Huygens Professional Deconvolution Guide

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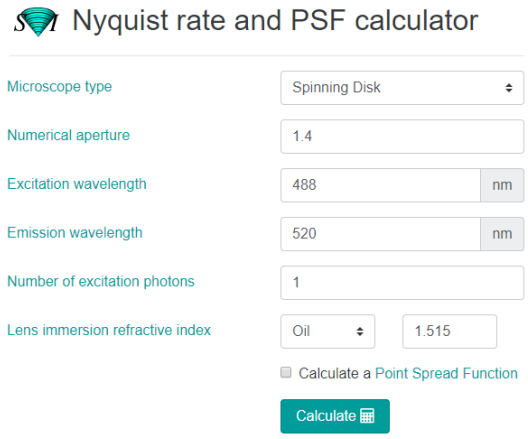
A note on sampling density

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.



(a) Microscope parameters for the Nyquist calculator.

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
— Set your zooms and scanning steps so that each pixel covers a x-y area of 43 nm × 43 nm (or smaller)
— Calibrate and set your z-stepper so that it takes steps of 130 nm when acquiring a 3D stack (or smaller)

(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-μm and 100-μm pinholes) and Yokogawa (Table 2) spinning disk confocal microscopes, and for the LSM 510 and 710 confocal laser-scanning microscopes (Table 3). 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-μm and 100-μm 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 XY (nm)	Optimal XY (nm)	Standard Z (nm)	Optimal Z (nm)	Backprojected pinhole radius, 50-μm pinholes (nm)	Pinhole spacing, 50-μm pinholes (μm)	Backprojected pinhole radius, 100-μm pinholes (nm)	Pinhole spacing, 100-μm pinholes (μm)
10x	0.45	559.6							
20x	0.75	276.3	76	1200-800	360	1125	13.23	2421	26.07
40xW	1.15	140.2	50	1000-400	186	562.5	6.615		
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 TIRF	1.49	93.7	40	300-200	98	375	4.42	831.5	8.872
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 (nm)	Optimal XY (nm)	Standard Z (nm)	Optimal Z (nm)	Backprojected pinhole radius (nm)	Pinhole spacing (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	41	400-200	130	250	5

Table 3: 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× 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× larger; when using large pinhole diameters (> 4 Airy disk units), these may be up to 2× 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.

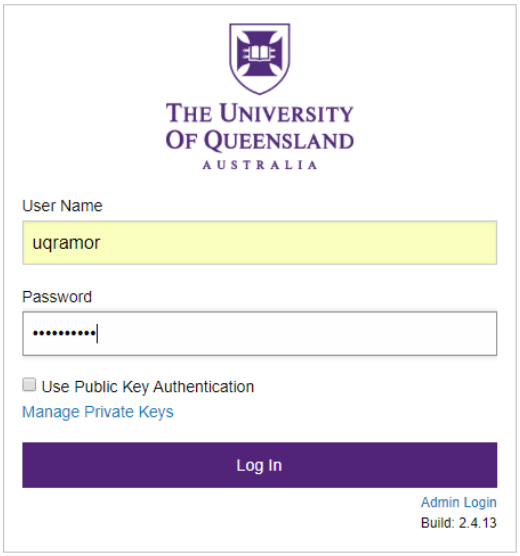
Objective	N.A.	Actual XY (nm)	Optimal XY (nm)	Standard Z (nm)	Optimal Z (nm)
10x	0.45	600-400	135	3000	1140
20x	0.8	300-200	76	1200-800	305
32xW	0.85	200-100	71	900-600	257
40xW	1.2	200-100	50	1000-400	163
63xW	1.2	100-50	50	600-300	163
63x oil	1.4	100-50	43	400-200	130

Connecting via XFast/MATE (VirtualGL)

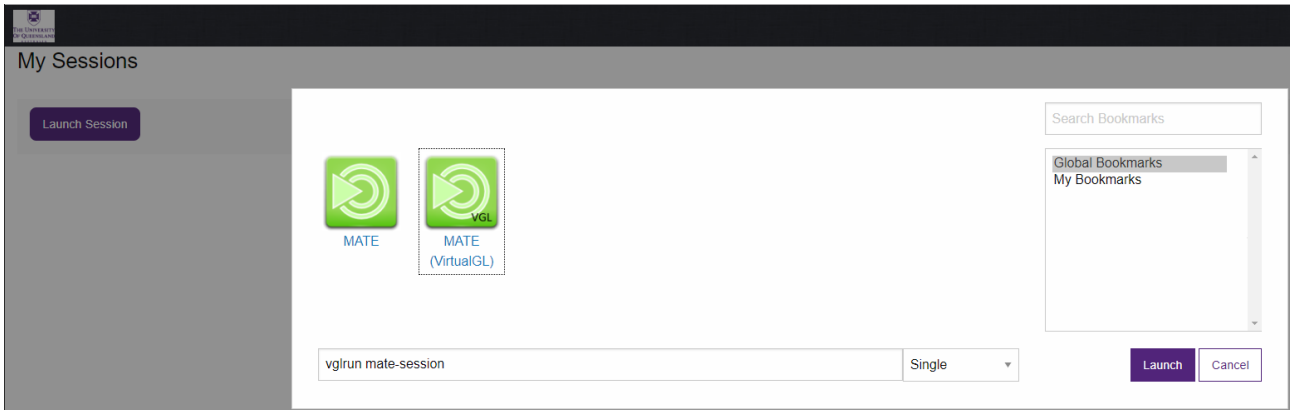
1. XFast is a lightweight desktop environment that incorporates a display manager and a window manager and allows access to a remote graphics hardware [5]. The MATE Desktop Environment, the continuation of GNOME 2, which featured a simple desktop where users can interact with virtual objects [6], provides 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.

2. 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).



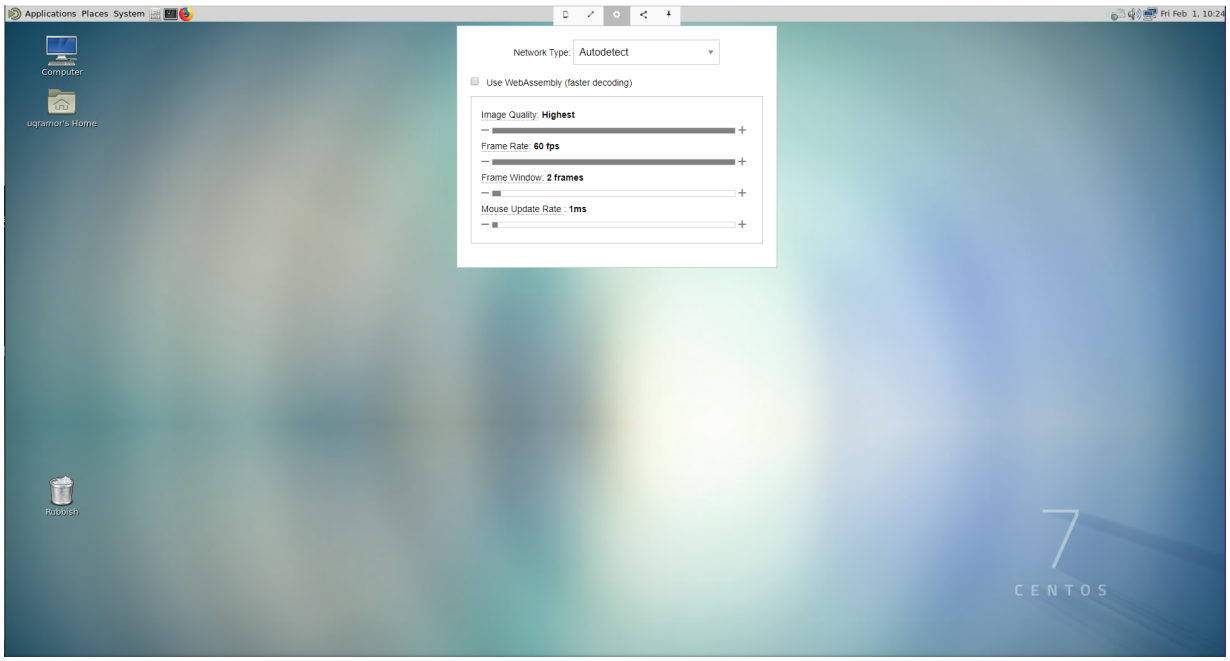
(a) XFast login prompt.



