SCANNING MICROWAVE MICROSCOPY IN BIOLOGY

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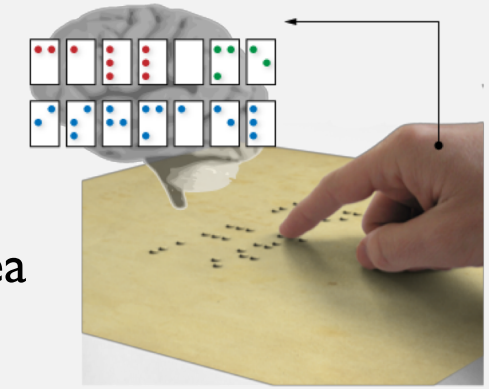
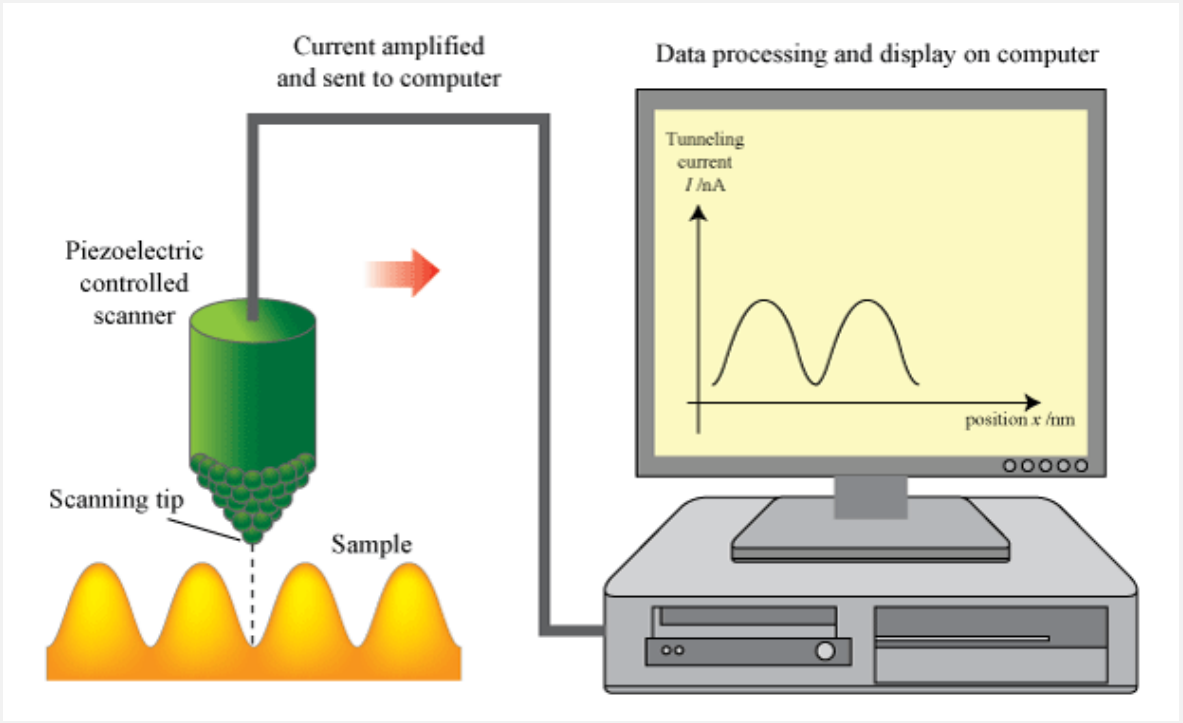
SCANNING PROBE MICROSCOPY

Optical Microscopy limit

In 1986 Gerd Binnig and Heinrich Rohrer (IBM in Zurich) won the Nobel Prize in Physics for inventing it.[1,2]

