

Harwell-MICe OPT Manual

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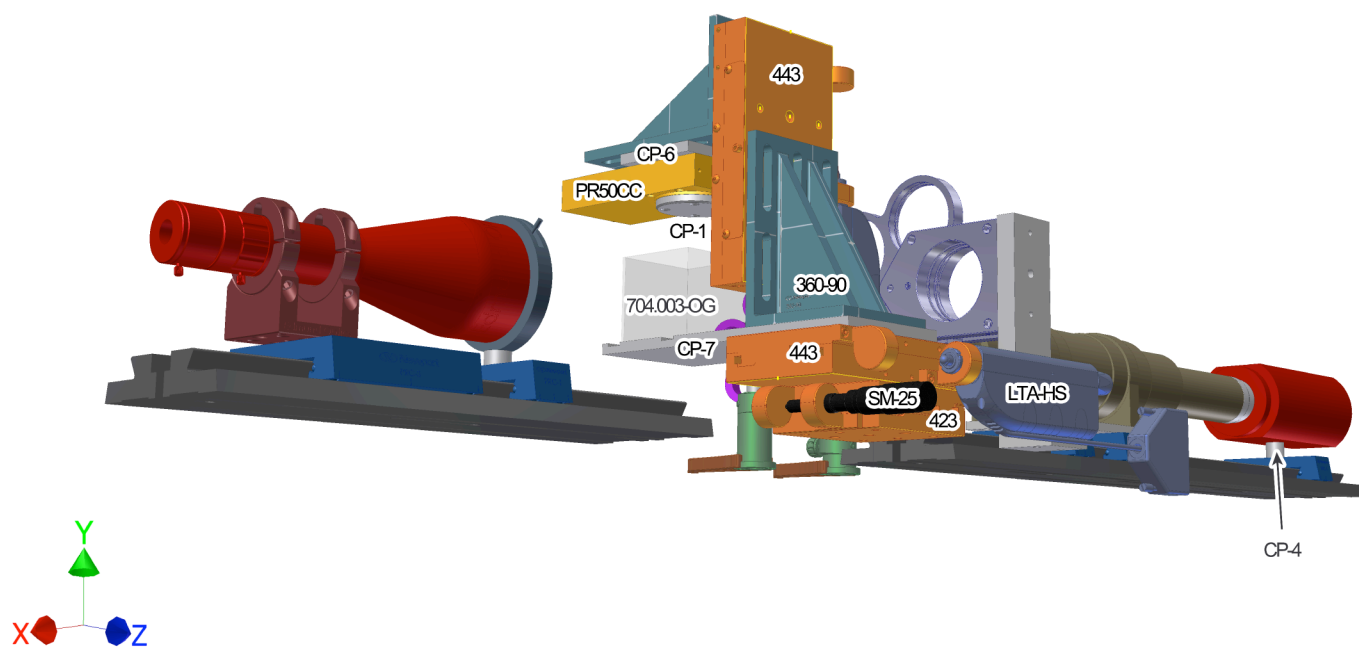
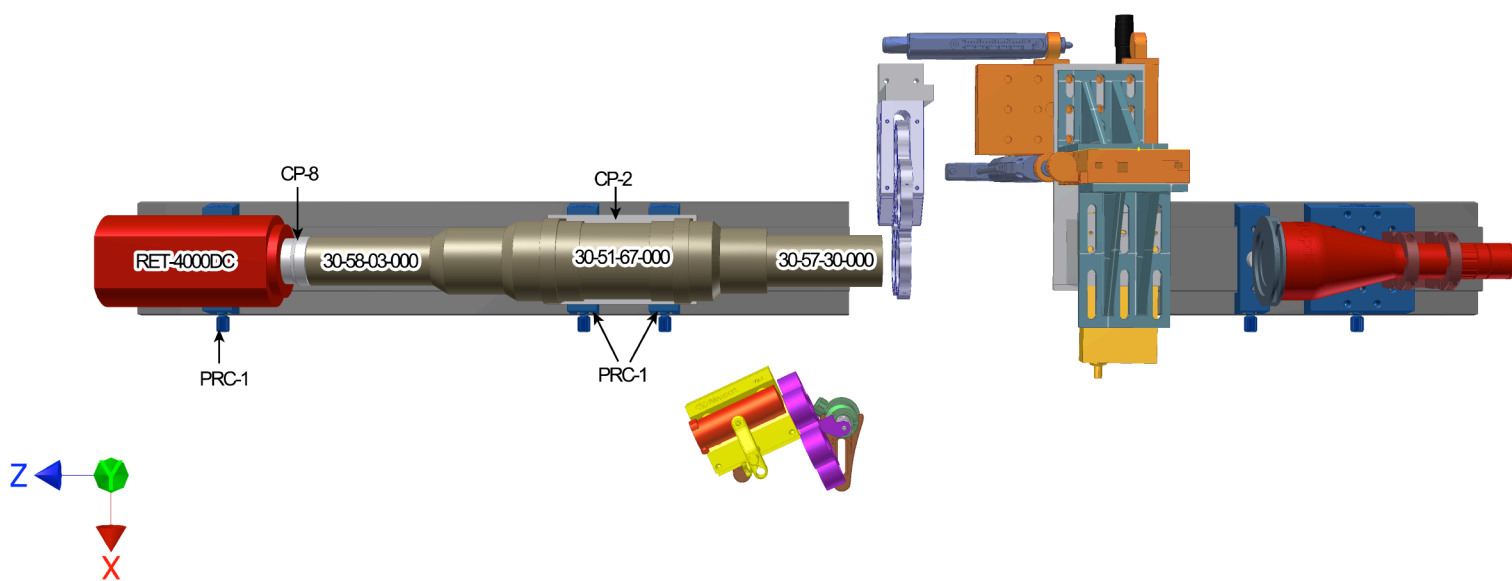
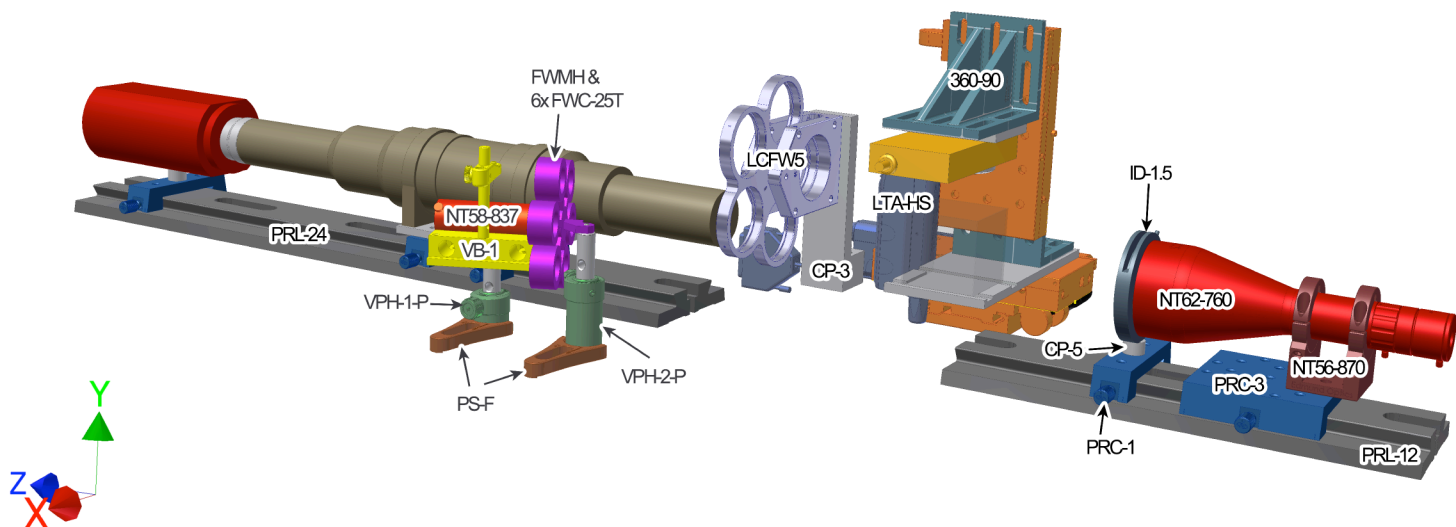
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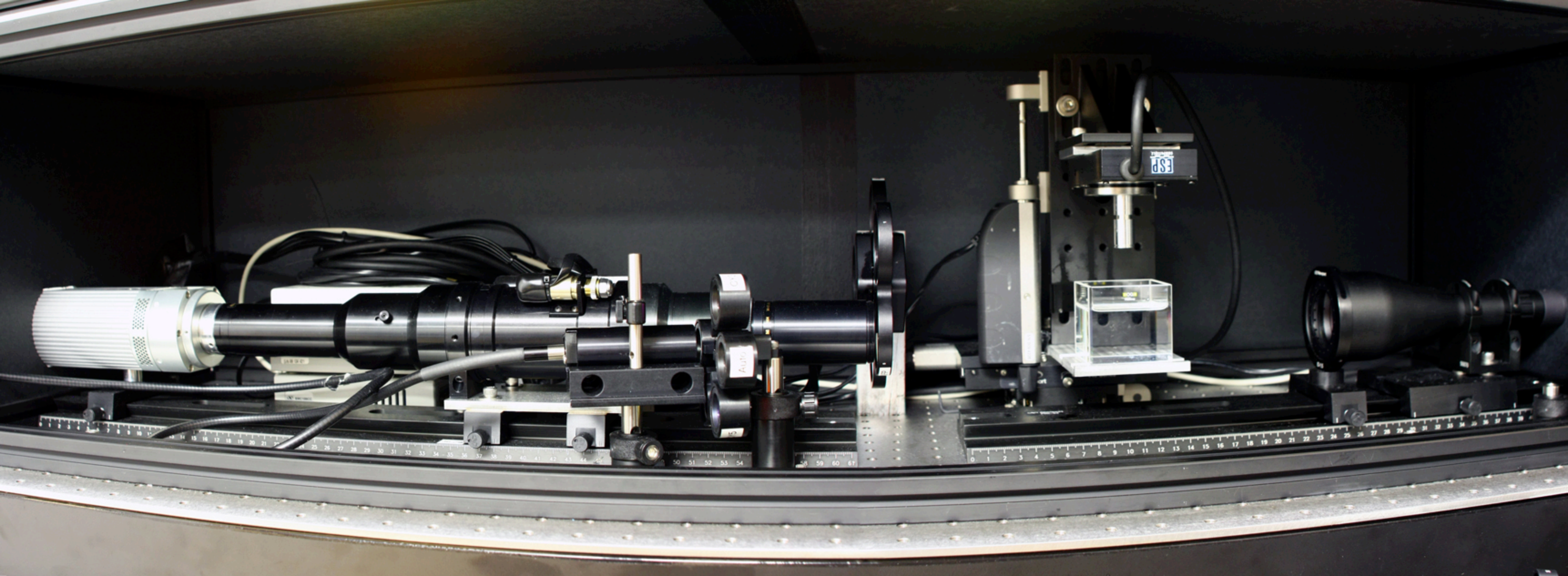
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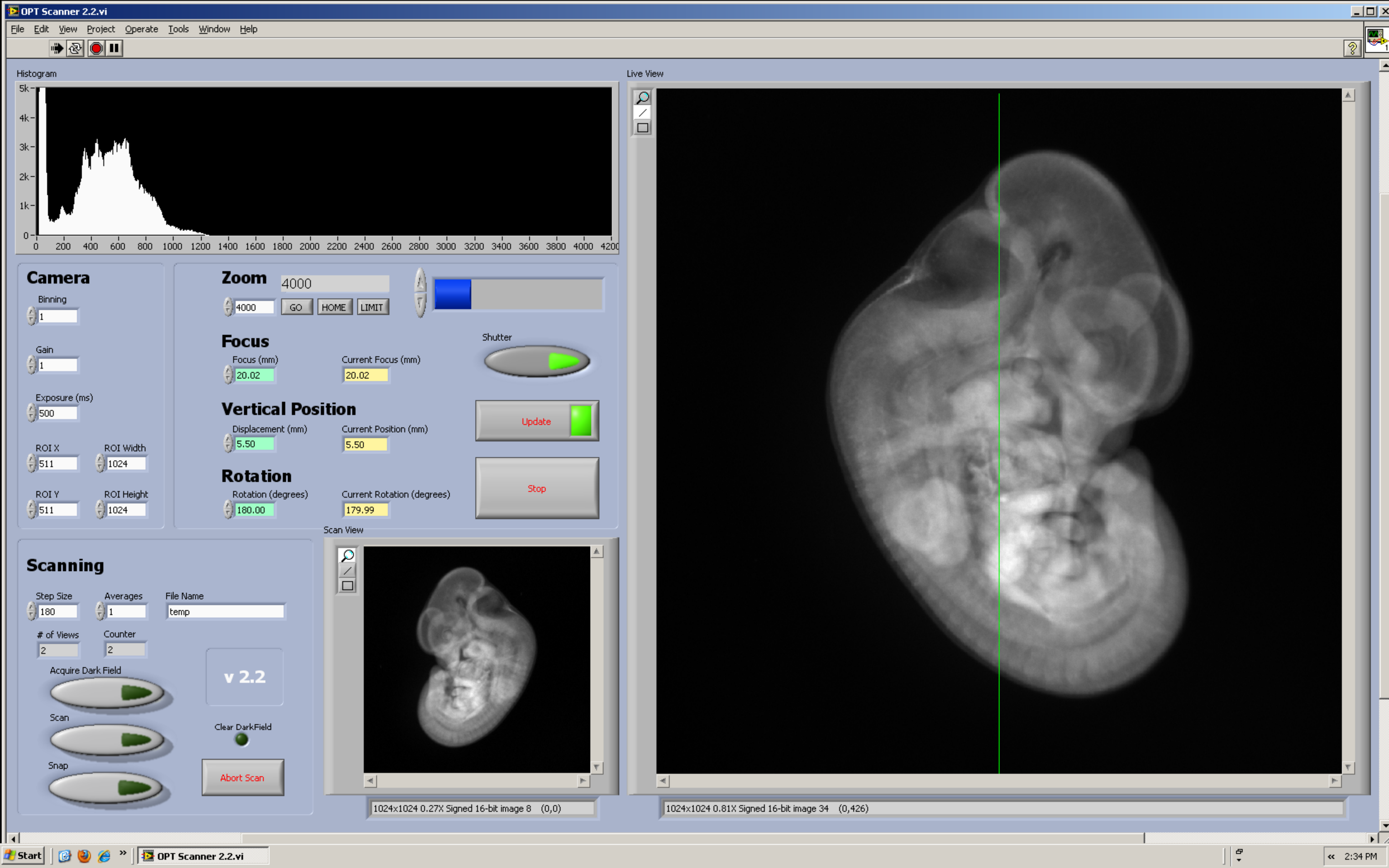
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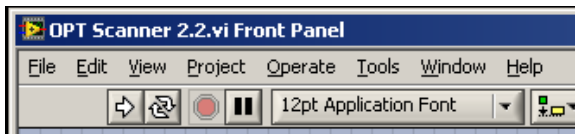


