**TN455: What’s New in Volocity 5.5?**

**Topic**

This is a description of the major changes in Volocity 5.5 and contains guidance for users upgrading from a recent, previous version. For a full list of all the changes in Volocity 5.5 please see the release notes (available from [http://www.cellularimaging.com/downloads/release_notes/VolocityNotes.html](http://www.cellularimaging.com/downloads/release_notes/VolocityNotes.html)). For information on how to use Volocity see the Volocity User Guide (available from [http://www.cellularimaging.com/pdfs/manuals/VolocityUserGuide.pdf](http://www.cellularimaging.com/pdfs/manuals/VolocityUserGuide.pdf)) or Help within the application.

**Volocity**

**Movement Correction**

Movement Correction automatically corrects for microscope drift or specimen movement. Drift can be caused by thermal effects or mechanical inaccuracies. Movement can cause artefacts in measurements, especially FRAP analysis and tracking. Movement correction does not adjust for major changes due to growth or changing morphology, nor can it correct for drift when structures change shape over time.

From the Tools menu select ‘Movement Correction’.

![Movement Correction dialog box](image)

Set a channel in which no movement should be seen as the guide channel. Other channels will be adjusted by the same amount as the guide channel.

Use the first timepoint as the anchor for most accurate results but this is also least tolerant of changes between timepoints.

Use the previous timepoint as the anchor for when the data changes more. The result will show more persistent drift as it will accumulate over time.

Correct using the whole field or an ROI. Use an ROI when a detail in the image can be used as a form of fiduciary mark. The ROI must contain the detail to be aligned throughout the experiment. Correcting using an ROI will be faster than correcting using the whole field.

Lateral movement in X, Y and Z is always corrected. To correct for rotation, check the “Correct rotation” box. Note that this may increase processing time substantially. Movement correction creates a new image sequence with the text “(movement corrected)” appended to the name. The new sequence will only contain voxels that contain data after corrections, so it will vary in dimensions from the original.
Region of Interest (ROI)
Drawing, modification and viewing of ROIs has been improved in to allow easier selection and measurement of multiple areas of the field and more accurate selection of areas of particular sizes.
Multiple, non-contiguous ROIs are considered separate, they may overlap and be moved individually.
Control points allow rectangular and circular ROIs to be resized. ROI dimensions are shown during drawing a resizing.

Multiple ROIs can be drawn without holding the shift key. Select ROIs to delete or modify them. The Edit -> ROI submenu has been extended to include new tools for selecting ROIs and combining them to make new ones. Fine movement of selected ROIs is possible using the arrow keys on the keyboard.
Choose Edit -> Clear to remove selected ROIs. Click outside the image or choose Edit -> Select None to clear all selections including ROIs.
Note that the new ROI drawing is not available in the Colocalization view.

Volocity Quantitation
Find Objects Task
The new “Find Objects” task allows you to find objects more accurately and quickly.
As with all Find tasks select the channel containing the objects of interest.

An automatic threshold and exclusion of objects below a minimum size is applied. Object(s) in your image will be highlighted.

To modify the selection open the secondary dialog for the task.
Threshold using: Automatic

Automatic thresholding uses Otsu’s method (http://en.wikipedia.org/wiki/Otsu%27s_method) on the histogram of intensities in the image to separate signal from background. An offset from the calculated threshold can be applied to adjust the objects that will be found. The threshold is recalculated for each timepoint, so automatic thresholding is ideally suited to multi-timepoint data and batch processing of multiple datasets.

“Find Objects” in automatic mode is only suitable for objects where the signal is brighter than the background, such as images generated by fluorescence microscopy.

Change from “Automatic” mode to use Intensity, % or SD. A value determined by Otsu’s method will be used as a default threshold in all cases.

Find objects in automatic mode is only suitable for objects where signal is higher intensity than background such as images generated by fluorescence microscopy.

The calculate button sets the threshold in any mode back to the value determined by Otsu’s method.