SPM Idea

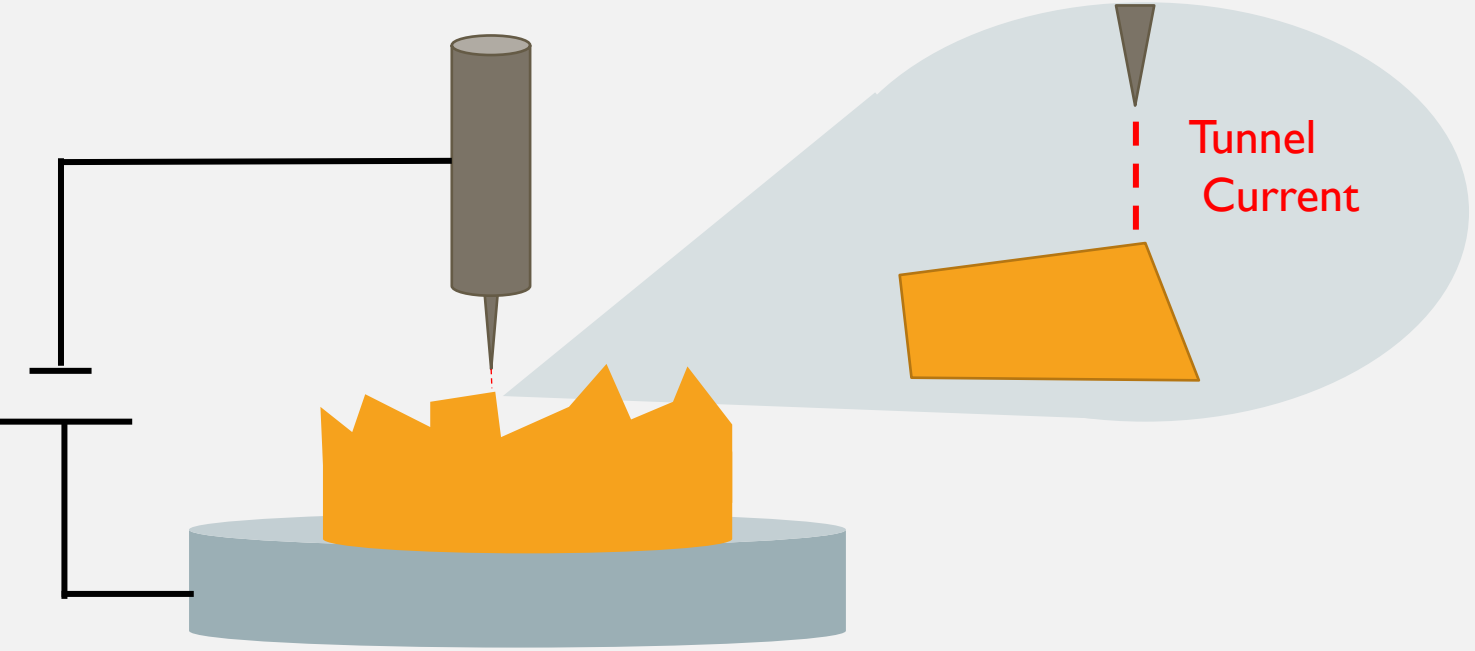


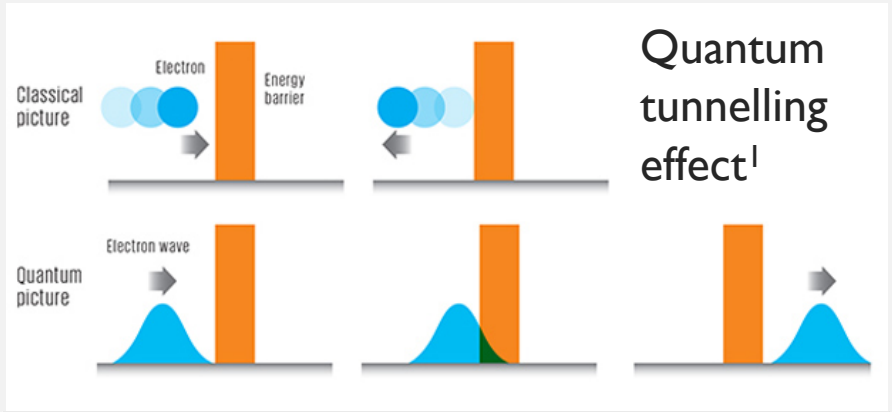


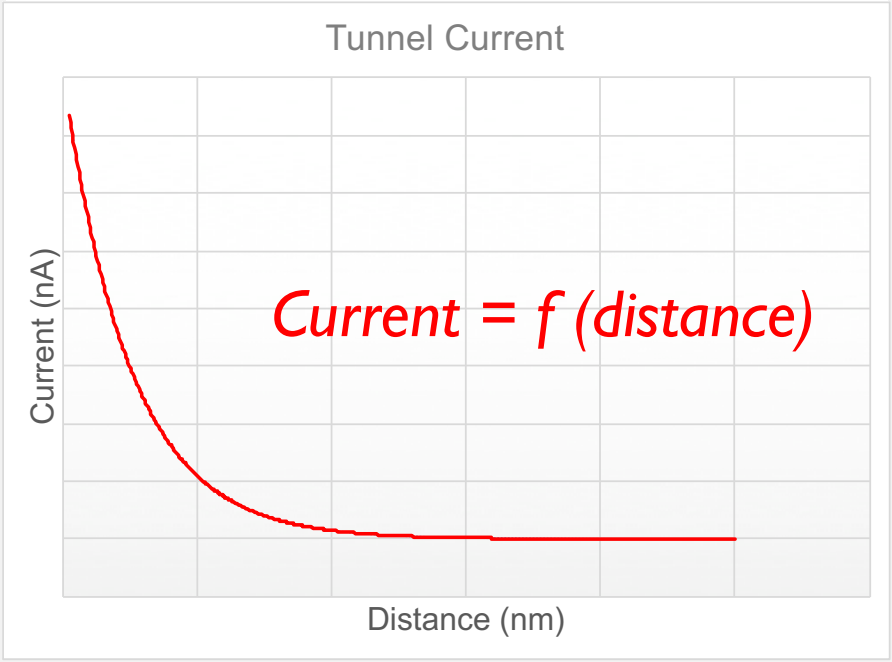
- A probe is raster scanned while detecting interaction
- Generally piezoelectric membranes displace the sample or the probe at sub-nanometric scale
- Membranes are driven by some feedback system

1 G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Phys. Rev. Lett. 50, 120 - 123 (1983)
2 G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Phys. Rev. Lett. 49, 57 - 61 (1982)

SCANNING TUNNELING MICROSCOPY







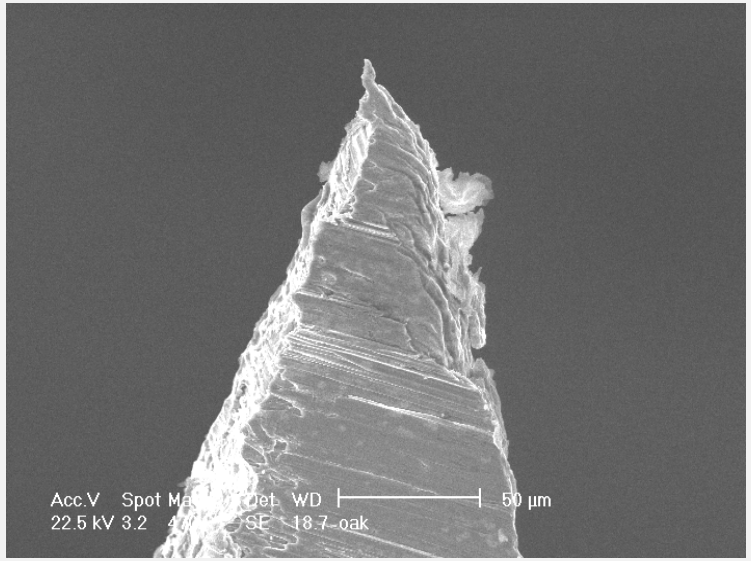
Tip-sample biasing and recording tunnel current;

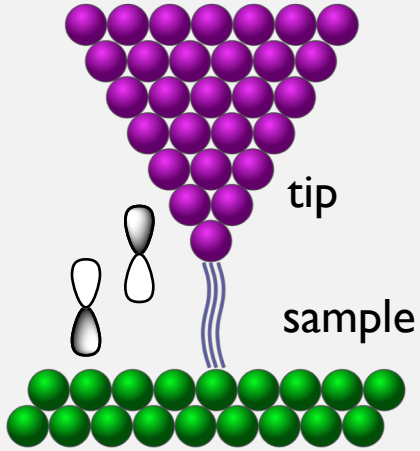
Scan "constant current" or "constant height";

Easy to get atomic resolution but would theoretically need a "conductive" sample.

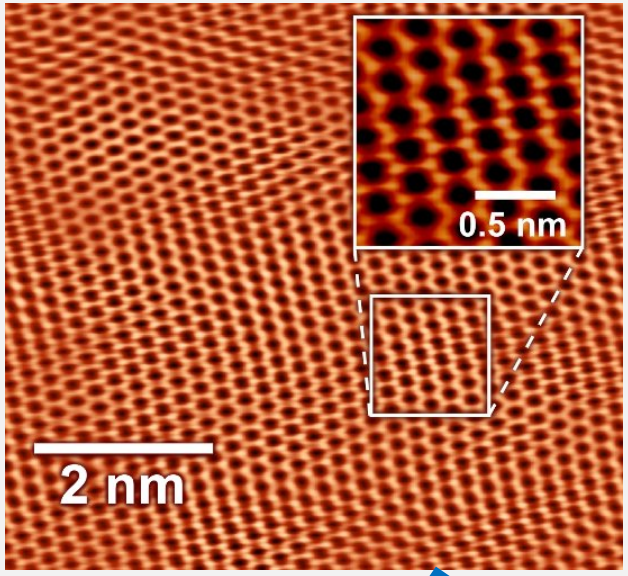
1 S. Kalinin and A. Gruverman, Scanning Probe Microscopy, Vol 1, Springer, 2007

SCANNING TUNNELING MICROSCOPY





The resolution of an image is limited by the radius of curvature of the scanning tip.



Advantages

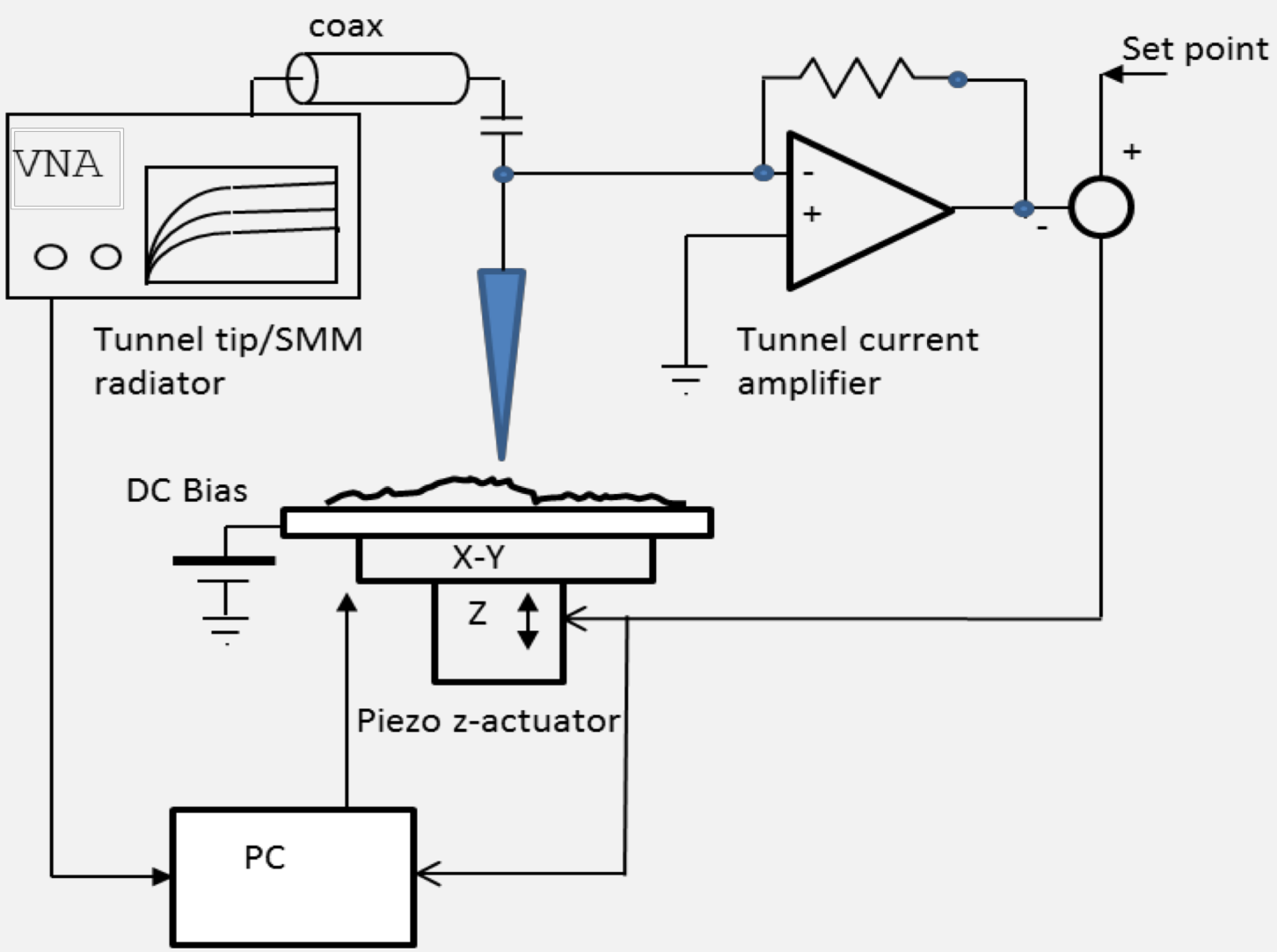
- Atomic resolution
- STM probe can be used also as microwave probe
- Low parasitics in the microwave system

