bmseed
BioMedical
Sustainable Elastic Electronic Devices

Innovation in Neural Interface Technology

OPERATING INSTRUCTIONS FOR THE IMAGING MODULE

Basler Camera: acA2440-75µm

BMSEED LLC
Center for Entrepreneurial Innovation, Suite 135
275 N. Gateway Drive
Phoenix, AZ 85034

Web: www.bmseed.com
Email: info@bmseed.com
Phone: 609-532-9744

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1. Protocol to Connect the Camera

BMSEED currently offers MEASSuRE-Mini systems with the **Basler acA2440-75µm camera**, which has the following specs:

- Sensor: Sony IMX250
- Sensor type: CMOS
- Sensor size: 8.4 mm × 7.1 mm
- Shutter: Global shutter
- Max image circle: 2/3”
- Monochrome system
- Pixel image resolution: 2448 px × 2048 px (5MP)
- Pixel size: 3.45 µm × 3.45 µm
- Frame rate at full image resolution: 75 fps
  Note: Higher frame rates can be achieved for smaller frames.

The images below show, from left to right: front of the acA2440 camera, back side of the acA2440 camera, and a USB 3.0 Micro B to USB A cable.

(a) (b) (c)

Fig. 1: (a) front of the acA2440 camera, **(b)** back side of the acA2440 camera, and **(c)** a USB 3.0 Micro B to USB A cable.

To connect the camera, do the following:

1) Once the camera has been mounted on the MEASSuRE frame, connect the USB 3.0 Micro B end of the USB cable to the back of the camera.

2) Connect the USB A end of the USB cable to the computer. The green LED on the back of the camera should light up.

2 Protocol to Download and Setup the Camera Software: pylon Viewer

The software **pylon Viewer** is used to control the Basler camera.

1) If you need to download the pylon Viewer software, go to the following website: https://www.baslerweb.com/en/sales-support/downloads/software-downloads/

2) If running in a Windows PC, select **pylon 6.3.0 Camera Software Suite Windows**.

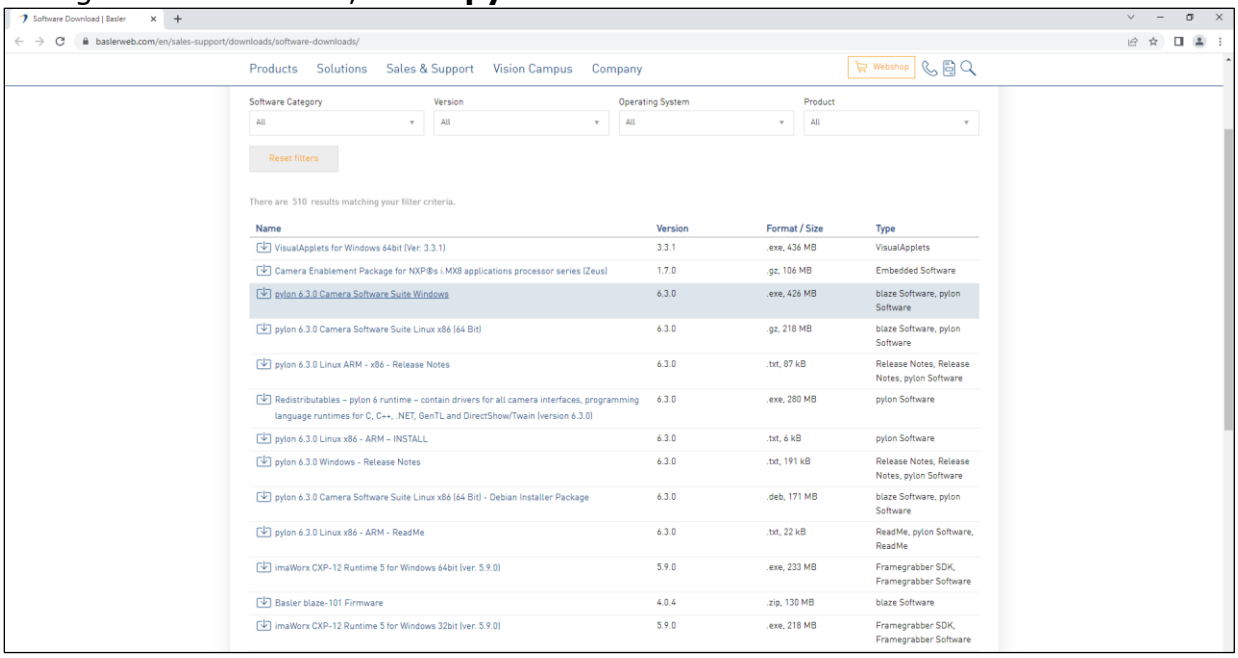


Fig. 2: Basler website with available software downloads (screenshot taken on 04/25/22).

3) Fill out the form and click the "Start the Download!" orange button.

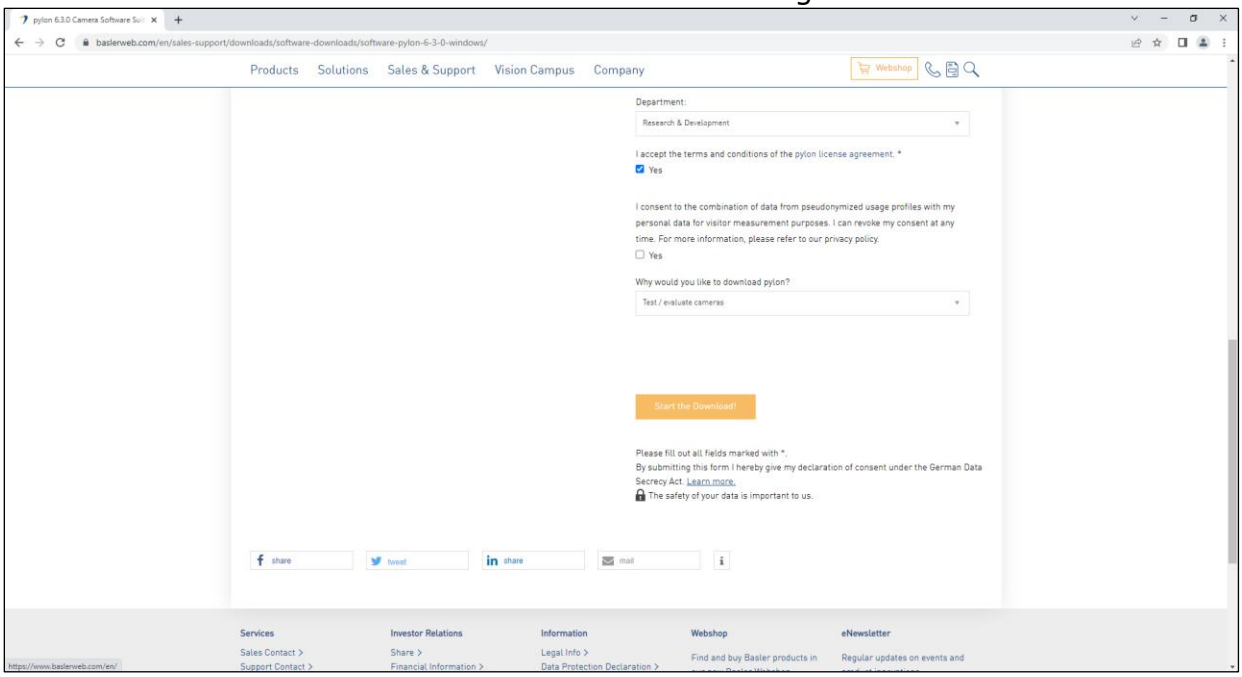


Fig. 3: End of software download form with "Start the Download!" button (screenshot taken on 04/25/22).

4) Run the "Basler_pylon_6.3.0.23157" executable file.

Fig. 4: pylon Viewer software loading banner.

5) In the pylon 6 Welcome window, check the box "I agree to the pylon Terms & Conditions" and click "Next."

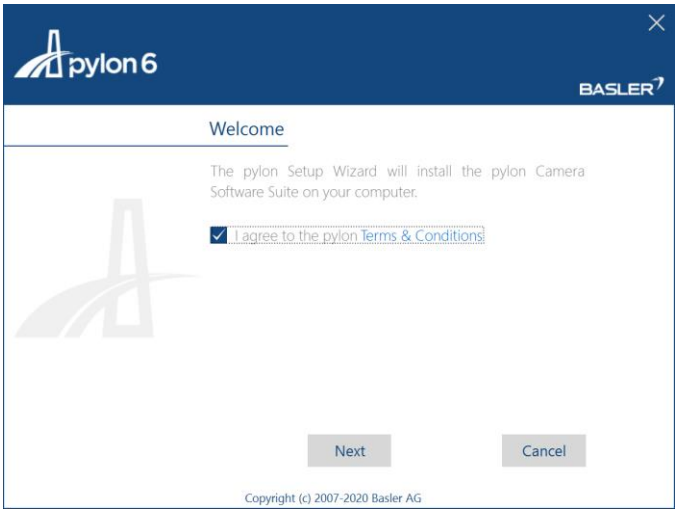


Fig. 5: pylon Viewer software welcome window.

6) In the pylon 6 Profiles window, select "Camera User" and click "Next."

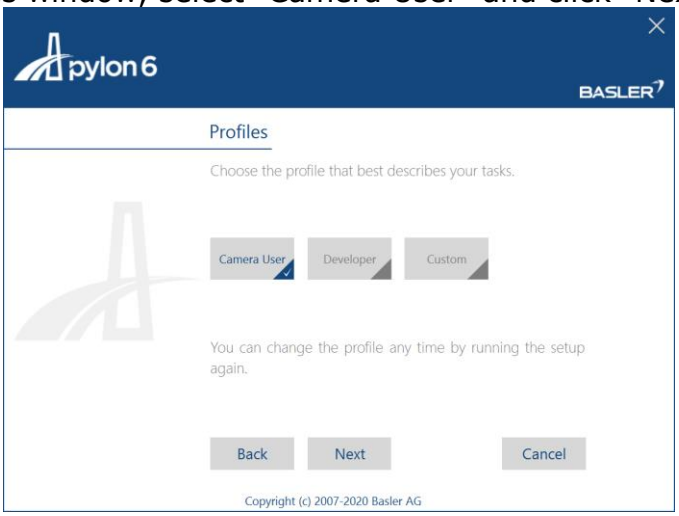


Fig. 6: pylon Viewer software profile selection window.

7) In the pylon 6 Interfaces window, select "USB" and click "Next."

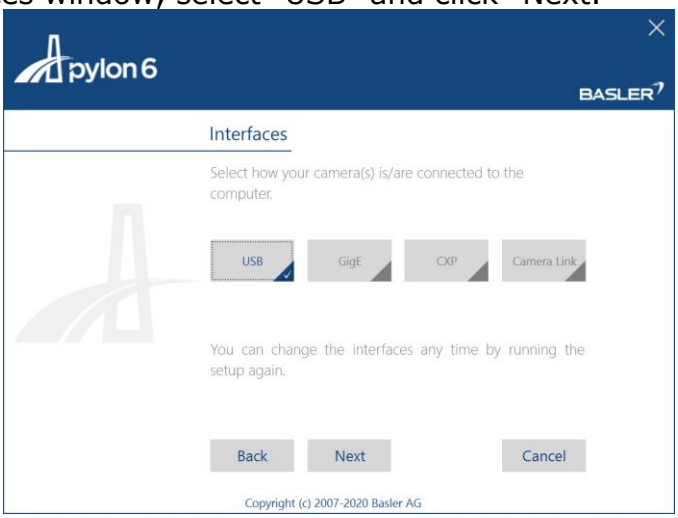


Fig. 7: pylon Viewer software interface selection window.

8) In the pylon 6 Destination Folder window, note the path and click "Next."

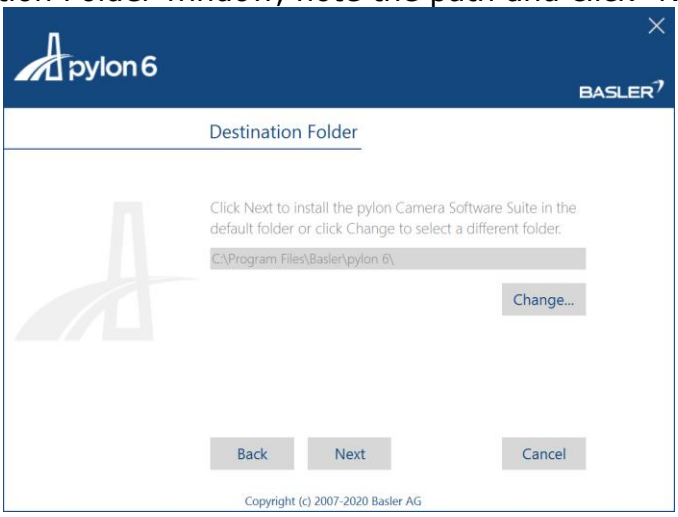


Fig. 8: pylon Viewer software destination folder selection window.

9) In the pylon 6 Ready to install window, click "Install" to start the installation.

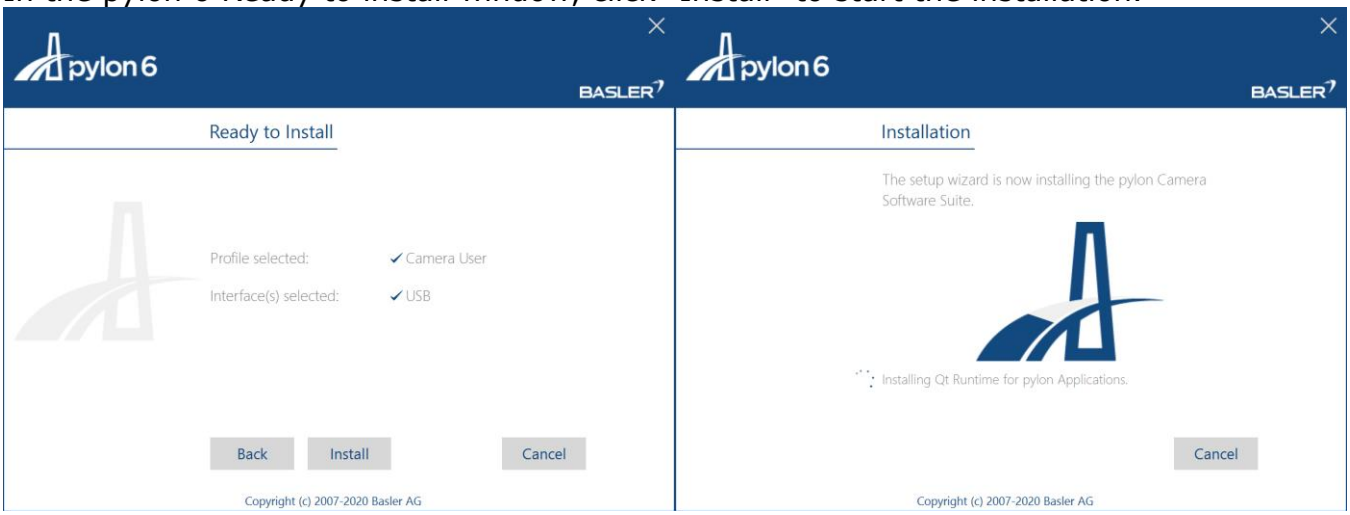


Fig. 9: pylon Viewer software installation windows.

10) Once the installation is completed, the Enjoy pylon window will open. Click "Close."

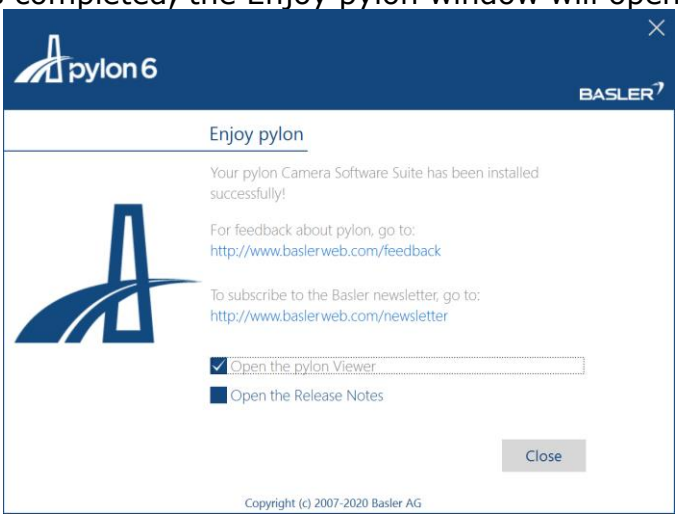


Fig. 10: pylon Viewer software installation success window.

11) If the "Open the pylon Viewer" box was checked prior to closing, the pylon Viewer will open. If this is the first time the software is loaded, select either "Yes" or "No" to participate in the product improvement program and click "Apply."

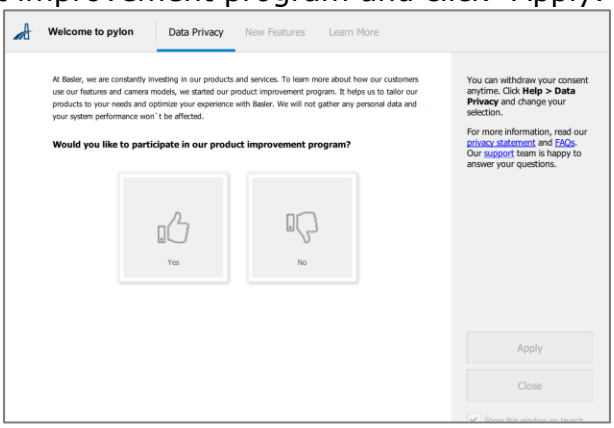


Fig. 11: pylon Viewer software improvement program participation window.

12) The pylon Viewer main window will open. Detected USB cameras will be listed in the Devices pane under the USB header, as shown in the screenshot below.

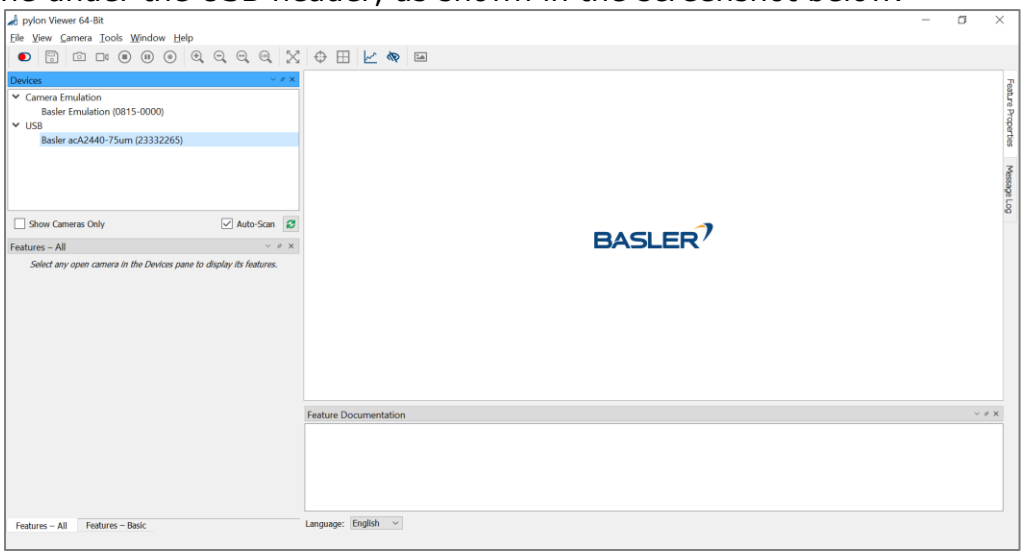


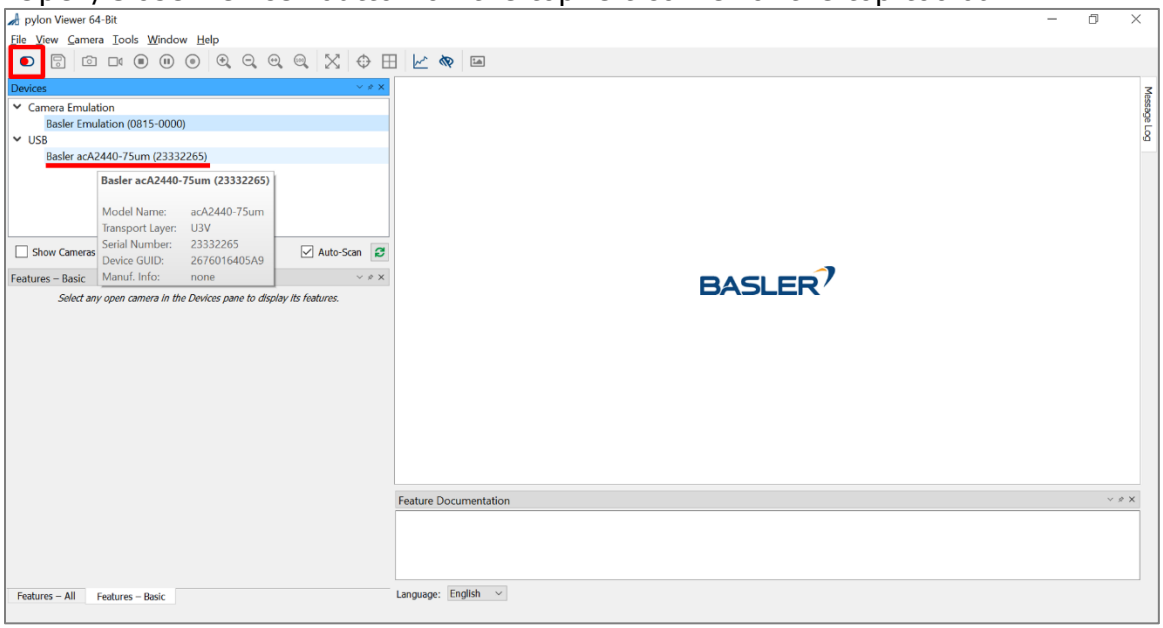
Fig. 12: pylon Viewer software main window.

