

User Manual
Visionary
Instrument control software

Read the main Clarity/Clarity^{HS} Operating Manual, in particular the Health and Safety information, before operating Visionary with the Clarity confocal microscopy system.

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1. INTRODUCTION

Visionary has been designed to provide a user friendly, simple to use, graphical user interface for instrument control of the Aurox Clarity and Clarity^{HS} laser free confocal devices as well as compatible auxiliary equipment such as microscopes, light sources, XY stages, Z drives and digital cameras.

This Software User Guide is suitable for installations of Version 4.3 or later. For information on previous installations of Visionary, please contact Aurox or read the release notes for your current software version.

For a list of supported auxiliary devices, please refer to the following Aurox web-page:
<http://aurox.co.uk/aurox-confocal-microscope-downloads-supported-devices.php>

2. INSTALLATION OF VISIONARY

WARNING



Windows 10 operating system is required for the installation and correct running of the Visionary software.

2.1. INSTALLATION PROCEDURE

The Aurox Visionary software can be found on the USB stick included with your Aurox Clarity system or downloaded direct from the Aurox website at <http://www.aurox.co.uk/aurox-confocal-microscope-downloads-software.php>.

1. Insert the USB stick into a USB port on your computer and copy the files from the USB stick onto a suitable folder on your computer.
2. Launch the VisionaryInstallRunner.exe application
3. Click Visionary. This will install .Net and Java first, use default settings everywhere. You will then be presented with a menu to install Fiji and Visionary, don't select any options, just click install. Do not launch the application upon the installation, continue installing all the remaining elements
4. Click Micromanager, use default location/settings, create desktop link but do not launch the application upon installation
5. If you are using an Andor Zyla, PCO Edge or Photometrics detector, click the relevant button to install the camera drivers.
6. If you are using Nikon Ti-E, click Nikon Ti-E and install the relevant control application. Once this is complete finish installing Nikon Ti-E drivers by attaching the stand, finding a driverless USB device in Device Manager and pointing to C:/ProgramFiles/Nikon/shared/drivers for driver update
7. If you are using a CoolLED pe300 light source, please install the drivers from the manufacturers website
8. If you are using a Hamamatsu Orca Flash 4 detector, install the DCAM driver package for Hamamatsu Orca Flash 4.0 (USB driver only) by downloading the package from the Hamamatsu website

Once installation is complete, two icons will be present on the computer desktop, MicroManager and Visionary



NOTE

For information regarding procedure of installation of drivers for other supported auxiliary devices, please contact Aurox.

2.2. Software updates

Visionary is regularly updated with new features and bug fixes. The latest release can be downloaded by registered users from the Aurox website (<http://www.aurox.co.uk/aurox-confocal-microscope-downloads-software.php>). It is recommended that users monitor the website and use the latest software.

2.3. Feature Requests and Bug Reporting

Visionary users are encouraged to report any software bugs by sending details to support@aurox.co.uk. Only by receiving bug reports from experienced users can these be successfully identified and repaired. Ideas for software improvement are also very much welcomed, and all are considered when creating new modules for future releases of Visionary.

3. STARTING AND CLOSING VISIONARY

3.1. Starting Visionary

To start Visionary, double click on the icon on the Windows desktop. When the programme is started on a PC connected to an Aurox Clarity confocal device, Visionary will start-up in hardware set-up window awaiting the input of additional equipment as shown in Figure 3.1.1



Figure 3.1.1 The Visionary hardware set-up window

3.2. Closing Visionary

To close Visionary, click on the X icon in the top right corner of the Visionary window.

4. VISIONARY LAYOUT AND CONTROLS

4.1. General Layout

The Visionary GUI, comprises of two areas. The main area of the software displays either the hardware set-up screen, the live or the recorded image depending upon which coloured triangular tab is selected in the Workflow Toolbar shown in Figure 4.1.1. A border around the triangular tab indicates that it is selected and active.

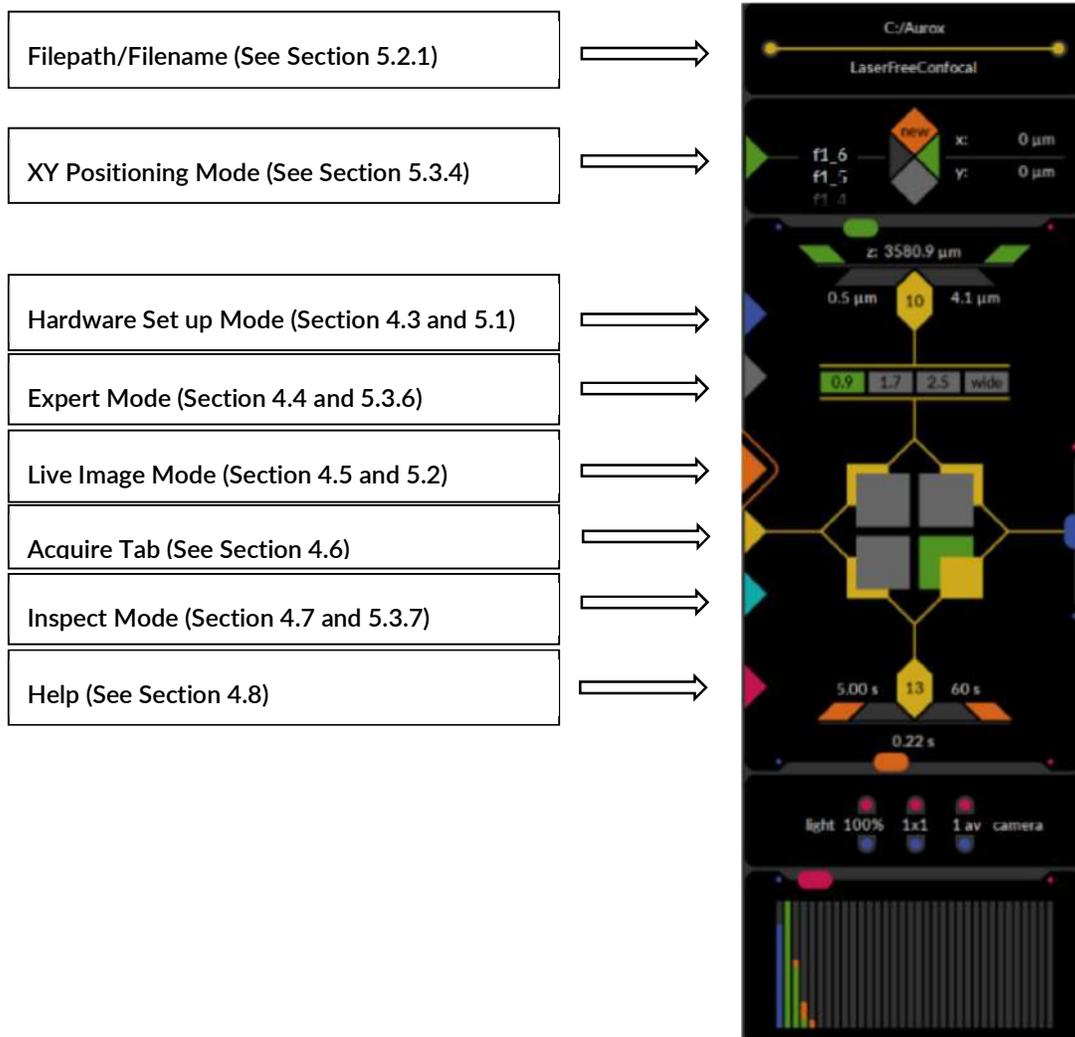


Figure 4.1.1 Workflow toolbar triangular tabs

4.2. Workflow Toolbar Control Icons

All experimental parameters are set up using the Workflow Toolbar in Live Mode. The various sections control stages in setting up your experiment as shown in Figure 4.2.1 and described in more detail in later sections of this manual. In Inspect Mode, the icons have a different function and these are described in section 5.3.7

