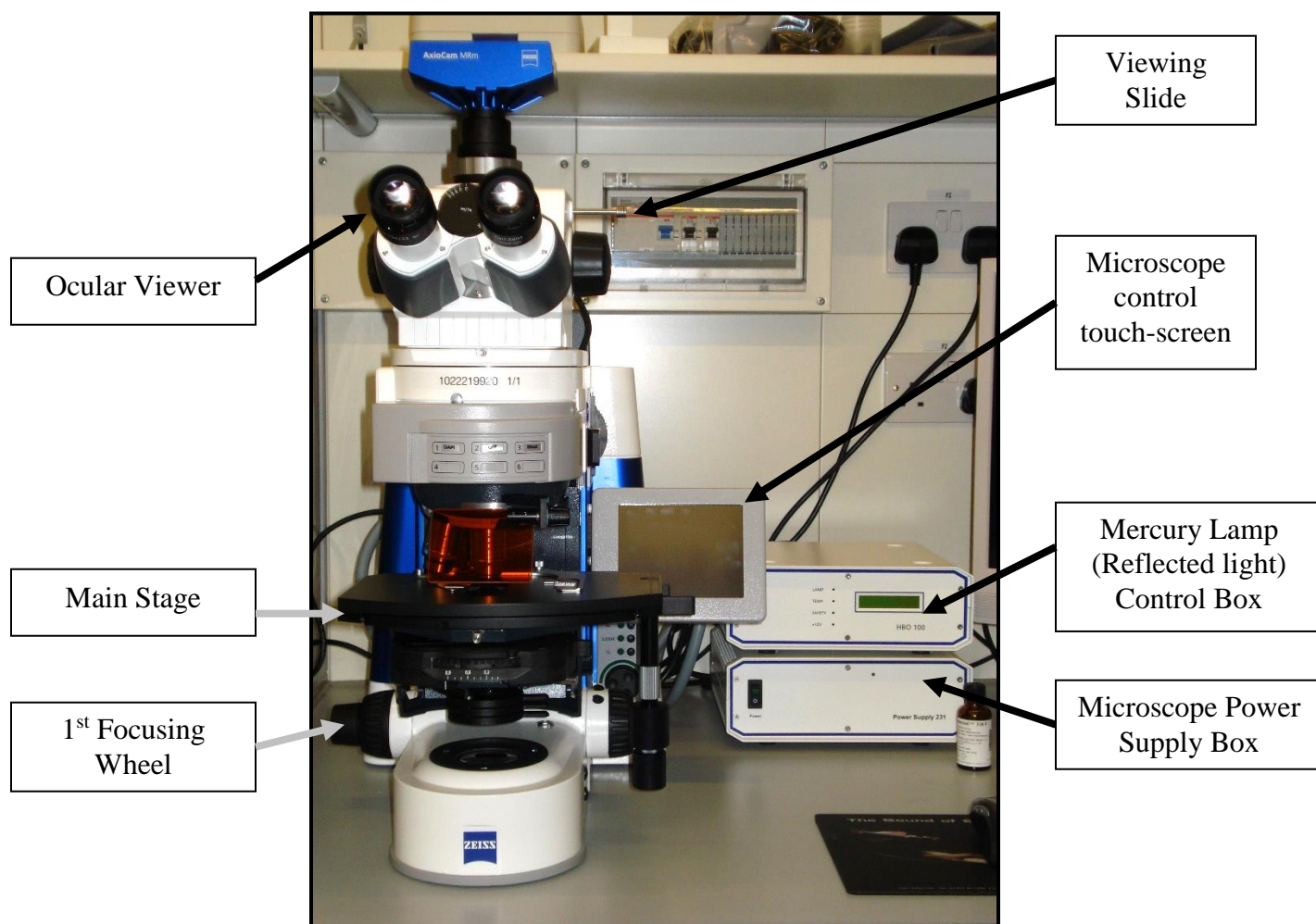# ZEISS AXIO IMAGER A1 AND M1
A USERS GUIDE

This is intended to provide a basic coverage of use of the Zeiss Axio Imager M1 for multi-channelled fluorescent acquisition. For more complex usages please contact

Fig.1 – Front View of Microscope

Viewing Slide

Ocular Viewer

Microscope control touch-screen

Main Stage

Mercury Lamp (Reflected light) Control Box

1st Focusing Wheel

Microscope Power Supply Box

1) SWITCHING ON

⚠️ CHECK MICROSCOPE LOG BOOK OR WITH PREVIOUS USER PRIOR TO SWITCHING ON. 30 MINUTES MUST HAVE PASSED BETWEEN TURNING OFF REFLECTED LIGHT LAMP AND TURNING BACK ON IN ORDER FOR MERCURY BULB TO COOL.

