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Rogers PMN Movie - Background Information

George McNamara

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George McNamara, Mary David, Tom Stossel, Tom Coates 2010 Rogers PMN neutrophil chasing bacteria panorama movie – text 20100920M – updated with new web links 20120203F

I am posting downloadable content on my http://works.bepress.com/gmcnamara web site.

Original video and text from http://www.biochemweb.org/neutrophil.shtml

This video is taken from a 16-mm movie made in the 1950s by the late David Rogers at Vanderbilt University. It was given to me via Dr. Victor Najjar, Professor Emeritus at Tufts University Medical School and a former colleague of Rogers. It depicts a human polymorphonuclear leukocyte (neutrophil) on a blood film, crawling among red blood cells, notable for their dark color and principally spherical shape. The neutrophil is "chasing" *Staphylococcus aureus* microorganisms, added to the film. The chemoattractant derived from the microbe is unclear but may be complement fragment C5a, generated by the interaction of antibodies in the blood serum with the complement cascade, and/or bacterial *N*-formyl peptides. Blood platelets adherent to the underlying glass are also visible. Notable is the characteristic asymmetric shape of the crawling neutrophil with an organelle-excluding leading lamella and a narrowing at the opposite end culminating in a "tail" that the cell appears to drag along. Contraction waves are visible along the surface of the moving cell as it moves forward in a gliding fashion. As the neutrophil relentlessly pursues the microbe it ignores the red cells and platelets. However, its leading edge is sufficiently stiff (elastic) to deform and displace the red cells it bumps into. The internal contents of the neutrophil also move, and granule motion is particularly dynamic near the leading edge. These granules only approach the cell surface membrane when the cell changes direction and redistributes its peripheral "gel." After the neutrophil has engulfed the bacterium, note that the cell's movements become somewhat more jerky, and that it begins to extend more spherical surface projections. These bleb-like protruberances resemble the blebs that form constitutively in the M2 melanoma cells missing the actin filament crosslinking protein filamin-1 (ABP-280) and may be telling us something about the mechanism of membrane protrusion.

Thomas P. Stossel (Brigham and Women's Hospital and Harvard Medical School), June 22, 1999.

This movie is in QuickTime format and was digitized from the analog footage and made available on the Internet by Philip G. Allen (Boston University) and Gabriel Fenteany (University of Connecticut). The movie can also be viewed in compressed form as an MPEG-4 or a WMV file.

Title: Thomas P. Stossel, Thomas D. Coates, George McNamara 2010 David Rogers 1950s panorama movie of human polymorphonuclear leukocyte (PMN, neutrophil) chasing 3 bacteria.

Mary David of Molecular Devices / Universal Imaging Corp, www.moldev.com, submitted this video for us for ASCB CellDance 2010

http://ascb.ascb.org/meetings/celldance_winners.cfm

http://ascb.org/index.php?option=com_content&view=article&id=761&Itemid=305

http://ascb.org/files/Past-AM-Meetings/2010-Celldance/Rogers_PMN.swf

The original 16mm film and digitized video (first movie) jumped between fields of view to follow the neutrophil. The new video panorama reveals the entire chase and the presence of 3 bacteria. Four video segments are displayed – the panorama, panorama with outlines, topographic surface map (TSM), and temporal area map (TAM). The TSM shows when the neutrophil first covered a pixel. The TAM, a quantitative map of how much time the neutrophil covered a pixel. TAM was developed by R.P. Futrelle and G. McNamara in 1985, inspired by the dshape analysis of Verschueren and Van Larebeke (1984).

The use of temporal area map histograms (not shown) enables comparison of a cell, or populations of cells, over time, e.g. before and after stimuli such as application of a chemoattractant, chemokinetic, or phagocytosis.

Magnification: the red blood cells are ~8 um diameter. The original 16mm movie film and video digitized by T.P. Stossel, P.G. Allen and G. Fenteany (1999) did not have scale bars. Human red blood cell diameter is ~8 um (Puig-de-Morales-Marinkovic 2007; www.bionumbers.org), presuming healthy adult human (e.g. D. Rogers). Some illnesses can affect RBC size (Aarts et al 1983).

