AFM enhancing traditional Electron Microscopy Applications

Dr. Johannes H. Kindt
What is AFM?

- Microscopy technique based on local mechanical contact
- Resolution <1-5nm
- Sample topography
- Surface mechanical properties
- Ambient & fluid conditions
- Established in: Materials Science, Semiconductors, Biological & other Academic Research
- About 18,000 tools WW
## Comparison EM / AFM

<table>
<thead>
<tr>
<th></th>
<th>TEM</th>
<th>SEM</th>
<th>AFM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principle</strong></td>
<td>El. Shadowing, diffraction</td>
<td>El. scattering, sec. electrons</td>
<td>Mechanical (Lennard-Jones,..)</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>&lt;0.1nm</td>
<td>&lt;1nm</td>
<td>20pm-5nm. ~30pm in z.</td>
</tr>
<tr>
<td><strong>Max. Field of view</strong></td>
<td>10s of um</td>
<td>mm</td>
<td>Typ. 30-200um</td>
</tr>
<tr>
<td><strong>Field Depth</strong></td>
<td>Samp.Th. 100nm</td>
<td>um..mm</td>
<td>3 - 20 um (Tip/Piezo)</td>
</tr>
<tr>
<td><strong>Material contrast</strong></td>
<td>Density</td>
<td>Element</td>
<td>Young’s mod., adhesion, deform..</td>
</tr>
<tr>
<td><strong>Environments</strong></td>
<td>High Vacuum</td>
<td>Vacuum</td>
<td>Ambient, fluids, reagents, gases</td>
</tr>
<tr>
<td><strong>Speed (hi res scan)</strong></td>
<td>&lt; 1 min</td>
<td>&lt; 1 min</td>
<td>&lt; 1 min (FastScan)</td>
</tr>
<tr>
<td><strong>Sample prep</strong></td>
<td>&lt;100nm thick</td>
<td>Dry&amp; conductive for high resolution</td>
<td>On a horizontal surface</td>
</tr>
</tbody>
</table>

**AFM-strengths:**
Understanding AFM resolution

**Lateral:**

- AFM tip
- Contact Area
- "Hard" Material (e.g. Mineral, Metal)
- "Soft" Material (e.g. Gel, Protein)

\[ R_{\text{tip}} = 2\text{nm} \]

Lateral Resolution: \(<< 2\text{nm} \)  
Lateral Resolution: \(\sim 2\text{nm} \)

Some factors that can further affect achievable lateral resolution:
- Moisture layer on the surface (in air)
- Ionic screening charges (in fluid)
- Tip engage robustness (AFM’s ability to preserve the tip during engage)

**Vertical:**

Vertical AFM resolution is limited by position noise (with mechanical, electrical and fundamental (thermal) contributions)

\[ \text{e.g. Dimension FastScan: } < 0.3 \text{ Angstrom (30pm)} \]
FastScan Resolution example: Hard Surface

Muscovite Mica (a: atomic layers, b: lattice)

Scan Size.

13 Seconds
20μm, 20 lines/s

5.3 Seconds
2μm, 48 lines/s

Stability.

157 Seconds
1024x1024 pixels
20nm, 6.5 Lines/s.
FastScan Resolution example: Soft Surface

2D crystal of Bacteriorhodopsin trimeric complexes

Data Details:
- Dimension FastScan™
- Probe: Broadband– C (special)
- Mode: PF-Tapping in fluid
- PFT-Frequency: 8kHz
- PFT-Amplitude: 2nm
- Line rate: 8.8 lines/s
- Tip velocity: 3.2um/s
- Pixel resolution: 512x512

58 Seconds per Frame

S. Hu, (Bruker) in collaboration with I. Medalsy, D. Mueller (ETH D-BSSE, Basel, Switzerland)

The 2D-FFT reflects the hexagonal symmetry of the protein crystal. The Spectrum shows good symmetry (low distortion). The inner maxima represent the unit cell, the outer ones the trimeric sub-structure, as expected from the XRAY crystallography data (zoom, PDB model). The central pore is well resolved.
Recent AFM advances
(and how they make the AFM interesting for EM environments)

- Simplified operation: Self-tuning AFM imaging & feedback (ScanAsyst(*))
  
  Operation now comparable to state-of-the-art SEM

- AFM-resolution mechanical property imaging (PeakForce QNM(*) )
  
  Material-contrast imaging complementary to Elemental-contrast in SEM (e.g. EDX). Especially useful for contrasting organic material.

