

STANDARD OPERATING PROCEDURE Deltavision Elite Deconvolution Microscope

PURPOSE

This document describes the operational setup and procedures for the Deltavision Elite Deconvolution microscope (Deltavision). This document offers users, particularly beginners, a brief step-by-step instruction on how to take images using the Deltavision microscope with its acquisition software Softworx 6.5.2.

The Deltavision microscope is a high-end wide-field fluorescence inverted microscope featuring a softwarebased restorative deconvolution process. The system is equipped with optimised solid illumination light with fast excitation switching (microsecond) that allows fast and bright multicolour 3D live cell imaging resulting in reduced specimen fading and photobleaching. Dual cameras (CoolSnap HQ and EMCCD) provide high resolution and high sensitivity options. Features include speed, sensitivity and high-resolution of images making it suitable for small/thin and faint fluorescence samples as well as living cells.

SCOPE

The procedure covers preparing the system, start-up & basic acquisition, advanced acquisition options including sectioning (Z stacking), multiple channels, time lapse, multiple positions etc., as well as basic image visualization & processing.

Users are to refer to the full manual provided by the vendor for more detailed operational instructions. The full manual can be found on the WRH Cell Imaging website.

SAFETY

The operator must exercise caution during initialisation of the stage. Never put fingers inside the incubator while the stage is moving or initialising.

Safe operation of the instrument requires the awareness of risks e.g. biological spills, glass cuts, fatigue and gesture discomfort/injury etc. Refer to the "WIMR-SWP-WHS-GEN-16.01 Use of fluorescence and laser microscopes" for details:

https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

Acquisition of samples on the Deltavision microscope must only occur after the approval of an associated project in PPMS. Any projects to be run on the Deltavision involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply.

TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by facility staff with competency demonstration necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

EQUIPMENT & SUPPLIES

The Deltavision microscope is located in J2.12A, Level 2 of WIMR.



PROCEDURE

Important notes. Please keep the following default set-ups of the microscope in position after your use:

- System should always be left on (do not power off unless you are advised otherwise by the Imaging staff)
- Coolsnap HQ2 camera attached to the microscope (not the EMCCD)
- Filter wheel set: Standard set (not the Live Filter Set). Also make sure in the "Settings" in Resolve 3D, the Standard filters are selected as shown below

	Resolv	e3D Setting	gs	-	×
Display Files	Imaging	Autofocus	Misc		
- Stage Motion					
Allow Lost Motion	Compensat	ion (LMC)			
- Stage View Option	s				
Spiral Mosaic 5x5	-				
🖌 Show stage trails					
🔽 Show stage thumb	nails Re	solution Lo	W 🔻		
🖌 Show point numbe	rs				
- Filter Wheel Sets -					
Excitation filter wheel	Standard	-			
Emission filter wheel	Standard	-	•		
Eyepiece filter wheel	Standard	-			
Activate Filter Sets.					
Done Save Setting	js 🛛			ŀ	Help

- Dichroic mirror/filter set for the red channel: "1" Position ("2" is for Alexa 594)
- "1X" Optivar is pushed in (Pull out: "1.6X") and the "Aux Mag" is turned off in Resolve 3D

	Resolve3D _ ×
	<u>File View Options Calibration Help</u>
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	XYZ (μm) -4479.66 -7707.01 4.54
	Min 102 Max 1194 Mean 209.3 Io 📒

1. Start up

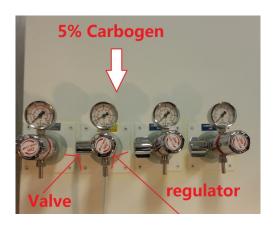
1.1 Turn on. The system should be left on all the time so no need to turn on any parts if you are just imaging fixed samples.

For live cell imaging settings, turn on additional components for CO2 supply and temperature control. **Note**: Warm up the weather station for at least 3 hours in advance to equilibrate the temperature and CO2 (Do not forget to place the appropriate oil in the Incubator so it will be warmed up as well).

• Open the carbogen gas valve on the wall and then turn on the regulator clockwise to the marked line.

