Binding Mechanism of Tau on Mica

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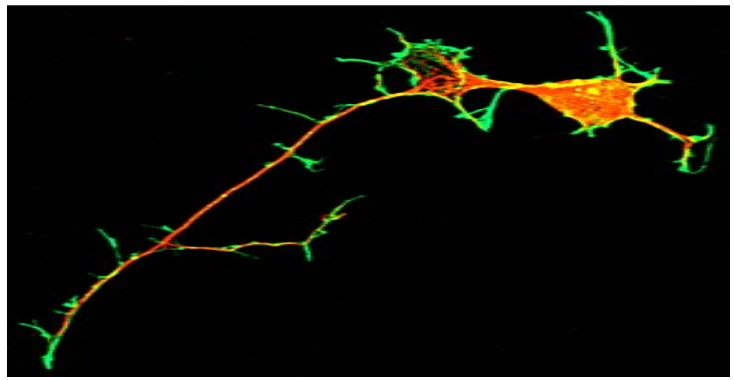
Implications for Jenny Ross' Research

Tau to Tau oligomerization is an important ingredient in microtubule strength in eukaryotic cells.

- Axon growth and strength is a part of healthy nerve cells in the brain.



But too much oligomerization can lead to **Tauopathies**, tau associated neurodegenerative diseases such as Alzheimer's and frontotemporal dementia.

- Accumulation of abnormal tau filaments.
- Tau plaque sequesters tau from performing its function.

How Does Tau Deposit On Mica?

What are the deposit heights, diameters, and volumes for each of the three tau concentrations?

- **2 μg/mL**
- **10 μg/mL**
- **20 μg/mL**

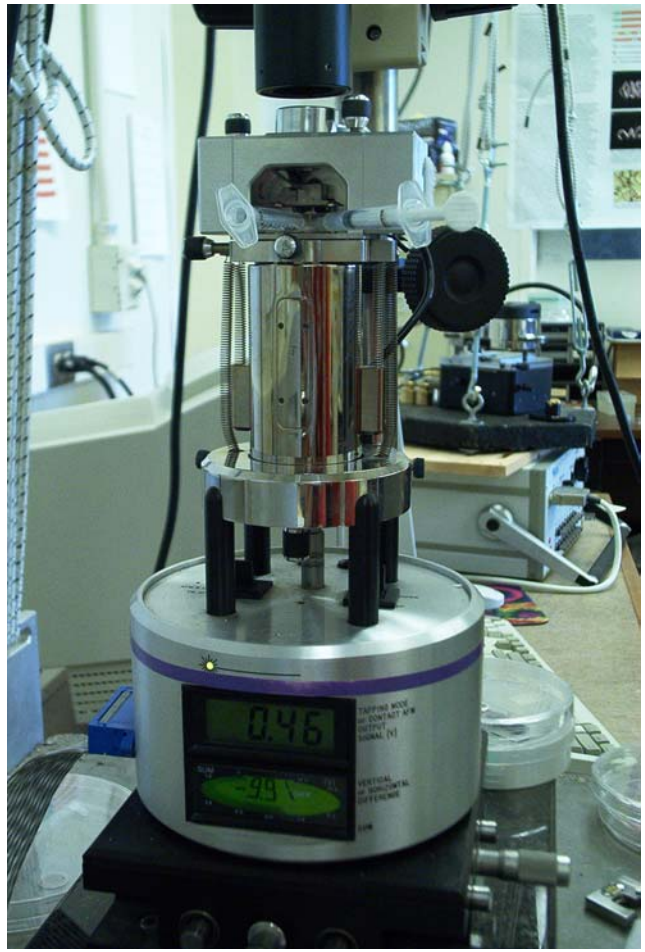
ATOMIC FORCE MICROSCOPE (AFM)

•**Advantages** over electron microscopy

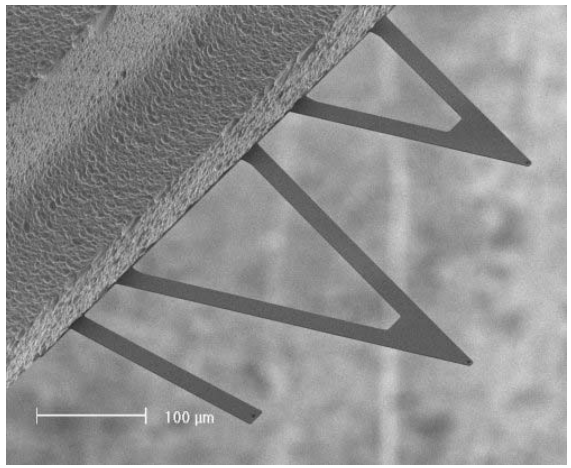
- Image hydrated protein molecules in an aqueous environment.
- Image insulated samples such as organic molecules.
- Minimal sample preparation.

•Types of **Forces** analyzed for imaging

- Interatomic interactions.
- Magnetic.
- Electrostatic.



