



**Offices of Health Science Research
Faculty of Medicine Ramathibodi Hospital**


Support Document

**Title: Instructions for Using the ZEISS Axio Imager M2
Microscope and ZEN Pro Software**

Total pages: 27

Issued Date: 9th June 2025

Document code: WI-MI-16:01		Signature	Date
Prepared/Compiled by:	Pawarit Innachai Research assistant	<i>Pawarit Innachai</i>	13 June 2025
Revised by:	Tassanee Lerksuthirat Researcher	<i>Tassanee Lerksuthirat</i>	12 Jun 2025
	Chalisa Jaturapaktrarak Research assistant	<i>Chalisa Jaturapaktrarak</i>	13 JUN 2025
Approved by:	Thidarat Rujirawat Head of the Microbiology Unit	<i>Thidarat Rujirawat</i>	13 Jun 2025

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 3 of 27

1. Equipment/Related Items:

1.1 Axio Imager M2 Microscope (Fully Motorized Microscope)

1.2 Computer and Monitor

1.3 LED light source

1.3.1 White light source: LED

1.3.2 Fluorescence light source: LED Colibri 7, Type R[G/Y]B-UV

1. UV (385/30 nm - 370-400 nm) for excitation of DAPI, Hoechst 33342, Hoechst 33258, Alexa Fluor 350, Alexa Fluor 405, Indo-1, eBFP / BFP, eGFP (wt), True Blue and similar dyes.

2. Blue (469/38 nm - 450-488 nm) for excitation of FM1-43, Cy2, eGFP, NBD, MitoTracker Green, Alexa Fluor 488, BCECF, Calcein, DiO SNAFL, YO-Pro-1, Nissl, LysoSensor Green, honeydew, FITC/ Fluorescein, Kaede (green /red), PerCP, YoYo-1, FuraRed and similar dyes.

3. Green (555/30 nm - 540-570 nm) for excitation of TRITC, 7-AAD, Cy3, tdTomato, Alexa Fluor 546, Alexa Fluor 555, DsRed, mOrange, TagRFP, SNARF, DyLight 549, Spectrum Orange and similar dyes.

4. Yellow (590/27 nm - 576-603 nm) for excitation of MitoTracker RED FM/CMXRos, txRed, mCherry, mRFP1, Cy3.5, Rhodamine B, Alexa Fluor 568, Dylight 594, Alexa Fluor 594, Bodipy TR and similar dyes.

5. Red (631/33 nm - 614-647 nm) for excitation of Alexa Fluor 633, Alexa Fluor 647, Cy5, DRAQ5, ToTo-3, ATTO-655, MitoTracker DeepRed, APC, ATTO-647N and similar dyes.

1.4 Filter

1.4.1 Filter set 02 DAPI G 365 FT 395 LP 397

1.4.2 Filter set 44 FITC EX BP 475/40, BS FT 500, EM BP 530/50

1.4.3 63 HE mRFP EX BP 572/25 (HE) FT 590 (HE) BP629/62 (HE)

1.4.4 90 HE DAPI/ GFP/ Cy3/ Cy5 EX BP 613/33

Beam splitter: QBS 405+493+575+653

Emission: QBP 425/30+514/30+592/25+709/100

1.4.5 Cy 3 Chroma EX AT540/25x, BS AT565DC, EM AT605/55m

1.5 Objective Lens:

1.5.1 N-Achroplan 5x/0.15 M27 (FWD=12.0mm)

1.5.2 N-Achroplan 10x/0.25 M27 (FWD=6.5mm)

1.5.3 N-Achroplan 20x/0.45 M27 (FWD=0.63mm)


1.5.4 EC Plan-Neofluar 40x/0.75 M27 (FWD=0.71mm)

1.5.5 EC Plan-Neofluar 100x/1.30 Oil M27 (FWD=0.20mm)

1.6 Camera

1.6.1 Color - ZEISS AxioCam 305 color

1.6.2 Mono - ZEISS AxioCam 807 mono

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 4 of 27	

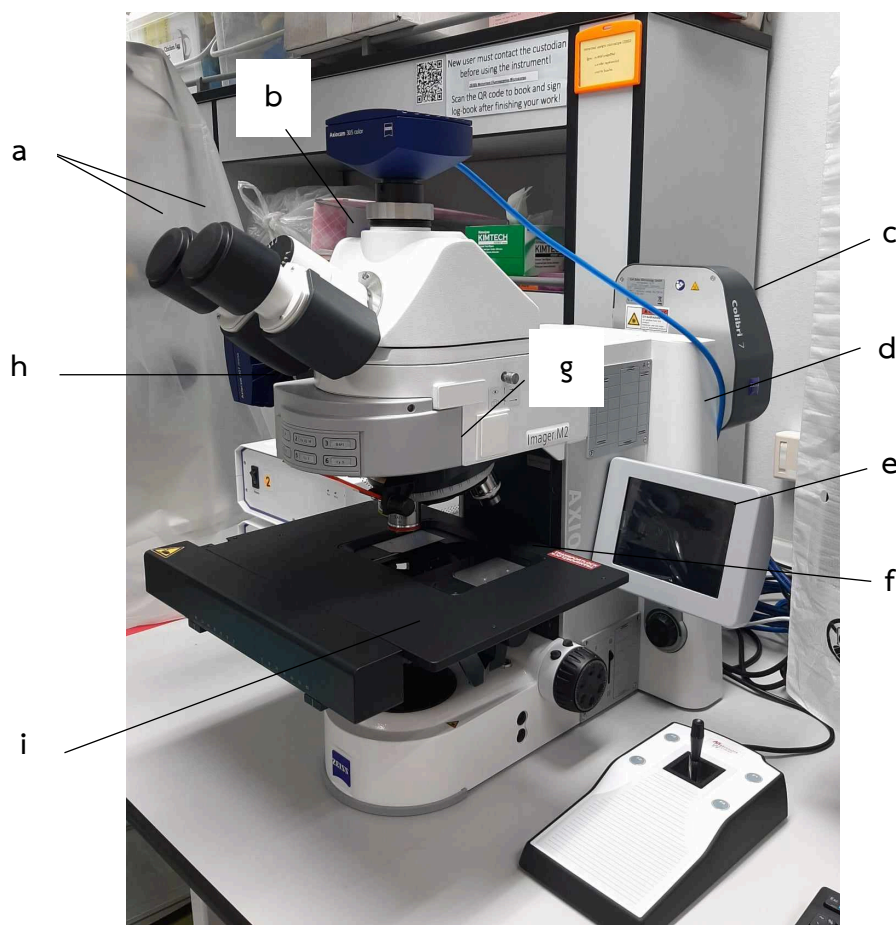



Figure 1. Overall view of Axio Imager - motorized

- a. Eyepieces
- b. Binocular phototube
- c. Fluorescence light source: LED Colibri 7
- d. Microscope stand, motorized
- e. TFT display
- f. Motorized stage
- g. Nosepiece
- h. Reflector turret
- i. White light source: LED

2. Scope of Work

Custodians:	2.1 Tassanee	Lerksuthirat	Position: Researcher
	2.2 Pawarit	Innachai	Position: Research Assistant
	2.3 Chalisa	Jaturapaktrarak	Position: Research Assistant

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 5 of 27

3. Method of Operation

3.1 Turn on the Uninterruptible Power Supply (UPS) (No.1)



Image showing the status of the UPS


3.2 Turn on the microscope, starting with the power supply of the microscope (No.2 and No. 3). Then, press the power button (No. 4) located on the left side of the user.



Power supply of the microscope



The Power On/Off Button

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 6 of 27

3.3 Turn on the computer (No. 5) and the monitor.



Power on the computer

3.4 Launch the software by double-clicking the ZEN Pro icon.

3.5 Start using the ZEN software. The program will detect the devices connected to the microscope system, displaying a screen as shown in Figure 2. Click on the "Calibrate Now" option.