a) Switch on mercury lamp control and Microscope power boxes located proximally to main microscope stage. Turn on Microscope switch (located behind 1st focusing wheel)

b) Turn on computer and Activate AxioVision Program
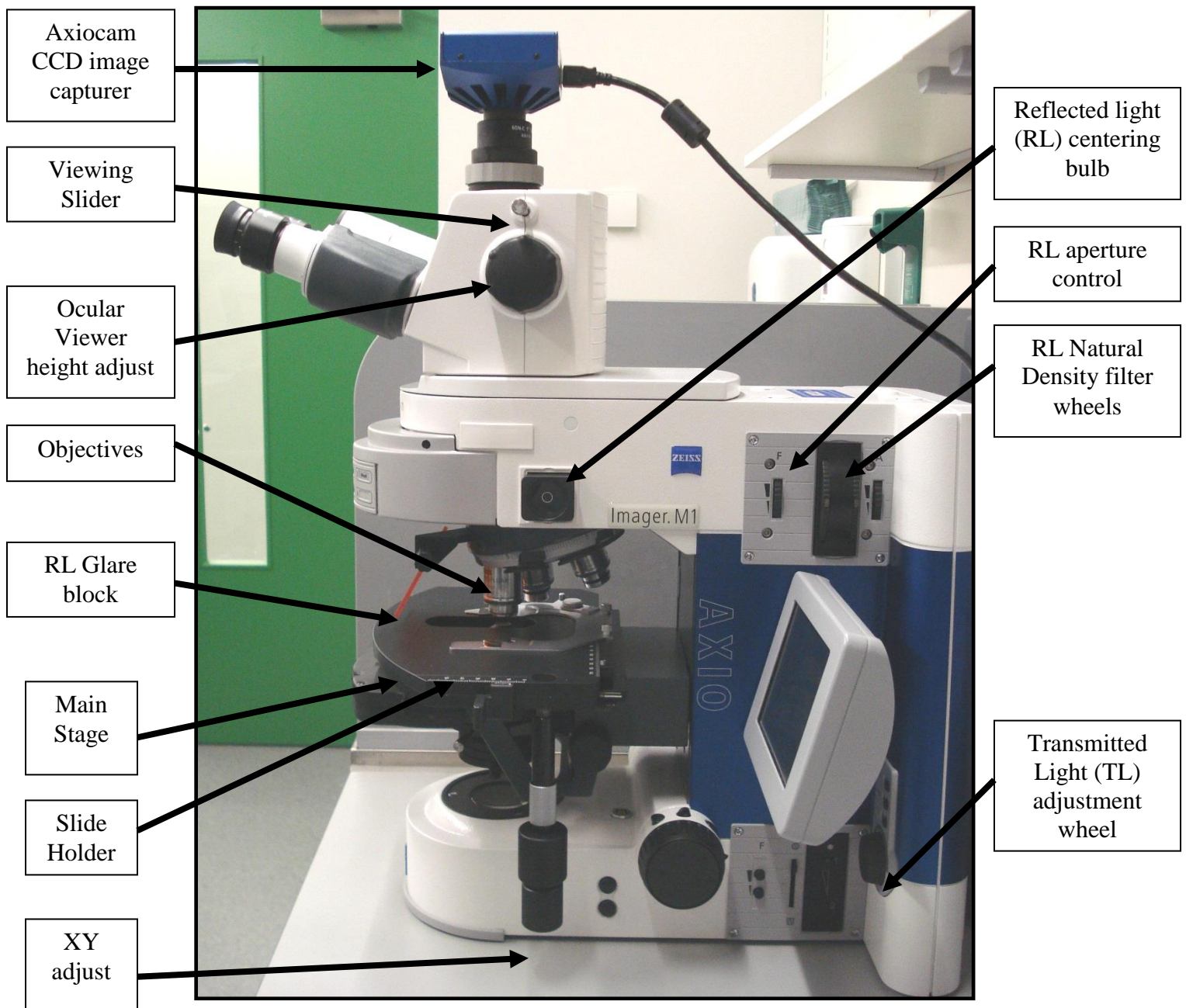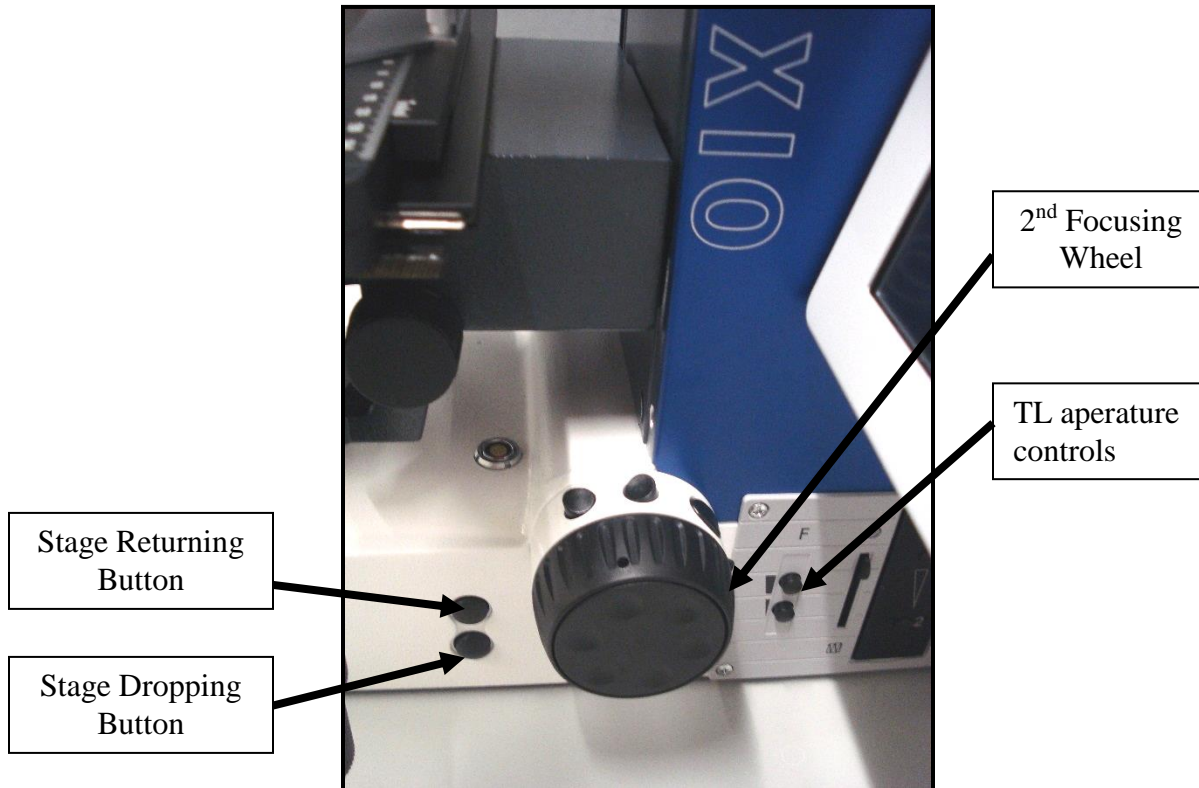
Fig 2 – Side View of Microscope