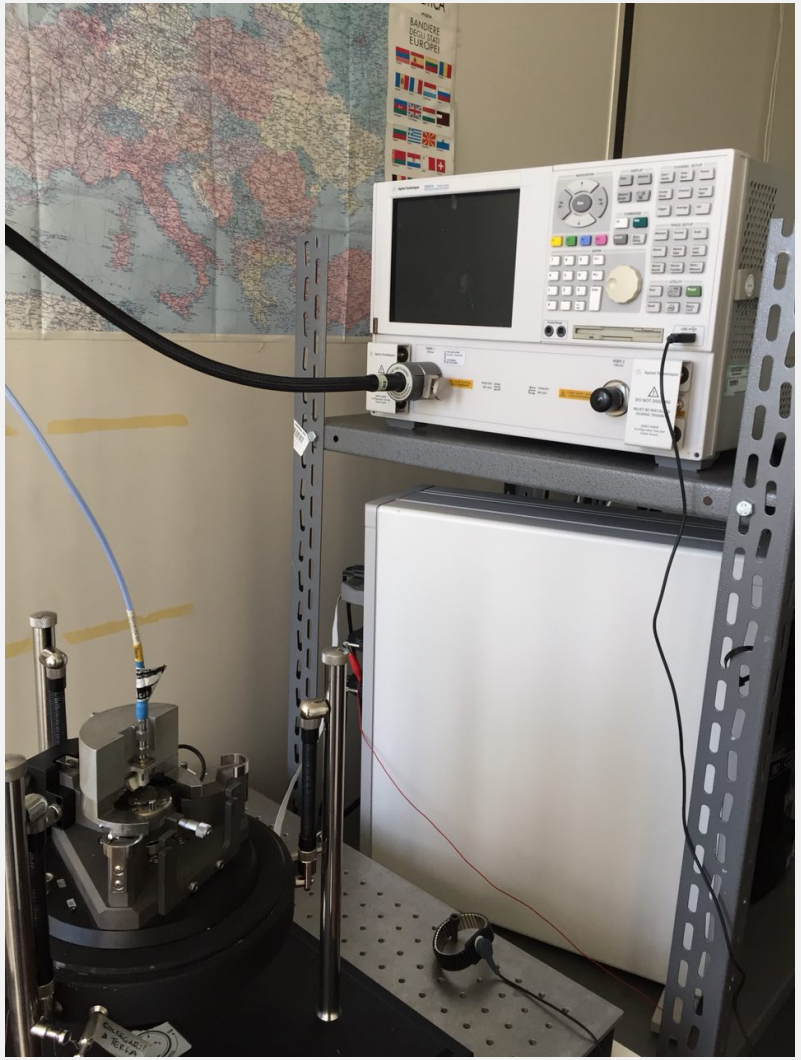
Disadvantages

- Not purely topographic (DoS)
- Difficult on poorly conductive samples
- Very difficult in salty liquids
- Needs bias

public/news/blog/2006-2009/2009-01-23_atomic-resolution-stm.jpg

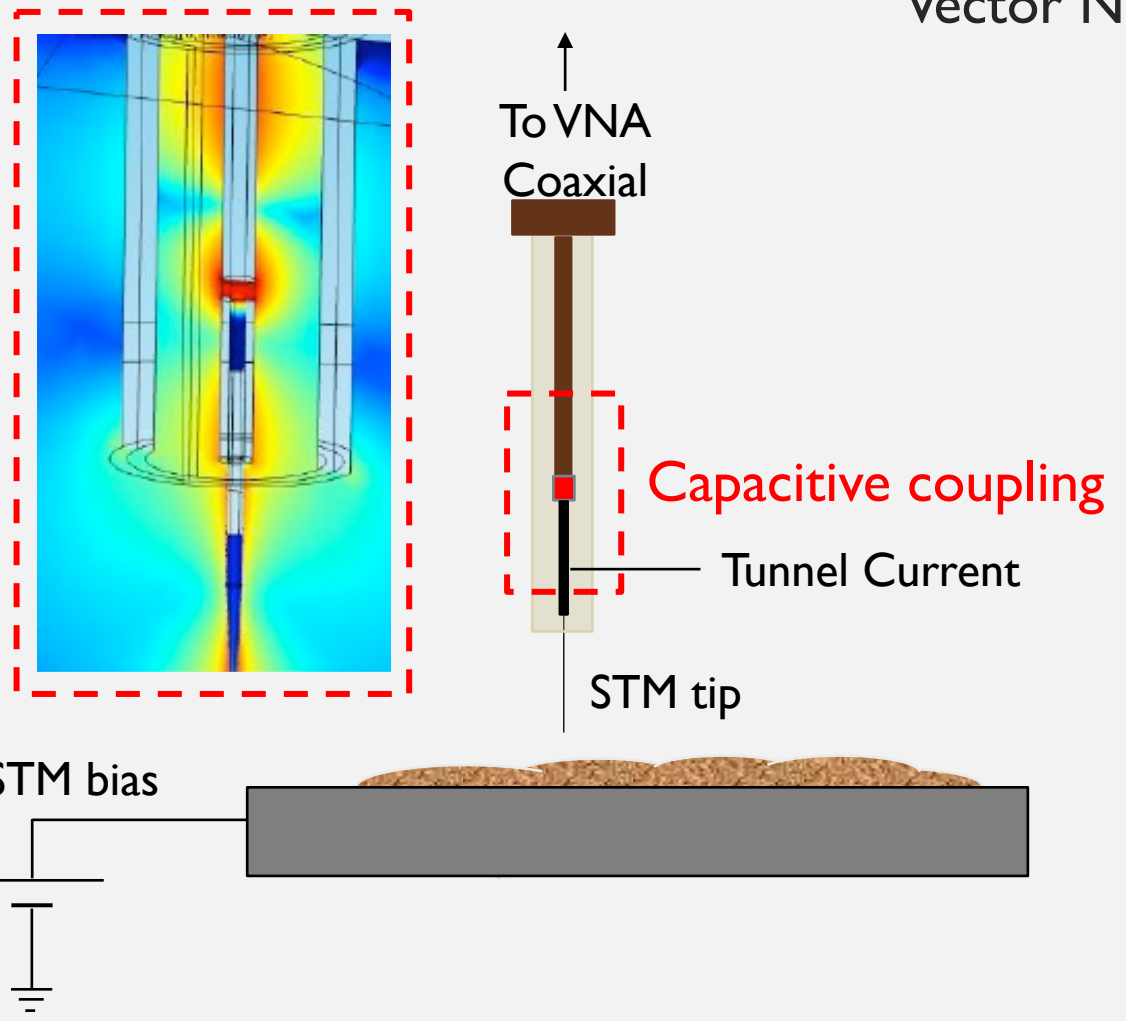
SMM COUPLED WITH STM





SCANNING MICROWAVE MICROSCOPY

Short range interaction can be "near-field" (quasi-static) microwave field generated by a Vector Network Analyzer (VNA)



