Huygens Professional Deconvolution Guide

A. Thompson, A. Gaudin, and R. Amor QBI Microscopy Facility, Queensland Brain Institute Research Lane, The University of Queensland St Lucia, 4072 QLD, AUSTRALIA <r.amor@uq.edu.au>

A note on sampling density

When we acquire images using a microscope and store these in a computer, we are using the technique of "sampling", that is, we are converting an analog signal (continuous in time or space) into digital form (discrete steps) [1, 2]. Ideally, when acquiring images, one should aim for a sampling density that satisfies the Nyquist criterion: there must be two samples for every structure one wishes to resolve [3].

Scientific Volume Imaging have a Nyquist calculator online [4]. To use the calculator, choose the appropriate microscope type, the numerical aperture of the imaging objective, the excitation and emission wavelengths, the number of excitation photons (1 for wide-field fluorescence, laser-scanning confocal and spinning disk confocal; 2 for two-photon microscopy), and the refractive index of the immersion medium. For example, for images of the Alexa 488 channel acquired on the Yokogawa spinning disk confocal microscope using the 63x/1.4 NA oil-immersion objective, the parameters should be what are shown in Fig. 1a. Click "Calculate". The calculator then shows the results and for this particular example, a sampling rate of 43 nm is required in the lateral (X and Y) direction and 130 nm in the axial (Z) direction (Fig. 1b). The sampling rate in X and Y is a function of the optics in the light path and the relay optics in front of the detectors and therefore is fixed, but the sampling rate in Z is user-defined.

S Nyquist rate a	and PSF calculator	Parameter	Value
		Microscope type	Spinning Disk
Microscope type	Spinning Disk	Numerical aperture	1.4
		Excitation wavelength	488
Numerical aperture	1.4	Emission wavelength	520
Excitation wavelength	488 n	n Number of excitation photons	1
		Lens immersion refractive index	1.515
Emission wavelength	520 n	n The optical axis lays along z. You	r Nyquist sampling is:
Number of excitation photons Lens immersion refractive index	1 Oil ÷ 1.515 Calculate a Point Spread Function	x: 43 nm y: 43 nm z: 130 nm — Set your zooms and scannii of 43 nm × 43 nm (or smaller)	ng steps so that each pixel covers a x-y area
		acquiring a 3D stack (or smalle	pper so that it takes steps of 130 nm when er)

Parameter	value				
Microscope type	Spinning Disk				
Numerical aperture	1.4				
Excitation wavelength	488				
Emission wavelength	520				
Number of excitation photons	1				
Lens immersion refractive index	1.515				
The optical axis lays along z. Your Nyquist sampling is:					
x: 43 nm					
y: 43 nm					
z: 130 nm					

(a) Microscope parameters for the Nyquist calculator.

(b) Calculator results.

Figure 1: Scientific Volume Imaging's Nyquist calculator.

We have compiled tables of optimal and actual sampling rates for the Diskovery (Table 1; note 50- μ m and 100- μ m pinholes) and Yokogawa (Table 3) spinning disk confocal microscopes, and for the LSM 510 and 710 confocal laser-scanning microscopes (Table 4). The backprojected pinhole radii and pinhole spacing are required in the deconvolution step (please refer to the succeeding sections).

Table 1: Diskovery spinning disk confocal microscope deconvolution parameters, $50-\mu m$ and $100-\mu m$ pinholes. For images with ~10,000–20,000 grey levels, start with an SNR value of 40 and check the deconvolution result for noise and artefacts (please refer to the succeeding discussion of the deconvolution process). Lower-intensity images would require lower SNR values. ***Please keep your settings consistent if you wish to compare labelling intensity. ***

Objective	N.A.	Actual	Optimal	Standard	Optimal	Backprojected	Pinhole	Backprojected	Pinhole
		XY	XY	Z (nm)	Z (nm)	pinhole ra-	spacing,	pinhole ra-	spacing,
		(nm)	(nm)			dius, $50-\mu m$	50 - μm	dius, 100-	$100\text{-}\mu\mathrm{m}$
						pinholes	pinholes	μm pinholes	pinholes
						(nm)	(μm)	(nm)	(μm)
10x	0.45	559.6							
20x	0.75	276.3	76	1200-	360	1125	13.23	2421	26.07
				800					
40xW	1.15	140.2	50	1000-	186	562.5	6.615		
				400					
60xW	1.27	93.7	50	600-300	133	375	4.42	831.5	8.872
60x oil	1.4	93.4	43	400-200	130	375	4.42	831.5	8.872
60x oil	1.49	93.7	40	300-200	98	375	4.42	831.5	8.872
TIRF									
100x oil	1.45	56.03	42		113	225	2.652	479	5.287

Table 2: Yokogawa spinning disk confocal microscope deconvolution parameters. Please follow the same guidelines as those discussed for the Diskovery spinning disk confocal.