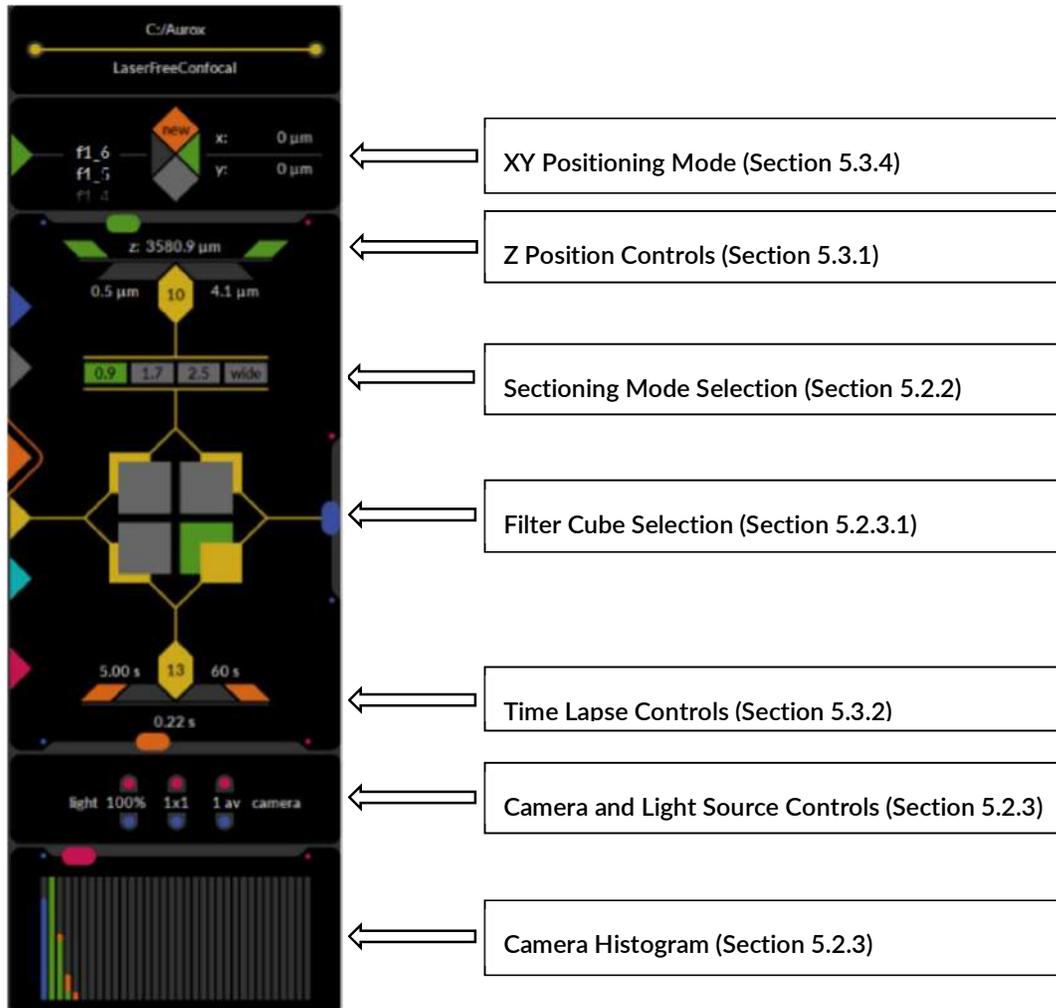


Figure 4.2.1 Key to Workflow Toolbar Control Icons

4.3. Hardware Set up screen

The hardware set-up screen, shown in Figure 4.3.1, is the initial screen you will see when starting Visionary and can be accessed at any time by clicking on the blue triangular tab of the Workflow Toolbar. If you are setting up a new system or have changed the hardware configuration, you must specify the hardware configuration in this screen as described in section 5.1 before proceeding.

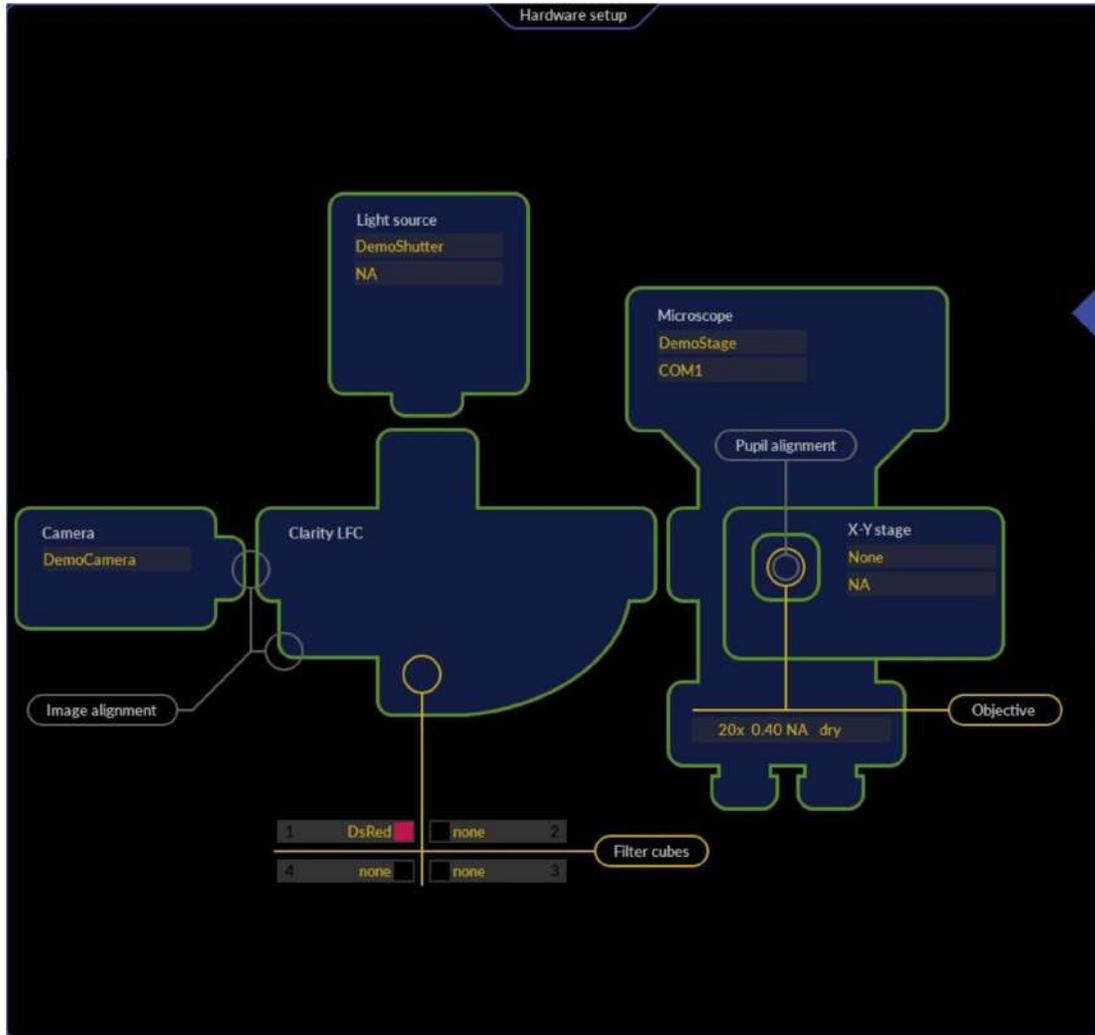
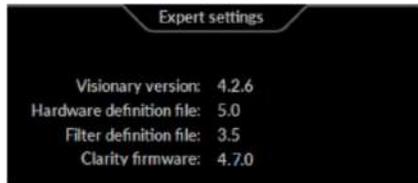