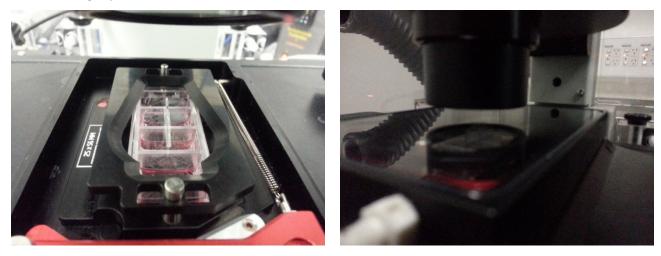


• Press the "Heat" button and choose "Hi" for fan speed of the Weather Station to blow the heated air into the incubator. Slowly adjust the flow till it reaches "3".





• Set up the specimen like this (for beforehand warm up, you can use a used/empty chamber to seal CO2 leakage and later replace with your own sample container) (below left picture) and cover with the lid (below right picture).



More information for live cell imaging settings is provided in "CO2 and temperature settings for live cell imaging with the Deltavision microscope": https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

- 1.2 Log in to the Workstation
- **1.3** Start Softworx: "Applications"/"GE Healthcare"/"Start Softworx".
- 1.4 Open up Resolve 3D and other components of Softworx: "File"/"Acquire". Then the following 4 subwindows will be brought up as shown below:

<u>Resolve 3D</u>: set up the microscope, imaging conditions and experiment (pre-acquisition) <u>**Data collection**</u>: live window to view what's being seen on the camera (live imaging) <u>**Softworx**</u>: image viewing, processing and analysis (post-acquisition)

<u>Filter monitor</u>: display the eyepieces filters including excitation and emission as well as illumination intensity



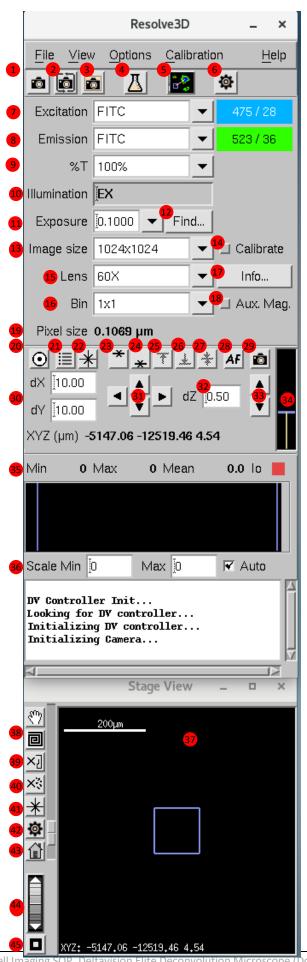
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2. Procedures

- 2.1 Resolve 3D features (P5)
- 2.2 Preparation (P6)
- 2.3 Basic image acquisition (P9)
- 2.4 Z sectioning (P10)
- 2.5 Time lapse (P10)
- 2.6 Points (P11)
- 2.7 Simple post-acquisition processing and analysis (P12)

2.1 Resolve 3D features





3.1 Resolve 3D features

- 1. Acquire an image of the current channel
- 2. Continuous acquire images to display changes in the live window i.e. manual adjustment of the focusing knob
- 3. Save snapshot
- 4. Open the Experiment Designer window
- 5. Bring stage view window (37) to the front
- 6. Open the Resolve 3D Settings window
- 7. Excitation wavelength
- 8. Emission wavelength
- 9. Percentage illumination intensity
- 10. Illumination type (EX: fluorescence; Trans: transmission)
- 11. Exposure time (seconds): can be selected from drop down list
- 12. Find the optimal exposure time for a specific max intensity
- Specify image size (must be ≤ 512X512 if binning larger than 1X1 is to be used)
- 14. When selected, will apply calibration file (if it exists). A calibration file must be created for each combination of image size, channels, objective and camera used
- 15. Objective lens. Must be changed when changing objective
- Binning size (must be 1X1 if > 512X512 image size is used) signifies how many pixels on the camera translate to each pixel in the image
- 17. Display lens information including optical conditions and recommended oil refractive index needed to be used after inputting the required information
- 18. Auxiliary magnification should be selected if the auxiliary magnification slider is being used
- 19. Show the pixel size in micrometres
- 20. Centring tool: click on the tool then click on a spot in the image that should be in the centre. The stage will move to the appropriate location
- 21. Show point list window
- 22-14. Visit Z positions of Centre of Z selection (22), Top (23) and Bottom (24) respectively
- 25-27. Mark stage positions of Top (25), Bottom (26) and Stage Coordinate (27) respectively
- 28. Software-based or contrast-based Autofocus
- 29. Acquire an image of the current channel
- 30. Step size for X & Y movement when arrows (31) used.
- 31. X & Y movement arrows
- 32. Step size for Z movement when arrows (33) used
- 33. Z movement arrows
- 34. Visual indicator of Z position (blue bar: current stage position; yellow bars: marked top & bottom of sample
- 35. Image intensity values: Min, Max, Mean
- 36. Set min and max values of histogram
- Stage window: visual indicator of XY stage position (blue box) and XYZ stage coordinates (bottom left corner)
- 38. Pan the stage view (37)
- 39. Clear stage trails from stage view area
- 40. Clear thumbnails of the sample in a single channel
- 41. Mark the current stage coordinate to add it to the Marked Point list
- 42. Settings (same as 6)
- 43. Restore the stage View to its original position
- 44. Zoom in and out stage view
- 45. Zoom factor 1: return stage view area to 1X zoom