Objective	N.A.	Actual XY	Optimal XY	Standard Z	Optimal Z	Backprojected	Pinhole
		(nm)	(nm)	(nm)	(nm)	pinhole ra-	spacing
						dius (nm)	(μm)
10x	0.45	625	135	3000	1140	2500	50
20x	0.8	313	76	1200-800	305	1250	30
40xW	1.2	156	50	1000-400	163	625	15
63xW	1.2	99	50	600-300	163	416.7	8.33
63x oil	1.4	99	43	400-200	130	416.7	8.33
100x oil	1.4	63	43	400-200	130	250	5

Objective	N.A.	Actual XY	Optimal XY	Standard Z	Optimal Z	Backprojected	Pinhole
		(nm)	(nm)	(nm)	(nm)	pinhole ra-	spacing
						dius (nm)	(μm)
10x	0.45	650	135	3000	1140	2530	50
20x	0.8	325	76	1200-800	305	1170	25.21
30xS confocal	1.05	216.67	58	1000-400	259	765	16.63
30xS SoRa	1.05	67.71	58	1000-400	259	240	5.23
40xS confocal	1.25	162.50	48	600-300	160	555	12.51
40xS SoRa	1.25	50.78	48	600-300	160	200	3.91
60x oil confocal	1.42	108.33	42	400-200	123	390	8.36
60x oil SoRa	1.42	33.85	42	400-200	123	125	2.60
100x oil confocal	1.45	65	42	400-200	113	235	4.98
100x oil SoRa	1.45	20.31	42	400-200	113	85	1.57

Table 3: Olympus SpinSR10 spinning disk confocal super-resolution microscope deconvolution parameters.

Table 4: LSM 510 and 710 laser-scanning confocal microscope deconvolution parameters. When imaging with a pinhole diameter of 1 Airy disk unit, lateral sampling distances may be up to $1.6 \times$ that of the recommended Nyquist criteria without significantly compromising image quality. When small pinhole diameters are used (< 0.5 Airy disk units), these may be up to $1.3 \times$ larger; when using large pinhole diameters (> 4 Airy disk units), these may be up to $2 \times$ larger. An SNR value of 15-20 is typical for laser-scanning confocal. For images with ~10,000–20,000 grey levels, start with an SNR of 20 and check the result for noise or artefacts. Lower-intensity images would require lower SNR values.

Objective	N.A.	Actual XY (nm)	Optimal XY (nm)	Standard Z (nm)	Optimal Z (nm)
10x	0.45	600-400	135	3000	1140
20x	0.8	300-200	76	1200-800	305
32xW	0.85	200-100	71	900-600	257
40xW	1.2	200-100	50	1000-400	163
63xW	1.2	100-50	50	600-300	163
63x oil	1.4	100-50	43	400-200	130

Connecting via XFast/MATE (VirtualGL)

1. XFast is a lightweight desktop environment that incorporates a display manager and a window manager and allows access to a remote graphics hardware [5]. The MATE Desktop Environment, the continuation of GNOME 2, which featured a simple desktop where users can interact with virtual objects [6], provides an intuitive desktop environment for Linux and other Unix-like operating systems and is actively developed to support new technologies [7]. VirtualGL is an open source toolkit that gives any Linux or Unix remote display software the ability to run OpenGL applications with full hardware acceleration, virtualising GPU hardware and allowing GPUs to be shared among multiple users, making it possible for large 3D workstations to be replaced with laptops and, more importantly, eliminating the workstation and the network as barriers to data size [8].

We will use XFast and MATE (VirtualGL) to connect remotely to QBI's deconvolution server, visnode1, using a laptop or PC, making the PC a terminal that interacts with visnode1 and forwards the display from visnode1 to the PC.

Open a web browser and go to https://visnode1.hpc.net.uq.edu.au:3443 and log on using your UQ credentials (Fig. 2a). Click Launch Session > MATE (Virtual GL) > Launch (Fig. 2b).

THE UNIVERSITY OF QUEENSLAND AUSTRALIA				
User Name				
uqramor				
Password				
••••••				
Use Public Key Authentication Manage Private Keys				
Log In				
	Admin Login Build: 2.4.13			

(a) XFast login prompt.

The Exercise of the Constrainty			
My Sessions			
Launch Session			Search Bookmarks
	MATE MATE VirtualGL)		Global Bookmarks My Bookmarks
	vglrun mate-session	Single •	Launch Cancel

(b) Launching a MATE (VirtualGL) session.

Figure 2: Launching a MATE (VirtualGL) session using XFast.