Figure 4.3.1 Hardware Set-up Screen

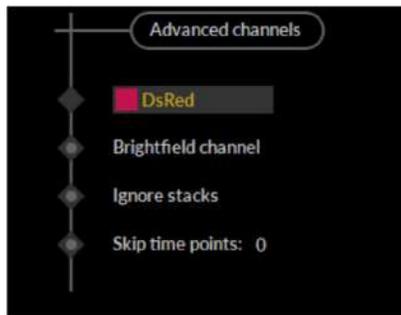
4.4. Expert Mode

Expert mode may be activated by clicking on the grey triangular tab of the Workflow Toolbar. This mode is used to list the current version information associated with the system and to access some advanced features. It is also where the tools needed for alignment procedure are located. Details of the expert mode functions and icons are listed in Figure 4.4.1



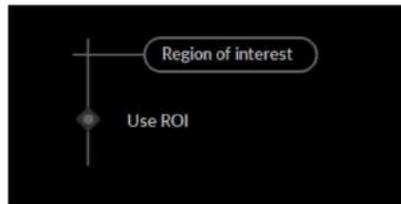
Software Versions

This section lists the current version numbers of the Visionary software and associated hardware and filter cube definition files as well as the Clarity firmware version.



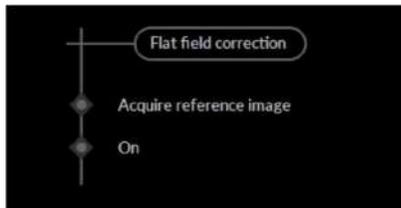
Advanced Channels

Allows advanced control of the colour channels. Described in further detail in Section 5.3.6



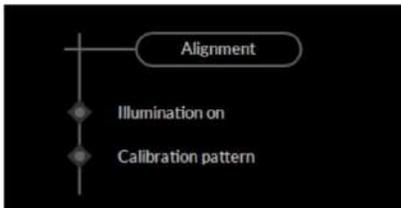
Region of Interest

(This feature is still under development.)



Flat Field Correction

Place a flatfield reference sample on your microscope stage. Click acquire reference image to save a reference flatfield image. Click the 'On' radio button to enable flatfield correction.



Alignment Tools

Click these radio buttons to turn on the illumination and/or calibration pattern when aligning the Clarity system. Further details of how to align and calibrate the Clarity system are described in the Clarity Operating manual and beyond the scope of this manual.

Figure 4.4.1 Expert Mode Functions

4.5. Live Mode

To enter live mode, click on the orange triangular tab in the WorkFlow Toolbar. If you enter this mode from the hardware set-up screen or have just aligned the system, the Clarity will go through an automatic calibration procedure.

The live image window will display a live image of the sample on the microscope stage as shown in Figure 4.5.1

To pause the live image (to reduce photobleaching for example) simply click the live image orange triangular tab again, which will turn off the illumination.

All operations for setting up the experiment will be carried out in this mode. For further instructions on setting up a data collection, see section 5.2 and 5.3.

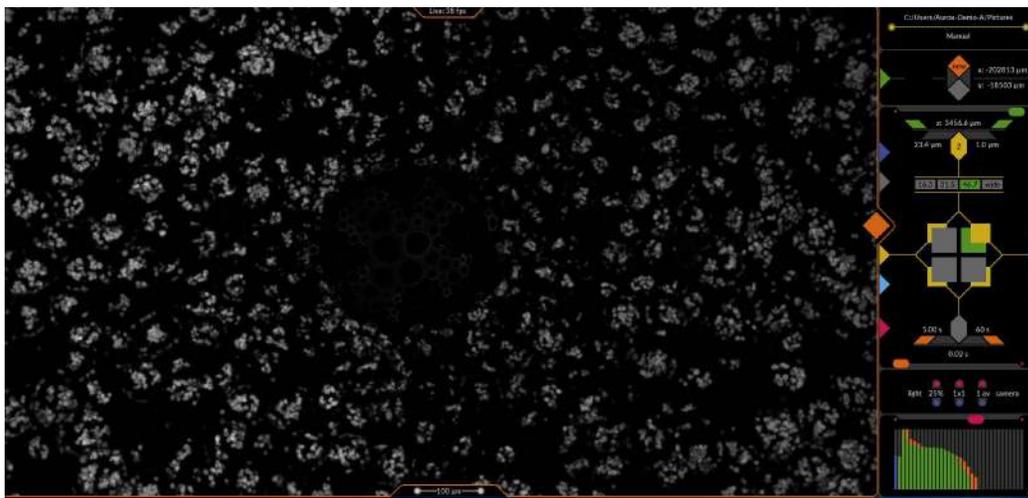


Figure 4.5.1 Live Mode Window

4.6. Acquire Tab

Once the system is aligned and the desired capture parameters have been set in the Visionary Live Mode Workflow Toolbar, click the yellow triangular tab begin data collection.



NOTE

If you want to stop data collection in Visionary at any point just click on the live image triangular tab which will take you back to live mode. This will result in the loss of all acquired data in that data set.

4.7. Inspect Window

When Visionary has completed the acquisition of all data it will automatically switch to inspect mode shown in Figure 4.7.1 allowing you to review all the data before exporting the images. This is described in more detail in section 5.3.7

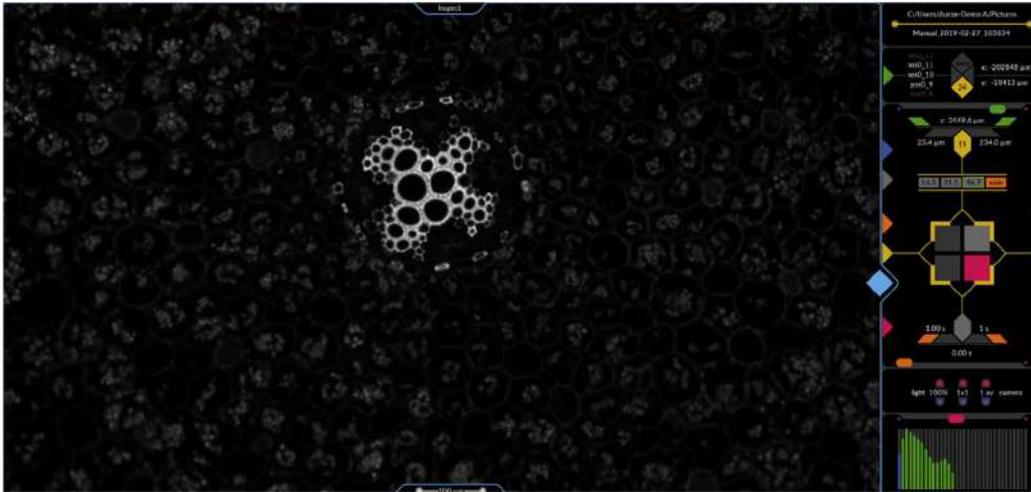


Figure 4.7.1 Inspect Mode Window

4.8. Help Tab

If any errors occur during set-up or operation of Visionary this tab will display error messages or details on the problem so that they can be resolved. To close error messages once they have been read and resolved, please click on the red square tab. Clicking on the red triangular tab at any time will open the Visionary Help window shown in Figure 4.8.1