Graphical User Interface (GUI)

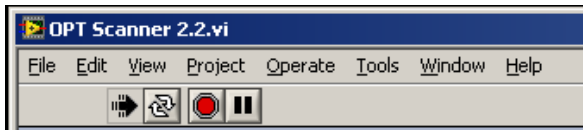
Labview Toolbar

On the top toolbar you will find the 'Run'  and 'Abort Execution' . To turn on the OPT software, click the 'Run' button. If the 'Run' button is greyed out, the software is inoperable because the background code does not compile correctly. This should not happen in normal use. Please check that you are running the correct software version if this does happen. **The 'Abort Execution' button should not be clicked in normal use.** This button 'exits' the algorithm no matter where the software is in the process i.e. (Live view, Scanning Etc.) **Clicking it breaks all communication with the hardware and they must be reset individual before you can run the software again.** It should only be used as a last resort if the OPT software cannot be turned off by clicking the larger 'Stop' button that is in the program.

Off

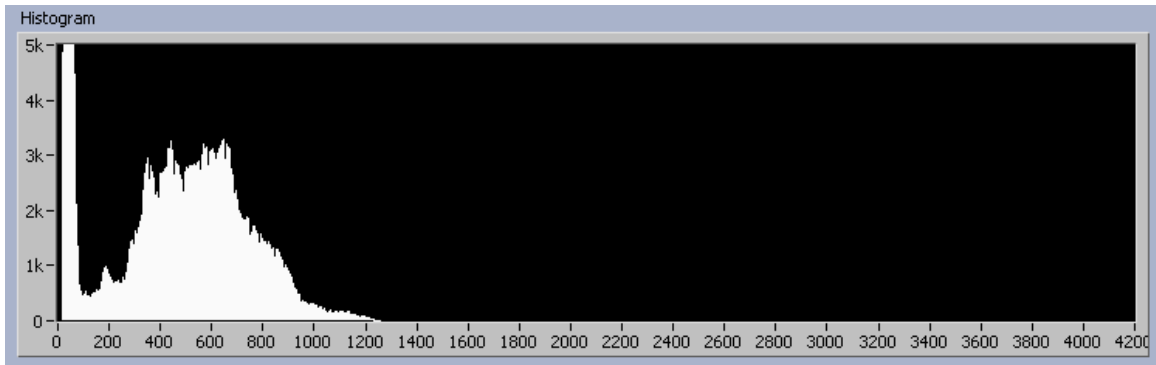


On

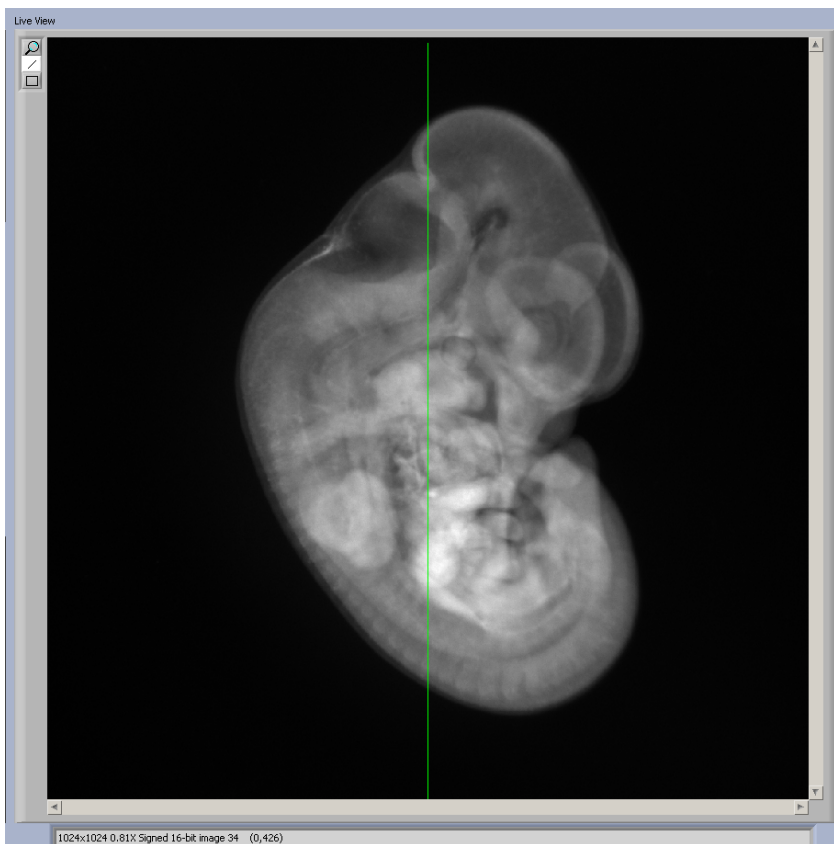


Histogram

The histogram displays how many pixels on the CCD (y-axis) have a specific 'brightness' value (x-axis) within the camera dynamic range. The values range from 0-4095 because the camera is 12-bit (i.e. $2^{12} = 4096$). For example, the left of the histogram is usually a high number (in UV mode) because that accounts for the majority of the dark pixels in the background. The histogram is used to determine what exposure time to use. Basically you want to adjust the exposure time such that the brightest value that the CCD picks up is close to but not more than 4095. This is called 'exposing to the right' (ETTR). In practice, the exposure should be increased until the histogram reaches about 3000-4095 on the x-axis.