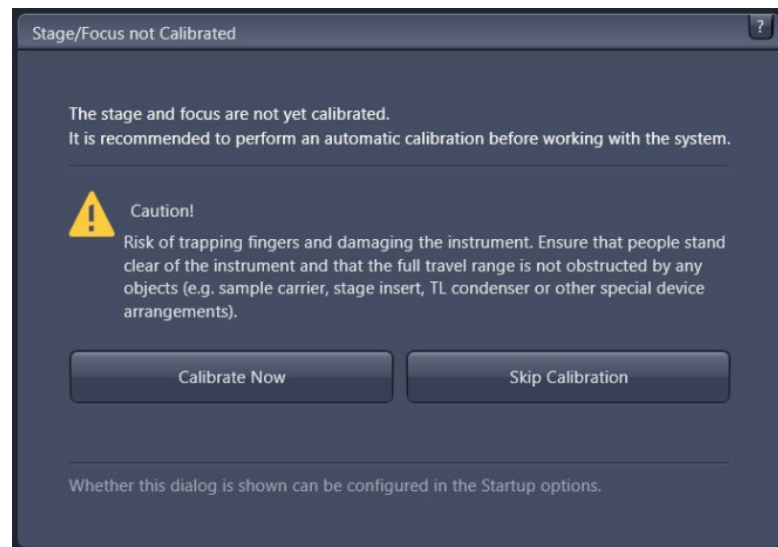



Figure 2. Calibrate stage

3.6 Locate mode: Select the area of interest in the sample through the eyepiece or digital camera.

Choose the "Favorites" button to select the desired technique (see Figure 3, yellow frame).

3.6.1 **DAPI**: Fluorescent dye in the blue emission range, including DAPI, Hoechst 33342, Alexa Fluor 405, or other similar colours.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 7 of 27	

3.6.2 **FITC**: Fluorescent dye in the green emission range, including FITC, GFP, eGFP, Alexa Fluor 488, or other similar colours.

3.6.3 **Cy3**: Fluorescent dye in the orange-red emission range, including Cy3, TRITC, Alexa Fluor 555, or other similar colours.

3.6.4 **Cy5**: Fluorescent dye in the far red emission range, including Cy5, APC, Alexa Fluor 647, or other similar colours.

3.6.5 **TexRed**: Fluorescent dye in the red emission range, including MitoTracker RED FM/CMXRos, Alexa Fluor 594, TexasRed, mCherry, Cy3.5, or other similar colours.

3.6.6 **BF**: Brightfield

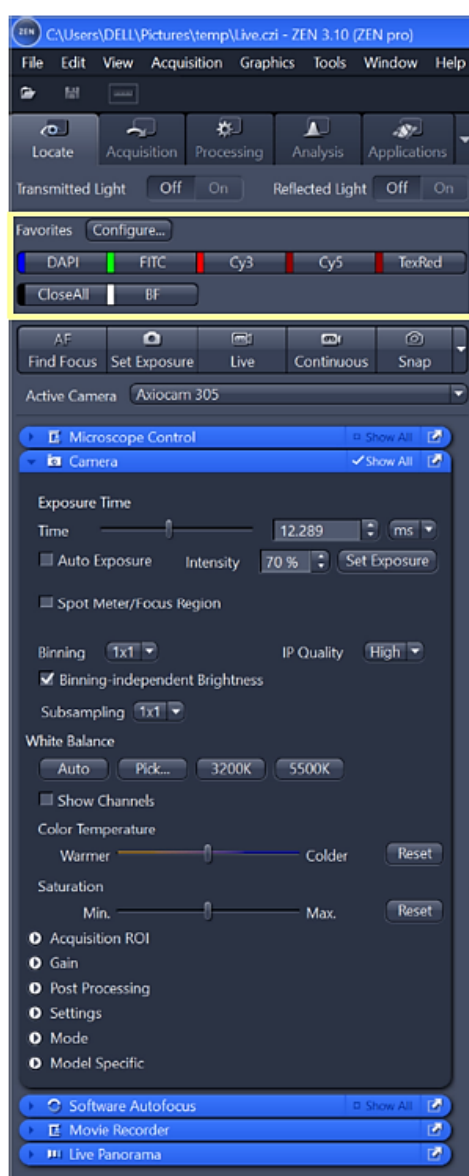



Figure 3. Locate mode

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 8 of 27	

3.7 Change the objective lens used for imaging by selecting from the left side or the Microscope window on the right side (Figure 4).

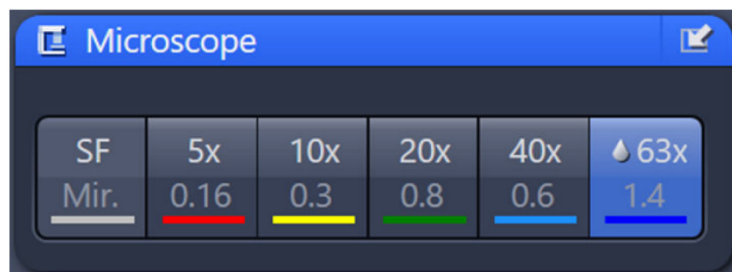


Figure 4. Magnification of the Objective Lens

3.8 Preview the image and capture images from the digital camera (see Figure 5).

3.8.1 Select the appropriate camera for use under Active Camera:

Axiocam 305

- For colour images, **push** the silver knob on the camera head all the way in (you will still be able to see the image through the eyepiece).

Axiocam 807

- For black and white images, **pull** the silver knob on the camera head all the way out.


3.8.2 Click the "Live" button to display the image.

3.8.3 Adjust the brightness of the light and image (Exposure Time) through the Camera window, and for colour cameras, adjust the white balance using the **Auto** or **Pick** button to set the colour tone of the image.

3.8.4 The displayed image can be saved by following these steps:

- Click the **"Snap"** button to capture the current image.
- To save the captured image, navigate to **File** in the menu bar.
- Select **"Save As with options"** to open the save options dialog.
- Enter a name for your file in the **File Name** field.
- Choose the desired **File Type** from the dropdown menu.

Note that if you do not specify a file type, the image will be saved in the default format, which is **.CZI** (Carl Zeiss Image format).

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 9 of 27

4. Fluorescence Imaging (FL, Figure 6)


4.1 Go to the **Acquisition** tab and select the imaging settings from an experiment that has been predefined in the Experiment Manager or retrieve previously set values from a .czi file using the **Reuse** button.

4.2 Select the channel you want to image by checking ☒ the box corresponding to the desired colour.

4.3 Choose the colour range for the Live View by left-clicking in the colour box you wish to view. For example, if you want to see the green light, click on **FITC**. The FITC box will turn grey, and then click the **Live** button at the top left of the screen to display the image.

4.4 The program can automatically adjust the camera exposure settings by clicking ☒ **Auto Exposure**, or you can enter a fixed value in the Time field. Adjustments can be made separately for each colour channel.