3 Protocol for Software Initial Setup

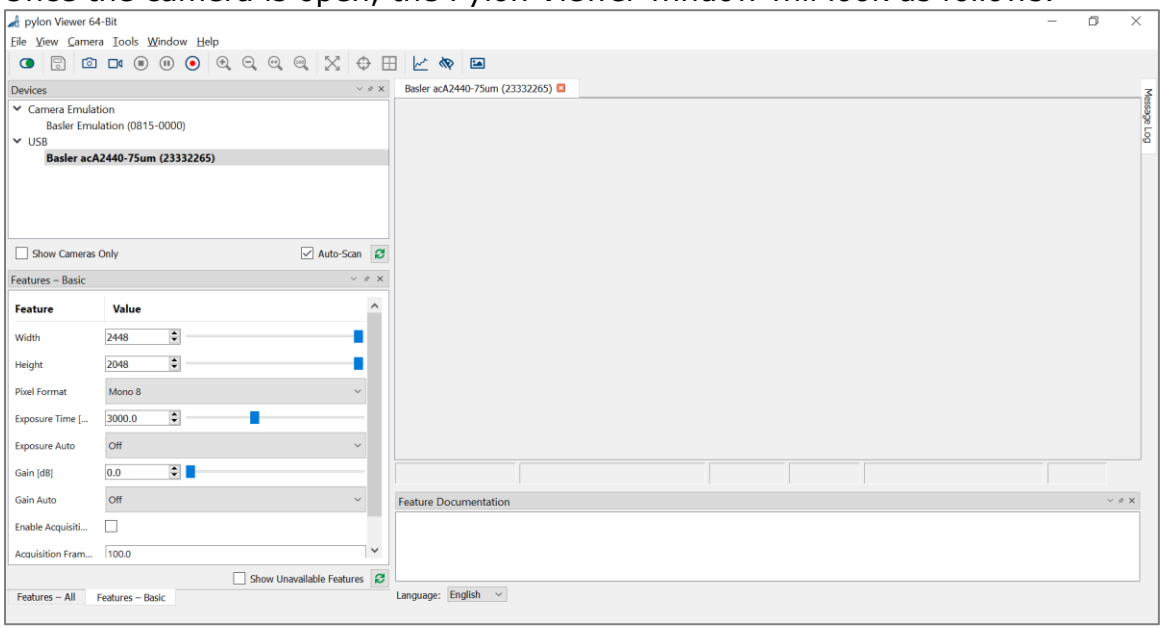
Follow these steps for the initial setup of the camera and software.

1) Load the pylon Viewer software.

2) In the "Devices" pane select "Basler acA2440-75um" under USB, and click the "Open/Close Device" button on the top left corner of the top toolbar.

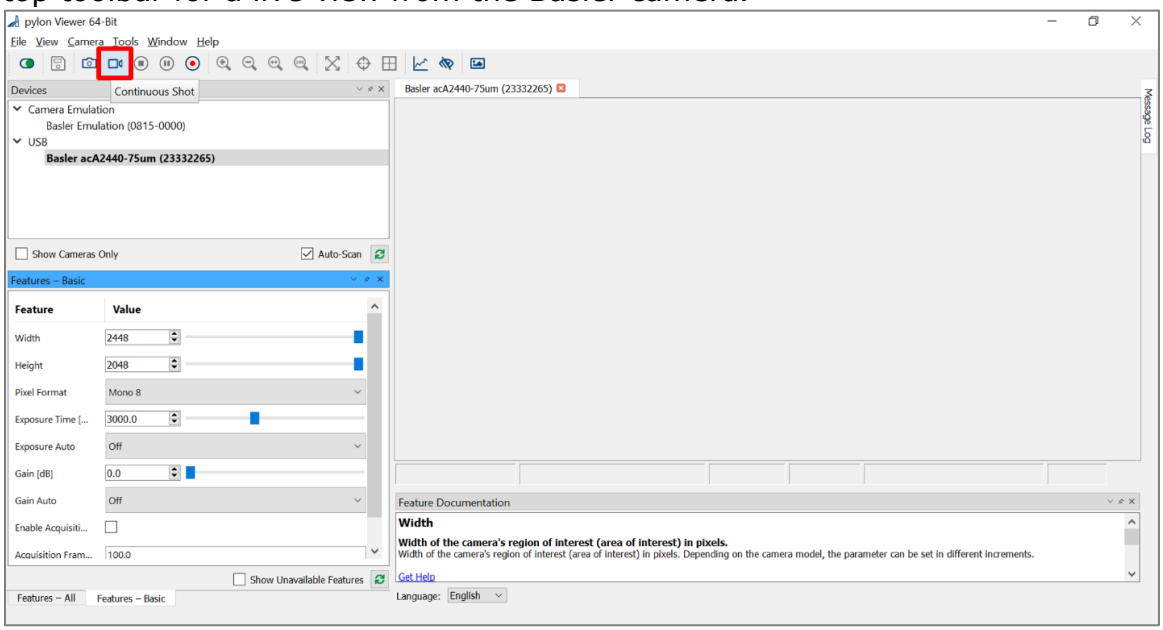


Once the camera is open, the Pylon Viewer window will look as follows.

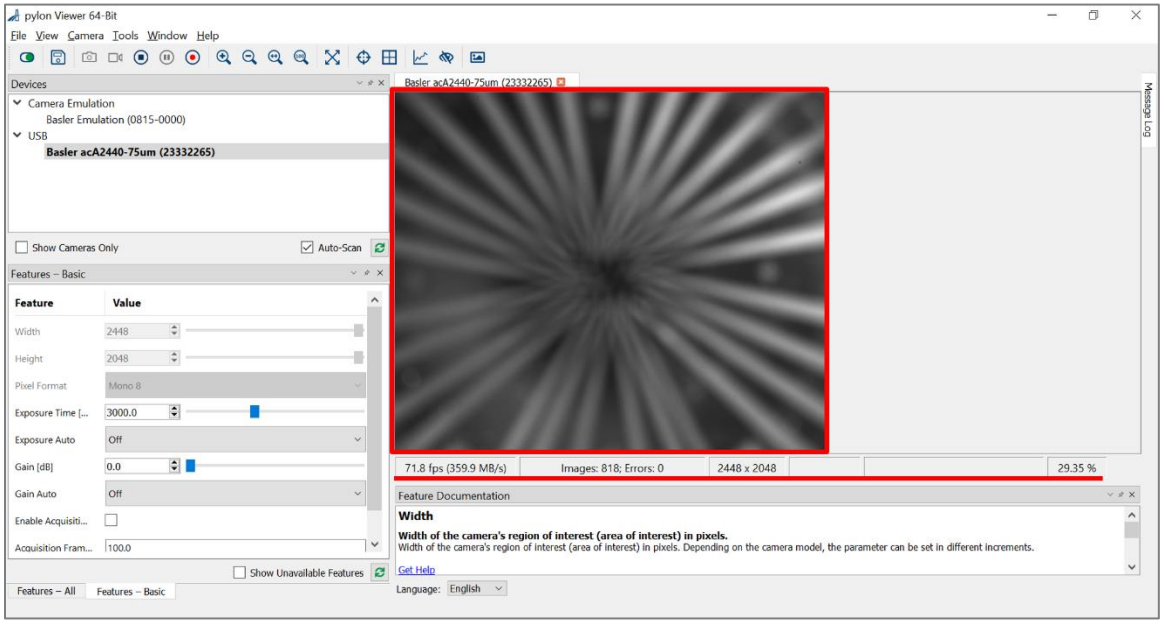


Note: When the selected camera is closed, the icon will have the following appearance  and with the label "Open Device" when hovering above the icon. When the selected camera is open, the icon will have the following appearance  and with the label "Close Device" when hovering above the icon.

3) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for a live view from the Basler camera.



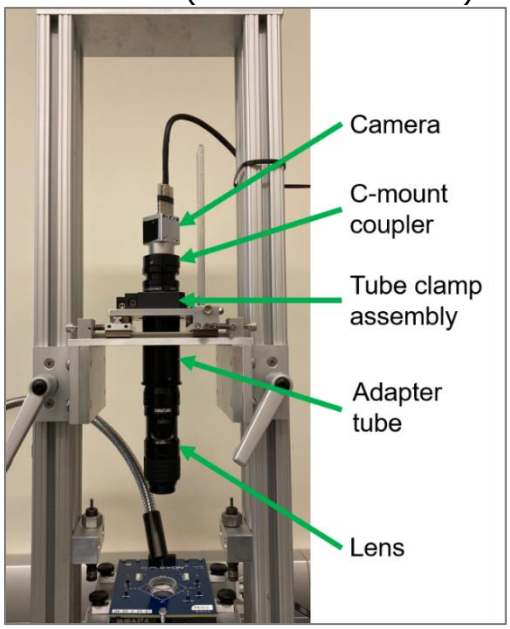
The live feed from the camera will be displayed in the main section of the Pylon Viewer window.

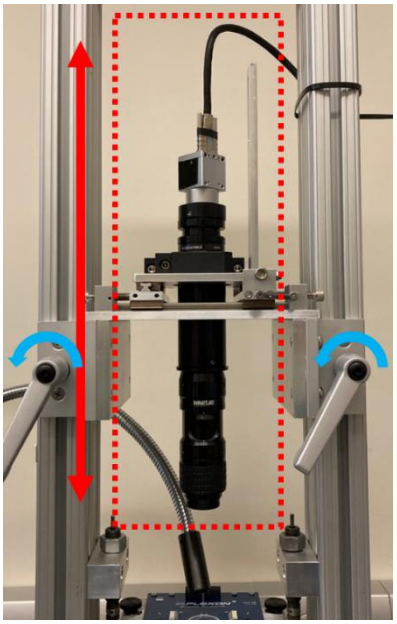


Note: Some parameters (shown above with a red underline) will be displayed under the live video: actual frame rate in fps; frames/images collected from camera since the "Continuous Shot" button was enabled; image resolution in pixels; and window zoom level.

4) With the Zoom control dial of the lens at its lowest magnification level (i.e., 0.7× for the Navitar Zoom 6000 and UltraZoom 6000 lens), **adjust the focus** by turning the focus knob. In some cases, you will need to adjust the vertical position of the camera stage holding all imaging components.

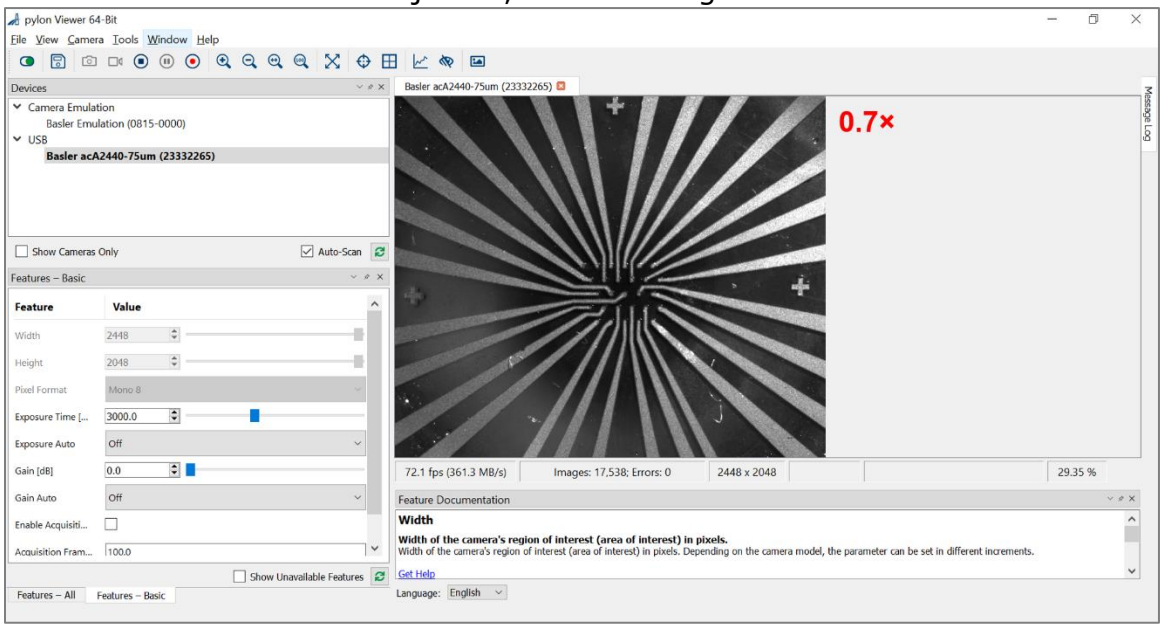
The camera will be mounted into the frame as shown in the left photo below. The zoom control dial and focus knob of the lens are shown in the center image. If the distance between the camera and sample needs to be adjusted, loosen the two handles (counter-clockwise rotation) and move the camera stage up or down. Once adjusted, re-tighten the handles (clockwise rotation) to hold the vertical position.



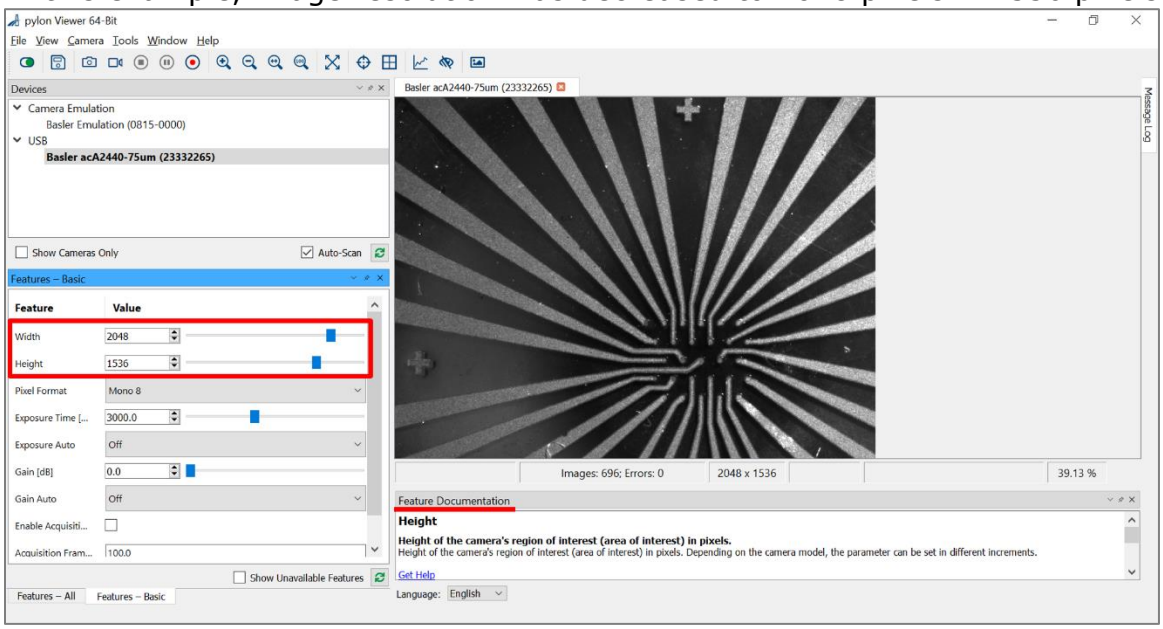


Note: See **Appendix A** for the protocol to swap camera lenses and adapter tubes if needed and **Appendix B** for working distances for various imaging hardware configurations, which serve as references when finding an optimal vertical z-position.

Once the focus has been adjusted, the live image should look as shown below.



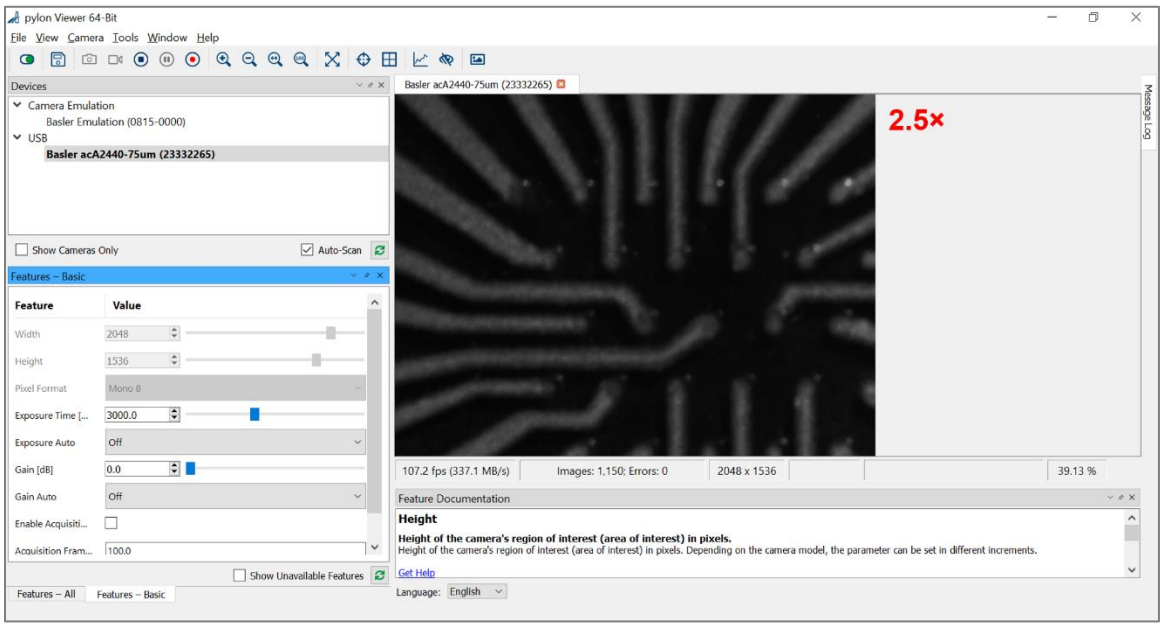
5) When the Basler camera is opened, the image resolution is set to the camera's max values by default, i.e., 2448 pixels wide × 2048 pixels tall. If the **camera image resolution** needs to be changed (e.g., to achieve a higher frame rate), type in the new pixel width and pixel height under the "Features – Basic" pane. Note that the "Features – Basic" tab at the bottom must be selected.
In this example, image resolution was decreased to 2048 pixels × 1536 pixels.



Some remarks:

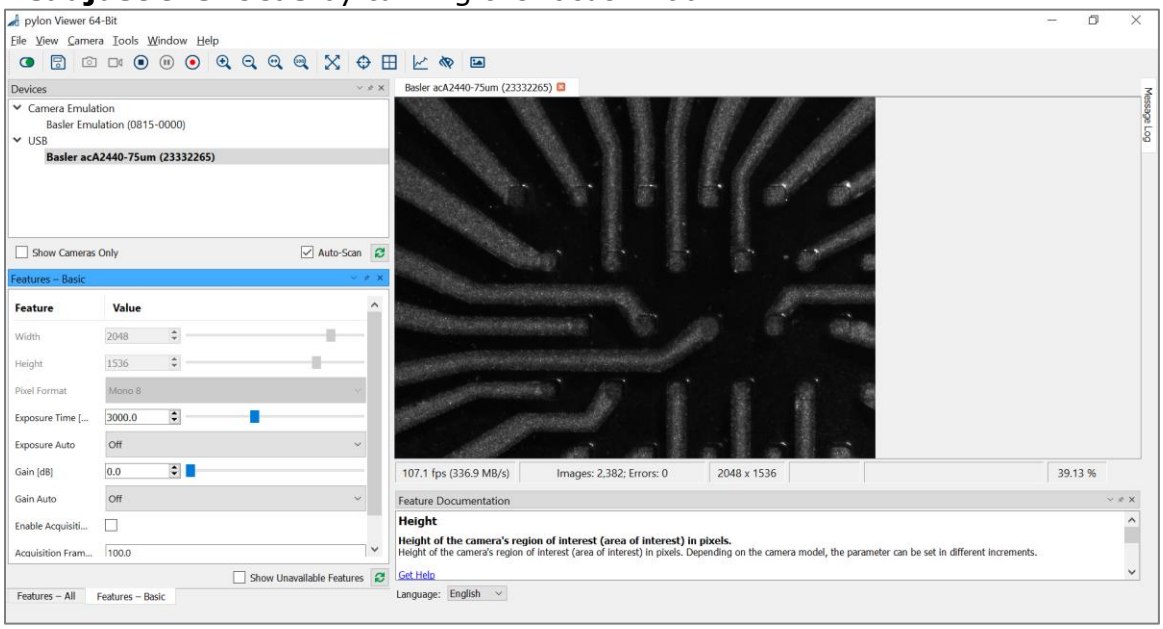
- After updating the image resolution, the image might need to be re-centered since the bottom right of the image is the section being "cut off" from view.
- By default, Pylon Viewer software will zoom-to-fit the image. For example, in our screenshots, the zoom level of the software increased from 29.35% to 39.13%.
- As different parameters are selected, the "Feature Documentation" pane below the live image will provide a brief description of the selected parameter.