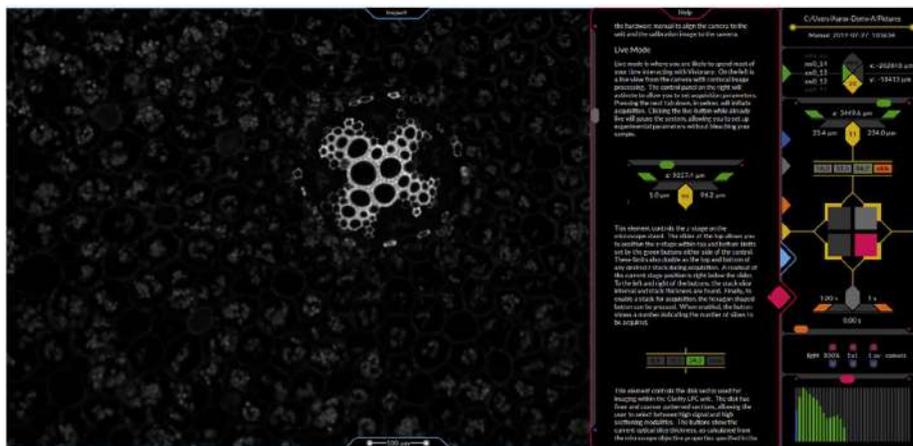


Figure 4.8.1 Visionary Help Window

5. OPERATION

5.1. Hardware Set up

The hardware set-up screen, shown in Figure 5.1.1, is the initial screen you will see when starting Visionary. If you are setting up a new system or have changed the hardware configuration, you must specify the hardware configuration in this screen before proceeding

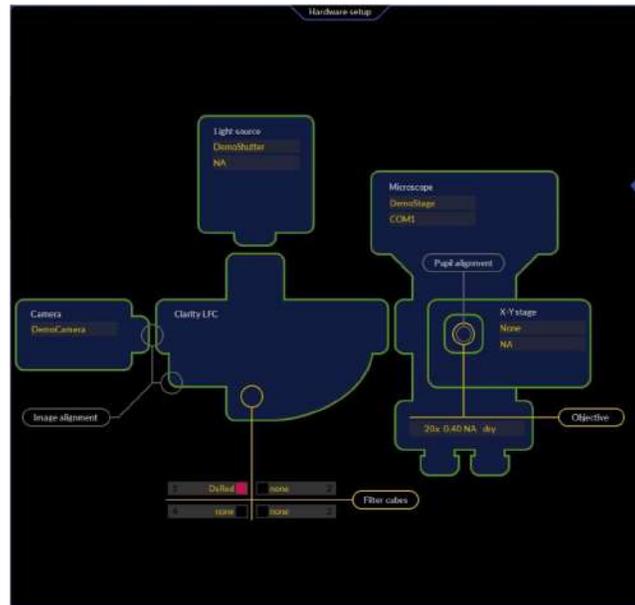


Figure 5.1.1 Hardware Set-up Screen

1. Ensure that all of your Visionary compatible hardware are connected via a suitable USB, Serial, Firewire or CameraLink cable and are powered on.
2. Change the active port on your microscope to eyepieces or shield the sample
3. Start the Visionary Software by double clicking on the desktop icon.
4. In the region marked Camera, click on DemoCamera to expose a pull down menu, shown in Figure 5.1.2. Select your connected camera from the pull down list. The Green boundary will turn red for a short moment and then green again when the camera is connected to the Visionary software.
5. In the region marked Light source, select your light source from the pull down menu shown in Figure 5.1.3 and select the COM port to which it is attached by clicking on NA and selecting the correct port. When connected, the boundary will turn green.

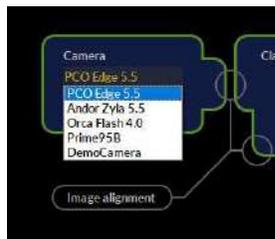


Figure 5.1.2 Camera Selection Pull Down Menu



Figure 5.1.3 Light Source Pull Down Menu



NOTE

Step 5 is only valid for light sources that can be controlled by Visionary software. For other light sources, leave the light source settings as DemoShutter/NA

6. In the region marked Microscope, click on DemoStage and select your microscope model or Z-drive controller from the pull down menu shown in Figure 5.1.4. Select the correct COM port using the pull down menu or leave as NA for a microscope connected by serial connection
7. In the region marked XY Stage, click on None and select your motorized XY stage from the pull down menu shown in Figure 5.1.5 and click on NA to select the relevant COM port



Figure 5.1.4 Microscope Z Stage Pull Down Menu



Figure 5.1.5 XY Stage Pull Down Menu



NOTE

If you do not have a Visionary compatible motorized XY stage, please leave the settings as None/NA

8. Enter the details of the objectives installed on your microscope into the objective list by clicking on the pull down menu, shown in Figure 5.1.6. Click on add objective and type in the new details including numerical aperture and immersion conditions. The details of the objectives will be saved on entry and can be accessed/changed via the pull down menu at any time when required.

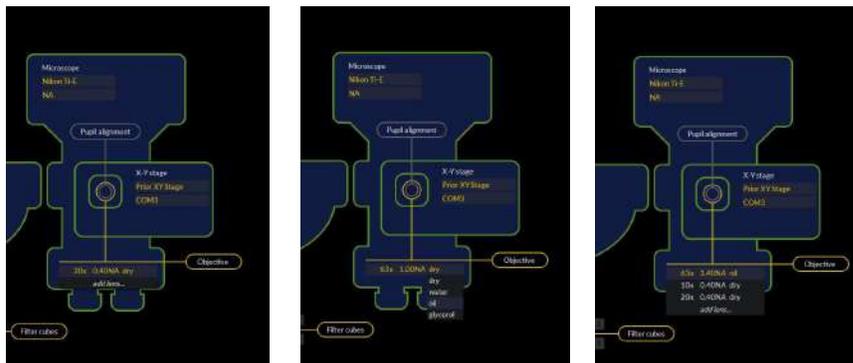


Figure 5.1.6 Entering details of objective lenses

9. At the bottom of the hardware set up screen in the filter cube section, shown in Figure 5.1.7, click on the grey box marked '1' to expose a pull down menu listing the available filter cubes. Select the filter cube from the list that is installed in position 1 in your Clarity device. Repeat for positions 2,3, and 4
10. When the outlines for each device turn green then they have successfully connected to Visionary
11. Proceed to expert mode by clicking on the grey triangular tab and follow the instructions in the Clarity Operating Manual to align your system (Section 5.4.6).



Figure 5.1.7 Adding filter cubes

	<p>NOTE</p> <p>At this point a restart of the Visionary software is recommended to ensure secure and correct connections.</p>
	<p>NOTE</p> <p>If a region does not turn green in the Hardware set-up window, a successful connection has not been made to the hardware. If this is the case, please refer to the Troubleshooting section of this manual (Section 6.2)</p>

5.2. First images

The next section assumes that you have correctly set-up all hardware parameters in the hardware set-up tab and that your system is fully aligned and calibrated. For alignment procedure, please refer to section 5.4.6 of the Clarity Operating Manual.

The following operations are carried out in live mode unless stated otherwise

5.2.1 Set the File-path for your Data



Figure 5.2.1.1 Save file path workflow icon

1. Click the orange triangular tab to enter live mode. The system will self calibrate
2. In Live Mode, click on the save file path workflow icon shown in Figure 5.2.1.1. A windows Explorer window will open.
3. Navigate to a suitable folder in which you wish your data to be saved
4. Type in a suitable root file name and click OK
5. You will see your file path and file name displayed in the file path workflow icon



NOTE

All data files collected into this folder will be named automatically with an auto-incrementing data and time stamp based on the root file name

5.2.2 Choose your sectioning resolution

Clarity and Clarity^{HS} contain a spinning disk. On this disk are three structured illumination patterns giving different levels of sectioning capability (Z-resolution levels). Visionary enables easy interchange between 3 disk positions to access the 3 sectioning modes: High Signal/Low Sectioning, Medium Signal/Medium Sectioning and Low signal/High Sectioning. Visionary also allows a widefield image to be acquired. These 4 different modes are accessed by clicking the tabs shown in Figure 5.2.2.1 in the Workflow Toolbar. When a sectioning mode is selected, the corresponding box will turn green. If widefield is selected, the corresponding box will turn orange. The number in the box corresponds to the theoretical Z resolution in microns for the chosen combination of disk position setting and objective lens combination.