4.5 After making your adjustments, click **Stop** and then select **Snap** to capture the image. The resulting image will combine all colours that have been checked ☒.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 10 of 27	

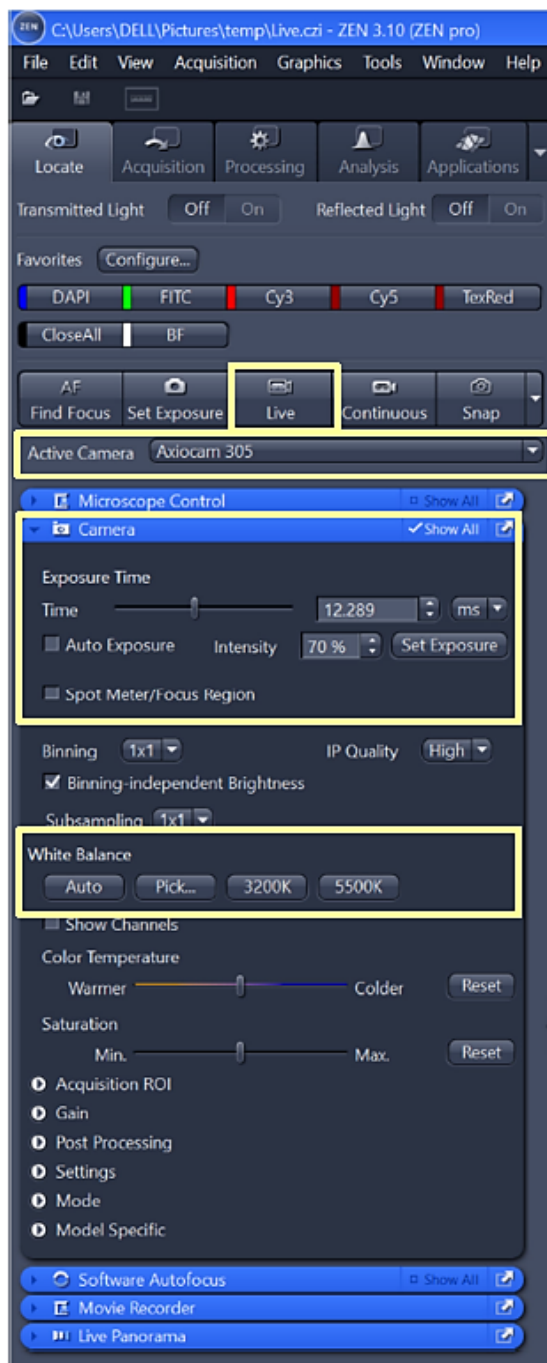

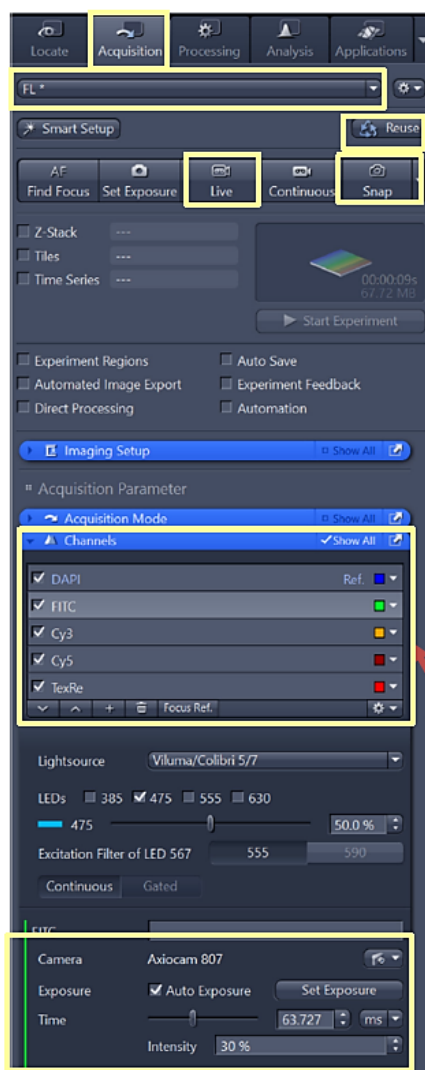



Figure 5. Image Preview and Capture

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 11 of 27	



If the selections are made as shown in the example image ☒, the resulting image will capture all 5 wavelength ranges in a single frame.

Figure 6. Fluorescence Image Capture

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 12 of 27

5. Z-Stack Imaging (Figure 7)

5.1 Select the Z-Stack mode as shown in the image on the left. The settings for Z-stack imaging will automatically expand.

5.2 Choose to capture images in Z-stack mode by defining the uppermost and lowermost positions (First/Last) for samples where the thickness is not precisely known. Click **Live** to display the image on the screen.

5.3. Rotate the focus to the uppermost position, then click **Set First** to save this position.

5.4. Next, rotate the focus in the opposite direction and click **Set Last** to save that position.

5.5 Select Optimal, and the program will automatically calculate the number of layers based on the appropriate parameters. Alternatively, you can manually set the thickness in the Interval field.

5.6 Once everything is set, stop the image by clicking **Stop**, and start capturing the Z-Stack images by clicking **Start Experiment**.


6. Time Series Imaging (Figure 8)

6.1 When the **Time Series** mode is selected, the settings for time series imaging will automatically expand. You can capture multiple fluorescence colours and Z-Stacks along with the time series imaging.

6.1.1 **Duration:** This sets the total time for capturing images, which can be defined in cycles or in time (units can be milliseconds, seconds, minutes, hours, or days). If cycles are not specified, you can manually stop the experiment by selecting **As Long as Possible**.

6.1.2 **Interval:** This defines the time interval between image captures during the specified duration (units can be milliseconds, seconds, minutes, hours, or days).

6.2 Start capturing the time series images by clicking **Start Experiment**.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 13 of 27	

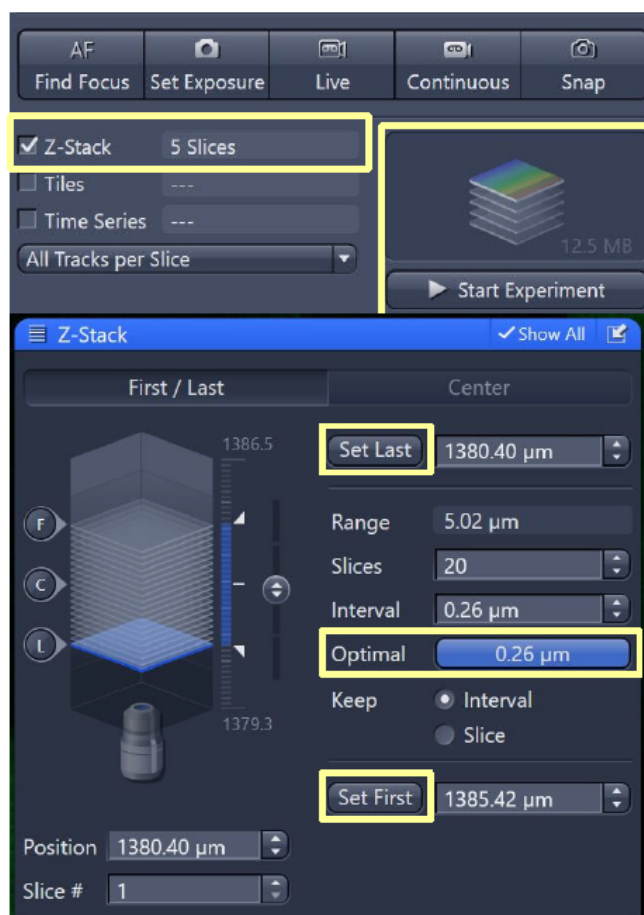



Figure 7. Z-Stack Imaging

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 14 of 27	

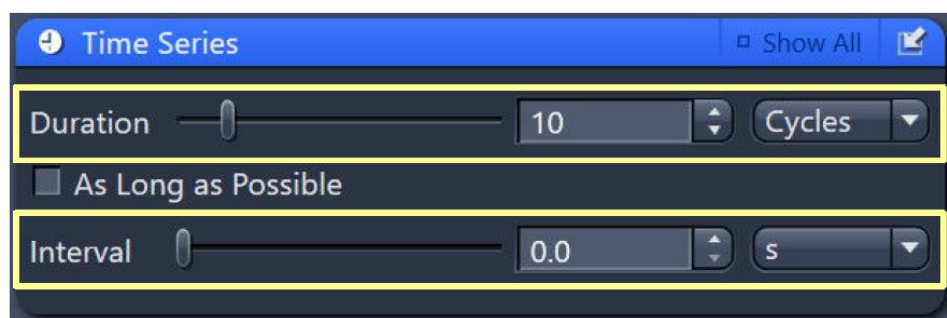
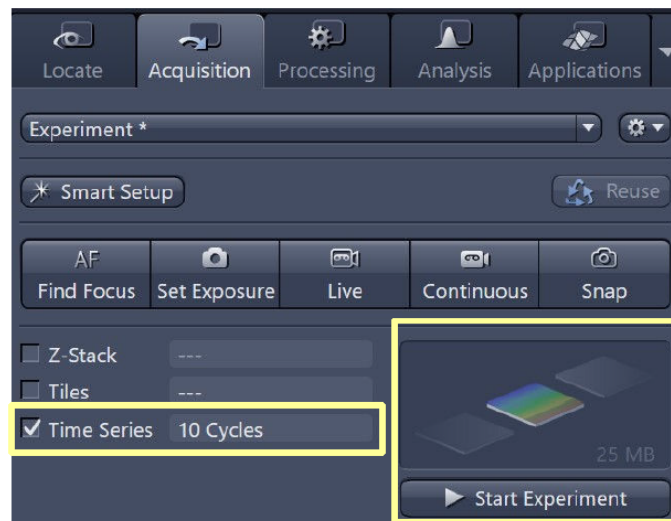