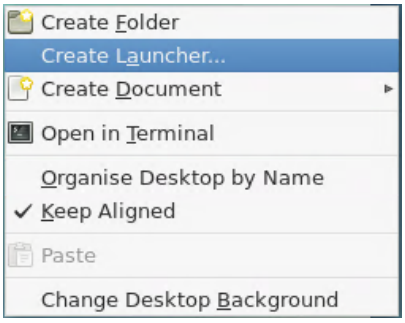
(b) Launching a MATE (VirtualGL) session.

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

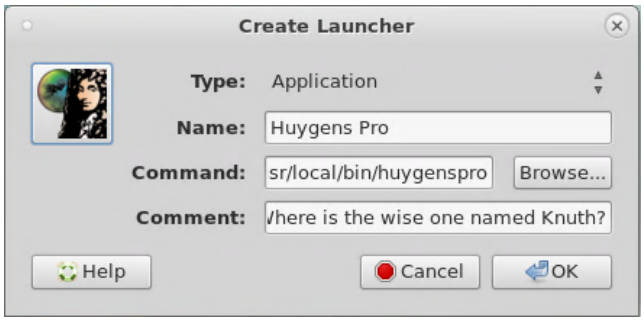
3. This launches the MATE (VirtualGL) desktop (Fig. 3a). In Settings, Image Quality, Frame Rate and other properties can be tweaked, especially if working on a VPN. To create a shortcut to Huygens Professional, right-click on the desktop and choose "Create Launcher" (Fig. 3b). Give the launcher a name,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



(a) MATE (VirtualGL) desktop environment.



(b) Creating a launcher.



(c) Launcher 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.

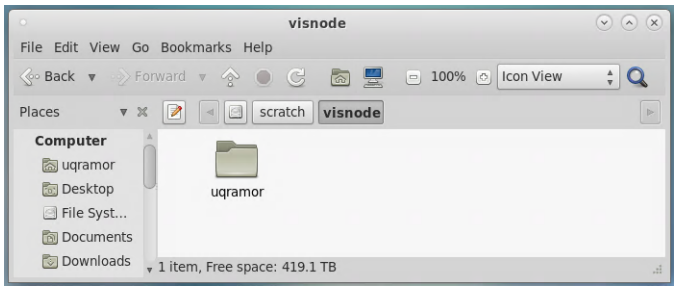


Figure 4: All data should be stored on the `/scratch/visnode/` directory.

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.

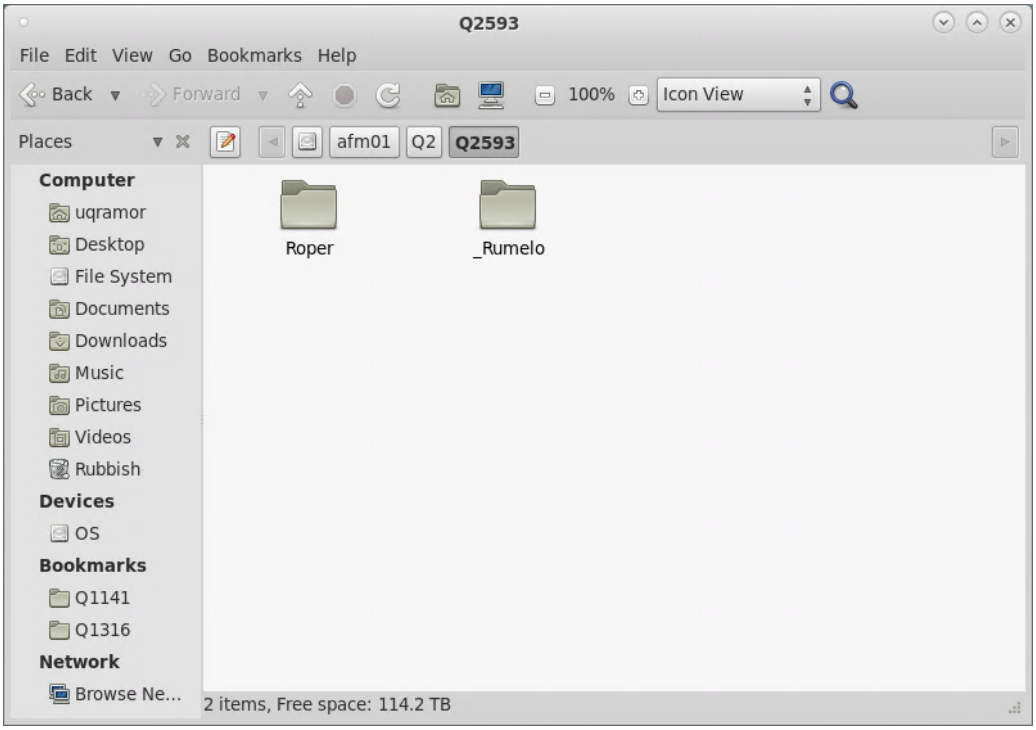


Figure 5: Bookmarking one's RDM collection.

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).

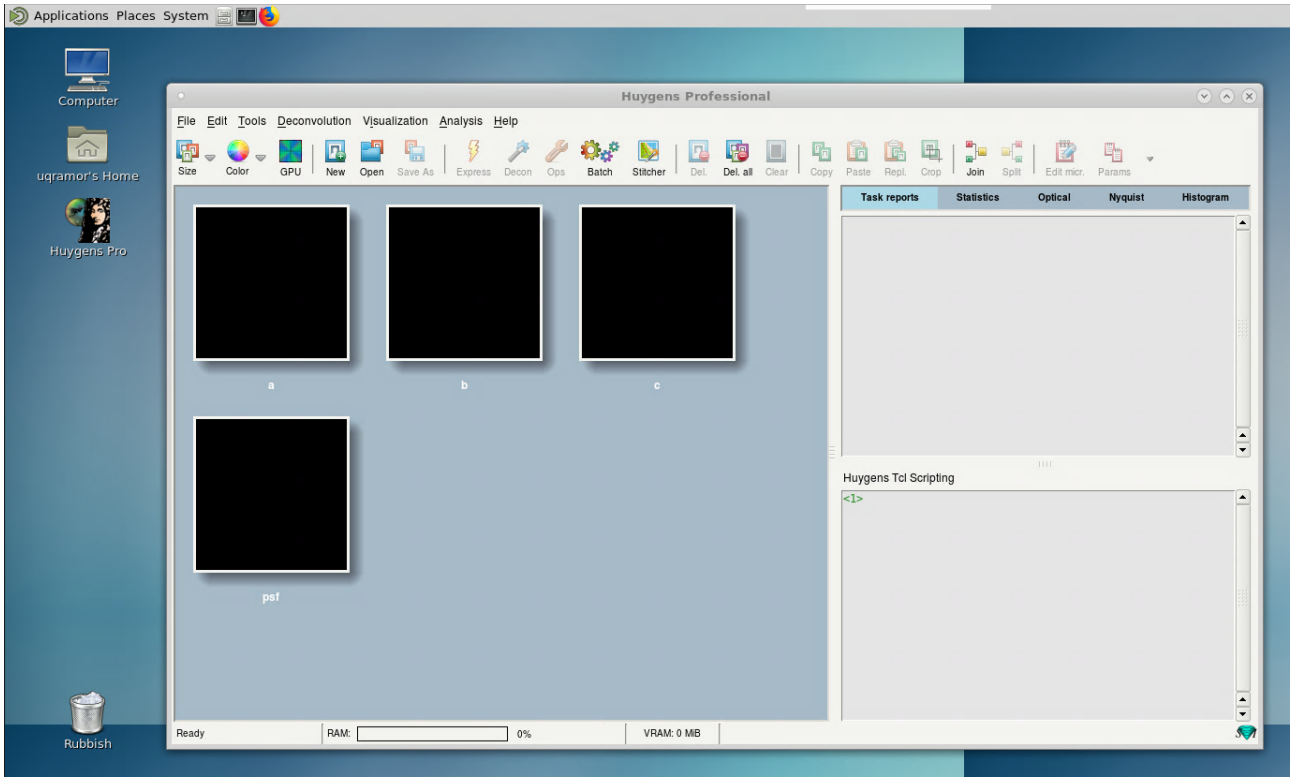
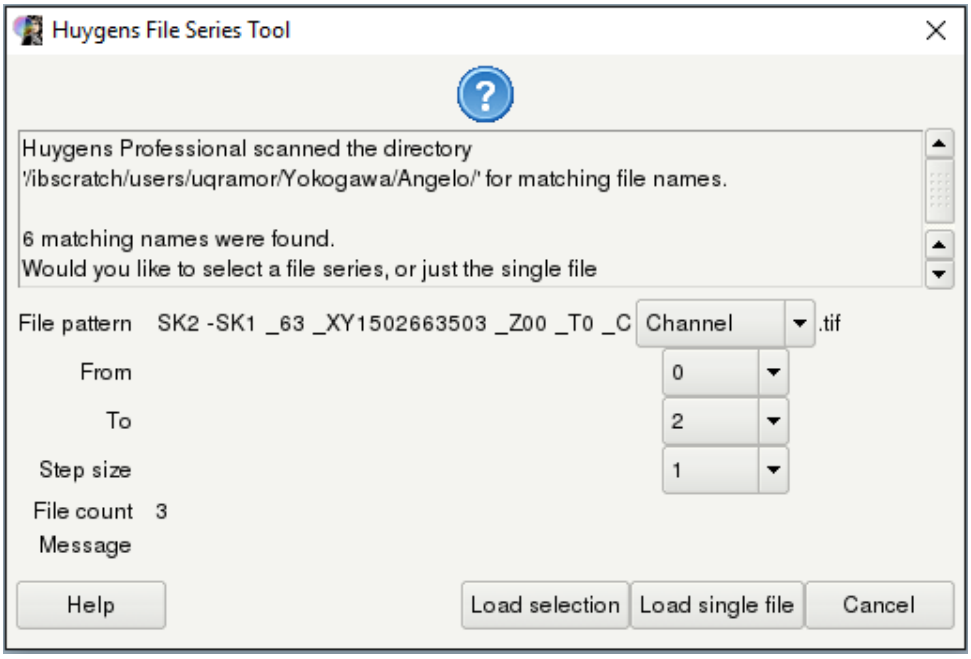
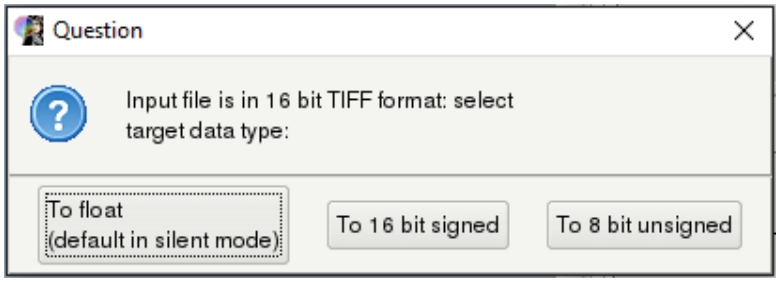