- 20x increased imaging speed, same quality (FastScan)
  
  Imaging times (e.g. 25s for a high quality image) now same range as SEM

- Large-sample & multi-sample automation, at highest resolution
  
  Simplified Sample prep on many substrates; capacity for industrial applications (e.g. wafer level screening)

(*) Based on Bruker’s proprietary PeakForce™ Tapping
Bruker’s unique PeakForce™ Tapping mode

What can be measured

Stiffness: DMT model

\[ F_{\text{tip}} = \frac{4}{3} E^* \sqrt{R} d^{3/2} + F_{\text{adh}}, \]

Johannes.Kindt@Bruker-nano.com
Separate image channels for standard material properties, at nm resolution.
Full System Transfer Function
Equivalent Tip-Sample Force

- >10x faster than a standard AFM
- Same image quality, same force control
The foundation of speed
FastScan™ Probes

- Probe Details

<table>
<thead>
<tr>
<th>Style</th>
<th>L (um)</th>
<th>W (um)</th>
<th>fr (MHz)</th>
<th>k (N/m)</th>
<th>t (um)</th>
<th>Tip Height</th>
<th>ROC</th>
<th>TSB</th>
<th>Backside Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Style A</td>
<td>27</td>
<td>32</td>
<td>1.25</td>
<td>17</td>
<td>0.6</td>
<td>2.5 um</td>
<td>5 nm</td>
<td>5 μm</td>
<td>Yes</td>
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<tr>
<td>Style B</td>
<td>30</td>
<td>32</td>
<td>0.40</td>
<td>4</td>
<td>0.3</td>
<td>5 nm to 8 um</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Style C</td>
<td>40</td>
<td>40</td>
<td>0.25</td>
<td>1.5</td>
<td>0.3</td>
<td></td>
<td></td>
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</tbody>
</table>

Typical Probe and Lorenz Best Fit

\[
F \propto \frac{k}{Q} \\
BW \propto \frac{fr}{Q}
\]
Celgard™ battery Separator membrane.

Real time view, from Dimension FastScan™ AFM.
Survey through fast high resolution imaging

One scan. All the detail.

Sample: PTFE

8 Minutes per 16M pixel Image

Johannes.Kindt@Bruker-nano.com
For speed leading to throughput and productivity, it must be combined with data quality, robustness, self-optimization, and programmability.

The Dimension FastScan system combines the stability and multi sample automation capabilities of the Dimension ICON platform with the self optimization for different samples enabled by ScanAsyst, and rapid sample engages through SmartEngage.
AFM unique strengths

- High resolution on non-conductive samples
- True 3D metrology
- Surface Material Properties
- Conductivity
- Minimal prep
- Changes under environmental influence
- In fluids
- Live samples

Examples...
True 3D metrology
Example: roughness on a Glass HD substrate

- 4 Runs
- 8 Angles
- 3 Tracks (at 20%, 50%, 80%)
  96 measurements total

Individual Images: 1um, 512x512 pixels

2 Hours for the entire data set
(incl. Navigate, Engage, Capture, Withdraw)

A glass substrate for 2.5” HD media was imaged repeatedly at multiple sites, automated, to determine nm roughness variations. Consistent HD substrate roughness is critical to enable today’s storage capacities.
True 3D metrology
Example: roughness on a Glass HD substrate

The data can now be analyzed in different ways, to verify repeatability, determine and geometric dependencies, and feed these back to control/ optimize the Chemo-mechanical polishing process.

**Raw data:**

<table>
<thead>
<tr>
<th>Site #</th>
<th>Ra roughness</th>
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<tbody>
<tr>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
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<td>0.24</td>
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<td>0.25</td>
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<tr>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Roughness vs. Angle**

Variations localized, no clear trend.

**Roughness vs. Radius**

~2% dependency.

**Tip wear?**

No decreasing trend.

Johannes.Kindt@Bruker-nano.com
Thermally induced Polymer Film De-Wetting

In-situ recording of a 5 nm thick polystyrene film (molecular weight 4.2 kg/mol) de-wetting a hydrophobic Si wafer at 55, 65 and 75°C.

M. Lessel, K. Jacobs (Universität des Saarlandes), J.H. Kindt (Bruker Nano Inc.)

Bruker FastScan™
Dimension Heater/Cooler
Probe: Broadband –A
Mode: Tapping in Air
Scan size: 1.5um
Resolution: 1024x512
340 frames

22 Seconds per Frame
Dynamics: Fast PeakForce Imaging of Purple Membrane Background

- Purple Membrane is a two-dimensional protein crystal formed by the membrane protein Bacteriorhodopsin (BR)
- BR consists of three identical protein chains, arranged by 120 degree rotation
- BR converts light into chemical energy, by pumping ions across the cell membrane
- High resolution imaging of membrane protein crystals, like Purple Membrane, is one of the most taxing samples for the performance of an AFM, with regard to resolution and force control in fluid.
- The AFM image on the right, from a 2008 review, represents the pinnacle of a decade of membrane protein research by AFM, with regard to its quality and resolution.

