

Asylum MFP-3D Standard Operating Procedures

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The Asylum MFP-3D is an extremely versatile, research-oriented scanning probe microscope on an inverted optical microscope platform. Unique capabilities of the MFP-3D (and its associated modules) include:

- Precise cantilever stiffness measurements and mechanical force curves
- Conductive probe AFM via the ORCA module
- Piezo force microscopy (PFM), including high-voltage, Dual AC Resonance Tracking (DART), and Band Excitation (imaging and switching spectroscopy)
- Kelvin Probe (SKPM/KPFM), EFM, and other electrical techniques
- Localized sample biasing via the probe station stage
- Measurements in fluids (liquid droplet or gas/liquid in a sealed cell), including temperature control (via Petri Dish Heater)
- Measurements in controlled humidity environments via the humidity sensing cell
- In-situ measurements of growth processes via the electrochemical cell
- Measurements requiring optical excitation of the sample and/or inverted viewing through a transparent substrate, including fluorescence imaging
- Nanolithography and related techniques
- Automated sample property (force curve, I-V curve, etc.) point mapping on grids or defined points
- High-resolution scanning (up to 4096 points and lines)

All users should receive general system training, which will cover basic topographic scanning as well as principles of operation and other necessary information, and should demonstrate basic proficiency with the system before moving on to other techniques. Facility staff will assist with advanced techniques, but users are expected to pursue this somewhat independently using documentation provided by Asylum.

This document includes general info and procedures for all users, as well as specific procedures for topographic scanning in AC tapping mode and basic image analysis.

Additional Resources

If you need help while using the MFP-3D or have general questions about its operation and/or capabilities, please contact the facility staff:

- Primary contact – Steve Kriske (kriske@cornell.edu, D21F Clark Hall, 255-2367)
- Secondary contact – Dr. Jonathan Shu (jbs24@cornell.edu, 633 Clark Hall, 255-9833)

All users (particularly those without prior AFM experience) are encouraged to watch Asylum's "Understanding AFM" DVD (60 minutes), which can be borrowed from the facility staff. This is an

excellent resource which includes background on the history of scanning probe microscopy and the operating principles and advantages of the Asylum system.

A PDF copy of Asylum's system manual is available on the AFM computer desktop for use in the lab or to be copied. Please note that at least some sections of the manual refer to older versions of the software, so specific instructions may not be applicable on a word-by-word basis.

Asylum has also produced a "Procedural Operation Manualette" which is more task-oriented and includes more figures than the main system manual. A PDF copy of the manualette is stored on the computer desktop and at <https://cornell.box.com/MFP3D>, and a printed copy is kept in D15. All users should read Chapters 1 and pages 6.1 – 6.13, and are strongly encouraged to browse the manualette for tips and more details on specific procedures. Figures in this document are taken from the manualette.

In addition to the two above documents, the folder "MFP3D_manuals" on the desktop includes individual application notes on several specialty techniques, many of which are compiled in a longer "Applications" document.

There is a fairly extensive help index within the Asylum software. The question mark icon to the right of each item in the software will bring up the help blurb for that specific item.

Reservations and Logs

Before using the AFM, users must make a reservation in CCMR Coral. At this time, there are no restrictions on number or lengths of reservations. The AFM is listed under the Surface Analysis and Characterization category. All users must enable the AFM in Coral at the start of use (Equipment Actions – Enable; note that the left computer monitor is interlocked to Coral enabling) and disable when finished (Equipment Actions – Disable). In addition to enabling in Coral, users must fill out a log sheet for each use session and place it in the appropriate binder.

Software & Files

Asylum's control and analysis software is built on WaveMetrics Igor Pro architecture, which allows great freedom for customization and programming (including control and analysis macros, input and output of signals to/from external instruments, and advanced programming). The current Asylum software is Version 14. The software is available for any user to install for offline analysis, but it requires an Igor license for analysis operations. A full Igor manual is available in PDF format on the computer desktop. There is a computer in D15 which is configured for off-line analysis; the Asylum software and all documentation mentioned above is available on this computer in addition to the AFM computer.

When the software is initialized, the Mode Master (also accessible by clicking Ctrl-F2 at any time) directs the user to their desired experiment type. This will configure the software with generic parameters for any given experiment type. Users may also save an "experiment" (.pxp) file, which essentially captures the entire open workspace, including parameters, data channels, captured images, and other data files and application windows.

