Scanning Near Field Optical Microscopy (SNOM): Applications in Material Science and Biology

Paolo Perfetti
Istituto di Struttura della Materia
CNR-ROMA

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MICROSCOPY

OPTICAL MICROSCOPY
The observation is made possible by the reflected or transmitted light through different optical systems (objectives and lenses). The resolution limit for the Distance of two nearest points is $\lambda/2$.

DIFFRACTION LIMIT

to increase resolution

SCANNING NEAR FIELD OPTICAL MICROSCOPY (SNOM)

SCANNING ELECTRON MICROSCOPY (to reduce $\lambda$)
Summary:

• Near-field microscopy (SNOM) in a nutshell

• Practical examples of results:
  – PtSi/Si
  – Spectroscopic SNOM: Diamond films
  – Biological growth medium
  – Pancreatic cells
  – LiF
  – Internal photoemission, Schottky Barrier
  – Boron nitride
  – Fluorescent markers in cells

• The future: new sources -- 4GLS etc.
Thanks to all the partners of this work:

- Monterotondo - ISM-CNR: F. Cattaruzza, A. Flamini, T. Prosperi
- EPFL: D. Vobornik, G. Margaritondo, S. Catsicas, P. Steiner, H. Hirling
- Frascati - ENEA: G. Baldacchini, F. Bonfigli, F. Flora, T. Marolo, R. M. Montereali
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- University of Roma: A. Congiu Castellano

Nashville country music
Swiss cheese & Frascati wine
The scanning near-field optical microscope (SNOM): like the stethoscope

Heart:
- Frequency $\approx 30$-100 Hz
- Wavelength $\lambda \approx 102$ m
- Accuracy in localization $\approx 10$ cm $\approx \lambda /1000$

SNOM resolution: well below the “diffraction limit” of standard microscopy ($\approx \lambda$)
The diffraction limit and how we can beat it

Far-field microscope:

Diffraction by the lens aperture blurs the images of the two objects: the minimum separation $\Delta y$ that can be resolved is $\lambda/2$

Near-field scanning microscope: diffraction does not play a role

Tapered optic fiber
The electromagnetic field generated by an electrical dipole contains terms proportional to $1/r$, $1/r^2$, and $1/r^3$.

For $r >> \lambda$ (far field) only the term $1/r$ survives and the Poynting vector $S$ and its flux have a well definite value and the traveling wave can be detected far from the dipole.

For $r \approx \lambda$ (near field) The terms $1/r^2$ and $1/r^3$ do not contribute to $S$, they represent the near field whose intensity decay exponentially to zero.
A simple approach to SNOM

Task: resolving two small objects illuminated by a light-wave in this case two slits separated by a distance $x_0$

The uncertainty principle applied to the wave requires: $\Delta x \Delta k_x > 2\pi$

the minimum $\Delta x$ corresponds to the maximum $\Delta k_x$, but

$$k_z = (k^2 - k_x^2)^{1/2} = [(2\pi/\lambda)^2 - k_x^2]^{1/2}$$

we may have two cases

$k_z$ is real, then $-2\pi/\lambda < k_x < 2\pi/\lambda$
the maximum value for $\Delta k_x$ is $4\pi/\lambda$
and the minimum value for $\Delta x$ is, from the uncertainty principle $\lambda/2$, far field limit
second case

$$k_z = (k^2 - k_x^2)^{1/2} = [(2\pi/\lambda)^2 - k_x^2]^{1/2}$$

$k_z$ is imaginary (evanescent waves): then $\Delta k_x$ can be larger than $4\pi/\lambda$ and one can have $\Delta x < \lambda/2$ (near field limit)

How to get an imaginary $k_z$

After a pinhole of width $a$, the wave will have a strong Fourier component along $x$-direction, with $k_x = \pi/2a$, corresponding to

$$k_z = (k^2 - k_x^2)^{1/2} = [(2\pi/\lambda)^2 - (\pi/2a)^2]^{1/2}$$

if $a < \lambda/4$ then one has an evanescent wave with imaginary $k_z$
SNOM transmission mode

sample

plane wave

small aperture
\( a \ll \lambda \)

E. H. Synge Philos. Mag. 6, 356 (1928)
SNOM transmission mode

sample

plane wave

small aperture

\(a << \lambda\)

DETECTOR
Multi-purpose SNOM module built at the ISM-CNR (Istituto di Struttura della Materia), Frascati [Antonio Cricenti et al.]