Figure 8. Time Series Imaging


7. Tiles Imaging (Capturing Large Sample Images, Figure 9)

7.1 When the **Tiles** mode is selected, the settings for tile imaging will automatically expand.

7.2 Click **Show Viewer** to begin selecting the area you want to capture. The **Tiles – Advanced Setup** window will appear on the right side, and click **Live** to view the current image.

7.3 In the Tiles imaging mode, **select mode 3 (X,Y)**. Then, navigate to the uppermost corner of the desired area and press the **+** button to designate the starting point for capturing. Next, move to the other corners and continue pressing the **+** button until the entire area of interest is marked.

7.4 Once you have completed marking the area, stop the image by clicking **Stop**, and start capturing images by clicking **Start Experiment**.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 15 of 27

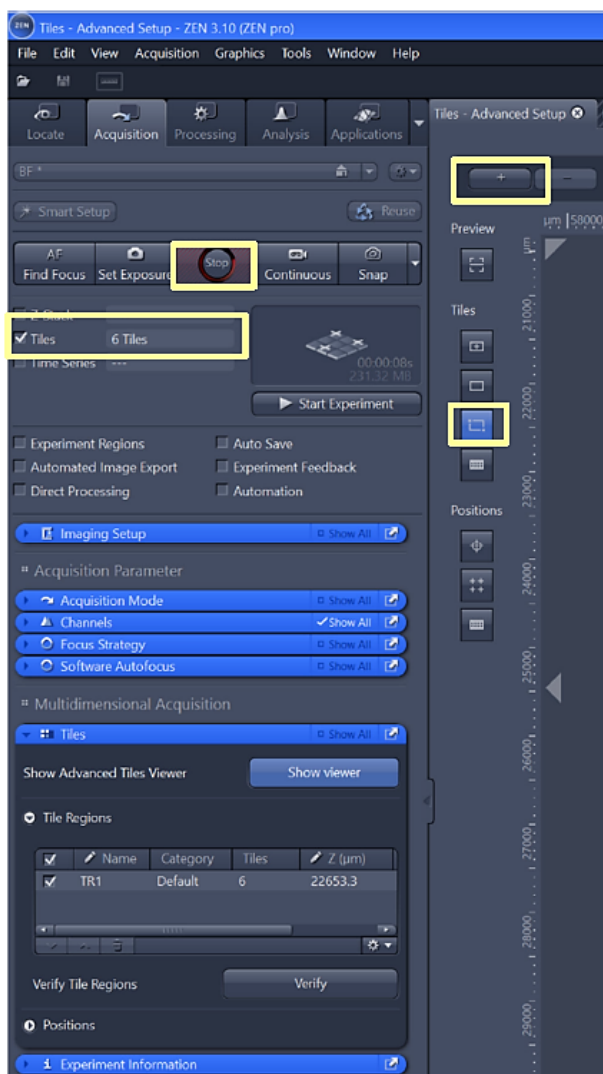



Figure 9. Tiles Imaging

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 16 of 27	

8. Image Processing Mode: (Figure 10)

8.1 Adjusting the **Histogram** (RGB values) to modify the colours of the image can be done by sliding the bars in the histogram located at the bottom of the image.

8.2 For fluorescence (FL) images, each channel can be adjusted by selecting **Single Channel**. Click on each colour in the image and adjust the corresponding graph. Once adjustments are complete, uncheck the **Single Channel** option to restore the view with all channels displayed as originally captured.

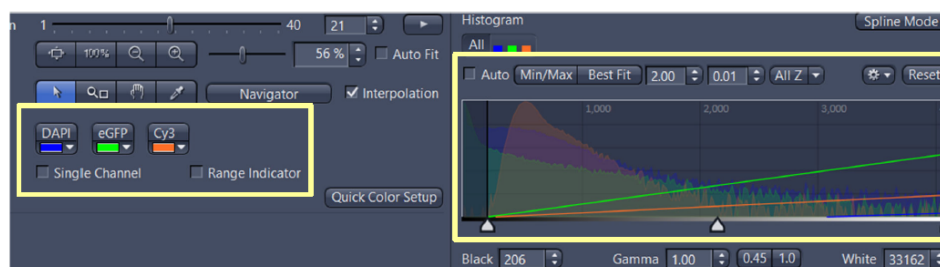


Figure 10. Image Processing Mode

8.3 Adjusting Deconvolution Images (for FL images, see Figure 11)

8.3.1 Go to the **Processing** tab and select the method as **Deconvolution**.

8.3.2 Choose the desired input for the deconvolution process.

8.3.3 Adjust the parameters as desired, as each setting will yield different results and processing times.

8.3.4 Click **Apply** to proceed, and a new image will be displayed in the final image tab.

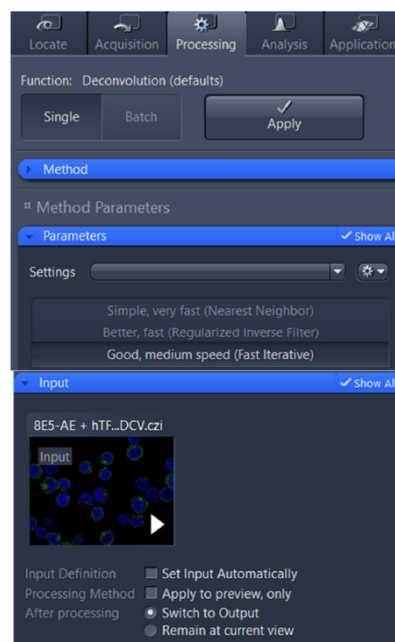



Figure 11. Adjusting Deconvolution Images

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 17 of 27

9. Image Analysis Mode: (Size Measurement, Scale Bar Addition, Figure 12)

9.1 Select the image to be measured and go to **Graphics**. Various measurement tools will be available, such as selecting the ROI (Region of Interest), adding text, using a scale bar, measuring distances, adding arrows, and measuring dimensions of rectangles or circles.

9.2 Various values can be added by accessing **Frequent Annotations**, which include details such as capture time, focus position, and exposure time.

9.3 Prior to saving the image, click **Burn-in Annotations** to merge the scale bar and measurement values with the image. If saved as a standard file, the measurement elements will not be visible in the saved image

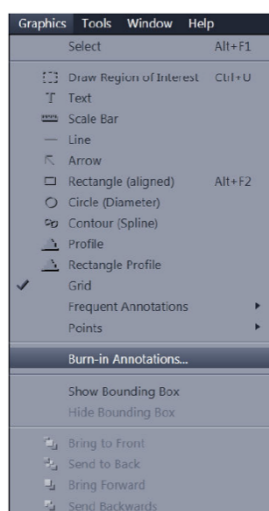



Figure 12. Image Analysis Mode

9.4 Export Table (Figure 13) - Go to the **Measure tab**, then in the **Measurement tab**, click **Create Document** to generate a table. To save the table, go to File > Save As (the file can be saved in three formats: .czt, .csv, and .xml).

