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MetaMatters

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Inside this issue:

FOCUS: Quantifying Cell Motility Using IMA Through Journaling

George McNamara, Ph.D., Image Core Manager
Analytical Imaging Core Facility, University of Miami

In part one, I described making a panoramic movie of the classic timelapse film by David Rogers of a neutrophil chasing bacteria. In part two, I described the three tracking options, Track Points (manual), Track Objects (using threshold), Multi-Dimensional Motion Analysis (using threshold). Here, I discuss using the Integrated Morphometry Analysis (IMA) in a journal to measure a stack of images. Things you want to consider when using the IMA module in a journal are:

1. Pick your parameters – in the Measurements tab (Figure 1, left) and only pick the parameters that you are interested in seeing in your data tables or sending to Excel.
2. Pay attention to your preferences – IMA Preferences tab (Figure 1, right).
3. Test that the parameters make sense before logging your data.
4. You will need to record IMA into a journal in order to "loop for all planes".
5. Turn on separate object log, summary log and, if you want data histogram, data log. Be sure to open each to a separate log or some of your data will get overwritten, ex. "Object", "Summary", "Data" worksheets in Excel (alternatively, save to three different comma separated text files).

In part two, I explained how to use *Trace Region* and *Graphics – Paint Region* to paint the neutrophil intensity level 255 for easy tracking. For a uniform intensity object – especially one that you selected the intensity - the Average Intensity, Total Intensity, Intensity Center X and Y, and Radial Dispersion are not very interesting. When tracking fluorescent objects they are useful. If you are unfamiliar with any of these measurement parameters, select the IMA dialog and press the F1 function key, or press the question mark icon in the IMA window.