Cantilevers and Tips



Tip radius (Nominal) 20 nm

Physics behind the AFM

- Mechanically scan over a surface.
- Nanoscaled cantilever with a high resonant frequency (~12 kHz).
- Tapping Mode "taps" specimen in liquid at a high rate.
- Over 1000 pixels of information per second.
- Deflection of the cantilever allows one to take force vs. height measurements according to Hooke's Law.
- Small spring constant allows one to non-destructively image object.

Imaging Tau on Mica

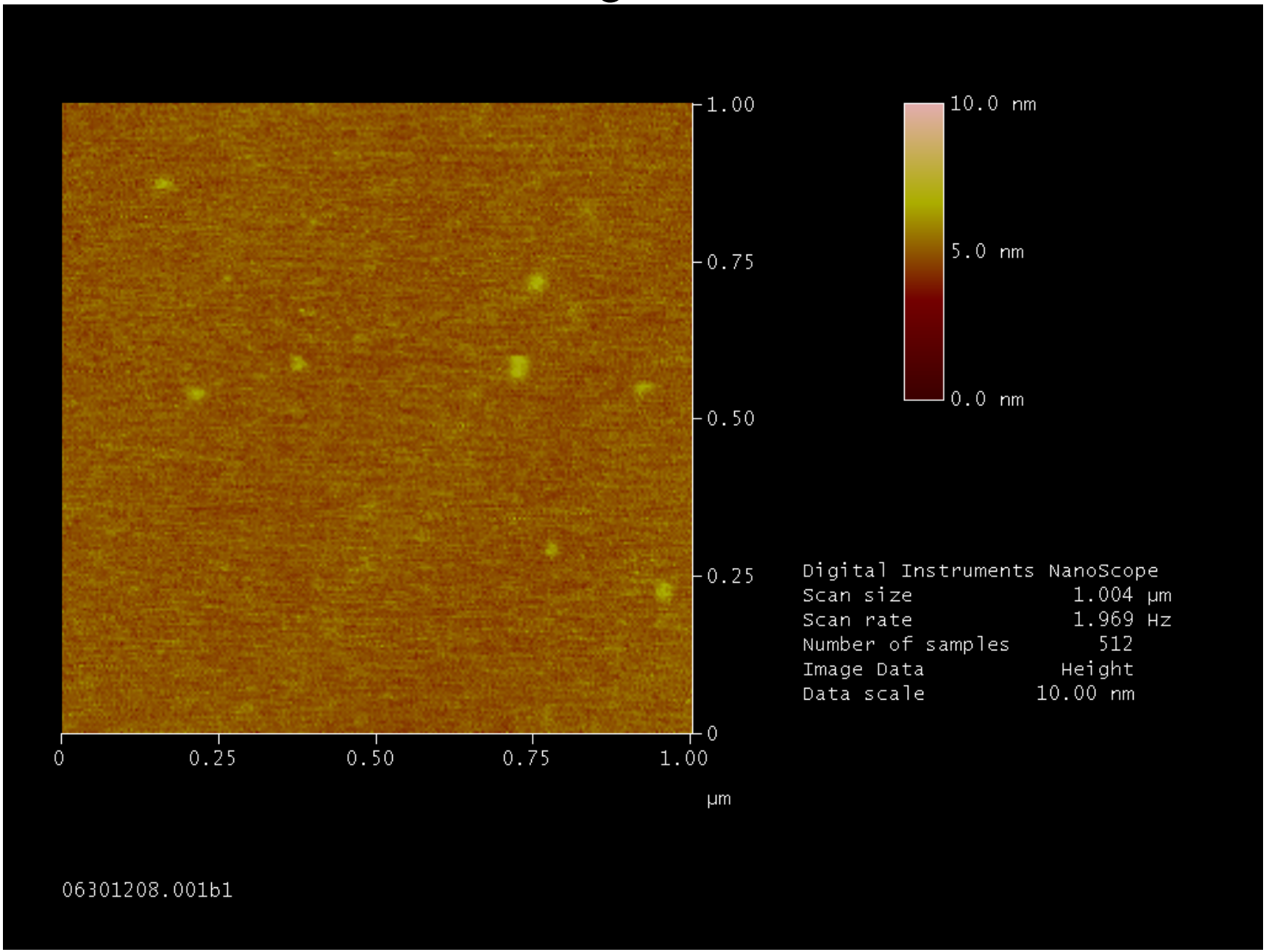
•Sample Preparation

- Cleave mica.
- Deposit 20 ul of a particular Tau concentration.
- Allow 15 minutes for electrostatic attraction to take place between the binding domain of the tau and the mica.
- Rinse sample with buffer solution.
- Place on microscope stage.

•Atomic Force Microscope (AFM) Preparation

- Seat cantilever in flow cell.
- Place flow cell with buffer on top of sample.
- Maximize laser reflection and zero photon detector.
- Adjust scan size, frequency, gain, etc.