Figure 6: Launching the Huygens Professional graphical user interface on a MATE (VirtualGL) desktop.

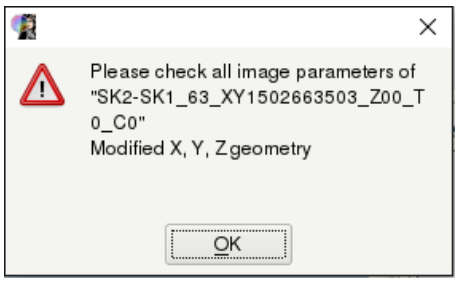
2. Open your raw dataset by going to File > Open. If the dataset is multi-channel, or is a mosaic or a time series, Huygens Professional will automatically recognise this (Fig. 7a). Click on "Load selection" to open all of the dataset. When prompted for the target data type, choose "To float (default 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.



(a) Opening a file series.



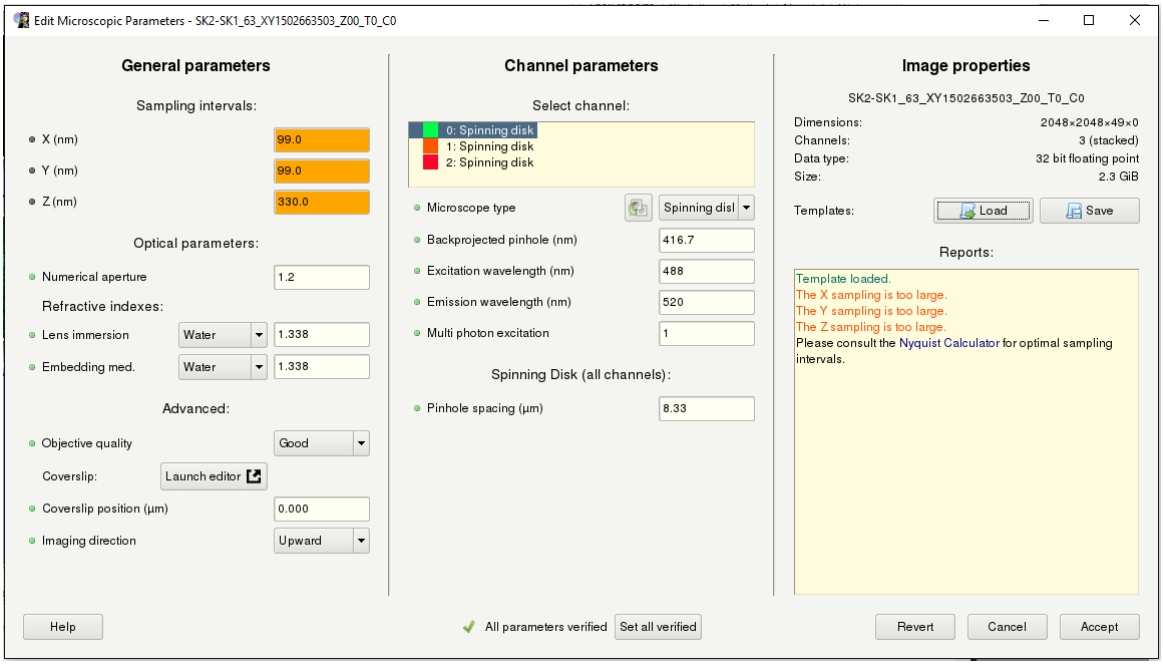
(b) Target data type.



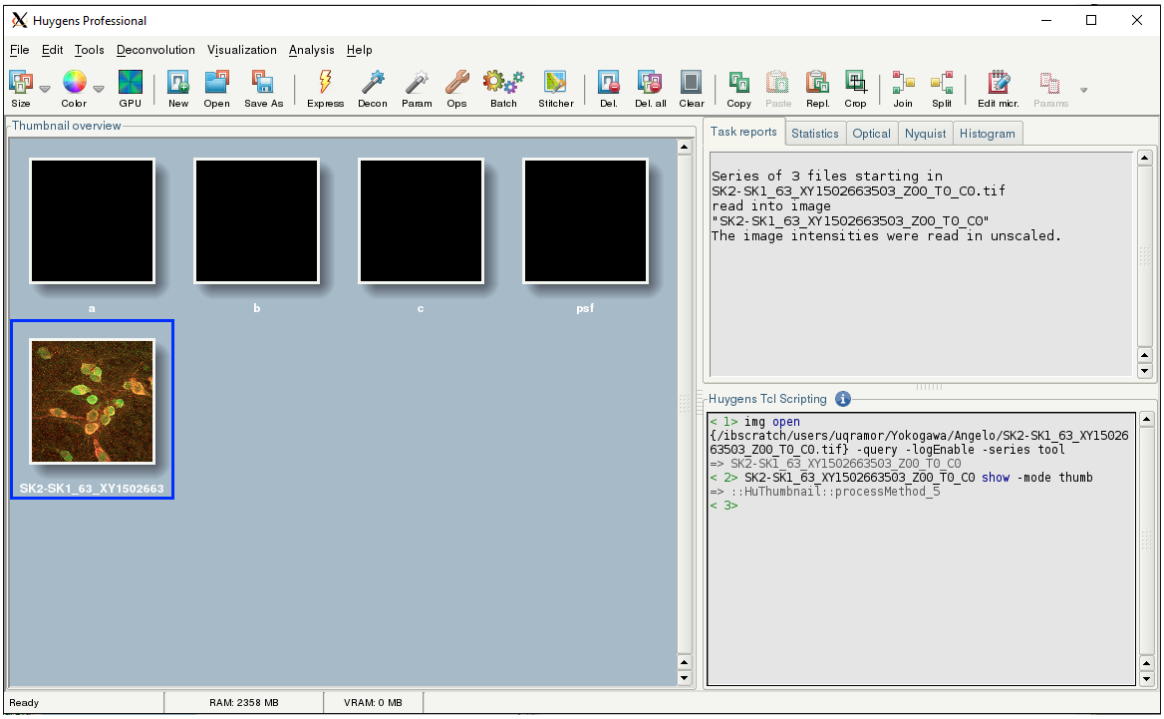
(c) Check image parameters.

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).



(a) Editing microscope parameters.



(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”.