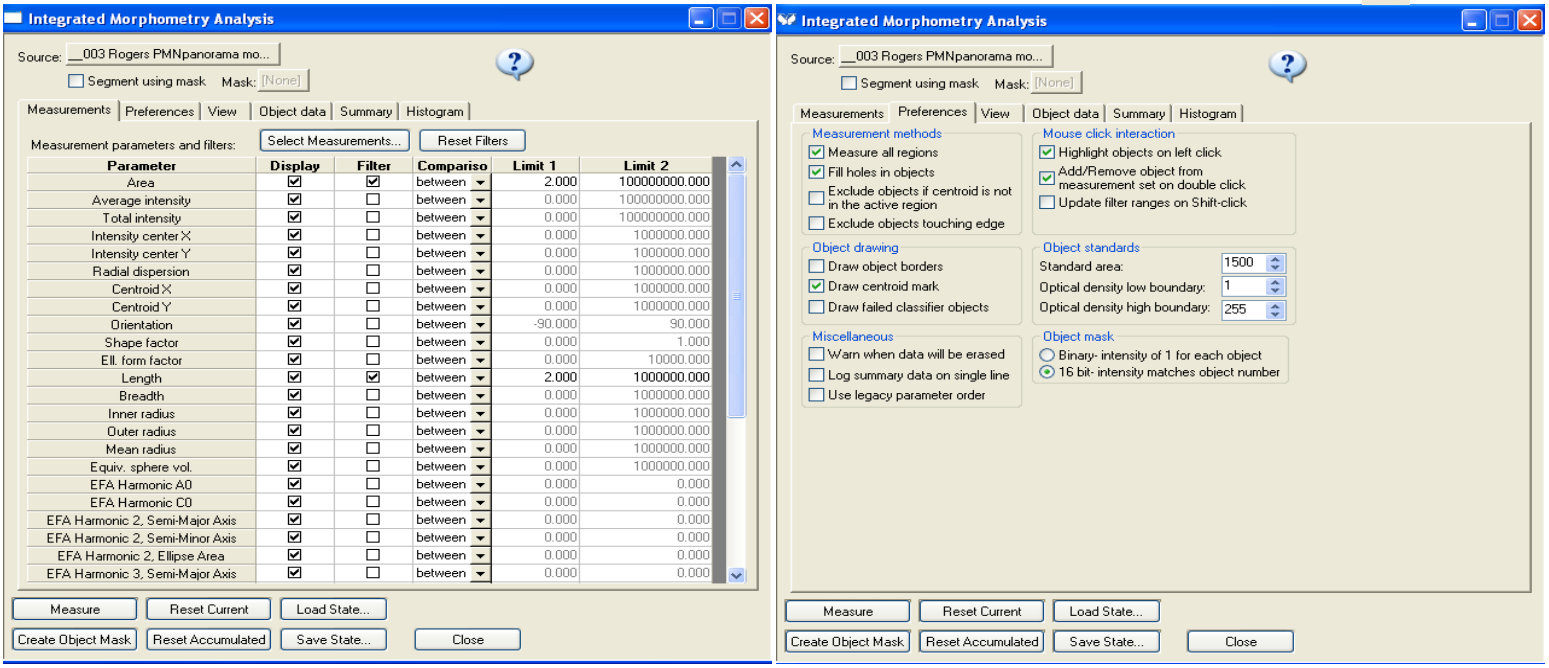

Figure 1. Integrated Morphometry Analysis (IMA) Measurements tab parameters list useful for cell motility, and Preferences tab.

To journalize IMA, have your image (stack) open and thresholded, then open the IMA window. Select from the *Journal menu - Start Recording*, and click *Measure* in the IMA window. If you want your data saved to a log, select the IMA window Object, Summary, or Histogram tab, and click *Record* (if the log file is not open), or *F9: log Data* (if the log file is open). You need to log each data type separately (and be sure not to put them in the same worksheet). To finish recording, select *Journal – Stop Recording*, then save the journal and optionally place it on the Taskbar (if you do not use journals and Taskbar(s), you are missing out on one of MetaMorph software's strengths).

(***continued on page 3***)

FOCUS: Quantifying Cell Motility Using IMA Through Journaling

(***continued from page 2***)

Figure 2 shows a screenshot from *Journal – Journal Editor* displaying two identical looking *Integrated Morphometry – Log Data*, commands (or you can also edit a journal by alt-clicking on its Taskbar button). The Command #3 is highlighted and in the Function Settings section (bottom right quadrant of window), the word SUMMARY appears. This is one way to see more details about each journal command. Another is to click on the Edit Functions Settings, which will bring up the IMA window and relevant tab. This journal is set to measure an image and log to both the Objects log and the Summary log.

To run the journal on the stack, have the stack ready to go (opened & thresholded), have the log(s) open, and choose from the *Journal menu: Loop – Loop for All Planes*, and select the IMA… journal that you just created. This will measure all the planes in the stack. If you prefer, you can leave the Log Data steps out of the journal, and log the accumulated data after the stack is measured by going to the Object Data or Summary tab, selecting *Display mode = Accumulated*, and pressing *F9: Log Data* yourself (I prefer logging current measurements so I do not need to remember to "Reset Accumulated").

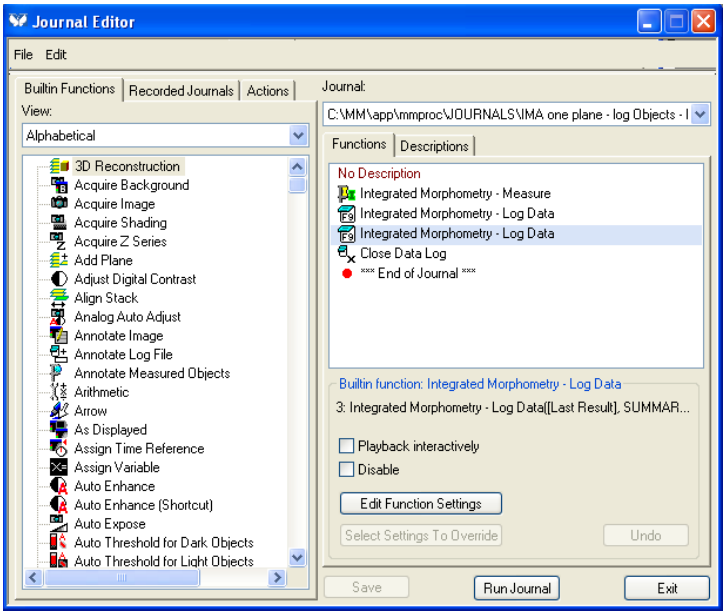

Figure 2. Journal Editor window showing "IMA – one plane – log Objects – log Summary" journal.