2 ug/mL


1.00
0.75
0.50
0.25
0
0
0.25
0.50
0.75
1.00
µm
10.0 nm
5.0 nm
0.0 nm
Digital Instruments NanoScope
Scan size 1.004 µm
Scan rate 1.969 Hz
Number of samples 512
Image Data Height
Data scale 10.00 nm
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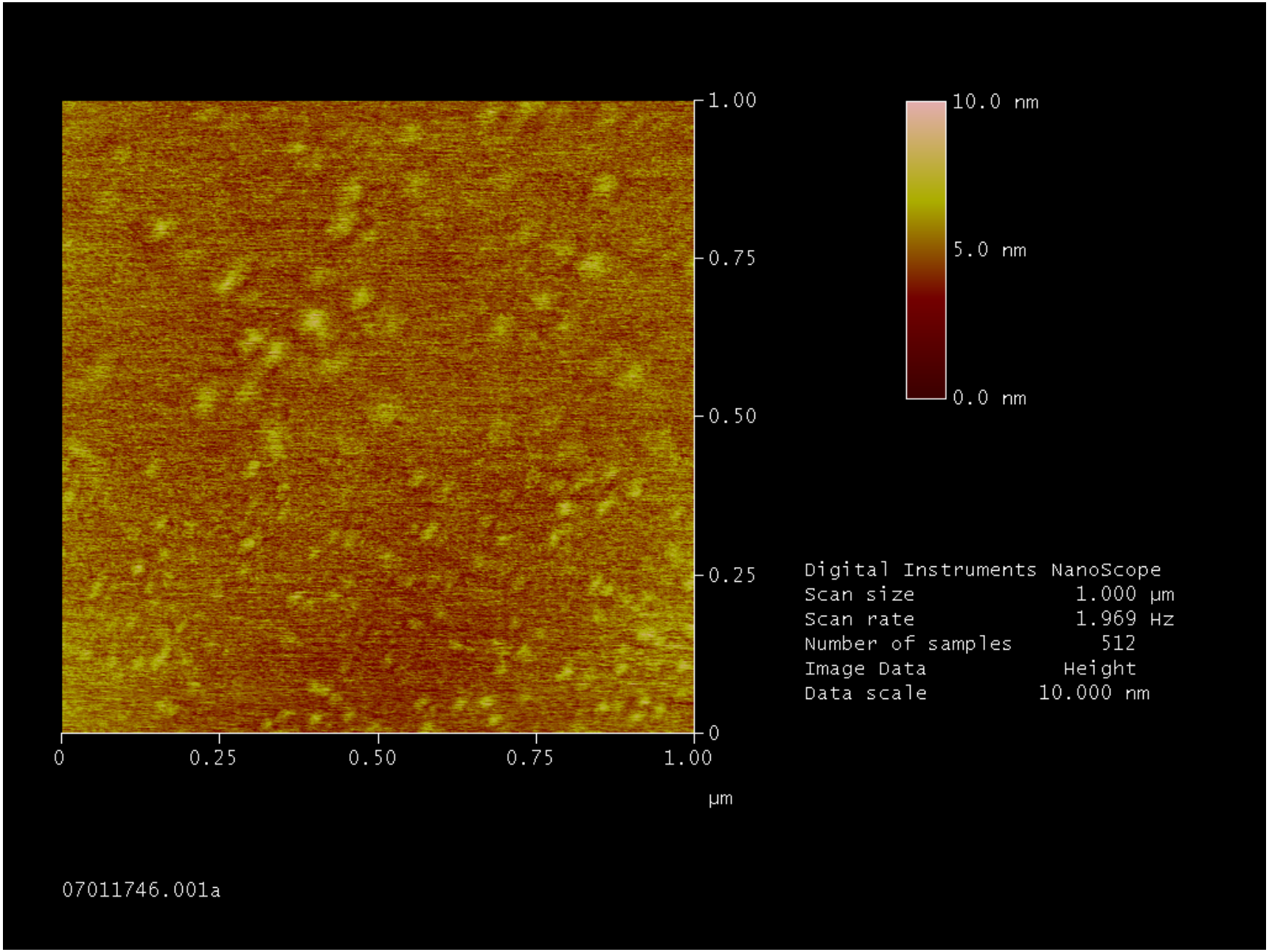
10 ug/ml