- Short range interaction can be the near field of a probe: detect changes in reflection coefficient
- Imaging resolution: tip radius (edge effect)
- Quantitative detection of electromagnetic parameters
- Probe can be the tip of an STM, or of an AFM

SCANNING MICROWAVE MICROSCOPY

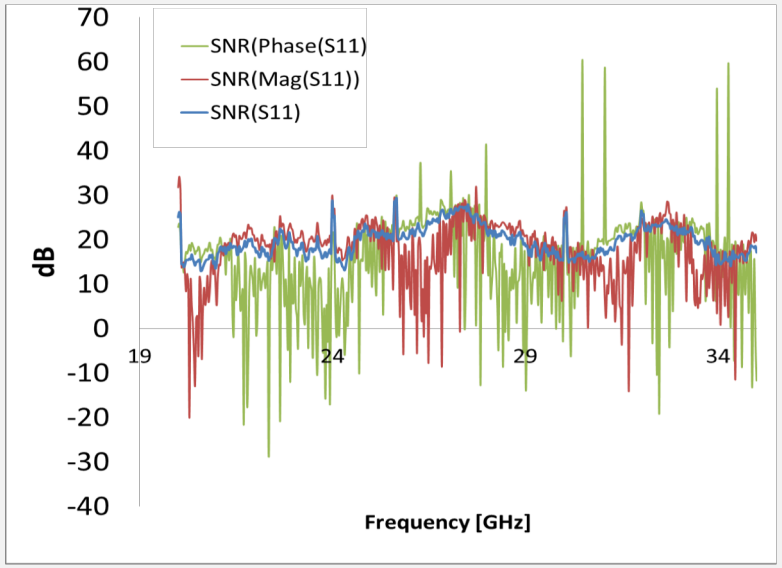
Broadband

- Broadband approach allows "frequency spectroscopy"
- Time-domain analyses
- Lower sensitivity in the single frequency
- Slower

Resonating

- Higher sensitivity in single frequency
- Faster

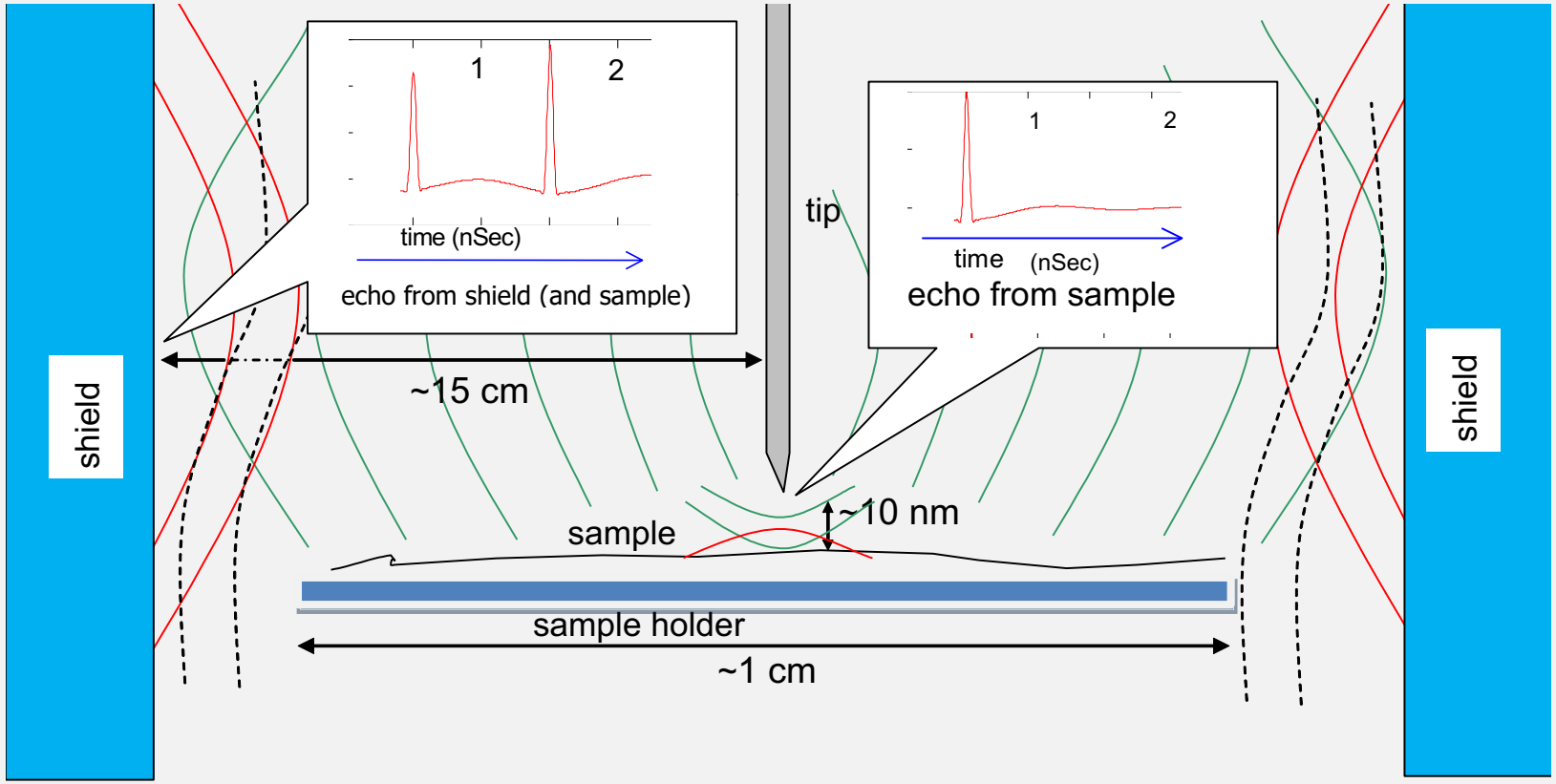
Selection of the frequency range



$$SNR(p(f)) = 20Log\left(\frac{\left|\bar{p}_1(f) - \bar{p}_2(f)\right|}{\sigma(p(f))}\right)$$

- $p(f)$ is either the magnitude or phase or complex value of the microwave reflection coefficient S11,
- $p1(f)$ and $p2(f)$ are $p(f)$ measured under two distinct conditions