Figure 5.2.2.1 Choosing your sectioning mode

1. Choose your chosen sectioning level or widefield mode by clicking the corresponding box shown in Figure 5.2.2.1. The disk will move into position.

5.2.3 Setting up Channels

You must now set up each colour channel you wish to record with a suitable exposure time. You may also adjust the light setting and camera settings if necessary. The exposure time, and camera and light source settings are set individually for each filter cube

Repeat steps 5.2.3.1 to 5.2.3.5 for each filter cube to be used in your experiment

5.2.3.1. Choosing and selecting Filter Cube

The filter cubes installed in your Clarity system are related to the fluorophores present in the sample. They are visualised in Visionary as four boxes in the Workflow Toolbar, shown in Figure 5.2.3.1. The box representing the filter cube currently in position in the turret is coloured and boxes are grey when not active.

1. Click on the large inner grey square corresponding to the filter cube of interest to place it in an active position in the turret. The cube will move into position.

	<p>NOTE</p> <p>Clicking on the outer yellow boundary will select the cube for use when acquiring images and display a yellow square in front of the grey box. Up to a total of four cubes can be selected simultaneously. This should be done after setting up the exposure time and other parameters for all channels before clicking acquire and is described later</p>
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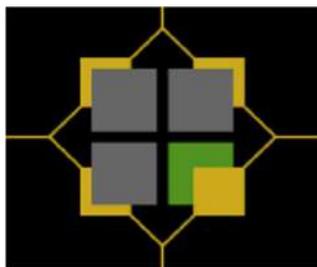
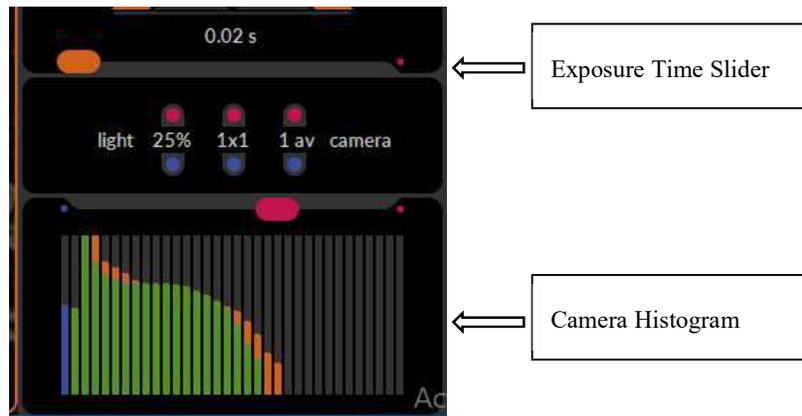


Figure 5.2.3.1: Filter Cube Selection

5.2.3.2. Setting the Exposure Time

1. Focus your sample using the manual focus knob of the microscope and using the eyepieces of the microscope.
2. Change the active microscope port to the 100% left port (or the port to which your Clarity device is attached)
3. Select a suitable filter cube as described in Section 5.2.3.1
4. You should see a live image of your sample on the screen, Adjust the focus if necessary.
5. Increase or reduce the exposure time of the camera as necessary by dragging the orange slider (Figure 5.2.3.2 Top) left/right until the histogram (showing the dynamic range of the camera) (Figure 5.2.3.2 bottom) is no more than half filled and the image is not over saturated. The minimum exposure time for the Clarity LFC is 20ms and for the Clarity HS is 10ms.



Blue Bar	Located on left hand side. The amount of subtraction occurring during the creation of a confocal image
Green Bar	The Confocal Signal (What Visionary displays on the screen in confocal mode)
Orange Bar	The total signal received by the camera (What Visionary displays on the screen in widefield mode)
Red Bar	Located on right hand side. Over-saturation (Always reduce the exposure to remove this)

Figure 5.2.3.2 Changing the Exposure Time

	<p>NOTE</p> <p>In widefield mode, only the orange and red bars of the histogram are active</p>
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5.2.3.3. Set your light source intensity (optional)

If your sample is very bright and causing over saturation, even at short exposure times, adjust the light intensity by clicking on the blue radio button shown in Figure 5.2.3.3. The light intensity may be reduced to 50%, 25% or 12.5%

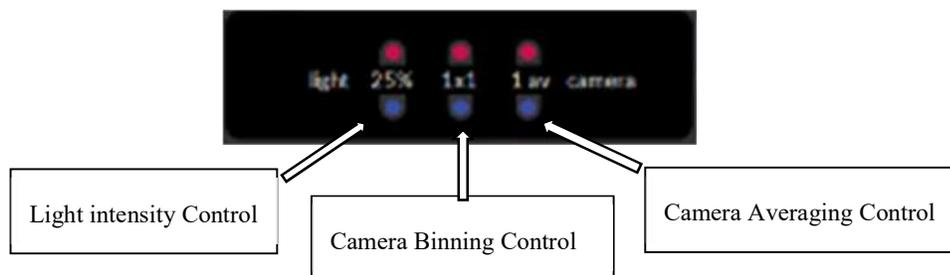


Figure 5.2.3.3 Light Intensity, Camera Binning and Camera Averaging Controls

**NOTE**

If your sample is sensitive to photo bleaching or photo toxicity, it may be useful to reduce the light intensity and increase exposure time to reduce these unwanted effects

5.2.3.4. Set your camera Binning parameter (optional)

If your sample is very weak or you are looking to use very short exposure times to record fast events, camera binning may be useful. Increase the camera binning parameter by clicking on the red radio button shown in Figure 5.2.3.3. Decrease the binning parameter by clicking on the blue radio button. Binning modes of 1x1, 2x2, 4x4 and 8x8 are available

**NOTE**

For more information about binning, please refer to the documentation of your camera manufacturer

5.2.3.5. Set your camera averaging parameters (optional)

If your sample contains noise/background caused by, for example, moving debris in cell culture media, camera averaging may be useful. Increase the camera averaging parameter by clicking on the red radio button shown in Figure 5.2.3.3. Decrease the camera averaging parameter by clicking on the blue radio button. Averaging modes of 1, 2, 3 and 4 are available. Camera averaging will take a number of images per frame therefore your resultant frame rate will be slower.

**NOTE**

For more information about camera averaging, please refer to the documentation of your camera manufacturer

5.3. Data Collection Set-up and acquisition

The instructions in this section assume the user has correctly set up the system as described in the Hardware Manual and set up the colour channels, as described in section 5.2

5.3.1 Acquiring a Z-Stack in a Single position

In order to obtain 3D information from your sample or to create a rendered maximum projection image of your sample, you should collect a Z-Stack (a series of confocal images acquired at different Z positions). There are two methods for setting up a Z Stack collection which are described in sections 5.3.1.1 and 5.3.1.2

5.3.1.1. Setting up a Z stack by defining a top and bottom limit of your sample

This method is useful when you do not know how thick your sample is and you wish the software to calculate your z range from chosen microscope positions.

