber

SNOM uses optical fibers whose end is tapered into the sharpest possible tip (Figure 2). This tapered end slowly scans the surface of the sample, collects the light through a very small aperture at its end (at this moment the smallest apertures we are able to get with silica fibers have around 50 nm diameter) and conduct the light from the sample to the detector through the fiber (in our experiments the sample was illuminated from the outside, like shown in the Figure 2). During the scanning, the fiber tip is at a constant distance of just a few nanometers from the sample, without ever touching it.
Finally, instead of getting superposed diffraction patterns of many features on the sample, we get only the signal from the very small portion of the sample under the tip. Any feature that is at least as large as the fiber tip is resolved. The diffraction doesn't limit the resolution any more, but only the size of the optical fiber tip (4-9).
It is worth to mention that a new SNOM technique has been developed recently: aperturless SNOM. The aperturless SNOM is not based on optical fibers, but on a sharp metallic tip scanning the sample and scattering the light from its surface. The technology allows much sharper tips with metal (atomic size STM tips for example) than with glass fibers (~50 nm), so that this technique can achieve much better resolutions. Yet, the work in a liquid environment, which is our final aim for biological samples, is still impossible with aperturless technique. That is why we used a more classic, optical fiber, approach to get the results presented here.

SNOM WITH THE INFRA-RED LIGHT (IR-SNOM)

There are two strong reasons that motivated us into the development of the IR-SNOM.
-First, the diffraction limit is more severe in the infrared spectral domain than in the visible: The resolution of classic, lenses based microscopes is proportional to the wavelength of the light used to illuminate the sample. So, with relatively long wavelengths of the infrared light the resolution is limited to no better than a few microns, which is at least an order of magnitude worse than for the visible light. So, we gain much more by overcoming the diffraction limit in the infrared region than in the visible spectral region.
-Secondly, a huge number of molecules have distinct absorptions spectra in the infrared region. In this sense, infrared absorptions at certain wavelengths are real fingerprints of a specific molecule. An IR-SNOM study of a sample shows not only the presence but also the exact position of the specific molecule inside the sample. IR-SNOM gives us images which are basically highly resolved chemical maps of the samples (10-13).
With optical fiber based approach, the main challenge for successful IR-SNOM setup was to find an adequate optical fiber, being able to conduct the infrared light. Then a good tapering technique had to be found in order to get the sharpest possible tips. The most suitable fibers for our experiments, in the 1 to 10 m wavelength spectral region, are Chalcogenide glass fibers (4). We tapered the Chalcogenide fibers by chemical etching and then evaporated gold on them, leaving only a small aperture, ideally of the order of 50 nm, at theirs tip (Figure 2).
An infrared (IR) absorption of a biological system can report on fundamentally important micro chemical properties. For example, molecular IR profiles are known to change during increases in metabolic flux, protein phosphorylation, or proteolytic cleavage. However, practical implementation of intracellular IR imaging has been problematic until now because the diffraction limit of conventional infrared microscopy results in low spatial resolution. We have overcome this limitation by using an IR spectroscopic version of scanning near-field optical microscopy (SNOM), in conjunction with a tunable free-electron laser (FEL) source. The results presented here clearly reveal different chemical constituents in biological cells. The space distribution of specific chemical species was obtained by taking SNOM images at IR wavelengths corresponding to stretch absorption bands of common biochemical bonds, such as the amide bond. In our SNOM implementation, this chemical sensitivity is combined with a lateral resolution of 0.1 μm. In some images we got a resolution of the order of λ/70, well below the diffraction limit of standard infrared microscopy, improving the existing resolution 70 times.
The potential applications of IR-SNOM touch virtually every aspect of the life sciences and medical research, as well as problems in materials science, chemistry, physics, and environmental research.

SHEAR FORCE SCANNING

We are using the shear-force approach for the scanning. The fiber scans the sample line per line without ever touching it. During scanning it always stays at a constant distance of just a few nanometers from the surface of the sample.
If one wants a nanometer precision resolution first he must develop a nanometer precision scanning. This was

achieved with the piezo-electric ceramics. Application of high voltages, controlled very easily and precisely, causes a few nanometers expansions or contractions of the piezo-electric materials. Moreover, these movements are almost perfectly reproducible and reversible. So, the use of these piezo-electric ceramics allows a nanometer precision controlled movements for our scanning.

