Volocity ver. 6.3.0 (2013)

Standard Operation Protocol

A. Content

B. Overview

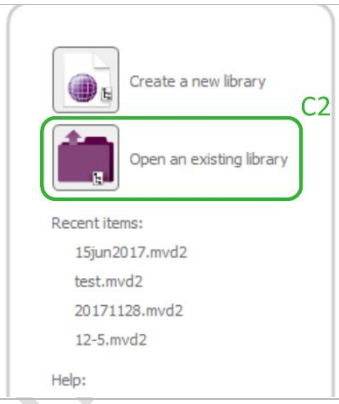
Volocity is an image acquisition and manipulation software designed by PerkinElmer. The software accepts proprietary *.mvd2 files libraries. *.mvd2 files are multi-dimensional image data file.

C. Start up

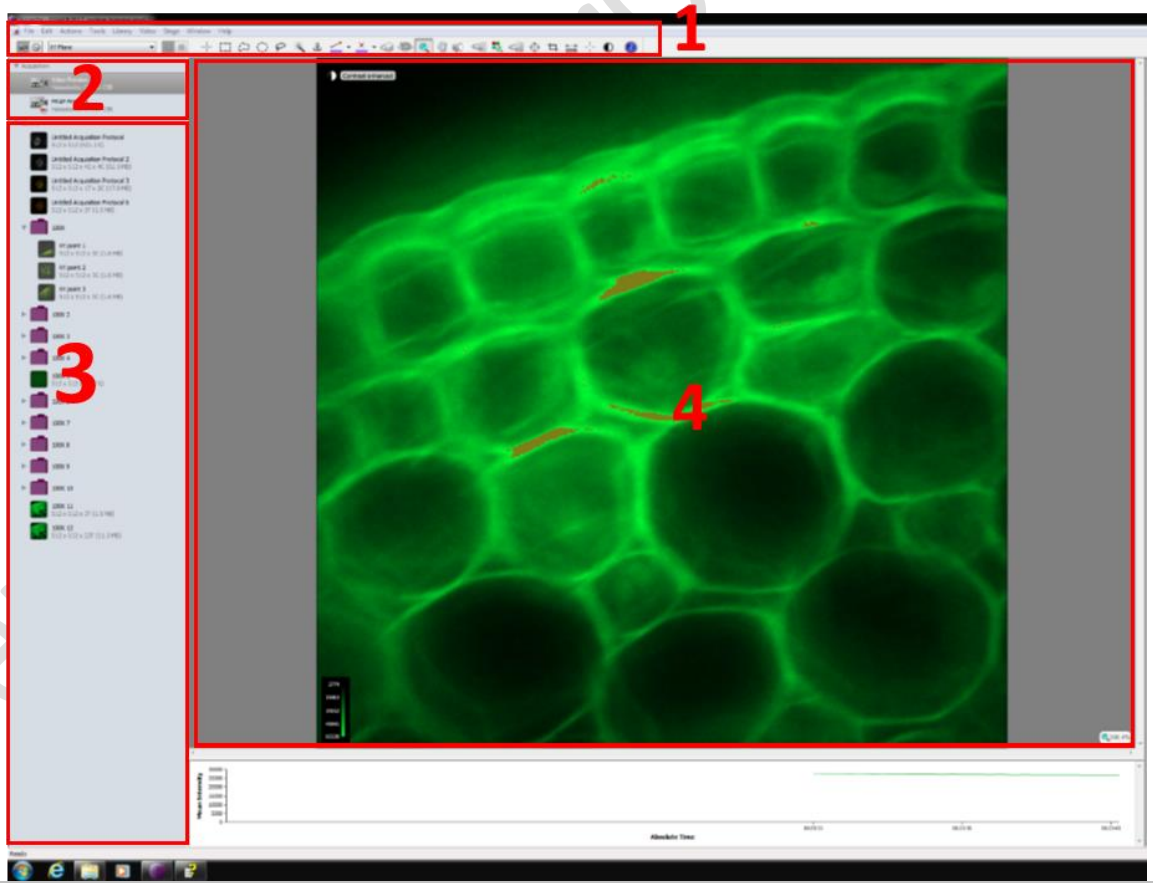
1. Double click on the program icon on desktop.

   Or
   double click on any files with .mvd2 suffix to activate software.

2. (Optional) If the software was turned on alone. A window will prompt for “Open existing library”



Graphical user interface description:
The terms will be used throughout this document to locate different buttons.