2.2 Preparation

2.2.1 In Resolve 3D

Click "Settings" (6) and visit the following Tabs:

<u>"Misc" Tab</u>: make sure filters are correctly selected in Resolve 3D settings and physically placed in position on the microscope.

<u>"Imaging" Tab</u>: chose the camera suitable for your sample/imaging requirements. Coolsnap: high resolution and applies to most of applications. EMCCD: high sensitivity good for faint staining or to meet fast imaging requirement.

"Files" Tab: users are required to follow:

Data management policy: all data must be saved here (kept for 7 days before auto deleting: Data1/Home/"your folder". Your data will be transferred to the server instantly: Scientific Platforms\Cell Imaging\Deltavision\"your folder" (kept for 14 days before auto deleting). Your images can also be found on the Deltavision analysis computer (kept for 14 days before auto deleting): data

Resolve3D Settings _ ×	Resolve3D Settings _ ×	Resolve3D Settings _ ×
Display Files Imaging Autofocus Misc	Display Files Imaging Autofocus Misc	Display Files Imaging Autofocus Misc
- Stage Motion	Camera CoolSNAP_H02/H02-ICX285	Data folder //data 1
Allow Lost Motion Compensation (LMC)	Frames to average CoolSNAP_HQ2/HQ2-ICX285	Experiment macros folder //data1/home/hong.yu
Stage View Options Spiral Mosaic 5x5 Show stage trails Show stage thumbnails Resolution Low Show point numbers	Gala Cascades STE Conv. EEV CCD 978 Gala Cascades STE Conv. EEV CCD 978 Transfer speed 10.00 MHz T Target temperature 30.00 Current temperature -20.35 Refresh	Data folder is temporary Auto-increment file names Discrement to 2 byte signed integer
Filter Wheel Standard Exotation filter wheel Standard Emission filter wheel Standard Eyepiece filter wheel Standard Activate Filter Sets.	✓ Use photosensor (* Settings change based on Excitation Filter)	
Done Save Settings Help	Done Save Settings Help	Done Save Settings Help

- Make sure an appropriate lens is selected in the lens area ((),
- Choose the Image Size (13).
- Click the "Info" button (1). Enter the information under current optical conditions including: <u>"Distance from coverslip to</u> <u>sample"</u> (you can start with an estimate)

<u>"Coverslip thickness"</u> (i.e. 1.5# coverslip: 170 um)

<u>"Temperature"</u> (reading can be found on the Weather Station metre)

		Lens Information	_ ×
<u>File L</u> ens			<u>H</u> elp
Lens ID	10612	Name [Olympus 60X/1.42, Plan Apo N, UIS2, 1-	U2B933
Manufacturer	[Olympus		
Manufacturer P/N	[1-U2B933	APLLC P/N 34-018019-110	
Magn	ification [60.0	NA [1.42	
Working Distan	ce (µm) (150.0	Focal Length (μm) [3000.0	
Standard Refractiv	e Index 1.515	Recommended Refractive Index 1.515	
- Optical Condition	ons		
Distance From Co	verslip to Specime	n (µm ([0.0 overslip Thickness (µr ([170.0	
	Temperatu	ure (C) (25.0	
Sp	ecimen Refractive	Inder 1.470 1.470 - Pure Glycerol	-
-Resolution Calo	ulations		
Resolution Ra	atio (Z/XY) 1.5	Depth of Field (µm)	
Maximum XY Pixel	Size (µm) 0.08	Recommended Z Step Size (µm) 0.20	

<u>"Specimen refractive index"</u>. In the screenshot, it recommends 1.515 (as all the Refractive Indices of the provided oils end in even numbers so in this case you can start with either 1.514 or 1.516.