All data files, including raw and processed images (specifically, these are numerical data files represented as images) as well as curves (force curves, surface profiles, etc.) are saved as .ibw (Igor binary wave) files or “waves”. These are intended for use in Igor, and can be converted to more standard image or data formats. All files should be saved in Asylum User – My Documents – Asylum Research Data – [user folder].

Samples & Substrates

In order to allow bottom-up optical access to samples, the stage is designed so that samples are suspended over a gap, typically attached to standard microscope slides. Samples larger than the gap can simply be placed on the stage; any sample that fits between the three legs of the scan head and is shorter than the fully-extended leg height is acceptable, but for samples larger than the X-Y scanner (silver), it may help to elevate them slightly in order to avoid friction with the outer base of the stage (black).

Small samples can be rigidly mounted on a microscope slide; multiple samples may be attached to one slide, but please note that the mechanical stage motion range is fairly limited (~1 cm). Alternatively, the Sample Puck Holder can be used for magnetically attaching the small steel sample prep discs, or the SEM Mount Holder can be used for SEM-mounted samples. For nanoparticles or other samples requiring a non-integrated substrate, it is advisable to use an atomically smooth surface such as mica rather than applying the sample directly to the microscope slide, which is not flat a small scale. For more tips on sample preparation and mounting, consult the facility staff.

Additionally, most application modules have a particular sample holder and/or mounting technique. Leg extenders are available for thick/tall samples.