SCANNING MICROWAVE MICROSCOPY TIME DOMAIN



"Disentangling" time

In our SMM implementation there are radiated fields

we gate-out unwanted non-local contributions

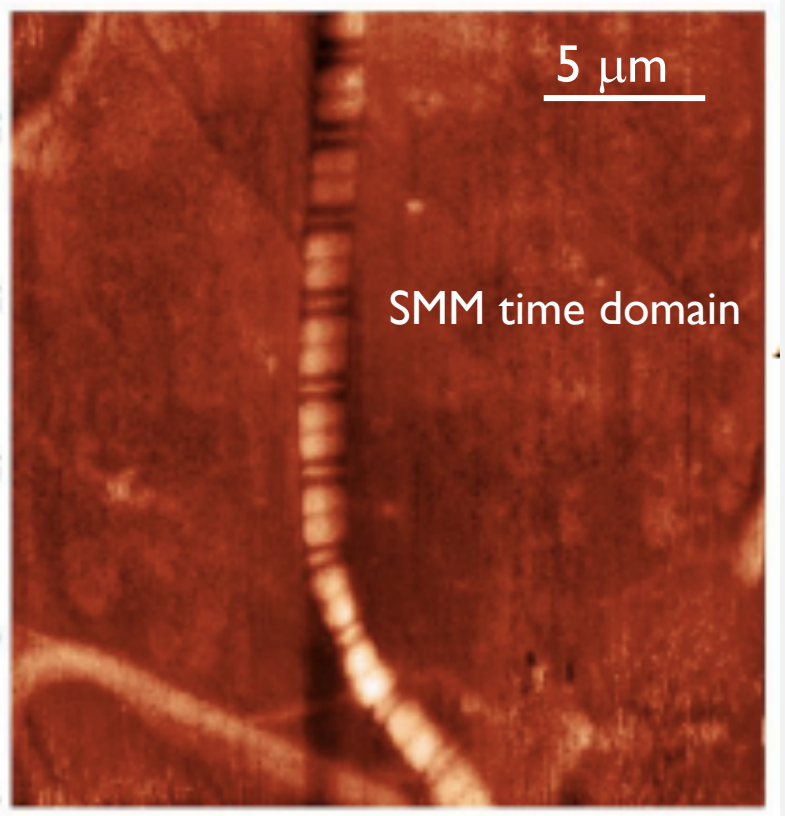
STM/SMM BIOLOGICAL APPLICATION

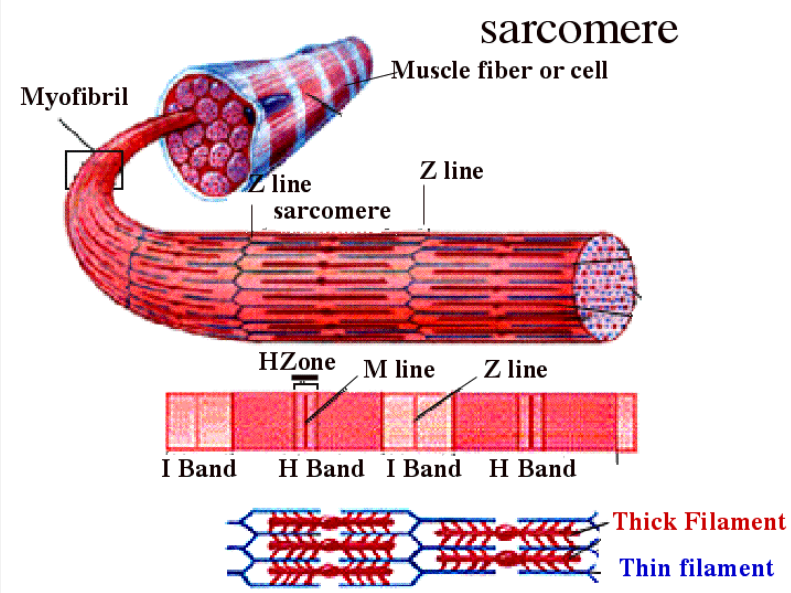
CONDITION:

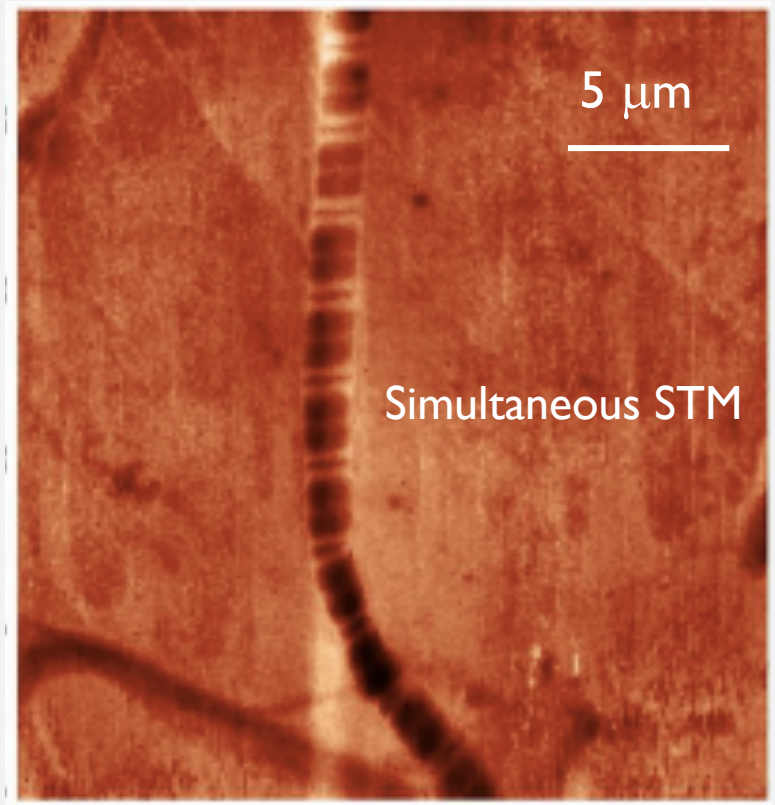
- Dried samples / Samples scanned while drying;
- Conductive substrate to hold the sample;
- Fixed sample (using Poly-Lysine) scanned in physiological environment (liquid buffer):

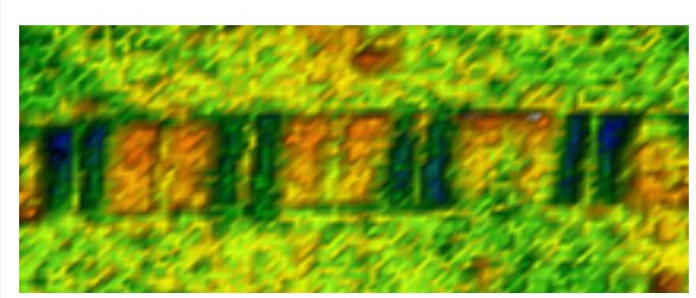
- STM probe can be used also as microwave probe
- Low parasitics in the microwave system
- Broadband approach allows frequency spectroscopy
- Time-domain analyses and improvement of image quality