2.2.2 Get the microscope ready

- Turn the objective turret to select the lens. Objectives can be identified by the colours of the rubber band around the objective neck (refer to below table): 100X (purple), 60X (green), 40X (blue), 20X (empty).
- Make sure condenser position matching your objective (i.e. Position "3" for 40X as shown below)

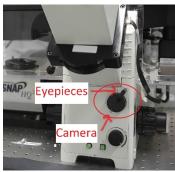


Objective	Rubber band	Condenser
	colour	position
20X	NA (empty)	3
40X	Blue	3
60X	Green	4
100X	Purple	5

- Clean the lens with provided 100% ethanol and cotton swabs.
- Place one drop of oil (with recommended RI) onto the coverslip (for fixed sample) or the objective (for chambered slides/coverslip when imaging live samples).
- Mount the sample (coverslip side down) in the slide holder.
- Rotate the eyepiece filter wheel to an appropriate channel. Make sure the Excitation and Emission filers are the same, i.e. for transmission (or BF) channel, Ex: POL; EM: POL.

Filter M	Monitor _ ×
POL (TRANS)	T = 100
EM = POL	EP = POL

Rotate the port selector knob to the eyepieces



- 2.2.3 Focus on sample through eyepieces
 - Bring objective up to sample by rotating course focus knob located on the front of the microscope base) towards you just until you see the oil spread.
 - Open the shutter i.e. transmission shutter from the joystick keypad
 - Look through the eyepieces and slowly bring the sample in focus by adjusting the fine focus knob.

Note: special attention needs to be paid to avoid bringing the objective too high to push the slide off the sample holder.

• Use the joystick to scan your slide in X and Y to find a region of interest.



2.2.4 Check out 3 things

<u>Koehler Illumination</u> (Recommended to run this procedure once at the beginning of your booking session). Detailed instruction on Koehler Illumination procedure can be found on Imaging site page: <u>https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/765397549/Tips+Tricks</u>.

- Close the field diaphragm (at the top of the microscope where the light is coming in) so you can see its polygonal contour in the eyepiece
- Focus the edges of the brighter polygon-shaped contour by adjusting the condenser height (the knob on the right side of the microscope back pole).
 Note: you should not adjust focus knob to achieve the in-focus polygon edges!
- Centre the condenser by fiddling with two centering pins until the polygon contour locates in middle of field-of-view.
- Open the field diaphragm until it is just outside the edge of the field of view.

<u>DIC</u>

- Push the Polariser in (the silver part at the top of the microscope under the field diaphragm).
- Push the DIC Slider in (beneath the stage) and if necessary, twist the DIC Slicer to get a satisfactory DIC effect.

Fluorescence

- Open the fluorescence shutter
- Choose a filter to view your channel i.e. DAPI channel. Repeat this to view all fluorescence channels. **Note**: leave DIC slider and Polarizer out while imaging fluorescence. If DIC image is needed, it must be acquired separately.



2.3 Basic image acquisition

2.3.1 Set up the experimental conditions for all to-be-imaged channels

- Rotate the port selector knob to the camera.
- In Resolve 3D, "Acquire" an image (49) to view the current channel first in live window based on the current settings (illumination intensity exposure time etc, etc).
- Use the "Centring Tool" (20) to centre the specimen in the image window.
- If necessary, adjust focusing using either "AF" (autofocus (28)) or Up/Down arrows (33) or manually adjust the focus knob on the microscope while "Continuous acquire" is turned on in Resolve 3D (22).
- If necessary, adjust exposure time (1) and illumination intensity (3) to get the right saturation for the image to achieve (if you can) "Max" 2000-2500 for fixed samples and 3-5 times the background ("Min" intensity) for live samples (1).
- Switch "Excitation" filter () to the next channel (After you choose the "Excitation" filter in the drop down list, the corresponding "Emission" filter [Design/Run Experiment: * (modified) _____ ×

(8) will be selected automatically). Repeat the following steps to set up focusing,

exposure time and illumination intensity etc.