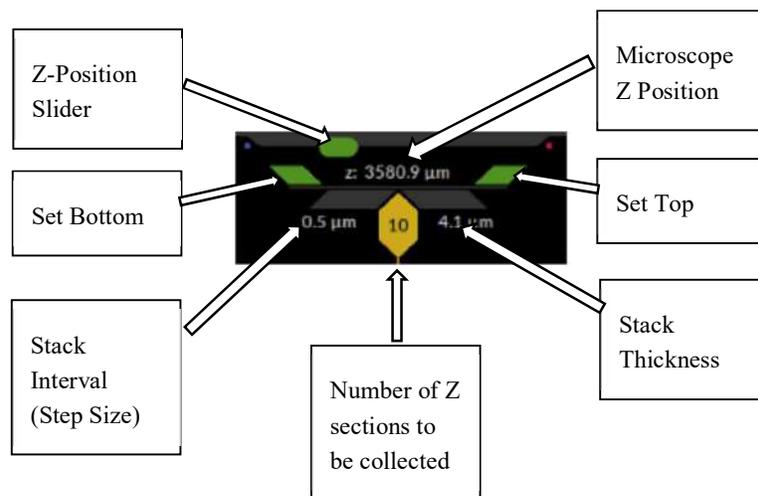


Figure 5.3.1.1 Z-Position Set-up Controls

1. Navigate to the region you want to image using the x-y stage of the microscope.
2. Use the manual focus knob of the microscope to navigate to the bottom of your sample (the value of Z position displayed in Visionary and on the microscope should decrease until the sample is just out of focus) then click the left green 'set bottom' chevron shown in Figure 5.3.1.1. This will set the bottom limit of your Z-stack.
3. Use the manual focus knob of the microscope to navigate through to the top of your sample (the value of Z position displayed in Visionary and the microscope will increase until sample is just out of focus) then click the right green 'set top' chevron as shown in Figure 5.3.1.1.
4. Click the central hexagon to activate Z-stack collection. The hexagon will turn yellow and display the number of images to be collected in the Z-stack as shown in Figure 5.3.1.1. The stack thickness is displayed on the bottom right.



NOTE

If the central Hexagon is not selected and yellow in colour, images will be collected in one Z plane at the microscope Z position displayed.

5. The stack interval (bottom left value) is automatically calculated with Nyquist sampling. To change this (to oversample or undersample for example) double click the number and enter the desired value using your keyboard.
6. Select the filter cubes to be acquired as described in section 5.2.3.1 by clicking on the yellow outer boundary
7. Check all your experimental settings and when ready click on the yellow triangular Acquire tab to begin acquisition.

5.3.1.2. Setting up a Z Stack by Defining a Mid Point and a Z-Range

This method is useful if you want a defined stack thickness for your Z stacks

1. Use the manual focus knob of the microscope to navigate to the centre/mid point of the focus range of your sample
2. Click on the stack thickness number shown in Figure 5.3.1.1 and type in the desired stack thickness (e.g 20 microns). The Z stack will be defined to collect 50% above your mid point and 50% below your mid point
3. Follow steps 4 to 7 in Section 5.3.1.1

5.3.2 Acquiring a Time-Lapse in a Single Position

This section applies when trying to acquire a series of images over a period of time. The time lapse control region of the Workflow Toolbar is shown in Figure 5.3.3.1:

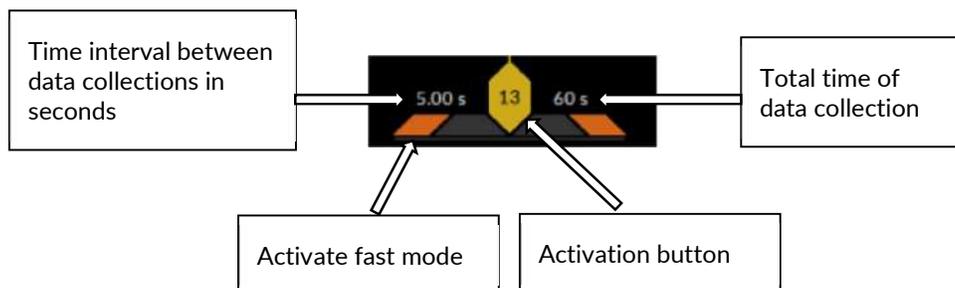


Figure 5.3.2.1 Time lapse Controls

1. Click on the time interval number and type in the desired value for the interval between the start of one data collection and the start of the repeated data collection using the keyboard
2. Click on the total time number and enter the total desired duration of the time-lapse experiment using the keyboard.
3. Click on the central activation hexagon. It will turn yellow and display the total number of data sets that will be acquired.
4. Check your other experimental settings and when ready click on the acquire tab to begin acquisition.



NOTE

In the example shown in figure 5.3.2.1, Visionary has been set to acquire one image every five seconds for sixty seconds giving a total of thirteen images.

	<p>NOTE</p> <p>If the time interval is too short for Visionary to complete a full data collection cycle then an error message will appear after the first cycle is complete and Visionary will instead gather the data as fast as it can</p>
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5.3.3 Fast Video acquisition

This section covers the creation of fast videos within Visionary. Visionary will capture images as fast as is possible with the combination of camera capability and exposure time

	<p>NOTE</p> <p>Unlike when creating a time-lapse this process can only be used with a single filter cube in a single XYZ position.</p>
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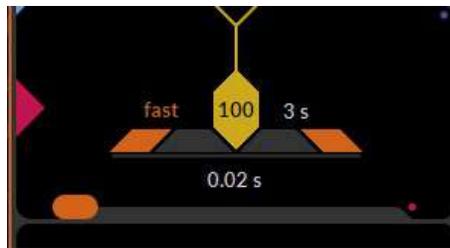


Figure 5.3.3.1 Fast Acquisition Mode Active

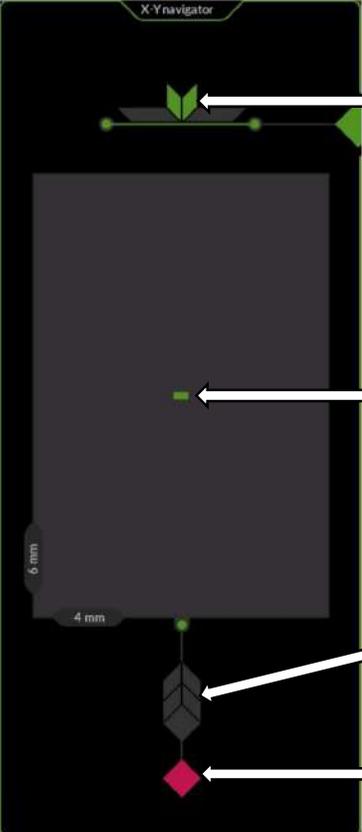
1. Click on the left orange chevron shown in figure 5.3.3.1. The display will read 'Fast' rather than a numerical value
2. Click on the Total time value and enter the total duration of the video.

	<p>NOTE</p> <p>Note that due to the image size and quantity of the images involved long duration videos are not recommended.</p>
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3. Click on the central hexagon which will select fast video mode for acquisition.
4. Check your settings and when ready click on the acquire tab to begin acquisition.

5.3.4 Multi-Position Data Collections

	<p>NOTE</p> <p>In order to collect tilescan or multiple position data, a Visionary supported motorised XY stage must be fitted to the microscope and integrated into Visionary.</p>
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The screenshot shows the XY Navigator window. At the top, it is labeled 'X-Y navigator'. The main area is a dark grey rectangle representing the live mode viewer. A green arrow points to a green selection area at the top of the viewer. A green arrow points to a green selection area on the left side of the viewer. A green arrow points to a green arrow icon at the bottom of the viewer. A red diamond icon is located at the bottom of the viewer. A green arrow points to the red diamond icon. The dimensions '6 mm' and '4 mm' are visible on the left side of the viewer.