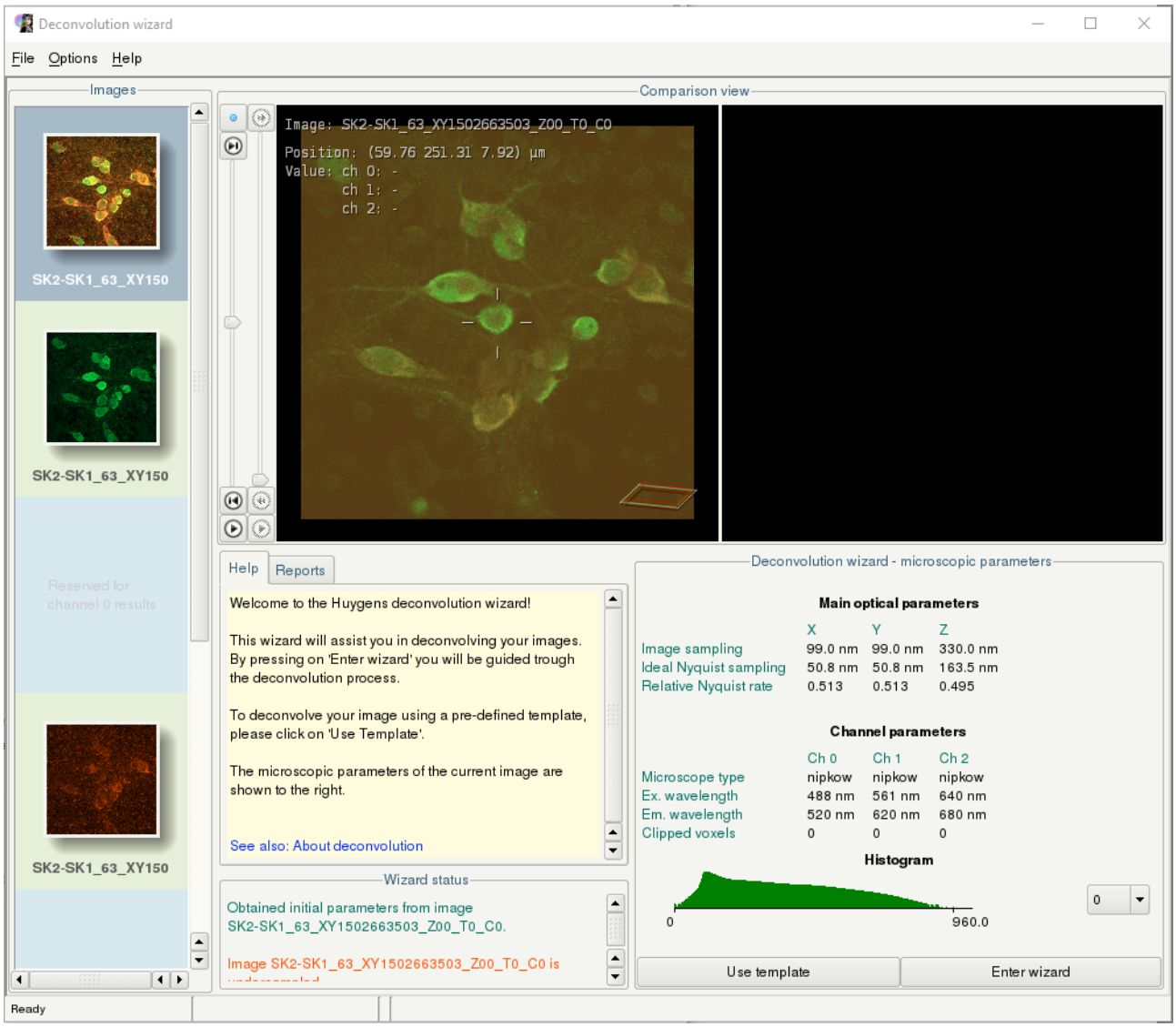
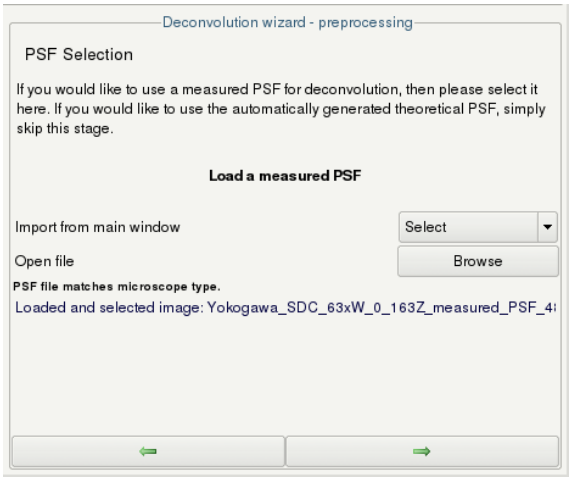
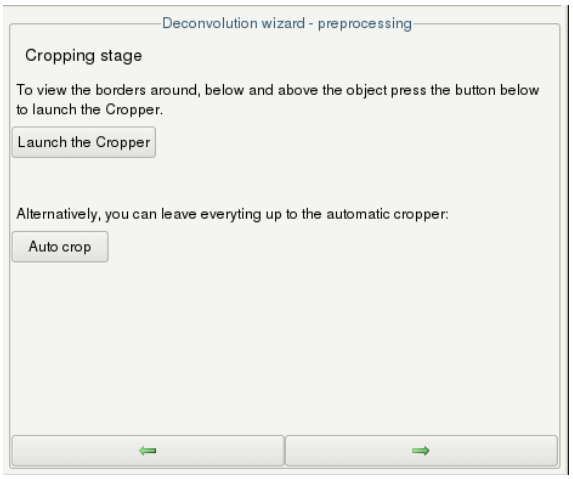


Figure 9: The deconvolution wizard.

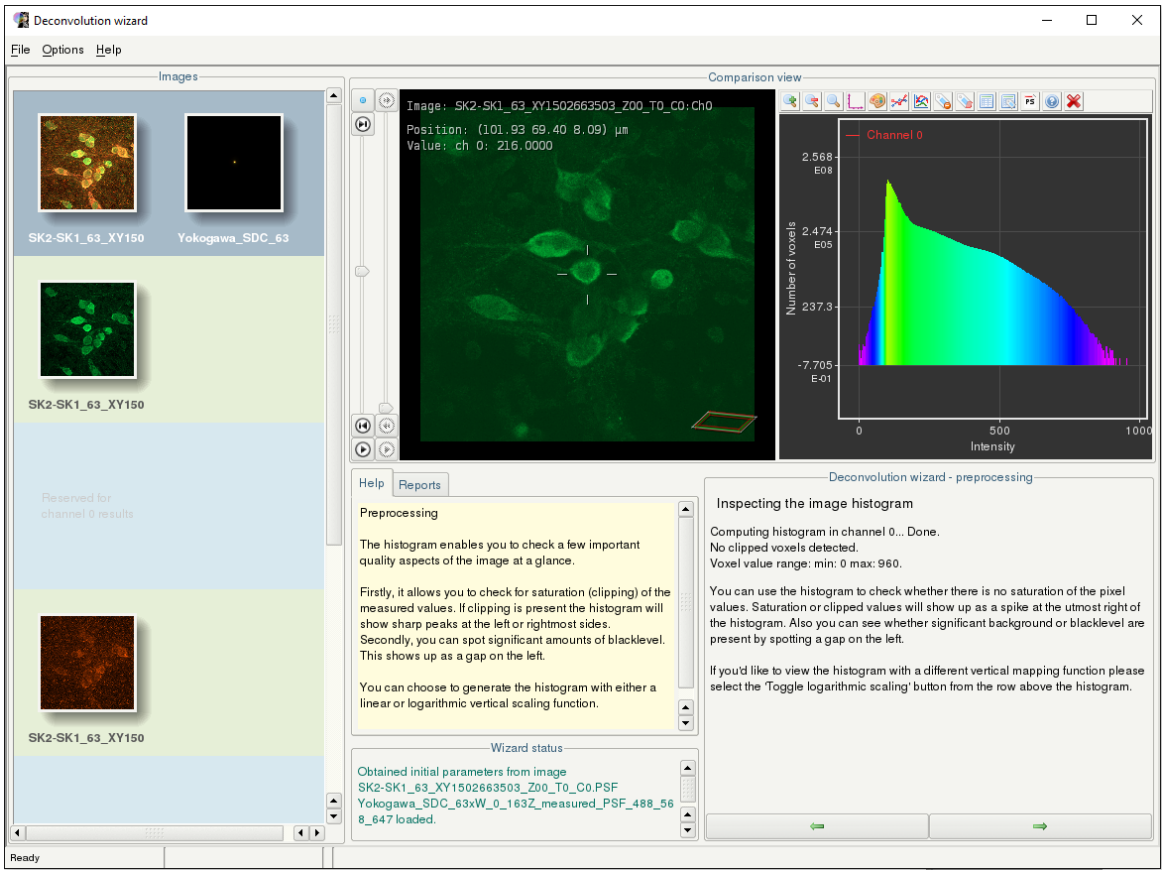
5. Inside the wizard, choose a measured PSF file for the specific objective used when the image was acquired (Fig. 10a). Measured PSF files can be obtained from \group_microscopy\Templates for Huygens Deconvolution\Measured PSF files. Click next (green arrow). Define a crop window if required by launching 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.