2.3.2 2D multi-channel image acquisition

- Click "Experiment" (4) in the Resolve 3D. Go to "Channels".
- Click "+" to add channels you have just set up in the Resolve 3D (Section 2.3.1).
- IF DIC/BF is to be used, select POL for the filter sets.
- Load the channel settings by clicking "Refresh exposure conditions" in the Design/Run Experiment".
- Select "Run" tab. When prompted, click "ok" to save the experiment macro.
- Enter an image name (no space allowed in the name).
- Select the "Play" button (green arrow) to initialise image acquisition.

		Design/Run Experime	ent: * (mod	lified)	_ ×
File					Help
228 4	/ 🖻 🜔	Current experimer	nt (None)		
Design Run					
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Image	title				
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Change next time la	pse 0.00		Do It		
Show images a	during acquisition				
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<u>Fi</u> le	<u>H</u> elp
🔁 🔂 🔛 🥢 🖻 🕒 Current experiment: (None)	
Design Run	
Experiment name Resolve3D	
Estimated file size 2.00 Mb (1739.14 Gb Available)	
Use Fast Acquisition Fast Acquisition Options	
Sectioning Channels Time-lapse Points Panels Actions	
Image Scan Sequence Wavelength then Z	
Exp EX Filter EM Filter %T Frequency	8
- 0.2000 - FITC - FITC - 100% - 1	
- 2.0000 V TRITC V TRITC V 100% V 1	-
+	
Reference Image	
Z Position Middle of Sample	
+ [0.1500 V POL V POL V 100% V [1	
Refresh exposure conditions	



2.4 Z sectioning

2.4.1 Set up experimental conditions in Resolve 3D

- Set up channels as described in Section 2.3.1.
 - In Resolve 3D, use the Z bar (1) or Z movement arrows (1) to scroll up until you reach the top of your sample. Mark the Top (1). Same way to scroll down until you reach the bottom of your sample. Mark the Bottom (1).
- 2.4.2 Design and Run Z sectioning experiment
 - Click the Experiment button (4) in the Resolve 3D. Go to "Channels".
 - Click "+" to add channels you have just set up in the Resolve 3D (Section 2.3.1).
 - Click "Z Sectioning" tab. Optical section spacing: 0.1-0.3 um is okay. This Z step-size will determine your Z sampling rate. Clicking "select thickness" will load the Z thickness you set in Section 2.4.1.
 Note: Make sure that you have elected to start from the Z position of your sample i.e. Middle of the Sample in this case as shown below.
 - Select "Run" tab. When prompted, click "ok" to save the experiment macro.
- Design/Run Experiment: × Help 22 🛛 🖉 Current experiment: (None) Design Run Experiment name Resolve3Dį Estimated file size 128.01 Mb (1739.14 Gb Available) Use Fast Acquisition Fast Acquisition Options... Sectioning * Channels Time-lapse Points Panels Actions 🖌 Z Sectioning Z Scan Options... Image Scan Sequence Wavelength then Z -Focus point when scan starts Middle of Sample -Optical section spacing (µm) [0.20 Number of optical sections [64 Sample thickness (µm) [12.80 Get thickness 💷 Enable OAI Scan
- Enter an image name (no space allowed in the name).

2.5 Time lapse

- Set up channels as described in Section 2.3.1.
- In "Experiment" designer, activate the time lapse tab and enter the time between points and total time you wish to collect.
- Tracking and focus options:
 Cell tracking should be turned on if observing an isolated cell tat is expected to migrate in X and Y. It will keep the cell withing the filed of view by moving the stage to compensate for cellular movement.

<u>AutoFocus</u> will use contrast in fluorescence or transmission light images to find the focal plane.

Note: if there is significant time between points of your time lapse,

Design/Run Experiment: * (modified)	_ ×
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Design Run	
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Hours Minutes Seconds Milliseconds	
Time-lapse 10 51 10 10	
Total Time 2 10 10	
Time Points 25	
Cell Tracking Cell Tracking Options	
✓ Image-based Autofocus before imaging Autofocus Options	

you may want to select the "deconvolution during experiment" option in the Run Experiment Tab.



