Volocity Quick User Guide
ver 6.3 *Revised 1-05-15*

These instructions are for a PC. The Mac version is slightly different.

Import Data
File - Create New Library – Choose location and Name it
>Note you must name a 'library' before you can import your data.

Drag files/folders into library: drag a .lei or .lsm, or .czi, or .lif or a folder of tifs
Or File->Import: click on .lsm or .czi or .lei or .lif
Click (once!) on desired series
>You will see max projections of a merged image

Drag to make window large
For a folder of tifs: use Actions – Create New - Image Sequence
>You will get an empty window. Drag the folder or series of tifs into it

IN UPPER LEFT CORNER find small drop down menu
3D Opacity is best for 3D viewing
On right of image are color channels and choices for each.
>**Fluorescence** – good for 2-3 colors when you want to look at interactions between colors and may want one color to dominate over the other.
>Adjust Brightness, Density, Black Level as follows:
>>First adjust the 3rd option, the Black Level, to get rid of background.
>>Then adjust the 2nd option, Density or Opacity
>>Also adjust the Brightness along with Opacity, there are interrelated
>>Adjust the other color channel as desired

>**Max Intensity**—like a Max projection in confocal, is best for viewing small details
>>There is no Opacity control here
>>Adjust Brightness and Black Level for each color channel

>You can use Max Intensity for one channel and fluorescence for the other.
>You can do whatever gives you the info you want to see.

>**IsoSurface**- this looks only at the surfaces and blocks the interior
>>Adjust Brightness and Black Level and **wait** to see. This takes time to render.
>>Keep black level above 0
>>Adjust light source angle with Lamp icon in top toolbar.

ICONS:
At top are icons for rotating, zoom, pan, light source angle, etc.
Rotate (either one) - click and drag on your image to rotate your image. Click and throw to make a continuous rotation. Force of 'throw' adjusts speed.
Zoom – click on image and drag down for increasing zoom, up to decrease In some modes, use the Ctrl key and mouse click to bring zoom down or up.
Pan (hand) – to pan when zoomed

Image Menu-Display
Choose to show or hide the floor, volume ball, orientation, scale (grid), etc.

UPPER LEFT CORNER other options:
3D Ray Tracer
>This is not interactive and takes a LONG time, but will give you a very nice image for presentation.

3D slice

Shows the image as a box. You only see one plane at a time. The backside is opaque, the front is not there. This is more useful than you think.

Look for slider at right of image to change planes, clicking is faster than sliding.

You can change the angle at which you section your image.

Icons at top:

Use the Blue Rotate Slice or Rotate about Slice Icon to change the angle or Select X,Y,Z angle at bottom. Go back to regular rotate tool to rotate entire image box.

Play thru slices with slider.

Image-Reslice - Image to folder will create new images of sections taken at any angle, eg create a cross section series of images

XYZ

Click anywhere in image to see a cross section at that point in X and Y. Click in XZ or YZ windows to change plane in XY window

Change size of windows as desired or

XY plane, XZ plane and **YZ plane** and **3D Plane** to see the windows chosen in XYZ

Extended Focus

This is a Maximum projection. You can change Brightness of each color channel only

Zplanes

This is a Gallery

Bookmarks and Movie Making

For quick, Pre-Made movies:

Movie – Restore - Highlight one and just hit OK, Play

To make your own movie:

Image menu in top program menu, Click on:

Show bookmarks

Show movie

To make a movie:

Bookmark first image with **Ctrl B**

Change image by rotating, zooming etc,

Add bookmark

Repeat for as many bookmarks as you want

Drag bookmarks into time sequence at bottom (shift click to get all of them), adjust the time/ by changing the width of the bookmark (=#frames)

Hit play to see your movie

To save you must first make a movie, then export it.

Movie Menu:

Make movie creates a high resolution library sequence you can export.

Use current resolution or the highest

To Export, Highlight movie in library sequence folder

File menu – Export, choose AVI or Quicktime (Right-click Export might work better)

Options to choose compression, quality, speed.

Compression options are somewhat dependent on your computer. Try a few.

You can return to any bookmark (double click)and the image will go there. You can delete bookmarks, add bookmarks, etc.

If you want to use the same sequence of bookmarks on another file, use Save movie in the movie menu and use Restore to add it to another sequence.

Saving Images
Image -- **Capture Snapshot**
To save any rotated, zoomed or rendered image on the screen.
Rename and export as .tif

Voxel Spy
This color pixel icon is active in XYZ thru Extended Focus options
A small window opens and shows you the x-y-z- coordinate of the pixel at your cursor and the 'grey' value of each channel. Click on little image to change between 3 zooms.

Measurements
Note this section has not been updated for the newest version.
Click on Measurements just above the image window. A measurements menu item will appear at the top.
Make a list of commands by dragging a task from the lower to the upper window on left.
   Note that measurements will be calculated automatically, which could take a long time.
   Uncheck 'automatically update feedback' under the top Measurements menu.
   Then when you want to see results, you must click on 'Update feedback' or use Ctrl U.
Strategy
First you need to define your objects, here are suggestions:
        Find Object Using Intensity
                Slide black level bar for bright objects– unclick and wait for the program to catch up

                Fill Holes
                Exclude by size.
                Separate touching objects - Uses average volume
To get sizes or volumes, run update and look thru the table. Data can be sorted by clicking on any column header. You can change the number of columns with Measurements-- Columns
To see what you have go to **Image**
Rotate to find best angle for light or change light direction with lamp icon at top
Use Ctrl A to highlight all objects

Measure distances
        Choose line tool - click and Shift-click to get a line and distance. To add to table, must have the command in the upper window.

To Save measurements
        Measurement menu at top
                Make measurement item – puts it into the library
                File – export as .cvs or .txt

To Edit measurements
        Edit – select none deletes all from table
        Edit clear deletes highlights item from the table but not from the image.
        If you now save, it is gone from the measurement item but it can come back into the measurements window.

Regions of Interest (ROI)
First draw in Extended Focus View (or XYZ?)
Choose shape and draw

Then switch to XYZ and limit depth, etc with these:

     Shift – for multiple regions or to add to a region

     Shift+Crtl – to subtract part or all of a region

     Ctrl – uses the intersection of 2 regions (Boolean AND)

     Move - click inside region

Can't resize except 1 voxel at a time:

Edit – ROI - grow or shrink

     Repeat for next voxel

Edit – ROI - Undo to delete ROI

Once you have an ROI defined, you can choose not to show it so you don't have a big opaque box in your 3D image. Go to the very top image menu and find View options, choose ROI and unclick Show ROI. You can also change the opacity here. You will find yourself showing and unshowing the ROI as you work.

When measuring, you can limit measurements to the ROI.

Filters

Edit, duplicate image sequences before running filters! You original file will be overwritten!

Remove noise – 4 levels for background noise

     Fine=weak, very coarse is aggressive (3x3 5x5 7x7 9x9)

     Do this before measuring to reduce the number of objects

Correct registration

Correct background – collect background image first

DO NOT FORGET TO EXIT THE PROGRAM WHEN YOU ARE DONE!