3. This launches the MATE (VirtualGL) desktop (Fig. 3a). In Settings, Image Quality, Frame Rate and other properties can be tweaked, especially if working on a VPN. To create a shortcut to Huygens Professional, right-click on the desktop and choose "Create Launcher" (Fig. 3b). Give the launcher a name, for example, "Huygens Pro," and in the "Command" field, type in "/usr/local/bin/huygenspro" (Fig. 3c).

🔊 Applications Places System 🗮 🔟 😓	0 / 0 < +		🔊 🏟 🛒 Fri Feb 1. 10:2
	Network Type: Autodetect *		
Computer	Use WebAssembly (faster decoding)		
ugramor's Home	Image Quality: Highest		
	Frame Rate: 60 fps		
	Frame Window: 2 frames		
	Mouse Update Rate : 1ms		
Rubbish			
(a) MA	TE (VirtualGL) desktop env	ironment.	
Create <u>F</u> older	· c	reate Launcher	×
Create L <u>a</u> uncher			
Create Document	▶ Type:	Application	*
Open in Terminal	Name:	Huygens Pro	
Organise Desktop by Name	Command:	sr/local/bin/huygenspro	Browse
✓ <u>K</u> eep Aligned	Comment:	/here is the wise one nar	ned Knuth?
Paste			48.0%
Change Desktop <u>B</u> ackground	Снер	Cancel	₩ OK
(b) Creating a launchor	(a) La	uncher parameters	
(b) Creating a faulteller.	(C) La	anonoi parameters.	

Figure 3: MATE (VirtualGL) desktop and creating a shortcut to Huygens Professional.

4. Create your "uq_username" folder in /scratch/visnode/ (Fig. 4). You can then point Huygens Professional to this location to open files to be deconvolved, and save outputs.



Figure 4: All data should be stored on the /scratch/visnode/ directory.

5. To streamline data transfer to /scratch/visnode/uq_username from your RDM collection and back, bookmark your RDM collection by opening "Computer" on the desktop and navigating to your collection at /File System/afm01/Qn/Qwxyz and clicking on Bookmark > + Add Bookmark at the top-level menu (Fig. 5). You can bookmark your /scratch/visnode/uq_username folder in the same way. Note that /afm01/Q0 contains collections Q0001-Q0999, /afm01/Q1 contains Q1001-Q1999, and so on.

0		Q2593				\odot \otimes \otimes
File Edit View Go	Bookmarks Help					
🖑 Back 🔻 💮 Fo	rward 🔻 🎓 🕒 🤂	🗟 💆 🕒 10	00% 💿 Icon	View	Q	
Places 🔻 🗶	📝 🖪 afm01 Q	2 Q2593				
Computer uqramor Desktop	Roper	Rumelo				
File System Comments Co						
Music						
🛅 Videos 🛞 Rubbish						
Devices OS						
© Q1141						
Network	2 items, Free space: 114.2	ТВ				al

Figure 5: Bookmarking one's RDM collection.

6. Transfer your raw datasets to your /scratch/visnode/uq_username folder. Sub-folders can be created if required.

Deconvolution using Huygens Professional

1. Go back to the MATE (VirtualGL) desktop environment and double-click on your recently-created Huygens Professional Launcher. This launches Huygens Professional (Fig. 6).



Figure 6: Launching the Huygens Professional graphical user interface on a MATE (VirtualGL) desktop.

2. Open your raw dataset by going to File > Open. If the dataset is multi-channel, or is a mosaic or a time series, Huygens Professional will automatically recognise this (Fig. 7a). Click on "Load selection" to open all of the dataset. When prompted for the target data type, choose "To float (default in silent mode)" (Fig. 7b). Huygens will prompt "Please check all image parameters ... " (Fig. 7c), click "OK", as defining the acquisition parameters will be the next step.

	🙀 Huygens F	ïle Series Tool				×	
			?				
	Huygens Pro /ibscratch/us	fessional scanned the directo ers/uqramor/Yokogawa/Ange	ory elo/' for matching fik	e names.			
	6 matching n Would you lik	ames were found. se to select a file series, or jus	st the single file				
	File pattern	SK2 -SK1 _63 _XY1502663	503 _Z00 _T0 _C	Channel	▼ .tif		
	From			0	-		
	To			2	-		
	Step size			1	-		
	File count	3					
	Message						
	Help]	Load selection	Load sing	le file Can	cel	
		(a) Oper	ning a file series.	_			
📱 Question	1		×	\$			×
? Ir ta	nput file is in 16 arget data type:	bit TIFF format: select			Please check a "SK2-SK1_63_ 0_C0" Modified X, Y, 2	ll image paramet XY1502663503_ Zgeometry	tersof _Z00_T
To float (default i	n silent mode)	To 16 bit signed	8 bit unsigned			<u>DK</u>	
	-						

(b) Target data type.

(c) Check image parameters.

Figure 7: Opening a dataset in Huygens Professional.