6) Set the Zoom 6000 or UltraZoom 6000 **lens magnification** to the desired level by turning the zoom control dial. In this example, magnification was increase to 2.5×.

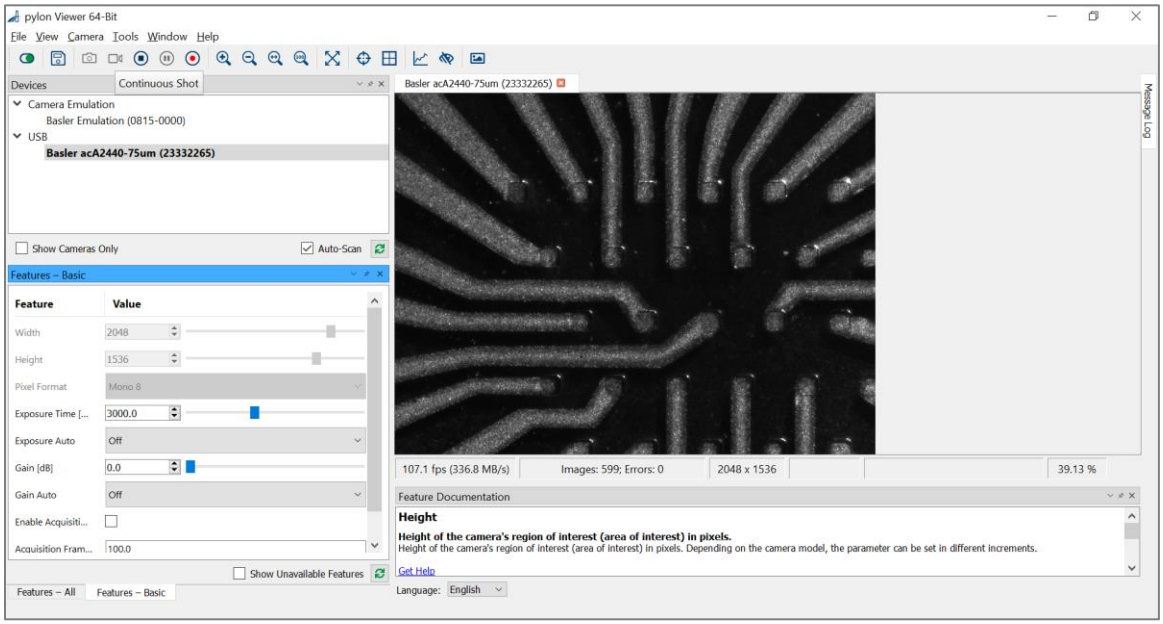


Note: If the image gets out of focus when the zoom level is changed, we recommend to adjust the parfocal of the imaging system (see Appendix F for details). When the parfocal

7) **Readjust the focus** by turning the focus knob.



8) Adjust the illuminator or **light source intensity** as needed to increase the image lighting. In this example, light intensity was increased from "9" to "10."

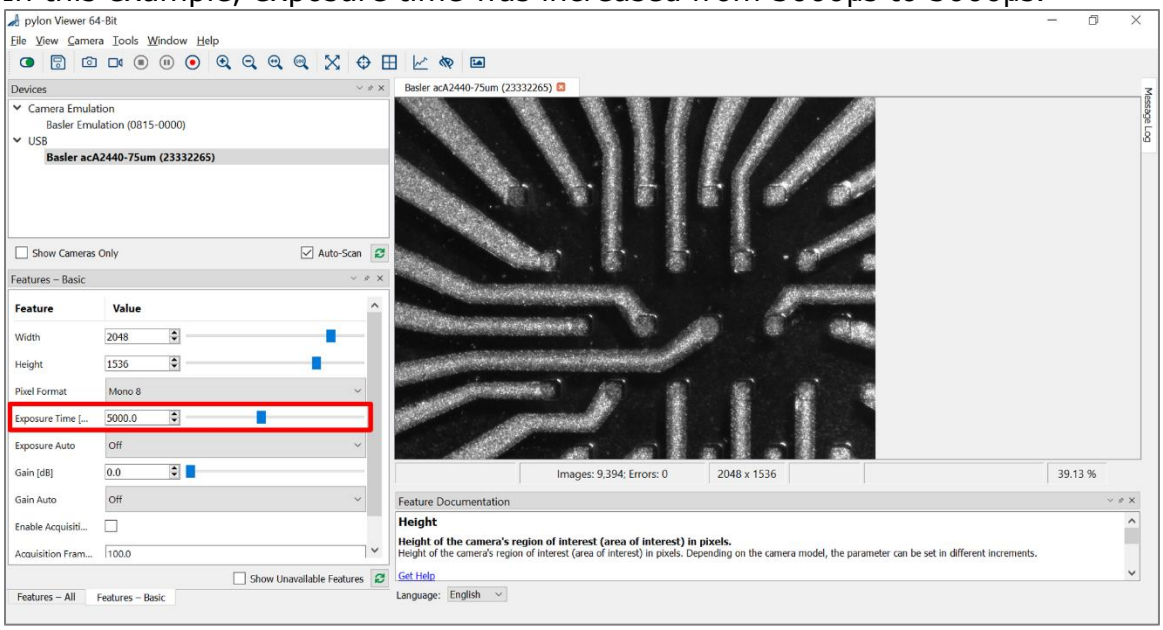

pylon Viewer 64-Bit
File View Camera Tools Window Help
Devices
Continuous Shot
Camera Emulation
Basler Emulation (0815-0000)
USB
Basler acA2440-75um (23332265)
Show Cameras Only
Auto-Scan
Features – Basic
Feature
Value
Width
2048
Height
1536
Pixel Format
Mono 8
Exposure Time [...
3000.0
Exposure Auto
Off
Gain [dB]
0.0
Gain Auto
Off
Enable Acquisiti...
Acquisition Fram...
100.0
Show Unavailable Features
Features – All
Features – Basic
Basler acA2440-75um (23332265)
Message Log
107.1 fps (336.8 MB/s)
Images: 599; Errors: 0
2048 x 1536
39.13 %
Feature Documentation
Height
Height of the camera's region of interest (area of interest) in pixels.
Height of the camera's region of interest (area of interest) in pixels. Depending on the camera model, the parameter can be set in different increments.
Get Help
Language:
English


DIMMING
OFF
ON
POWER
MIN
MAX
LED-50W

9) Exposure Time:

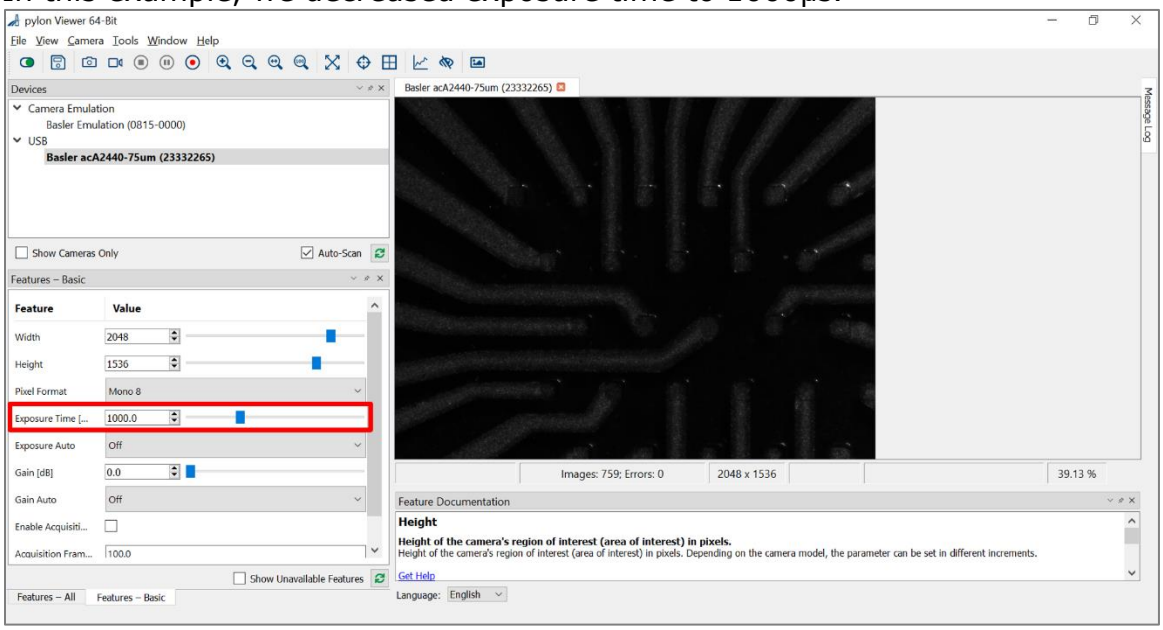
In addition to the illuminator's settings, lighting on the sample can be improved by **adjusting the exposure time**.

In this example, exposure time was increased from 3000µs to 5000µs.



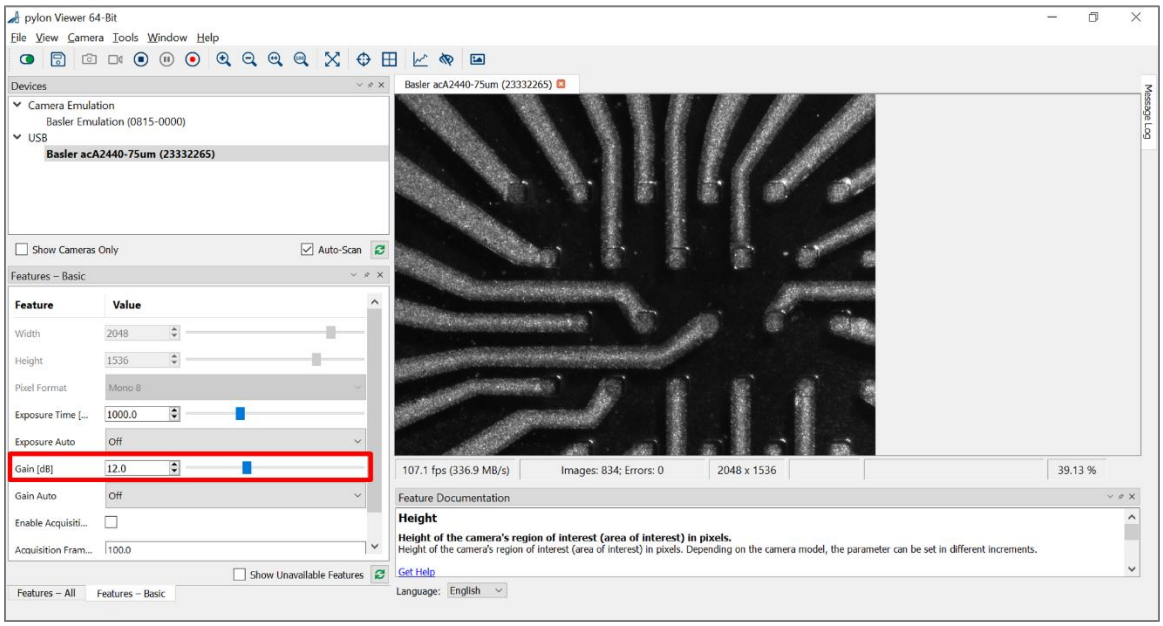
In some cases when recording video at high frame rates, it is not recommended to set long exposure times. Long exposure times result in worse motion blurs during recordings. In these situations, **it is recommended to decrease exposure time to an acceptable level and then increase the gain**

In this example, we decreased exposure time to 1000µs.



Note: In this example, the target frame rate is set to 100fps. Thus, the maximum allowable exposure time is 10ms or 10000µs. While we could still use an exposure time of 5000µs, we are decreasing exposure time to highlight the effects of increasing the gain.

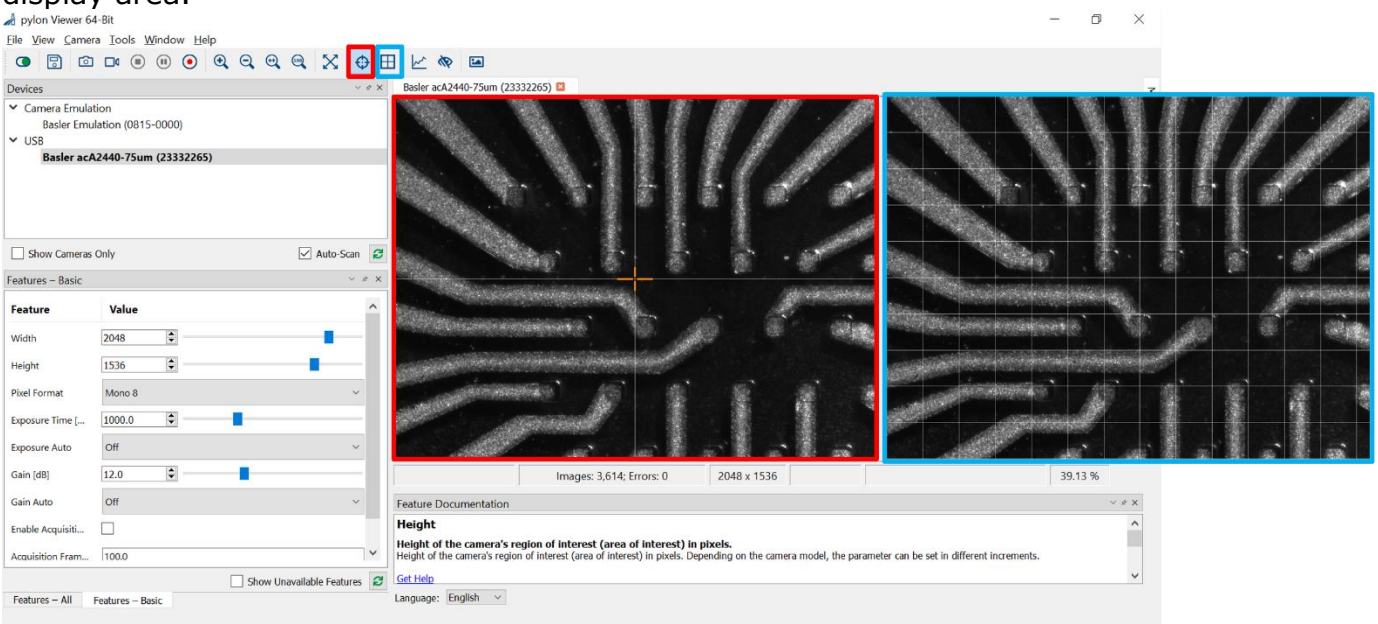
In this example, gain was increased from 0dB to 12.0dB while keeping the exposure time at 1000 µs.



10) Markers on Image

Pylon Viewer allows the user to overlay two types of markers on the camera image that could help in aligning or setting the camera orientation.

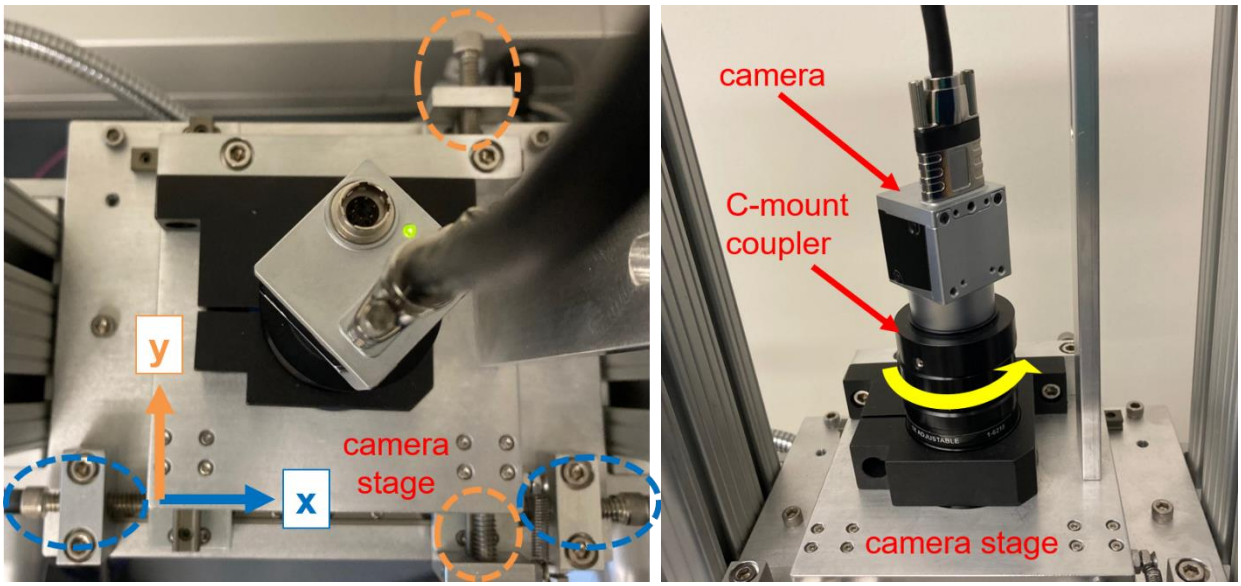
Press either a) the "Show/Hide Crosshair" button (red square in the screenshot below) in the top toolbar to display the crosshair marker on the middle of image; or b) the "Show/Hide Grid" button (cyan square) in the top toolbar to display the grid in the image display area.



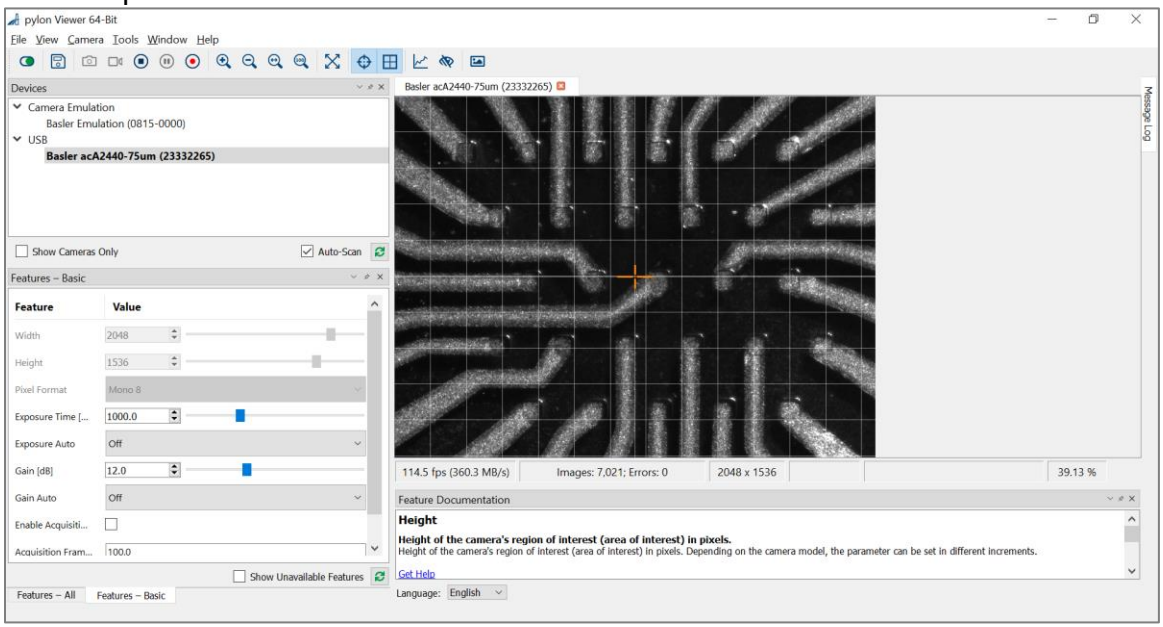
Note: Both markers, i.e., the crosshair and the grid, can be displayed simultaneously in the image display area. This is shown in step 12 below.