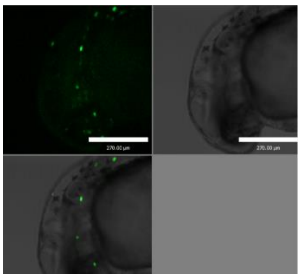
1 Menu bar
2 Acquisition
3 Library
4 Image Preview window

Tool bar list:

3. View options
   Frontal view = XY plane
   Maximal projection = Extended Focus

4. Toggle between merged channel view 
   or tile channel view 

Tile channel view:



5. Creating region of interests

6. Line and point marker tool.

7. 3D view manipulation tools


= manual rotate 3D rendered object

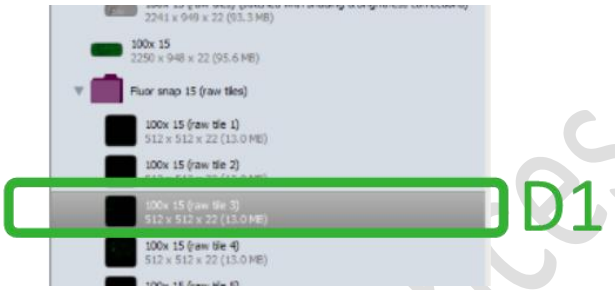

= spin the object around.(continuous)

8. Left panel channel relative brightness adjust

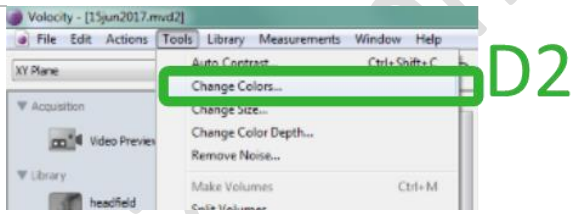
D. Change pseudo-colour

All fluorescent imaging data are encoded as 16-Bit monochrome images. .mvd2 files will assign a pseudo-colour on each channels to make the signal stand out from others. You can change the assigned pseudo-colour.

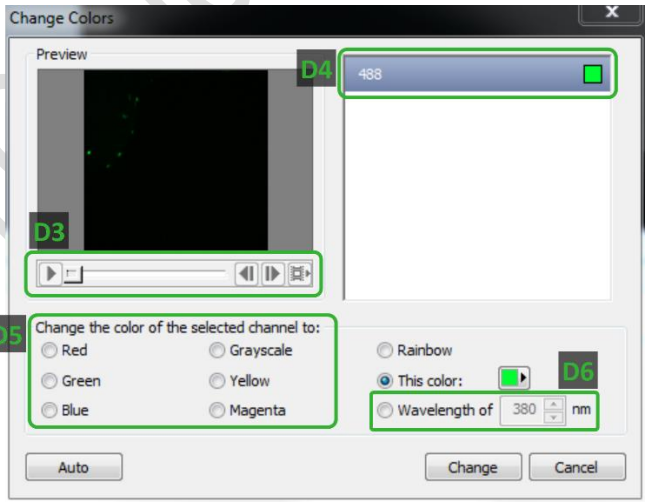
1. Select an image file from library panel (left side).



2. Go to "Tools" on Menu bar.
   Click on "Change Colors…"



3. A new window will be shown with setting for each channel.
   You can scroll through a z-stack or time series with a slider under "Preview"
4. Select the channel you want to modify
5. There are a few pre-set colours to choose from.
6. Alternatively, one can also assign a colour base on wavelength in the visible spectrum. (E.g. 580 nm ~ yellow.)

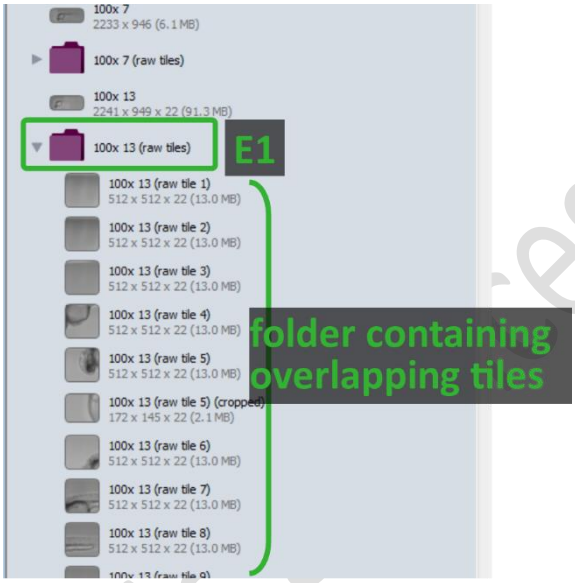


7. Click "Change" to save settings.

E. Stitching images

For multi-tiled images, the images are saved in separate blocks. It is necessary to piece together tiles to give a complete image. This function is applicable to both single optical sections and z-stack images.