Protein structure of Bacteriorhodopsin, native conformation (Source: Protein Data Bank)

AFM image of Purple Membrane.
A Engel, HE Gaub, Annu. Rev. Biochem., 2008
Dynamics:
Fast PeakForce Imaging of Purple Membrane Bacteriorhodopsin patch edge kinetics

Data Details:
- **Dimension FastScan™**
- **Probe:** Broadband– C (special)
- **Mode:** PF-Tapping in fluid
- **PFT-Frequency:** 8kHz
- **Line rate:** 11.2 lines/s
- **Tip velocity:** 24.6um/s
- **Resolution:** 256x256
- **75 Frames in the movie**

H. Stadler, J.H. Kindt (Bruker).

BR self-assembles into Purple Membrane patches on a mica surface under the right buffer conditions. The movie shows the dynamic edge of two adjacent patches, integration of small patches, and the addition of further BR during continuous imaging.
Dynamics:
Fast PeakForce Imaging of Purple Membrane
Fast High Resolution Imaging

Data Details:
- Dimension FastScan™
- Probe: Broadband– C (special)
- Mode: PF-Tapping in fluid
- PFT-Frequency: 8kHz
- PFT-Amplitude: 2nm
- Line rate: 8.8 lines/s
- Tip velocity: 3.2um/s
- Resolution: 512x512

58 Seconds per Frame

The acquired BR data, after background subtraction. The 2D-FFT reflects the hexagonal symmetry of the protein crystal. The Spectrum shows good symmetry (low distortion). The inner maxima represent the unit cell, the outer ones the trimeric sub-structure, as expected from the XRAY crystallography data (zoom, PDB model). The central pore is well resolved.
Dynamics:
Fast PeakForce Imaging of Purple Membrane

Conclusions

High Speed PeakForce Tapping (PFT) was used to image BR:

• to study Patch dynamics, with 22s time resolution

• on the lattice level, with 58s/frame, with good resolution of the three BR domains, using a PFT Amplitude of only 2nm.

The data was obtained by the combined capability of low amplitude (2nm) PFT in Fluid, and the increased imaging bandwidth enabled by the Dimension FastScan AFM, and small FastScan cantilevers.

• PFT achieved resolution typical for ContactMode, and beyond TappingMode

• PFT simplified force control, due to it’s immunity to thermal force drift

• FastScan speed enabled the study of sample dynamics
Electron Microscopy and AFM

Complementary Application Examples
Diatoms (SEM and AFM)

Imaging of phytoplankton cell wall with a BioScope Catalyst AFM. Top left: SEM image of a diatom, sample courtesy of Dennis Kunkel, Astrographics.
Protein particle analysis

Structure and Function of Purified Monoclonal Antibody Dimers Induced by Different Stress Conditions
Pharm. Res. Published online 4/2012

250 nm scan taken at low temperature demonstrates the AFM resolution on individual "Y"-shaped IgG antibody molecules. Image Courtesy of Shao lab, University of Virginia.
Herpes Simplex 1 virus

AFM images by Liashkovich et al., nuclear pore group (Victor Shahin) in Münster, Germany
Mineralized Tissue

From:
Mineralized Tissue
In situ chemical dissection & AFM

A) Fracture surface of Bovine trabecular Bone presents fibrillar structures with a particulate surface appearance.

B) 5 second exposure to dilute EDTA dissolves hydroxyapatite, reveals underlying collagen

JH Kindt et al., UC Santa Barbara, 2003
Polymers

TEM BF thin section

AFM Phase Shift blockface

Example courtesy of N. Matsko, TU Graz
Vitrified tissue specimens learning from TEM preparation techniques

Sample Prep:
- High Pressure vitrification
- Freeze substitution
- Ultra-Microtome

On many, especially soft, samples, the resolution is determined & limited by the prep, not the instrument.

