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MetaTool Tips: Journaling Shortcuts



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Recording
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Pause
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From the Desk of Chris Kier

*The MetaMorph®
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To efficiently utilize our support and development resources we will be curtailing support for MetaMorph® software versions earlier than version 6. There are two main reasons for this decision:

- The last update for version 5 was issued more than seven years ago, and the sheer numbers of changes to the software make it difficult for our staff to possess the knowledge base to sufficiently support these older versions.
- Windows operating systems compatible with early versions of MetaMorph software are no longer available for purchase, and Microsoft no longer supports these older operating systems.

Existing information, application, and technical notes for these earlier versions of MetaMorph software will remain on our web site. We encourage all of our customers to upgrade to the current version of MetaMorph software and benefit from the additions and improvements that have been made to the package over the last several years. **To make this transition easier, we will be offering upgrades from all earlier versions of MetaMorph Premier software for \$2000.** This is a 20% discount from list price. Orders can be placed directly with MDS Analytical Technologies or through any of our partners. A list of our distribution partners and details of the latest version 7 release of MetaMorph software can be found on our web site, link information is located on the back page of this issue.

This discount is applicable on one system per customer.

FOCUS: Multidimensional Cell Tracking with MetaMorph® Software

by Srikanth Polusani and Edward Kalmykov

*Department of Biochemistry, Dr. Bruce Nicholsons laboratory
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Directed cell migration affects many aspects of life, from embryonic development to proper functioning of the immune system. Failure of cells to migrate, or migration of cells to inappropriate locations, can result in life threatening consequences. Cell motility is central to homeostatic processes such as mounting an effective immune response and the repair of injured tissues. Furthermore, it contributes to pathologies including vascular disease, chronic inflammatory diseases, tumor formation and metastasis leading to cancer propagation. Understanding the mechanisms underlying cell migration is also important to emerging areas of biotechnology, which focus on cellular transplantation, and the manufacture of artificial tissues, as well as for the development of new therapeutic strategies for controlling invasive tumor cells.

Cell tracking with MetaMorph software using the “multidimensional cell tracking” application module is not only quick and reliable, but also less tedious and less cumbersome than the single cell track feature. We have used the multidimensional tracking module to compare the rates of migration, after scrape wounding, of co-cultured cells labeled with different fluorescent lipid soluble carbocyanide dyes eg. Red fluorescent DiI (1,1',di-octadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) and green fluorescent DiO (3,3'-dioladecyloxycarbocyanine perchlorate) (Invitrogen).

(continued on page 3)

FOCUS: Multidimensional Cell Tracking with MetaMorph® Software

(continued from page 2)

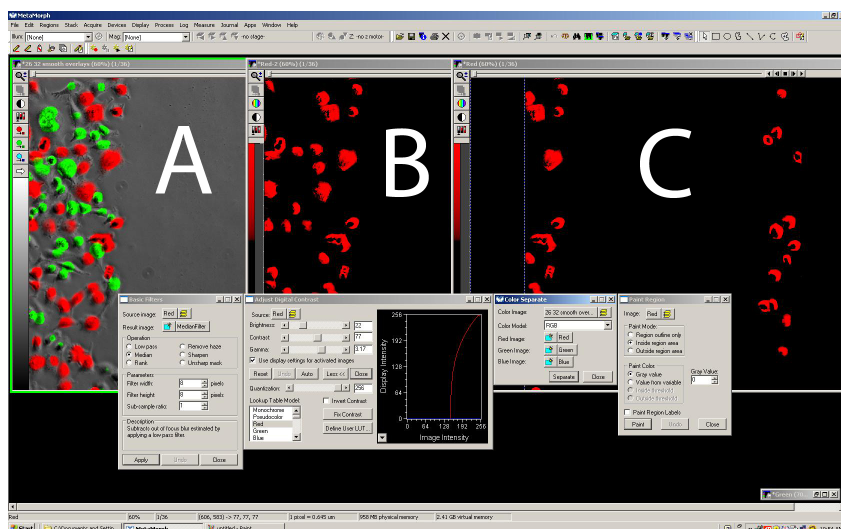


Figure 1

the color combined stack was composed of 24 bit color images, but the module requires them to be 16 bit, which is achieved using this feature. After parsing the stacks into separate colors, the blue one was deleted since it was not needed. To aid in cell selection at later stages, the images were scaled visually to see just the cells in each channel without any underlying background (Fig 1 B). Because we were only interested in tracking cells at the wound edge, we deleted all outlying cells by creating a region on both sides of the image away from the wound and masking this part of the image by going to “display,” “graphics,” and “paint region,” with the gray value set to “0” (Fig 1 C).