Axiocam CCD image capturer

Viewing Slider

Ocular Viewer height adjust

Objectives

RL Glare block

Main Stage

Slide Holder

XY adjust

Reflected light (RL) centering bulb

RL aperture control

RL Natural Density filter wheels

Transmitted Light (TL) adjustment wheel

Fig 3 – Side View of Lower Microscope

2) LOCATE FOCAL PLANE OF SAMPLE

   a) Using the microscope touch-screen open TL (Transmitted light) filter
   b) Reduce light level using TL adjustment wheel
   c) Place sample slide in slide holder.
   d) Select magnification
      - if using x40 or x60 addition of droplet of oil is necessary. This is achieved by pressing stage dropping button and adding a tear sized drop on top of slide (so cover slips are necessary) and returning stage by pressing stage returning button. Make sure at this point to press 'set working position' on the touch screen control.
   e) Using Ocular Viewer (viewing slide must be fully inserted) focus the sample using Focusing Wheel. (For finer focus use focussing 1st focusing wheel)
   f) Switch TL filter to closed on touch-screen controls

3) ACQUISITION OF IMAGES

It is possible to choose various methods of acquisition of an image and most actions capable from the software are possible. This will show you how to collect a Z-Stack. If you only desire a single multichannel image you can leave out to portions about Z-stack. Remember to not select a Z-stack (section f))
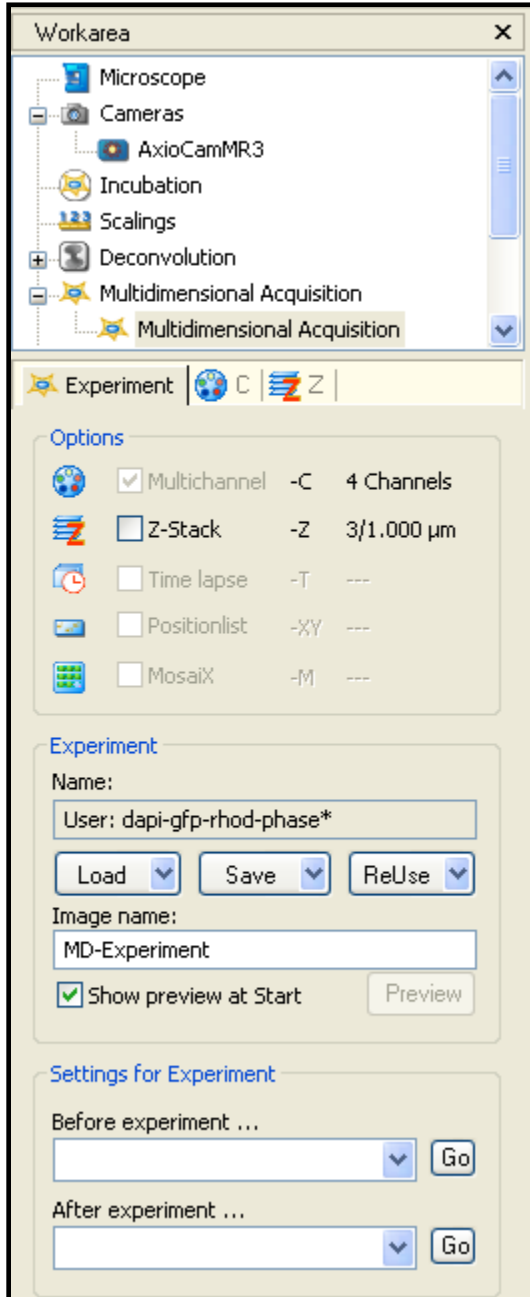
Fig 4 – Axiovision Tool Bar
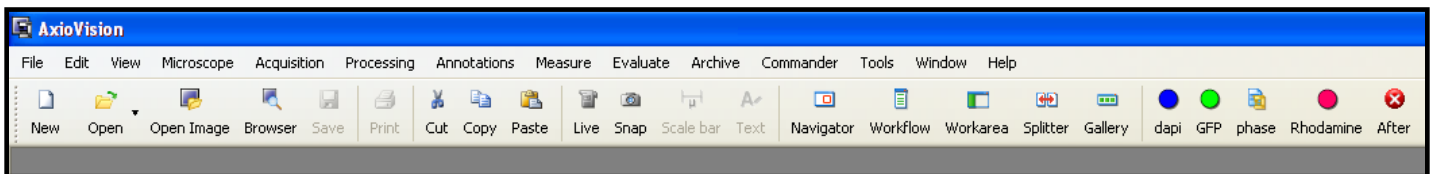
a) Select Workarea tool bar if workarea is not showing – Fig 4