3. The dataset is now opened in Huygens Professional. To edit the acquisition parameters, click on the "Edit microscope parameters" icon. For each channel, choose the appropriate microscope type and give the correct values for the backprojected pinhole radius, excitation and emission wavelengths, number of photons used for excitation, pinhole spacing, sampling intervals in X, Y and Z (in nm/pixel), objective numerical aperture, and lens immersion and embedding medium (Fig. 8a). Refer to Tables 1–3 for these values. Click on "Set all verified". At this point, one can then click "Save" to save these parameters for use later on with similar datasets, or for running Huygens Professional in batch mode. Click "Accept". The colours for the channels will now be updated to what they should be (Fig. 8b).

Edit Microscopic Parameters - SK2-SK1_63_XY1502663503_Z00_T0_C	0	- 🗆 X
General parameters	Channel parameters	Image properties
Sampling intervals:	Select channel:	SK2-SK1_63_XY1502663503_Z00_T0_C0
e X (mm)	0: Spinning disk	Dimensions: 2048×2048×49×0
	1: Spinning disk 2: Spinning disk	Data type: 32 bit floating point
• • • (nm) 99.0		Size: 2.3 GiB
● ∠ (nm) 330.0	Microscope type Spinning disl	Templates:
Optical parameters:	Backprojected pinhole (nm) 416.7	Reports:
Numerical aperture	Excitation wavelength (nm)	Template loaded.
Refractive indexes:	Emission wavelength (nm)	The X sampling is too large. The Y sampling is too large.
	Multi photon excitation	The Z sampling is too large.
● Embedding med. Water	Crimina Disk (all shares la).	intervals.
	Spinning Disk (all channels):	
Advanced:	 Pinhole spacing (μm) 8.33 	
Objective quality Good		
Coverslip: Launch editor		
 Coverslip position (μm) 0.000 		
◎ Imaging direction Upward ▼		
Help	All parameters verified Set all verified	Revert Cancel Accept
	(a) Editing microscope parameters.	
K Huygens Professional		- 🗆 X
<u>File Edit T</u> ools <u>D</u> econvolution V <u>i</u> sualization <u>A</u> nalysis <u>H</u> elp		
Size Color GPU New Open Save As Express Decom	Param Ops Batch Stitcher Del. Del. all Clear Copy Pa	👔 📴 🖳 📲 📲 📔 🗱 🖓 📑 🗸
-Thumbnail overview-	Task reports	s Statistics Optical Nyquist Histogram
a b	c psf	An a files starting in 63_XY1502663503_Z00_T0_C0.tif to image _63_XY1502663503_Z00_T0_C0* je intensities were read in unscaled.
~ 28	-Huygensic < 1> imq	open
	{/ibscrat 63503_200	ch/users/uqramor/Yokogawa/Angelo/SK2-SK1_63_XY15026 _T0_C0.tif} -query -logEnable -series tool
	=> SK2-SK < 2> SK2-	1_63_XY1502663503_Z00_T0_C0 SK1_63_XY1502663503_Z00_T0_C0 show -mode thumb
<u></u>	=> : :HuTh < 3>	umpnait::processMethod_5

(b) Colours update after microscope parameters are edited.

Figure 8: The Huygens Professional graphical user interface.

4. With your dataset ticked, click on "Decon" (represented by a wand icon). This will open the deconvolution wizard (Fig. 9). Click "Enter wizard".



Figure 9: The deconvolution wizard.

SK2-SK1_63_XY150

5. Inside the wizard, choose a measured PSF file for the specific objective used when the image was acquired (Fig. 10a). Measured PSF files can be obtained from \group_microscopy\Templates for Huygens Deconvolution\Measured PSF files. Click next (green arrow). Define a crop window if required by launching the cropper (Fig. 10b), or skip cropping by clicking next. Select the first channel to be deconvolved. For the histogram, choose "Logarithmic" for "Vertical mapping function" and click "Compute". This brings up a histogram of the current channel, which can be checked for clipped voxels and for the black level (Fig. 10c). Click next.

