AppliedPrecision®

Product Note

DeltaVision®

Integrated Cell Tracking

INTRODUCTION

Live cell research requires researchers to follow cells through time-lapse experiments that can last anywhere from a few minutes to several weeks. During live cell experiments, cells move continuously. Until recently, in order to keep the cell structure or region of interest (ROI) within the field of view, researchers were required to track cell movement by manually adjusting the position of the microscope stage throughout the experiment.

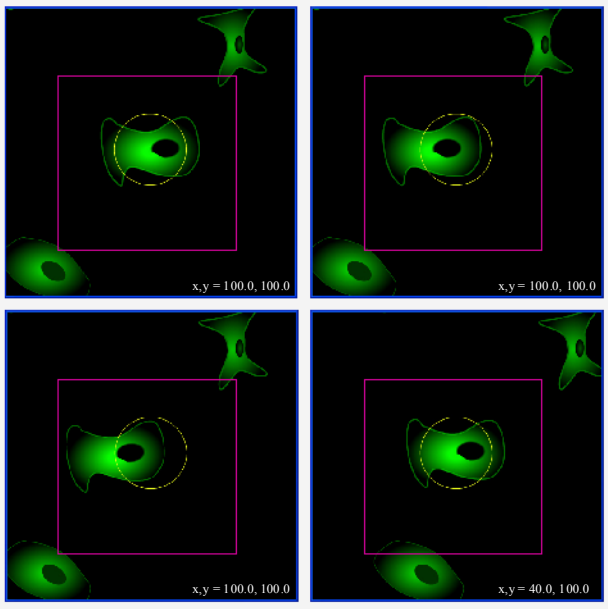

Figure 1. Live Cell Tracking
At Time Points 1 and 2, the cell is moving through the ROI (pink square), but the center is still within the Move Threshold (white circle). At Time Point 3, once the center of the cell reaches the threshold boundary, the stage moves to re-center the cell within the ROI. Note the new stage position displayed in the lower-right corner of Time Point 4.

The Integrated Cell Tracking feature of the DeltaVision High Resolution Imaging Systems automatically moves the stage to follow cells as they move during a time-lapse experiment. The user specifies an ROI around an object of interest. The software then determines the center of the cell and establishes a recognizable pattern within the ROI. In subsequent images, the software recognizes this pattern and recalculates the center of the cell. The position of the new center is compared with the position of the previous center and, if the cell has moved beyond a specified threshold, the system automatically moves the stage and re-centers the cell.

DeltaVision allows you to adjust the following cell tracking parameters:

- Tracking Method
- Threshold
- Reference Channel
- ROI Percent

TRACKING METHOD

The user chooses from two tracking methods when determining the center of the cell, as shown in Figure 2. The Center of Intensity method calculates the center based on signal intensity values, while the Center of Geometry method determines the center of the cell based on its shape.

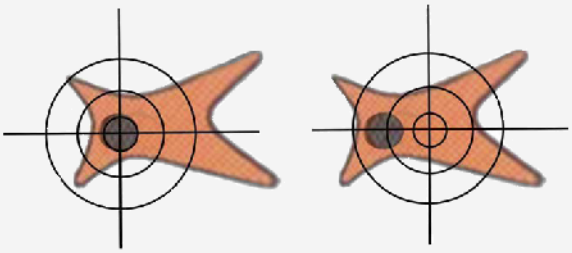

Figure 2. Live Cell Tracking Methods

REFERENCE CHANNEL

For image recognition, the user defines a single reference channel. For experiments that require data acquired from more than one channel, the user selects the channel that shows the most distinctive features.

MOVE THRESHOLD

The Move Threshold is the distance the center of the cell (as defined by the tracking method) must travel before the system resets the stage. When the cell moves beyond this threshold, the stage moves so that the center of the cell retains its original position in relationship to the center of the field of view.

By their very nature live cells are constantly on the move. When choosing a Move Threshold, it is important to select a region that is small enough to keep the cell in the field-of-view, yet large enough to buffer out stage movement that could result from numerous small cell movements.

ROI PERCENT

With rare exceptions, few live cells are loners. Most cells attach to and communicate with other cells. When performing live cell microscopy, it is important to define an appropriate region of interest within the field of view. This region of interest must be large enough to include the entire cell or structure of interest, yet small enough to exclude other cells or structures that may wander in and out of the field of view during the experiment.

The ROI Percent parameter defines how much of the field-of-view the software will use for image recognition. In the software, ROI units are specified as a percentage of the width of the field of view. For example, a 50% ROI has a width that is 50% of the width of the field-of-view. Any cell within that 50% ROI will be tracked. Cells outside that 50% ROI will be ignored (Figure 3).

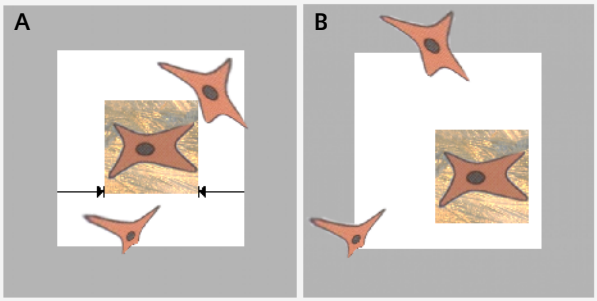

Figure 3. Specifying an appropriate ROI Percent
A) The width of the ROI is defined as 50% of the width of the total field of view (white square).
B) The stage has moved to keep the cell of interest within the ROI.Perimeter cells have also moved, almost entirely out of the field of view.

The automatic Cell Tracking feature of DeltaVision RT liberates researchers from the need to "baby-sit" lengthy, time-lapse experiments and frees up time for other research tasks.

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