2.6 Points

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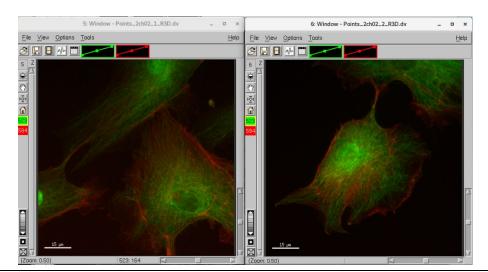
- Set up channels as described in Section 2.3.1.
- In "Experiment" designer, open the Points List in • Resolve 3D (38). Mark points using either (41) or the bottom right button "Point Mark" on the Joystick Keypad. You can also use the joystick to move to a position of interest and click "Mark" in the Position list. Repeat this step to look for all interesting positions/points and add each point to the list.

		Po	int List		-	×
Mark Point	Visit Point	1:	-4479.66 -4429.31	-7707.01 -7874.88	+7.05 +7.55	
Delete Point	Replace Point	4.	-4429.31	-7074.00	• 7.00	
Clear List	Compact List					
Save List	Open List					
	Optimize List					
Done	Help	50				

- You may "Visit" each marked point later and refine its focal plane using Z movement arrows (33) (you should not physically change the microscope focus knob). Click the "Replace" button in the points list to save the Z position.
- Open "Experiment" and go to "Points" tab. List the points you would like to use in your experiment i.e. 1-3, 5, 10-14 etc.
- You may add "Z sectioning" (refer to Section 3.3), and/or "Channels" (refer to Section 3.2), and/or "Time Lapse" (refer to Section 3.4.1). Autofocus can be applied (refer to Section 3.4.1).

Design/Run Experiment: * (modified)	_ ×
<u>F</u> ile	Help
🚰 🔂 🔛 🥢 🖻 💽 🌒 Current experiment: (None)	
Design Run	
Experiment name Resolve3D	
Estimated file size 200.02 Mb in 2 files (1739.14 Gb Available)	
Use Fast Acquisition Fast Acquisition Options	
Sectioning Channels Time-lapse Points Panels Actions	
Visit Point List	
₩ Image-based Autofocus before imaging Autofocus Options	

The resulting images will be saved separately for each point (below shows two 2D images for two • points for two channels).

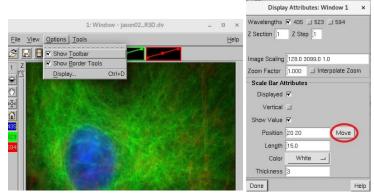


Cell Imaging SOP Deltavision Elite Deconvolution Microscope (Document Reference: WIMR-SOP-SP-CI-31.01)



2.7 Simple Post-acquisition processing and analysis

- 2.7.1 Add a scale bar
 - In the image window, select "Options"/"Display" (as shown below in the left screenshot). You will then have the "Display Attributes" window popping up for scale bar settings. If you click "Move" and then go to your image window, you can move the scale bar around in the image.



2.7.2 Save data

2.7.2.1 location

In Softworx", click "Data Folder", your images should be saved here: "Data1"/"your folder".

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Data Folder	s F <u>liter Model Measure Conversions Utilities Windows Help</u> age Windows: 1 21 (209 Gb free) Data Space 5% (1739 Gb free)		EM = POL	
< > (data1	hong.yu training		= -	• ×
⊘ Recent	Name Tluo01_R3D.dv		✓ Size 4.2 MB	Modified 28 Apr
Ĥ Home	fluo01_R3D.dv.log		2.5 kB	28 Apr
 Documents Downloads 	Nuc_imaging01_R3D.dv		4.2 MB	15:59
J Music	Fluo_imaging01_R3D.dv.log		2.6 kB	15:59
Pictures	jason01_R3D.dv		6.3 MB	4 May
Videos	jason01_R3D.dv.log		3.1 kB	4 May
圖 Trash	jason02_R3D.dv		6.3 MB	4 May
+ Other Locations	jason02_R3D.dv.log		3.1 kB	4 May
	jason03_R3D.dv		2.1 MB	4 May
	jason03_R3D.dv.log		2.0 kB	4 May
	jason04_R3D.dv		2.1 MB	4 May
	jason04_R3D.dv.log		2.0 kB	4 May



