High-Resolution Imaging and Nanomechanical Mapping of Virus Binding Sites to Animal Cells

BioScope Resolve™
Now with Fast Tapping

Alexander Dulebo, Ph.D. – Bio Application Engineer
BioScope Resolve™

- Complete cell mechanics data, accurate & repeatable
- The highest resolution imaging of any BioAFM
- Combine fluorescence and biomechanics, seamless & simple
- The only BioAFM enabling whole cell fast scanning studies

Now with Fast Tapping
The Only Comprehensive Solution for Quantitative Biomechanics

At the core of AFM biomechanics are quantifiable force distance measurements.

BioScope Resolve provides

• The only complete solution for accurate quantification, based on the gold standard, vibrometer calibration
• The widest range of ramp rates, from sub-Hz to 2kHz, with exclusive FASTForce Volume and PeakForce QNM
• The most flexible Ramp Scripting, complete with dynamic modulus and per segment optical integration

To enable a wide range of new experiments

• Live cell viscoelasticity studies as function of cell state and disease with Ramp Scripting
• Live cell receptor mapping with nm resolution and pN force control with PeakForce QNM
• Studies of single molecule interactions and dynamics
Widest Range of Ramp Rates
High Speed Quantitative Data with FASTForceVolume

- Highest speed linear ramping, to 300Hz
- All the data: pixel resolution up to 256x256x2048 or 956x956x256
- Real-time analysis: Instant property channels incl adhesion and modulus
- Force control with <50pN low force trigger
- Study time dependence – closes gap between traditional slow ramps and PeakForce Tapping
- Now also available on Dimension Icon and MultiMode
Accurate and Consistent Results
Open the door to the study of time dependent phenomena

No touch calibration
Accurate & no damage

Accurate spring constant
Calibrated by Vibrometer - the gold standard

17um tall tip
Avoids squeeze film artifacts on cell membranes.

Defined 65nm end radius
Critical for quantification. Optimized for live cell studies.

PeakForce Tapping 500Hz
PeakForce Tapping 250Hz
FASTForce Volume 156Hz
PeakForce Tapping 125Hz
Force Volume 1Hz

Measurements on 2.5% Agarose Gel
Ramp Scripting
Enabling Cell Mechanobiology Studies

- Cell mechanics of great interest in context of e.g. disease and tissue engineering

- Cells are highly viscoelastic i.e. show time-dependent response

- Ramp Scripting: direct point measurements of viscoelastic response e.g. quantify the relaxation time
  - With required dynamic range to address msec to sec relaxation times
  - pN force control with ms response time and stability over seconds
  - Synchronizing fluorescence per segment

- Ramp Scripting now also available on Dimension Icon

Creep response of a living fibroblast cell performed in fluid on the BioScope Resolve using MLCT-Bio-DC probes ($k \approx 0.011 \text{N/m}$).

Exponential Decay/Creep Fit: $y(X) = a + b \exp(-X/c)$
Highest Resolution Imaging
Obtained by an AFM on an Inverted Microscope

Resolving sub-nm structures in molecular imaging on individual molecules as well on virus capsid

Submolecular resolution of the major and minor grooves of the DNA Double Helix. Images obtained on an inverted microscope.

Herpes simplex virus capsid. Sample courtesy of Prof. Alex Evilevitch, University of Illinois at Urbana-Champaign, IL, USA
Individual Microvilli on Live Cells
Resolved by AFM for the First Time

- Microvilli are structures on epithelial cells. Involved in many processes.
- PeakForce Tapping enabled imaging of microvilli on live cells for the first time.
- Low Imaging forces of <30pN produced images of microvilli as upright cylindrical structures.

MIROView™ - New Overlay GUI
Quickly Find Feature of Interest & Scan

- Single view to control AFM operation based on optical data
- Seamless switching between imaging, force volume, and single ramps
- Set up scan and automated measurement sequence
- Continuous data capture for no loss of data
- User Rating system for captured data
- Data History for easy organization of multiple scan areas/data points
Membrane ruffling and actin cytoskeleton rearrangement are important dynamic processes in cell migration. Indications that cell is highly motile (i.e. loosely bound to surface).

Fast Tapping provides excellent force control as the loosely bound cell is not disrupted under continuous imaging. Dynamic processes not affected by tip interaction (see optical movie).