Deconvolution wizard - pre	eprocessing		Deconvolution wiz	rard - preprocessing
PSF Selection		Cropping stage		
If you would like to use a measured PSF for deconvolution, then please select it here. If you would like to use the automatically generated theoretical PSF, simply		To view the borders around, below and above the object press the button below to launch the Cropper.		
skip this stage.		Launch the C	ropper	
Load a measured	PSF			
Import from main window	Select 👻	Alternatively,	you can leave everyting up	to the automatic cropper:
Open file	Browse	Auto crop		
PSF file matches microscope type.				
Loaded and selected image: Tokogawa_SUC_b				
—	→		⇐	⇒
(a) Loading a measur	red PSF file.	(b)	Window for lau	inching the cropper. $ \Box$ \times
<u>F</u> ile <u>Options</u> <u>H</u> elp				
SK2-SK1_63_XY150 Yokogawa_SDC_63 SK2-SK1_63_XY150 Yokogawa_SDC_63	•••• Image: SK2-SK1_63_XY15026 •••• Position: (101.93 69.40 8 ••••• Value: ch 0: 216.0000	53503_200_T0_C0:Cl	Companied How D 2.568 E08 58 2.474 E05 C C C C C C C C C C C C C	> >
Reserved for channel 0 results	Help Reports Preprocessing The histogram enables you to check a few quality aspects of the image at a glance.	v important	Deconvo Inspecting the image hist Computing histogram in chann No clipped voxels detected. Voxel value range: min: 0 max:	Iution wizard - preprocessing ogram el 0 Done. 960.
	Firstly, it allows you to check for saturation measured values. If clipping is present th show sharp peaks at the left or rightmost s Secondly, you can spot significant amour This shows up as a gap on the left. You can choose to generate the histogran linear or logarithmic vertical scaling funct	n (clipping) of the e histogram will sides. ts of blacklevel. n with either a on.	You can use the histogram to c values. Saturation or clipped v the histogram. Also you can se present by spotting a gap on th If you'd like to view the histogra select the 'Toggle logarithmic s	heck whether there is no saturation of the pixel alues will show up as a spike at the utmost right of whether significant background or blacklevel are e left. In with a different vertical mapping function please caling' button from the row above the histogram.

(c) Inspecting the image histogram.

•

-Wizard status Obtained initial parameters from image SK2-SK1_63_XY1502663503_Z00_T0_C0.PSF Yokogawa_SDC_63xW_0_163Z_measured_PSF_488_56 % e17 locat

*

• •

Yokogawa_SE 8_647 loaded.

Figure 10: Deconvolution wizard preprocessing steps: PSF selection, cropping, and inspecting the histogram.

6. Determine the image background either manually by making the cursor hover on a background feature and reading the gray level on the display, or automatically by clicking "Auto" (Fig. 11a). The wizard will then show the background value it will use for the deconvolution. Click "Accept".

The last step is the deconvolution setup. Choose a maximum number of iterations (the default is 40), a quality threshold (a threshold of "0.1" requires a 10 % improvement between iterations), and a value for the signal to noise ratio (Fig. 11b).

A note on the signal to noise ratio:

The signal to noise ratio (SNR) is used in Huygens Professional as a parameter that controls the *sharpness* of the restoration, that is, the higher the SNR value used, the sharper the result [11]. However, using a value that is too high may result in the noise being enhanced and artefacts being generated. On the other hand, using an SNR value that is too low may lead to real structures being considered as noise and the result may be too smooth and lacking in details.

The SNR value can be estimated by getting the square root of the number of electrons measured by the detector in the brightest voxel of the image [12]. Incoming photons excite electrons in the detector which are then measured by the electronics hardware within. The number of electrons is defined as the intensity value (measured in grey levels or ADU – analog to digital units) multiplied by the conversion factor of the camera (given in e/ADU or ADU^{-1}). For example, the Diskovery spinning disk confocal microscope uses two Andor Zyla 4.2 sCMOS cameras, which have a black level of ~100 and a conversion factor of 0.45 e/ADU [13]. This means that if the brightest voxel in an image acquired using these cameras have ~2,000 grey levels, the estimated SNR would be:

$$SNR = \sqrt{N_{electrons,max}}$$

= $\sqrt{(maximum intensity - black level) \times conversion factor}$
= $\sqrt{(2,000 - 100) ADU \times 0.45 ADU^{-1}}$
= 29.2

Therefore one could use an SNR value of 29, but it is always best to check the deconvolution result in order to determine what SNR value is optimal. Imaging specialists from Scientific Volume Imaging often advise using an SNR of 20 even though a calculated SNR estimate yields a higher value. The following values can be a good starting point:

Noise-free wide-field image: SNR ~ 50 Spinning disk confocal image: SNR $\sim 20{-}30$ Confocal (laser-scanned) image: SNR $\sim 15{-}20$

After settling on a value for the SNR, choose "Optimized" for "Iteration mode" and "Auto" for "Brick layout". Click "Deconvolve". This begins the deconvolution run.

Estimating parameters			Deconvolution setup		
Background			Deconvolving the image		
In this stage the background value of channel 0 of the image can be automatically estimated or manually entered.			In this stage channel 0 of the original image will be deconvolved on the basis of the PSF and background as computed in the previous stages. Selected algorithm: CMLE.		
Automatic estimation			The result will be stored in Image de	scon.onu.	
Estimation mode	Lowest -		Below are the default values for dec	onvolving images. If necess	ary you can
Area radius (micron)	0.7		change these values.		
			Maximum iterations		40
			Signal to noise ratio		20
Manual mode Enter the background value of a specific region in the image. You will be able to inspect the intensity profiles of the background areas.			Quality threshold		0.1
			Iteration mode		Optimized 🔻
			Brick layout		Auto 🔻
Anual Manual	Auto		—	Deconvolv	e

(a) Determining the background.