(a) Loading a measured PSF file.



(b) Window for launching the cropper.



(c) Inspecting the image histogram.

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.

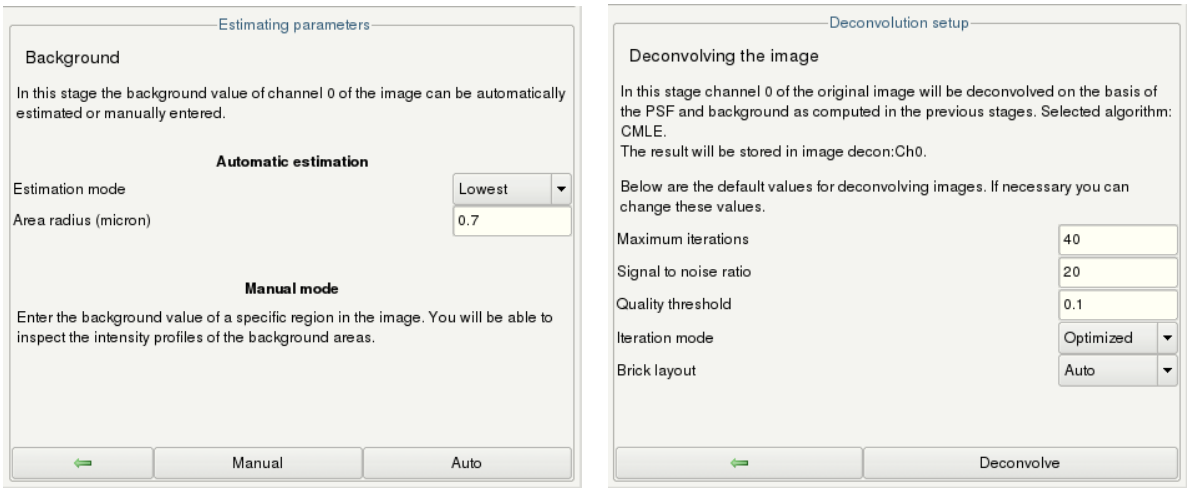
   The SNR value can be estimated by getting the square root of the number of electrons measured by the detector in the brightest voxel of the image [12]. Incoming photons excite electrons in the detector which are then measured by the electronics hardware within. The number of electrons is defined as the 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:

$$\begin{aligned} SNR &= \sqrt{N_{electrons,max}} \\ &= \sqrt{(maximum\ intensity - black\ level) \times conversion\ factor} \\ &= \sqrt{(2,000 - 100)\ ADU \times 0.45\ ADU^{-1}} \\ &= 29.2 \end{aligned}$$

   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 ∼ 20–30
   Confocal (laser-scanned) image: SNR ∼ 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.



(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`.

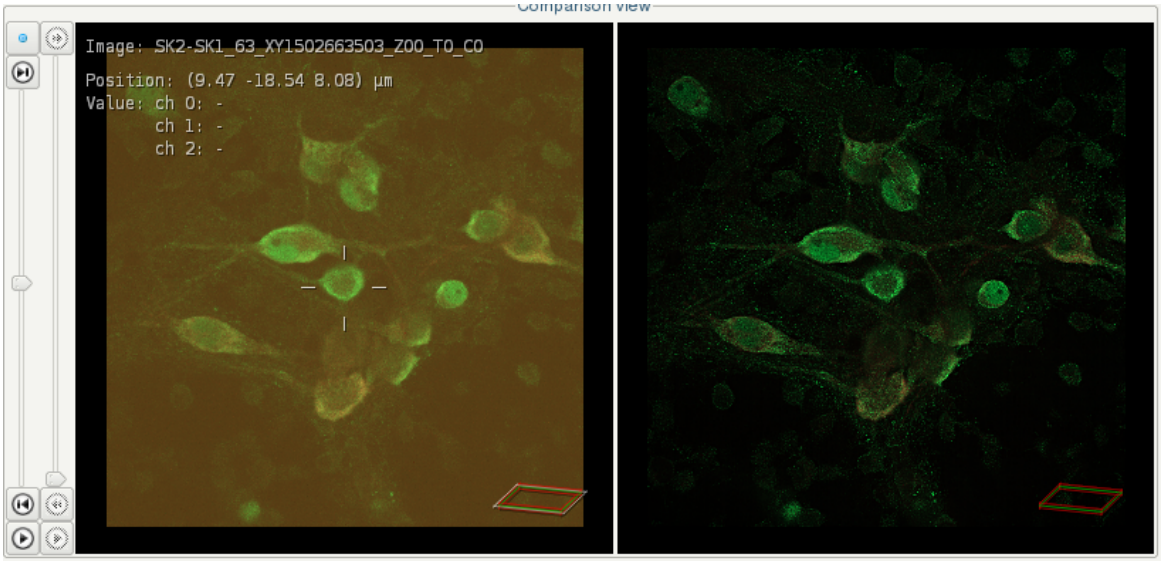


Figure 12: Raw multi-channel dataset (left) and deconvolution result (right).

A note on `clusterdata` storage and clearing the `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).

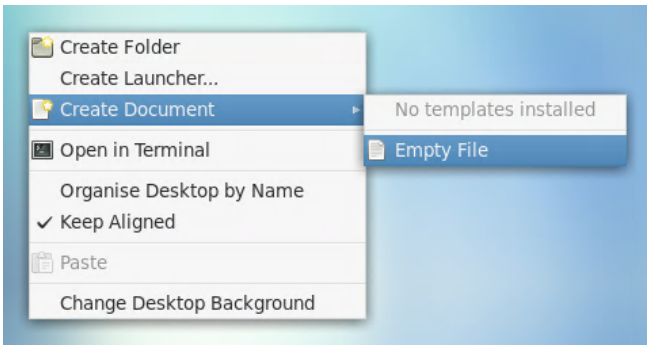


Figure 13: Creating a new document.