Data captured using full XYZ range (100µm x 100µm x 15µm)

35 seconds/frame (37µm scan) 12.5 seconds/frame (27µm scan)

Courtesy of Dr. Alexander Dulebo (Bruker) and Dr. Ivan Liashkovic & Dr. Hermann Schillers (Univ. Münster, Germany)
Resolve: Fast Tapping Mode
Live Cell Dynamics (HUVEC Cells)

- High resolution imaging of dynamic actin fiber arrangement in living cells
  - Fast Tapping provides excellent force control as the cell membrane and actin cytoskeleton are clearly not disrupted under continuous imaging.

Data captured using full XYZ range (100µm x 100µm x 15µm)

6 seconds/frame (7.2µm scan) 3.6 seconds/frame (7.2µm scan)

Courtesy of Dr. Alexander Dulebo (Bruker) and Prof. Stefan Zahler (LMU, Munich, Germany)
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- Complete cell mechanics data, accurate & repeatable
- The highest resolution imaging of any BioAFM
- Combine fluorescence and biomechanics, seamless & simple
- The only BioAFM enabling whole cell fast scanning studies
High-Resolution Imaging and Nanomechanical Mapping of Virus Binding Sites to Animal Cells

Dr. Andra Dumitru & Prof. David Alsteens

Université Catholique de Louvain
Institute of Life Sciences
NanoBiophysics group
INTRODUCTION
FD-based AFM imaging from single-molecules to cells

Alsteens et al., Nat. Rev. Mater., 2017
INTRODUCTION
FD-based AFM from single-molecule to cells

Alsteens et al., Nat. Rev. Mater., 2017
AFM evolves from SMFS to dynamic mapping:

- **RL interaction force:** Science, 1994, 264, 415.
- **Affinity imaging by FV:** J. Histochem. Cytochem., 2000, 48, 719.
- **Time-resolved mapping:** PNAS, 2010, 107, 20744.

Need for better temporal and lateral resolution!
INTRODUCTION
FD-based AFM technology

Is this method quantitative and able to map specific interactions at high-resolution?
**Biological Mapping**

Tracking single bacteriophage extrusion

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**Ff bacteriophages**

- Hi6
- pIX
- pVII
- pVIII
- pVI
- pIII

- Outer membrane
- Inner membrane
- PG
- ssDNA

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**Topographic imaging**

- Zoom #1
- 2 µm
- #1
- 300 nm

- Zoom #2

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**Adhesion**

- 356 ± 45 pN
- 500 pN
- 50 nm

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**Young Modulus**

- 20 MPa

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*Alsteens et al., Nat. Commun., 4, 2013*
**BIOLOGICAL MAPPING**

Spindle assembly abnormal protein 6 homologue (SAS6)

MAPPING AND QUANTIFYING SINGLE LIGAND-BINDING PROPERTIES

Mapping single ligand-binding

Alsteens et al., Nat. Methods, 12, 2015.
Mapping and Quantifying Single Ligand-Binding Properties

Mapping single ligand-binding

Alsteens et al., Nat. Methods, 12, 2015.
In collaboration with Prof. Cheng Zhang (University of Pittsburgh)

**Mapping and Quantifying Single Ligand-Binding Properties**

**Functional state vs. oligomerization**

**Complement component C5a**
- Anaphylatoxin and one of the most potent pro-inflammatory signaling peptides
- Involved in the pathogenesis of numerous inflammatory diseases.
- Most of the C5a functional effects occur through C5aR.
- Development of new anti-inflammatory therapies.

**C5a receptor**

**Receptor functional states vs. oligomerization of C5aR receptors**

**Dynamics of complement component C5a binding**

Peter A. Ward, Nat. Rev. Immunol, 4, 2004
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**Lipid bilayer Canlever Scansyst HR**

- $\omega_{\text{liquid}} \approx 35 \text{ kHz}$
- $k \approx 0.4 \text{ N/m}$
- $F_{\text{setpoint}} \approx 150 \text{ pN}$

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Dumitru et al. submitted
MAPPING AND QUANTIFYING SINGLE LIGAND-BINDING PROPERTIES
Functional state vs. oligomerization

In collaboration with Prof. Cheng Zhang
(University of Pittsburgh)

Endogenous ligand binding sites mapping

Histidine residues mapping

Dumitru et al. submitted
AFM Probe functionalization with tris-NTA

1. Cleaning by immersion in chloroform for 10 minutes, rinsing with ethanol, N₂ drying, UV-O (15 minutes).

2. Aminofunctionalization in an ethanolamine solution overnight (5 g ethanolamine in 10 ml DMSO).
AFM Probe functionalization with **tris-NTA**