Live View



The Live View displays a refreshing projection image that the CCD captures using the current camera settings. The green line shows the center vertical of the CCD FOV, which denotes the center of rotation of the system and the axis in which should co-align with the samples vertical axis. The field-of-view array (i.e. 1024 x 1024), the pixel brightness value (i.e. 34) and the current pixel your cursor is on (i.e. 0,426) are all displayed on the bottom of the Live View. This is beneficial when the user would like to know the brightest pixel within the projection image without using the histogram.

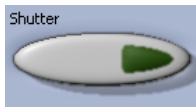
Update, Stop, and Shutter Buttons



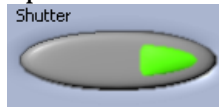
Shutter

To toggle on and off the shutter in the UV light source, click the shutter button. It will light up green if the shutter is open and revert back to dark green if the shutter is closed.

Closed Shutter



Open Shutter



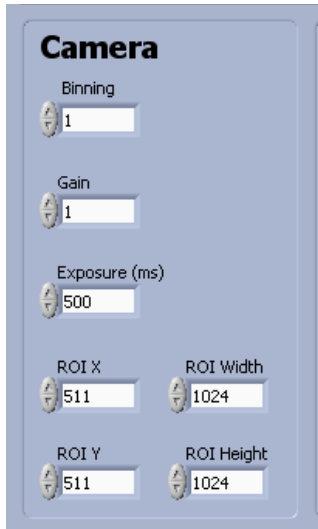
Update

The update button functions as a verification button for all camera setting changes and scan setting changes. Every time a change in camera settings occurs, or if one would like to start a scan or acquire darkfield, the update button must be clicked as confirmation.

Stop

To power off the software, click this 'Stop' button. This will not work while a scan is being acquired. To abort a scan, click the 'Abort Scan' button.

Camera Settings



Camera

Binning
1

Gain
1

Exposure (ms)
500

ROI X
511

ROI Width
1024

ROI Y
511

ROI Height
1024

Binning

This parameter determines how the array on the CCD is collecting photons. By default, the CCD is binned by 1, meaning each individual on the CCD is picks up photons and is responsible for one pixel on each projection. When you bin the camera by 2, 4 pixels in a square on the CCD is averaged and contributes to 1 pixel on projection image. For example, if the original projection image is 1024x1024 when binned by 1, it will become 512 x 512 when binned by 2. The advantage of binning is that you will increase the amount of photons gathered by this new bigger effective pixel, and you can use a lower exposure time and increase the signal to noise of your projection image. The disadvantage is that you lose image resolution by the amount you are binning the camera.

Gain

This parameter increases the electronic gain in the CCD. It acts as a multiplier on the native signal the CCD picks up. This could be used in instances where the exposure time need to ETTR is too long for the sample and you need to bump up the gain to ETTR. However, this increases the noise in your image dramatically. In practice this parameter should be avoided.

Exposure (ms)

This parameter is the exposure time. It is the amount of time in milliseconds that the camera allows photons to be recorded for each individual image it takes. A longer exposure time allows more photons to be recorded and moves the histogram to the right. The opposite is true. This is the parameter that is most tightly associated with photobleaching and scan time. The longer the exposure time, the

longer the sample is subjected to excitation light, and the longer the overall scan time.

ROI X, ROI Width, ROI Y, ROI Height

These parameters dictate how much of the CCD to use when imaging. At default, only the center 1024 x 1024 portion of the CCD array is being used. This is the best tradeoff between image quality, sample coverage in terms of depth of field, and exposure time. If one was to use the entire CCD and zoom into their sample such that it filled the entire Field-of-View, the optical depth of field will not be large enough to cover the sample. This means that a significant portion of your sample will be out of focus, which will make for a blurry final image. If one wanted to use the full CCD, they could enter 0 for ROI X and ROI Y and enter 2048 for ROI Width and ROI Height.

Entering Values

To change the values for the camera settings, the user must click the 'Update' button for it to take effect. When the update button is clicked, the green light will turn off and on again and at that point the new settings are applied.

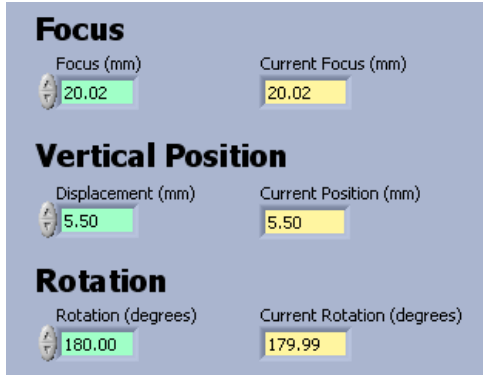


Zoom



The zoom level are a range of numbers from 0-19210 where 0 is minimum zoom and 19210 is maximum zoom. When the software is initiated, the zoom motor travels to minimum zoom at default. The current zoom level is in the grey field. To change the current zoom level, you enter a value in the white field and click 'GO'. You can move immediately to minimum zoom by clicking 'HOME' and maximum zoom by clicking 'LIMIT'. You can alternatively press the up/down arrows beside the blue indicator to zoom in and out.

Controlling the Stages



The screenshot shows a control interface with three sections: Focus, Vertical Position, and Rotation. Each section has a green input field for setting a value and a yellow output field for the current value. The Focus section shows a value of 20.02 mm. The Vertical Position section shows a value of 5.50 mm. The Rotation section shows a value of 180.00 degrees, with the current reading at 179.99 degrees.

Section	Control Field (mm/deg)	Current Reading (mm/deg)
Focus	20.02	20.02
Vertical Position	5.50	5.50
Rotation	180.00	179.99

Focus

To focus the sample, you can move the actuator along the optical axis (z-axis) by inputting a value in millimeters in the 'Focus' green field and then pressing 'ENTER' on the keyboard. The current focus reading is displayed in the yellow field. Alternatively, you can make fine adjustments by clicking the up/down arrows. The range of values is from 0 – 50mm.

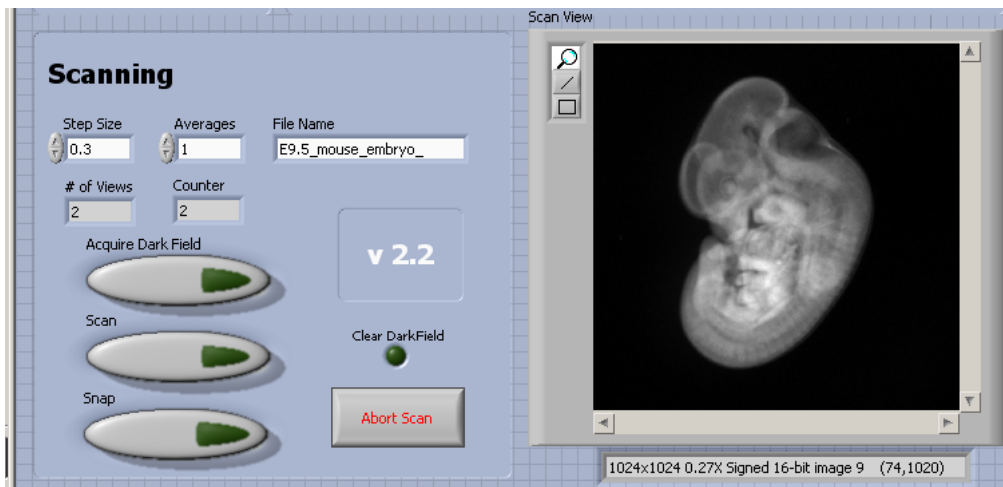
Vertical Position

To adjust the height of the sample, you can move the actuator along y-axis by inputting a value in millimeters in the 'Vertical Position' green field and then pressing 'ENTER' on the keyboard. The current vertical position reading is displayed in the yellow field. Alternatively, you can make fine adjustments by clicking the up/down arrows. The range of values is from 0 - 50mm.

Rotation

To rotate the sample, you can rotate in the xy plane along the optical axis (z-axis) by inputting a value in degrees in the 'Rotation' green field and then pressing 'ENTER' on the keyboard. The current rotation reading is displayed in the yellow field. Alternatively, you can make fine adjustments by clicking the up/down arrows. **Note: There is no ability to rotate the sample infinitely as in Bioptonics. A specific value must be entered.**

Scanning



Scan Parameters

You can enter the angle step size, number of projection averages, and file name in the white fields. Note: The filename should have no spaces and should always end with an '_'. For example, 'E9.5_mouse_embryo_'. The step size should always be divisible by 360. The number of views is calculated as $(360/\text{step size})$ and is displayed in the grey field. During an image acquisition, the user can tell how far along the scan is by looking at the 'Counter' grey field. Once the 'counter' value matches the # of views value, the scan is complete.

