Detailed specifications of Atomic Force Microscope

1. Standard Operating Modes which are must for the Instrument:

a) Contact mode (Lateral force, Topography, deflection, Feedback error, Sensor signal and auxiliary ports for both in air and liquid)
b) Dynamic or Tapping mode or AAC (Topography, Amplitude, Phase, Frequency shift, at least one auxiliary for both in air and liquid)
c) Magnetic Force Microscopy
d) Single molecule force spectroscopy
e) Scanning thermal AFM mode
f) Conducting Force Microscopy or Current-sensing Force Microscopy (Current, voltage, deflection, lateral, topography)
g) Nanolithography and Nanomanipulation
h) Plots and recordings of Force, cantilever deflection, raw-deflection with displacement (both piezo and sensor) and time can be viewed and recorder simultaneously.
i) The instrument should be capable of doing at least five force-clamp experiments on a same spot with five different force set-points. After one spot, instrument should be able to move automatically to a new spot without dragging the cantilever on sample surface and do similar five force-clamp measurements. This type of measurements should be possible at least for 256*256 matrices. The data should be saved automatically.
j) The experimental protocol as mentioned in (d) should also be possible for length-clamp experiments and dynamic force spectroscopies. In case of length clamp, one should be capable of setting at least 5 different tip-surface distances. For dynamic force spectroscopy, the instrument should allow users to define at least five different velocities.
k) Force curve based imaging system should be available. This mode should have automatic control over tip-surface interactions at every pixel without any need for set point or gain adjustment. . It should have in-built software compensation for any deflection drift during measurement.
l) Users must be able to set customized force-curve experiments by setting force ramps, temperature ramps, force feedback, Z-piezo position, Z-piezo position with time etc. Users should be able to initiate the Force-distance measurement externally using synchronised TTL inputs.
m) The atomic force microscope should have adapters to integrate and work on an inverted optical microscope without any loss in performance and any added noise. This integration should not block the use of the optical microscope.
n) The instrument should come with an inverted microscope along with bright-field imaging, phase contrast imaging DIC and side-view of the cantilever options. Demonstration on the simultaneous use of AFM-optical microscope is necessary after installation.

o) AFM must have the provision to localize the cantilever atop of laser spot coming from the inverted microscope for simultaneous AFM-fluorescence measurements.
p) A standard laser scanning confocal microscope (LSCM) should be integratable with the AFM. Demonstration or peer-reviewed publications of the use of a combined AFM-LSCM on this system is necessary. Please mention the references.
q) In the integrated AFM-optical microscope system, the precise localization of AFM cantilever on top of a Laser used in inverted microscope should not be done using objective scanning.
r) The AFM should have options to integrate with upright fluorescence microscope for opaque samples.
s) Cantilever holder should be removable, washable and autoclavable.
t) The instrument must have the features to enhance imaging quality in liquid with any commercial cantilevers and should be able to use extremely small oscillation amplitudes of 1nm and less.

2) <u>Scanner Specifications (Range, Resolution and Noise level):</u>

a) X, Y and Z scanner should operate in both open loop and closed loop and decoupled with independent actuators.
b) The AFM should be a tip-scanner. All x, y and z should be in scanner head itself but decoupled. All piezo-scanners (x, y and z) should have with flexure design.
c) X, Y scanning range should be ≥ 90-µm X 90 µm and Z scan range ≥ 15 µm in both open loop and closed loop configuration. Selecting or switching between open loop and closed loop should be user-defined.
d) DC height noise or Z -noise level or tip-surface distance noise should be ≤ 50 pm (RMS) in a bandwidth of 0.1 Hz to 1 kHz. While quoting please mention the method of noise measurements along with the cantilever specifications.
e) Noise for cantilever deflection detection system should be ≤ 3 pm (RMS) in a bandwidth of 0.1 Hz to 1 kHz.
f) Z- closed-loop sensor noise should be ≤ 70 pm (RMS in a BW of 3 kHz) with sensor non-linearity of ≤ 0.05% at full-range scan. **(Please mention the type of sensors used for all 3 axes)**
g) The position noise should be ≤ 200 pm in closed loop x and y piezo with a sensor non-linearity of ≤ 0.5% at full scan.
h) A side camera view of the cantilever should be available.
i) The maximum bandwidth of deflection detection must be > 6 MHz.
j) Instrument should come along with an extra extendable closed-loop Z-scanner of ≥ 40 µm for live cell-live cell adhesion measurements. This piezo should also have closed-loop functioning option.
k) The Instrument should demonstrate an atomic lattice resolution in all 3-axes during contact mode and tapping mode imaging.

l) The optical lever assembly and the cantilever holder should be built on a single frame to eliminate artifacts due to relative motion between cantilever and the optical lever along with the detector.
m) The Instrument should have IR deflection detection light source with low coherence for interference-free measurements.