ISOLATED MYOFIBRILS



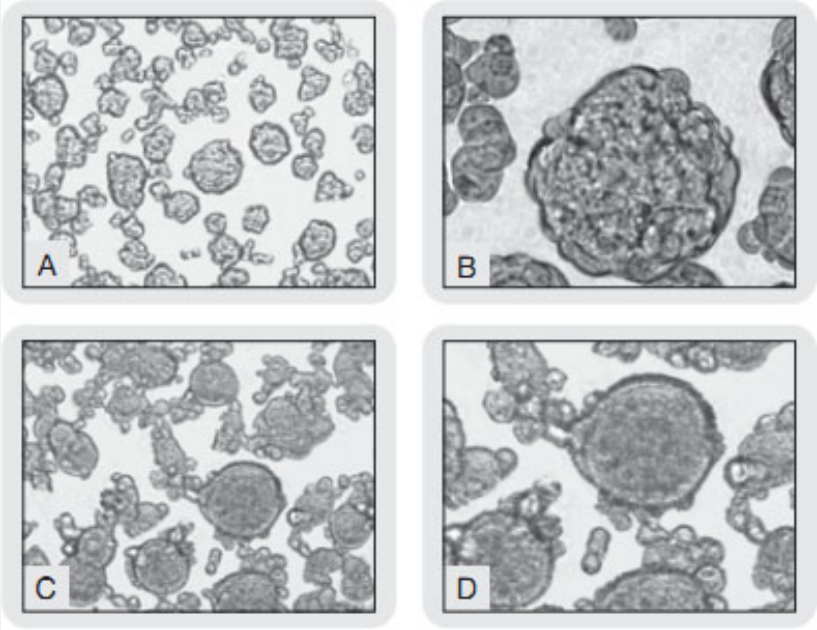




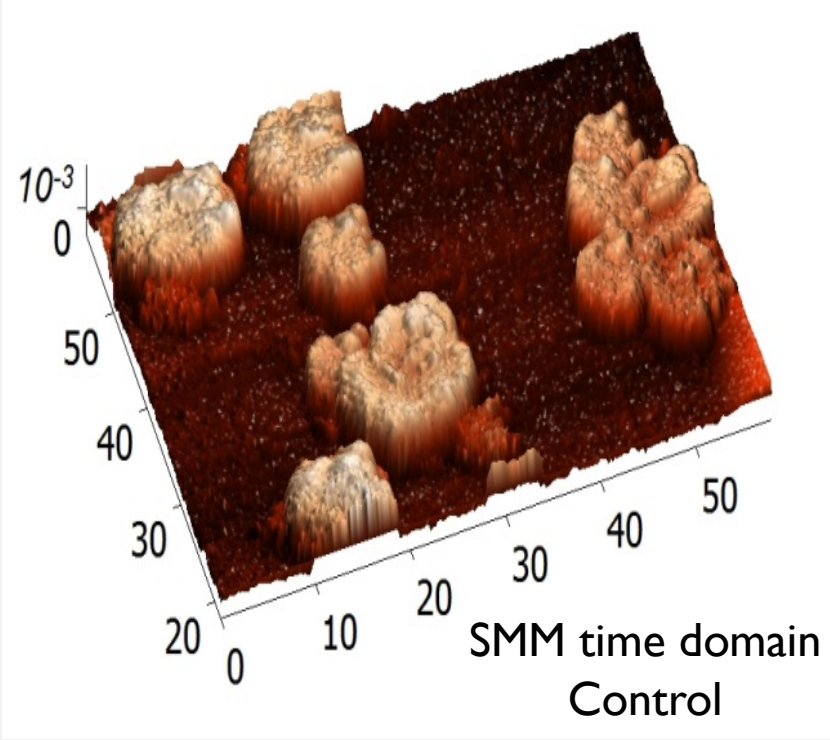


SMM frequency domain

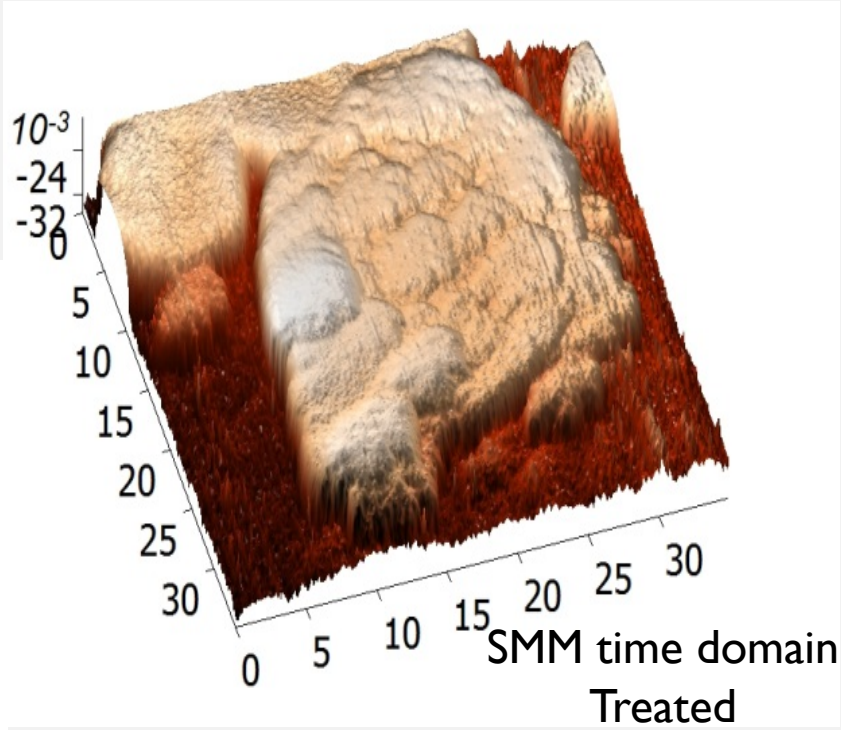
BREAST CANCER CELLS MCF-7 (DRIED)



Typical tumor spheres, formed from adherent populations[1,2]



SMM time domain Control

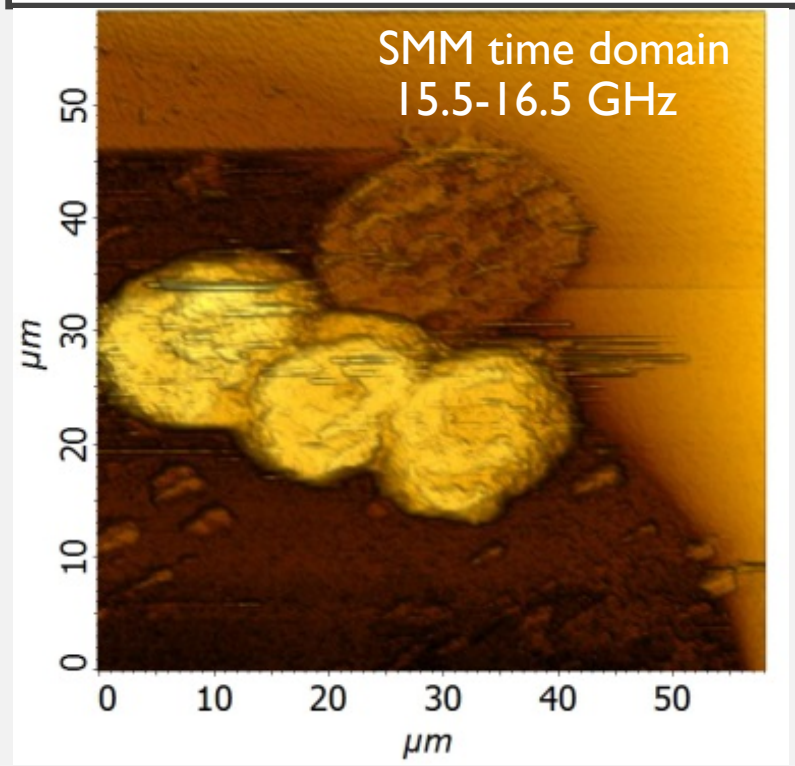


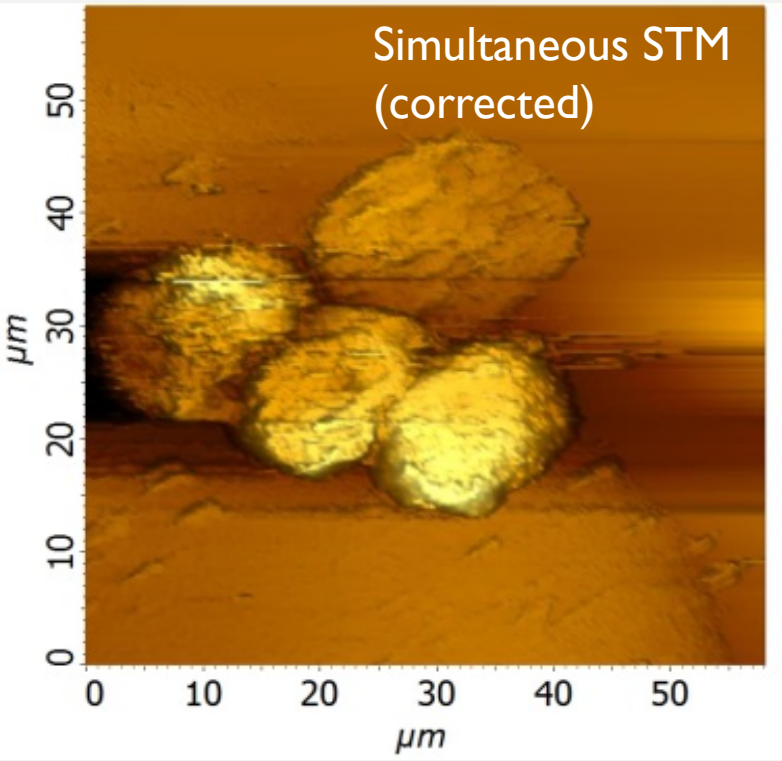
SMM time domain Treated

1 M. Farina et al., Investigation of Fullerene Exposure of Breast Cancer Cells by Time-Gated Scanning Microwave Microscopy, IEEE Trans. Microwave Theory Techn., 2016, 64, 4823-4831.