Comments: Full details are in McNamara (2010a, 2010b, 2010c, 2010d). The Stossel et al (1999) QuickTime movie was exported to 24-bit TIFF image files. Images were opened in MetaMorph as a stack and the Color Separate command was used to separate the green channel (all three channels were identical). A large gray level zero image was created to use as a template for the panorama and made into a 439 plane stack. All planes of the 8-bit stack were copy/pasted into the template. The MetaMorph *Align Stack* was used to align the planes. Arithmetic subtract 5 intensity levels was used to make sure no intensity level 255 pixels were present. Threshold image – Clip to gray level 109 was used to make the background match the video. Draw text was used to add white text. The stack was saved as segment 1. A MetaMorph active region of interest and Duplicate Stack was used to trim the panorama stack. The neutrophil was traced in each image plane using a Trace region of interest and Paint Region 255 was applied. The Binary operation Binarize stack, intensity 255, was used to create a binary stack. The Binary operation Outline stack was used to create the outline, this was dilated one time (3 pixels wide), Arithmetic Maximum (Segment 1, Outline), was used to combine the first segment and the outline, saved, and used as segment 2. The binary stack was converted to 8-bit with the cell at intensity level one (background zero), and topographic surface map created. The MetaMorph standard pseudocolor lookup table (LUT) was applied to the topographic surface map image and duplicated as displayed to 24-bit color. The 3-pixel wide dilated Outline stack was merged with the topographic surface map image using Arithmetic, and saved as segment 3. The binary 8-bit intensity level one stack was processed using Stack Arithmetic Sum, and saved as a 16-bit quantitative temporal area map. The MetaMorph pseudocolor LUT was applied, duplicated as displayed to 24-bit color, the 3-pixel wide dilated Outline stack was merged, and the temporal area map with 3-pixel outline was saved as segment 4. The four segments were merged using Add plane. The stack is 1,633,906 Kb (1.6 Gb) and is available from Dr. McNamara along with the topographic surface map and 16-bit temporal area map. The stack was exported as TIFF images (100% quality), imported into Apple QuickTime Pro using Open Image Sequence, and saved as a 16 Mb QuickTime movie using H.264 compression, 30 fps.

References

Aarts PA, Bolhuis PA, Sakariassen KS, Heethaar RM, Sixma JJ 1983 Red blood cell size is important for adherence of blood platelets to artery subendothelium. *Blood* 62: 214-217. PubMed PMID: 6860793.

McNamara G 2010a MetaMorph Software Method: Part 1 - Making a Panoramic Movie from a 16 mm film. *MetaMatters* 2(3): 2-3.

McNamara G 2010b MetaMorph Software Method: Part 2 - Quantifying Cell Motility Using Tracking Applications. *MetaMatters* 2(4): 2-3.

McNamara G 2010c MetaMorph Software Method: Part 3 - Rogers Neutrophil PMN Quantifying cell motility using morphometry IMA Through Journaling. *MetaMatters* 2(5): 2-3.

McNamara G 2010d MetaMorph Software Method: Part 4 - Temporal Area Maps and more. *MetaMatters* 2(6): 2-3.

Puig-de-Morales-Marinkovic M, Turner KT, Butler JP, Fredberg JJ, Suresh S 2007 Viscoelasticity of the human red blood cell. *Am J Physiol Cell Physiol* 293: C597-c605. PubMed PMID: 17428838.

Stossel TP, Allen PG, Fenteany G (1999) Crawling neutrophil chasing a bacterium. http://www.biochemweb.org/neutrophil.shtml

Verschueren H, Van Larebeke N 1984 A new model for the quantitative analysis of cell movements in vitro: definition of a shape change factor. *Cytometry* 5: 557-561.

Note: MetaMorph MetaMatters newsletters are available online at (web links current as of 20120203F):

http://mdc.custhelp.com/app/answers/detail/a_id/18689/~/metamatters-newsletters

MetaMatters Volume 2

MetaMatters Volume 2 Issue 6 (November 2010)
Highlight of this issue:
Utilizing Temporal Area Maps to Trace an Object Path

MetaMatters Volume 2 Issue 5 (September 2010)
Highlight of this issue:
Quantifying Cell Motility Using Morphometry IMA

MetaMatters Volume 2 Issue 4 (July 2010)
Highlight of this issue:
Quantifying Cell Motility Using Tracking Applications

MetaMatters Volume 2 Issue 3 (May 2010)
Highlight of this issue:
Making a Panoramic Movie From 16 mm Film Using Stack Align

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