Fig 5 – Work area
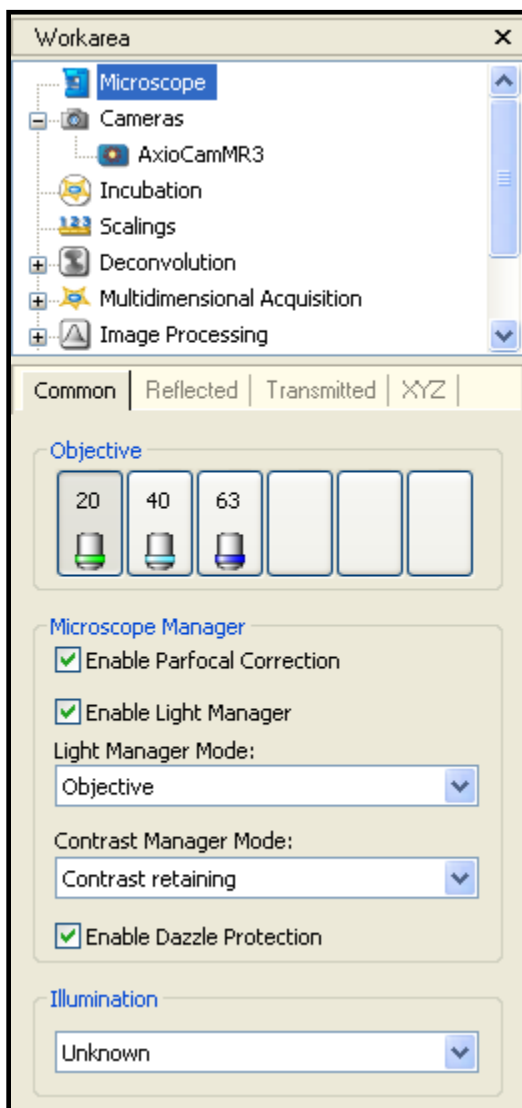
b) Select Objective of choice (ensuring oil is used as previously described above in 2c) or leave as presently chosen - Fig 5

c) Select 'Multidimensional Acquisition' sub tab (as shown also in Fig 6)

Fig 6 – Multidimensional Acquisition Experiment Tab

d)   Select Experiment tab and click on ReUse button – Fig 6

e)   Select a .zvx file normally found in directory;

'my documents/Carl Zeiss/data/experiment'

These files are labelled with the combination of channels that will be examined by microscope if chosen. For example in the Experiment box shown in Fig 6 the name shown is 'dapi-gfp-rhod-phase*' – This file will make the microscope acquire images through all 4 channels
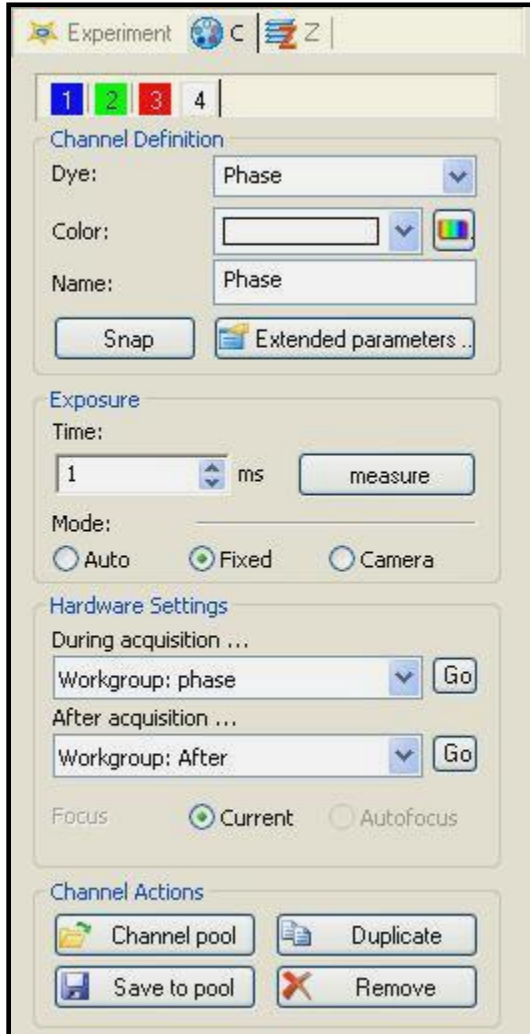
It is also possible to reselect settings from a saved image to replicate the settings from that. This is achieved in the 'ReUse' drop down menu and choosing to load from image.

f)   If you desire to create a Z-Stack click the Z-Stack button in the 'Options' box

g)   Select the C tab – Fig 6

Fig 7 – C tab of Multidimensional Acquisition

h) Ensure the correct condenser filter is selected for application – Fig 8

H – Brightfield
2 – Phase contrast in x20 objective
3 – Phase Contrast in x40 and x60 Objectives