After these steps were completed, we opened the “multidimensional tracking” module from the “apps” menu and selected one of the stacks, in this example the red one. In order to set up the tracking criteria, we opened “search options”, selected “adaptive threshold”, and entered empirically determined values for the user-input fields such as the minimum and maximum X and Y cell dimensions and the intensity threshold above background to ensure capture of all cells (Fig 2 A). These values were obtained using the line region tool and mouse-over intensity values found at the bottom of the screen. Although we were not able to select all the cells in the field, our increased stringency allowed for better tracks of the positively identified cells, to ensure optimal quality by eliminating any potential for including non-cellular artifacts (Fig 2 B). The positive identification of cells was possible using the “preview” feature after entering all parameters for tracking. Once we were satisfied with the cells to track, we hit the “start-tracking” button. After the tracking was completed, we logged the data into Excel for quantitative analysis (Fig 2 C).

The accuracy and speed of this module made it a very favorable tool for us to use in the motion tracking analysis of our co-cultured cells, reducing work-load to a single image processing event and providing unbiased quantitative measurements, which was not possible when tracking the cells individually by the user. This allowed quantitative and statistically significant comparisons of the effects of different treatment in the rate and directionality of cell motility in a wound healing culture model. The same basic approach has also been adapted to following migration rates in sparse cultures.

Using this app module, we have determined the velocity of migration, distance travelled in a defined direction (X - perpendicular to the wound), and directionality (X/Y - ratio of distance travelled perpendicular and parallel to wound) of each different cell type in the co-culture. Briefly, after scrape-wounding the monolayer, we took images of migrating cells over time (1 image/6 minutes) for 48 hrs and created a user-defined time-lapse stack to show the movement after overlaying the different colors using the “overlay images” feature (Fig 1 A). Before using the multidimensional cell-tracking app, we used filters such as “median” and “top hat” to smooth cells and fill in the empty spaces where the cells were not completely labeled with the dyes. Next, we made separate stacks of the red and green channels using the “color separate” (RGB) feature from the “display” menu (Fig 1 B). This was necessary because

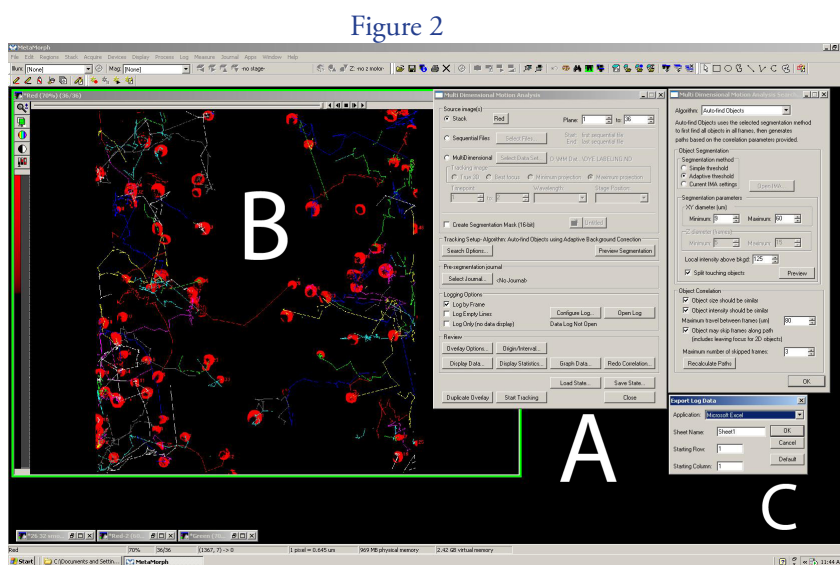


Figure 2

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About MetaMorph® Software MetaMorph® software is the leading, world-class image acquisition and analysis software. Combining the most flexible and powerful tools for image acquisition, processing, and analysis, MetaMorph® software offers a complete solution for even the most demanding live-cell imaging needs.

About MDS Analytical Technologies

MDS Analytical Technologies, a business unit of MDS Inc., is focused on the research, design, manufacture and marketing of state-of-the-art tools for mass-spectrometry, drug discovery and bioresearch. MDS Analytical Technologies' products are designed to help accelerate the complex process of discovering and developing new drug compounds, and are sold to research scientists around the world. The mass-spectrometer product lines are also sold globally through joint ventures with two of the world's leading analytical instrumentation and life sciences companies, Applied Biosystems, Inc. and PerkinElmer, Inc. Find out more at www.mdssciex.com or www.moleculardevices.com.

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