2.7.2.3 Save as Tiff

- If you run image acquisition in "Experiment", your images will be automatically saved to your defined folder in the natural format .dv.
- To save as Tiff. In the image window, <u>Wavelength</u>: Choose the channel(s): "435" represents DAPI Ch, "523" represents FITC/green Ch, "594" represents "TRITC/red Ch. If you need individual channel Tiff images, you need to save one at one time separately.

<u>Output size</u>: either Grey scale or RGB for fluorescence images. For BF image, only grey scale format works (either 8 bit or 16 bit).

2.7.2.4 Save as Movie

In the image window, select "File"/"save as movie". Select the preferred file format i.e. MPEG, AVI or Quicktime, and Frame Rate then select "Preview". When the speed of the file looks ok, click "Ok" to confirm.

2.7.3 Image processing

2.7.3.1 General tips

• Loading files into processing tools i.e. deconvolution. First, open the tool, then you can either:

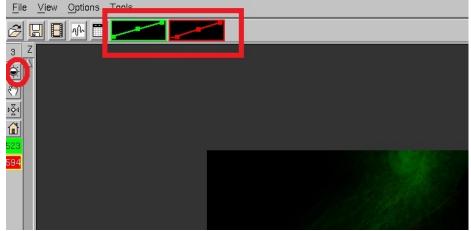
	Save As TI	FF		-	×
Input 2					
Select Region	Reset Details				
Wavelength	523 😿 594 🖯 🖵	□			
Output Folder	j/data1/hong.yu/training				
File Prefix	Fluo_imaging01_R3D				
- TIFF Options	·				
Scale Using Min	/Max/Exp Values Below [
	Compression (
	Short File Names				
	Vala File Naming	_			
	Destination Computer	8-Bit Grey	or Linux		
	Output Size	16-bit Grey			
- Wavelength S		32-bit Grey			
Wave 1 Color	r _ G 💶 Min/Max/Exp	24-bit RGB	1234.0	[1.00	00
Wave 2 Colo	r R 🔟 Min/Max/Exp	208.0	[1045.0	[1.00	00
Done Do It	1			Н	elp

<u>Drag and drop</u> the window ID number of the file into the Input line of the tool <u>Or Drag and drop</u> the image file icon from the data folder into the Input Line of the tool <u>Or Type</u> the window ID number of the open image file into the Input line of the tool

	Deconvolve
<u>File View Options Tools</u>	Input /data1/hong.yu/training/Z_2ch01_R3D.dv
중 🛛 🗋 🕂 🗂 🗾	Output /data1/hong.yu/training/Z_2ch01_R3D_D3D.dv
Can drag the window ID i.e. "3" and drop to Input	Select Region Reset Details
line to load the current image to the prcoessing	Wavelengths 🗹 523 🗹 594 🔟 🔟
Tool i.e. deconvolution tool in this case	- Deconvolution Options
ÞŽ	OTF File /usr/local/otf/Olympus_60X_142_10612.ot[
Image: State of the state	Method Enhanced Ratio (aggressive)
523	Number of Cycles 110
<mark>594</mark>	Noise Filtering Medium (200 nm)
	Apply Correction
	Deconvolve Projections
	Run Options More Options RV Show image when finished
	Done Do It



- Not all tools automatically save their output. You will be prompted to save unsaved files before closing.
- Histogram. Select the histogram button (red circle in the below screenshot) or use the small histograms located on the top of the image window (square in the below screenshot). This will adjust display scaling but will not alter your experimental data.