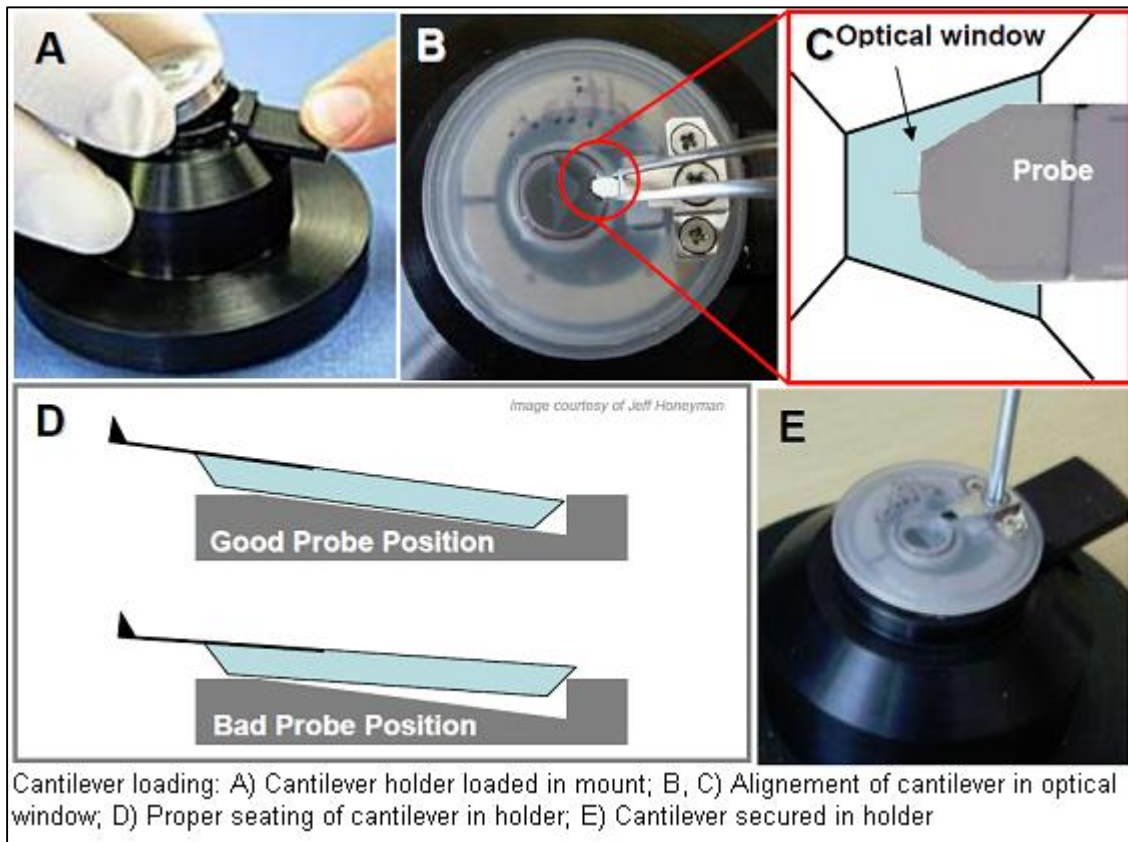
Probes

The MFP-3D uses the same standard probes as the Dimension 3100 and most other AFMs. CCMR keeps a supply of Olympus AC160TS-R3 and AppNano ACCESS-NC tapping mode probes which are available to users at \$20 each, as well as a variety of contact mode and specialty cantilevers. Due to the use of an infrared laser, silicon cantilevers need a reflective metal coating on the backside since silicon is transparent to IR light. Check with the facility staff to buy stocked cantilevers, or for help selecting and purchasing the best cantilever for your application.

We currently have four different probe holders, each of which is appropriate for various applications. Each holder has its own case and should be stored securely when not in use, and cleaned as necessary after using with fluids or other contaminants. [Note – Cleaning with KimWipes can scratch the optical surfaces, so Asylum recommends using cotton swabs or lens paper.] The standard holder can be used for most standard imaging and force measurements, as well as most electrical applications requiring tip biasing. The PEEK holder is configured with no metal parts and is more appropriate for fluid, biological, and electrochemical cell applications. The ORCA holder is used with the ORCA module, allowing current measurement. The AC Mode PFM holder is compatible with the high voltage module.

Operating Procedures

Parts 1-3 and 7 are applicable for all applications except for closed cell operation. Part 4 is specifically for AC Mode imaging. Parts 5 and 6 are written for AC Mode imaging, but should largely apply to all imaging modes.



Part 1 - Load the probe

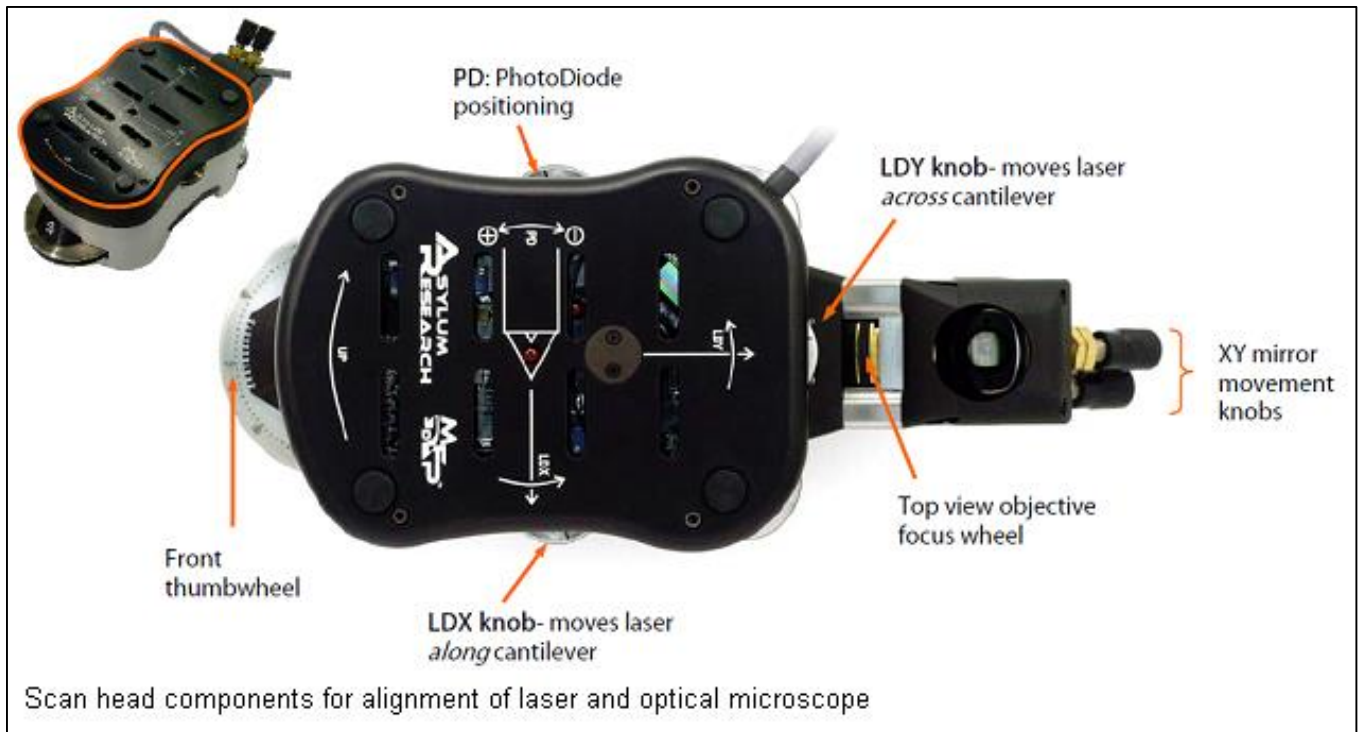
- 1) Place the probe mounting device on the table with the spring-loaded lever facing right.
- 2) Select the appropriate probe holder and place it in the mounting device by pressing the lever down, inserting the holder with the screws on the right side, and releasing the lever.
- 3) Use the provided Phillips screwdriver to loosen the screw on the probe holder clip by $\sim 1 \frac{1}{2}$ turn. [Note – Use the green-handled screwdriver when using PEEK holder to avoid damage to the Teflon screw.]
- 4) Using fine tweezers (curved tweezers provide a better handle for this operation), grab a probe by the body edges, closer to the cantilever end, and remove it from its gel pack.
- 5) Carefully place the probe on the window and slide the probe under the clip, then tighten the screw. [Note - AppNano AC probes have an etched line which should still be visible after the