2 From http://www.promab.com/services/cancer-stem-cell/cancer-stem-cell-media/

BREAST CANCER CELLS MCF-7 (DRIED)

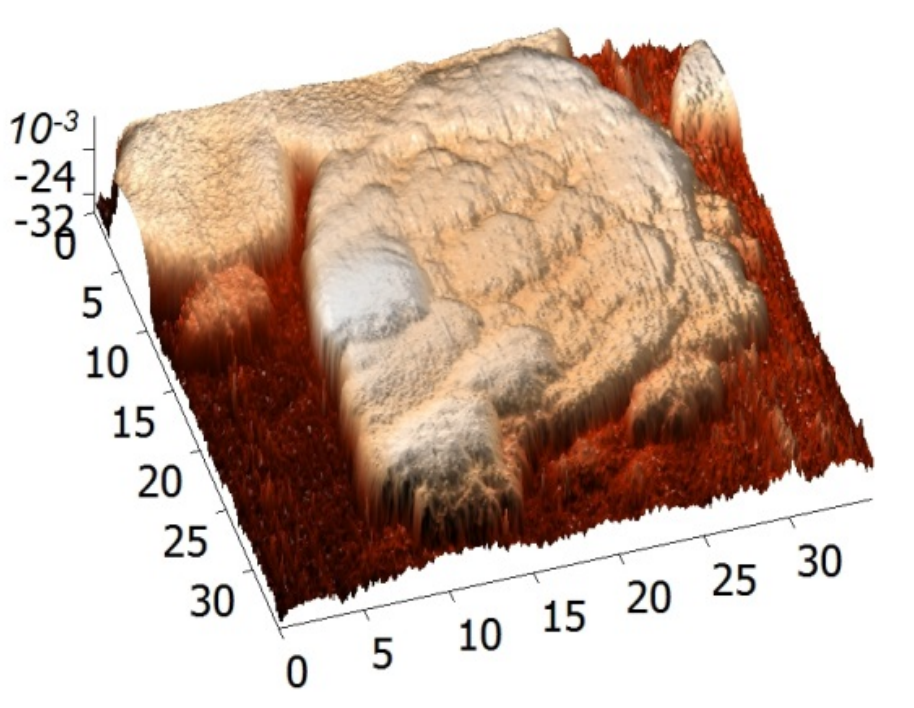


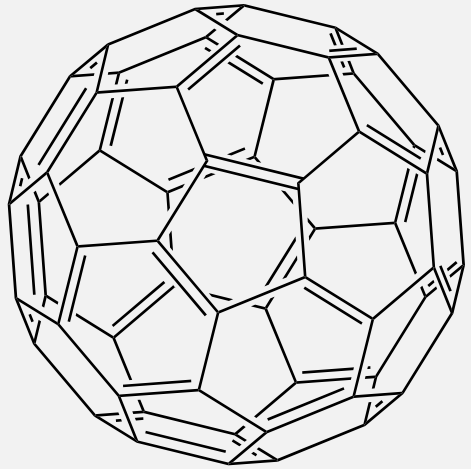


STM image was corrected to remove the systematic tilt of the underlying substrate; moreover it is never truly a topographic image because the tunneling current is highly dependent on the local atomic structure. SMM image did not need to be corrected for systematic tilt and it showed negligible cross talk with the z displacement of the piezoelectric actuator. Brighter cells appear more reflective to microwave SMM. [1]

1 M. Farina et al., Investigation of Fullerene Exposure of Breast Cancer Cells by Time-Gated Scanning Microwave Microscopy, IEEE Trans. Microwave Theory Techn., 2016, 64, 4823-4831.

BREAST CANCER CELLS MCF-7 (DRIED)





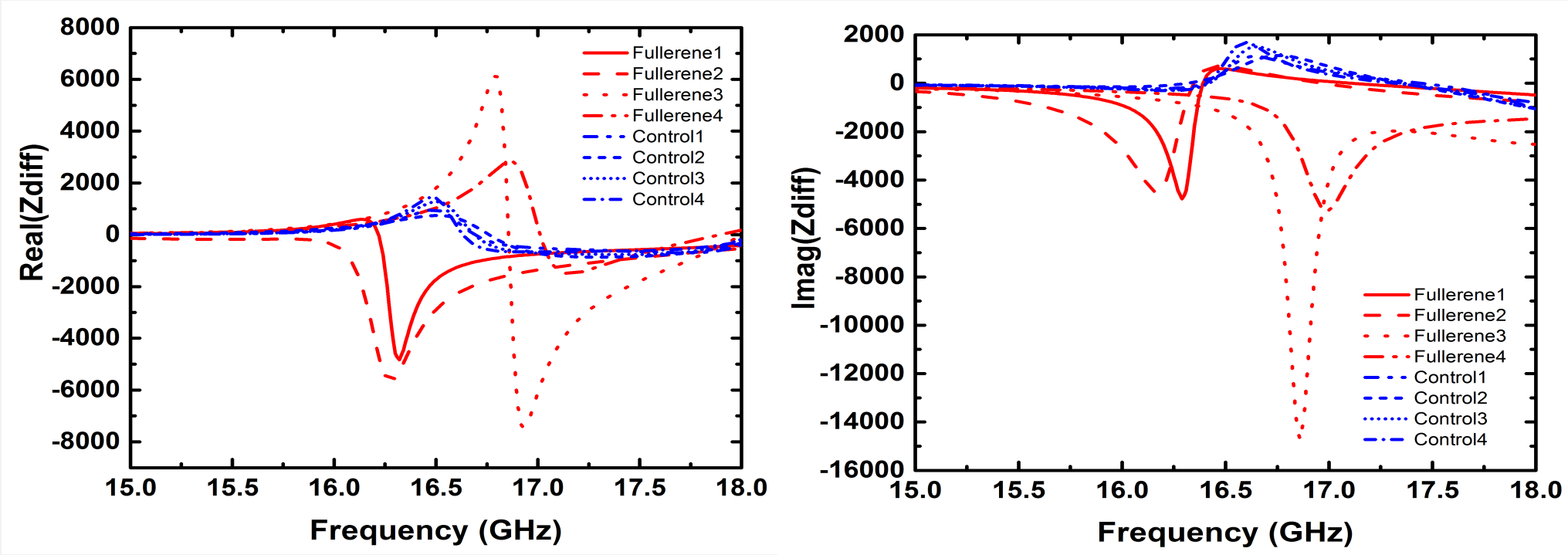
C_{60}
Fullerene

- Lipophlic: incorporation in cell membranes
- Potential for use either as anticancer or as inducing apoptosis
- Drug incorporation and release[1]

Needed a technique to assess penetration of fullerene (in literature AFM has been used recording changes in membrane elasticity in red blood cells)

1 M. Farina et al., Investigation of Fullerene Exposure of Breast Cancer Cells by Time-Gated Scanning Microwave Microscopy, IEEE Trans. Microwave Theory Techn., 2016, 64, 4823-4831.

BREAST CANCER CELLS MCF-7

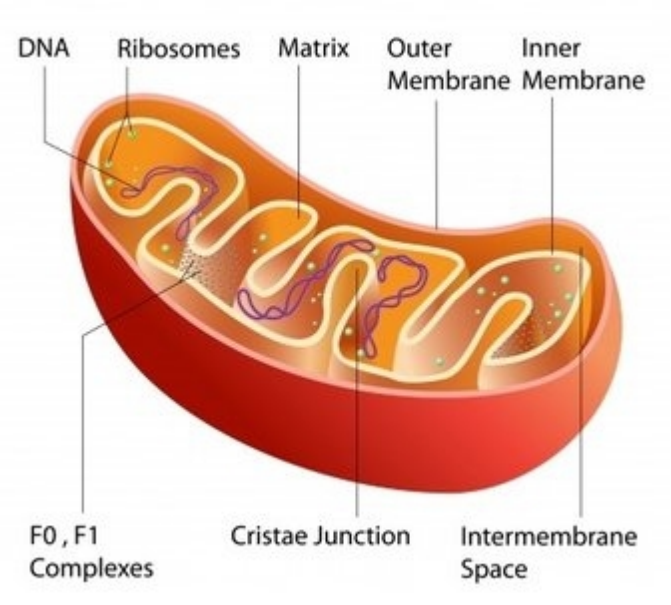


Real and imaginary relative impedance of MCF-7 cells with and without fullerene treatment.