2.7.3.2 Deconvolution

• Open the Deconvolution tool in Softworx Section in the acquisition software: "Process"/"Deconvolution"

Excitation POL -50 / 28 Emission POL -50 / 0 %T 100% Illumination TRANS Exposure 1.0000 Inage size 1024x1024	Applications	Places				
File View Options Calibration Help Image size 1 1 1 1 Image size 1024x1024 Image size 1024x1024 Image size 1 1		Resolve3D	_ ×			
Excitation POL -50 / 28 Emission POL -50 / 28 Scratch Space Flat Field Correction (Resolve3D) %T 100% Illumination TRANS Exposure 1.0000 1.0000 Find Image size 1024x1024	File Viev	v Options Calibrati	on Help		softWoRx 6.5.2 Running On	Host: cn62532
Excitation POL -50 / 28 Emission POL -50 / 0 %T 100% %T 100% Illumination ITRANS Exposure 10.000 - Find Image size 1024x1024 - I Calibrate 3 Z Align Image Align Image		- <u>-</u>	ø	<u>File E</u> dit <u>V</u> iew	Process Filter Model Measure	<u>Conversions</u> <u>U</u> ti
Emission POL -50 / 0 Flat Field Correction (Resolve3D) 5% (1739 - 100%) %T 100% Flat Field Correction (Ultra) 3. Win Illumination ITRANS File View Optio Nearest Neighbor Exposure 1.0000 < Find File View Optio Nearest Neighbor Image size 1024x1024 < Calibrate 3 Z Align Image Align Image				📐 🔗 Data Fold	Correct	1
%T 100% Illumination Flat Field Correction (Ultra) 3: Wir Illumination ITRANS Eile View Optio Nearest Neighbor Exposure 1.0000 - Find Equalize Time Points Align Image Image size 1024x1024 - I Calibrate 3 Z Align Image Align Image				Scratch Space	Flat Field Correction (<u>Resolve</u> 3D)	5% (1739 0
Illumination ITRANS File View Option Nearest Neighbor Apply Constrained Exposure 1.0000 Find File View Option Nearest Neighbor Image					Flat Field Correction (Ultra)	3: Win
Exposure 10000 Image Find Image Imag]	File View Ontin	Deconvolve	Apply Constrained
Image size 1024x1024 Calibrate 3 Z Align Image)^ -	1		Nearest Neighbor	
	Exposure	1.0000 T Find		∠ □ □ ∞	E <u>q</u> ualize Time Points	
Lens 60X	Image size	1024x1024 💌	🔲 🗆 Calibrate	3 Z	<u>A</u> lign Image	
	Lens	60X 🗸	Info	Θ^{Δ}	Task Builder	
Bin 1x1 🗾 Aux. Mag. 🕎 OMX SI Reconstruction	Bin	1x1 •	🔲 Aux. Mag.	<u>₹"</u> ?	OMX SI Reconstruction	100000
Pixel size 0.1069 µm P∑4 OMX PSF to OTF	Pixel size	0.1069 µm		ÞŽd	OMX PSF to OTF	
O IE ★ ★ ★ ★ ★ AF I ★ AF I I ★ AF I I ★ AF I I I ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓		· ┤╳╷┰╷╷╪	AF 💼 🗖		$Create\;OM\underline{X}\;Image\;Alignment$	A States
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dY 10.00 Image: Constraint of the second se					Legacy >	

- Load the image to the Deconvolution tool using one of the methods described in Section 2.7.3.1. The information of the image will be loaded to the tool from the raw image file. Default settings are usually adequate for most applications.
- Process deconvolution. Click "Do it" to run deconvolution.

2.7.3.3 Quick projection

Open the Projection tool in Softworx Section in the acquisition software: "View"/"Quick projection". This will project your 3D data into 2D format by either Max intensity, Average or Sum method. 2.7.3.4 Volume Viewer

Open the Volume viewer tool in Softworx Section in the acquisition software: "View"/"Volume viewer".



DATA & RECORDS MANAGEMENT

All data must be saved onto the local computer (kept for 7 days before auto deleting):

Data1\"firstname.surname". Your data gets transferred to the server automatically at mid night every day: Scientific Platforms (<u>\\Scientific Platforms\Cell</u> Imaging\Deltavision\"firstname.surname" (kept for 14 days before auto deleting). NO USBs are allowed on the acquisition computer to minimise virus risk.

REFERENCES

The hardcopy of the full manual provided by the instrument vendor is available on the shelve above the microscope and on the Westmead Imaging site

DOCUMENT CONTROL

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Enquiries Contact:	Name: Hong Yu
	Position: Advanced Cell Imaging Specialist
	Email: hong.yu@sydney.edu.au
	Phone: 8627 3211

REVISION HISTORY

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