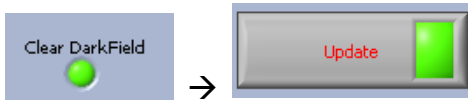
Acquire Dark Field

Before the scan starts, the user needs to acquire background images that will be later subtracted from each projection. Once the user has set the exposure time which establishes ETTR of the histogram, the user will click on the Acquire Dark Field Button and it will light up green. Then the user must press the 'Update' button and then the sample will be lifted out of the field of view, 10 background images will be taken, and the sample will be placed back to where it once was. Now in the Live View, the update projections will have the background images subtracted from them. **This step must be done before every scan. The dark field must be updated every time the File Name has been changed, and every time the exposure time has been changed.**



Clear DarkField

If the user does not want to see the effects of the darkfield in the Live View image, the user can press the Clear Darkfield button and then press 'Update'.



Scan

Once the user is content with all the scan, camera, and stage parameters and the darkfield has been acquired, the user can click the 'Scan' button and then the 'Update' button. At this point, the Live View screen will freeze and the Scan View screen will update for each projection that is taken during the scan.

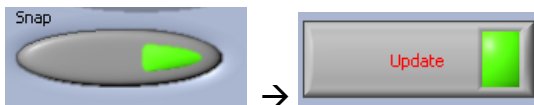


Abort Scan

If the user chooses to abort a scan, they can click the appropriate button "Abort Scan".

Snap

If the user wants to take a snapshot of the current projection that is displayed in Live View they must click the 'Snap' button then the 'Update' Button



Optical Projection Tomography Scanning Walkthrough

1. Power on hardware components

Power on the CCD Camera, Optem Zoom Controller, and X-CITE UV Light Source.

CCD:



Optem Zoom Controller:



Note: The switch on the right should be switched to DISABLE at all times.

X-CITE UV Light Source



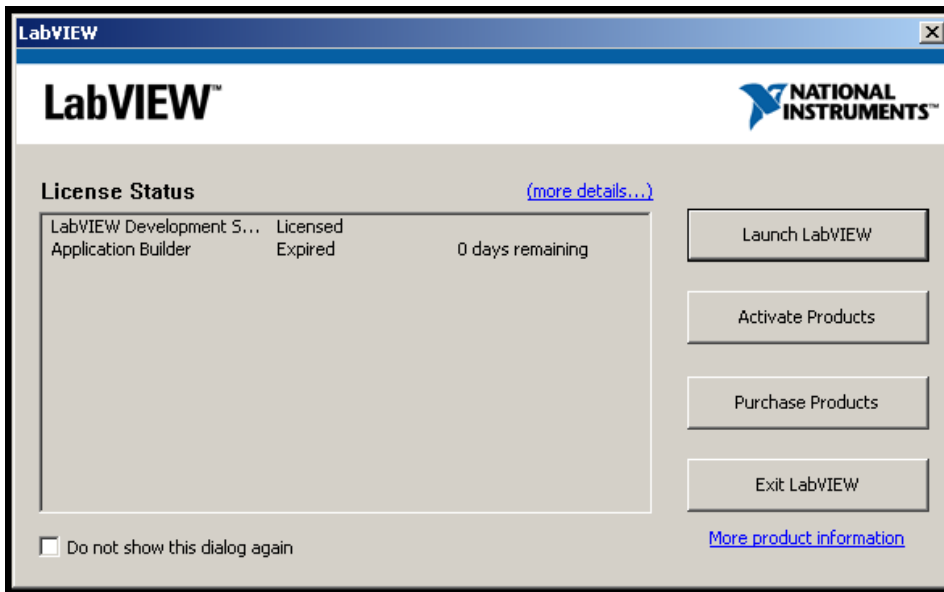
The UV light source takes a few minutes to power up. Refer to the user manual for more information about the light source. When it is fully powered, it will look like the above picture with '000' in digital screen. This is the current percentage intensity of the UV light. Press the 'UP' button until it reads '100'. Lastly, you can manually open and close the shutter by pressing the 'SHUTTER' button. This can be done with the Labview software so do not press it unless necessary.


2. Run the LabView software

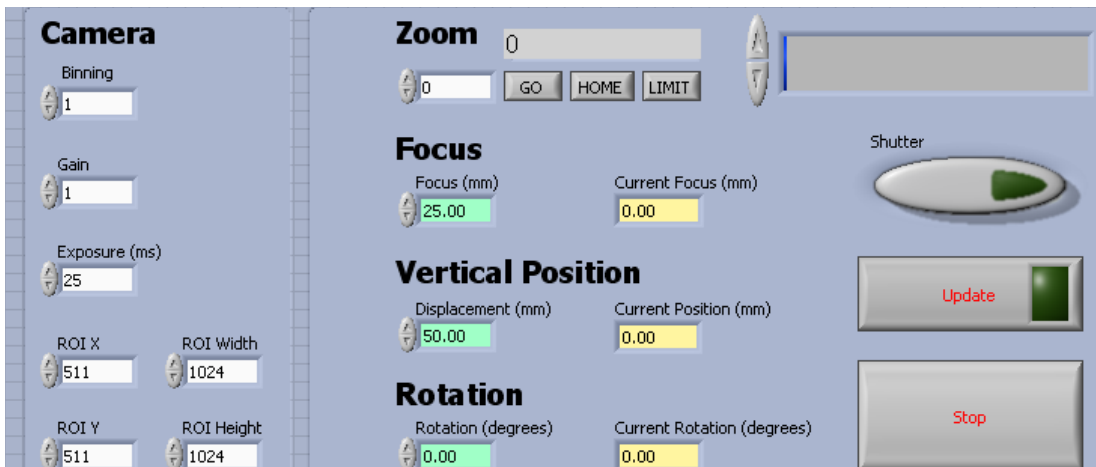
Double-click on the OPT Scanner 2.2 icon on the computer desktop.



This will launch a Labview Window like the one below. Click on the Launch LabVIEW button.



You will then be presented with the LabVIEW graphical user interface described in the last section. To power on the software, click the 'Run'  button on the top toolbar. At this point in time, the camera and all the stages are initialized and reset to default values. They will eventually arrive at the values as seen here:

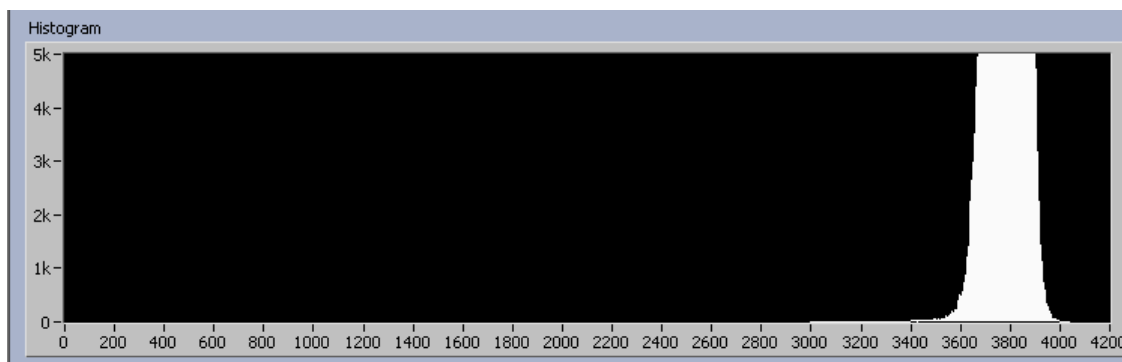


3. Power on white light source.

Turn on the white light source box.