(b) Deconvolution setup.

Figure 11: Background estimation and deconvolution parameters.

7. Once the wizard finishes deconvolving the first channel, click "Resume" to resume the iterations starting from the current result, "Restart channel" to run the deconvolution again using different background settings, or "Accept, to next channel" to accept the result and proceed to the next channel. Repeat the steps above for the next channel, and so on.

After all the channels have been deconvolved (Fig. 12), select "All done". Click "Save template" to save the same deconvolution parameters to use on similarly-acquired datasets, or for running in batch mode. Click "Done". To save the result, click on its thumbnail and go to File > Save as, then choose "ICS2 image format". Choose the target directory and click "OK". The deconvolved dataset will now be saved and can be retrieved using FileZilla. Huygens Professional works with the 32-bit float data format and the ICS2 file format preserves the data as 32-bit float and also preserves the metadata. The 32-bit .ics2 image data file can be read by Imaris, FIJI and other software packages. For more details on the ICS file format, please visit https://svi.nl/ICSfileFormat.



Figure 12: Raw multi-channel dataset (left) and deconvolution result (right).

A note on clusterdata storage and clearing the $\ensuremath{\texttt{ExecLog}}$

- 1. All data for deconvolution should be stored on the /scratch/visnode directory, and should be removed periodically as this directory is not meant for long-term storage. However, when users perform deconvolution on these files, Huygens Professional will generate log files on a *per user* basis on their individual folders in \clusterdata\uq_username\SVI\ExecLog. For users performing multiple deconvolution runs, the log files can accumulate rapidly and cause issues by having users exceed their storage quota on clusterdata. One can automate the removal of old log files by following the steps below.
- 2. Right-click on the desktop and create a new "Empty File" (Fig. 13).



Figure 13: Creating a new document.

3. Give the document a name such as "ClearExecLog". Right-click on the file and open it with the "Pluma" text editor. Edit by adding the text below, replacing "uq_username" with your own UQ username (Fig. 14): #/bin/bash

```
find /clusterdata/uq_username/SVI/ExecLog/* -mtime +14 -exec rm -fr {} \;
```



Figure 14: Editing "ClearExecLog" in Pluma.

4. Right-click on the new file and go to Properties > Permissions. Tick "Allow executing file as program" (Fig. 15)

	🖻 Open		• CI	earExecLog Properties	۲
	Open With Other Application		Basic Emblems	Permissions Open With Not	tes
Computer	<mark>⊮</mark> Cut ₱ Copy		Owner:	uqramor - Rumelo Amor - 562	2309
uqramor's Home	Make Link Rename		Access:	Read and write	₹
	Copy to	•	Group:	users	* *
Huygens Pro	Move to Rubbish	-	Access:	Read-only	Ŧ
	8 Delete	- 1	Others		
	Resize Icon		Access:	Read-only	Ţ
Restore Icon's Original Size		-1	Execute:	Allow executing file as provide the second secon	ogram
	Compress		SELinux context:	unknown	
#/1 fi	Roperties		Last changed:	unknown	
FIJI ClearE	ixecLog		🙄 Help	[X Close

Figure 15: Executing "ClearExecLog" as a program.

5. At the top-level menu, go to System > Preferences > Personal > Startup Applications (Fig. 16).



Figure 16: Adding a new startup application.

6. On the Startup Applications Preferences window, add a new "ClearExecLog" startup program and point the command to the ClearExecLog file on the desktop (Fig. 17). This will delete files in the \clusterdata\uq_username\SVI\ExecLog folder that are older than 14 days. This duration can be changed by editing the "+14" argument in ClearExecLog using Pluma.

		Startup Applications Preferences			
		Startup Programs Options			
		Additional startup programs:			
		Automatic Bug Reporting Tool ABRT notification applet			
		Certificate and Key Storage GNOME Keyring: PKCS#11 Component			
	Add Startup Program	ClearExecLog No description			
Name:	ClearExecLog	Input Method starter			
Command:	/clusterdata/uqramor/E Browse	MATE Settings Daemon No description			
Comment:	Clear log files.	Network 🔻			
	● Cancel ♣ Add	Close X Close			

Figure 17: Executing "ClearExecLog" at each startup.