Data & method courtesy of N. Matsko, TU Graz
Cross section of ant antenna

High pressure vitrified

Freeze substituted

Ultra-microtomed

In collaboration with N. Matsko, TU Graz

25MPixel image

30x30um

6 nm pixel resolution

PeakForce Tapping

8k pixels/s
Vitrified tissue specimens
Vitrified tissue specimens

N. Matsko, TU Graz
Dynamics: Bacteria attacked by Antimicrobial Peptides (AmP)

Introduction

- AmPs are naturally occurring small molecular weight molecules (<50 aa residues) that are part of the innate immune response of all classes of life.

- With increasing problem of antibiotic-resistant bacteria and the selectivity of AmPs for microbial cells, AmPs are now being evaluated as potential therapeutic agents.

- AmPs permeabilize the microbial cell membrane through membrane disruption and pore formation. Three main mechanisms:
  - Barrel-Stave Model
  - Toroidal Pore Model
  - Carpet Model

- Cecropin-melittin (CM15).
  - Milani et al. (2009) *Molecules*


*From Prof. Yechiel Shai (http://www.weizmann.ac.il/Biological_Chemistry/scientist/Shai/)*
Dynamics:
Bacteria attacked by Antimicrobial Peptides (AmP)
Effects of CM15 on *E. coli* Cells

- Cells exposed to 10 µg/mL CM15.

- Dramatic changes to cell morphology with increase in roughness and corrugation of cell surface.
  
  Andra et al. (2008) Biochem. J.

- Onset of changes to individual cells occurred at different times.

Data acquired on the Dimension FastScan™ system operated in TappingMode™ in fluid, using 8µm wide, 150kHz (fluid) EBD tipped CL (Nanotools). 18 sec/frame, 2.5 µm scan, 1024 x 256 pixels. Phase data channel is shown.
Dynamics:
Bacteria attacked by Antimicrobial Peptides (AmP)
High-Resolution Imaging of Cell Membrane Morphology

High-resolution TEM image of S-layer and AFM of the surface of individual *E. coli* cells in fluid reveal similar structure, with different degree of order.

Comparable resolving power of the features. However, the AFM sample is **ALIVE**.
Dynamics:
Bacteria attacked by Antimicrobial Peptides (AmP)
High-Resolution Imaging of Cell Membrane Morphology

- Cells exposed to 20 µg/mL CM15.
- Onset of disruption of cell membrane surface observed within 30 seconds after CM15 exposure.
  - Membrane rippling
  - Membrane protrusions consistent with toroidal model (micellization)
  - Formation of defects (<15nm width)

Data acquired on the Dimension FastScan™ system operated in TappingMode™ in fluid, using 8µm wide, 150kHz (fluid) EBD tipped CL (Nanotools, Munich). 8 sec/frame, 300nm scan, 1024 x 256 pixels. Total elapsed time is 358 sec. Height Sensor data channel is shown.
Dynamics:
Bacteria attacked by Antimicrobial Peptides (AmP)
Effects of CM15 on *E. coli* Cells

- Imaging of *E. coli* cell confirmed localized changes not induced by AFM probe.
- Reduced size of CM15 treated cells was reported previously from SEM studies. (CM 15 was speculated to inhibit Bacteria growth.)
- AFM before & after exposure shows size difference is due to CM15-induced shrinkage.

![Phase images shown.](image-url)
Dynamics:
Bacteria attacked by Antimicrobial Peptides (AmP)
Conclusions

The morphological response of e. coli to CM15 antibacterial peptide was time resolved, on multiple length scales, using 8um wide EBD tipped probes:

- **On the multi-cell scale:** The onset of morphological responses to CM15 varied between cells (consistent with the literature)
- **At high resolution:** The initially observed well ordered S-layer was disrupted as exposure permeabilized the membrane, with topography increase consistent with micellization (toroidal pore model). Direct S-layer observation on bacteria was only reported recently (2009).

This work was enabled by the Dimension FastScan’s:

- Ability to image in fluid
- High speed (18s/frame) over large XYZ scan range
- High speed (8s/frame) at high resolution
- Small, soft (8um wide, 0.2N/m, 150kHz in fluid) EBD tipped cantilever support
- Large sample (glass slide) capacity
Review of the Application examples, and how they relate to the recent advances benefitting for EM environments

Simplified operation: Self-tuning AFM imaging & feedback (ScanAsyst(*))
Operation now comparable to state-of-the-art SEM
Examples: Bacteriorhodopsin imaging, 6 Samples in 10 Minutes

AFM-resolution mechanical property imaging (PeakForce QNM(*) )
Material-contrast imaging complementary to Elemental-contrast in SEM (e.g. EDX).
Especially useful for contrasting organic material. Example: Polymer brushes

Large-sample & multi-sample automation, at highest resolution
Simplified Sample prep on many substrates; capacity for industrial applications
Examples: 6 Samples in 10 Minutes, HD Substrate roughness, Pharma Screening

20x increased imaging speed, same quality (FastScan)
Imaging times (e.g. 25s for a high quality image) now same range as SEM
Example: All.