1.00
0.75
0.50
0.25
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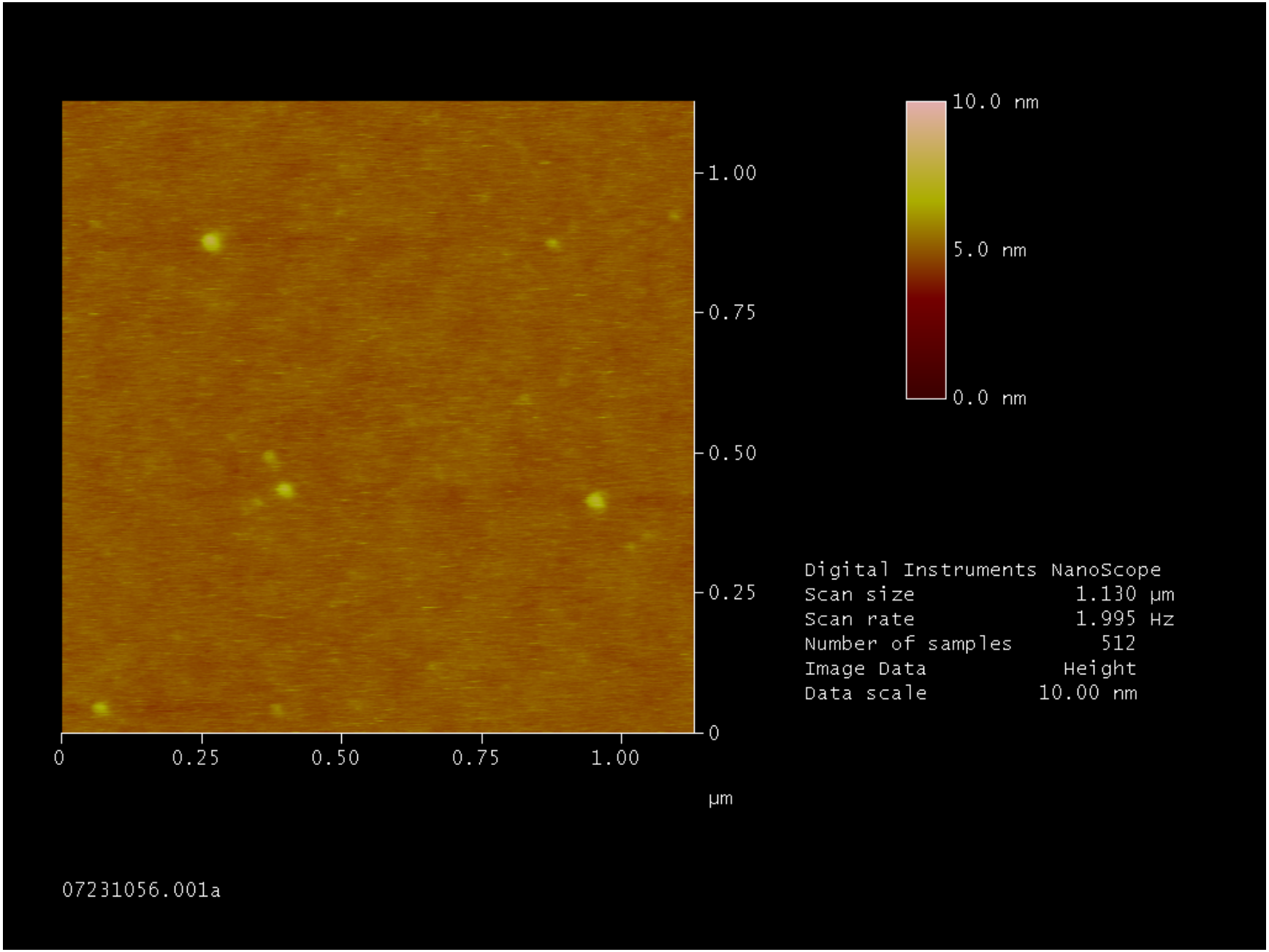
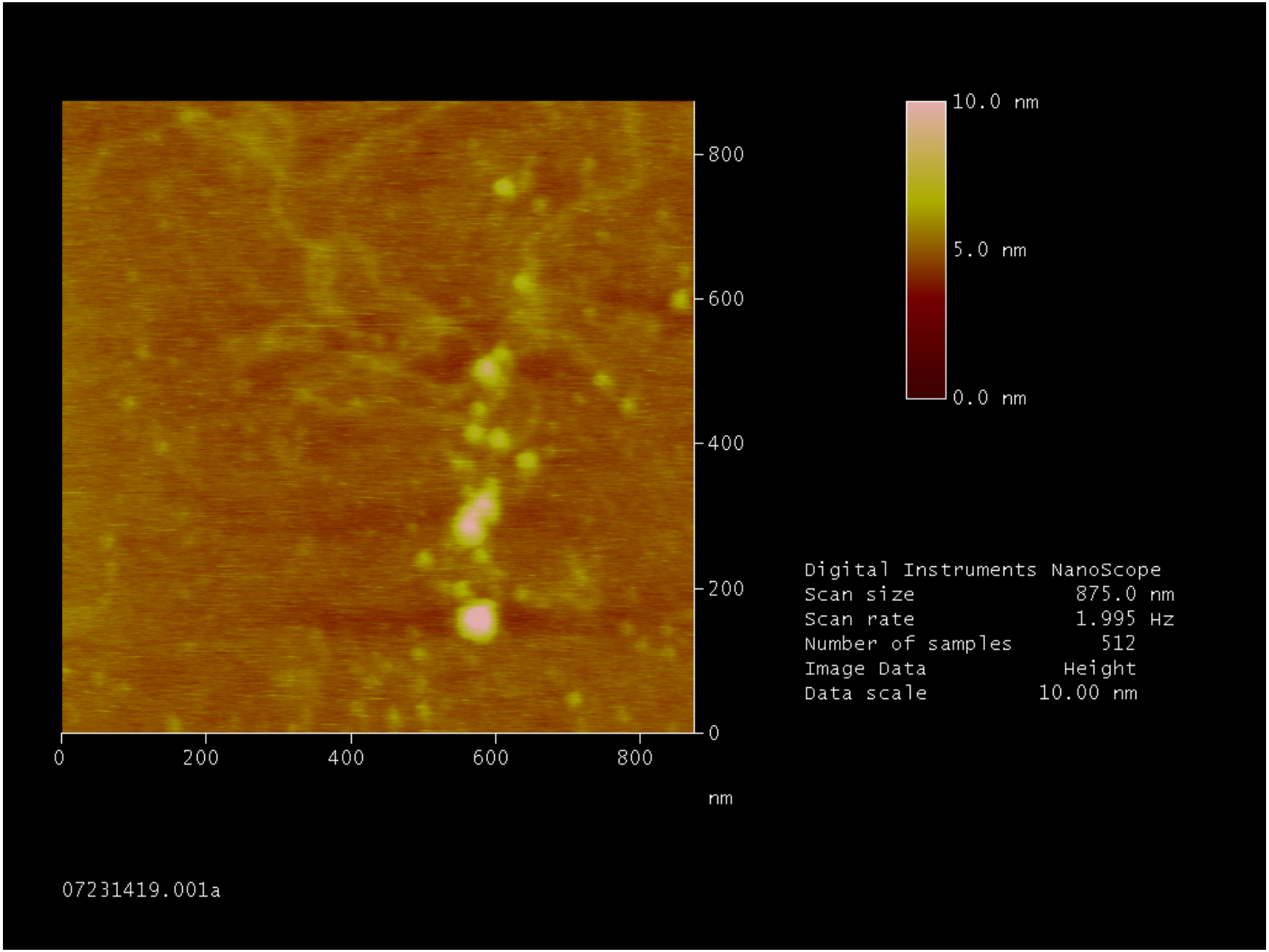
0
0.25
0.50
0.75
1.00
µm
10.0 nm
5.0 nm
0.0 nm
Digital Instruments NanoScope
Scan size 1.000 µm
Scan rate 1.969 Hz
Number of samples 512
Image Data Height
Data scale 10.000 nm
07011746.001a