11) Image Centering and Orientation

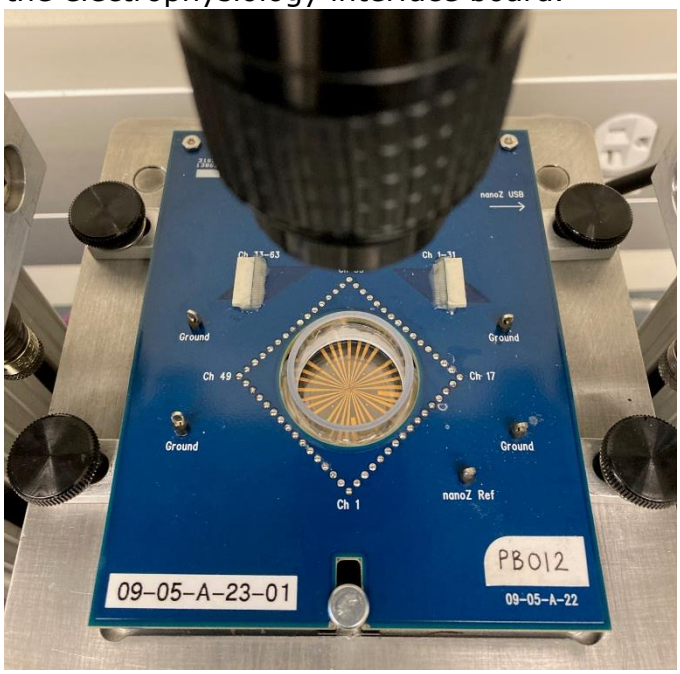
To center the image, use the display marker on the image and adjust the position of the camera xy-stage. The image on the left shows a top view of the camera and where to adjust the xy-stage position by tightening/loosening the screws inside the dashed circles. The image on the right below shows a side view of the camera on the imaging stage. If the camera orientation needs to be adjusted, loosen the three set screws on the C-mount coupler (PN 1-6010) which connect the camera to the adapter tube. Once loose, rotate the C-mount coupler (as shown by the yellow arrow) to re-orient the camera. Once the orientation has been readjusted, tighten the three set screws on the C-mount coupler.



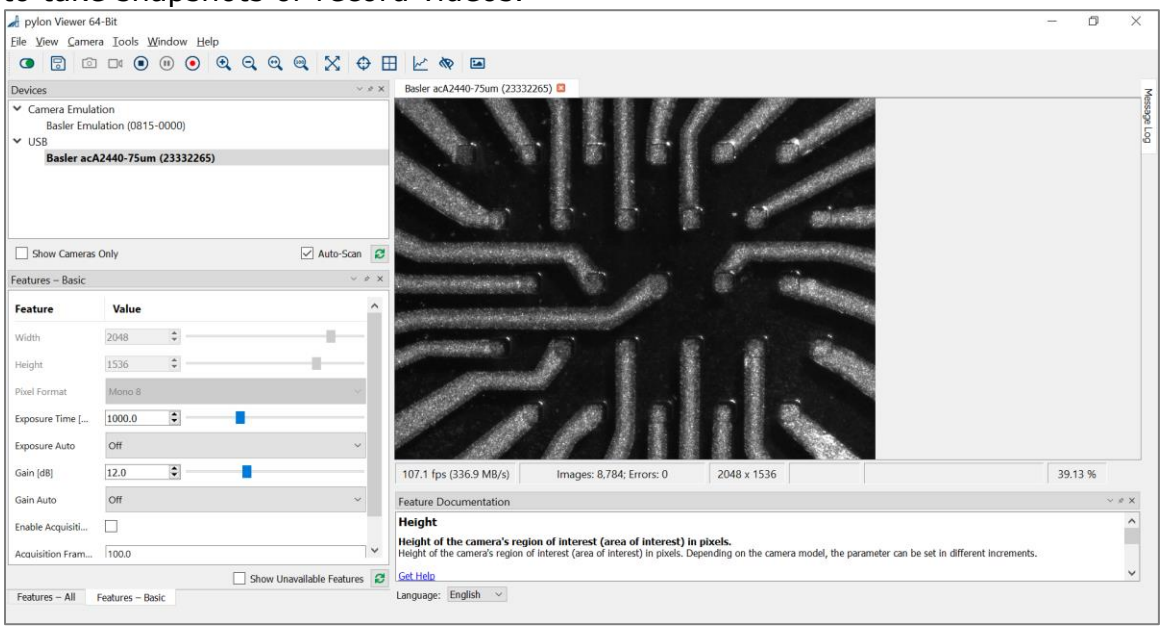
Once the camera has been centered on the sample, the captured image should appear as in the screenshot below. Note how the crosshair and grid can help when adjusting the camera position and orientation.



Note that the camera is oriented diagonally to mimic the orientation of the sMEA loaded in the electrophysiology interface board.



12) The camera image is now set with the desired parameters (i.e., resolution, exposure time, gain, recording frame rate) and is centered on the area of interest. The camera is ready to take snapshots or record videos.



4 Protocol to Save Snapshots

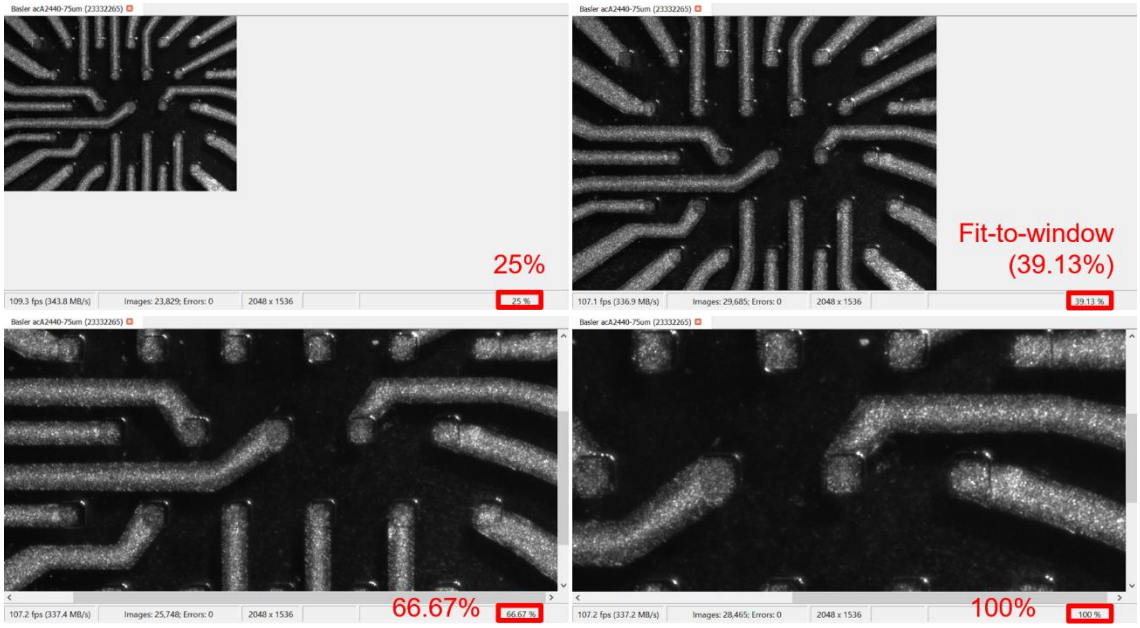
Pylon Viewer allows the user to take and save snapshots.

1) Once the software has loaded, select "Basler acA2440-75um" under USB, and click the "Open/Close Device" button on the top left corner of the top toolbar.

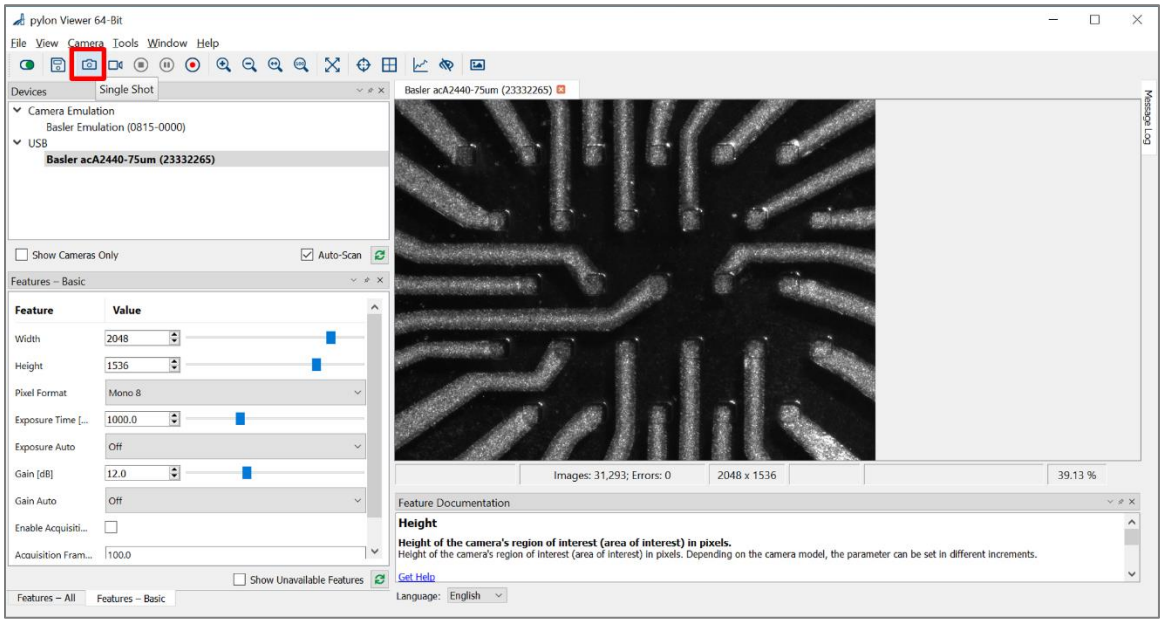
2) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for a live view from the Basler camera.

3) Perform the **initial setup of the camera** as described in **Section 3** of this document.

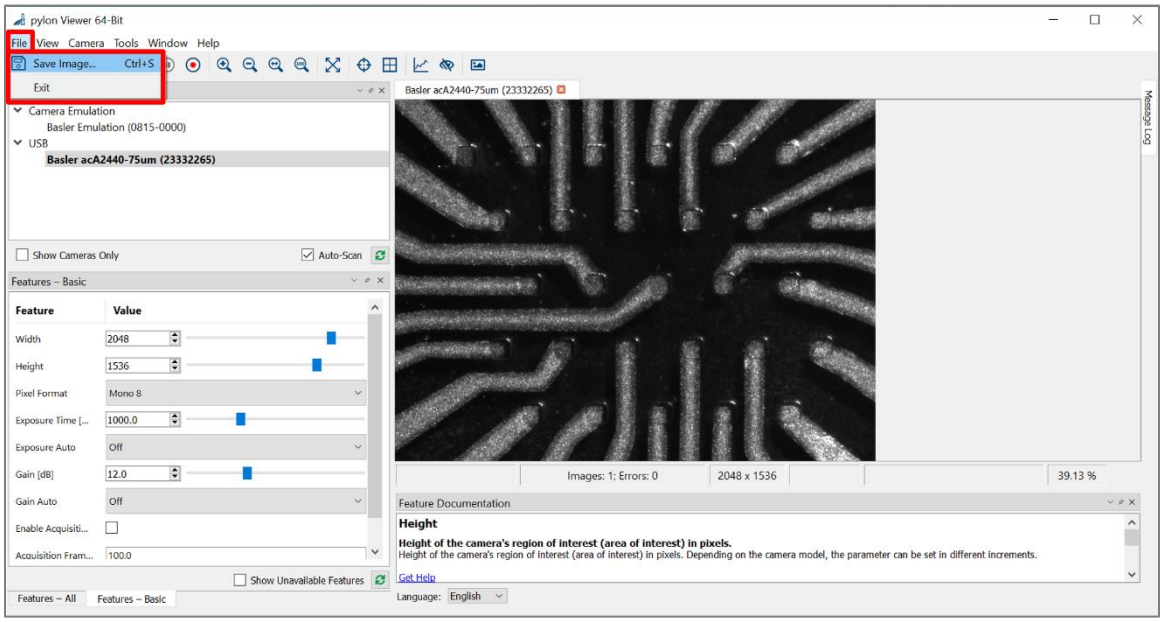
4) Once the camera and software have been set, adjust the software magnification if necessary.
The screenshots below show the same camera image at various software zoom levels.



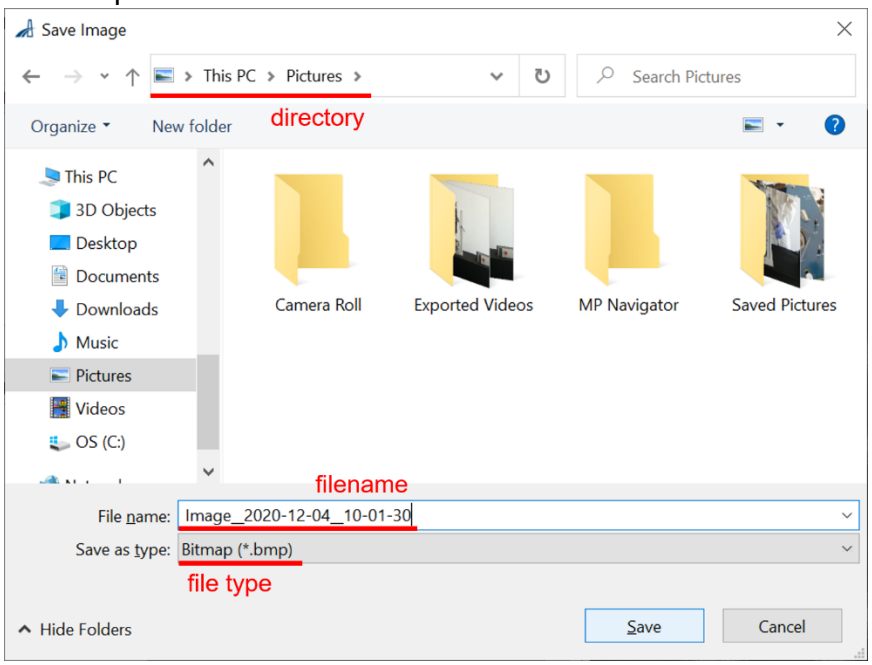
5) Click the "Single Shot" button (i.e., camera icon) on the top left corner of the top toolbar for the software to take snapshot.



6) On the menu bar, click "File" and then click "Save Image…"



7) In the "Save Image" pop-up window, specify the directory, file type, and filename for the snapshot.



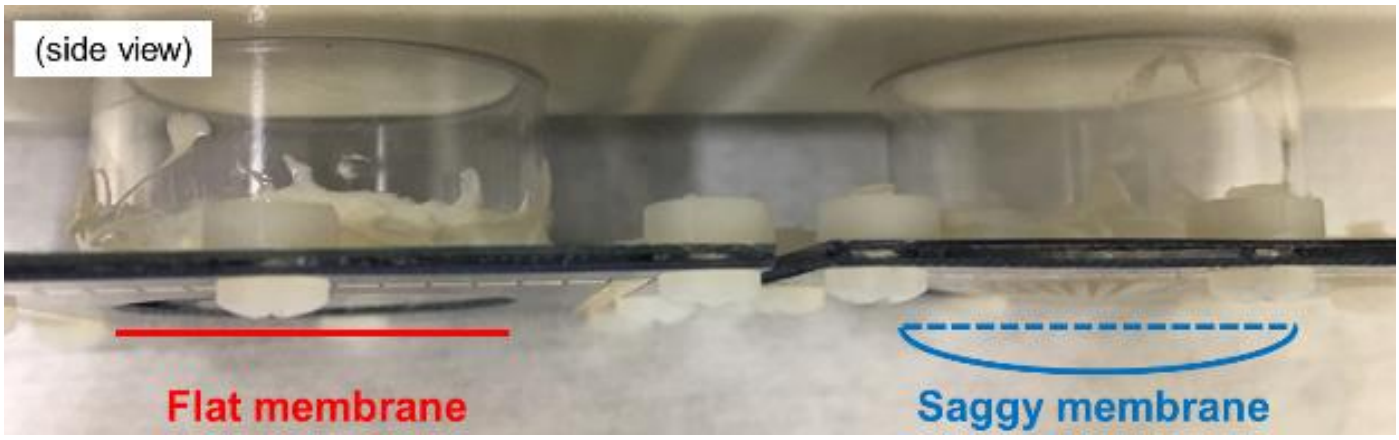
8) The snapshot should now be saved in the specified folder.

5 Protocol to Find the Flush Position

The flush position is the position at which:

a. the sample and the indenter are in direct contact;
b. the sample is at 0% strain, and where any movement of the sample in the negative z-direction (i.e., towards the indenter) would result in a non-zero strain; and
c. the focus will remain constant as strain is applied to the sample.

The latter two conditions are especially important since some sMEAs might have a little sag in their PDMS membrane. This bulging could be a result from previous stretching of the sample or fabrication variability.



BMSEED provides aluminum blocks with calibrated heights to be used for homing the VCA and to aid in finding the flush position. For **MEASSuRE-X and MEASSuRE-Premium** systems, the aluminum homing blocks have a **calibrated height of 1 inch**. For **MEASSuRE-Mini** systems, the aluminum homing blocks have a **calibrated height of 22 mm**.

Use the calculations in **Appendix C to get an initial estimate of the flush position. It is highly recommended to verify the flush position using the voice coil actuator (VCA) and camera** to avoid any discrepancy between the desired applied strain and the actual measured strain. In addition, the VCA and camera can be used to find a more accurate flush position.

For the following protocol to **verify or find the flush position**, use MotionLab to control the actuator and Pylon Viewer to monitor the sample.

1) Once the Pylon Viewer software has loaded, select "Basler acA2440-75um" under USB, and click the "Open/Close Device" button on the top left corner of the top toolbar.
2) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for a live view from the Basler camera.
3) Perform the **initial setup of the camera** as described **in Section 3** of this document to adjust the camera position, zoom, focus, and lighting to obtain a clear image of the area of interest.
4) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for the software to start the camera live view.

5) Using the computer connected to the voice coil actuator, open the MotionLab software and home the VCA.

   i. Open the MotionLab software.

   ii. Select the "Jupiter" or "Pluto" drive depending on which drive is being used.

   iii. Click on "Motion" to open the Motion sub-window.

   iv. Click on the "Homing" tab.

   v. Set the homing method to "[-3] Negative mechanical limit" and click on the [Enable Motor] button, to set the reference frame. The actual position should be close to 0 µm.

   vi. Click on the [Disable Motor] button to finish homing the VCA.

   IMPORTANT: Refer to **Mechanics Module Manual** for further details on how to operate the VCA.

6) In MotionLab, click on the "Position" tab and set the manual increment to 100 µm. The software might automatically change it to a nearby value (depending on the conversion between "counts" and microns, as set by the "stroke" motor parameter).

7) Click on the [Enable Motor] button.

8) Click on the [+ Target Position] button five times to raise the platform holding the sample by ~500 µm.

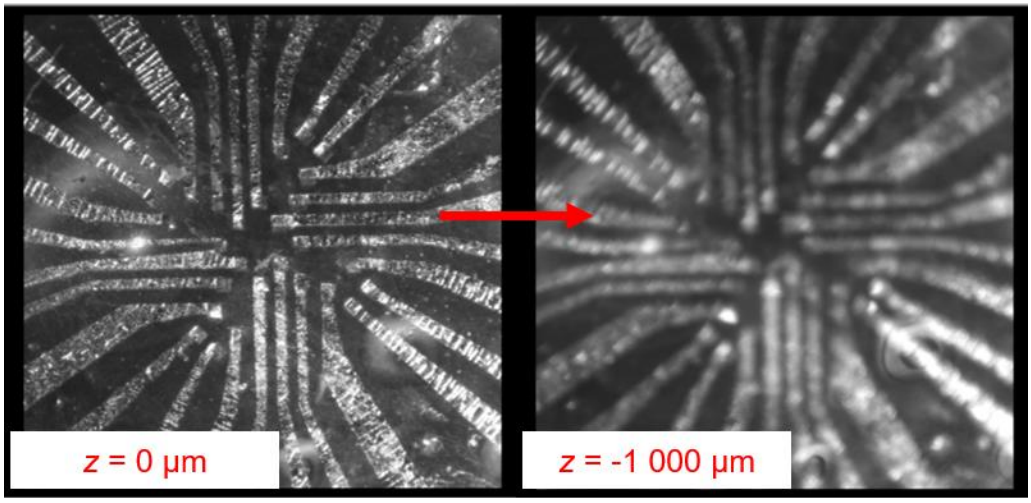
9) Remove the aluminum homing block underneath the platform.

10) Click on the [- Target Position] button five times to lower the platform back to ~0 µm.

At this point, we are ready to find the flush position. To determine the flush position, there must be no need to re-adjust the focus as you lower the platform holding the sample and the indenter stretches the PDMS membrane.

11) Lower the sample by 1000 µm by entering a value of "-1000" in the target position (in µm) or pressing the [- Target Position] button ten times.

12) Compare the 'current' image (i.e. at the position prior to lowering the platform) and the 'lowered' image (i.e. at the position post lowering the platform by 1000 µm).