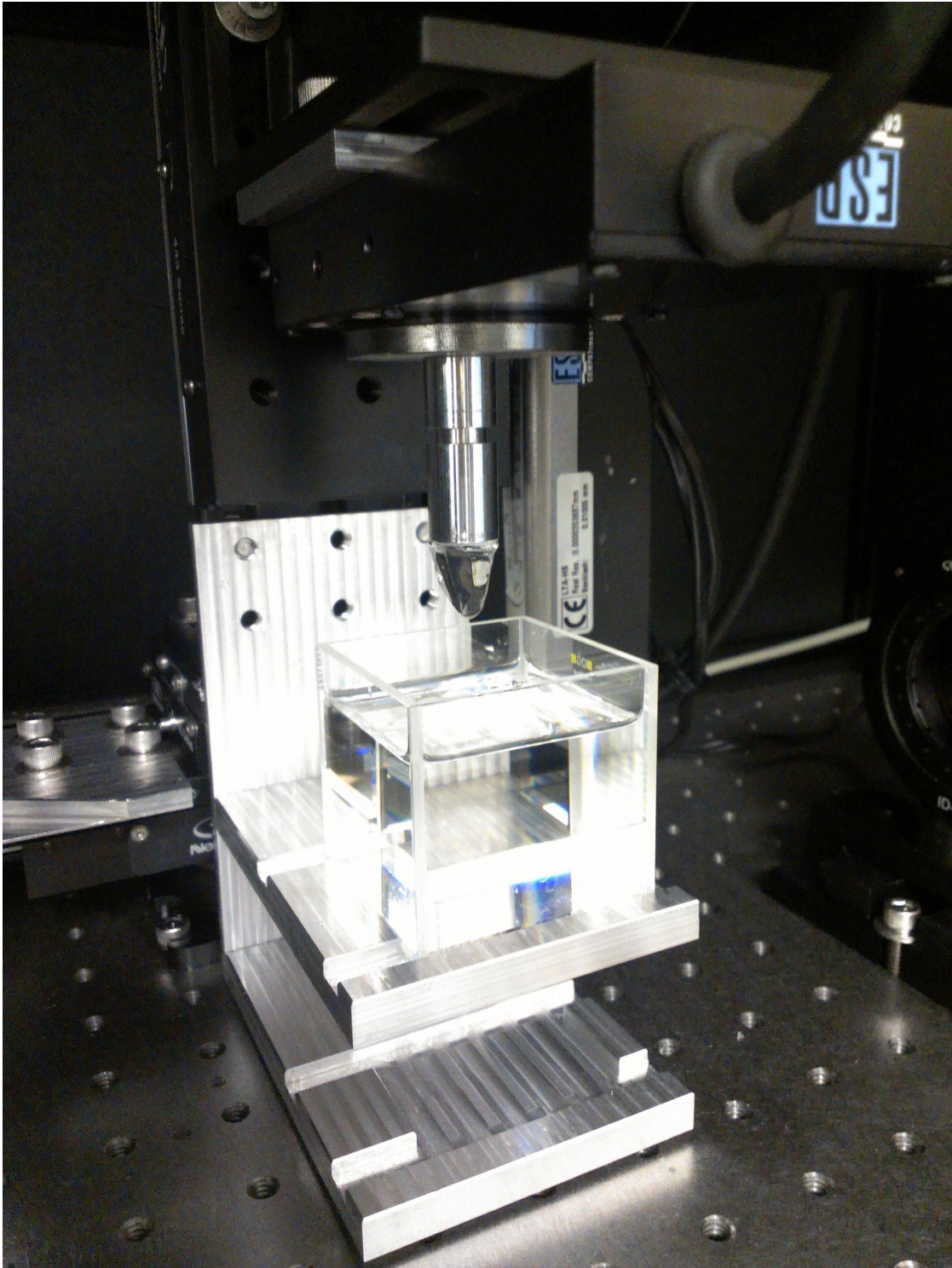


Keep the exposure time as default (25 ms) and increase the intensity of the white light using the hardware knob seen above. Adjust the intensity until the histogram is ETTR as seen below.



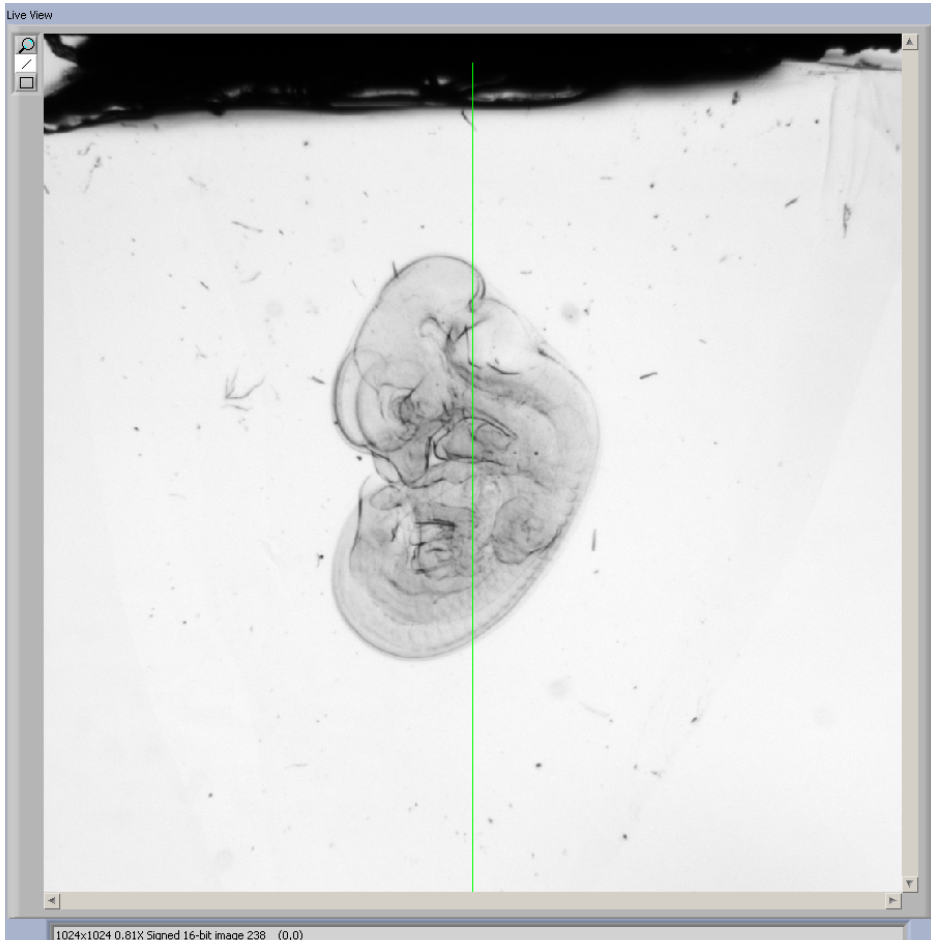
4. Place the sample onto the rotation stage.

Place the magnetic chuck onto the rotation stage such that it is located in the middle of the circular plate as seen below.



5. Position the sample.

Lower the specimen using the Vertical Position fields until the sample is in the center of the Live View Screen.



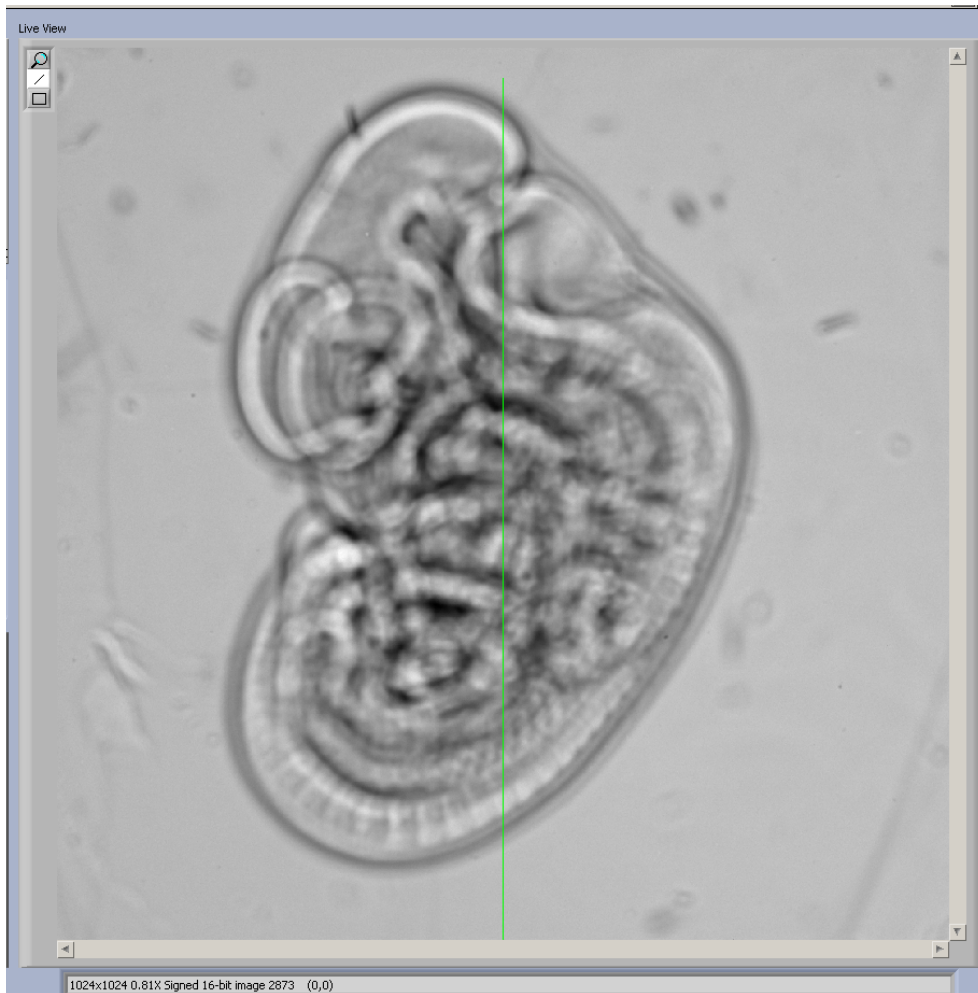
The user must manually move the sample on the rotation stage along the x-axis such that the central axis of the specimen is on the green line.

6. Zoom to appropriate magnification.

Change the zoom by manipulation the values in the zoom panel.