20 ug/mL


10.0 nm
5.0 nm
0.0 nm
1.00
0.75
0.50
0.25
0
0
0.25
0.50
0.75
1.00
µm
Digital Instruments NanoScope
Scan size 1.130 µm
Scan rate 1.995 Hz
Number of samples 512
Image Data Height
Data scale 10.00 nm
07231056.001a

20 ug/mL


10.0 nm
5.0 nm
0.0 nm
800
600
400
200
0
0
200
400
600
800
nm
Digital Instruments NanoScope
Scan size 875.0 nm
Scan rate 1.995 Hz
Number of samples 512
Image Data Height
Data scale 10.00 nm
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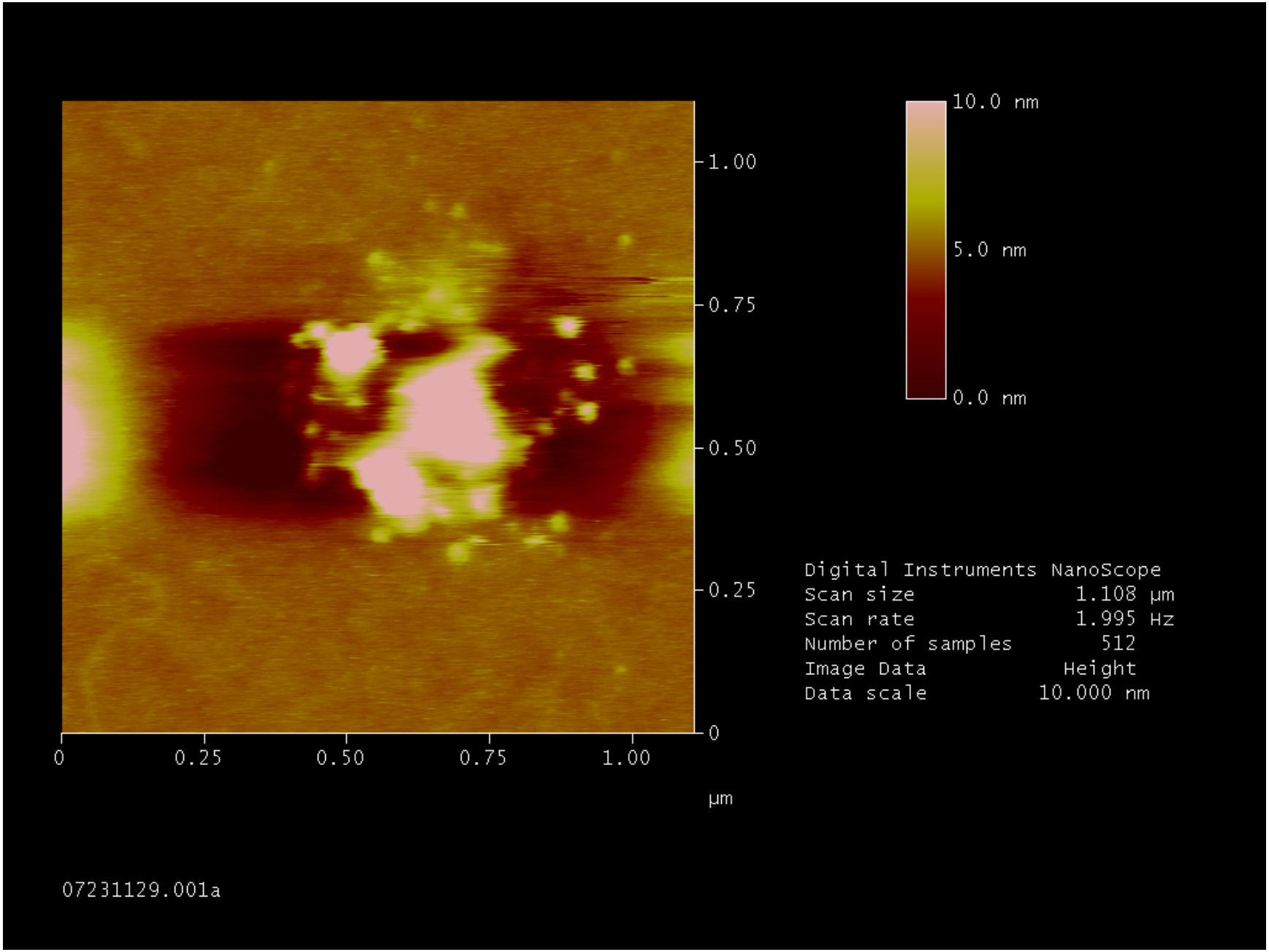
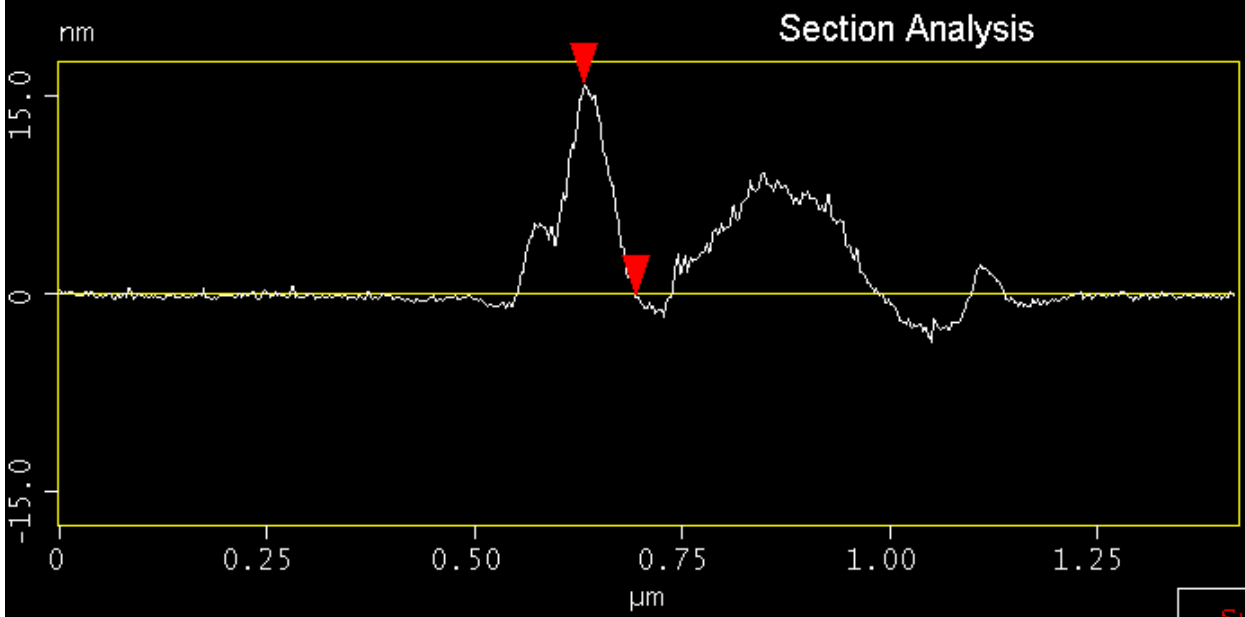
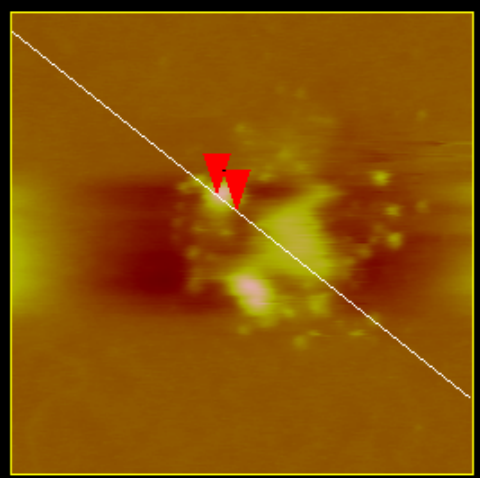
20 ug/mL