cantilever has been inserted into the clip. Pushing too far can cause it to slide up off the holder surface.] Do not over-tighten the clip to the point of bending the metal.

- 6) Press the mounting device lever down and remove the holder.
- 7) Open the enclosure door (or, if maximum thermal stability is desired, the window[s]).
- 8) On the AFM head (which should be on the shelf inside the enclosure, facing up), press the button on the right face of the probe holder cone, insert the probe holder, and release the button. Make sure the holder is securely in place and flat.

Part 2 - Load the sample and head onto the stage

- 1) Place the sample on the stage and secure it in place with the magnets.
- 2) Check to verify that the front leg of the head is extended. If necessary, turn the dial in the UP direction to extend it (see below for details), particularly if you have a tall/thick sample/substrate.
- 3) Lift the head off the shelf, turn it over face down and place it on the stage with the three legs in the appropriate holes in the sample stage. [Note - Always turn it with the bottom towards the stage to load, and opposite to unload. Never turn the bottom of the head away from the stage; twisting the cables can cause failure of the cable shielding.] Be careful to avoid bumping into the top optical scope with the mirror assembly on the back of the head. You should be able to feel the back feet settle into the depressions on the Olympus stage.



Part 3 - Initialize the software and align the laser

- 1) Open the Asylum Research software.
- 2) In the Mode Master, select the desired operation mode (Standard... - Topography... - ACAirTopography for AC imaging), or open a desired experiment file.
- 3) Click the camera icon in the bottom of the software to initialize the video window. If necessary, click Inputs and select the first SVideo input to use the top-view camera. [Note - The first Composite input corresponds to the bottom-view camera.]
- 4) Using the Dolan-Jenner Fiber Lite Remote Intensity Control, turn on the optical illumination and verify that light can be seen in the video window.
- 5) Find the cantilever by (if necessary) moving the field of view, i.e. turning the XY mirror movement knobs. Typically, the field of view will need to be moved down; the border between the frosted and clear section of the window provides a good reference for approaching the probe.
- 6) If necessary, adjust the focus wheel in order to focus on the cantilever.
- 7) Turn the key switch on the front of the ARC2 controller to turn the laser on. Use the LDX and LDY dials (on the right and back sides of the head, respectively) to move the laser near the end of the cantilever. The IR laser can be best seen by the camera if the Fiber Lite intensity is around 20%.
 - a. Move the laser spot onto the probe body. Note that it will not be visible very close to the edge. The Sum (Sum and Deflection Meter window) should be greater than 4 at this point, depending on the probe type.
 - b. Turn the LDY dial to align the laser spot with the cantilever.
 - c. Turn the LDX dial to move the spot near the end of the cantilever.
 - d. Optimize the spot location. You want the spot to be far enough out in order to get maximum deflection/amplitude sensitivity, but not too far out into the tapered section, which decreases reflected intensity. Move LDX, then LDY to maximize the Sum.
- 8) Turn the PD dial on the left side of the head to the opposite sign of the Deflection reading (Sum and Deflection Meter window) in order to zero the Deflection. Note that the Deflection scale only goes from -10 to 10, so there may not be an immediate response when turning the PD dial if the Deflection is initially outside this range.
- 9) At this point, the tip should be far above the sample. Turn the front dial ("Front thumbwheel" in the image above) counter-clockwise to retract the front leg and lower the cantilever into the vicinity of the sample (~1-2 mm separation is good at this point; for reflective samples, the reflection of the fibers in the illuminator become clearly visible in this range). Follow the lateral probe motion by adjusting the field of view as described in step 5. One full turn of the dial corresponds to 100 μm of vertical motion, each number on the dial is 1/10 turn or 10 μm vertically, and each small mark is 1 μm vertically.

