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MetaMatters

FOCUS: Tracking Migrating Cells with MetaMorph® Software

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The "scratch assay" or "wound-healing assay" is a commonly used method for measuring motility rates of cells in vitro. However, use of this method assumes several conditions which often are not true. For instance, cells frequently do not move uniformly. Tracking the actual paths of multiple single cells in motility assays provides a much more accurate assessment of cell migratory behavior. We have found that MetaMorph® software can be used successfully 1) for control of our automated microscope, 2) for acquisition of time-lapse images from multiple areas of interest in parallel and 3) for tracking of migrating cells in a highly quantitative manner[1]. This article outlines two separate methods that use MetaMorph® software to track the paths and velocities of migrating cells from sequential time-lapse images of cells from a scratch in either a confluent monolayer or sparsely plated cells. It is assumed that sequential control and experimental images have already been acquired and saved.

In the MetaMorph® program, press Ctrl-Q. This will bring up the "Build Stack: Quick" window. Find the folder in which your images are saved and select the first image of your time-lapse series. Click "Open". MetaMorph® software will now load all of the images in that series and organize them, in numerical order, into a stack of images. The stack of images can then be saved for later use by choosing "File", "Save As" and renaming the stack appropriately as either a MetaMorph multi-plane TIFF or in MetaMorph Stack File Format.

At this point, either "Track Points" or "Track Objects" can be performed. So far, these applications have been aimed at tracking subcellular particles, but they work equally well for tracking cells. Track Points must be used when it is necessary to follow the migrating cells manually because they are part of a group with no clearly discernable edges, such as confluent cells along a scratch edge.

To use Track Points, click on the "Apps" menu and choose the "Track Points" application. In the new dialog box, click on the "Add Track" button at the top to begin tracking. Once you click this, you will then either: (FOCUS *continues on page 3*)

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MetaMorph® software is available through our distributors: www.moleculardevices.com/pages/distributors.

FOCUS: Tracking Migrating Cells with MetaMorph® Software

(continued from page 1)

(1) See the images automatically progress as you continue clicking on a specific point in the target cell (e.g. subcellular organelle such as the nucleolus)
(2) Advance the images by holding the cursor over the specific point while depressing "5" continuously on the number pad.

Either way, once you have worked through all of the images, it will reset. You can now press "Add Track" again for the next cell. Only track those cells that remain in view throughout the experiment. If a cell divides, you can choose one of the daughter cells and track it for the remainder of the images. In general, we track about 15 to 20 cells in each stack of images. But, the more the better! To see the paths that the cells took, click on "Set Overlay". In the next dialog box, check the "Display all Track Points" and "Display Track Path" boxes and click OK. This will give you the cell paths (red) on the image (see Fig. 1 below). To save the image, right click on the image and then click the option "Copy to Clipboard". Then, the image can be pasted into another application for use in publications or presentations. Once all cells have been tracked, click on "Open Log". A dialog will open asking where to log measurements. Make certain Dynamic Data Exchange (DDE) is selected and click OK. Another window will open up asking where to export the data – here, give a name to the log and decide which application to use, usually Microsoft Excel. Then, click OK. This will open an Excel worksheet. The button that said "Open Log" will now say "F9:Log Data". Click this button. This will log all of the data into the spreadsheet. Once the logging is finished, the file can be saved into the folder of your choice.

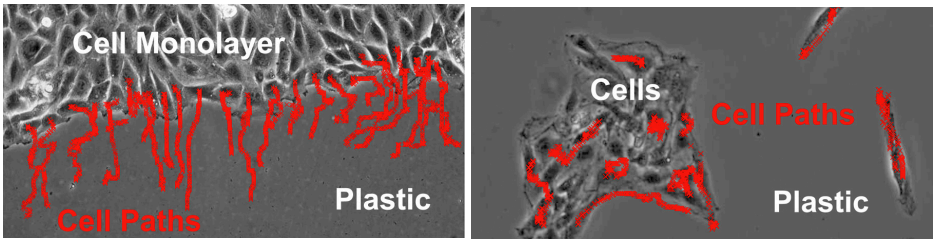

Fig. 1 (Left): A scratch was made in a cell monolayer to produce a denuded area into which cells could migrate. Time-lapse phase contrast images were acquired every 5 minutes, and cells along the scratch edge were tracked using Track Points, with nucleoli as targets. The red lines are the series of red "Xs" of cell position in each frame, thus showing their paths during the experiment. Velocities over time can be plotted for each cell and for the population from the tabulated data. (Right): Cells plated at a lower density and tracked for random migration patterns using Track Points as described for the left panel.

The Track Objects function can be used to easily track fluorescently labeled cells plated at low density, where there is high contrast between the labeled cell and the darker background, even when the cells are plated on top of another cell monolayer[1]. To use "Track Objects," click on the "Apps" menu and choose the "Track Objects" application. Click on the "Track" button. Next, press and hold down the "Ctrl" key and proceed to click on the cell of interest. A box containing a smaller box will appear. Click OK. The MetaMorph® software will now automatically track the cell. The inner box is called the "Object Box" and is used to contain the object that the user wishes to track. It distinguishes objects via contrast and works for either light objects on a dark background or dark objects on a light background. The outer box is the "Search Box" and is used to search for the selected object in the "Object box" in the event that the MetaMorph® program can no longer find what was contained in the "Object Box". Once found, the program will reorient the boxes around the object and continue tracking. If not found, the tracking will stop and the user can either reposition the box manually, skip the frame, or stop the tracking and start over. Both boxes can be resized, however the "Object Box" must be smaller and inside the "Search Box". Below is an example showing the Object and Search boxes and the tracked paths of 2 cells. Once all cells have been tracked, click on "Open Log" and log data as described above for Track Points. Graphs of cell velocities can then be plotted from tabulated data.

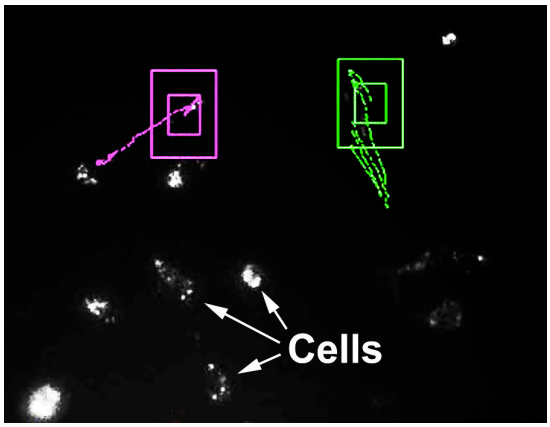

Fig. 2: Cells were labeled in suspension with a fluorescent membrane dye (DiO) and plated at low density. By the next day, the dye was internalized and appeared as bright clustered intracellular puncta. Fluorescent time-lapse images were collected as described[1] and cells were tracked using Track Objects. The double Search and Object Boxes are shown around 2 cells that were tracked. Also shown are the paths of migration that the cells took during the course of the experiment. Cells labeled with DiO photobleach somewhat over the course of the experiment[1], however contrast is sufficient for the Track Objects boxes to accurately follow the cells.

[1] Fotos, J.S., V.P. Patel, N.J. Karin, M.K. Temburni, J.T. Koh, and D.S. Galileo (2006). Automated time-lapse microscopy and high-resolution tracking of cell migration. Cytotechnology, 51(1), 7-19.

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