Protocols from previous versions of Volocity that use “Find Objects by ...” tasks are supported in Volocity 5.5 and the tasks themselves are available in Volocity 5.5 under the “Thresholding” group.

Local contrast adjustment

This option is useful where intensity varies across the field. Local contrast adjustment modifies the selection so that it is made up of voxels that are significantly brighter than the mean intensity within the radius entered. In other words a local background intensity is used when selecting an object rather than one background intensity value for the whole field.

Once a selection is made by thresholding automatically or manually, selection of a voxel depends on the similarity of intensity to its neighbours. Enter the radius of the neighbourhood i.e. the approximate size of the object to be found. Voxels that are dissimilar to the local neighbourhood intensity will be removed from the selection.

Enabling local contrast adjustment will increase the processing time for creating selections.
The calculate button analyzes the image to estimate a good value for the local contrast adjustment radius. Internally a histogram of object sizes, based on the existing selection, is created. Otsu’s method is applied to determine the significant size for that group of objects. The local contrast adjustment radius is set to the radius of a circle (2D data) or sphere (3D data) of that volume.

**Minimum size**
This option filters objects by size. By default, objects smaller than 9 voxels will be rejected.

The calculate button analyzes the image to estimate a good value for the local contrast adjustment radius. Otsu’s method is used on object sizes as described above.

**Find Spots Task**
The new "Find Spots" task finds local intensity maxima in both 2D and 3D. It is ideal for finding small punctuate objects.
Local intensity maxima are bright objects against a dark background, this task is therefore only suitable for darkfield images such as those generated by fluorescence microscopy.
As with all “Find” tasks, select the channel containing the spots of interest. Open the secondary dialog to adjust additional settings.

By default the minimum spot intensity will be set to 2 x the threshold intensity value for the data as determined by Otsu’s method. The calculate button will reset the threshold to this value.
Only the brightest spot within the radius set will be selected. Adjust the radius to match the expected spot size.
Users of previous versions of Volocity will find ‘Find 2D Spots’ and ‘Find 2D Nuclei’ in the ‘Thresholding’ group.
For all “Find Spots” tasks the intensity at each point location is now recorded.
Points may be operated on by other tasks in a protocol, for example ‘Dilate Objects’ can be used to turn spots into objects.

Volocity Acquisition

Live Video Plot
The “Live Video Plot” plots mean intensity for the whole field or ROIs over time for live video. The plots help the user to set up acquisition with appropriate settings and also show detailed information on the progress of experiments.

A separate trace is created for each channel. A separate graph is created for each ROI. When working with multiple XY points a separate graph is created for each point.

Hovering over the plot will show information on time, mean intensity and % of max intensity.
The plots may be hidden to save space on the screen.
The plots in the Ratio and FRAP views have been updated to have the same functionality.
Plots are for display only; they are not saved or exported. Use the measurement tools in Volocity Quantitation to generate data for export.

**New Hardware Support**
Volocity 5.5 adds support for the Leica “Adaptive Focus Control” (AFC) on the DMI6000 microscope. With this, Volocity supports hardware autofocus systems from all the major microscope manufacturers.
See the supported hardware list and technical note 454 for more information.

**Photometrics Evolve 512 EMCCD (NOT AVAILABLE ON ULTRAVIEW)**
This camera is only supported on Windows as Mac OS X drivers are not available.
See the supported hardware list for driver version requirements and more information on setting up this camera with Volocity.
The Evolve is not compatible with the UltraVIEW.

**Orca-Flash2.8**
See the supported hardware list for driver version requirements and more information on setting up this camera with Volocity.
The Orca Flash2.8 is not compatible with the UltraVIEW.

**FRAP Monitor Region**
In FRAP experiments, bleaching regions and observing the degree to which these regions recover also requires an understanding of whether intensity changes that are not due to the bleach/recovery cycle have taken place. The FRAP monitor region can assist with this by allowing the creation of an ROI that is not to be bleached but is shown as a mean intensity in the live video plot.
To specify a monitor region draw an ROI and choose “Set Monitor Region” in the FRAP menu.