1. Cleaning by immersion in chloroform for 10 minutes, rinsing with ethanol, N₂ drying, UV-O (15 minutes).

2. Aminofunctionalization in an ethanolamine solution overnight (5 g ethanolamine in 10 ml DMSO).

3. Incubation in a solution of 1 mg acetal-PEG27-NHS linker dissolved in 0.5 ml chloroform and 30 μl trimethylamine.

4. Addition of citric acid 1% for 10 minutes to convert the acetal in aldehyde.

Acetal-PEG₂₇-NHS
AFM Probe functionalization with **tris-NTA**

1. Cleaning by immersion in chloroform for 10 minutes, rinsing with ethanol, N\(_2\) drying, UV-O (15 minutes).

2. Aminofunctionalization in an ethanolamine solution overnight (5 g ethanolamine in 10 ml DMSO).

3. Incubation in a solution of 1 mg acetal-PEG27-NHS linker dissolved in 0.5 ml chloroform and 30 μl trimethylamine.

4. Addition of citric acid 1% for 10 minutes to convert the acetal in aldehyde.

5. Incubation 1 hour in 100 μl tris-NTA-NH\(_2\) 80 uM and 2 μL sodium cyanoborohydride 1 M.

![tris-NTA-NH\(_2\)](image-url)
**AFM Probe functionalization with ** **His-tagged protein**

1. Cleaning by immersion in chloroform for 10 minutes, rinsing with ethanol, N₂ drying, UV-O (15 minutes).

2. Aminofunctionalization in an ethanolamine solution overnight (5 g ethanolamine in 10 ml DMSO).

3. Incubation in a solution of 1 mg acetal-PEG27-NHS linker dissolved in 0.5 ml chloroform and 30 μl trimethylamine.

4. Addition of citric acid 1% for 10 minutes to convert the acetal in aldehyde.

5. Incubation 1 hour in 100 μl tris-NTA-NH₂ 80 uM and 2 μL sodium cyanoborohydride 1 M.

6. Addition of 5 μL of ethanolamine 1 M pH 8.0 (10 minutes).

7. Rinsing with PBS 3 x 5 minutes.

8. Immersion in a 40 mM NiSO₄ solution for 30 min prior to experiments.

9. Incubation of tris-NTA tips in 100 μl 1 μM His₆-Complement C5a premixed with 40 mM NiSO₄ for 2 hours.
Mapping and Quantifying Single Ligand-Binding Properties

Functional state vs. oligomerization

Histidine residues mapping

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Histidine residues mapping

Topography

Adhesion

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Endogenous ligand binding sites mapping

Dumitru et al. submitted
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Functional state vs. oligomerization

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Control experiments

Topography
Bare tip

Adhesion

Ethanolamine tip

Dumitru et al. submitted
**Mapping and Quantifying Single Ligand-Binding Properties**

Functional state vs. oligomerization

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Control experiments

Topography

NTA Ni^{2+} tip

Adhesion

EDTA 10 mM 30 min

Dumitru et al. submitted
Control experiments

Topography

Adhesion

C5a tip

After C5a block

Dumitru et al. submitted
BIOLICAL MAPPING ON LIVING CELLS
Mapping binding sites of single-virus

Collaboration with Prof. Botond Roska

Mapping of interaction at the single virus level in physiological conditions

BIOLOGICAL MAPPING ON LIVING CELLS

AFM chamber

Cells are dividing for several days under the AFM

**Biological Mapping on Living Cells**

Controls Expression of the TVA receptor

MDCK express cell surface TVA receptors

BIOLOGICAL MAPPING ON LIVING CELLS
Controls_Virus assembly

Virus is able to specifically infect MDCK(TVA)

BIOLOGICAL MAPPING ON LIVING CELLS

Controls

Confocal and SEM of single viral particle on the AFM tip apex

BIOLOGICAL MAPPING ON LIVING CELLS
Combination AFM-confocal

BIOLOGICAL MAPPING ON LIVING CELLS
Combination AFM-confocal

BIOLOGICAL MAPPING ON LIVING CELLS
Combination AFM-confocal

BIOLICAL MAPPING ON LIVING CELLS
Combination AFM-confocal

BIOLOGICAL MAPPING ON LIVING CELLS

Energy landscape

BIOLOGICAL MAPPING ON LIVING CELLS

Energy landscape

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Energy landscape

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Energy landscape

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Energy landscape

BIOLOGICAL MAPPING ON LIVING CELLS
Energy landscape

FD-based AFM is a powerful method to...

... Localize at high-resolution binding sites

... Extract kinetics and thermodynamics parameters

... Study complex binding events on living mamalian cells
ACKNOWLEDGMENTS

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PhD and post-doc positions
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