I,II and III are for DIC applications which are not currently installed, treat as brightfield

1 is intended for phase contrast using an x10 objective which is not currently installed

i) It is now possible to select the optimal settings for each channel by clicking on each channel separately and selecting measure in the exposure box – This will automatically select an optimal exposure time, if you desire a known exposure time to generate comparable images select the desired exposure time. The Exposure Measurement window will open. Adjust exposure here or click ok
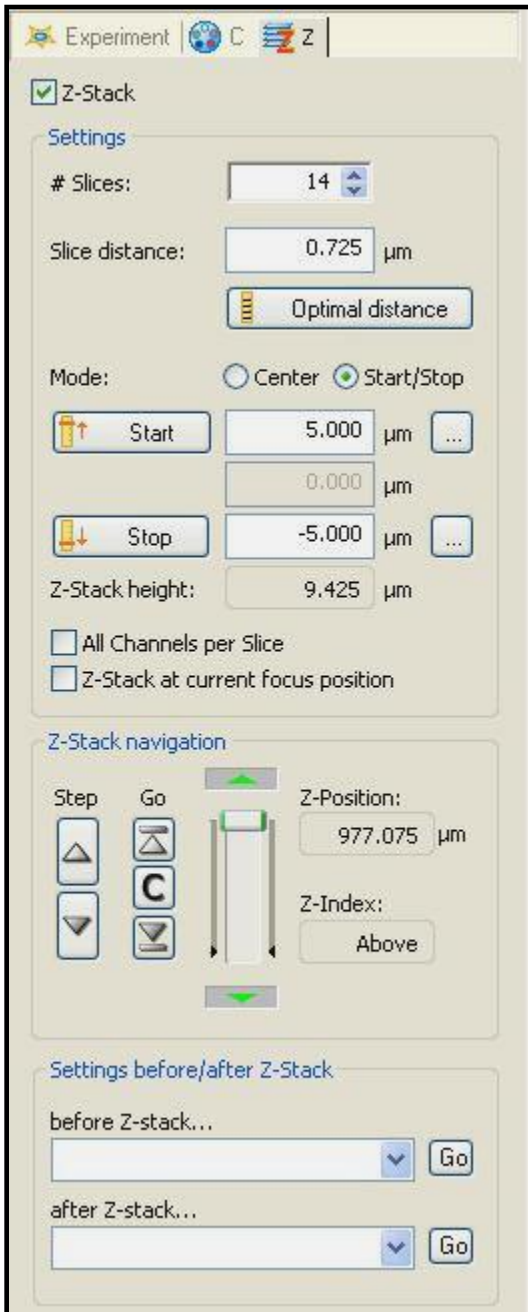
Figure 8 – Condenser Controls.

If you do not desire to collect a Z-stack ignore sections j) to o)

j) Open the Z tab – Fig 9

Fig 9 – Multidimensional Acquisition Z tab

k)   if you desire a collection of images through the Z axis click on Z button at top of tab – as shown fig 10. Select Start/Stop mode as shown.

l)   Using ocular viewer focus (slide viewing slider in) to the top of your sample and click on start button – the computer now marks where imaging will begin

m)   Focus to beneath sample and click on stop

n)   Select a slice distance so that z slices are at desired distance from each other.

o)   Return Viewing slider to the original position (pulled out)

p)   Click on Start – located at bottom of screen near left side.

q)   Once the series of images (or image) is collected it is prudent to save immediately. Saving as a .zvi file is recommended as ALL data about how it was collected by the microscope is saved. These files are larger but you can return to the file and process to a much greater proportion to other file formats. It is possible once you have finished creating desired images to export as a smaller file type of choice.