3) <u>Specifications for Conductive AFM:</u>

a) The system must allow conductive measurements while scanning as well as at user specified locations (I-V curves).
b) A sample bias of -10V to 10V must be possible.
c) The bandwidth of the trans-impedance amplifier must be at least 17 kHz.
d) The software must allow user-specified waveforms for I/V spectroscopy (square, sine, triangle, pulse, or user defined).
e) The software must allow user-specified waveforms for loading and unloading, including multiple user specified trigger-points, while simultaneously monitoring current.
f) The system should preferably include automated mechanism for reducing contact resistance due to surface contamination in I/V curves.
g) The current sensing range must be 1pA to 120 nA.
h) For measuring sample is inert and controlled environment, Enclosed Volume sample holder must be provided.

4) <u>Hardware Specifications (Including Controller and Electronics):</u>

a) The AFM controller should have 100% digital operations.
b) The instrument must have software operated digital Q-control of the cantilever's Q-factor.
c) The instrument must have software-controlled switches for user-defined signals.
d) The instrument must monitor and display the tip-sample interactions and extract, display and save the adhesion, elasticity modulus, and dissipation during AC mode operation.
e) The controller must have modular design with both FPGA and PPC technology.
f) The instrument should have the ability to adjust all scanning parameters (scan rate, scan size, scan offset, gains and others) in-situ during scanning.
g) The instrument must come with an adapter for the integration of AFM with an advanced inverted optical microscopy. The design should not compromise the performance of AFM (scan range, noise level, Piezo speed etc) as well as the optical microscope.
h) The adapter designs for AFM-inverted optical microscope should be able to perform for Laser-scanning confocal microscopy, phase contrast, DIC, Total Internal Reflection Fluorescence Microscopy, FRAP, FRET, epi-fluorescence microscopy and tip-enhance microscopy.
i) The instrument should also be able to perform as stand-alone basis and on top of an inverted microscope.

j) The instrument must perform thermal tuning of the cantilever at frequencies of 3 MHz or more.
k) The instrument must be able to record power spectrum of the cantilever and the cantilever spring constant following thermal noise method.
l) The instrument must have BNC output for all major signals, e.g., raw deflection, sum signal, friction or lateral deflection, phase, amplitude, X, Y, Z sensors, three user outputs, X, Y, Z piezo drive voltages,
m) The instrument must have number of user input-output channels with BNC connectors.
n) The instrument must include auto-configuration of external hardware and accessories. Device parameters must be stored and read each time the instrument is turned off or on.
o) The instrument must have high-speed lock-in amplifiers for precise amplitude and phase detection.
p) High-speed data acquisition with optional burst mode should be available.
q) The instrument should have signal access and signal generators with analog and digital input-outputs.
r) The controller should have options to generate TTL pulses. Software should be able to send TTL pulses to BNC outputs that can be fed to external instruments.
s) The controller should have gated photon counters.

5) <u>Specifications for the Light Source and Position sensitive photodetector:</u>

a) The instrument must use super-luminescent diode as a low-coherence light source to eliminate artifacts from optical interference.

b) The instrument should use beam-bounce technology.
c) The IR SLD can be used to eliminate optical cross talk with light used for fluorescence imaging. The AFM must include a detector/camera for aligning the invisible IR laser to the cantilever in both air and liquids. Real-time monitoring of Laser spot on cantilever would be an added advantage to have.
d) The bandwidth of deflection detection must be > 6 MHz.