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 18 of 27	

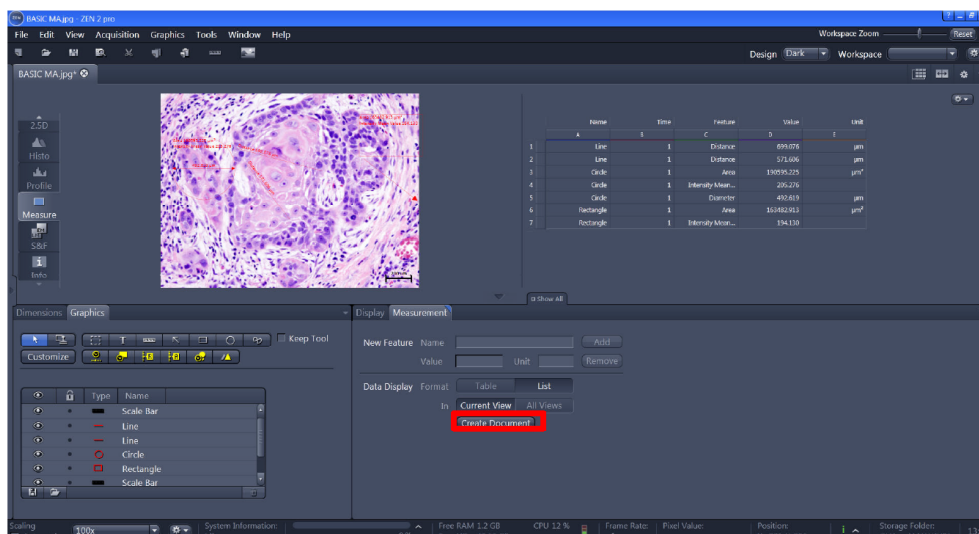


Figure 13. Export Table

10. Saving Image Files (Figure 14)

10.1 To save an image, go to **File** and select:


- > **Save**: This saves the file with the .CZI extension or overwrites the existing file.
- > **Save As CZI**: This saves the file with the .CZI extension.
- > **Save As with Options**: This allows saving in a different file format.

10.2 To export as an image file in a different format, go to **File > Export/Import > Export**, select the **input image**, choose the **file type**, click **Burn-in Graphics**, select the location to save the file, and then click **Apply**.

11. Saving Video files (Figure 15)

11.1 Go to **File > Export/Import > Movie Export** then select input image

11.2 Select the file type for the movie > choose the video size > select the playback speed > choose the storage location for the file, and then click **Apply**.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 19 of 27

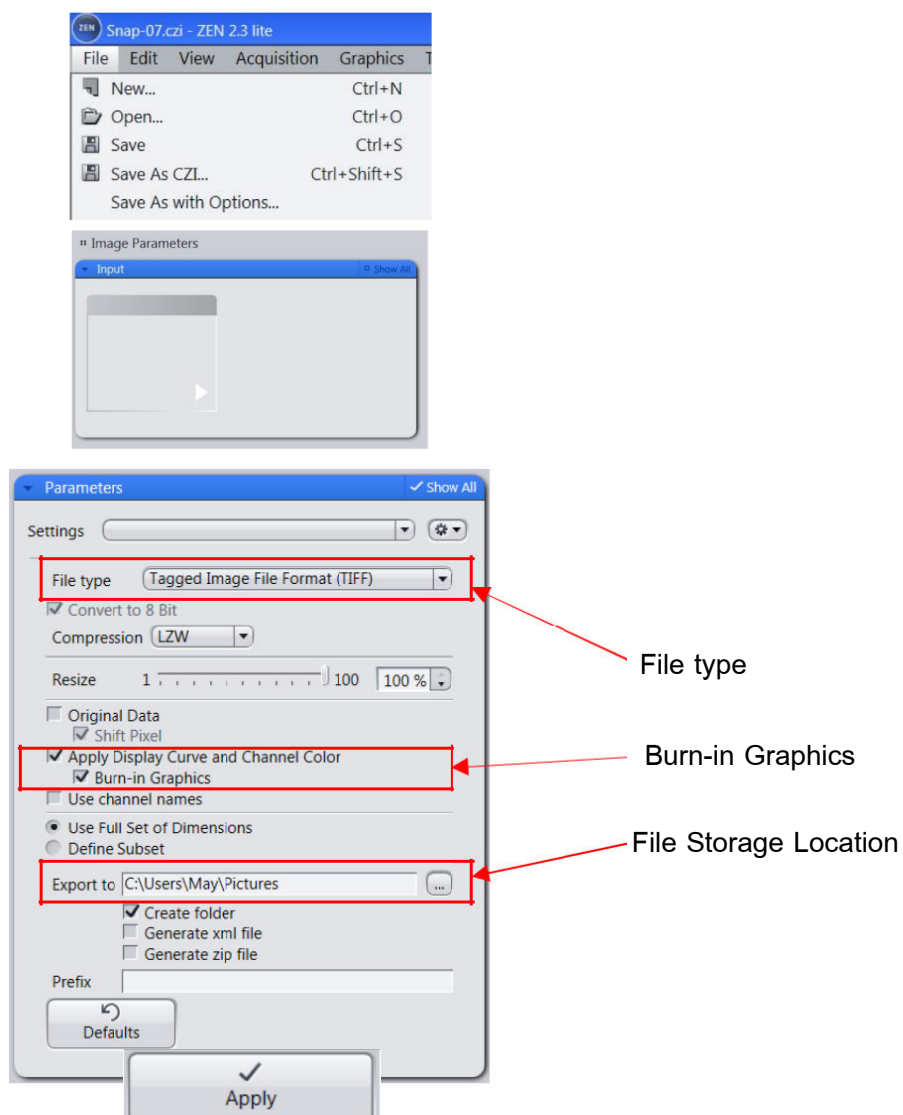



Figure 14. Saving Image Files

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 20 of 27	

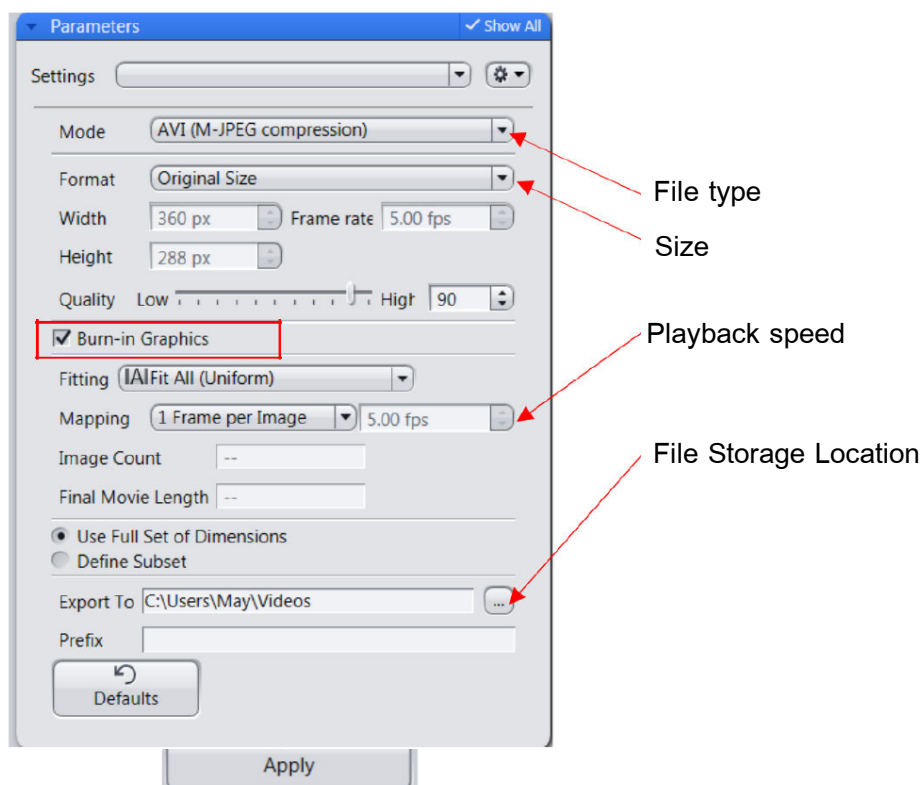


Figure 15. Saving Video Files


12. Image Analysis Mode: Automatic Cell Counting with AI (Figure 16)

12.1 Go to the **Analysis** tab, select **Bio Apps**, and then choose **Cell Counting**.

12.2 Select the desired Settings.

12.3 Choose the image for cell counting in the Input tab.

12.4 Click **Run Analysis** to start the counting process. Once completed, the program will display the counting results and a counting table in the **Bio Apps** tab of the image.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 21 of 27