Modes of operation:

- **Fiber tip**
  - Transmission
- **Sample**
  - Reflection
- **Collection**
IR SNOM -- the strong points:

- It beats the diffraction limit thus reaching high lateral resolution
- It can work in any environment and (almost) without any particular sample preparation
- It provides real optical signal
- It can perform spectroscopic measurements
- It does not touch the sample: totally non-destructive technique
Fiber Preparation:

- Silica fibers for $\lambda = 400 \text{ nm} - 2 \mu \text{m}$
  (tips: produced by pulling & CO$_2$ laser cutting)
- Chalcogenide (As sulphide) fibers for $\lambda = 2 - 10 \mu \text{m}$
  (tips: produced by pulling & thermal cutting)

### Chemical etching for IR fibers:

- **Tampon solution**
  (Tetra-methyl-penta-decane)
- **Piranha Solution**
  70% H$_2$SO$_4$ + 30% H$_2$S$_2$
Final structure
(tapered chalcogenide fiber with Au coating):

190 µm

As$_{38}$Se$_{62}$

Au

As$_{40}$Se$_{60}$

120 µm
Managing the distance fiber tip-sample:

When the tip approaches the sample, it feels the shear forces and the oscillation is damped:

- Optical fiber
- Dieter piezo
- Laser beam
- Sample
- Detector
- Amplitude
- Oscillation (several kHz)

Optimum: 60-70% of maximum amplitude
...our final component -- the Vanderbilt FEL!
WHAT IS A SASE-FEL RADIATION SOURCE?

SPONTANEOUS RADIATION:

\[ \lambda_{ph} \cong \frac{\lambda_u}{2\gamma^2(1 + K^2)} \]

Beam divergence \( \cong \frac{1}{\gamma} = \frac{m_e c^2}{E_e} \cong 1000 \mu\text{rad} \)

\[ I_{ph} \cong N_e \]

\( N_e \) number of electrons (\( \cong 10^9 \))

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THE INTERACTION OF THE ELECTRONS WITH THE SPONTANEOUS RADIATION CAUSES MICROBUNCHING AND THE SELF-AMPLIFICATION OF SPONTANEOUS EMISSION (SASE)

IN THE SASE MODE THE INTENSITY:

\[ I_{ph} \approx N_e^2 \]

SINCE \( N_e \) number of electrons (\( \approx 10^9 \))

THE AMPLIFICATION GIVES AN EXTRAORDINARY HIGH PHOTON FLUX

Beam divergence (few \( \mu \text{rad.} \))

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The penetration depth of photons inside the material depends on the wave-length of the incident laser beam. In first approximation the bigger is \( \lambda \), the bigger is \( d \).

\[
\lambda = \frac{c}{\nu} = \frac{hc}{hv}
\]

The penetration depth of electrons is limited to the surface of the sample. The electrons are not a good probe for investigating the bulk properties.
SNOM IN THE SPETTROSCOPIC MODE

Take the image at $\lambda$ values where the sample has a spectroscopic fingerprint

- reflectivity
- absorption
- fluorescence
- .......

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This is an example of how we can use a SNOM to investigate bulk optical properties with lateral resolution well below the diffraction limit.

12 \mu m by 10 \mu m images of PtSi/Si obtained with free electron laser radiation in near-field condition.
Absorption Spectrum of Artificial Diamond Films

$\lambda = 3.5 \, \mu m$
Artificial CVD Diamond Film

Near Field Optical images

Topographic Images
SNOM/FEL Images of Biological Growth Medium

• Surfaces immersed in water under physiological conditions are rapidly covered with biofilms, which consist of microorganisms embedded in a matrix of extracellular substance.

• Intensive research is in progress to elucidate the influence of biofilm activity on a variety of industrial, medical and environmental processes.

• The first step of this research is to study the growth medium of biofilms.