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 {} \;
```

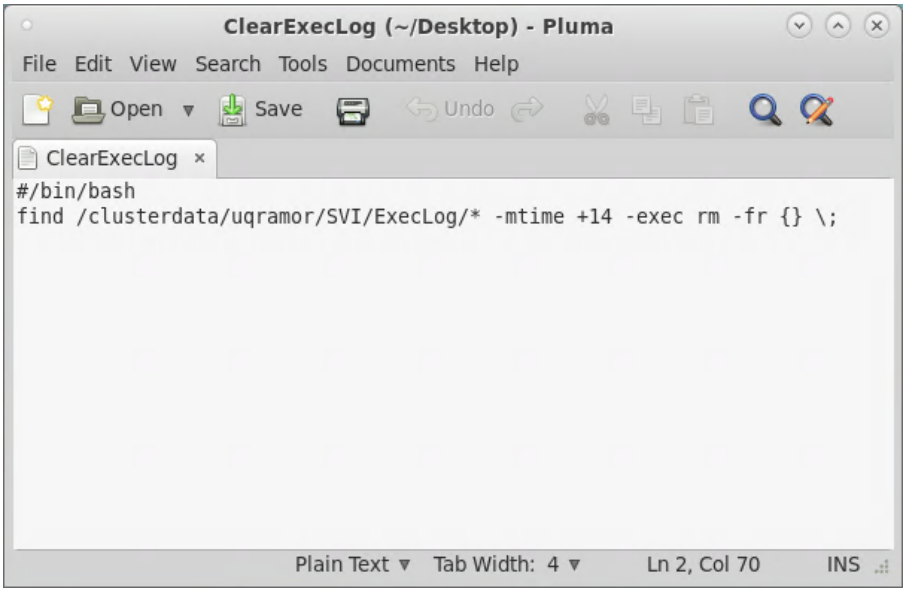


Figure 14: Editing "ClearExecLog" in Pluma.

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

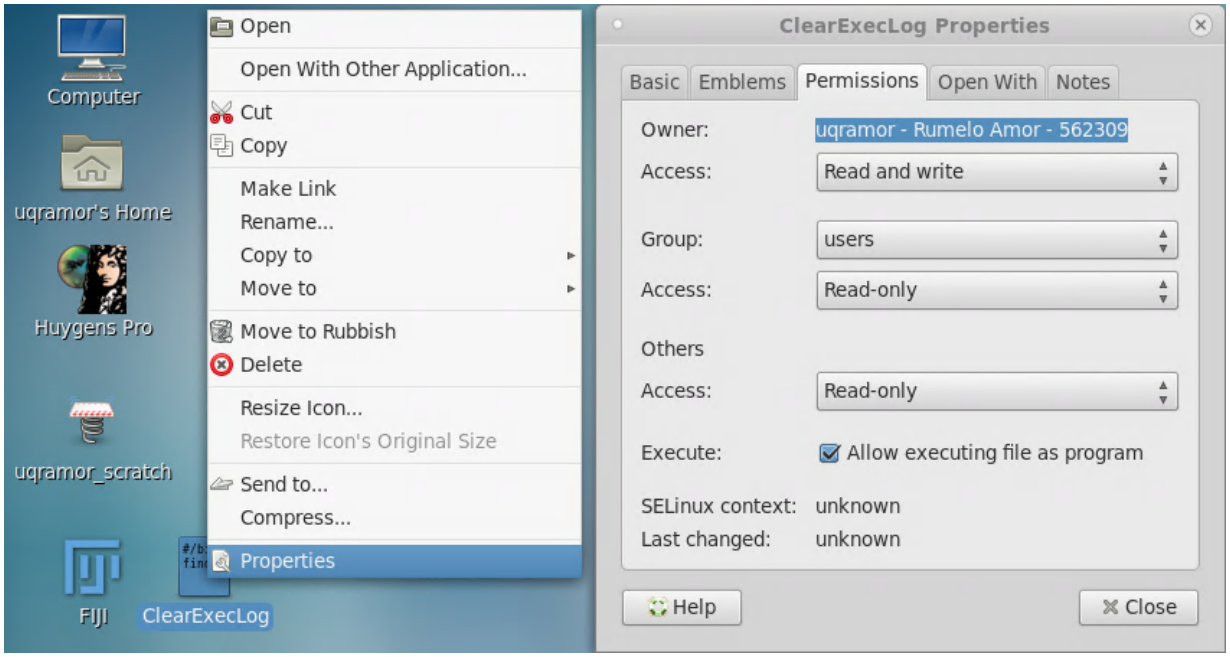


Figure 15: Executing "ClearExecLog" as a program.

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

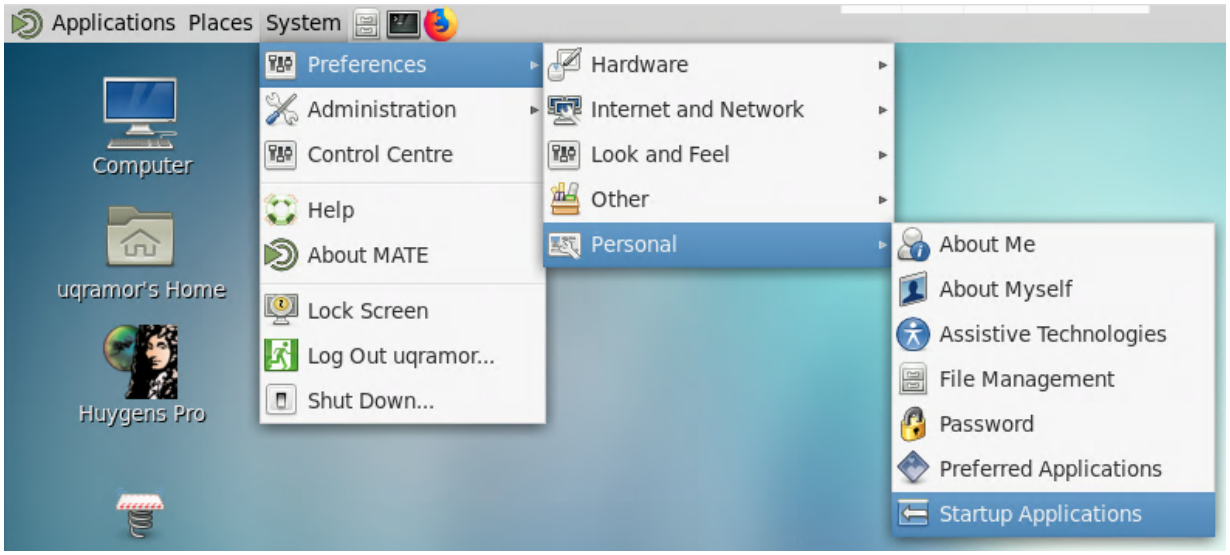


Figure 16: Adding a new startup application.

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.

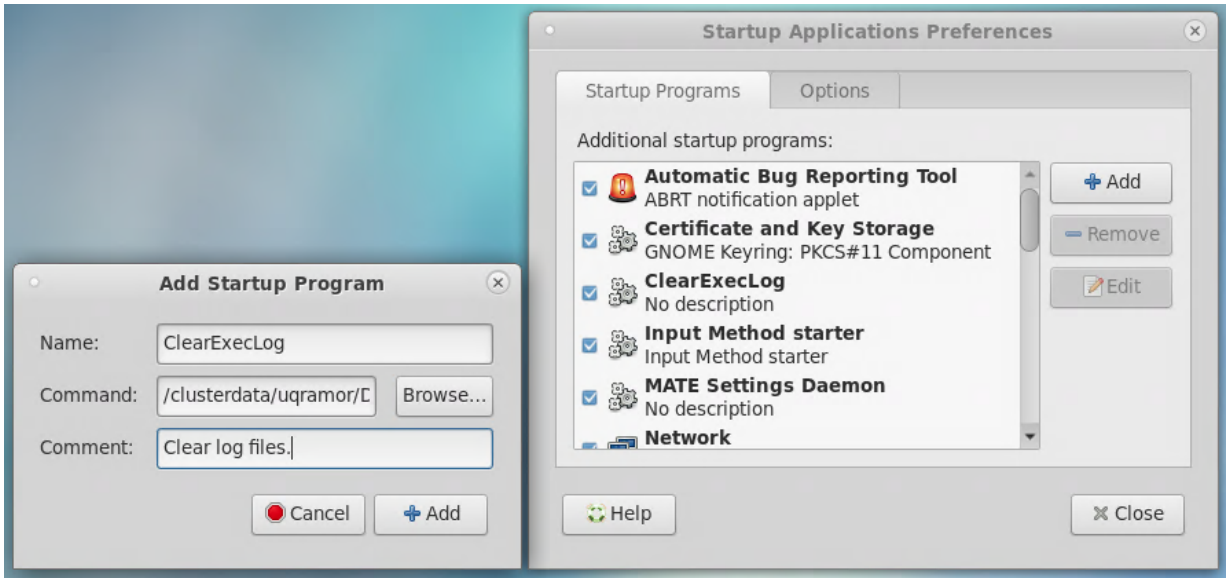
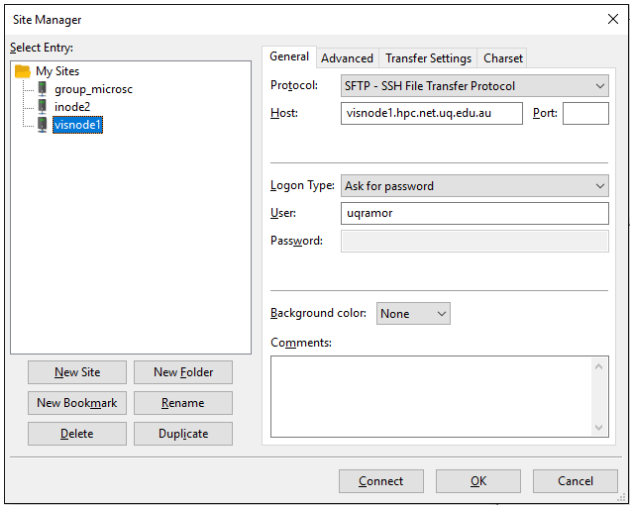


Figure 17: Executing "ClearExecLog" at each startup.

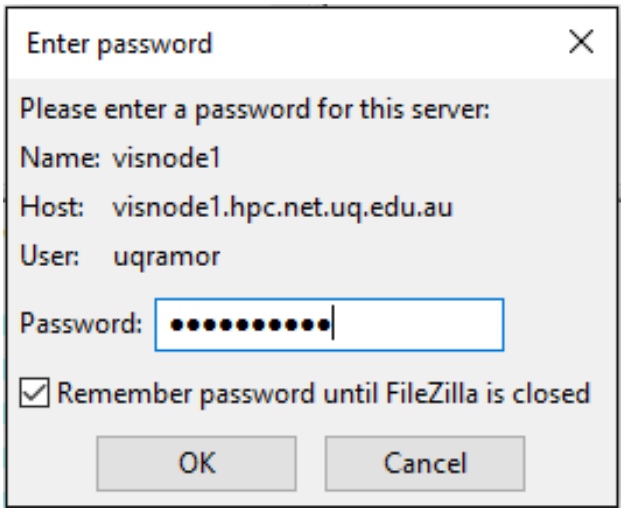
Old FileZilla instructions

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.

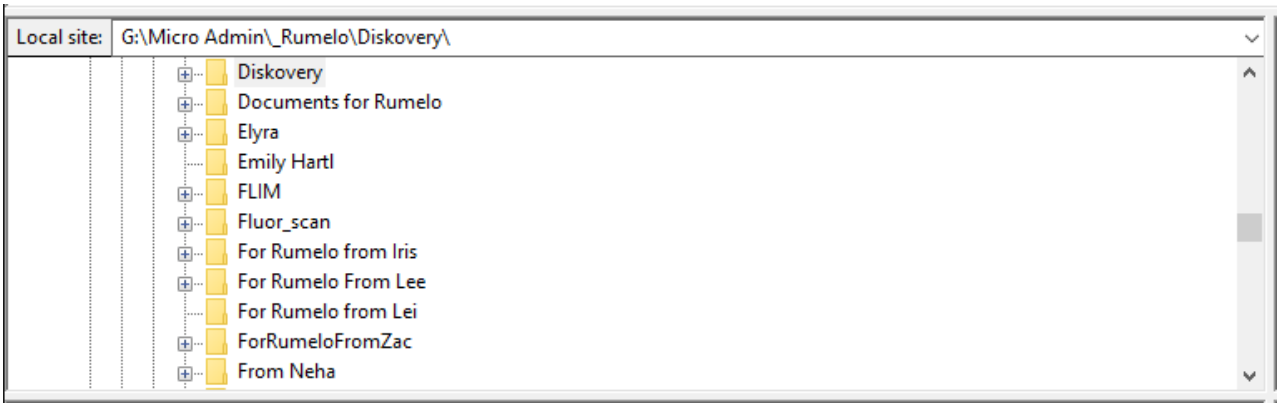