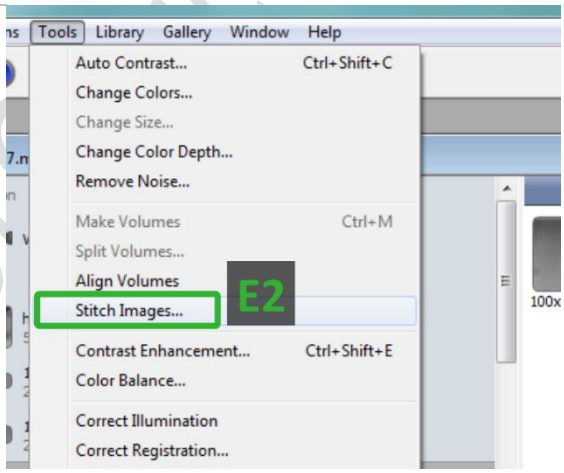
1. In library panel (left side).
   Select a folder containing raw image tiles



2. Go to “Tools” in Menu bar and click on “Stitch Images…”

   Or

   right click on the folder containing images to be stitched and select “Stitch Images…”



3. There are a few parameters to be considered. Please try stitching with no correction (un-check all of the boxes)
   If the first image is not optimal,
   - Correct for shading
     (mainly for Bright-field images)
   - Correct for Brightness
     (mainly for Fluorescent images)

4. Alternatively, select “Create multiple stitched images with all possible combinations of corrections”. Volocity will generate 4 stitched images with following parameters [None] / [Shading] / [Brightness] / [Shading + Brightness]
   ***Note: this will take longer.

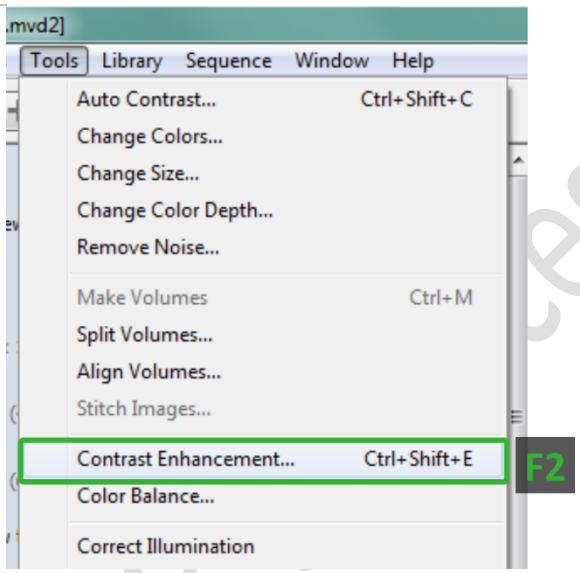
5. Click “Stitch”.