(*) Based on Bruker’s proprietary PeakForce™ Tapping

Johannes.Kindt@Bruker-nano.com
Thank you for your attention.

For more information about FastScan™, please visit:

www.bruker.com/fastscan

contact me at:
Dr. Johannes H. Kindt
johannes.kindt@bruker-nano.com

More Info: nanoscaleworld.bruker-axs.com
Backup
AFM is a contact measurement, and electrical measurements can be performed together with topography imaging, for example to locate functional device defects.
Dynamics:
Fast PeakForce Imaging of Purple Membrane
Fast High Resolution Imaging

Data Details:
- Dimension FastScan™
- Probe: Broadband– C (special)
- Mode: PF-Tapping in fluid
- PFT-Frequency: 8kHz
- PFT-Amplitude: 2nm
- Line rate: 8.8 lines/s
- Tip velocity: 3.2um/s
- Resolution: 512x512

58 Seconds per Frame

Real-time view of the FastScan system imaging the purple membrane lattice, using PFT.
Channel: Height Sensor, Z range 1.5nm.
The PFT Amplitude of only 2nm is combining the high resolution of small amplitude tapping with the robustness of the PFT mode.

S. Hu, (Bruker) in collaboration with I. Medalsy, D. Mueller (ETH D-BSSE, Basel, Switzerland)
Screening
Amorphous Formulation Stability

Environmental stress testing and subsequent XRPD detection of API crystals can take *Weeks to Months*.

**AFM detects SMALLER crystals EARLIER** *(Hours - Days).*

An AFM based stability assay can be predictive for XRPD based stability testing.

AFM Phase image of an amorphous formulation, after a few hours of aging.

*Re-crystallized API (10-20nm)*

(Courtesy of M. Lauer, F.Hoffmann–La Roche)
Screening
Amorphous Formulation Stability

Different Excipients

API: NK1(1) and Excipients

API: CETP(2) and Excipients

Pharmaceutical Research (November 2010)
“Atomic Force Microscopy-Based Screening of Drug-Excipient Miscibility and Stability of Solid Dispersions”
(1) Hoffmann La-Roche, Discovery Technologies, (2) Bruker Nano GmbH, (3) Biozentrum Basel

Johannes.Kindt@Bruker-nano.com
Phase maps recorded with tapping mode AFM on miscible NK1(1):excipient combinations (a–d), and on corresponding CETP(2) combinations (e–h) after exposure to stress conditions (RH=75%, T=40° C) for 2 h. The scale bars: 1μm.
Amorphous Formulation
Stability Assay

Outcome of AFM-based Stability Assay (2h storage under stress conditions)

Formulate NK1(1) w. HPMCAS MF
- Well miscible with NK1(1)
- Low potential to restructure in humid environment
- Stable even at T=80°C

Formulate CETP(2) w. PVP VA64
- Well miscible with CETP(2)
- Low potential to restructure in humid environment
- Stable even at T=80°C

Verification with bulk methods (6-8 month storage under stress conditions)

<table>
<thead>
<tr>
<th>Api:Excipient 1:1</th>
<th>Excipient:</th>
<th>Initial State:</th>
<th>after 6-8 mo. (40°C/75%RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>API:NK1(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVP VA64</td>
<td>amorphous</td>
<td>crystallized</td>
</tr>
<tr>
<td></td>
<td>PVP K30</td>
<td>amorphous</td>
<td>crystallized</td>
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<tr>
<td></td>
<td>HPMCAS MF</td>
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<td>PVP PF17</td>
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<td>Eudragit L100</td>
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<td>API:CETP(2)</td>
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<td>PVP VA64</td>
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<tr>
<td></td>
<td>HPMCAS MF</td>
<td>amorphous</td>
<td>crystallized</td>
</tr>
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</table>
(data courtesy of M.E. Lauer, F. Hoffmann-La Roche)
Amorphous Drug Formulations.
Samples courtesy of M.E. Lauer, O. Grassmann, F. Hoffmann-La Roche, Basel, Switzerland

Repeat, using the new Bruker FastScan Miscibility & Stability screen

12 Samples, 60 Sites. Automated.

60 Minutes for the entire data set
(incl. Navigate, Engage, Capture, Withdraw)