6) <u>Specifications for Sample Holders and Environmental Chambers:</u>

a) The instrument must allow to use cover slips, glass slides, plastic petri dishes and glass-bottom petri dishes.
b) The instrument should have an environmental chamber for live-cell studies. The chamber must include inlets and outlets for solutions as well as gas. An incubator like set-up for keeping live-cells happy is the must.
c) The instrument must have sample heater accessories for maintaining physiological temperature for live-cells as well as doing experiments at variable temperatures (range ~15° – ~ 60° C with 0.1° C precision in buffer).
d) It is advantageous if the instrument allows using all the above-mentioned sample holders for heating experiments.

e) Instrument should have facilities for perfusion and gas-flow to sample.
f) Instrument should have sample holders for high-NA immersion lenses and high resolution AFM down to the single molecule level.

7) Specifications for SPM Software:

a) Software must be free and should be accessible for multiple users.
b) Free lifetime software update
c) The system's software must include a one-click configuration tool that sets up the software for standard and user-defined operation modes, such as AC imaging in air and liquid, contact mode, EFM, force measurements etc.
d) The instrument must be capable of driving the cantilever simultaneously at two or more arbitrarily chosen excitation frequencies in AC (dynamic) mode, while simultaneously collecting and displaying the amplitude and phase signals and images from each of these frequencies, along with the height or Z-sensor data.
e) The Computer-to-Controller communication must be a simplified protocol like via USB2. This simplifies system set-up and allows easy and cost effective future upgrade of the system's computer.
f) Software for online Image analysis should be available free of cost.
g) The software should be user-programmable.
h) The software must include the functionality for the direct overlay of the optical microscope data obtained from the fluorescence microscope along with AFM data for correlated information.
i) The software must include the functionality for the direct overlay of the optical microscope data obtained from the fluorescence microscope with AFM data. This overlay procedure must include an integrated procedure to provide registration between both image data sets.
j) The instrument should have the ability to optimize automatically the critical imaging parameters (e.g., set point, gains, scan rates and Z limit in both air and liquid environment).
k) The software must be able to map the drive amplitude, drive phase, resonance frequency and quality factor of the resonance being tracked by the technique described above.
l) The software should provide the user the possibility of scripting to modify experiments.
m) During force-distance measurements, tip should be preserved automatically from damaging due to rough surfaces.

8) Instrument Isolation and Installation:

a) The system must include a thermally- and acoustically-isolating enclosure. The enclosure must provide at least 20dB of acoustic isolation.
b) The system must include active vibration isolation.
c) Active vibration control between 1 Hz to 200 Hz and passive for more than 200Hz.
d) The noise in terms of sound, low-frequency floor vibrations should not affect the

high resolution AFM imaging.

e) Onsite demonstration and Installation should be done. The demonstration and training should be rigorous (for 3 days or more) and useful for young PhD students.

9) <u>Guarantee, Warranty, Support and Service:</u>

a) The instrument should come with four years or more comprehensive warranty on all parts and labor. Please mention clearly warranty policies.

b) The instrument software upgradation should be free for the life of the instrument.

c) The vendor must clearly specify how the servicing will be done in case of any requirement. They should provide the contact details of the service engineer along with the quotation. **In case the purchase committee decides to go for a technical presentation after the tender period, the service engineer should be able to meet the committee for the presentation in a week's notice and should be able to address all the specifications mentioned here. Committee will conclude based upon presentations. This committee reserves the right to make the final decision.**

d) The company should have at least four installations/sales of their AFM in last two years (2012-2013) among the listed institutes; IISc, All IISERs, NISER, All IITs, NCL, IMTECH, CCMB, TIFR, NCBS, IACS and JNCASR.

e) Spot demonstration of the functions of combined microscopes (AFM and Fluorescence) is necessary.

f) A training session on the instrument is necessary for students after the installation.

g) Integration with TIRF microscope may require during installation.

10) Vendors are requested to quote for the following optional modes which will be purchased based on budget.

a) Piezo-response Force Microscopy, including Switching Spectroscopy PFM

b) Kelvin probe force spectroscopy.

11) Vendors should response to each points mentioned above and also clarify how their systems match with the required specifications.

12) The company should have trained service engineers locally based in India for after sales support at short notice. This aspect is extremely important when considering a quote and hence vendors must give details of the local after sales support in the quotation. Without this, quotes may not be considered. Vendors may also mention about the online support.