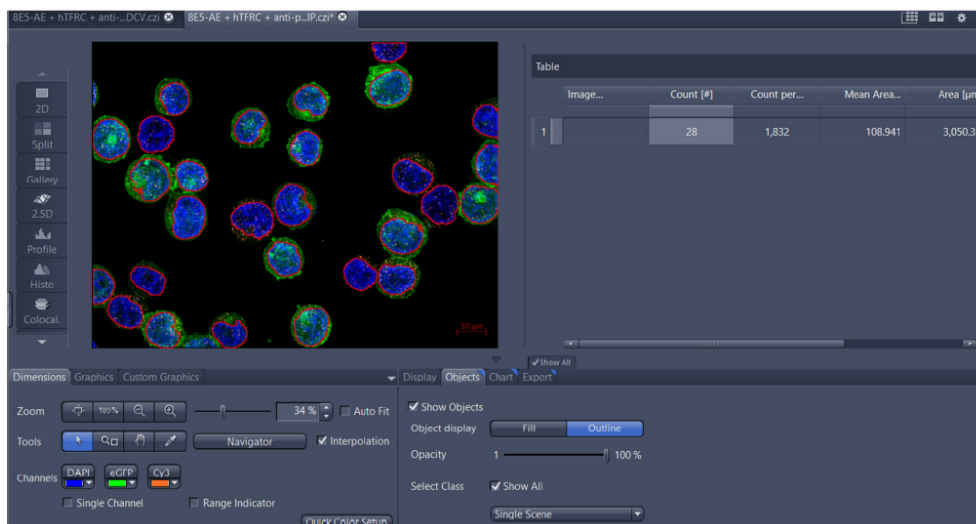



Figure 16. Automatic Cell Counting with AI

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 22 of 27

13. Image Analysis Mode: Custom Automated Measurement

13.1 Go to **Analysis > New** (To name the samples to be analysed) > **Set up** Image Analysis

13.2 The program will display a settings window for grouping the samples for counting (Classes).

13.2.1 Additional classes for counting can be added by clicking **Add Class**.

13.2.2 Classes can be removed by clicking **Remove Class**.

13.2.3 The colour of each class can be defined by selecting a colour using the **Color** button.

13.2.4 Click **Next** to proceed to the next step.

13.3 Select the area of the sample to be analysed.

13.3.1 Choose the tool to define the area for analysis.

13.3.2 Select the mode used to specify the area for analysis.

13.3.3 Click **Next** to proceed to the next step.

13.4 This step illustrates how to define the area of interest for analysis. This can be accomplished by selecting the desired sample to measure and adjusting the image analysis settings as needed before clicking **Next**.

13.5 Conditions for the sample can be specified by selecting **Edit**. The program will display a window with various condition parameters that can be applied to the sample analysis. These parameters can be selected by double-clicking, followed by clicking **OK** and then **Next**.


13.6 The settings for the sample can be modified from the toolbar, including options for separating or deleting samples. After making adjustments, click **Next**.

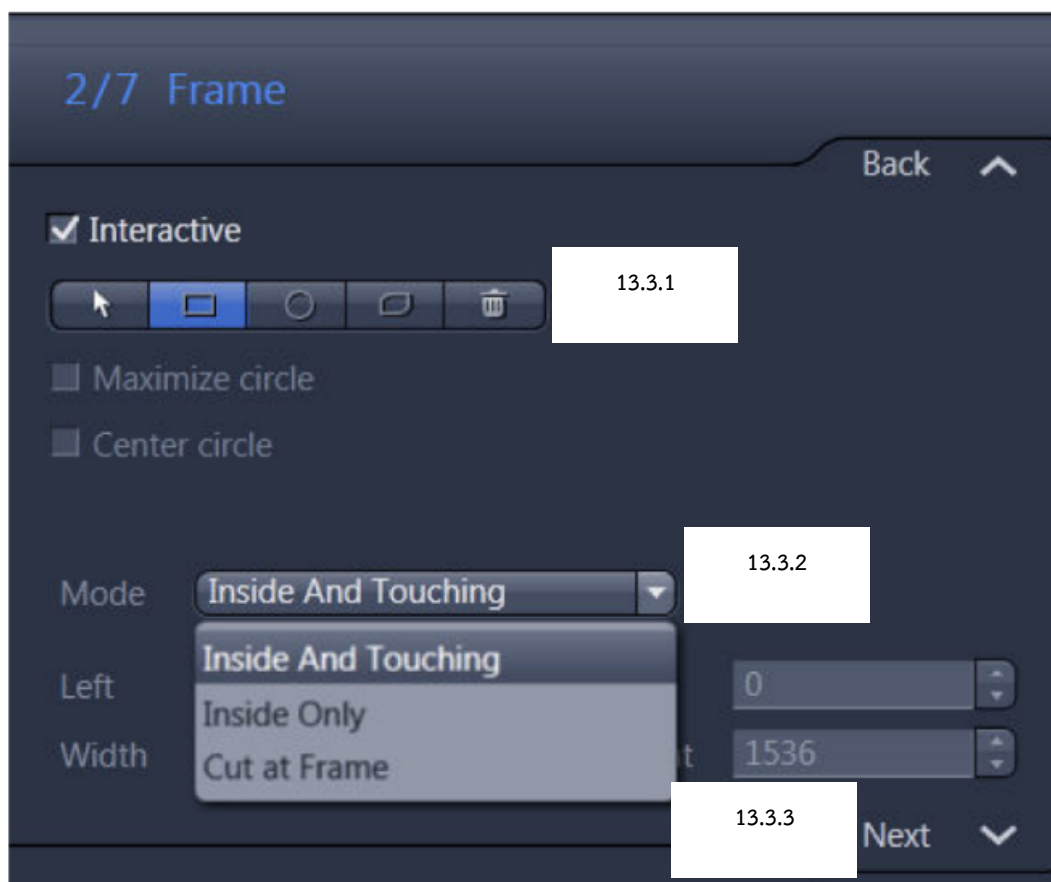
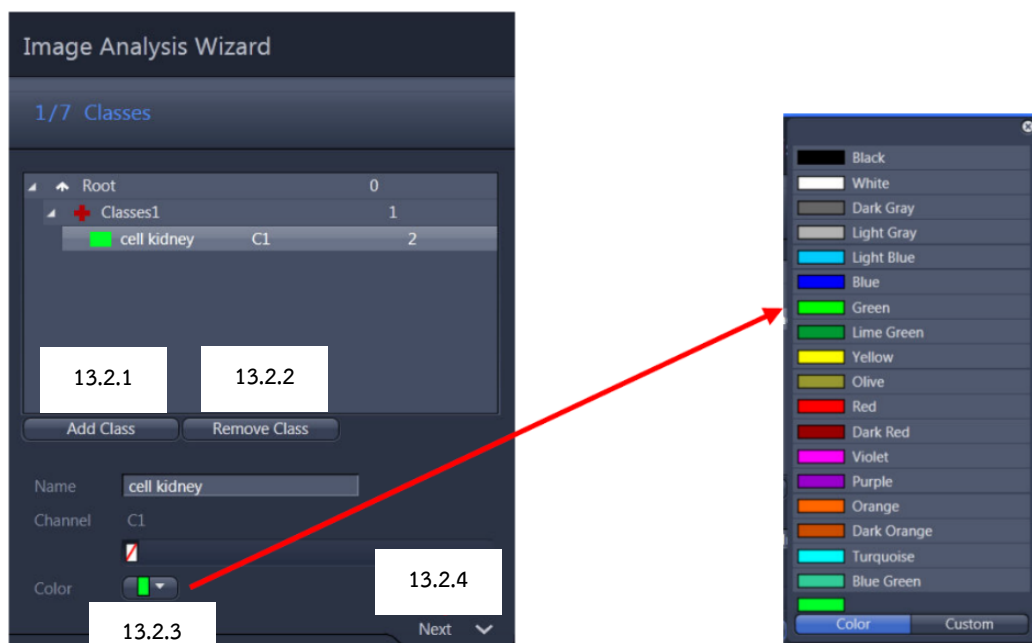
13.7 Parameters for analysis and the display of results can be adjusted as needed. Then, click **Next** to proceed to the next step