4) IMAGE PROCESSING AND ANNOTATION

VIEWING YOUR IMAGE OR Z-STACK

You should now have a Z-Stack of you sample in every channel desired - Fig 10. In this format the data is still raw and is best saved to be processed separately afterwards.
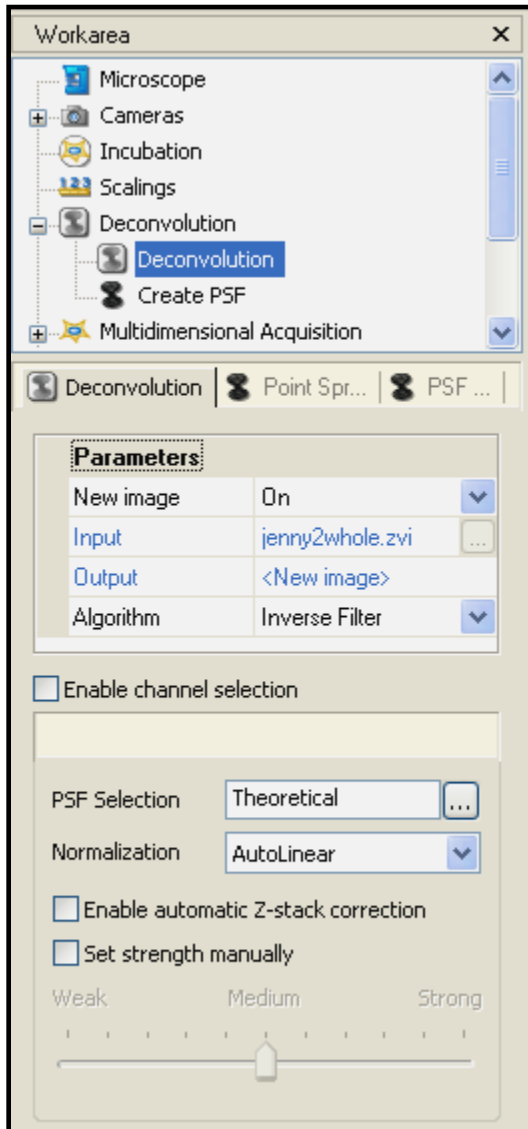
Fig 10 – Z-stack Window
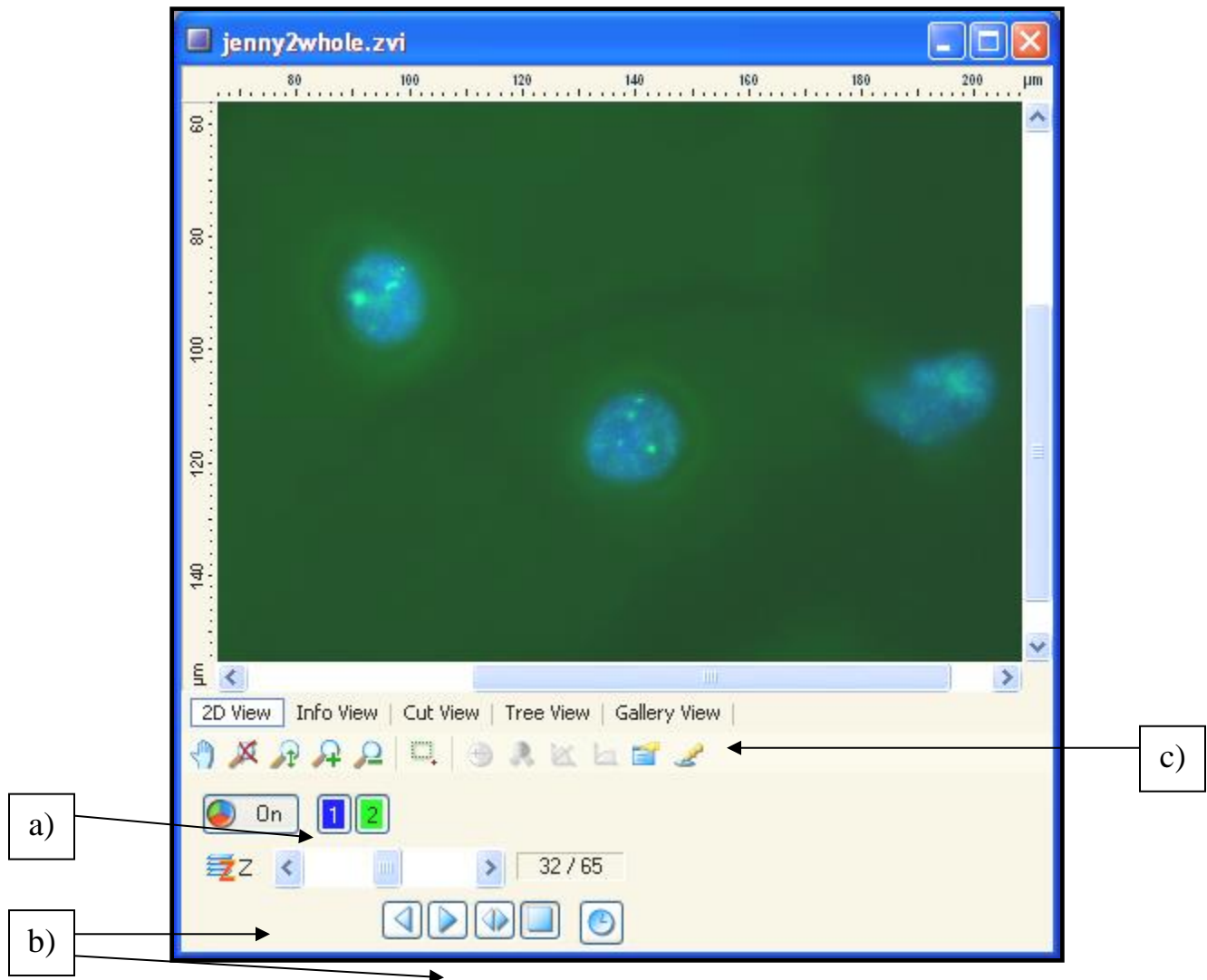
a)   You can turn each channel collected on and off using the   channel buttons.

b)   Scroll through the images top to bottom using the Z scroll bar or use the play button
        to   animate the scrolling.

c)   Choose a view with each channel tiled separately using gallery view.

