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MetaMatters

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Inside this issue:

FOCUS: Utilizing Temporal Area Maps to Trace an Object Path

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). In part three I discussed Measure – Integrated Morphometry Analysis (IMA) / Morphometry - Measure Objects (MO). Figure 1 shows the first plane of the panorama, with the cell thresholded (orange overlay) after I had subtracted 10 intensity levels, then painted the cell intensity level 255.

In this final installment I show how to make an outline of the path, and then how to create and use a quantitative image map of how long a cell covers each part of its path.

Using Process – Binary Operations, Binarize, low= 254, high=255, all planes, I now have a binary stack. The entire path can be obtained using Process – Stack Arithmetic – Maximum (see part 2, Figure 3, for image). The Binary - Outline, all planes, then Stack Arithmetic – Maximum, provides a different view (Figure 2).

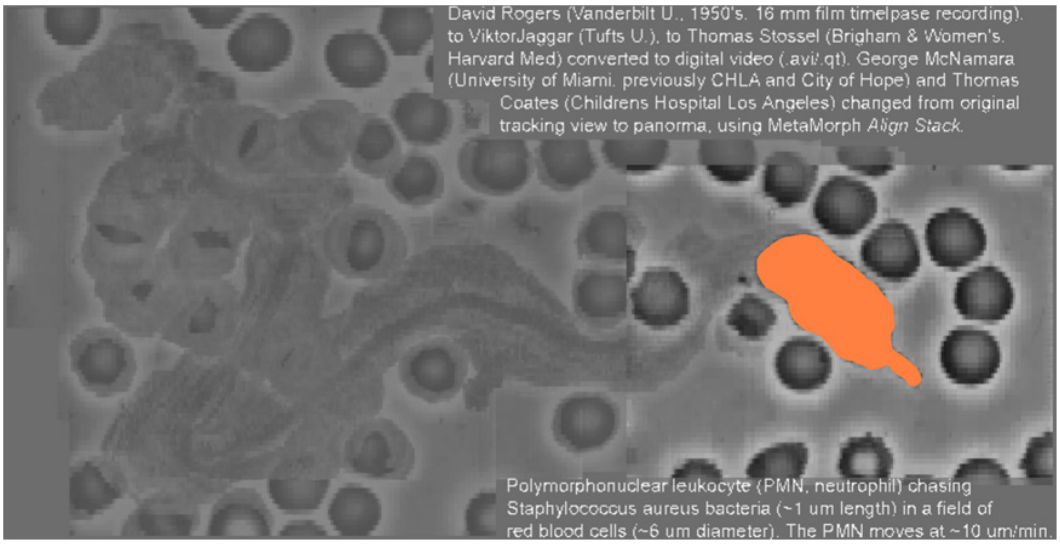

Figure 1. First plane of panoramic movie with cell thresholded orange.

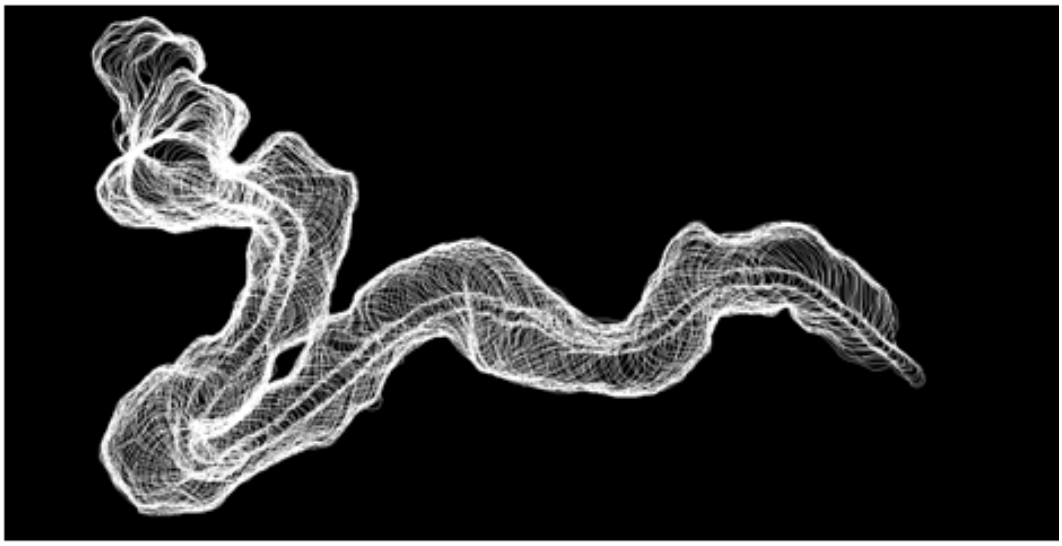

Figure 2. Binary outline stack arithmetic maximum, of neutrophil.

However, a problem with outlines from multiple time points is they overlap. Especially where the cell slows down, outline pileup presents a problem: how long did the cell spend at that location? Robert Futrelle and I came up with a solution, a temporal area map (TAM), that is very easy to do in MetaMorph using Stack Arithmetic: Sum of the binary stack.

To generate Figure 3, an intensity scale bar was made with Display – Graphics – Gray Wedge (starting=140, ending=0), the numbers 0 and 140 were added using Display – Graphics – Text, a pseudocolor lookup table (LUT) was selected from the image window toolbar, and Edit – Duplicate – As Displayed, was used to convert to 24-bit image for publication (the original 16-bit image was also saved).

The complete temporal area map (TAM) in Figure 3 shows the entire history of the neutrophil and how long it covered each pixel. Figure 4 shows the partial TAM's of the first and last segments, of 100 time points (MetaMorph planes) each.