F. Adjust Image Contrast

1. Select an image file from library panel (left side).

2. Go to “Tools” in Menu Bar.
   Select “Contrast Enhancement…”

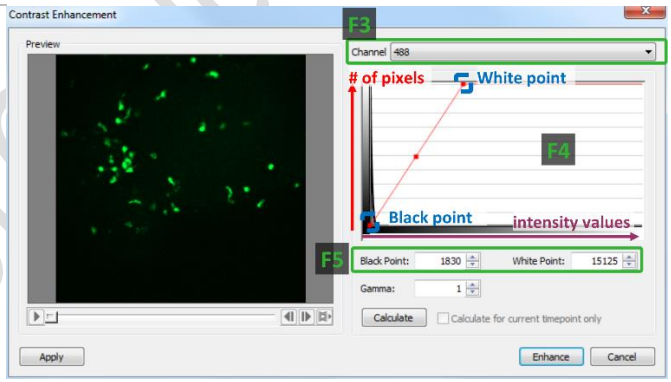
   or
   Press Control + Shift + E



3. In Contrast Enhancement window.
   Select the channel you want to modify.

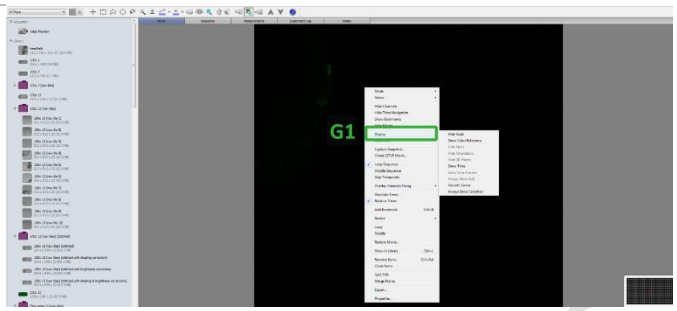
4. Histogram showing pixel count against intensity values.
   I.e. if the histogram is skewered towards the right = bright, left = dim.

5. Black point = intensity lower threshold
   White point = intensity higher threshold
   If you have multiple images to change contrast to the same value, note down and re-enter these values.

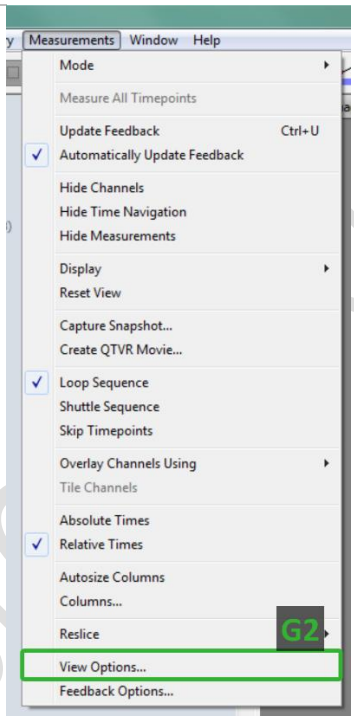


G. Annotation

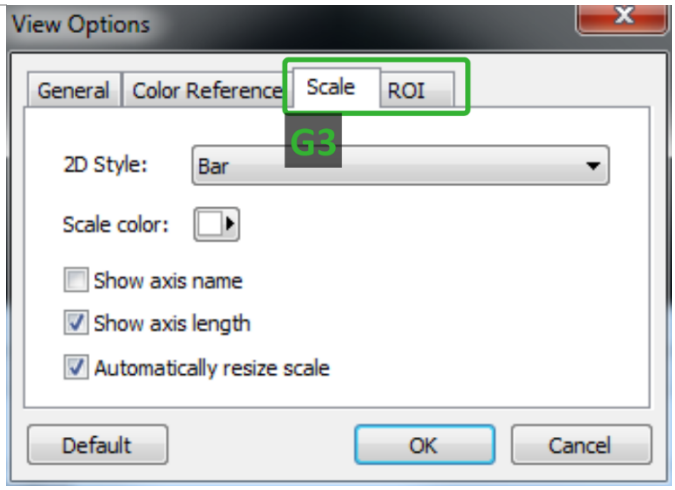
1. To show or hide annotations, right click on the image and go to “Display”.



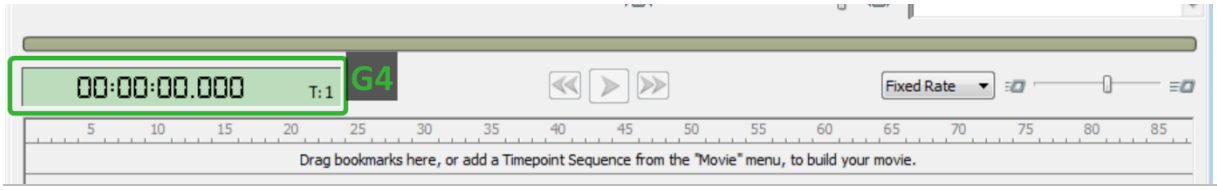
2. To change colours of different annotation elements. Go to “Measurements” on Menu bar. Then “View Options”



3. Navigate to Scale tab or ROI tab to adjust style and colours.



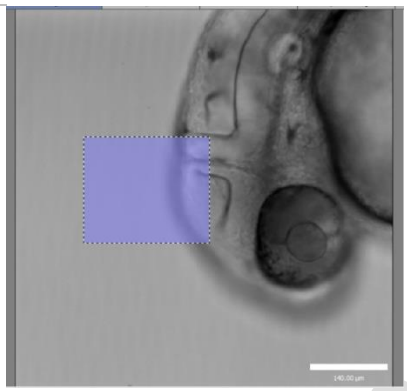
4. To change Time marking from absolute time (real time stamp) to relative time (t=0 at start of experiment). Click on green square.