• The growth medium is a minimal salt medium with several constituents like sulphur compounds and nitrogen oxide.
First IR spectroscopic SNOM results on bio-systems: postgate growth medium on silicon

FTIR results show absorption near 7 µm due to sulfur and nitrogen compounds:

- 6.6 µm
- 6.95 µm

Noise: <15% of signal
First IR spectroscopic SNOM results on biosystems: resolution tests

Line scan: Resolution < 150 nm = \lambda/40
Absorption of a Pancreatic Cell

\[ \lambda = 6.1 \, \mu m \]

\[ \lambda = 6.45 \, \mu m \]
Shear-Force and SNOM Images (20x20 μm) of Pancreatic Cells with photons of 6.1 μm

Shear-Force

Reflectivity

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IR spectroscopic SNOM results on bio-systems: fixed (with paraformaldehyde) cells in water from a rat pancreatic β cell line (INS-1)

- $\lambda = 6.95 \, \mu m$ (sulfide stretch)
- $\lambda = 6.45 \, \mu m$ (amide II)
- $\lambda = 6.1 \, \mu m$ (amide I, C=O stretch)

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IR spectroscopic SNOM study of metal (CdCl$_2$) contamination of pancreatic cells

\[ \lambda = 0.488 \, \mu m \quad 0.630 \, \mu m \quad 0.785 \, \mu m \quad 1.25 \, \mu m \quad 4.05 \, \mu m \]
First IR spectroscopic SNOM results on bio-systems: COS-7 cells (not fixed) in PBS

- Topography
- 20 µm
- Cell
- PBS crystal
- The nucleus is best seen here
- 8.05 µm (phosphates, DNA)
- 7.6 µm C-H bending
- 6.95 µm (sulfide)
- 6.45 µm (amide II)
- 6.1 µm (amide I)
- 20 µm
How important is IR SNOM, in particular for IR bio-research?

**Very important:** due to the long wavelengths, IR techniques are severely affected by the diffraction limit!

- Spectroscopic SNOM image of cell taken at 6.1 µm
- Same image with diffraction-limit blurring
First example of IR Spectroscopic SNOM: diamond films
Lithium fluoride (LiF) films on silicon

LiF - an interesting material for waveguides, microcavities, laser sources and generally optoelectronics devices:

- Non hygroscopic
- Irradiation by x-rays, e-beams and ion beams produces stable color centers (F-center) that can be used in device manufacturing. X-rays give high spatial resolution.

We need to clarify how the film growth parameters influence the coloring process and to compare film coloring with bulk coloring.
LiF crystal film on Si "colored" with X-rays through a 12.7 µm grid. Topography, fluorescence SNOM and reflectivity SNOM

The SNOM contrast at 9.2, 6.1, 5.8 and 5.5 µm reveals a coloration-induced change in the refractive index
LiF film pattern created with X-rays on a Si substrate

Shear-force (topography)

Reflectivity at 6.1 µm

The SNOM contrast at 9.2, 6.1, 5.8 and 5.5 µm reveals a coloration-induced change in the refractive index

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SNOM and Internal Photoemission (IPE) an improved local probe
The first steps: “FELIPE” (FEL Internal photoemission)

The Schottky Barrier is measured directly

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SNOM as a Useful Probe in Microelectronics

e.g. SNOM Images of Boron Doped Silicon (100)

Ion implantation is the key technique in integrated Circuits Technology

Depending on the Boron doses the post annealed samples may present clusters formation or other defects

SNOM/IPE Techniques may be used as powerful diagnostic tools

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IR Spectroscopic SNOM:
Boron-doped (ion-implanted, annealed) silicon

Topography
Reflectivity
Photocurrent

10 µm

Wavelength:
1.33 µm

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IR Spectroscopic SNOM: BN thin films (+ oxides)

Line scan: features narrower than $\lambda/9$

SNOM topography image

Wurtzite

Cubic

Hexagonal

SNOM reflection spectroscopic images

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A negative but important result: carboxylic acid terminated Si and silicon nitride surfaces (chemical processing for biological sensors)

Surface—\((\text{CH}_2)_n(\text{COOH})\)

IR spectroscopic images taken at this and other wavelengths show no lateral fluctuations

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Spectroscopic SNOM results on bio-systems: preliminary tests on internal cell structures labeled by a fluorescent marker