Old FileZilla instructions

- 1. For copying data to and from the cluster, we recommend using FileZilla, a free cross-platform File Transfer Protocol (FTP) application. Download FileZilla from https://filezilla-project.org/download.php and install.
- 2. When running FileZilla for the first time, go to File > Site Manager. Click "New Site". In the "General" tab, type in "visnode1.hpc.net.uq.edu.au" for "Host", choose "SFTP SSH File Transfer Protocol" for "Protocol" and "Ask for password" for "Logon Type" (Fig. 18a). Click "Connect". Log on using your UQ credentials (Fig. 18b). Once logged on, FileZilla will show a *Local site* (Fig. 18c) and a *Remote site* (Fig. 18c). The local site can be any location: a local drive or a network drive (for example, group_microscopy) as long as it has been mapped. For the remote site, go to /scratch/visnode/uq_username. You can then point Huygens Professional to this location to open files to be deconvolved, and save outputs.

Site Manager	×	
Select Entry:	General Advanced Transfer Settings Charset	Enter password X
group_microsc inode2 visnode1	Protocol: SFTP - SSH File Transfer Protocol v Host: visnode1.hpc.net.uq.edu.au Port:	Please enter a password for this server:
		Name: visnode1
	Logon Type: Ask for password V User: Uqramor	Host: visnode1.hpc.net.uq.edu.au
	Pass <u>w</u> ord:	User: uqramor
	Background color: None V	Password:
New Site New Eolder New Bookmark Rename		Remember password until FileZilla is closed
Delete Dupl <u>i</u> cate	×	OK Cancel
	Connect OK Cancel	Cancer

(a) FileZilla Site Manager.

(b) Password prompt.



(c) FileZilla local site.

Remote site:	/afm01/so	ratch/qbi/uqramor/Diskovery	~
	?	BF	^
	i	Diskovery	
		? Adahir	
		? Andrew	
		? James	
		? Lee	
		? Leon_3	- 10
		? Leon_4	
		? Leon_5_old	
		? Rosina	
		? Wei_W	~

(d) Remote site.

Figure 18: The FileZille user interface.

3. Upload your raw datasets to your /scratch/visnode folder. Sub-folders can be created if required. To upload, right-click on the file and click "Upload", or drag and drop.

Old Windows instructions [*PuTTY and Xming still work*]

- 1. When we connect to QBI's deconvolution server using a PC, we are essentially making the PC a terminal, that is, it interacts with the server using the X Window System, forwarding the display from the server to the PC [9, 10]. For this link to work, we use the PuTTY terminal emulator and the Xming X Window client. Download PuTTY from https://www.chiark.greenend.org.uk/~sgtatham/putty/ and Xming from https://sourceforge.net/projects/xming/ and install them by following the prompts.
- 2. After installing Xming, run XLaunch and confirm that the settings are the same as in Fig. 19.

X Display settings X	Session type X
Select display settings Choose how Xming displays programs.	Select how to start Xming Choose session type and whether a dient is started immediately.
Multiple windows	Start no dient This will just start Xming. You will be able to start local dients later.
One window	 Start a program This will start a local or remote program which will connect to Xming. You will be able to start local clients later too. Remote programs are started using PuTTY/SSH.
Display number 0	Open session via XDMCP This will start a remote XDMCP session. Starting local clients later is limited. This option is not available with the "Multiple windows" mode.
< Back Next > Cancel Help	< Back Next > Cancel Help
(a) XLaunch display settings.	(b) Session type.
X Additional parameters X	X Finish configuration X
Specify parameter settings Enter dipboard, remote font server, and all other parameters.	Configuration complete Choose whether to save your settings to an XML file.
Clipboard IN Access Control Start the integrated clipboard manager Disable Server Access Control	Click Finish to start Xming,
Remote font server (if any)	You may also 'Save configuration' for re-use (run automatically or alter via -load option).
Additional parameters for Xming	Save configuration Include PuTTY Password as insecure dear text
Additional parameters for PuTTY or SSH	
< Back Next > Cancel Help	< Back Finish Cancel Help
(c) Additional parameters.	(d) Finish configuration.

Figure 19: XLaunch settings.

- 3. Run PuTTY and for the category "Session", type in "visnode1.hpc.net.uq.edu.au" for "Host Name" and "22" for "Port" (Fig. 20a). For the category Connection > SSH, click on X11 and tick "Enable X11 forwarding" and type in "localhost:0.0" for "X display location" (Fig. 20b). Go back to Session, type in "visnode1" under "Saved Sessions" and click Save. This will save the configuration for visnode1.
- 4. For copying data to and from the cluster, we recommend using FileZilla, a free cross-platform File Transfer Protocol (FTP) application. Download FileZilla from https://filezilla-project.org/download.php and install. Follow the XFast/MATE (VirtualGL) instructions (Sec. 2) with regards to setting up and using FileZilla.



(a) PuTTY session configuration.

(b) X11 forwarding.

Figure 20: PuTTY configuration settings.