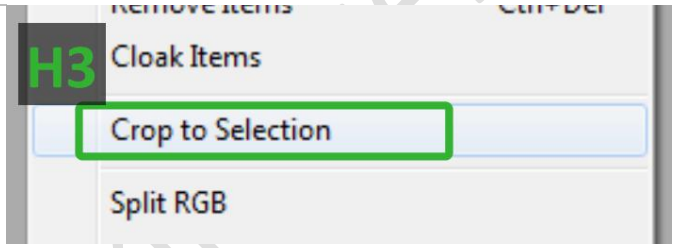
H. Cropping images

A function similar to the “Define Subset” function in Image export can be used to create *.czi files with a portion of the dimensions or a multi-dimensional image file.

1. Create an ROI on the image.



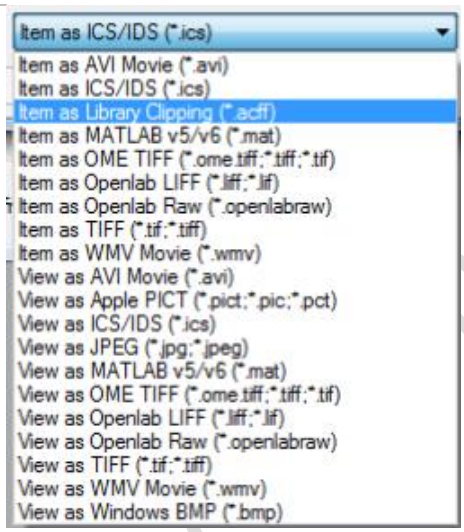
2. Right click on the image to show a put down menu.
3. Select “Crop to Selection”.



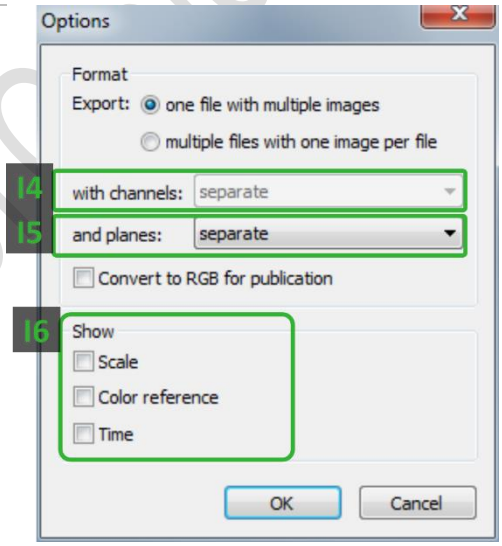
I. Image Export

1. To export images, right click on the image and select 'Export'
2. Alternatively, go to File\Export\
3. At "Save as type:" option, choose from a range of file types

   'Item as': same as raw data but different format
   'View as': direct export of image view (including contrast adjustment and annotations)



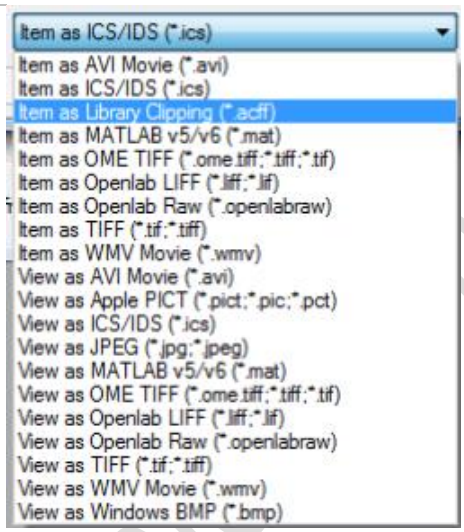
4. Separate / merged channels
5. Separate / merged z-planes
6. Label images with scalebar, color reference and time stamp.



J. Append libraries

To merge / transfer images from one library to another. Follow these steps.

7. Right click on the image and select 'Export'
8. Alternatively, go to File\Export\
9. At "Save as type:" option, choose "Item as Library Clipping (*.acff)"



10. Open a new library or open the target library to save the image to.
11. Go to "File" on Menu bar and click "Import…"