2. When running FileZilla for the first time, go to File > Site Manager. Click "New Site". In the "General" tab, type in "visnode1.hpc.net.uq.edu.au" for "Host", choose "SFTP - SSH File Transfer Protocol" for "Protocol" and "Ask for password" for "Logon Type" (Fig. 18a). Click "Connect". Log on using 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.



(a) FileZilla Site Manager.



(b) Password prompt.



(c) FileZilla local site.

(d) Remote site.

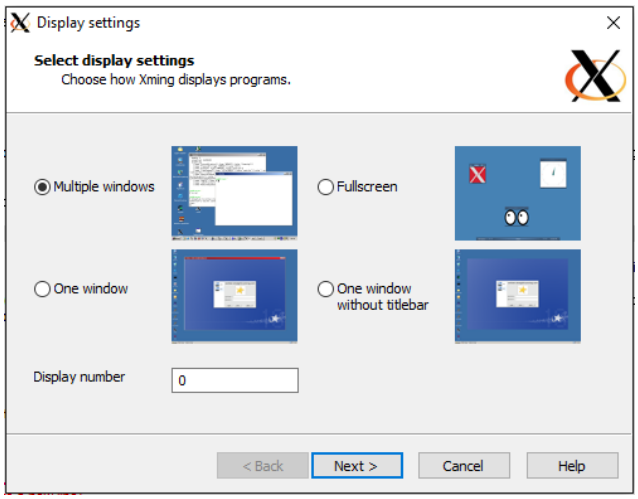
Figure 18: The FileZille user interface.

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.

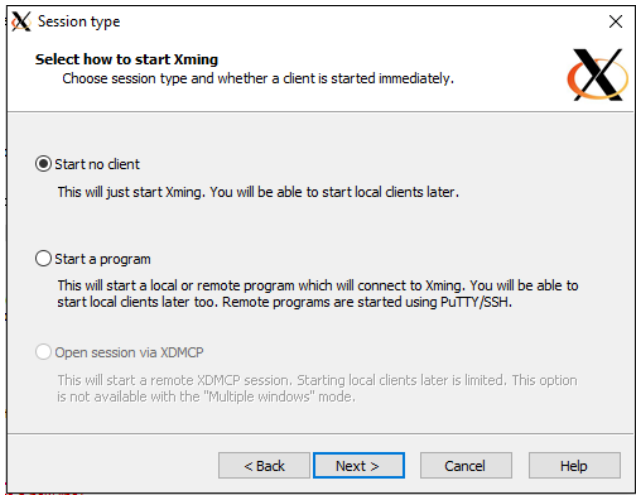
Old Windows instructions *[*PuTTY and Xming still work*]*

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.

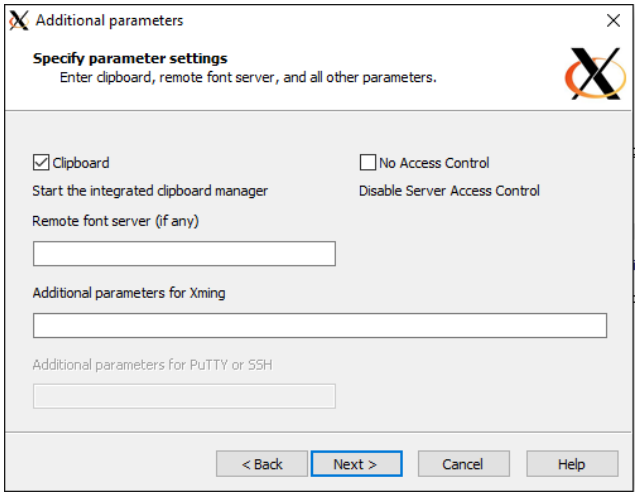
2. After installing Xming, run XLaunch and confirm that the settings are the same as in Fig. 19.



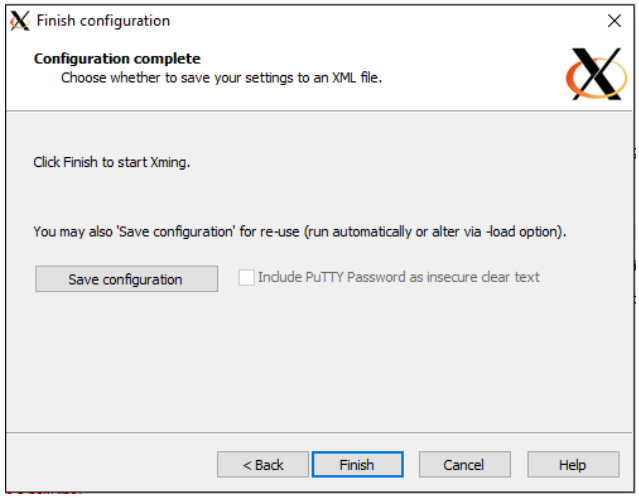
(a) XLaunch display settings.



