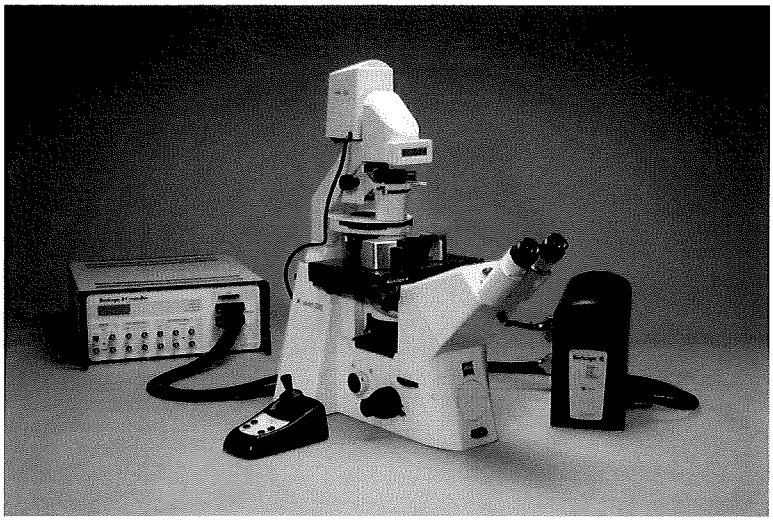
Bindley Bioscience Center

Biological Atomic Force Microscope (BioAFM) Laboratory

Bioscope II TRAINING



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The Bindley BioAFM Facility:

The Bindley Biosciences Center Biological Atomic Force Microscopy (Bio AFM) Laboratory was conceived from a need for the biological/life sciences community within Purdue to have access to this premiere tool of nanotechnology. As a research lab of the Bindley Bioscience Center within Discovery Park, the Bio AFM Lab will provide a university wide resource for interdisciplinary discovery and education using this state of the art technology. The Bio AFM lab provides AFMs specifically designed for research in the biological and medical fields. The newest instrumentation and techniques for AFM in fluids combined with the latest optical techniques are available.

AFM provides increased power of magnification/resolution coupled to real-time imaging of living samples - capabilities not shared with any other single imaging modality. Additionally, the unique versatility of the AFM also allows the user to 1) physically manipulate the sample, 2) provide precise quantitative measurements of distance, surface area and volume at the nanoscale if required, 3) derive physiologically significant data such as cellular membrane pressure and DNA or protein binding forces. The full impact of AFM in life sciences and medical applications is still maturing as new researchers are becoming involved and new techniques and methodologies are being developed.

The location of the Bio AFM lab within the Birck Nanotechnology Center provides collaboration opportunities among life science, medical and biological researchers with the traditional AFM development researchers of physics and mechanical/electrical engineering. As unique requirements develop for fluid applications of AFM the personnel, instrumentation and ideas necessary to meet these challenges with innovative and interdisciplinary approaches will be available.

Goals

- To provide AFM capabilities to the life science and medical community.
- To educate the Purdue research community of the AFM capabilities and the impact this technology can have in their research.
- To develop AFM techniques necessary for innovative experimental design.
- To integrate AFM technology with other analysis systems.
- To provide training and assistance to individual investigators
- To develop short courses for education of undergraduate and graduate students.

Equipment and Capabilities

In connection with the Bindley Bioscience Center and the Birck Nanotechnology Center, the Veeco Bioscope II AFM is available with various capabilities, enabling examination of single atoms and molecules as well as whole cell and tissue samples.

- Veeco (DI) Bioscope II Beta sight. This is the first placement of this latest technology instrument in the US.
- Optical capabilities include bright field, phase contrast and DIC. Fluorescence and confocal imaging capabilities must be procured. These can be completely integrated with the Bioscope II.
- Heating and cooling stages on the Bioscope II.
- Perfusion cell on the Bioscope II.
- Sample configurations include cover slip, microscope slides, 35mm, 55mm and 60mm petri dishes. Other sample arrangements may be accommodated as needed.
- Surface modifications, and specialized cleaning techniques.
- Wet labs, solvent and acid hoods for sample prep, cell culture rooms.
- Training of research students at all levels with individual instruction on specific equipment as necessary
- Training session via short courses.
- Specific consultation is provided for experimental design and unique requirements.

Current Research Projects

Molecules –
- Biosensor surface analysis and cantilever development.
- DNA conformation and surface attachment analysis.
- Protein conformation and surface attachment analysis.

Whole Cells –
- Membrane and lipid domain mechanics and organization.
- Investigations of neurogenesis, neurotrauma and regeneration.

AFM technology development –
- Nonlinear dynamics in fluid applications
- Frequency modulation technique
- Torsion Mode in fluid applications

AFM integration with other analysis systems –
- Integration with vibrating probe technology.
- Integration with RAMAN spectroscopy

Education and Training: To meet the full potential of AFM throughout the Purdue community two sides of the educational component must be addressed.

1) Investigator Understanding of AFM Capabilities and Requirements
2) Student Education and Training

A general knowledge of AFM and its capabilities must be provided to the Purdue research community. In turn, the requirements of these interested researchers must be conveyed to the AFM research community. The first has already been accomplished by a number of investigators who have found a need for AFM in their research and actively searched out the capabilities of this technology which will accomplish their requirement. Their students have been trained through group and individual training sessions on the basics of AFM and its applications

into the fluid environment. Assistance in sample preparation and experimental design was/is also provided.