<p>Load XY CSV Template: Load a pre-defined tile layout or multiple position list.</p>
<p>Tile Selection: The green area is what Visionary is currently displaying in the live mode viewer window.</p> <p>Define an area or number of positions by clicking and dragging a region of interest.</p> <p>The mouse wheel can be used to zoom in/out.</p> <p>This window can also be used to move to an existing saved position by simply clicking on it</p>
<p>Generate Tiles: After defining an area a green arrow will appear in this space. Clicking on the green arrow will save the tile positions into the position list in the Position Controls area in the Workflow Toolbar (figure 5.3.5.5).</p>
<p>Clear List: Clicking this will clear the current selection.</p>

Figure 5.3.4.1 XY Navigator Window

	<p>NOTE</p> <p>Visionary automatically maintains a CSV file within C:\ProgramData\Visionary containing the current/last positions used. The file is named XYPositions.CSV and if you want to generate a default CSV file to load simply copy this file to another location and rename it.</p>
---	--

The second work area containing the Position Controls is located in the Workflow Toolbar and can be seen in Figure 5.3.4.2

		<p style="text-align: center;">New Position</p> <p>This button can be used to add new positions outside of the XY Navigator. Use the motorised X-Y stage to navigate to a region of interest and click this button to add those XY coordinates to the position list for acquisition</p> <p>If the XY Navigator is open the position set is the location of the green rectangle in the centre.</p>
		<p style="text-align: center;">XY Acquisition</p> <p>This hexagonal button will activate the list of XY positions in the list to be acquired on clicking the acquire Yellow Triangular Tab. When active this button will turn yellow and the number of positions to be collected will be displayed.</p>
		<p style="text-align: center;">Move to Position</p> <p>After saving an XY position to the list you can move to the position by selecting it from the position list and clicking this button.</p>
		<p style="text-align: center;">Modify Position</p> <p>This allows you to overwrite a selected position with the current position of the XY stage.</p>
		<p style="text-align: center;">Saved Positions</p> <p>These are all the positions selected within either the XY Navigator or the buttons above.</p> <p>Scroll through the list using the mouse wheel.</p> <p>To edit the name of the position, click on a position in this list and type in a new name.</p> <p>Positions may also be deleted from this list by simply deleting the position name</p>

Figure 5.3.4.2 Position Controls

5.3.4.1. Generating a simple Tilescan

1. Focus on your sample using the manual focus knob of the microscope in live mode in Visionary and set up the colour channels/Z range as required
2. Open the X-Y navigator
3. Move the stage to the far left hand of your sample (or where you wish the left hand side of your image to be)
4. Click new position in the Position Control Work area
5. Move the stage to the far right hand side of your sample/image
6. Click new position
7. Repeat steps 5 and 6 for the top and bottom of your image



NOTE

You should now have 4 regions defined in the XY Navigator in a diamond shape as shown in Figure 5.3.4.3

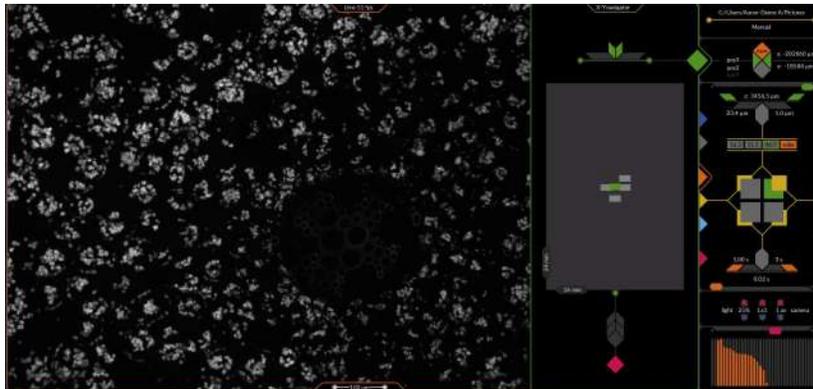


Figure 5.3.4.3 4 tiles defined in X-Y Navigator Screen

8. Using the mouse, drag and drop a region of interest that covers all 4 of these individual regions to create one larger region as shown in Figure 5.3.4.4
9. Click on the Green arrows in the generate tiles section of the XY Navigator to add the tiles to the Saved Position List as shown in Figure 5.3.4.5

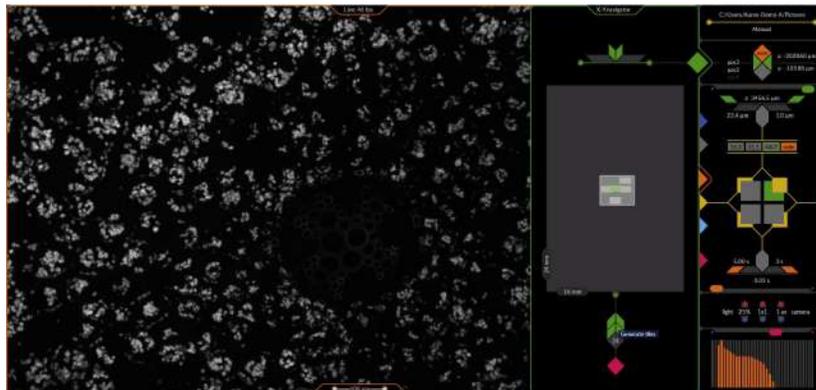


Figure 5.3.4.4 Generating Tiles in XY Navigator

10. Rename the positions as required

11. Click on the XY acquisition hexagon so that it turns yellow and displays the number of tiles to be collected as shown in Figure 5.3.4.5
12. Proceed with acquisition

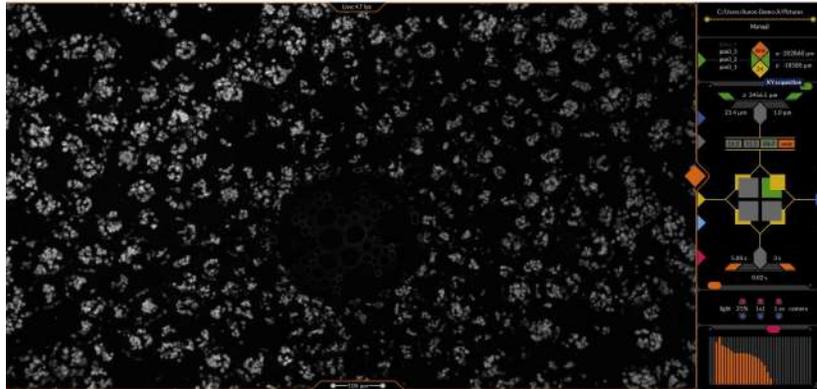


Figure 5.3.4.5 XY Positions Activated

5.3.5 Multi-dimensional Data Collections

Data collection parameters may be combined to form more complicated experimental conditions. For example a time lapse experiment including z-stack acquisition, x-y navigation and frame skipping. Simply click on the yellow 'activate' hexagonal button for each part of the experimental procedure to select which parameters should be collected. An example set-up is shown in Figure 5.3.5.1

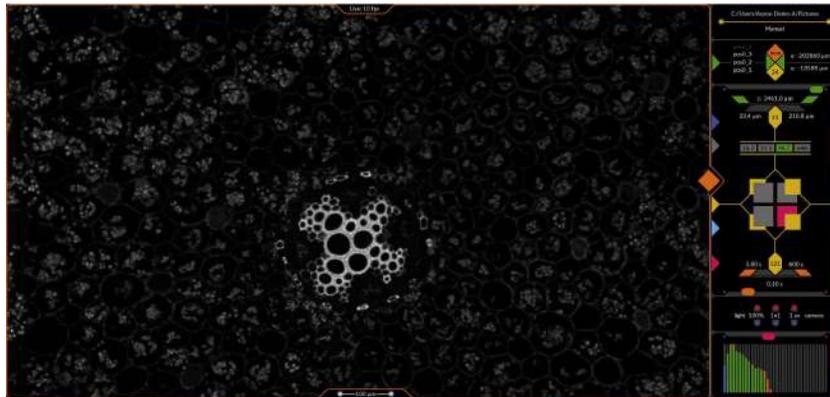


Figure 5.3.5.1 An example of a multi- dimensional experimental set-up

5.3.6 Advanced channels

In some cases, you may wish to alter the standard behaviour of colour channels within Visionary, for example you may wish to capture a brightfield image with the transillumination turned on instead of a fluorescence image. Up to 3 advanced channel settings can be set for each colour channel simultaneously