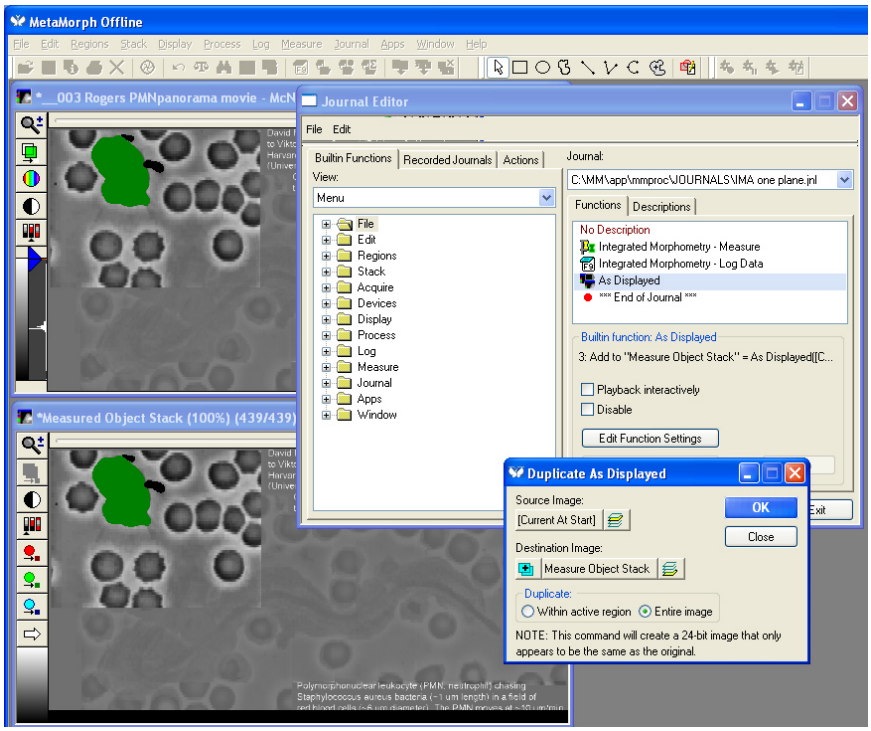

Figure 3. Measured Object Stack with journal editor and Duplicate As Displayed command.

One of my favorite MetaMorph capabilities is creating a new image of just the measured objects – optionally with *Edit menu – Preferences - Preferences tab – "Draw Centroid Mark"*. To create a new image with the IMA overlay, in a journal use, *Edit – Duplicate – Duplicate Image as Displayed*, specifying the "Current at Start" and "Current Plane" (not stack) as the source, with Destination Image "Add To" for the image selector, and "Measured Object Stack" as the specified name. (Figure 3) Optionally, a second journal can be used to record and save with *Journal – Loop for All Planes*. Using *Measure - Set Color Threshold*, set the measured object(s) to Red 0..0, Green 127..128, Blue 0..0, followed by *Process – Binary Operations, Binarize,* to create a binary image stack of the green measured object(s). Using the same steps, the centroid(s) can be set to Red 254..255, Green 254..255, Blue 254..255, followed by *Process – Binary Operations, Binarize,* to create a binary image stack of the centroid(s).

This concludes part 3. I hope you have a better understanding of how to journalize IMA and use journaling to automate object or cell measurements over time. Coming in part 4: Temporal Area Maps and more!

Molecular Devices

402 Boot Road
Downingtown, PA 19335

Phone: 800-635-5577
Fax: 610-873-5499
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