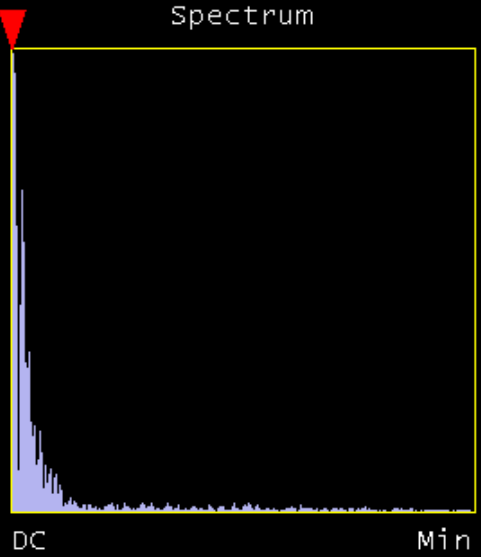

10.0 nm
5.0 nm
0.0 nm
1.00
0.75
0.50
0.25
0
0
0.25
0.50
0.75
1.00
µm
Digital Instruments NanoScope
Scan size 1.108 µm
Scan rate 1.995 Hz
Number of samples 512
Image Data Height
Data scale 10.000 nm
07231129.001a

Cursor Marker Spectrum Zoom Center Line Offset Clear



L	62.779 nm
RMS	5.449 nm
lc	DC
Ra(lc)	0.571 nm
Rmax	2.892 nm
Rz	1.797 nm
Rz Cnt	8
Radius	43.398 nm
Sigma	3.800 nm





07231129.001a

Surface distance	67.537 nm
Horiz distance(L)	62.779 nm
Vert distance	16.142 nm
Angle	14.419 °

Surface distance
Horiz distance
Vert distance
Angle
Surface distance
Horiz distance
Vert distance
Angle
Spectral period
Spectral freq
Spectral RMS amp

Cursor: fixed Zoom: 2:1 Cen line: Off Offset: Off

How Tall Do We Expect Each Tau to Be?

- Tau is found in six different isoforms in humans.

- The isoform **4RM** typically has 91 amino acids in the projection domain, i.e. those sticking up away from the mica or microtubule binding domain.

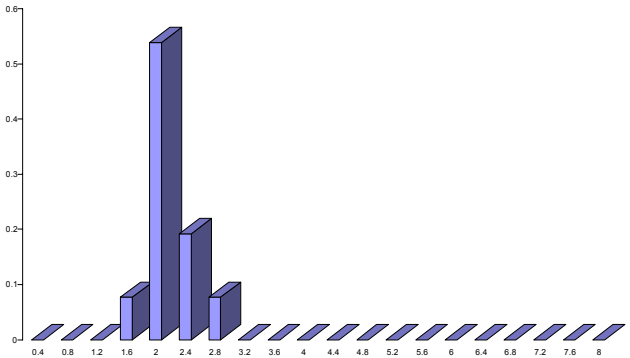
- Using the Radius of Gyration Calculation according to Teraoka (2002)

$R_g \approx bN^{(0.59)}$; where N is the # of amino acids and b is the diameter for each

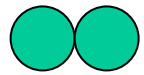
Approximately, **6 nm**

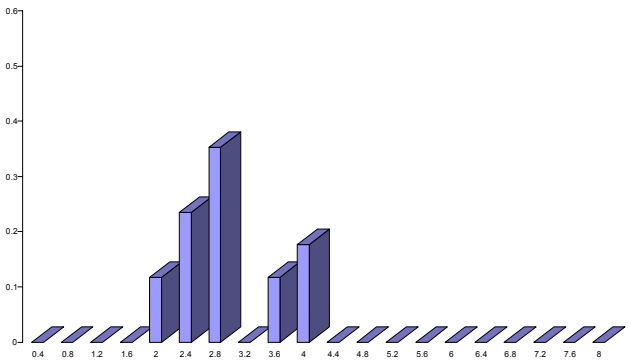
Frequency of Measured Heights

Frequency

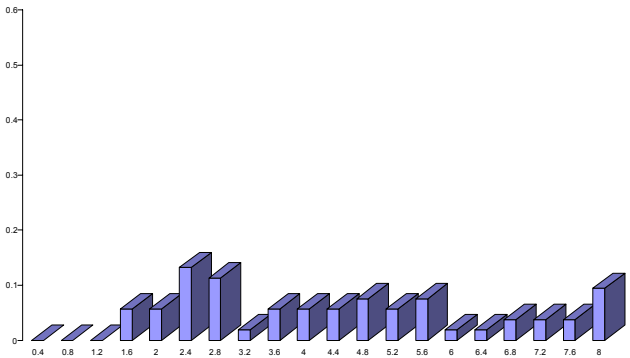


2 ug/mL

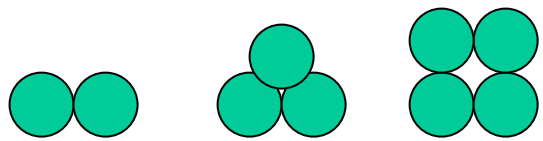




10 ug/mL



20 ug/mL



Measured Height [nm]

How Wide Do We Expect Each Tau to Be?