To access Advanced Channels, shown in Figure 5.3.6.1, enter expert mode by clicking on the grey triangular tab

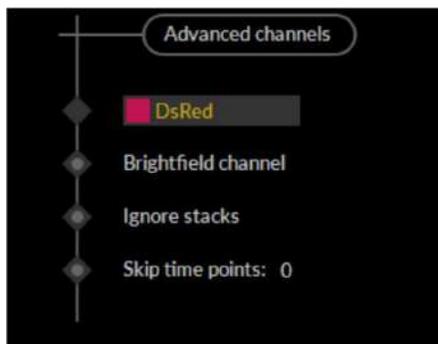


Figure 5.3.6.1 Advanced channels

5.3.6.1. Setting up a brightfield channel in Advanced Channels

If you wish to look at your sample in brightfield imaging mode, as well as fluorescence, one channel must be set to be a brightfield channel.

	<p>NOTE</p> <p>When a channel is set up to be a brightfield channel, the transillumination lamp will be switched on when that channel is active instead of the fluorescence lamp</p>
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1. Click on the filter cube to be changed to brightfield in the Workflow Toolbar.
2. Open expert mode by clicking on the grey triangular tab. In advanced channel region, you will see this filter cube listed
3. Click the radio button marked Brightfield channel as shown in Figure 5.3.6.1. The button will turn yellow.
4. Close expert mode and enter live mode by clicking on the orange triangular tab.

5.3.6.2. Ignore Z stacks for a channel in Advanced Channels

In some cases you may wish to capture a channel in one Z plane rather than collecting z stacks (for example if the channel is set as a brightfield channel)

1. Click on the filter cube to be changed to one z plane only in the Workflow Toolbar.

2. Open expert mode by clicking on the grey triangular tab. In advanced channel region, you will see this filter cube listed
3. Click the radio button marked Ignore Stacks shown in Figure 5.3.6.1. The button will turn yellow.
4. Close expert mode and enter live mode by clicking on the orange triangular tab

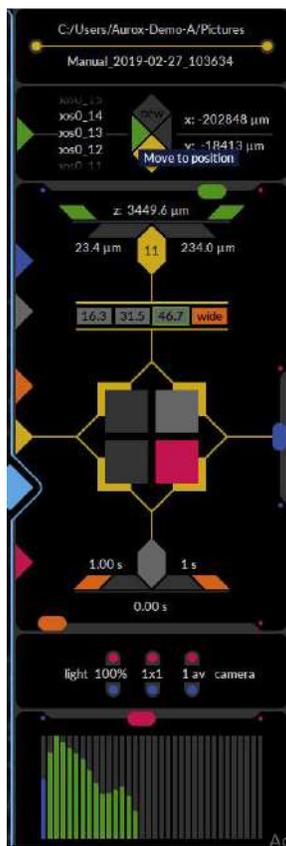
5.3.6.3. Frame skipping in Advanced Channels

In some cases you may wish to capture a fluorescence channel less frequently than other channels (if your sample bleaches faster at a certain wavelength for example)

1. Click on the filter cube to be changed to skipping mode in the Workflow Toolbar.
2. Open expert mode by clicking on the grey triangular tab. In advanced channel region, you will see this filter cube listed
3. Click the radio button marked skip time points shown in Figure 5.3.6.1. The button will turn yellow.
4. Click on the number '0' and enter the number of frames you wish to skip for this channel
5. Close expert mode and enter live mode by clicking on the orange triangular tab

5.3.7 Inspect Mode

In inspect mode, the function of the Workflow Toolbar Control Icons change compared their functions in Live Mode. These are summarized in Figure 5.3.7.1



Function in Live Mode	Function in Inspect Mode
Filename/filepath Selection	One click export to Image-J/Fiji
XY Positioning Mode	Navigate through recorded tiles
Z Position Control	Navigate through recorded Z range
Sectioning Mode Selection	Displays sectioning mode used in data collection
Filter Cube Selection	Change the displayed recorded channel
Time Lapse Control	Shows the number of time lapse images recorded
Exposure time Slider	Plays through the recorded timelapse

Figure 5.3.7.1 Inspect Mode Tab

5.3.7.1. Exporting Data to ImageJ/Fiji

While in the Visionary Inspect window if you are satisfied with the captured data then it can be easily exported to ImageJ/Fiji by clicking the file-path again as shown in Figure 5.3.7.2. Visionary will now open ImageJ/Fiji.

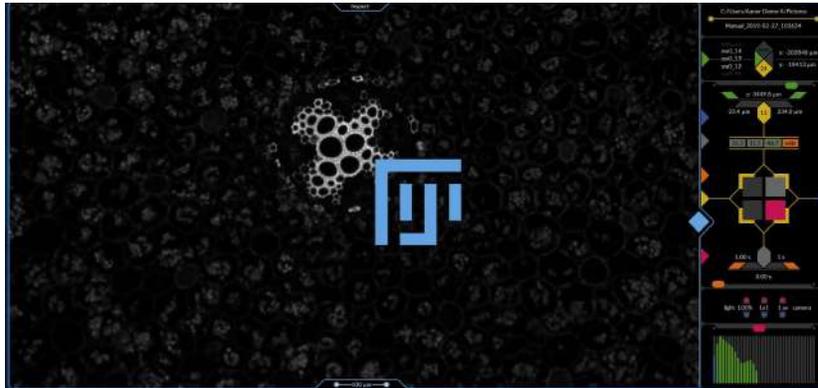


Figure 5.3.7.2 One click export to Fiji

6. APPENDIX

6.1. Directory Structure

The Visionary program system is installed in the following directory structure;

Windows program files directory (typically C:\)

Visionary root (typically C:\Program Files)

Applications root (\Visionary)

*.exe, *.dll, *.ini*,.dat,*.lib,*.xml,*.exp*,.predefines.

This directory contains the Visionary executable and drivers.

Exported Data File Format

All exported data is stored in .ome.tiff, .companion.ome & .position.csv formats.

Definitions root (typically C:\ProgramData\Visionary)

This is the location of the files containing your current equipment set-up. Any files deleted here will be generated again when Visionary opens.

Hardware Definition File: HardwareDescriptions.json.

This file contains all the data on all equipment compatible with Visionary.

Filter Definition File: ClarityFilters.json.

This file contains all the filter cubes currently sold by Aurox.

Log File: Visionary.log.

This file is where the log of all errors encountered by Visionary will be stored.

Flatfield Reference Image: Flatfield.raw.

This file contains the flat field correction data exported from Visionary.

6.2. Troubleshooting

Problem	Possible Reason	Possible Solutions
In Hardware set up Mode, my device is not turning green and not connecting	No communication between Visionary and device	Power cycle the PC, and the device and restart Visionary. Ensure device is properly connected by USB or serial cable and switched on. Ensure correct device is selected in Visionary
	Wrong COM port selected in Visionary	Open Device manager and check which COM port your device is connected to. Change the COM port in the Hardware set up tab
The system will not calibrate	Camera or mirror misalignment	Refer to the Clarity Hardware Operating manual for information regarding calibration and alignment procedure
	Too much light entering the system	Change microscope active port to eyepieces or shield the sample from room light during calibration step