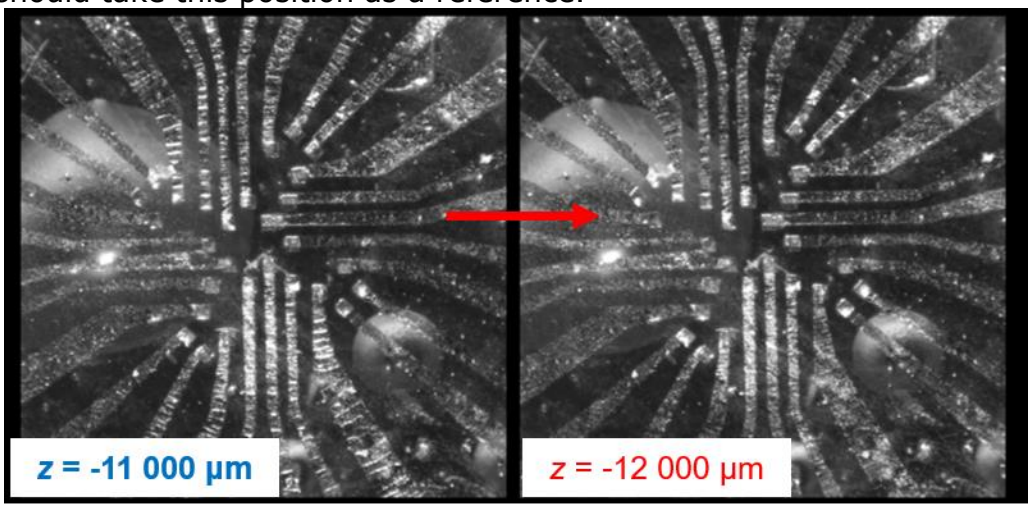


13) If the lowered image gets out of focus, the position of the current image is not the flush position. Thus, you should do the following:

   i. Lower the platform from the current position by 100, 200, or 300 µm. You can decide this offset based on how much worse the focus gets. For example, if the focus changes quite a bit, a larger shift/correction might be more efficient.
   ii. Treat this as the new 'current' position/image.
   iii. Re-focus the image.
   iv. Repeat steps 8 and 9.

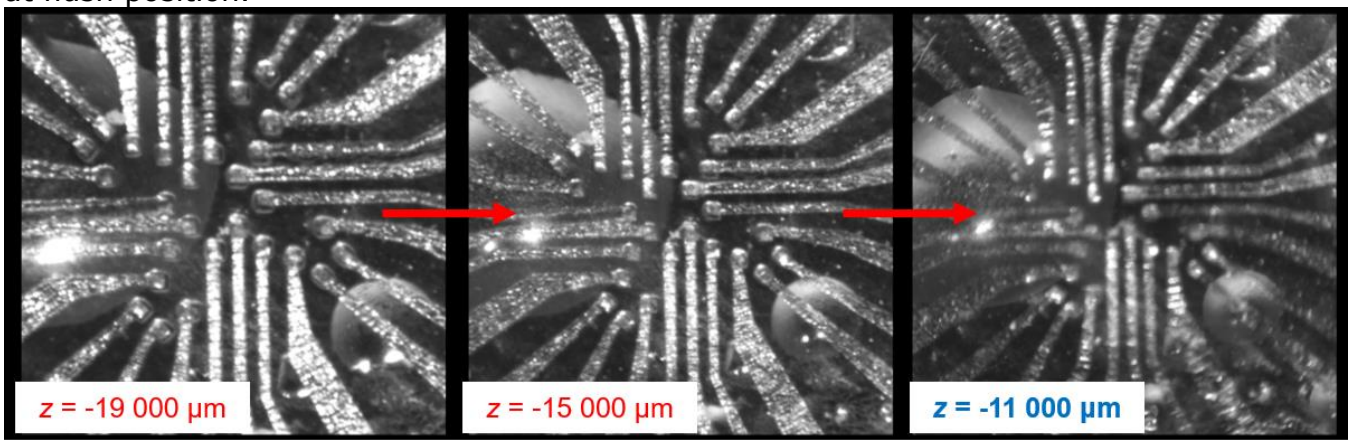
   If the lowered image remains in focus, proceed to step 11.

14) **The position of the current image is the flush position.** Take note of this position as this represents the position for 0% strain, and further displacements of the VCA should take this position as a reference.



For instance, in our example the flush position was determined to be z = -11000 µm. In this example, we were using the "no Ephys module" configuration, a 33.1 mm tall indenter, and supporting rods that were 85.80 mm tall.

The following images show the camera image with the sequence: 8 mm below the flush position, 4 mm below flush (releasing strain from the previous image), and back at flush position.



Note: See **Appendices D** and **E** for two additional examples on how to determine the flush position.

6 Protocol to Record Videos

6.1 Setting Up the VCA prior to Video Recording

Set the desired motion of the VCA and determine the **flush position** as shown in the manual for the Mechanics Module. **Any prescribed motions of the VCA should be based on this flush position**. Adjust the VCA parameters accordingly. The two main types of mechanical stimulations are:

a. Impulse stretching: The platform moves quickly to a target position and then back again to the flush position. The user can specify the target position, profile parameters (i.e. velocity, acceleration, deceleration), and controller parameters (i.e. PID gains). Typically, this type of stimulation is 'very fast' and runs once for a very short period of time (e.g. <100 ms).
b. Cyclic stretching: The platform moves following a sinusoidal profile. The user can specify the baseline position, amplitude, and frequency of the oscillations. Typically, this type of stimulation is 'slow' but runs for longer periods of time, ranging from a few hours to days.

The user may control the VCA through the MotionLab GUI or pre-programmed macros.

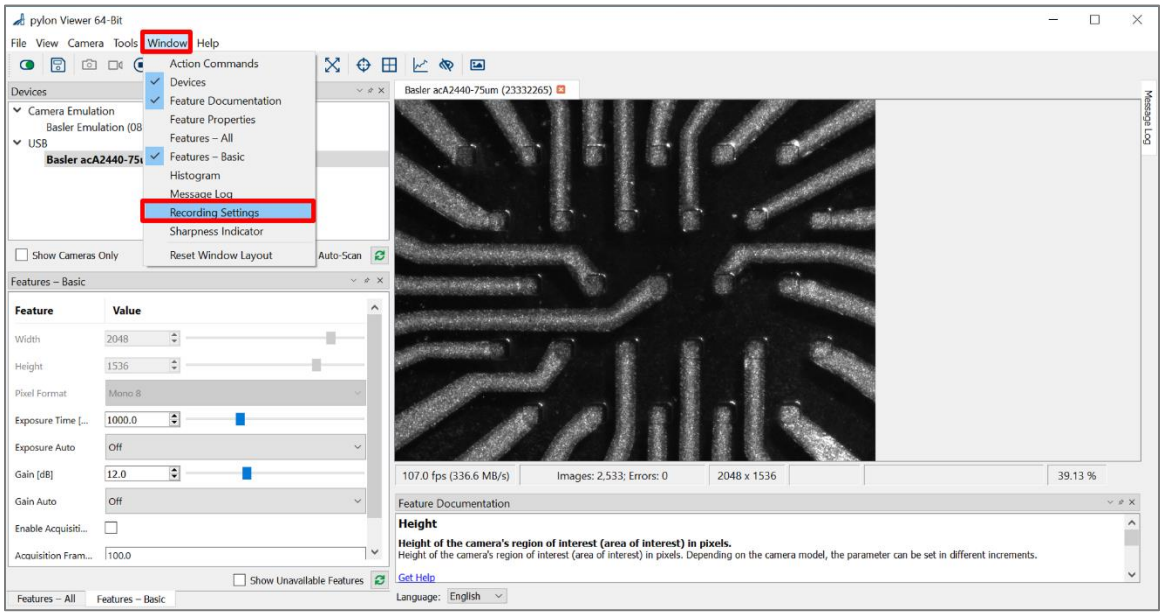
6.2 Using Pylon Viewer to Record Videos

Once the VCA has been set to apply the desired mechanical stimulus, a video can be recorded using Pylon Viewer.

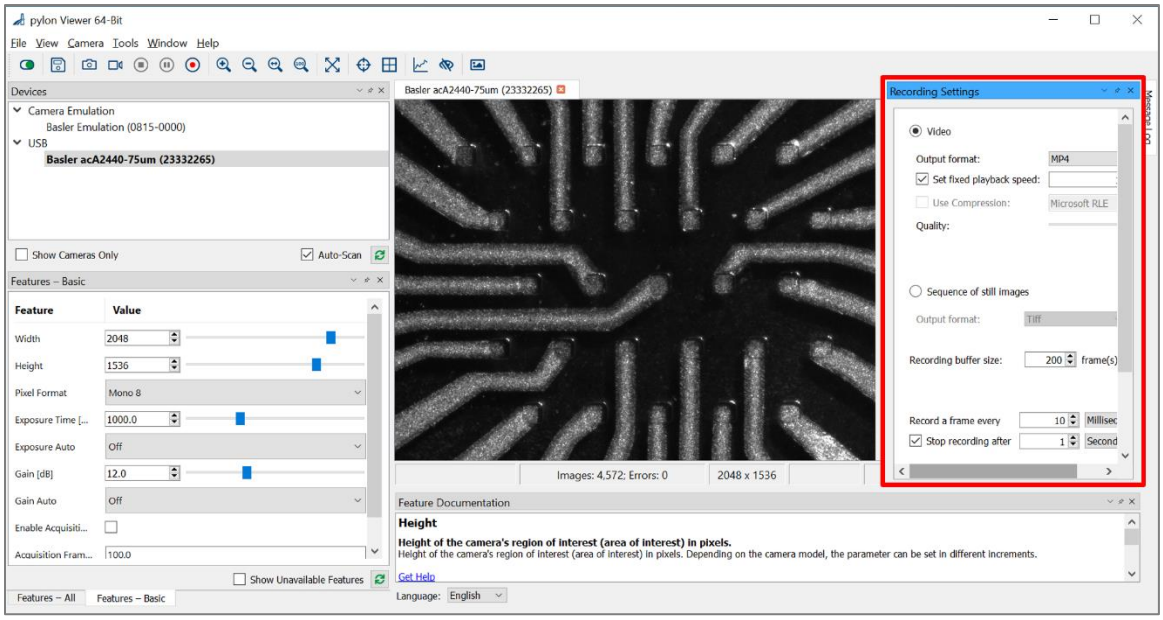
It is highly recommended to perform one or multiple test runs to make sure that the area of interest is always captured by the camera and displayed in the Pylon Viewer window. If not, make sure to make any adjustments with the movable stage and lens zoom control.

1) Once the Pylon Viewer software has been loaded, select "Basler acA2440-75um" under USB, and click the "Open/Close Device" button on the top left corner of the top toolbar.
2) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for a live view from the Basler camera.
3) Perform the **initial setup of the camera** as described **in Section 3** of this document to adjust the camera position, zoom, focus, and lighting to obtain a clear image of the area of interest.
4) Click the "Continuous Shot" button (i.e., video recorder icon) on the top left corner of the top toolbar for the software to start the camera live view.

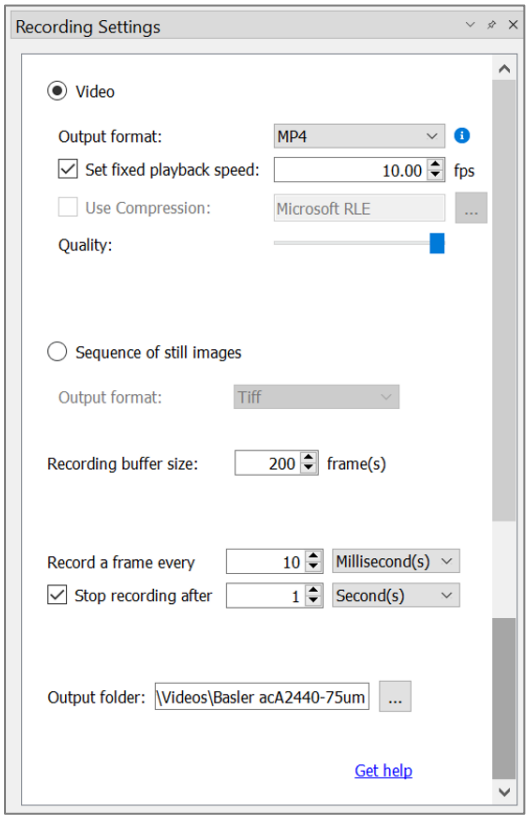
5) On the menu bar, click “Window” and then click “Recording Settings.”



The “Recording Settings” pane will open.



6) Set the recording parameters.



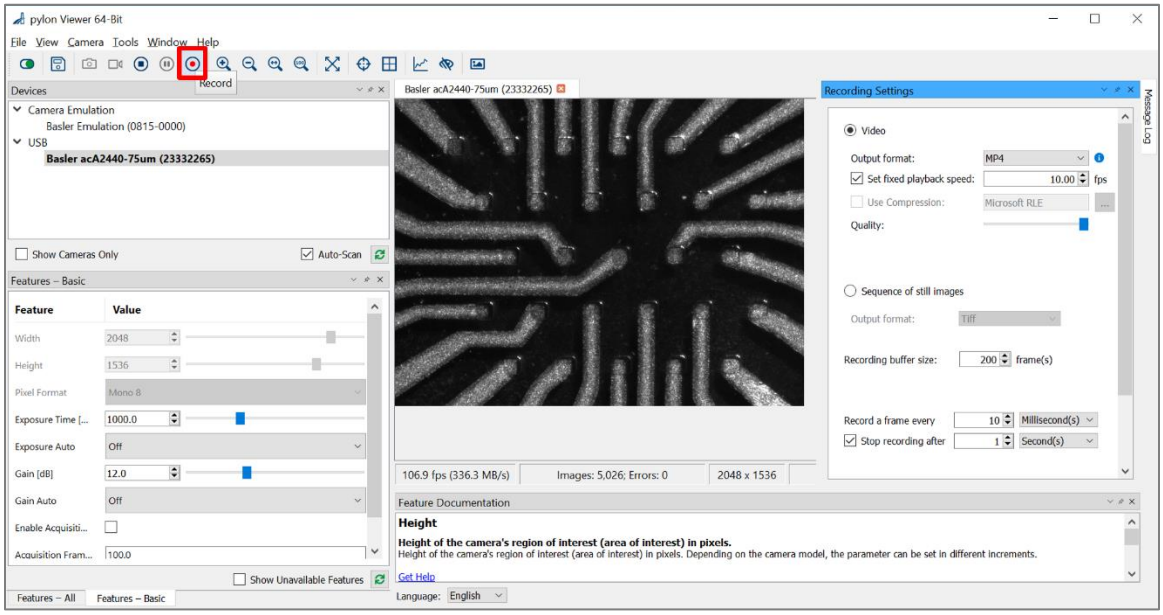
The recording can be saved as either a video or sequence of still images. The parameters for each file type are listed here.

- Video: - output format: either MP4 or AVI
  Note: AVI files allow the user to record without compression, resulting in high quality, lossless videos. However, resulting video clips can be large (i.e. tens or hundreds of MB). Keep in mind that avi files size is limited to 2GB. MP4 files are always compressed resulting in loss of detail but much smaller files.
  - set fixed playback speed: in frames per second (fps);
  Note: if this option is not enabled, the clip will be played at the same speed that it had been recorded
  - use compression: only available when saving as .avi
  - quality: only available when saving as .avi
- Sequence of still images: - output format: either bitmap or tiff

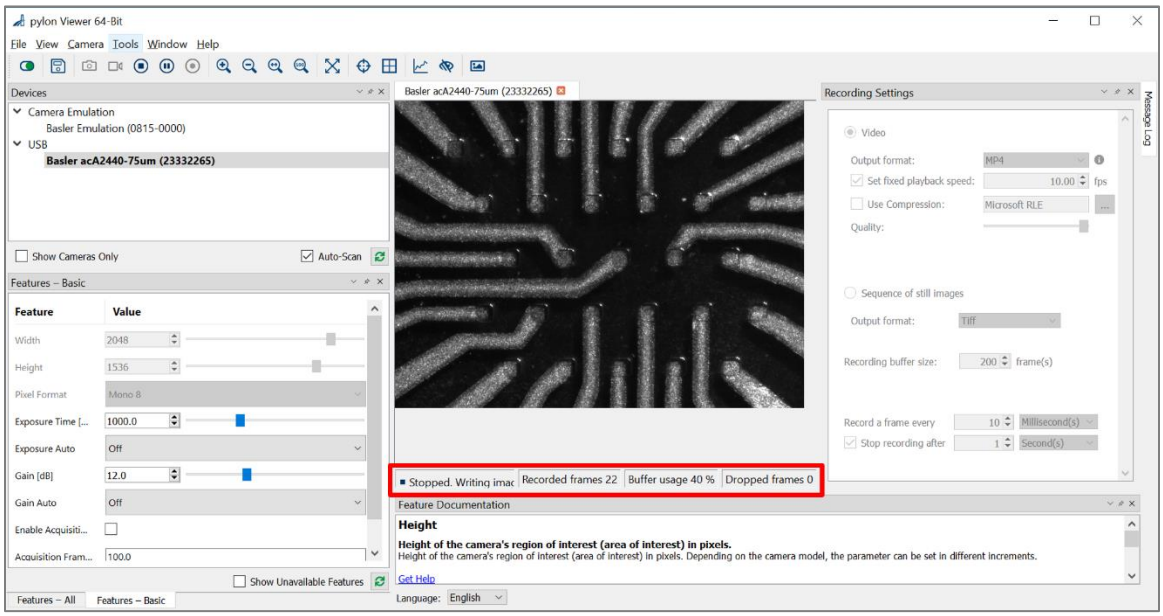
Other recording parameters, regardless of the type of output file, are:

- Recording buffer size: The number of images stored in the buffer during recording. The image buffer acts like a cache to store images if they cannot be read out of the camera quickly enough.
- Record a frame every …: Rate at which frames are recorded.
- Stop recording after …: To stop a recording after a certain period of time has passed or a certain number of frames have been recorded.
- Output folder: Location where resulting video clips and images will be saved to.

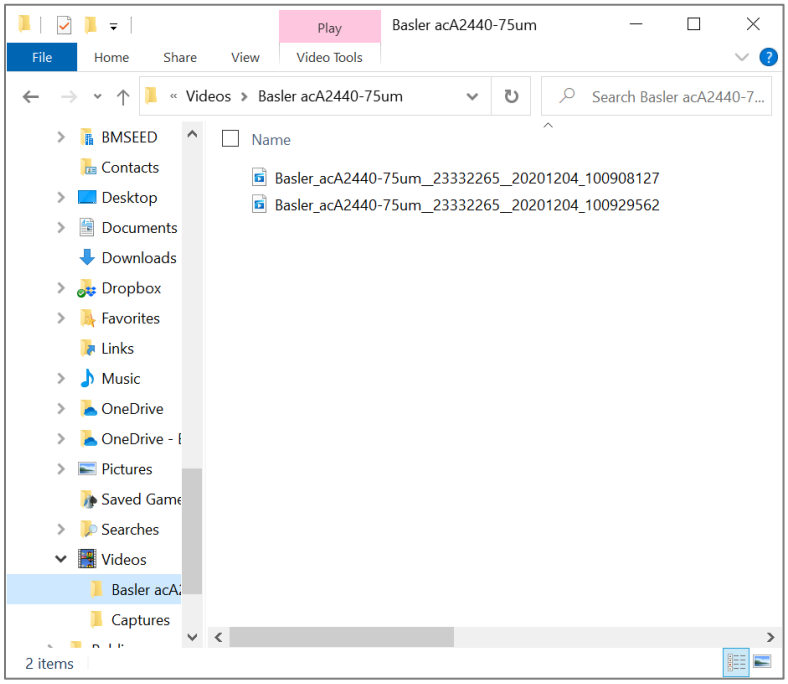
7) Once the recording parameters have been set, click the "Record" button on the top toolbar.



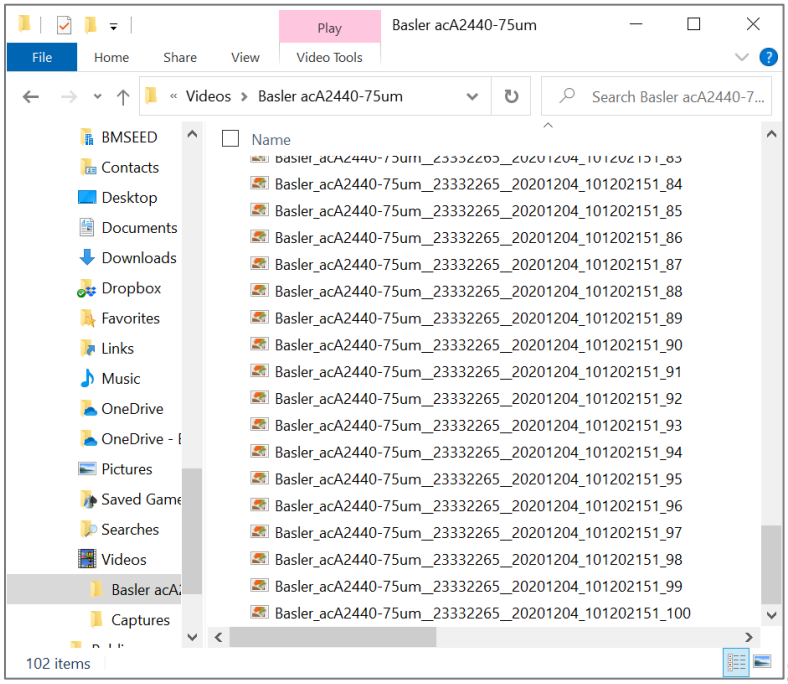
The video or sequence of images will start recording. The status bar in the image display area will show how much of the buffer is being used.