[Note – If you have a homogenous surface and/or do not need to locate any particular feature or sample area for scanning, steps 10-12 can be skipped.]

- 10) Turn the rubberized focus dial in the mirror attachment counter-clockwise until the sample surface is in focus.
- 11) Use the sample stage X and Y adjustment knobs (small knobs on the front-right of the stage) to locate the desired area on the sample. Note that the area in the field of view at this point may not be the exact area where the probe tip will touch down.
- 12) Turn the focus dial clockwise until the cantilever is in focus.

Part 4 - Tune the cantilever

- 1) In the Master Panel, open the Tune tab.
- 2) Under "Auto Tune", select the appropriate frequency range based on the cantilever specifications and use a Target Amplitude of 1 V and a Target Percent of -5%. [Note – these are recommended settings for tapping mode; for non-contact mode, use 5%.]
- 3) Click the Auto Tune button. After a few seconds, a tone will sound and a graph of amplitude and phase vs. frequency will be displayed. The peak amplitude and resonant frequency will be displayed at the top of the window, and the lower frequency with 5% lower amplitude is exported for use as the drive frequency. At this point, the cantilever is oscillating and the peak (free air) amplitude will also be displayed in the Sum and Deflection Meter.
- 4) Save the tune data if desired, and close or minimize the graph.

Part 5 - Set up the scan and data parameters and engage

Scan parameters are set in the Main tab of the Master Panel. Data to be displayed in real time and saved is set in the Master Channel Panel. Each experiment (manually saved or via the Mode Master) has a default set of parameters and data channels.

- 1) In the Main tab of the Master Panel, set the Scan Size, Scan Rate, and Points & Lines (resolution). See the Asylum manual for definitions and limits of each parameter.
- 2) In the Master Channel Panel, select the data to be displayed using the Input drop-down for each channel (up to 8 channels, each represented in one tab).
- 3) If desired, adjust the Image Display and Image Modification for each channel; these may also be adjusted once scanning has begun, at which point the appropriate values will be more apparent. Images will likely need to be modified in post-processing in any case, and choosing the 'wrong' setting for real-time will not prevent correct modification later.
- 4) In the Main tab of the Master Panel, set the Set Point to ~85% of the free air Amplitude (as displayed in the Sum and Deflection Meter), and the Integral Gain to 10.
- 5) Click the Engage button. The Z Voltage increases to 150 V, meaning the Z piezo is active and fully extended.
- 6) Slowly lower the head by turning the dial on the front of the head counter-clockwise. This is actually tilting the head forward since you are only retracting one of the three legs, and therefore will cause the tip to move laterally within the camera view. To compensate for this, you can either move the back corners down by turning their dials counter-clockwise, or

- continue to adjust the field of view as described above.
- 7) As you get close to the surface, it will nearly be in focus, and you will probably see shadows of the cantilever and/or probe body. If you can see that the tip will be touching down other than where you want to scan, move the head back up slightly and manually re-adjust the X-Y sample stage position. Repeat this step as necessary.
 - 8) Continue moving the head down very slowly and watch the Sum and Deflection Meter to eventually see the Amplitude decrease. Once it reaches the Set Point value, a tone sounds to indicate that the tip has engaged on the surface. Continue lowering the head until the Z Voltage is around 70 V, which is the center of the Z piezo range.
 - 9) Click the Do Scan button to begin scanning. The data channels now display their respective images and traces.

