Microfibril Analysis

Dr Steve Barrett, Department of Physics, University of Liverpool, UK

Overview of MIASMA

MIASMA is Microscopy Image Analysis Software for Medical Applications, the collective name for a number of projects involving image analysis in which I am collaborating with medics. I am the author of software for image analysis of scanning microscopy images, principally for applications in nanoscience and related disciplines. The software that I have written, and continue to develop and expand, is *Image SXM*. Although written for scanning microscopy applications, I have found that *Image SXM* is an excellent platform on which to develop specialist image analysis solutions for the specific needs of users, including those who obtain images from light microscopes. *MIASMA* is the result of a number of these specialist applications having some common ground and so benefiting from being considered as part of a larger, overarching project.

Microfibril Analysis

Image SXM contains routines that have been written to speed up the processing and analysis of microfibril images. In the following pages the process by which microfibril beads are identified and the options available to the user when carrying out the analysis are explained.

For more information on MIASMA see the web page For help using the other functions of *Image SXM* see If you have any problems using *Image SXM*, email me http://www.liv.ac.uk/~sdb/MIASMA http://www.ImageSXM.org.uk S.D.Barrett @ liv.ac.uk



Overview of Microfibril Analysis

- The Microfibril Analysis (MFA) routines have been written for use with atomic force microscopy (AFM) images.
- Selecting MIASMA > Microfibril Analysis starts the analysis process.
- For each AFM image in each folder in the folder selected, the image is loaded with line-by-line compensation to ensure that the microfibril beads stand out against the background clutter. The image is inverted (dark beads on a light background) to make the beads easier to see.
- The user starts by clicking on a bead at the start of a microfibril. The MFA routine then identifies the bead predicted to be the next bead in the microfibril and circles it.



Left: Raw AFM image. Centre: Increased contrast after line-by-line compensation for variations in AFM scan. Right: Inverted for clarity. The image shows line segments between beads already selected and a circle around the bead predicted to be the next bead in the microfibril.

• The user then has a number of options:

Hit space bar to accept the suggested bead as the next bead in this microfibril Click on another bead if the suggested bead is not the correct one Hit the delete key to remove the last bead Hit the enter key to end the analysis of this image and continue to the next image Hit the escape key to abort the MFA analysis

- If either the suggested bead is accepted or another bead is clicked on, a line segment is drawn to this bead and the process of predicting the next bead and awaiting user input is repeated.
- For each bead, a number of results are calculated: the xy coordinates of the bead; the distance from the previous bead; and the angle formed with the two beads on either side.
- The results are printed to a text output file 'MFA-image-filename.txt'.

Menu Location

By default the 'Microfibril Analysis' menu item appears in the menu structure of *Image SXM* in a series of sub-menus:

Analyze > Specialist Analysis > MIASMA > Microfibril Analysis

Most users of *Image SXM* will not use this menu item and so it is tucked away where it will not get in anybody's way. Those of you who intend to use MFA extensively will probably prefer to have it available directly from the menu bar. If you press the option and control keys and select the MFA sub-menu you will find an extra item 'Move This Menu To Menu Bar'. This creates a new 'MIASMA' menu in the menu bar, which will appear every time you run *Image SXM* (on that Mac). If you want to move it back, repeat the process.

Appendix 1

History of changes to Microfibril Analysis routines in Image SXM

v1	Beta-testing of the MFA code (<i>Image SXM</i> v193 β)	Feb 2012
v2	Improved contrast of microfibrils by excluding salt crystals from line-by-line fit Added unsharp mask to improve discrimination of beads from background	18 Mar 2012
	Public release of <i>Image SXM</i> v193	28 Apr 2012
v3	Resize image window on smaller display (such as MacBook Pro) Finding beads now uses threshold that varies with image size	26 Feb 2013