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# MetaMatters

## *NEW in MetaMorph® Software version 7.7.1…*

### *Support for:*

- *The new Hamamatsu ORCA®–Flash 2.8 camera with the Scientific CMOS Image Sensor FL-280!*
- *The Olympus Soft Imaging Solutions IX2-FCB controller*
- *The Hamamatsu C9300-221 high speed VGA interline CCD camera*
- *The Zeiss Definite Focus z-drift compensator*
- *The Sutter Lambda XL extended life illumination source*

### *Improved support for:*

- *Hamamatsu C9100-13 high dynamic range EMCCD camera*

*To download this update, you must already be at version 7.x and have a current Software Maintenance Plan. Go HERE to initiate download.*

*For MetaMorph Software support, contact our Technical Support at 800-635-5577 x1820 or email us at support.dtn@moldev.com*

*For more information, contact info@moldev.com*

MetaMorph Software Webinar Series:
"Performing FRET with MetaMorph Software"
August 10, 2010
10:30 AM, EDT

Recording will be posted to our website for those who cannot attend live.

*Look for your invitation in your email in-box!*

**Inside this issue:**



MetaTool Tips:

Update vs. Upgrade

- An update is available to accounts with current software maintenance and is only good within a single version (i.e. 7.1 » 7.7).
- An upgrade is availabe to all customers who wish to go from an older version to a newer version (i.e. 6.x » 7.x).

## *Amazing Software Incentives!*

Submit a 500 - 800 word MetaMorph® Software method and receive a FREE software upgrade or application module.

Submit a 100 - 200 word MetaMorph® Software tip and receive a FREE 12 month software maintenance agreement.

The method description and tip paragraph will be published in *MetaMatters*.

*The free MetaMorph® Software Basics Training Course is September 21 & 22, 2010!*

*Email: Mary.David@moldev.com for more information on incentive programs.*

*For training courses click here:*

*MetaMorph Software Training*

*The MetaMorph® Software Advanced Training Course is September 23 & 24, 2010!*

# FOCUS: Quantifying Cell Motility Using Tracking Applications

*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 bacterium. The movie was acquired using phase contrast microscopy – a great method for visualizing transparent cells but a nightmare for fully automated tracking. I added the "human touch" by using the Trace Region tool and Display – Graphics - Paint Region, intensity level 255, to paint the neutrophils white in every plane. Prior to painting, I first used Process – Arithmetic; subtract constant 10, to make sure only the pixels I painted would be intensity level 255. Here, I will compare different tracking applications in MetaMorph Software:

1. Track Points (manual)
2. Track Objects (using Threshold algorithm)
3. MultiDimensional Motion Analysis (using either of two algorithms)
4. Integrated Morphometry Analysis (IMA) / Morphometry - Measure Objects (MO)

I used the original panoramic stack with Track Points, after Log – Open Data Log to enable saving to Microsoft Excel 2007 by Dynamic Data Exchange. I attempted to track the "center of mass" (centroid in image analysis terms). *Figure 1* shows the three planes from the original data, overlaid with Track points performed three times. The overlay was made by using Process – Arithmetic, maximum, with 24-bit result between bitmap image saved from Track Points – Graph Data and an image made by copying/pasting the three planes with regions of interest. If I did perfectly reproducible tracking, only one color cross would be visible. The positions are not identical and in some areas, wanders more than others.

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

# FOCUS: MetaMorph® Software Method - Making a Panoramic Movie from a 16 mm film

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

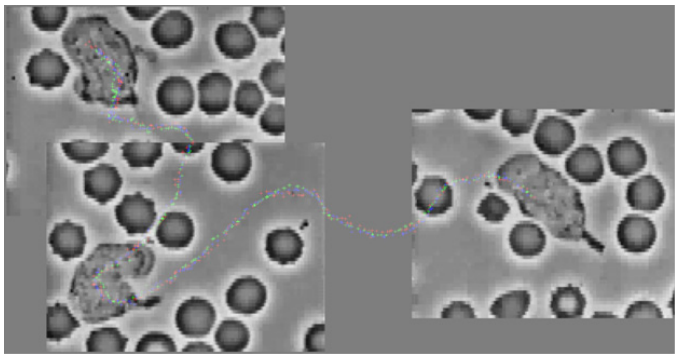

Figure 1. First (#1), middle (#220) and last (#439) planes merged into composite image, and overlaid with Track Points positions manually tracked three times (red, green, blue).

For Track Objects, I used Search Options–Algorithm = Threshold, with the tracking regions (40x40, 60x60 pixels) entirely inside the thresholded object in plane 1. *Figure 2* shows the path generated by Track Objects. The path is smoother than the manual Track Points method. Only one track is shown since multiple runs will generate the same result.

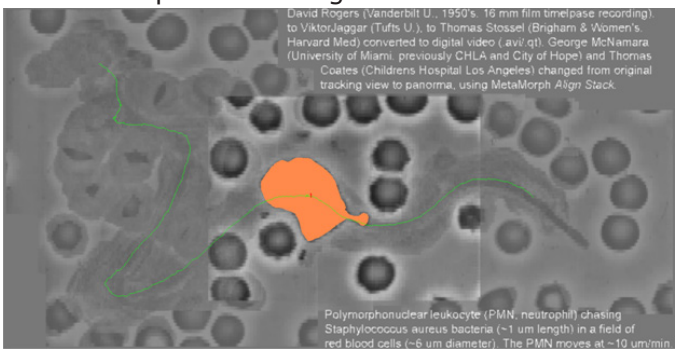


Figure 2. Panoramic movie plane #110 with threshold (orange) on for intensity 255 of the neutrophil; green trace is the Track Objects path, green X is the current position, and red "1" is Track Objects designating this as track 1.

MultiDimensional Motion Analysis has several algorithm options. The Threshold Result option and Template Match option use the Track Objects commands. The Auto-Find Objects algorithm, with simple threshold segmentation method, works fine with threshold (intensity 255) painted objects; though it is somewhat slower than Threshold Result. Auto-Find using the current IMA (Integrated Morphometry Analysis) morphometry setting is faster than simple threshold; though fine tuning settings is hardly needed if you have high contrast objects such as region painted cells as here.

The three Apps commands discussed above provide identical data display and graphing tools. If you do not need these tools, or do not have the trackobj drop-in, you can track using the morphometry commands. The Measure – Integrated Morphometry Analysis (IMA) and the Morphometry - Measure Objects (MO) are two interfaces to the same engine. When tracking objects with IMA/MO, I recommend having only one object per tracking cycle. If you are starting with more than one object present, you can often use Process – Binary Operations – Binarize, to make a binary stack, then Process – Stack Arithmetic, Maximum, to make a binary path image. If you need to set up to have only one object/track at one time, you can use a region of interest with Paint Region, intensity level 0, to black out all put one path or part of one path (Figure 3). You can then use the Process – Arithmetic, Logical And (stack all planes, binary path mask), to blackout everything but the object of immediate interest.


Figure 3. Complete binary path (left), Paint Region dialog box, and path truncated by blacking out part.

Once you have your one object stack, look closely at the IMA Measurements tab to make sure your parameter limits are usable. You may also want to enable more (or fewer!) parameters, by using the Select Measurements button.

In part 3, I will discuss IMA/MO in more detail. Here I just summarize the key points:

1. Pick your parameters.
2. Pay attention to Preferences.
3. Test the parameters make sense.
4. You will need to record IMA or MO into a journal in order to "loop for all planes".
5. Open log files to separate Excel worksheet tabs (or text files).

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San Diego, CA

DECEMBER 11 – 15, 2010

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