DECONVOLUTION OF Z-STACK

Fig 11 – Deconvolution Workarea

Axiovision software comes with a deconvolution image processing option.
The function of deconvolution is to 'clean up' images to remove positional and chromatic aberrations. There are a choice of 3 algorithms which each function in their own way. Simply click on the image you want to process and then click on start when deconvolution is selected. This process can take a long time as it is very processing dependant. More slices will take a lot longer to deconvolve, as will more channels.
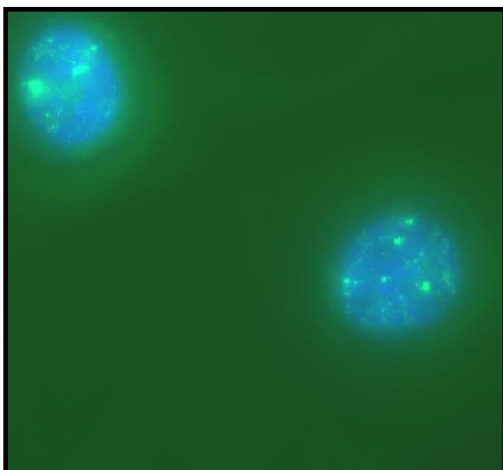
ORTHOVIEW

Fig 12 – Orthoview Workarea
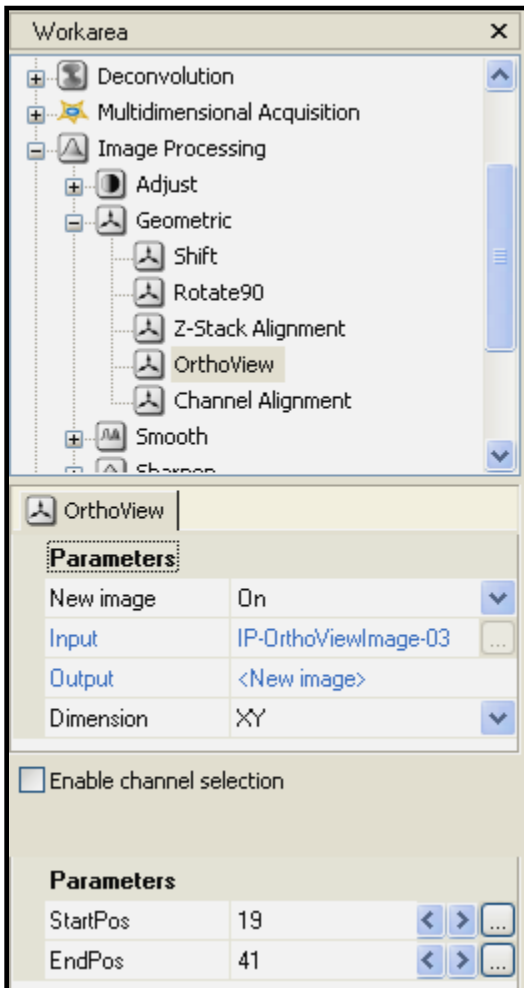
The Orthoview option will take a Z-Stack and average and overlay images so that all the information from the Z-Stack can be viewed at once in a 2D image.

a) select image to process

b) select initial and final frame of view desired and enter into parameters section of workarea
    (StartPos and EndPos – Fig 12)

c) Select dimension of view
    (default of ZY)

d) Click Start to create new image.

Fig 13 – Example of Orthoview projection from Z-Stack shown above

If you compare this image to Fig 10 you can see that more background green tone is present. This image was orthoview-processed but not deconvolved. The combination of both can produce much clearer images.
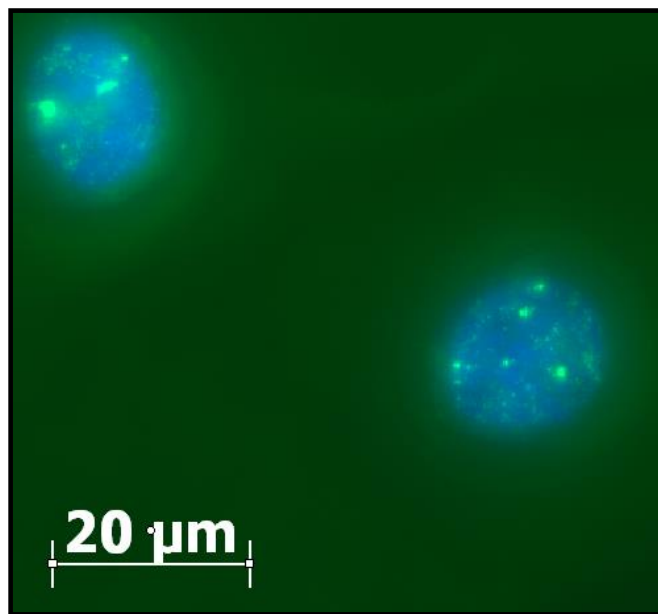
<u>ANNOTATIONS</u>

Axiovision can annotate images for publication. Things like Text, Arrows, Scale bars and geometric shapes can be added to your choice of colour, size and style. These can be chosen from the annotations tab in the tool bar – Fig 4

<u>Fig 14 – Scaling Tab</u>
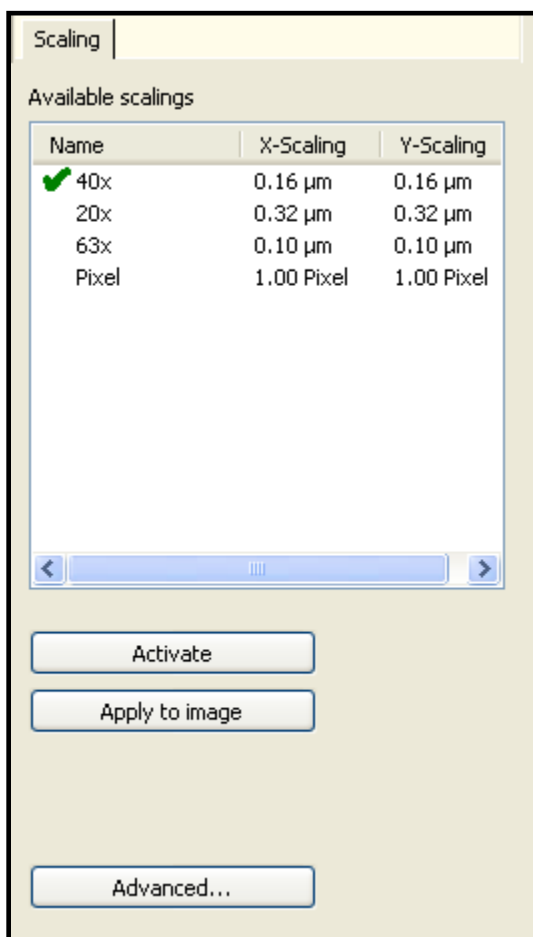
a) When adding a scale bar the scale of the pictures is not automatically chosen to be the right magnification. When you choose to annotate with a scale bar a scaling tab needs to be opened. This allows you to set the scale of the image