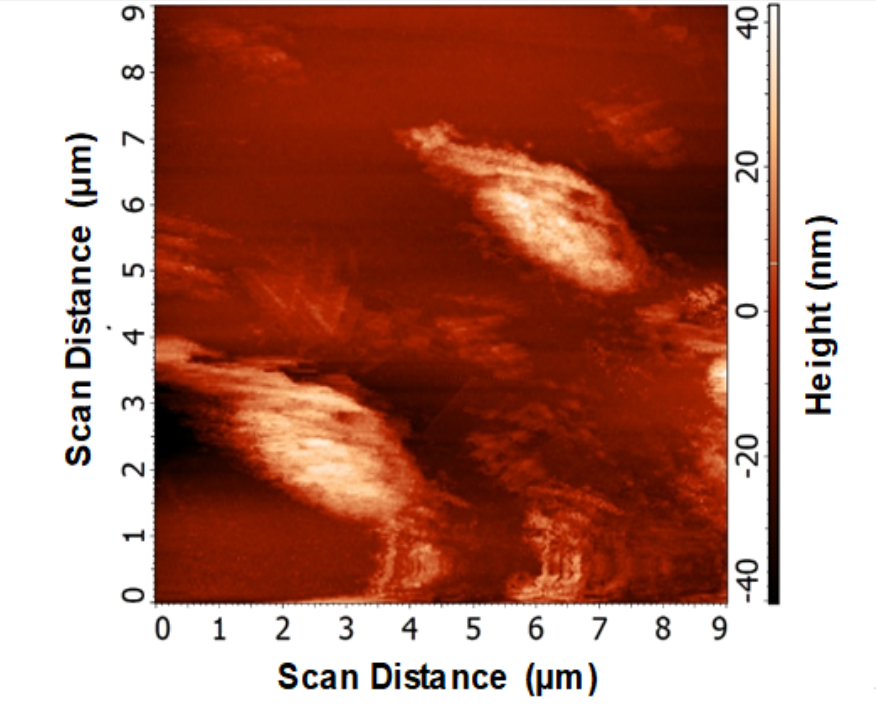
The relative impedance was calculated according to[1] and by extracting *Zc* from the top of a cell and *Zs* from a region without any cell. [2]

1 B. C. Yadav and R. Kumar, Structure, properties and applications of fullerenes, Int. J. Nanomed. Appl., 2008, 2, 15–24.
2 M. Farina et al., Investigation of Fullerene Exposure of Breast Cancer Cells by Time-Gated Scanning Microwave Microscopy, IEEE Trans. Microwave Theory Techn., 2016, 64, 4823-4831.

MITOCHONDRIA

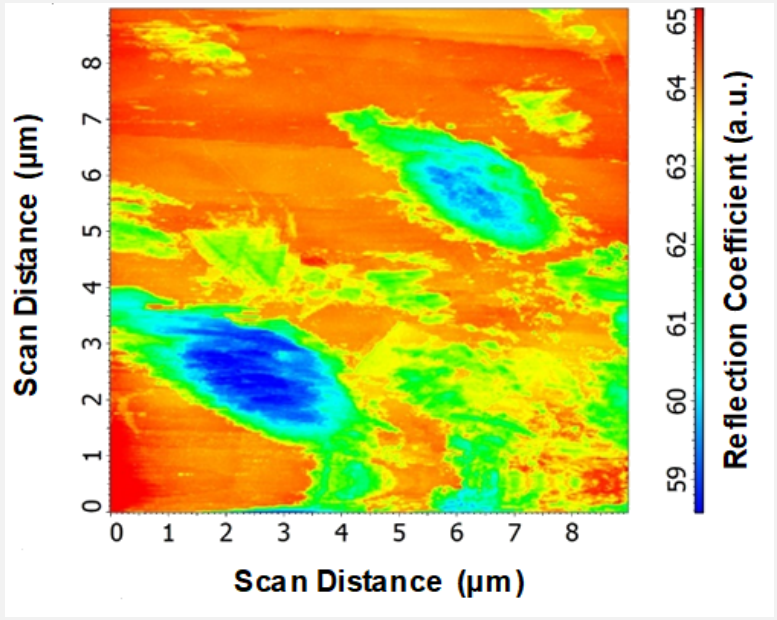


STM



- Double-membrane organelles generating most of a cell's energy supply, adenosine triphosphate (ATP)
- Implicated in many metabolic and degenerative diseases and cancers
- Fundamental role in controlling the cell suicide (apoptosis)

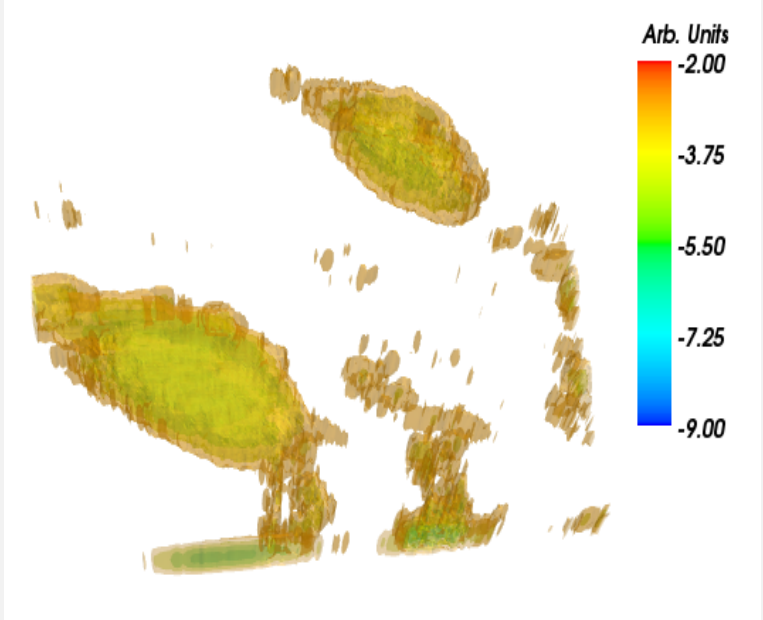
MITOCHONDRIA (COMPLETELY DRIED)



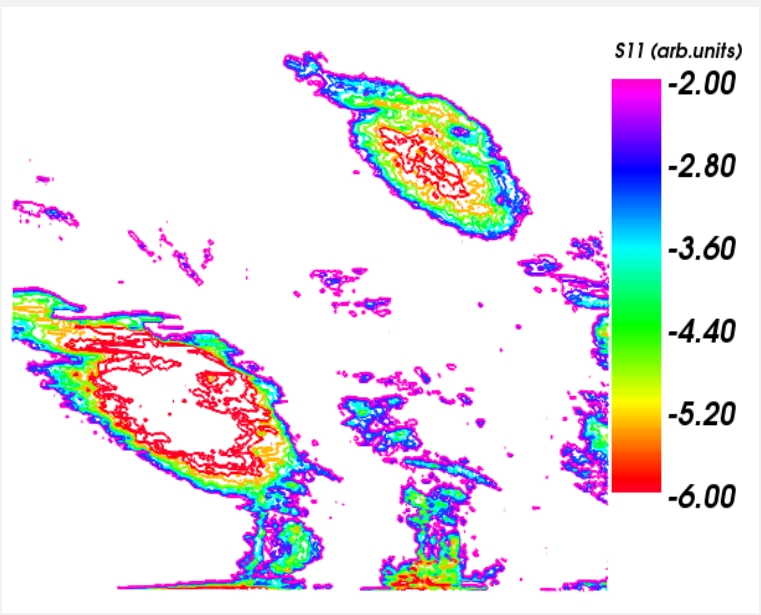
Time Domain

28-33 GHz

Keysight PNA E8361A



The time-domain data-cube (x,y, and time) is represented in 3D by plotting a set of points having the same reflection coefficient.



Section of the previous image: each curve represents a set of points with same value of reflectivity.

…Further investigation to estimate the dielectric constant of mitochondria…

SUMMARY

- Discussed STM-based SMM implementation
- Use of Time-Domain
- Special requirements biological applications
- Some results

ACKNOWLEDGEMENT

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THANK YOU FOR THE KIND ATTENTION