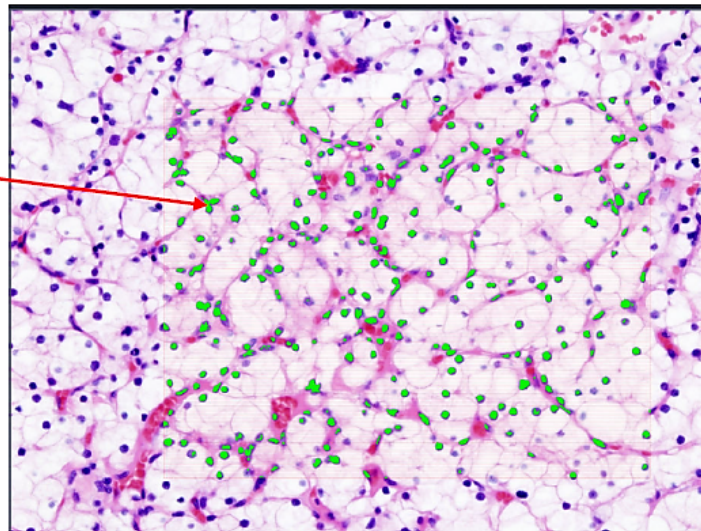
13.8 The program will display the analysis results. Click **Finish** to complete the sample analysis after configuring the settings.

13.9 Click **Analyze** to initiate the analysis, and the program will present the results.

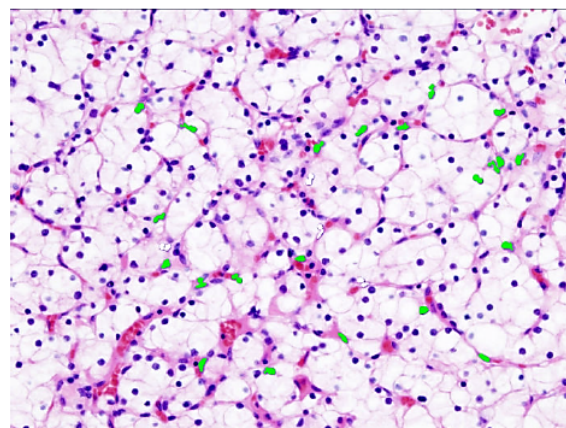
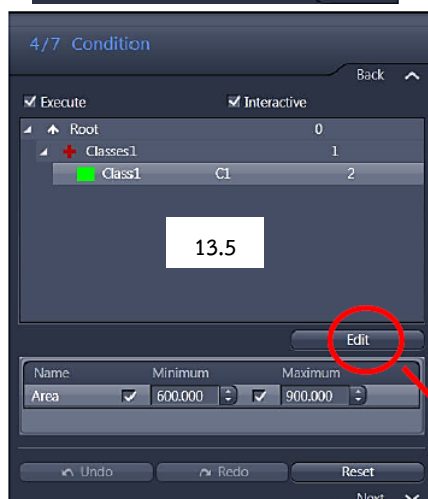
13.10 Results can be displayed in a table format and a summary of the count can be generated by clicking **Create Tables**.

	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software			
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 23 of 27	

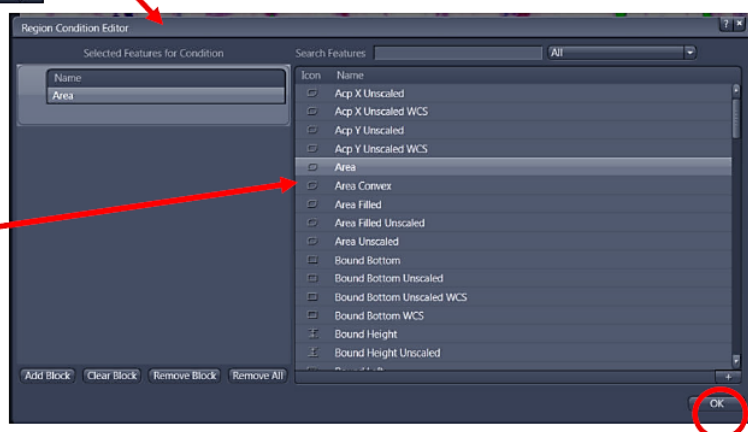


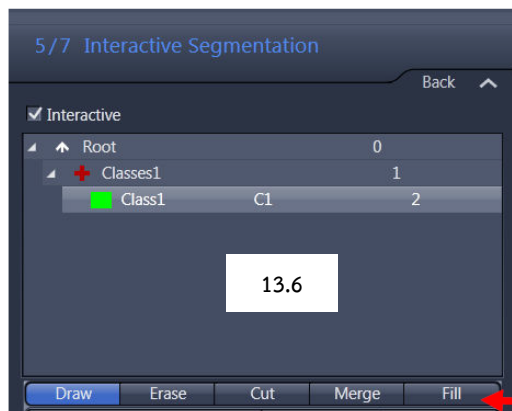


The function for adjusting the
settings for image analysis.

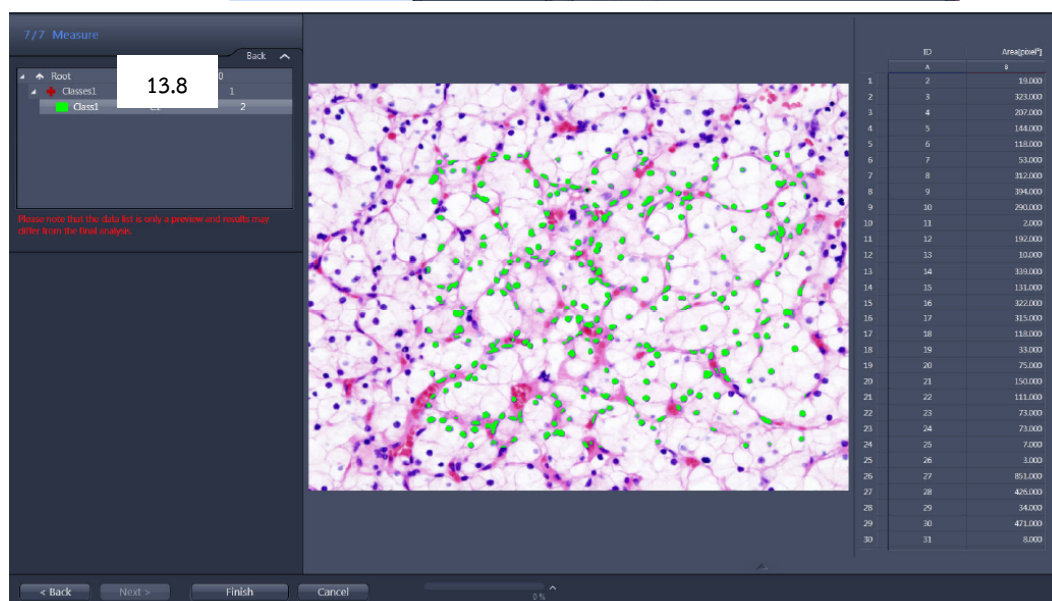
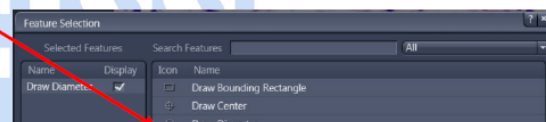
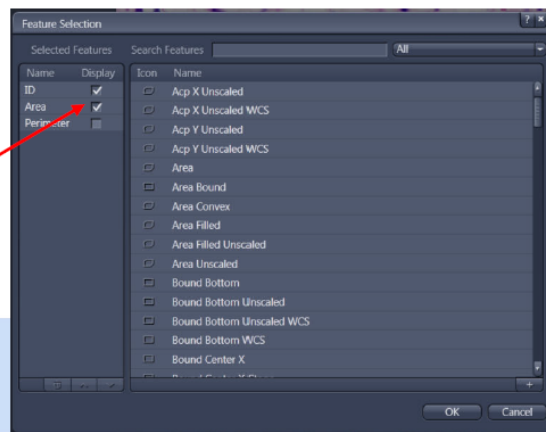
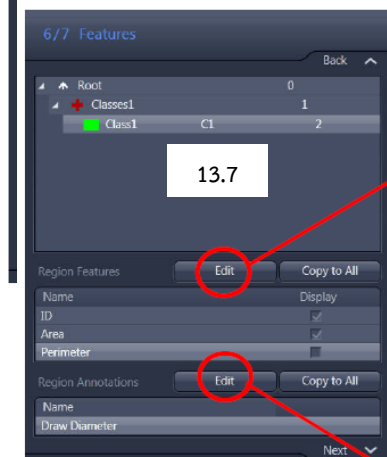