(continued on page 3)

FOCUS: Utilizing Temporal Area Maps to Trace an Object Path

(continued from page 2)

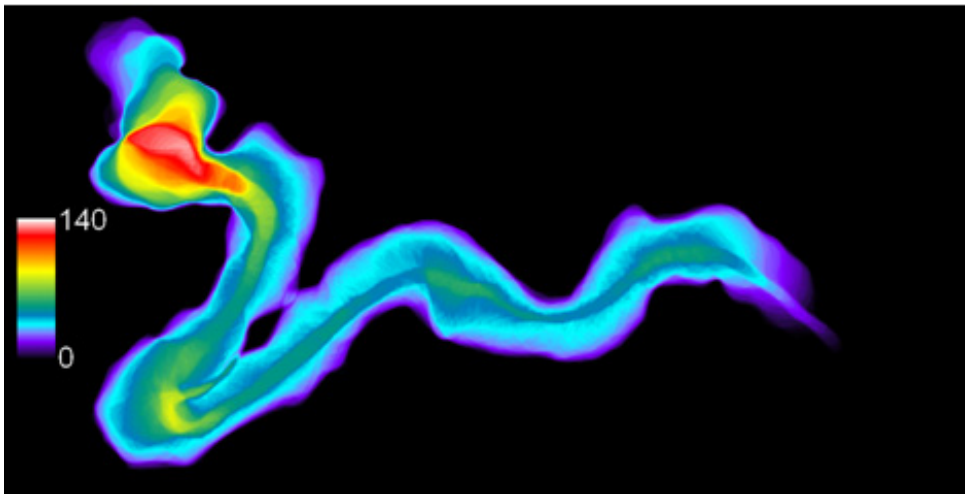

Figure 3. Temporal area map (TAM) of panoramic neutrophil. Maximum value is intensity level 134 (red area near top left).

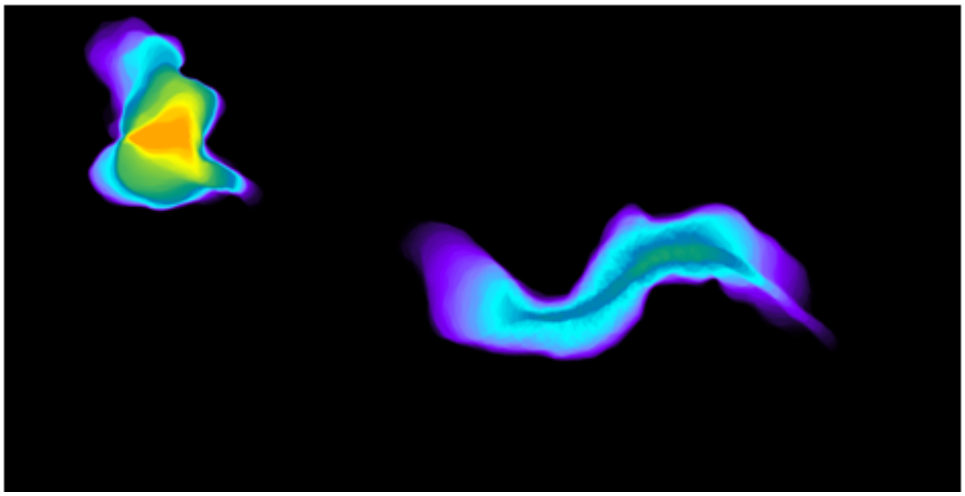

Figure 4. Temporal area map (TAM) of panoramic neutrophils, first 100 planes (right side, fast moving) and last 100 planes (left side, slow moving). Maximum value is intensity level 100 (orange area near top left). See Figure 3 for time coverage intensity wedge.

Figure 5 shows the temporal area map histograms (TAMgrams). It may appear surprising that the mode 100 value of the last 100 planes (bottom panel) does not appear as a spike in the "all planes" (top panel), but this is because the cell moved into the stationary spot and the values are distributed over a large range of the top panel (the vertical axes are different in all three panels – this could have been handled by graphing in Excel but I wanted to include the intensity color wedge in the histograms).

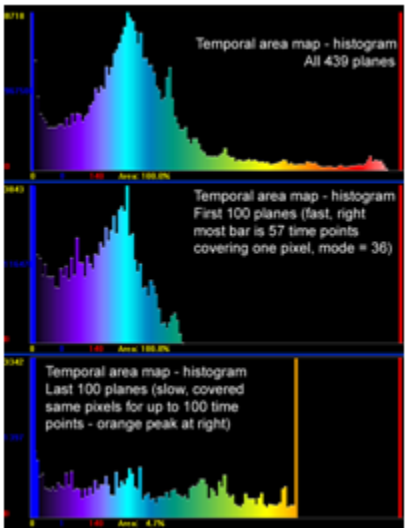

Figure 5. Temporal area map histograms of all planes (top, see Figure 3), first 100 planes (middle, see Figure 4), and last 100 planes (bottom, Figure 4 left side).

The cell makes a greater net displacement during the first 100 time points, than during the last 100 time points. This is apparent from the movie, but hard to show a movie in a printed page! The TAM images are useful in their own right – they give an instant overview of the history of the cell coverage. With 3D datasets, for example, fluorescence confocal (or nanoscope) volume data, TAM's can become TVM's – temporal volume maps. With fluorescent protein fusions, for example, GFP-myosin II, the TAM (or TVM) can be related to the time history of a cytoskeletal element. Any functional reporter, whether the classic Fura-2 calcium reporter or the latest FP biosensor, can also be related to dynamic cell morphology.

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