8) The recording has been saved and are ready to be viewed. The saved files can be found in the folder specified in step 6 above ("output folder" parameter).



MP4 recordings



Sequence of still images

7 Protocol to Extract Frames from Video

If a sequence of still images was recorded, there is no need to extract frames from videos. Simply select the frame that shows the sMEA at flush position (i.e., zero strain) and at the maximum applied strain.

If a video was recorded, you may use software such as **Motion Studio** or **VirtualDub**. The latter one is a free software that can be downloaded at https://sourceforge.net/projects/virtualdub/.

8 Protocol to Measure Strain from Images

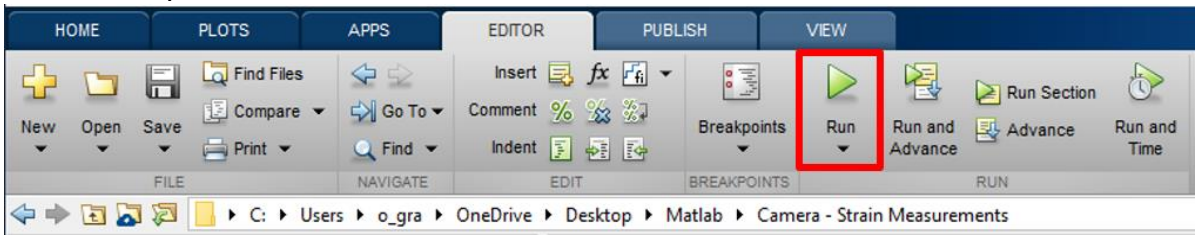
Strain is measured from images using **MATLAB**.

8.1 Running the MATLAB Code

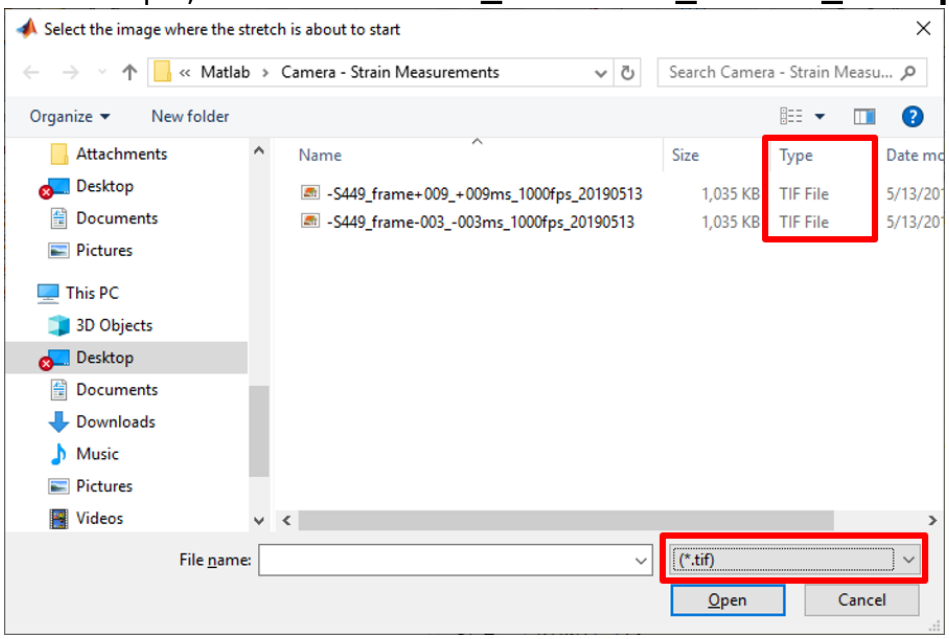
BMSEED provides Matlab code (originally created by the Neurotrauma and Repair Lab at Columbia University) to perform image analysis and measured applied strain.

1) Open the Matlab m-file with the code to calculate the strain between two images. **Contact BMSEED for the latest version of the strain calculations m-file.**

2) In the Editor tab, click the "Run" button. Alternatively, you may click anywhere in the Editor and press F5.

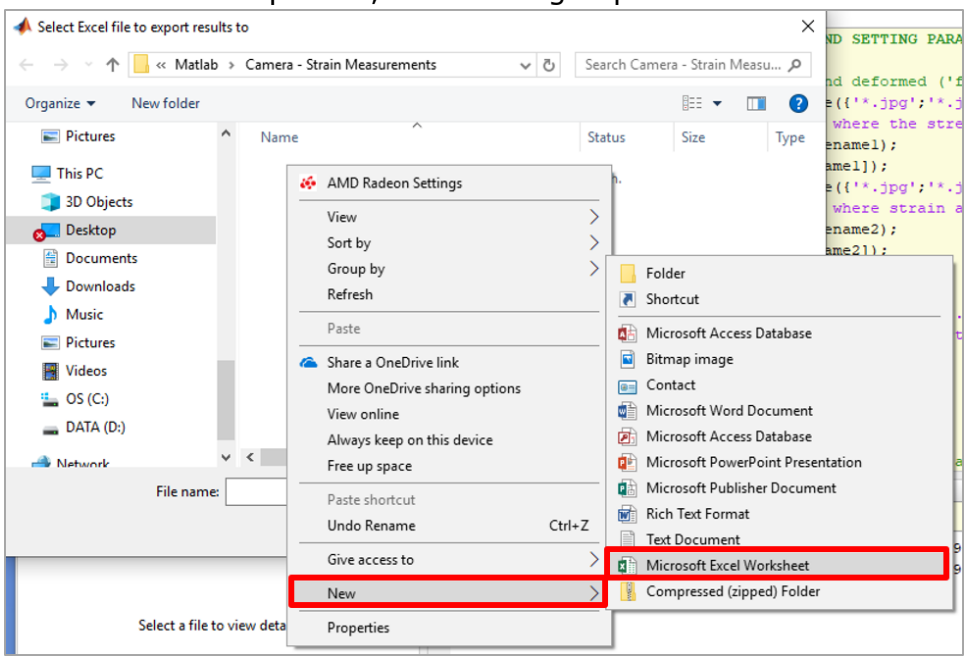


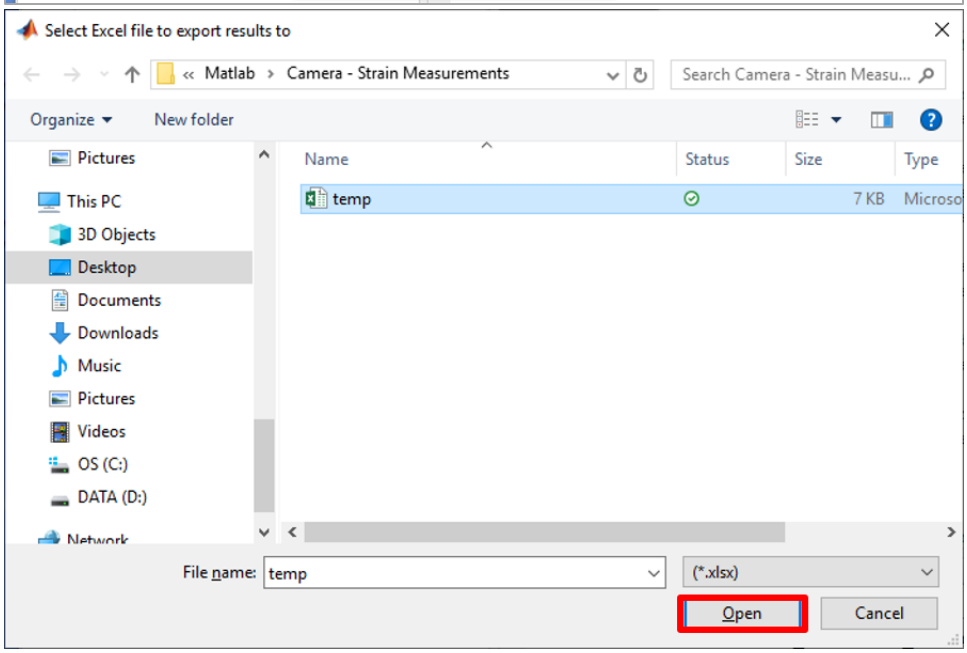
3) A pop-up window will ask to select the image where the stretch just begins. Specify the extension of the image file (e.g. tif, png, jpeg) that matches the extension of the image file of interest. Select the image and click "Open." Once an image has been selected, the window will close. The Command Window will display the name of the selected file.
For example, select file: "**-S449_frame-003_-003ms_1000fps_20190513.tif**".



4) A pop-up window will ask to select the image where strain appears to be at its maximum. Specify the extension of the image file (e.g. tif, png, jpeg) that matches the extension of the image file of interest. Select the image and click "Open." Once an image has been selected, the window will close. The name of the selected file will be displayed.
For example, select file "**-S449_frame+009_+009ms_1000fps_20190513.tif**"

5) A pop-up window will ask to select the Excel spreadsheet to export the results to. Select the corresponding spreadsheet and click "Open." Once a spreadsheet has been selected, the window will close. The Command Window will display the name of the file.
For example, select file: "**temp.xlsx**".
Note: If such Excel file does not exist, you may create it by: right clicking an empty space in the window; clicking "Microsoft Excel Worksheet" under the New menu; typing the name as "temp.xlsx"; and clicking "Open."





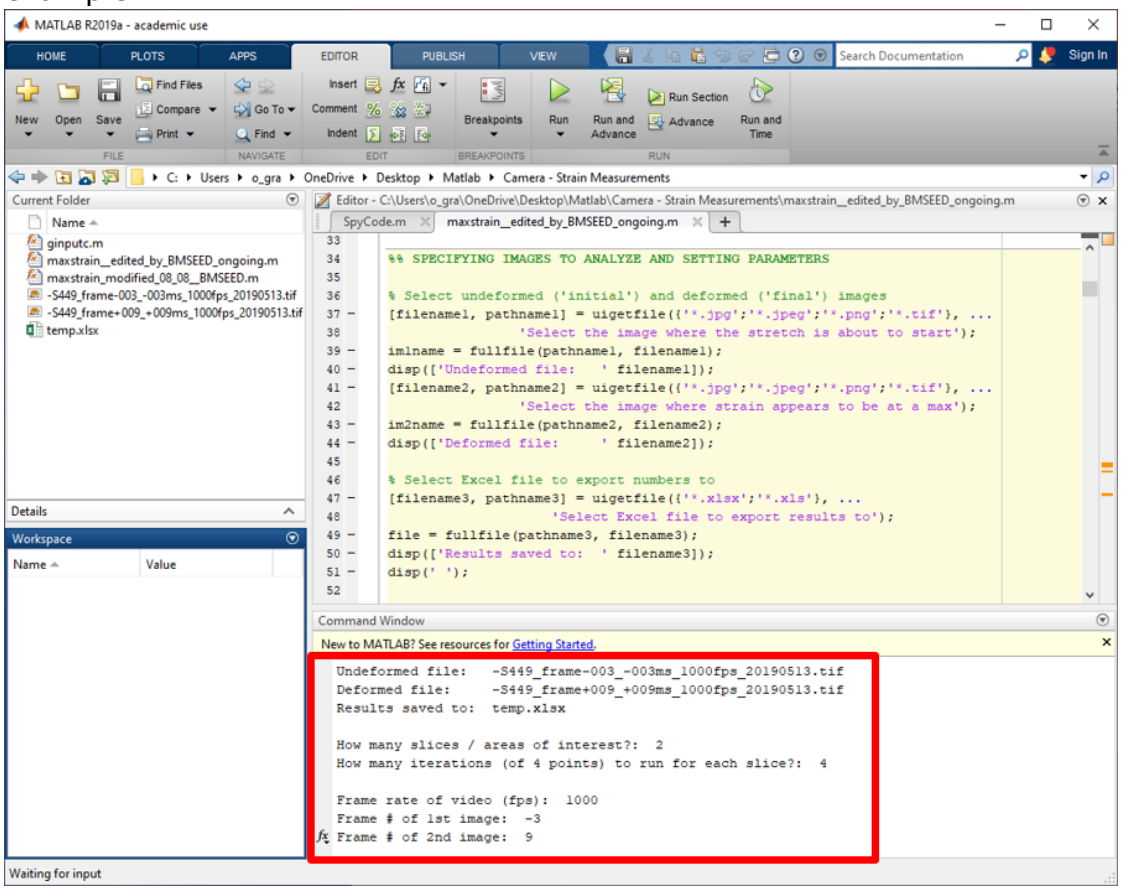
6) The program will ask you for the number of slices or areas of interest in the image. Type a number and press "Enter."
For example, input "**2**".

7) The program will ask you for the number of iterations to run per slice or area of interest. Type a number and press "Enter."
For example, input "**4**".

8) The program will ask you about the frame rate in frames per second (fps) of the video from where the images were extracted. This value will be used to calculate strain rate. Type the corresponding fps and press "Enter."
For example, input "**1000**" as specified by the image filenames in our example. The frame rate was defined under the acquisitions settings for the camera (described in section 4F, step 2 of this manual).

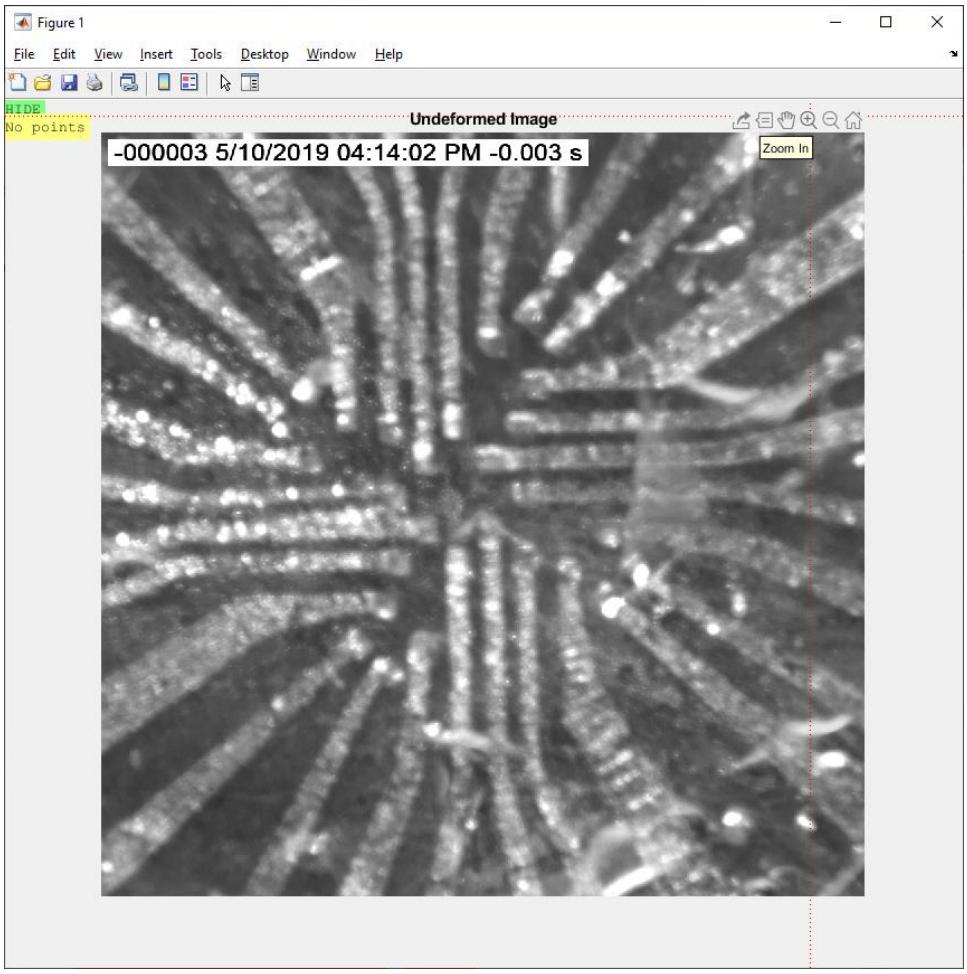
9) The program will ask you the frame # of the first image (i.e. right before stretch starts). This value will be used to calculate the number of frames elapsed, and thus, the strain rate. Type the corresponding frame number and press "Enter."
For example, input "**-3**" as specified by the filename of the first image in our example.

10) The program will ask you the frame # of the second image (i.e. at the maximum strain). This value will be used to calculate the number of frames elapsed, and thus, the strain rate. Type the corresponding frame number and press "Enter." The Command Window will now display the number of frames elapsed and the time elapsed.
For example, input "**9**" as specified by the filename of the second image in our example.



11) Matlab will now open the two images: undeformed image in Figure 1 and deformed image in Figure 2. Starting with Figure 1, locate a feature that is easy to track. Left-click it to select this point as Cartesian coordinates of Point #1 for the undeformed image.

- You can select the zoom-in ("+") or zoom-out ("-") function to zoom in or out, but you need to unselect this function before choosing the point on the image. You may find these options under the "Tools" menu.
- Similarly, you can use the "Pan" tool to move into selected areas of the figure. Unselect this function before choosing the point on the image.

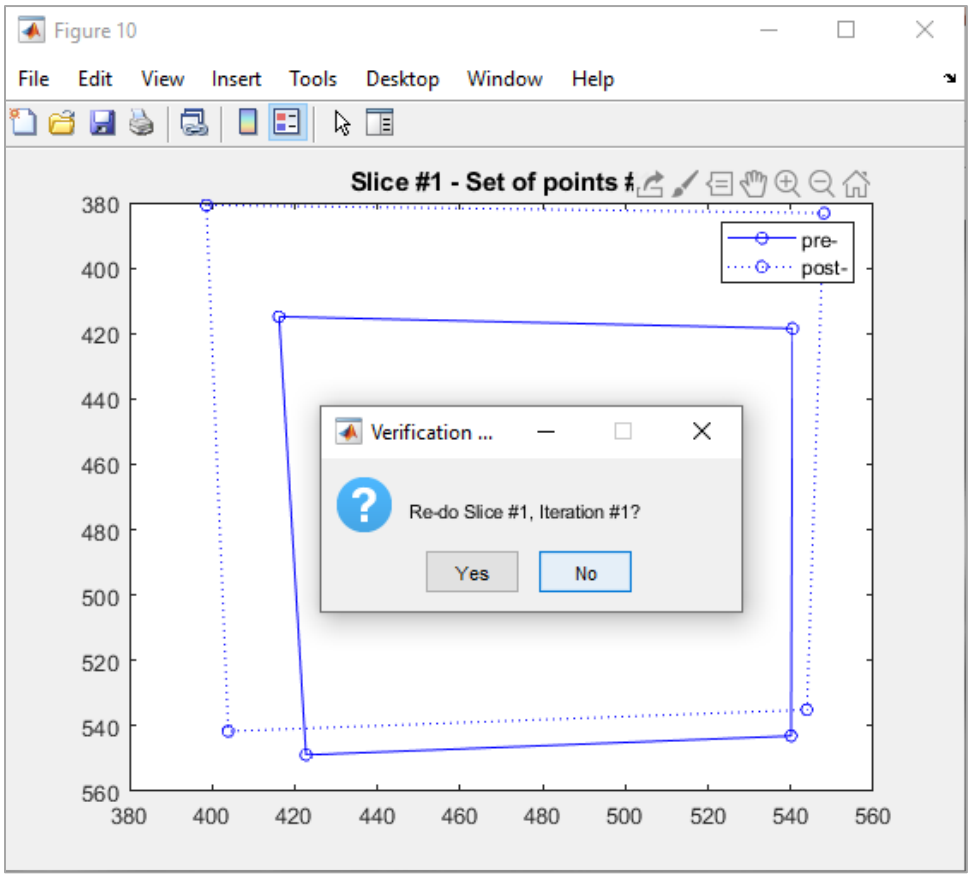


12) Locate the exact same feature in Figure 2 and left-click it to select this point as Cartesian coordinates of Point #1 for the deformed image.

13) Repeat steps 11 through 12 three more times to store x- and y-coordinates for Points #2 through #4 for both, the undeformed and deformed images.

14) The code will perform an automatic error check by verifying that the calculated strains in both directions (x- and y-) are positive. If a negative strain is calculated for either direction, the current iteration of selecting four data points in both images will be repeated since this should not be the case. A warning message will be displayed in the command window.
Note: If in the repetition of this iteration a calculated strain is negative once again, warning message will be displayed again, and the code will resume.

15) Once the iteration have passed the error check from the previous step, two windows will pop up. The first window will be a figure plotting the selected points of both images overlaid. This will allow the user to visualize how the selected points compare in both images.
The second window will ask the user if the current iteration should be repeated. The user will be given the chance to reselect the four points in both images if they believe they made a mistake when clicking on any of the points.