b) Select scale and click apply to image or both activate and apply to image for multiple images of same magnification.

c) Draw scale bar on image as desired

<u>Fig 15 – Example of Annotated Image</u>

Size of scale bars can be chosen by clicking
On end of bar and dragging it to a larger
size

Other annotations such a text or geometric
Shapes do not require setting to use.
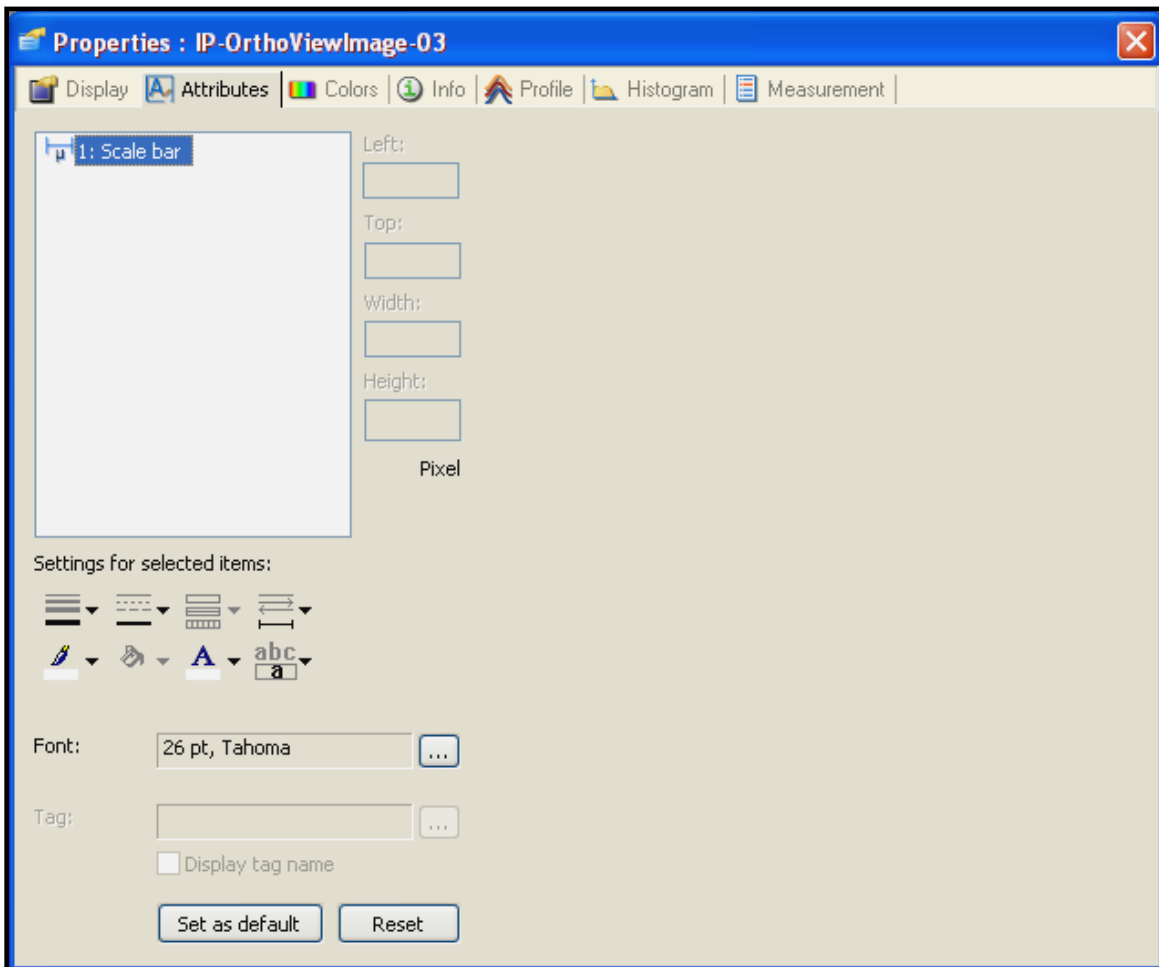
Fig 16 – Annotation / Image Properties Window

Figure 16 shows the properties window that can be accessed for any image by right clicking on the image and selecting properties. It is in this window that the Attributes tab can be selected where properties of annotations can be changed. This is particularly useful when annotation colour is not contrasting enough to background.

In the Display tab image properties such as brightness, contrast and gamma can be altered. These are useful when trying to bring out morphological data in an image.