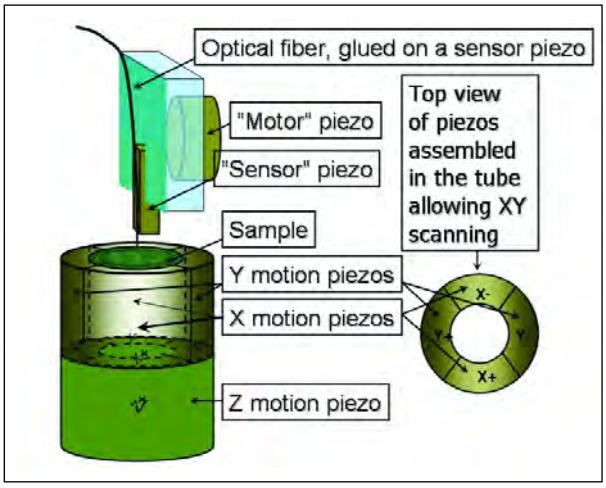

Figure 3: Scanning with piezo-electric materials

The scanning control is illustrated in the Figure 3, showing a cylindrical tube assembled from piezo-electric materials. The cylinder is divided in two: The upper half is hollow in the middle and divided in four vertical parts indicated with x+, x-, y+ and y-. In order to scan the sample we apply in the same time a voltage -V to the x- part of the tube and +V to the x+ part. In that way, in the same time the x- part gets shorter and the x+ part gets longer: all the sample stage bends and moves under the tip along the x axis in the desired direction. We can easily achieve the nanometer precision since the voltages are of the order of volts for just a few nanometers movements.

The part of the cylinder (z±) is made out of one piece piezo-electric material. Applying some voltage + or - V to it expends or contracts the cylinder in the z direction and in this way controls the height of the sample stage: the fiber tip follows the shape of the sample and stays always at a constant distance of just a few nanometers from the sample during the scanning.

The fiber tip is glued along the so-called "sensor" piezo (see Figure 4). We apply an AC voltage at the resonant frequency of the system to the "motor" piezo. The motor piezo starts to oscillate and induces a vibration to the whole system, including the fiber tip and the sensor-piezo. The sensor-piezo is partly attached to the holder, and partly free: the vibration makes it bend slightly. This bending of the sensor-piezo creates an AC electric signal (few volts of amplitude).

Then we make the vibrating tip approach the sample. At a few nanometers distance between the fiber and the sample, the intensity of the forces shown in the Figure 4 is sufficient to dump considerably the vibration. This dumping makes the sensor piezo bend less, which is translated into a decrease in the amplitude of the AC signal emanating from it.

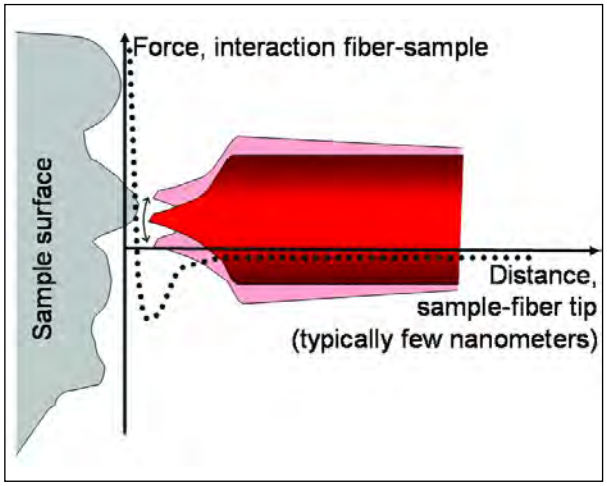

Figure 4: Interaction force between the vibrating tip and the sample (mainly Van der Waals forces) versus distance tip-sample at a few nanometers distance

Then the computer controlled feedback loop applies adequate voltages to z piezo in order to have always the same dumped value of the voltage coming form the sensor-piezo. This allows us to make a scan with the fiber at always the same constant distance of just a few nanometers from the sample.
Experimentally we have made numerous tests and we get the best quality images with an AC signal amplitude from the sensor piezo around 3V when we are far from the sample and some 10 percent decrease in amplitude when we approach. Some variations of these values are possible depending on the type of sample (biological cell or some semi-conductor film behave differently under the fiber-tip and other forces besides Van der Waals can influence the dumping).

We can chose the scan size (maximum with actual setup is 40×40 micrometers) and number of points of acquisition per line. All the results presented here are 20×20 micron scans with 200 acquisition points per line. That means that every 100 nm the fiber stopped for a few milliseconds and the light signal (intensity) from that point was acquired. With these settings our maximum possible resolution is 100 nm, but we still chose only 200 acquisition points and this limited resolution in these scans for the sake of scan time.