(b) Session type.



(c) Additional parameters.

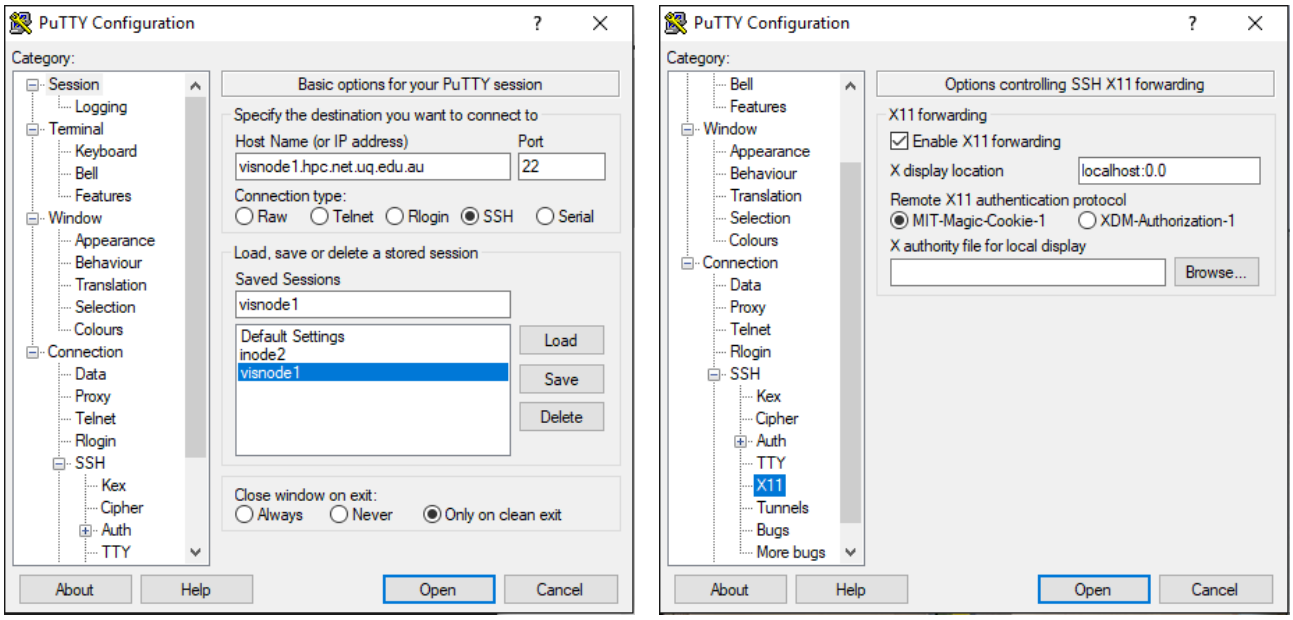


(d) Finish configuration.

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.

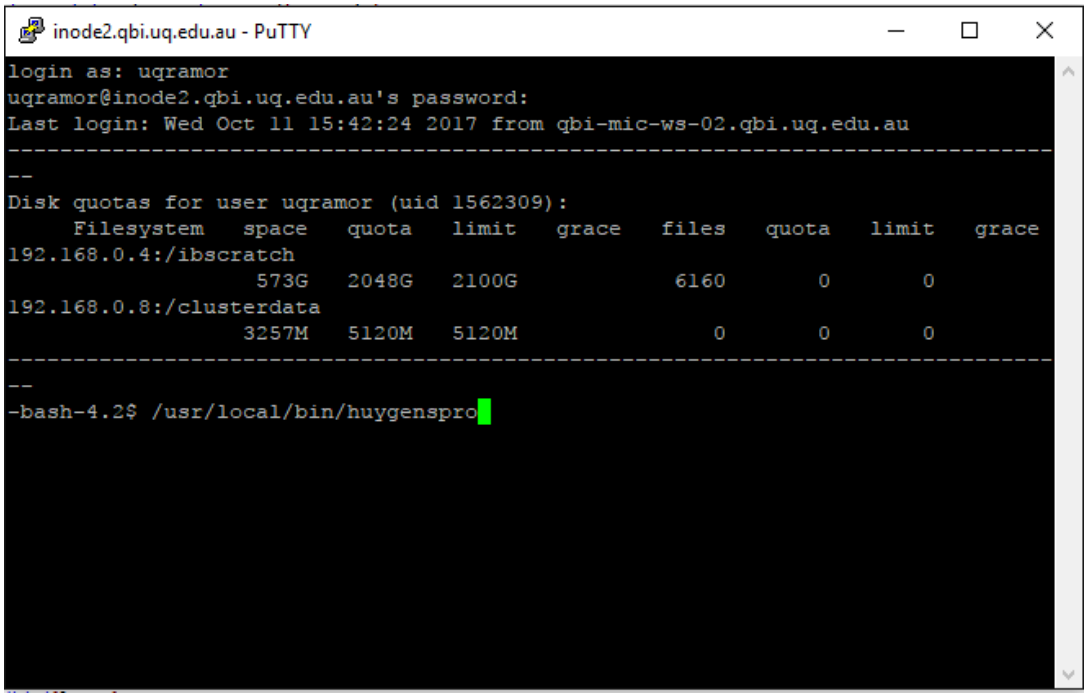


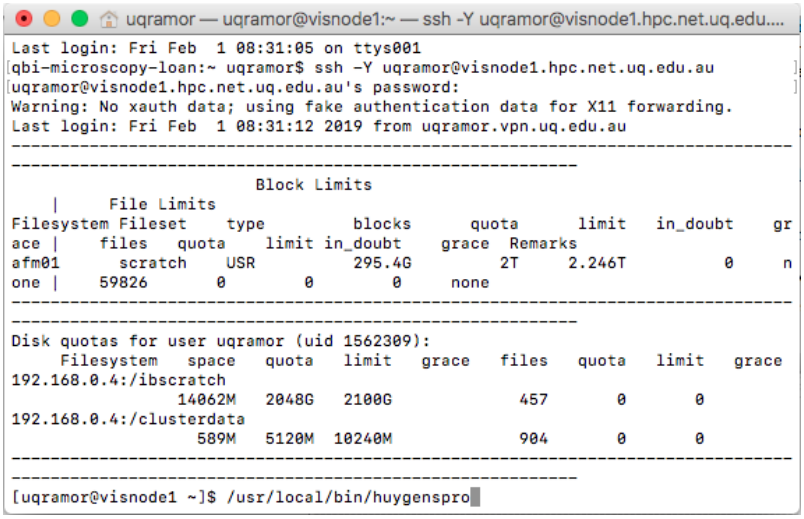
Figure 21: PuTTY terminal log on.

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

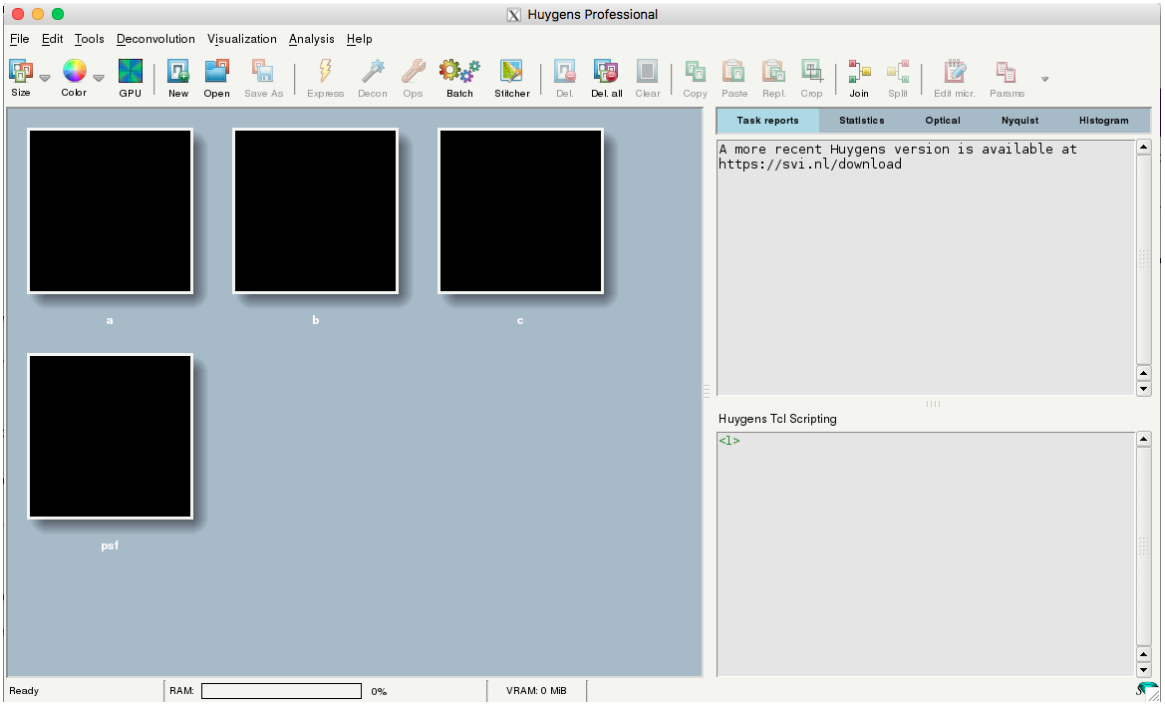
1. When we connect to QBI's deconvolution server using a Mac, we are making it a terminal, that is, we make it interact with the server, forwarding the display from the server to the Mac. The Mac is able to do this by using XQuartz. The XQuartz project is an open-source initiative to develop a version 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.



(a) XQuartz terminal on Mac OS X.



(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

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