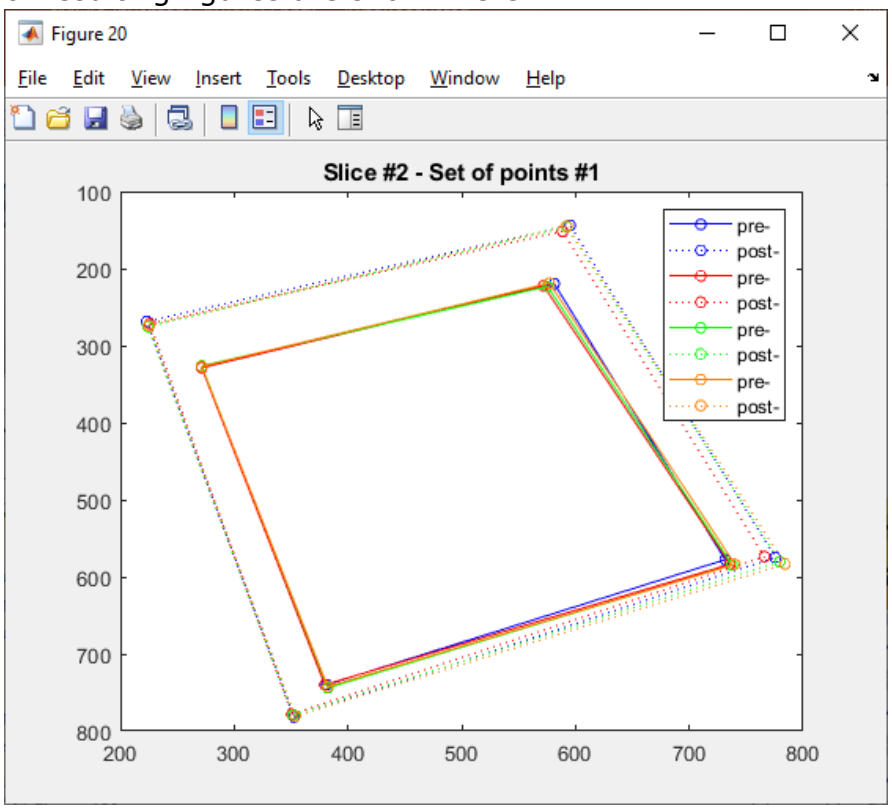
16) In this verification window you should click "Yes" or "No." If the window is closed or "Enter" is pressed, the program will interpret this as "No."

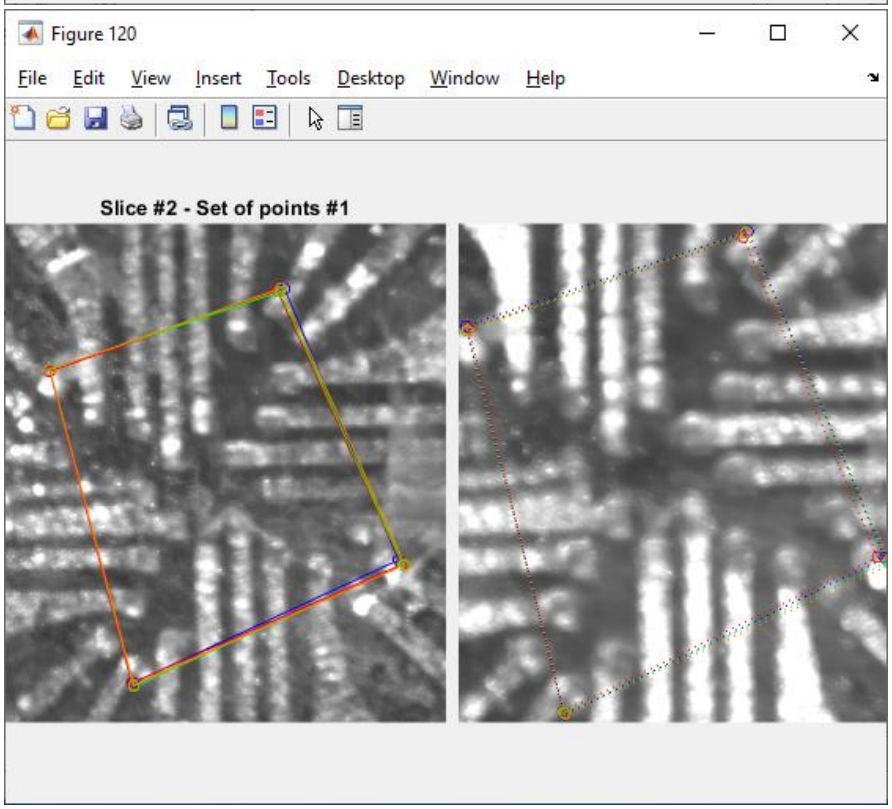
- Clicking on "Yes" means that the user wants to re-do the selection of four points for this iteration. Steps 11 to 15 will be repeated.
- Clicking on "No" means that the user is satisfied with the set of four points selected. Proceed to step 17.

17) If the number of iterations for this slice or area of interest has not been completed, repeat steps 11 through 16 for the remaining iterations. Otherwise, proceed to step 18.

18) Repeat the steps 11 through 17 for any additional slices or areas of interest that have yet to be completed.

19) Once all iterations for all slices have been completed, the program will end. Examples of resulting figures are shown here.



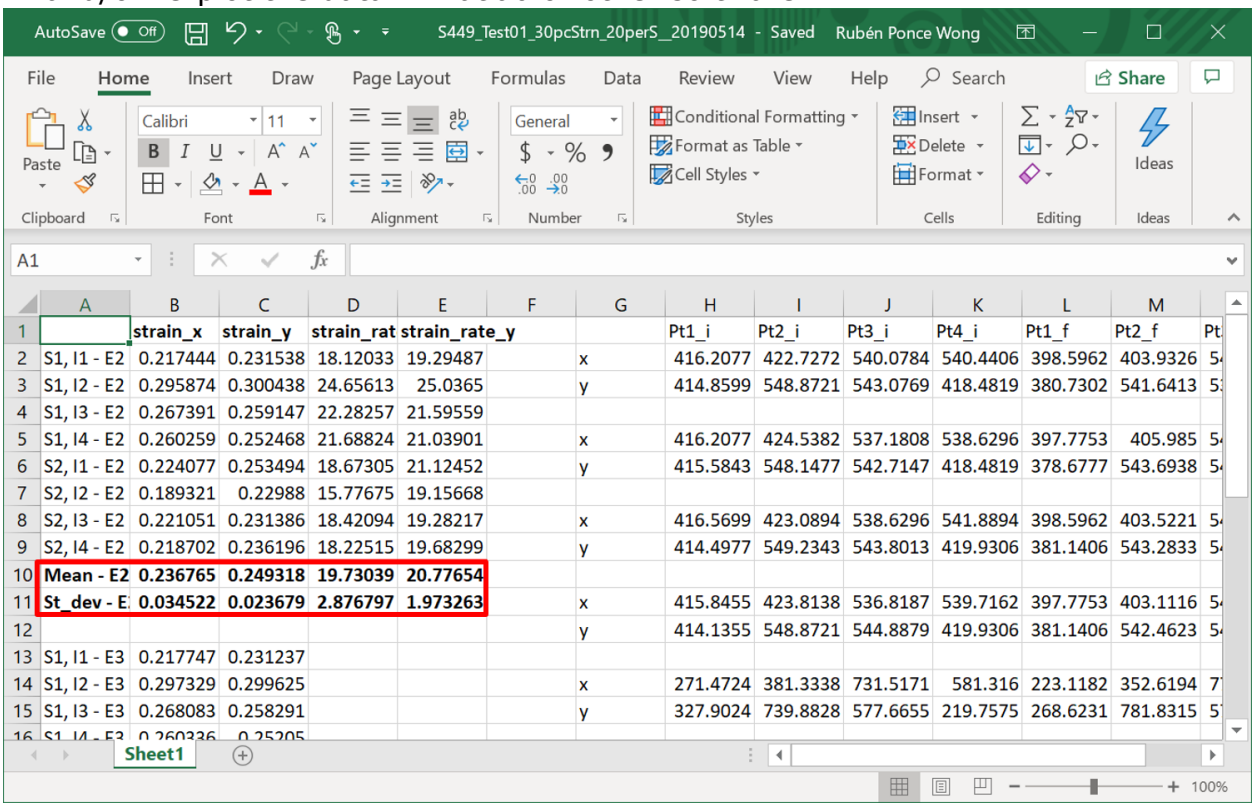


Note: Clicking on the Command Window and pressing "Ctrl-C" stops a program that is running.

8.2 Output from Matlab Code

1) **Excel spreadsheet:** The main output from running the Matlab code can be found in the specified spreadsheet (specified in **step 5 from Section 6.1** above). In this spreadsheet you will find results for each iteration of four points:

- **Method "E2"** to calculate strain: strains in the x- and y-directions; strain rates in the x- and y- directions.
- Method "E3" to calculate strain: strains in the x- and y-directions.
- Method "E4" to calculate strain: biaxial strain (method assumes biaxial strain, and no shear).
- Mean and standard deviations of the strain calculations for each of the three methods.
- Cartesian coordinates (x- and y-) for each of the four point for both the undeformed and deformed images. These values would allow us to re-calculate the strains and/or re-plot the data in Matlab or other software.



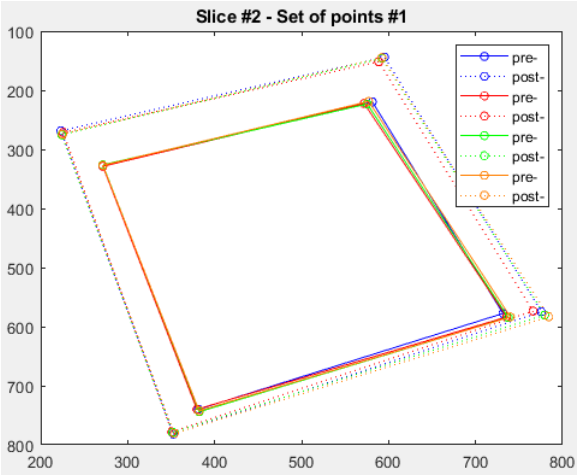
	A	B	C	D	E	F	G	H	I	J	K	L	M
1		strain_x	strain_y	strain_rat	strain_rate_y			Pt1_i	Pt2_i	Pt3_i	Pt4_i	Pt1_f	Pt2_f
2	S1, I1 - E2	0.217444	0.231538	18.12033	19.29487		x	416.2077	422.7272	540.0784	540.4406	398.5962	403.9326
3	S1, I2 - E2	0.295874	0.300438	24.65613	25.0365		y	414.8599	548.8721	543.0769	418.4819	380.7302	541.6413
4	S1, I3 - E2	0.267391	0.259147	22.28257	21.59559								
5	S1, I4 - E2	0.260259	0.252468	21.68824	21.03901		x	416.2077	424.5382	537.1808	538.6296	397.7753	405.985
6	S2, I1 - E2	0.224077	0.253494	18.67305	21.12452		y	415.5843	548.1477	542.7147	418.4819	378.6777	543.6938
7	S2, I2 - E2	0.189321	0.22988	15.77675	19.15668								
8	S2, I3 - E2	0.221051	0.231386	18.42094	19.28217		x	416.5699	423.0894	538.6296	541.8894	398.5962	403.5221
9	S2, I4 - E2	0.218702	0.236196	18.22515	19.68299		y	414.4977	549.2343	543.8013	419.9306	381.1406	543.2833
10	**Mean - E2**	**0.236765**	**0.249318**	**19.73039**	**20.77654**								
11	**St_dev - E**	**0.034522**	**0.023679**	**2.876797**	**1.973263**		x	415.8455	423.8138	536.8187	539.7162	397.7753	403.1116
12							y	414.1355	548.8721	544.8879	419.9306	381.1406	542.4623
13	S1, I1 - E3	0.217747	0.231237										
14	S1, I2 - E3	0.297329	0.299625				x	271.4724	381.3338	731.5171	581.316	223.1182	352.6194
15	S1, I3 - E3	0.268083	0.258291				y	327.9024	739.8828	577.6655	219.7575	268.6231	781.8315
16	S1, I4 - E3	0.260336	0.25205										

On the left columns of the spreadsheet, you will find the results grouped by method used to calculate strain. **The results (strain and strain rates for the x- and y-directions) provided by the top method, "E2", are the ones considered to be the strain measurement results**.

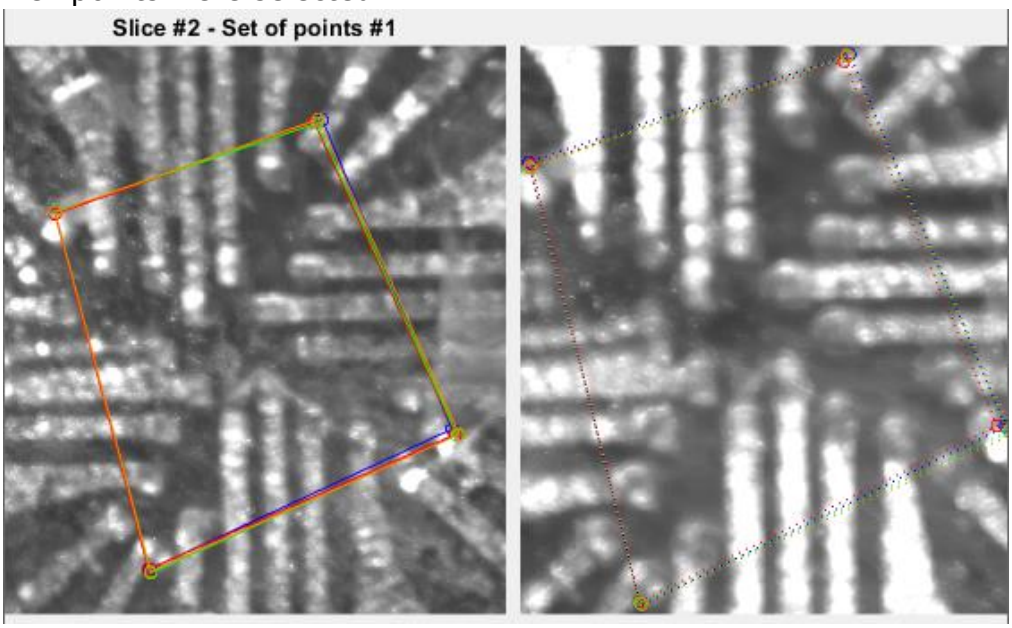
On the right columns of the spreadsheet, you will find the Cartesian coordinates of the four points for each iteration and for each slice/area of interest. For example, specifying 2 areas of interest and 4 iterations would result in eight sets of points.

2) **<u>Result figures</u>:** Figures 10's and 110's illustrate the selected points. These are saved as Matlab figures (".fig" extension) and as png or jpeg files.

- Figures in the 10's display in a single plot, the points selected for the undeformed and deformed images for the different slices or areas of interest. With these figures, you may compare how repeatable the selections of points were, as well have a general idea of the deformation of the membrane.



- Figures in the 110's show the two images side-by-side with the selected points for each iteration. Along with the previous figures, these allow the user to assess how well points were selected.



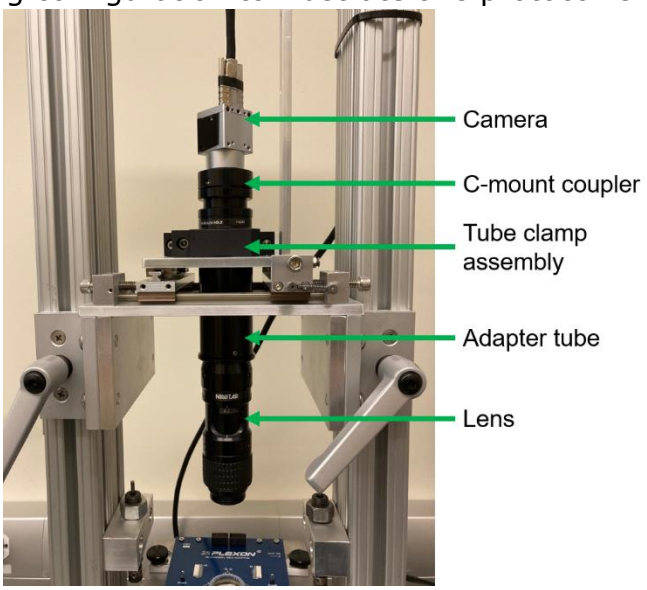
3) **<u>Matlab workspace variables</u>:** The Matlab (.mat) file allows the user to revisit results or perform further analysis using Matlab at a later time.

4) **<u>User input in Matlab</u>:** The Command Window allows the user to re-visit the inputs while the Matlab program was run.

<u>IMPORTANT</u>: If you want to save the obtained results, make sure to rename or move the Excel spreadsheet **"temp.xlsx"** resulting figures .mat files. Otherwise, the next time the code is run all files will be overwritten.

Appendix A: Protocol to Swap the Camera Lens and Adapter Tube on the Frame

This Appendix gives step-by-step instructions on how to replace the camera lens and camera adapter tube of the Imaging module of the MEASSuRE system. An example of a starting imaging configuration to illustrate this protocol is shown in the images below.



The specific imaging components used in this sample starting configuration are:

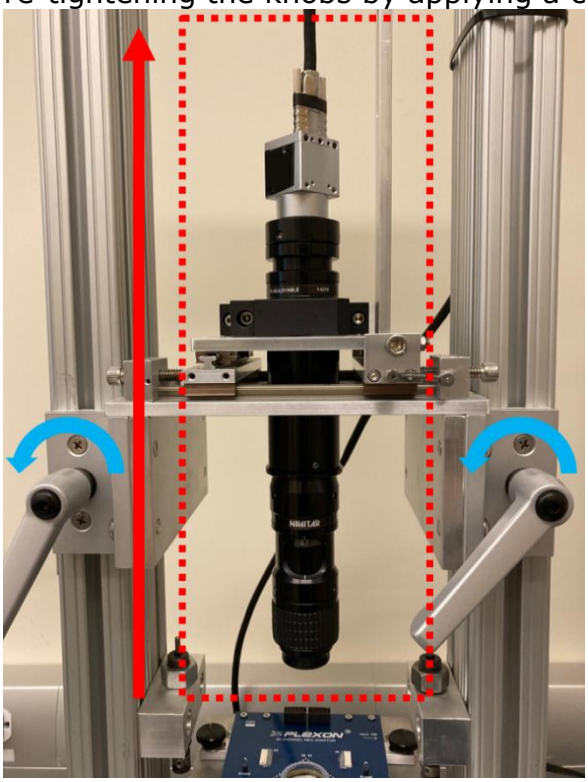
- Basler camera (acA2440 – 75µm with C-mount connector)
- C-mount coupler (Navitar 1-6010)
- 1× adjustable, standard adapter tube (Navitar 1-6218)
- Zoom 6000 lens (Navitar 1-60135)

The tube clamp assembly (Navitar 1-6270) is fixed to the MEASSuRE frame and is meant to always remain on the frame.

A.1 - Swapping the Camera Lens Only

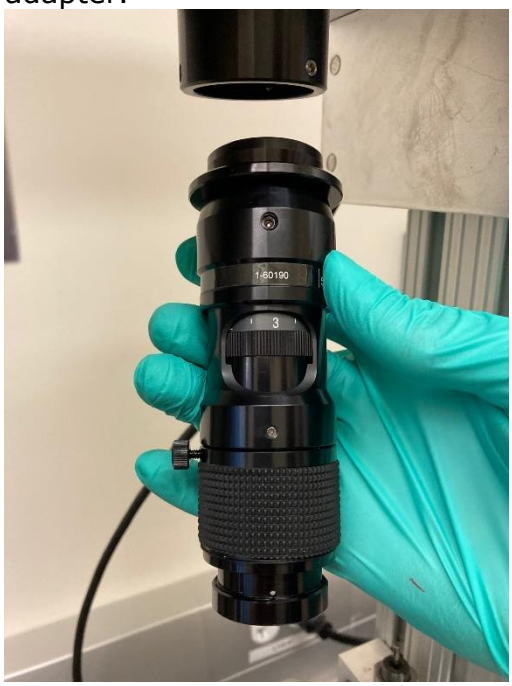
Here we show how to replace the Zoom 6000 lens with: an UltraZoom 6000 lens (Navitar 1-60190), a Mitutoyo objective coupler (3-60160), and a 10× Motic objective (1-62829) assembly. These components are shown, as listed, from left to right in the picture below.

1) Raise the camera stage assembly on the MEASSuRE frame by loosening the two knobs following a counter-clockwise motion (cyan), moving the camera platform up (red), and re-tightening the knobs by applying a clockwise motion.

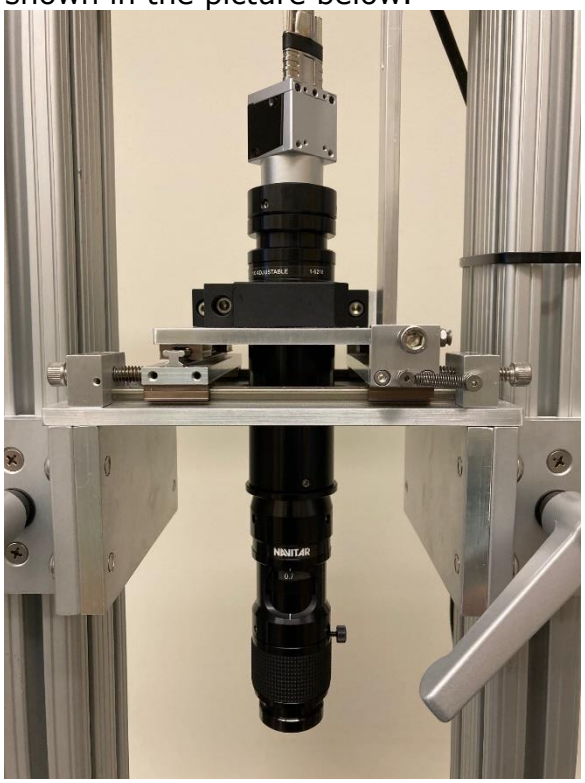


2) Loosen the three set screws (two of these shown in the red circles in the photo below) on the adapter that is securing the lens to the adapter and carefully remove the currently installed lens.
IMPORTANT: Make sure to always hold the lens with one of your hands while loosening the set screws.