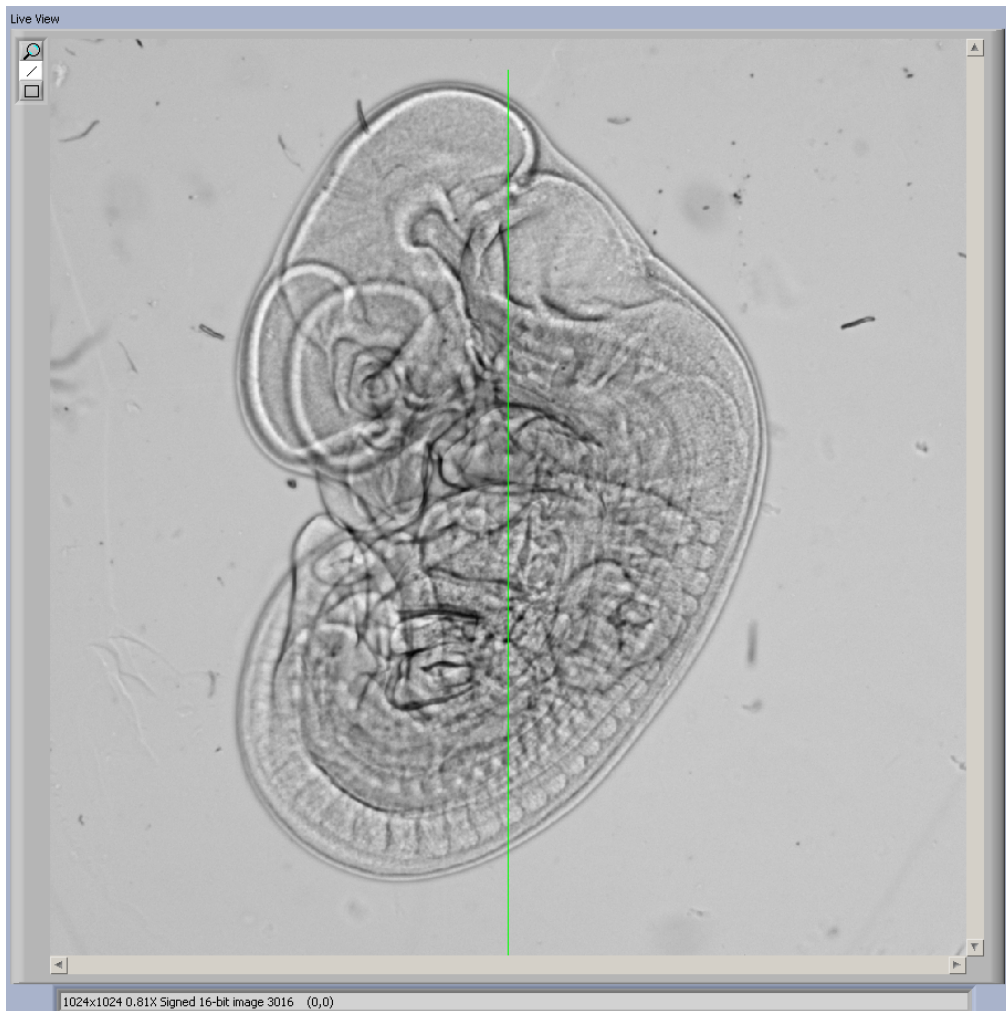


Zoom in such that the whole sample is a relatively tight fit within the field of view of the Live View screen.



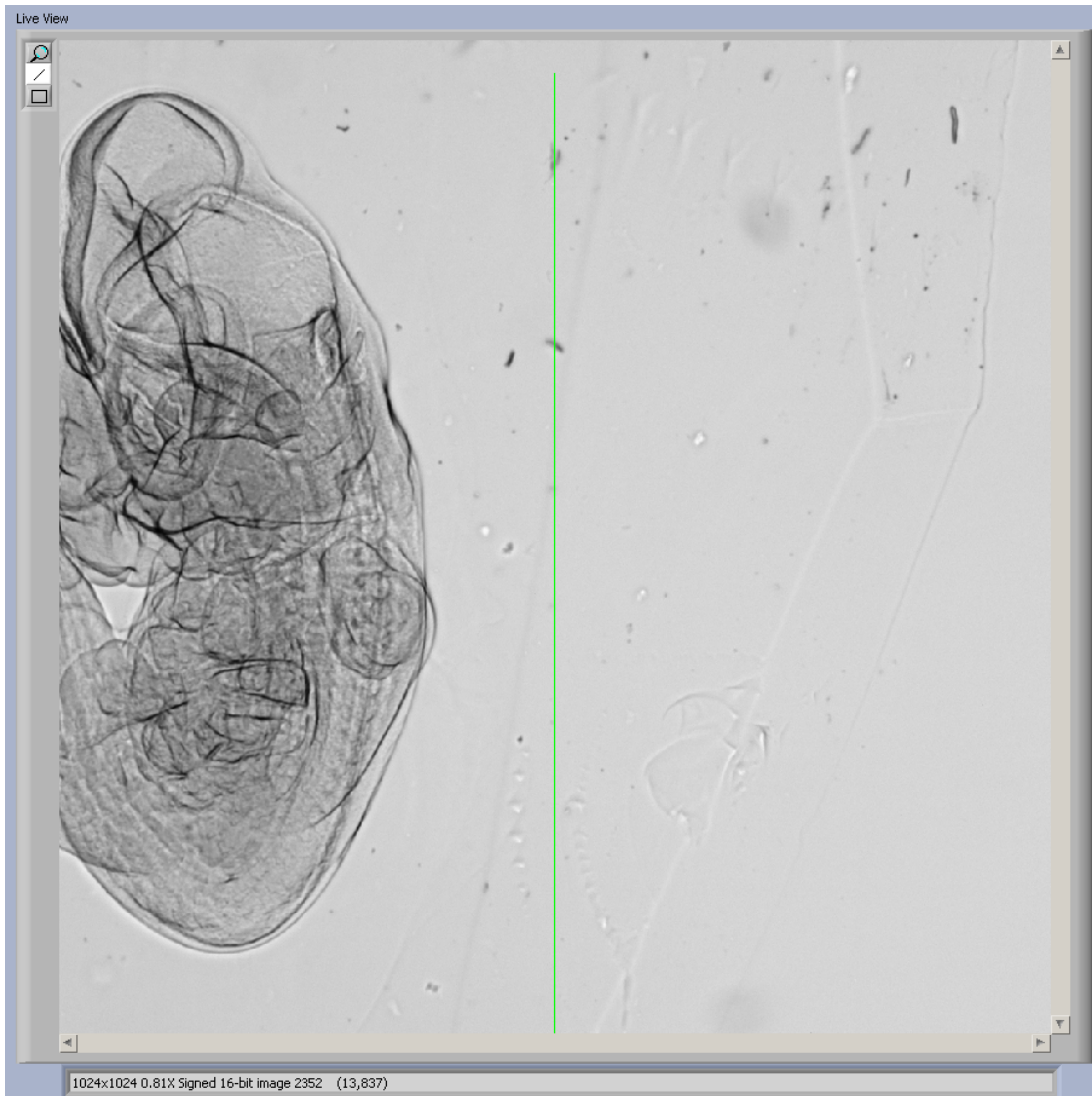
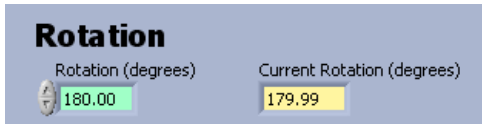
7. Focus the sample.

Focus the sample by moving the focus actuator with the focus panel.

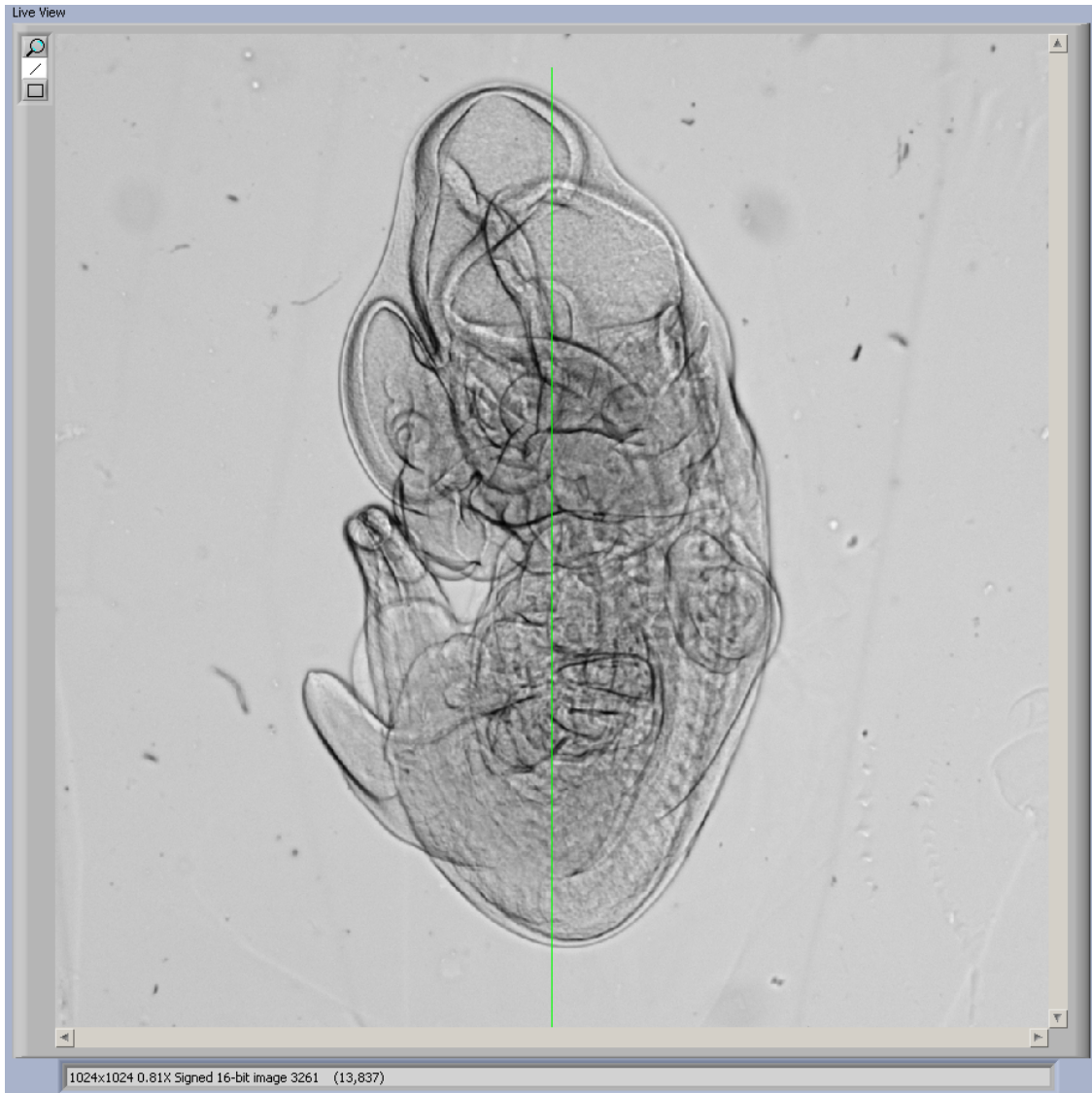


8. Center the sample onto the center of rotation.

Rotate the sample such that it is to the far left of the green line using the rotation panel. **Note: This is not like the Bioptonics scanner. Specific values have to be entered, the stage does not rotate infinitely.**



Use forceps to pull the sample towards you until the center of the sample resides on the center green line displayed in Live View.