RESULTS

A series of experiments (Fig. 5) was conducted on tissue culture cells from a rat pancreatic cell line (INS-1). Such cells were seeded on glass cover slides; after 24 hours the medium was removed and the cells were fixed in Paraformaldehyde and washed twice with PBS and twice with distilled water. The spectroscopic-SNOM images at 6.95 μm (sulfide stretch band) and 6.1 μm (amide I, C=O stretch band) show absorption within the cell, whereas the 6.45 μm (amide II band) image shows much less contrast. Both amide I and amide II absorptions being typical for all proteins, the near featureless images taken at 6.45 μm are quite surprising. We tested this result by taking many images with consistent results in all parts of the sample. Tentatively, the fact that absorption from the Amide II band is spread out throughout the sample could be due to uniform absorption from material on the coverslip, i.e., cells and precipitated crystals. Never the less, the resolution achieved is of the order of 100 nm, much better than the one of the standard microscopy.

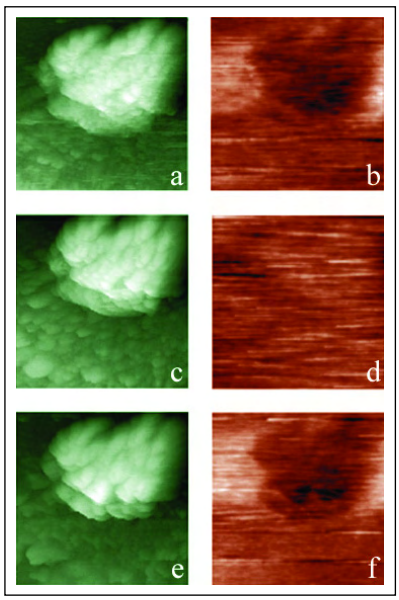

Figure 5: 20x20 μm SNOM reflection images of cells from a pancreatic INS-1 line in liquid. (a, b) topographic and corresponding optical image at a wavelength of 6.1 μm; (c, d) the same images for 6.45 μm; (e, f) the same images for 6.95 μm.

Figure 6 shows the results for a boron nitride (BN) film (with oxide components) LCVD grown on silicon. The objective in this case was to prove that spectroscopic SNOM could provide fine chemical and structural information on a microscopic scale in materials science. Specifically, the experiment targeted different vibrational modes corresponding to different crystallographic structures. The FTIR spectrum of Fig 6a reveals indeed several vibrational modes. Of these, the features at 7.4, 7.9 and 9.4 μm are directly associated to the hexagonal, wurtzite and cubic structures of BN. The spectroscopic (reflectivity) SNOM images of Fig. 6b, 6c and 6d clearly reveal differences in the lateral distribution of such structures. By comparison with the topographic image of Fig. 6e, we can see that, for example, the strongest contribution for the wurtzite structure, Fig. 6c, arises from one side of the big central grain. Figure 6f shows an intensity profile along the line marked in Fig. 6b. The intensity changes can be due to topographic or spectroscopic causes. However, changes occur in areas where they cannot be justified by topography. An extensive analysis of such changes in different line scans leads to a conservative estimate of a lateral resolution of at least λ/20, a resolution ten times better than that achieved by any other optical microscopic method.

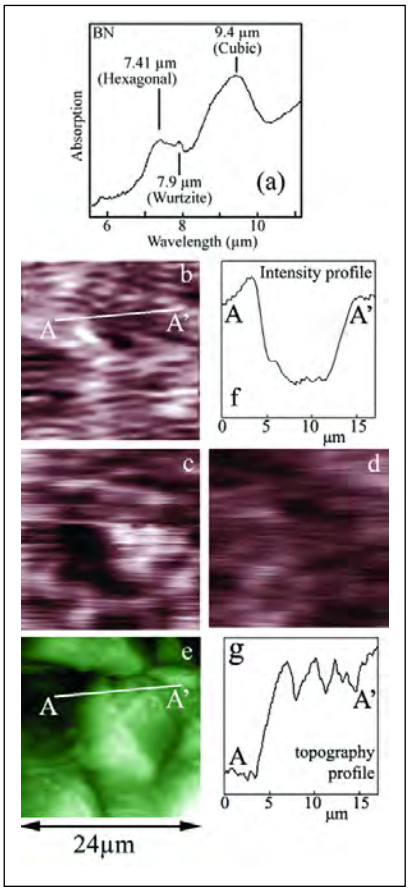

Figure 6: BN film. (a) FTIR spectrum showing vibrational modes corresponding to three crystallographic phases. (b-d) Spectroscopic (reflectivity) SNOM images at the wavelengths 9.4, 7.9 and 7.41 mm. (e) Topographic SNOM image. (f, g) Profiles for the marked line

CONCLUSION

Infrared Scanning Near-Field Optical Microscopy (IR-SNOM) makes high resolution chemical imaging possible. Still in the development and testing period, it already gives resolutions ten times better than the classic lenses based microscopy. It is a versatile, cost-effective and relatively simple technique. Completely non destructive for the samples, it works in any kind of environment (liquid, air …) and doesn't require any special sample preparation. Next steps in development will involve optimization of the technique for work with living cells. One of the critical parameters is a scan time, which is actually around 35 minutes for highly resolved (resolution around 100 nm) 30x30 μm scans. Following intracellular processes and metabolism requires much smaller times. This is an important direction for the future experimental development.