Diagram:
- Fluorescent marker
- Cells at Petri Dish bottom
- Detector (avalanche photodiode)
Intestinal adeno carcinoma epithelial cells labeled with Fluorescein Isothiocynate

Shear-force topography

Transmission $\lambda = 488.1 \text{nm}$

Fluorescence $\lambda = 512 \text{nm}$

25 x 25 $\mu$m

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Fibroblast cells (COS) with Oregon green fluorophore (excitation at 496 nm, emission at 522 nm) fixed to Transferrin

Neuron cells with Oregon green fluorophore directly attached to vesicles inside the cell
Fluorescence: SNOM vs far-field

Near-field fluorescence imaging of Osteosarcoma cells SAOS-2

Shear-force (topography)  Fluorescence $\lambda=512$  Fluorescence $\lambda=617$

CCD Camera fluorescence images

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SNOM Fluorescence study of magnetic field (24 hrs 50 Hz, 1 mT) effects on human keratinocyte cells

unexposed cells

exposed cells

Topography
SNOM fluorescence

Topography
SNOM fluorescence
New types of synchrotron sources:

- Ultrabright storage rings (SLS, new Grenoble project) approaching the diffraction limit
- Self-amplified spontaneous emission (SASE) X-ray free electron lasers
- VUV FEL’s (such as CLIO)
- Energy-recovery machines
- Inverse-Compton-scattering table-top sources
The 4GLS concept involves the use of an energy recovery linac (ERL) in a ring configuration. This is extremely flexible and will allow the easy incorporation of, for example, a single pass XUV-FEL that would allow the generation of coherent pulses of electron laser radiation up to several hundreds of eV in energy.
Conclusions:

1. SNOM experiments with an FEL are possible and yield resolution levels well beyond the diffraction limit
2. Vibrational spectroscopy SNOM provides chemical information on a microscopic scale
3. Results on biological systems finally open up the possibility to perform infrared microscopy of cells and internal cell structures
4. IR Spectroscopic SNOM tests were successfully performed in different SNOM modes
5. The experimental performances did not yet reach saturation and substantial improvements can be foreseen
6. In the near future, even better IR sources will further boost the capabilities of IR SNOM
A powerful international coalition:

Nashville country music

Swiss cheese & Frascati wine

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The first steps: “FELIPE” (FEL Internal photoemission)

Problem: the high value of the photon energy “mixes” the conduction band discontinuity and the local gap

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The first steps: “FELIPE” (FEL Internal photoemission)

The FEL removes the problem and the conduction band discontinuity is measured directly
Another problem, however, still remains: is the discontinuity the same for the entire interface?

Solution: FELIPE
(essentially, a near-field SNOM system)

First SNOM results with an FEL!

Lateral fluctuations!
SNOM: why does it work?

Uncertainty principle:
\[ \Delta y \Delta k_y > 2\pi \rightarrow \Delta y > \frac{2\pi}{\Delta k_y} \]
\[ \Delta k_y < k_y = (k^2 - k^2_x)^{1/2} \]
\( k_x \text{ real} \rightarrow \Delta k_y < k = \frac{2\pi}{\lambda} \)
\[ \Delta y > \lambda \]

( Abbé’s diffraction limit)

This conclusion is no longer valid if

\[ k_x \text{ is imaginary} \]

(absorbed, evanescent waves)
IR SNOM: a multi-purpose module built at the ISM (Istituto di Struttura della Materia, CNR), Frascati [Antonio Cricenti et al.]

Resolution
< 0.15 µm << λ
...our final component -- the Vanderbilt FEL!

- Laser Wavelengths 2.1 to 9.8 microns
- Mirror Bandwidths 2.0 - 3.0, 2.8 - 4.2, 4.0 - 6.0, 6.0 - 9.0
- Electron beam energy 25 to 45 MeV
- Electron macropulse length 8.0 microseconds
- Electron micropulse length <1 picosecond (approximately 0.7 ps)
- Electron bunch separation 10.5 cm, 351 ps
- RF power 12 to 30 MW
- RF pulse length 9.0 microseconds
- Klystron video pulse length 14 microseconds
- Repetition rate, up to 30 Hz
Thanks to all the partners of this work:

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