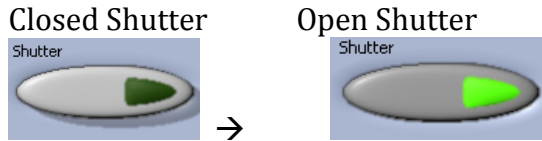
Rotate the sample by 90 degrees again and repeat the process until the sample rotates on its own axis on the green line.

9. Turn off white light source.
10. Implement desired excitation and emission filters.

Use the filter wheels to implement the desired filters for the excitation and emission light.

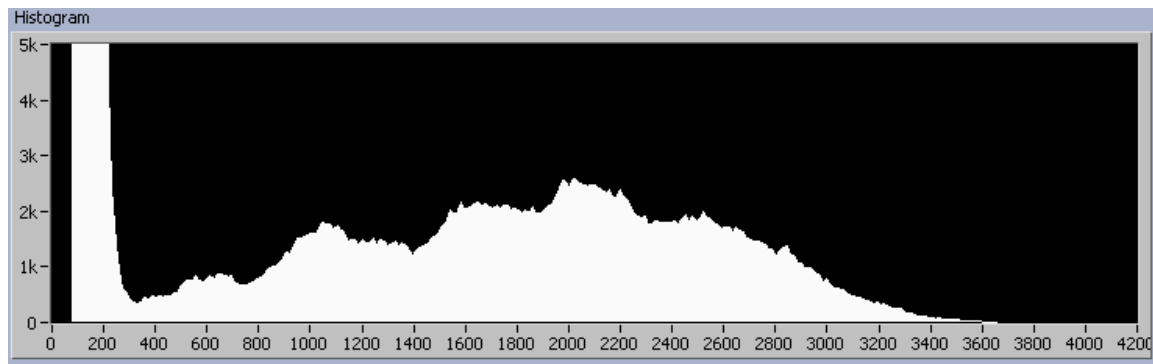
11. Open the UV light shutter.

Close the lid of the OPT enclosure. Open the UV light shutter by pressing the shutter button in the LabView software.



12. Adjust Camera Exposure Time

Adjust the camera exposure time such that the histogram is ETTR. You want the brightest pixel to be close to but less than the ceiling of the CCD dynamic range (i.e. 4095). **Note: The histogram changes at different projection angles. It is better to be safe and have the brightest pixel in your projection to be in the ~3500 on the histogram.**

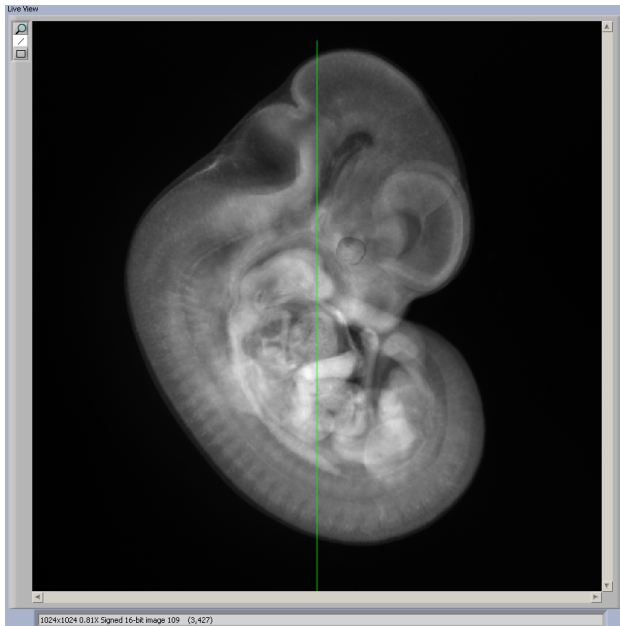


13. Re-focus the sample

The position of the sample and the wavelength of the light had been changed since the last time the sample was focused. The user must use their best judgment on what is the optical focus value for their sample.

14. Rotate the sample to preferred starting position

It is wise to scan all your samples the same way. The user should adjust the rotation value such that their samples are in the same orientation at the start of each scan. (I.E. embryos looking to the right)



15. Establish Scan Parameters

Once the sample is positioned and exposed correctly, the user can choose to change the default scan parameters.

16. Input File Name

For every scan the file name should be different. The filename must be entered before the dark field is acquired. If the name is not changed, the previous scan will be overwritten.

17. Acquire Dark Field

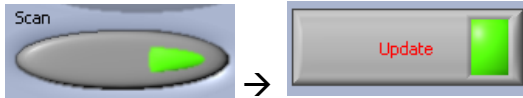
Click on the 'Acquire Dark Field' button and then click 'Update'.



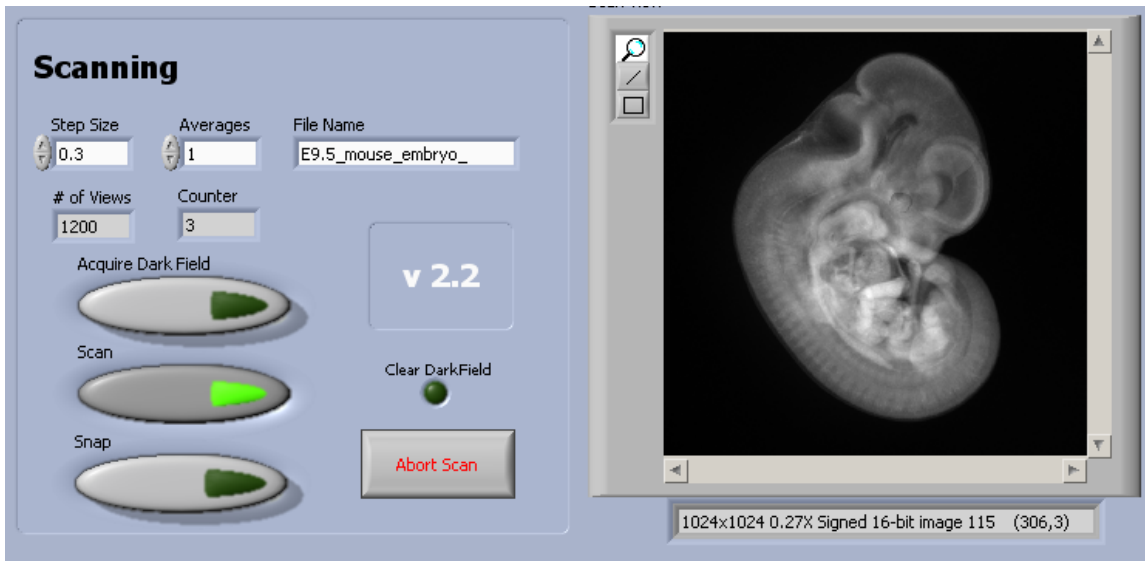
This will raise the sample out of the field of view, take 10 background images, average them, and then subtract them from each subsequent projection image. The sample will be returned to its previous position automatically.

18. Scan

Click on the 'Scan' button and then click 'Update'.



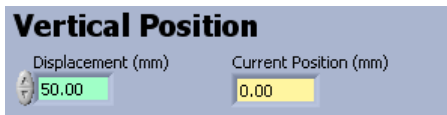
This will initiate the scan process and the scan progress can either be monitored through the Counter value or the Scan View screen.



The scan can be aborted by clicking on the 'Abort Scan' button. Once the scan is completed, the sample will return to its original position and the shutter will close to prevent photobleaching.

19. Unmount Sample

Raise the sample to 50mm and unmount the sample from the rotation stage.




If the user wants to image another sample, mount the new sample and repeat steps 3 through 16.

20. Power off Labview software.

To safely power off the software, click the 'Stop' button.



It is very important to ONLY power off the software in this fashion other than force closing the Labview window or clicking . If the software is closed in any other fashion, the hardware will be cut off abruptly and the camera will not be able to dump stored images within its buffer.

21. Power off hardware components.

Power off the CCD camera, OPT zoom controller, and X-CITE UV source. **Only power the hardware components once the LabView software has been powered off.**

Scanning Checklist

1. Power on hardware components
2. Run Labview software
3. Power on white light source.
4. Place sample on rotation stage.
5. Position the sample.
6. Zoom to appropriate magnification
7. Focus the sample.
8. Center the sample onto the center of rotation.
9. Power off white light source.
10. Implement excitation and emission filters.
11. Open the UV light shutter
12. Adjust camera exposure time.
13. Re-focus sample.
14. Rotate the sample to preferred starting position.
15. Establish Scan Parameters.
16. Input File Name.
17. Acquire Dark Field.
18. Scan.
19. Unmount Sample.
20. Power off Labview software.
21. Power off hardware components.