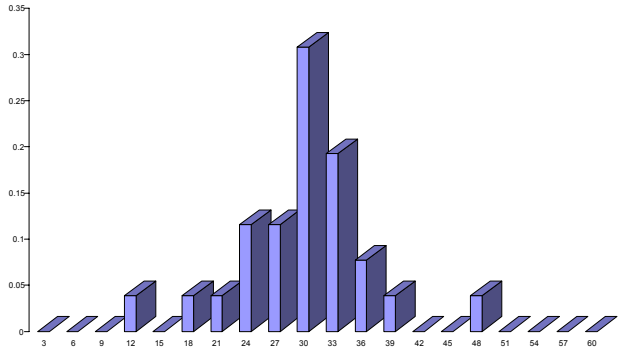
Western Blot Analysis

- Remove tau from mica with SDS page buffer.
- Run gel with tau.
- Transfer tau from gel to membrane.
- Expose membrane to antibodies.
- Analyze quantity of luminescence with gel analyzer.
- Compare light intensity with controls (known quantities of tau.)
- Calculate maximum radius of one tau molecule based on surface area of mica and molecular weight of tau.

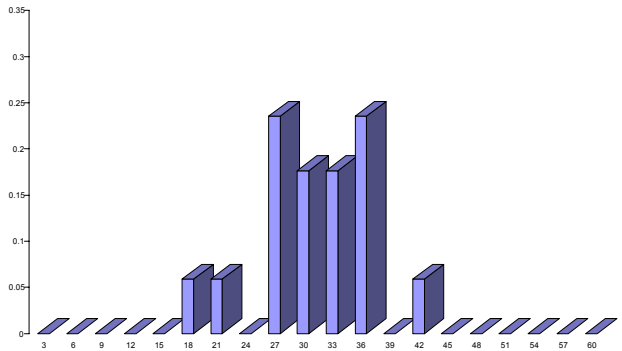
Approximately, **28 nm**

Frequency of Measured Diameters

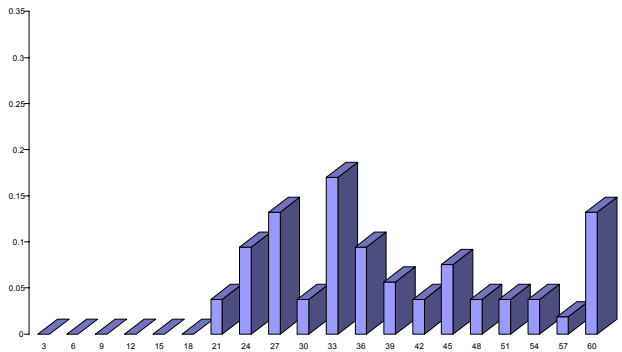
Frequency



2 ug/mL



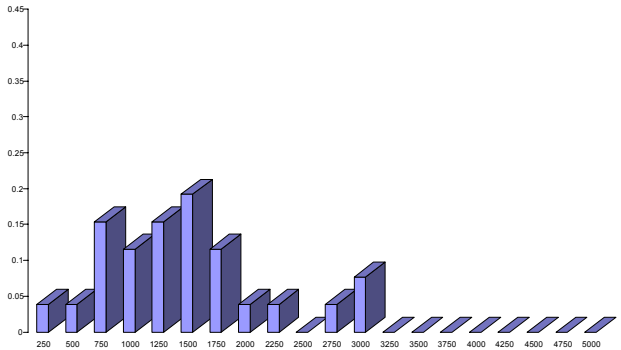
10 ug/mL



20 ug/mL

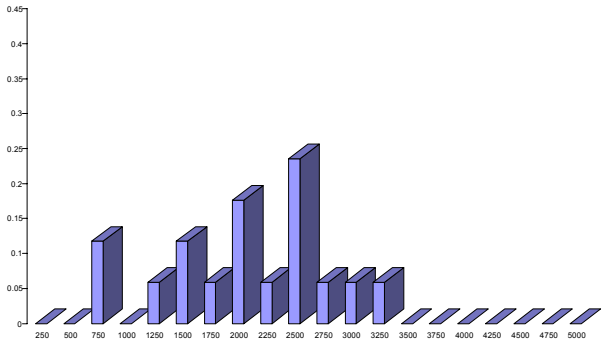
Measured Diameter [nm]

Frequency of Measured Volumes $(V \approx h \cdot \pi \cdot (d/2)^2)$

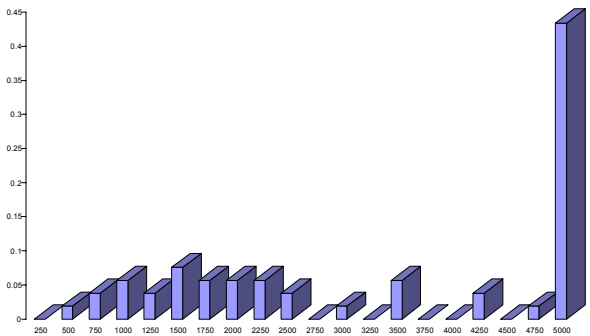


2 ug/mL

Frequency



10 ug/mL



20 ug/mL

Measured Volume [nm^3]

The Future

California State Physics Standards (grades 9-12) related to Atomic Force Microscope operation:

• Solving problems involving wavelength, **frequency**, and wave speed.
• Solving problems involving conservation of energy in simple systems with various sources of potential energy, such as capacitors and **springs**.

Also:

• Microscopic images can complement a Unit on **Measurement and Scale**