Present tests presented in this article demonstrate that the technique really works and offers a unique optical resolution (up to 50 nm) and possibilities both in materials science and biology.

REFERENCES

(1) O'Keefe J.A., Resolving power of visible light, J. Opt. Soc. America 1956; 46: 359-363.

(2) Ash E.A. and Nichols G., Super-resolution aperture scanning microscope, Nature 1972; 237: 510-520.

(3) Born M. and Wolf E., Principles of optics, 6th Ed. Pergamon, Oxford, 1987.

(4) Cricenti A., Generosi R., Barchesi C., Luce M., Rinaldi M., Coluzza C., Perfetti P., Margaritondo G., Schaafsma D.T., Aggarwal I.D., Gilligan J.M., and Tolk N.H., First experimental results with the free electron laser coupled to a scanning near-field optical microscope, Physica Status Solidi a-Applied Research 1998;170: 241-247.

(5) Cricenti A., Generosi R., Perfetti P., Gilligan J.M., Tolk N.H., Coluzza C., and Margaritondo G., Free-electron-laser near-field nanospectroscopy, Appl. Phys. Lett. 1998; 73: 151-159.

(6) Cricenti A., Generosi R., Perfetti P., Margaritondo G., Almeida J., Gilligan J.M., Tolk N.H., Coluzza C., Spajer M., Courjon D., and Aggarwal I.D., Interface applications of scanning near-field optical microscopy with a free electron laser, Physica Status Solidi a-Applied Research 1999;175: 317-329.

(7) Cricenti A., Generosi R., Herold G., Chiaradia P., Perfetti P., Margaritondo G., Gilligan J.M., and Tolk N.H. Chemical contrast observed at a III-V heterostructure by scanning near-field optical microscopy, Physica Status Solidi a-Applied Research 1999; 175: 345-349.

(8) Schaafsma D.T., Mossadegh R., Sanghera J.S., Aggarwal I.D., Luce M., Generosi R., Perfetti P., Cricenti A., Gilligan J.M., and Tolk N.H.. Fabrication of single-mode chalcogenide fiber probes for scanning near-field infrared optical microscopy, Opt. Eng. 1999; 38: 1381-1385.

(9) Schaafsma D.T., Mossadegh R., Sanghera J.S., Aggarwal I.D., Gilligan J.M., Tolk N.H., Luce M., Generosi R., Perfetti P., Cricenti A., and Margaritondo G., Singlemode chalcogenide fiber infrared SNOM probes. Ultramicroscopy 1999; 77: 77-81.

(10) Vobornik D., Margaritondo G., Cricenti A., Generosi R., Luce M., Tolk N.H., Perfetti P. Chemically resolved imaging of biological cells and thin films by Infrared Scanning Near-Field Optical Microscopy (IR-SNOM) JPEG, Poster presentation at EPIOPTICS International School of Solid State Physics, Erice-Sicily: 20-26 July 2002.

(11) Cricenti A., Longo G., Mussi V., Generosi R., Luce M., Perfetti P., Vobornik D., Margaritondo G., Thielen P., Sanghera J.S., Aggarwal I.D., Tolk N.H., Baldacchini G., Bonfigli F., Flora F., Marolo T., Montereali F.M., Faenov A., Pikuz T., Somma F. and Piston D.W. IR-SNOM on lithium fluoride films with regular arrays based on color centers. Phys. Status Solidi C 2003; 0 (8): 3075-3080.

(12) Cricenti A., Longo G., Luce M., Generosi R., Perfetti P., Vobornik D., Margaritondo G., Thielen P., Sanghera J.S., Aggarwal I.D., Miller J.K., Tolk N.H., Piston D.W., Cattaruzza F., Flamini A., Prosperi T., Mezzi A. AFM and SNOM characterization of carboxylic acid terminated silicon and silicon nitride surfaces, Surface Science 2003; 544 (1): 51-57.

(13) Cricenti A., Marocchi V., Generosi R., Luce M., Perfetti P., Vobornik D., Margaritondo G., Talley D., Thielen P. et al. Optical nanospectroscopy study of ion-implanted silicon and biological growth medium, Journal of Alloys and Compounds 2004; 362 (1-2): 21-25.