Part 6 - Optimize the scan and capture images

- 1) Initially, the tip will most likely be in "false engage", and the height trace (red line) and retrace (blue line) will not match or show the surface shape. Gradually reduce the Set Point until they match. (Note – Reducing the Set Point below 50% of the free air amplitude will likely damage the tip and/or sample.) The Set Point and other parameters in the Main tab can be adjusted in three ways:
 - a. Use the keyboard to type in a new value.
 - b. Click the up/down arrows to the right of the value to incrementally increase/decrease by 10%.
 - c. Use the "hamster wheel" - The outer ring toggles between parameters, and the inner wheel adjusts the value of the active parameter (indicated by the radio button to the right).
- 2) Adjust the integral gain to optimize the image quality. If the image is blurry and/or the tracking is still poor, increase the gain. If the amplitude image is noisy, decrease the gain. The optimal gain is the highest value with no noise visible in the amplitude image.
- 3) If necessary, adjust the X Offset, Y Offset, Scan Size, and Scan Angle in order to scan the appropriate area in the desired orientation. Values can be changed numerically in the Main tab, or graphically using cursors in the images. The scan cursor can be toggled between Box, Point, and Line by right-clicking on one of the images; right-clicking again allows the scan to be adjusted according to a drawn cursor, or the cursor to be erased.
- 4) Images are saved via the Main tab as well. If the Save Image box is checked, completed images will be saved as wave files with a name combining the Base Name and Base Suffix. Save Status indicates whether the current or next scan (if parameters have been changed within the current scan) will be saved. All active channels will be saved.

At any point during a scan, you can return the scan to the bottom or top of the scan area by clicking the Frame Up or Frame Down button respectively. Each image window indicates the time remaining in the scan. To stop scanning, click the Stop button or the Withdraw button in the Main tab of the Master Panel, or the Withdraw button in the Sum and Deflection Meter; this will stop the lateral scan and withdraw the Z piezo.

Part 7 - Finish

Image analysis (see below) may be done concurrently with scanning. Perform these steps only when completely finished or changing samples (steps 1-2).

- 1) Turn the front dial clockwise to raise the front of the head. Keep turning it so that the tip is at least 3 mm (~30 turns) above the sample surface. Move the field of view to follow the probe's lateral motion.
- 2) Remove the head, turn it over, and place it on the shelf inside the enclosure.
- 3) Turn the Fiber Lite intensity all the way down until it clicks off.
- 4) Flip the key switch on the controller to turn the laser off.
- 5) Remove the probe holder from the head and remove the cantilever from the holder by reversing the instructions in Part 1.
- 6) Remove your sample from the stage. Leave the magnets in place.
- 7) Save your experiment if desired. [Do not save changes to shared experiments for general use. Mode Master templates can't be modified.]
- 8) Close the software.
- 9) Shut and latch the enclosure doors and (if necessary) windows.

Analysis

To begin image analysis, open the AFM Analysis menu in the menu bar. "Browse Saved Data" opens a thumbnail gallery of the most recent capture folder. Double-click an image to open it or navigate to the desired folder. You can also open saved files by dragging them from Windows Explorer into the Asylum software. [Do not merely double-click a file in Windows Explorer, as this will open the file in a standard version of Igor rather than the Asylum software.] The image will be displayed in a new window with X and Y scale bars and a color scale for the data. Each saved channel is available as a tab in the image window.

Analysis notes and tips:

- Controls at the top of the window allow you to change the image scale and color palette.
- The small buttons at the top-right of the window do the following:
 - 3D - Three-dimensional surface plot of the open data channel. Extensive controls allow customization of viewing angle, illumination, color palette, etc. This mode can also be accessed directly from the menu bar (AFM Analysis - 3D Surface Plots) and applied to an active image in real time. The 3D shape and the coloring can be derived from two different data channels if desired.
 - Ly - Lay-out, an Igor report template
 - L - List Panel
 - N - Note Panel, which lists all image/capture parameters
 - A - Analyze Panel, with tabs for Roughness, Section, Histogram, and Particle Analysis

functions

- M - Modify Panel, with various image modification functions. Note that the Mask tab allows masks (automated and/or manual) to be applied to the image so that the modify functions are only applied to certain areas of the image.
- D - Offline Image Manager
- Any Modify operation creates a new channel/tab in the image window. The image must be saved via the File menu in order to keep the new modified channel.
- The Commands drop-down menu allows access to these modules as well as others, including image exporting. File – Save Graphics allows a graphic (image or plot) to be exported as an image or a PDF file (Note – This does not work for 3D renderings, but those can be saved with the Windows Snipping Tool).
- The main Asylum manual does not include information on analysis, so the in-program help and Chapter 12 of the manualette are the best resources for more info in this area.