A second and much larger wave of life science and medical AFM applications can be expected in the future. New researchers are contacting the Bio AFM facility everyday. Others will be enlightened to the AFM world through seminars and workshops provided by internal AFM researchers, outside experts and AFM manufacturers eager to demonstrate their products. As these new collaborations are identified, their requirements will be met with innovative experimental design and the development of new methodologies should the requirements exceed the current capabilities of the Bio AFM lab. Student training and education will again be conducted with group and individual session. As the use of AFM for life science and medical applications expands, short courses will be developed to increase the volume of student training and provide regular course offerings. Graduate and undergraduate level courses will be provided to meet the anticipated diverse background of potential users.

Organization and Approach: Administration of the Bio AFM lab falls under the Bindley Biosciences Center. Helen McNally will oversee the lab and provide technical expertise. An internal advisory committee will be formed to establish policy, determine operational guidelines, specify long-term maintenance requirements and mediate conflicts that might occur.

Bio AFM Location: Birck Nanotechnology Center, Room 1031

Scheduling:
https://engineering.purdue.edu/ECN/Resources/Tools/RAT/Entities/BioAFM
This is the website for scheduling the Bioscope II.
Sign on using your Purdue Career Account. Select "request a reservation", Select the AFM, your time and date and select "other" as a reason (this is required for some reason). Submit your request. It will come to me (or Elizabeth) for approval and then you will get an email that your reservation was either added or rejected. Let me know if you have any problems.

BNC Training:
The following training sessions must be completed prior to working on the Bioscope II independently.

BNC105
BNC208
Fume Hood Training

These can be accessed at http://www2.itap.purdue.edu/bnc/training/

Bioscope II Basics

Start Up:

- Check log in sheet and Sign In
- Open Enclosure – insure noone is still using the system
  - Turn red handle to the left
  - Flip hydraulics switch up
- Turn on Monitors
- Log into Computer
- Turn on Controller
- Turn on Microscope Lamp
- Start Nanoscope Program current version 7.0
  - Always insure the controller is turned on prior to opening the Nanoscope program.

Removal of Sample

- Withdraw scanner completely. **IMPORTANT**
- Push optical tower back
- Remove AFM scanner –"Carefully"
  - Insure no fluid drips
  - If so – clean it up.
- Place scanner onto cradle
- Remove Sample

Clean Up of Tip/Fluid Cell

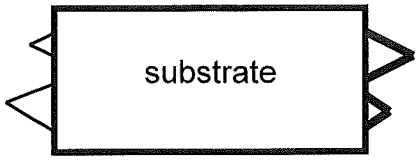
- Remove fluid cell from AFM head
- Rinse with DI water
- Wick it dry with paper
- Blow dry if necessary
- Store in clean and dry space
- *See Veeco notes for major clean of fluid cell.

Shut Down:

- Close Application program
- Turn off controller
- Turn off optical lamp
- Log off of computer
- Turn off monitors
- Check to insure everything is in proper place and clean
- Close Acoustic enclosure
  - Insure red handle is turned completely to the left
  - Flip the hydraulics switch down and lower the enclosure top.
  - Turn red handle completely to the right

Probe Considerations:

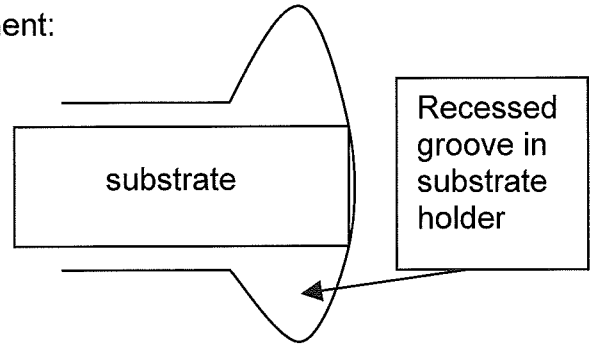
Tip Selection – review of various types



Veeco DNP
V-shaped cantilevers

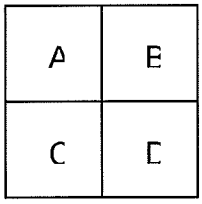
Veeco OTESPA
spring board cantilevers

Substrate Placement:



Laser Alignment:

- Easy Align
  - Cantilever location
  - Focus
  - Illumination
- Laser adjustments
- Dicrotic Mirror adjustments



Quad photo detector

Vertical (A+B) – (C+D)
Lateral (A+C) – (B+D)

Sample Considerations:

Sample Preparation – review of various possibilities

Sample Placement

- Coverslip
- Microscope Slide
- 35 mm Petri Dish
- 50 mm Petri Dish
- 60 mm Petri Dish

AFM Operations
- Imaging
  - Contact(Deflection Set point)
  - Tapping (Thermal Tune)
  - Jumping
  - Non contact
- Force Spectroscopy

AFM conditions
- Dry conditions
- Wet conditions
  - Look at clear bubbles
  - Adjust laser (to the left)
  - Adjust dicrotic mirror
  - Let stabilize!

Parameters to Adjust – Scan Parameters
- Scan size
- Scan angle
- Scan rate
- P&I gains
- Setpoint

OPTICAL MICROSCOPE

Olympus IX-71 Inverted Microscope

Objectives:
- 10X, 20X, 40X with adjustment collar
- Magnificent changer 1.6X

Condensor – 0.55NA
- Annulus Phase 1 – 10X, 20X
- Annulus Phase 2 – 40X
- Annulus 3 – brightfield

Filters:
- Frosted – diffusing filter
- Blue (daylight) – adjust yellow light of bulb
- Green – phase contrast

Camera
Flourescense, confocal, multiphoton

Follow on training:

- **Tapping Mode**
- **AFM wet conditions**
- **Force Spectroscopy**
- **Off-line Analysis**