5. Run Xming. Nothing happens visually but it will now be running in the background.

6. Run PuTTY. Launch the terminal by clicking on inode2, "Load", and then "Open". Log on using the credentials given by QBI IT. Once logged on, run Huygens Professional by typing /usr/local/bin/huygenspro (Fig. 21). Follow the XFast/MATE (VirtualGL) instructions with regards to opening datasets, editing the microscope parameters and the deconvolution process.

P inode2.qbi.uq.edu.au - PuTTY -		o x
login as: uqramor uqramor@inode2.qbi.uq.edu.au's password:		^
Last login: Wed Oct 11 15:42:24 2017 from qbi-mic-ws-02.qbi.uq.edu.au		
Disk quotas for user uqramor (uid 1562309):		
Filesystem space quota limit grace files quota limi	Lt	grace
192.168.0.4:/ibscratch		
573G 2048G 2100G 6160 0	U	
3257M 5120M 5120M 0 0	0	
-bash-4.2\$ /usr/local/bin/huygenspro		

Figure 21: PuTTY terminal log on.

Old Mac OS X instructions [*Terminal and XQuartz still work*]

- 1. When we connect to QBI's deconvolution server using a Mac, we are making it a terminal, that is, we make it interact with the server, forwarding the display from the server to the Mac. The Mac is able to do this by using XQuartz. The XQuartz project is an open-source initiative to develop a version of the X Window System that will run on OS X [14]. Download XQuartz from https://www.xquartz.org/ and install by following the prompts.
- 2. For copying data to and from the cluster, we recommend using FileZilla, a free cross-platform File Transfer Protocol (FTP) application. Download FileZilla for Mac OS X from https://filezilla-project.org/download.php?platform=osx and install. Follow the XFast/MATE (VirtualGL) instructions with regards to setting up and using FileZilla.

3. To log on to the cluster, open terminal by going to Applications > Utilities > Terminal (Fig. 22a). Type in ssh -Y your_username@visnode1.hpc.net.uq.edu.au and enter the password given by QBI IT. Once logged on, type in /usr/local/bin/huygenspro. This opens the Huygens Professional OS X graphical user interface (Fig. 22b). Follow the XFast/MATE (VirtualGL) instructions with regards to opening datasets, editing the microscope parameters and the deconvolution process.



(b) The Huygens Professional GUI on Mac OS X.

Figure 22: Logging on to the cluster and launching Huygens Professional on Mac OS X.

Acknowledgments

The QBI Microscopy Facility is grateful to Dr Angelo Tedoldi (Sah Lab) for the use of his dataset for screen captures, and to Dr Vincent Schoonderwoert (Scientific Volume Imaging) for helpful comments on the manuscript. We thank Mr Jake Carroll and Mr Irek Porebski (QBI IT) for their support and active development of QBI and UQ's supercomputing infrastructure.

References

- [1] Capturing images. (2017, October 18). Retrieved from http://microscopy.berkeley.edu/courses/dib/sections/02Images/sampling.html.
- [2] Nyquist-Shannon sampling theorem. (2017, October 19). Retrieved from https://en.wikipedia.org/wiki/Nyquist-Shannon_sampling_theorem.
- [3] R. H. Webb and C. K. Dorey. The Pixelated Image. In J. B. Pawley, editor, Handbook of Biological Confocal Microscopy, pages 41–51. Plenum Press, New York, 1990.
- [4] Microscopy Nyquist rate and PSF calculator. (2017, October 19). Retrieved from https://svi.nl/NyquistCalculator.
- [5] XFast. (2019, February 1). Retrieved from https://sourceforge.net/projects/xfast/.
- [6] GNOME. (2019, February 1). Retrieved from https://en.wikipedia.org/wiki/GNOME.
- [7] MATE Desktop Environment. (2019, February 1). Retrieved from https://mate-desktop.org/.
- [8] A Brief Introduction to VirtualGL. (2019, February 1). Retrieved from https://virtualgl.org/About/Introduction.
- [9] Installing/Configuring PuTTy and Xming. (2017, October 12). Retrieved from http://www.geo.mtu.edu/geoschem/docs/putty_install.html.
- [10] X Window System. (2017, October 13). Retrieved from https://en.wikipedia.org/wiki/X_Window_System.
- [11] Scientific Volume Imaging B.V. (2015). Huygens Professional: User Guide for version 15.05. Hilversum, The Netherlands.
- [12] Direct determination of the SNR. (2017, October 23). Retrieved from https://svi.nl/SignalToNoiseRatio#Direct_determination_of_the_SNR.
- [13] Dual Amplifier Dynamic Range. (2017, October 23). Retrieved from http://www.andor.com/learning-academy/dual-amplifier-dynamic-range-scmos-dynamic-range.
- [14] XQuartz. (2017, October 19). Retrieved from https://www.xquartz.org/.