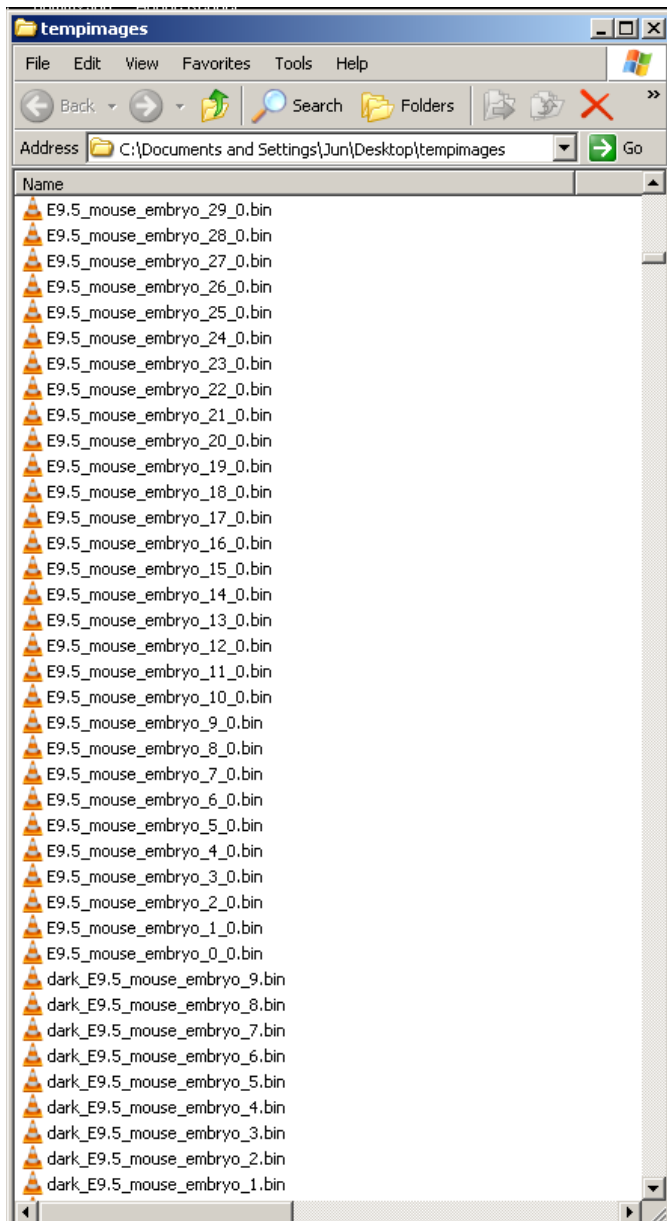
Post Image Acquisition

Raw Data Save Location

The raw data is saved in a folder called 'tempimages'.



Inside this folder you will find files such as the following:



The raw data is saved as raw binary files. This is too speed up the scan time and to not lose data in the Labview raw data to TIFF image conversion. The 10 background images are saved as 'dark_[filename][imagenumber].bin'. The raw projection files are saved as '[filename][projection number]_[averagenumber].bin'. In this example:

Filename: E9.5_mouse_embryo_
Averages: 1

Note: The number of averages is saved as [average - 1]

PostOPT.exe

This program:

- Subtracts the average of 10 background image from each projection image
- Averages the projections (if the user chose an average greater than 1)
- Convert the projection binary files to uncompressed 16-bit TIFF files so that Skyscan NRecon can read the files
- Export a dummy log file so NRecon will read the files
- Export a Scan log file with image properties in it



The program is essentially the Matlab script that runs in the background. **Note:** **This program must be run for NRecon to be able to reconstruct the raw data.**

Double click the icon and a command prompt will appear. It may take a few seconds for the first command to appear.

Step1. Enter the number of data set to be processed:

This is where you enter the number of scans you want to be converted for NRecon. Input the number and press ENTER.

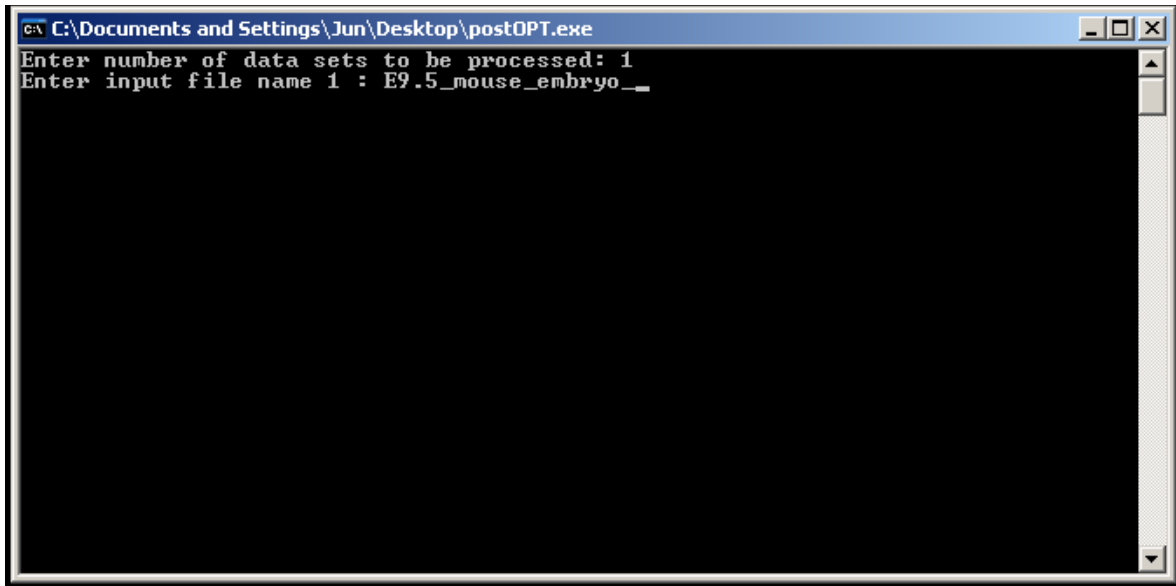
Step2. Enter input file name 1:

Enter the file name **exactly** how it was entered into the Labview software before scan and the press ENTER. This file name must be the **exactly** the same or the postOPT.exe script will exit out immediately.

If there is more than one scan to process, the program will prompt

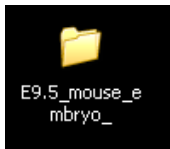
Enter input file name 2:

Repeat for as many scans you specified in **Step1**:



Once you press ENTER, the command prompt will remain open for as long as it is processing the data. If you enter the file name incorrectly, it will close immediately and you will need start again. This is true if the second/third/fourth... filename is incorrect. The program will convert all the scans that have correct filenames until it finishes or reads an incorrect filename.

Once the postOPT.exe script is finished, a new folder with the same name as the chosen file name will appear in the save location.



Inside this folder will be 1200 projection 16-bit TIFF files similar to the output of Bioptonics. They can be opened in NRecon and Dataviewer exactly in the same way as the Bioptonics files would. **Note: There are no filter specific folders and there is no filter information in the .log files. The filter information must be recorded in a lab book or be explicit in the file name.**

Also in this folder is a 'dummy' log file ('[filename].log') that is used such that NRecon will be compatible with the projection images. Do not use this file as a proper log file because most of the information does not pertain to the actual scan.

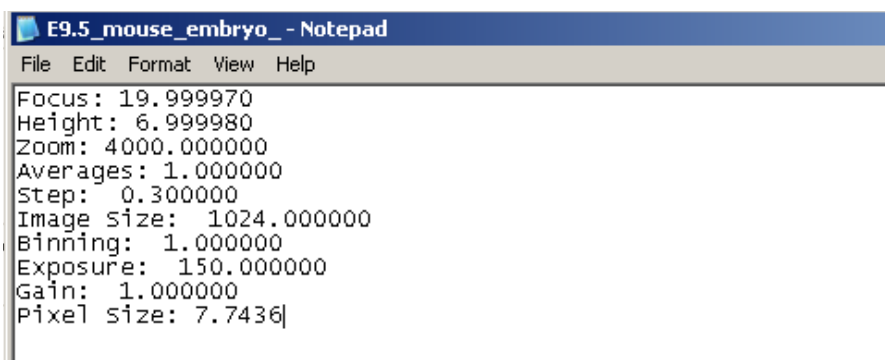


The true log file is named '[filename]'.



E9.5_mouse_embryo_

In this log file are all the relevant scan parameters that were specified during the scan of this sample:



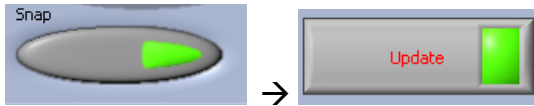
All the scan parameters are in the same units as specified in Labview. 'Pixel size' is the image resolution and it is in microns.

The projection files in this folder can now be opened and reconstructed in NRecon.



PostSnap.exe

If the user wanted to take a single snapshot of the LiveView screen in Labview, they will follow steps 1-17 in the scanning protocol but then click 'Snap' instead of 'Scan'.



There will now be a raw binary file in the 'tempimages' folder for this individual snap. It will have the file name as given in Labview.

To generate a TIF image of this file, double click on postSnap.exe



Double click the icon and a command prompt will appear. It may take a few seconds for the first command to appear.

Step1. Enter the number of snaps to be processed:

This is where you enter the number of snaps you want to be converted to TIFF. Input the number and press ENTER.

Step2. Apply Darkfield (y/n):

The user can choose to apply darkfield onto the snap or not. Type 'y' for yes or 'n' for no. Darkfield would have to had been acquired in Labview for this to work.

Step3. Enter input file name 1:

Enter the file name **exactly** how it was entered into the Labview software before scan and the press ENTER. This file name must be the **exactly** the same or the postSnap.exe script will exit out immediately.

If there is more than one scan to process, the program will prompt

Enter input file name 2:

Repeat for as many scans you specified in **Step1**:


```
C:\Documents and Settings\Jun\Desktop\postSnap.exe
Enter number of snaps be processed: 1
Apply Darkfield (y/n): y
Enter input file name 1 : E9.5_mouse_embryo_
```

Once you press ENTER, the command prompt will remain open for as long as it is processing the data. If you enter the file name incorrectly, it will close immediately and you will need start again. This is true if the second/third/fourth... filename is incorrect. The program will convert all the scans that have correct filenames until it finishes or reads an incorrect filename.

Once the process is complete, the new TIFF image will be in the OPT Snaps folder.