Condition parameter

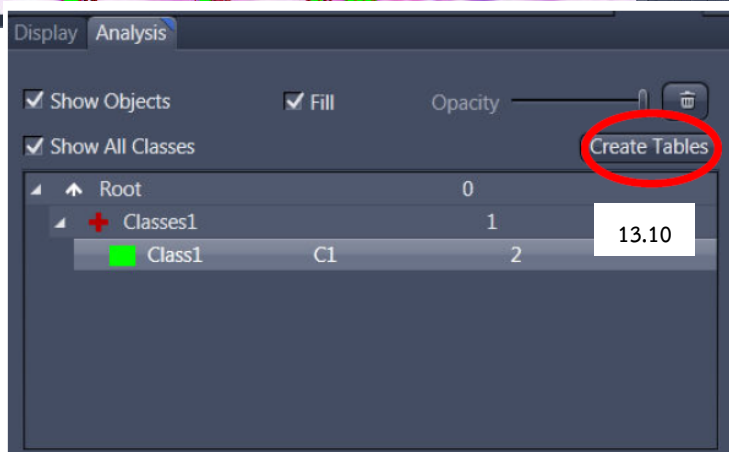
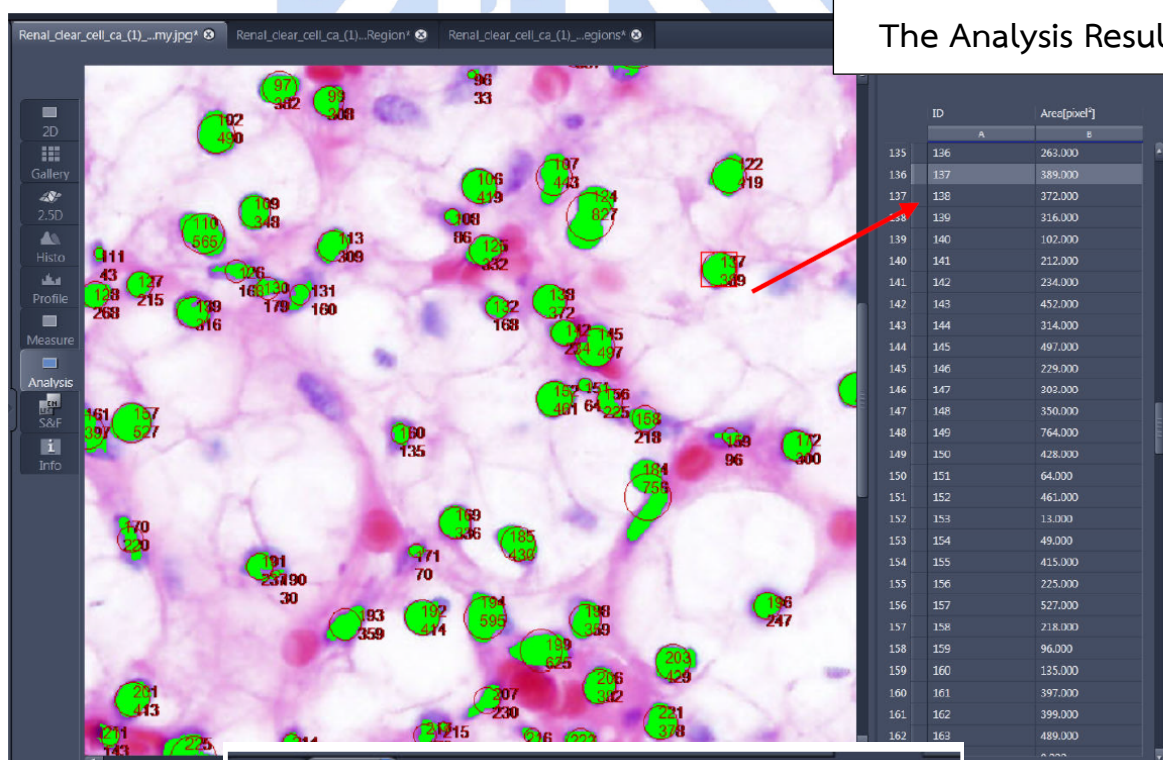
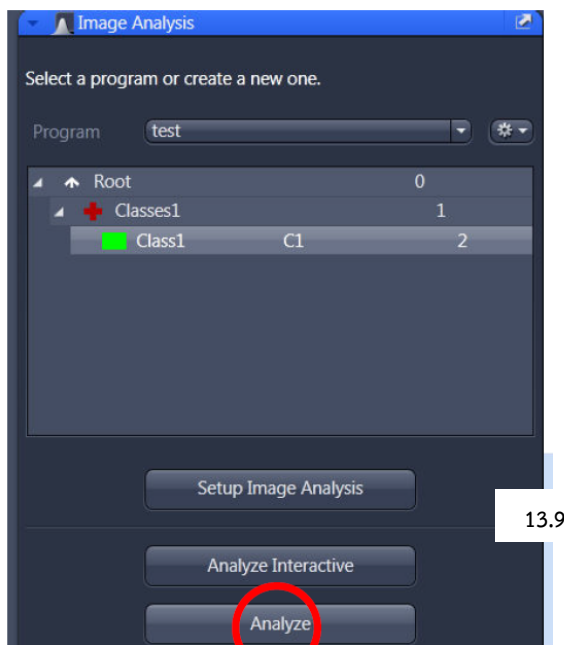





Toolbars



	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 26 of 27



	Offices of Health Science Research Faculty of Medicine Ramathibodi Hospital	Work Instructions Title: Instructions for Using the ZEISS Axio Imager M2 Microscope and ZEN Pro Software		
	Document code: WI-MI-16:01	No: 02	Date: 9 June 2025	Page: 27 of 27

*Note: - For additional information, visit <https://minicore-rarc.blogspot.com/2018/05/upright-fluorescence-microscope.html>

- Users must log their usage in the online logbook after each session, whether using the full system or only the computer.

Lens Cleaning Procedure:

To prevent residue from the immersion oil used with the 100x objective lens, first wipe off the oil using a dry piece of Kimwipes. Then, moisten a new piece of Kimwipes with 95% alcohol and gently wipe the lens surface 1–2 times in a straight motion. (Avoid rubbing in a circular motion)