3) Attach the replacement lens (in this example, Navitar UltraZoom 6000 lens) by inserting the lens into the adapter and tightening the three set screws at the bottom end of the adapter.



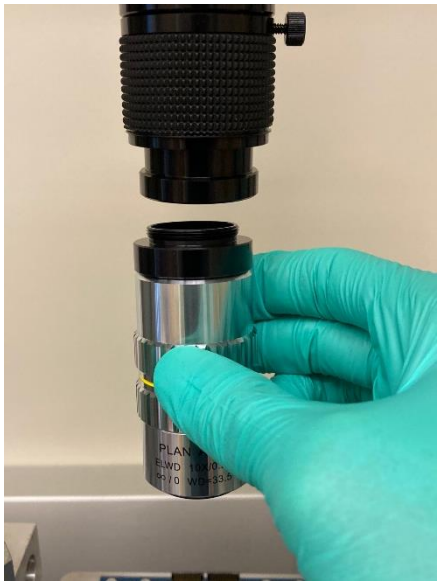
4) If no lens attachment will be mounted on the lens, the imaging assembly will be ready as shown in the picture below.

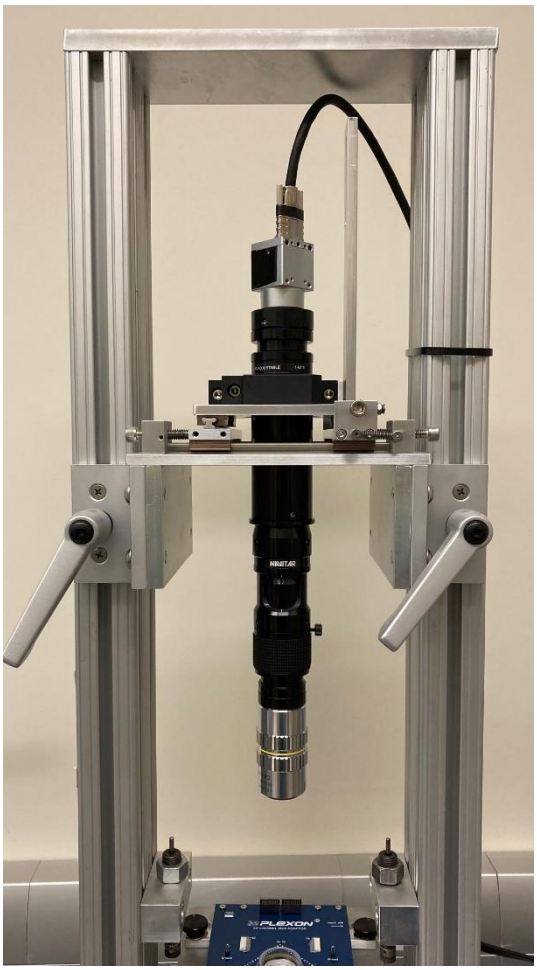


If a lens attachment will be mounted on the lens, proceed to the next step.
Note: Keep in mind that some lenses (e.g. the UltraZoom 6000 lens shown in this example) will only work with an objective attached.

5) For the UltraZoom 6000 lens, a coupler is needed to attach an objective lens. If this is the case, screw the objective coupler (in this example, Mitutoyo coupler) to the top of the objective lens (in this example, 10× Motic objective).

6) Screw the other end of the objective coupler to the bottom of the UltraZoom 6000 lens. The imaging setup is now ready to use. At this point, you will need to lower the camera stage (see **Appendix B** for working distances for different imaging hardware configurations) and re-focus on the sample.

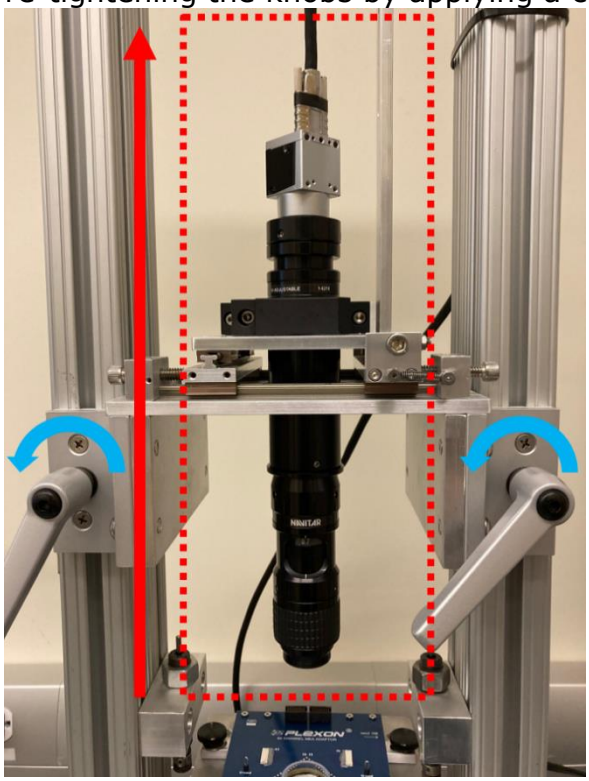




A.2 - Swapping the Camera Adapter Tube

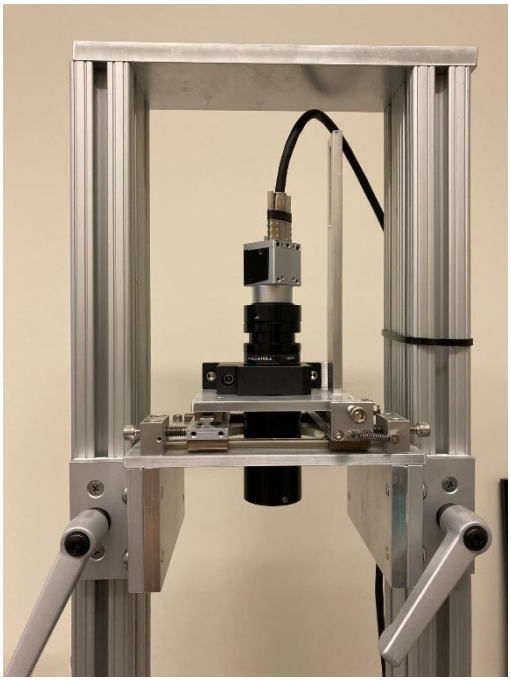
Here we show how to replace the 1× standard adapter with a 2× short adapter (1-6233). The two adapter tubes are shown side-by-side in the image below.

1) Raise the camera stage assembly on the MEASSuRE frame by loosening the two knobs following a counter-clockwise motion (cyan), moving the camera platform up (red), and re-tightening the knobs by applying a clockwise motion.

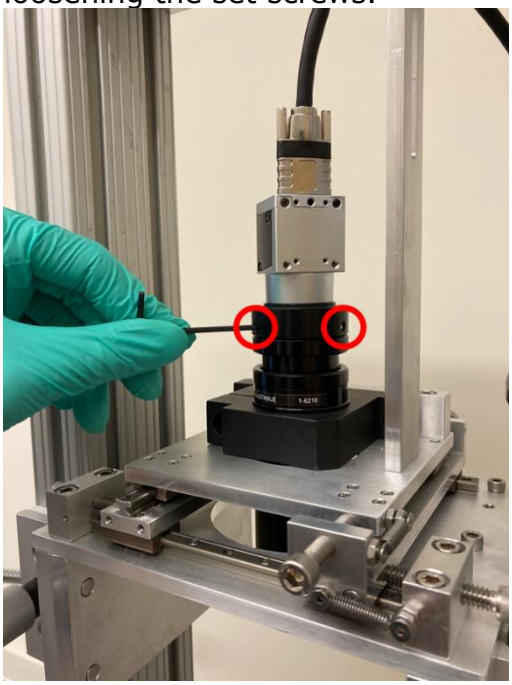
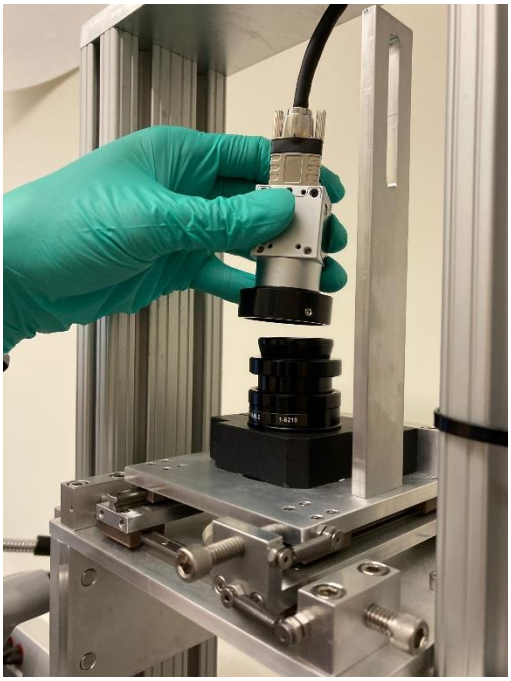


2) Loosen the three set screws (two of these shown in the red circles in the photo below) on the adapter that is securing the lens to the adapter and carefully remove the currently installed lens.
IMPORTANT: Make sure to always hold the lens with one of your hands while loosening the set screws.

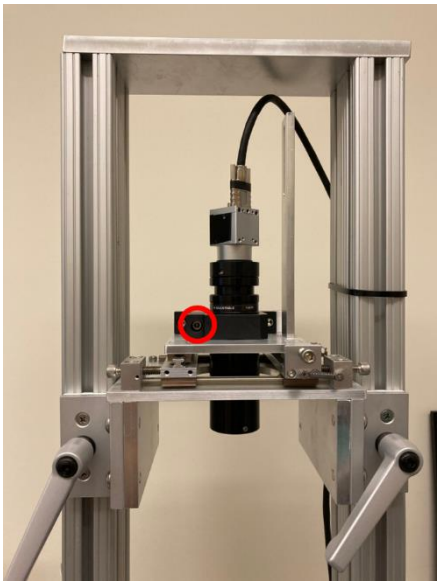


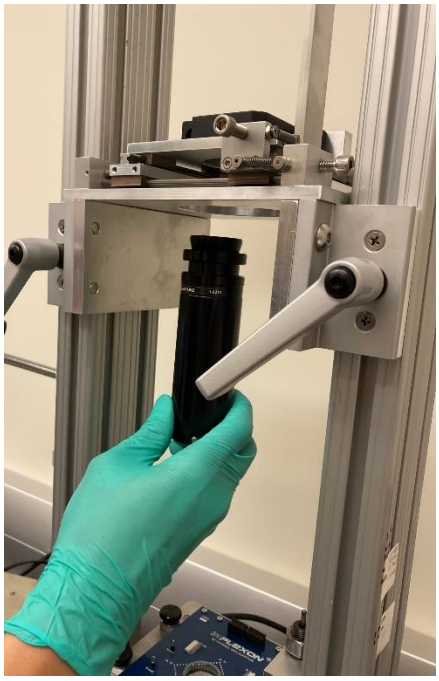


3) Loosen the three set screws (two of these shown in the red circles in the photo below) on the C-mount coupler mounting the camera and C-mount coupler to the adapter. Carefully remove the camera and coupler.
IMPORTANT: Make sure to always hold the camera with one of your hands while loosening the set screws.

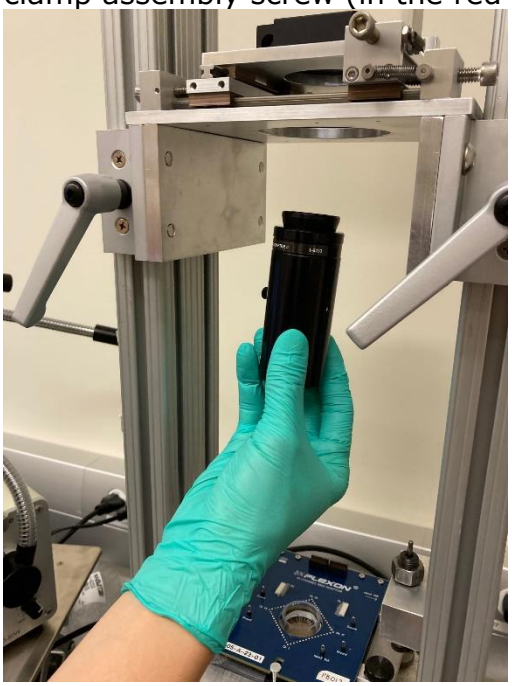
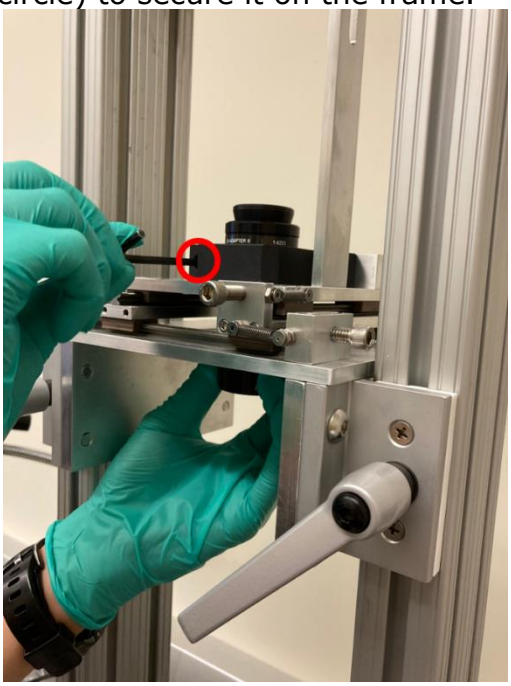



4) Loosen the screw (in the red circle in the photos below) clamping the adapter to the tube clamp assembly. Carefully remove the adapter. Note that there are two screws on the tube clamp assembly that are mounting it to the frame. These two screws must not be loosened.
IMPORTANT: Make sure to always hold the adapter with one of your hands to avoid damaging it.

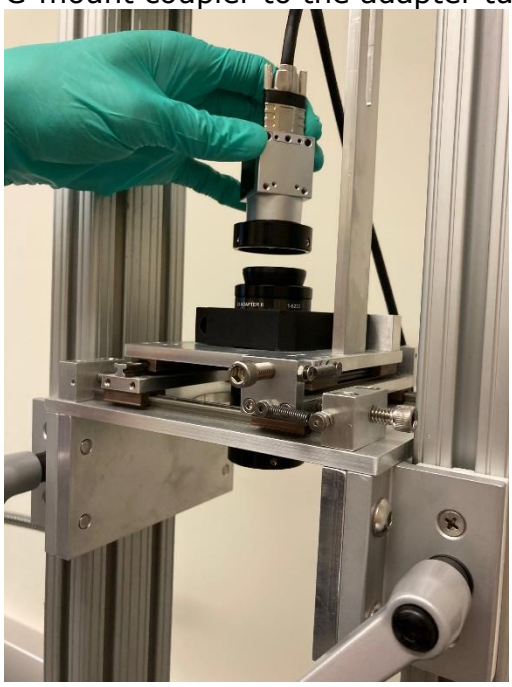


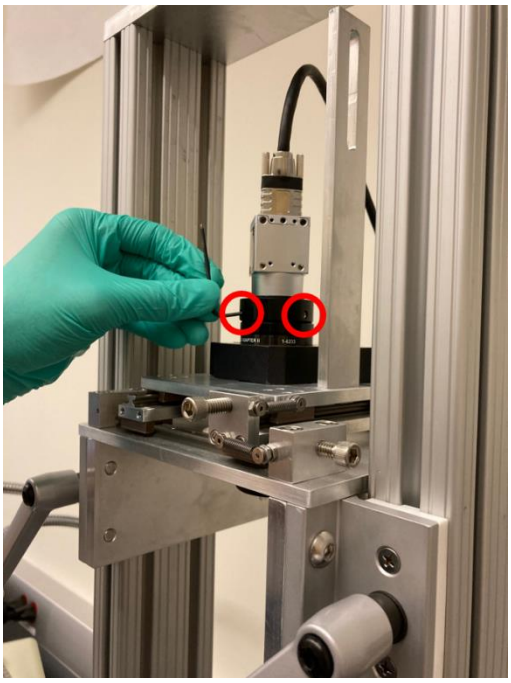


5) Insert the adapter into the tube clamp assembly from the bottom and tighten the tube clamp assembly screw (in the red circle) to secure it on the frame.

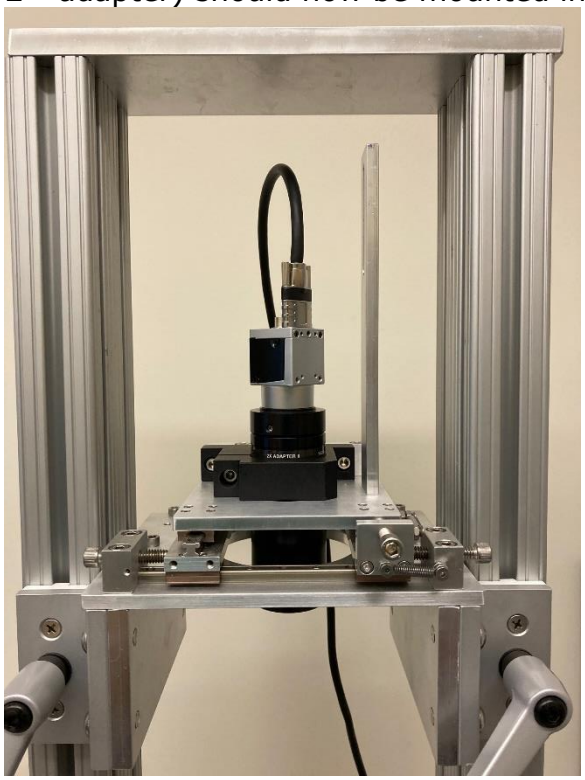



6) Place the camera and C-mount coupler on the top of the adapter tube and tighten the three set screws (two of these shown in the red circles in the photo below) securing the C-mount coupler to the adapter tube.





7) The camera, C-mount coupler, and replacement adapter tube (in this example, 2× adapter) should now be mounted in the MEASSuRE frame as shown here.



8) At this point, a camera lens should be attached by inserting the top of lens into the adapter and tightening the three set screws at the bottom end of the adapter. For further details on attaching the lens, go to **step 3) of section A.1** above.

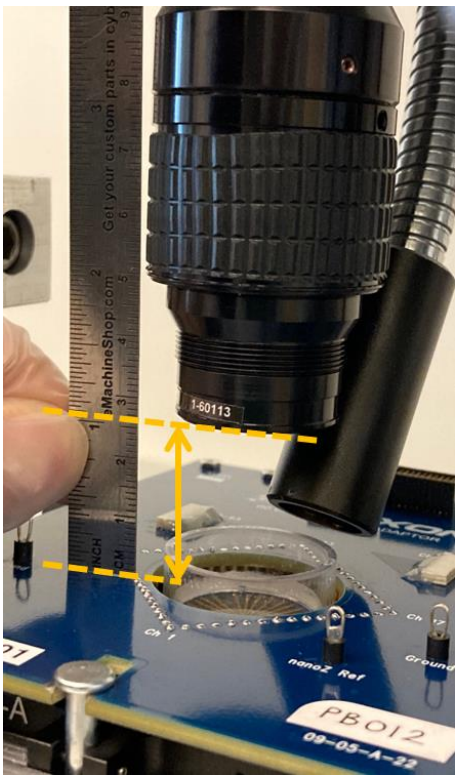
Appendix B: Working Distances for Various Imaging Hardware Configurations

	Config. #1	Config. #2	Config. #3	Config. #4	Config. #5	Config. #6
Adapter tube	1× adjustable, standard (Navitar 1-6218)	1× adjustable, standard (Navitar 1-6218)	2× adjustable, short (Navitar 1-6233)	2× adjustable, short (Navitar 1-6233)	1× adjustable, standard (Navitar 1-6218)	2× adjustable, short (Navitar 1-6233)
Lens	Zoom 6000 Lens (Navitar 1-60135)	Zoom 6000 Lens (Navitar 1-60135)	Zoom 6000 Lens (Navitar 1-60135)	Zoom 6000 Lens (Navitar 1-60135)	UltraZoom 6000 Lens (Navitar 1-60190)	UltraZoom 6000 Lens (Navitar 1-60190)
Lens attachment	n/a	2× lens attachment (Navitar 1-60113)	n/a	2× lens attachment (Navitar 1-60113)	10× objective (Navitar 1-62829)	10× objective (Navitar 1-62829)
Magnification[α]	1×	2×	2×	4×	10×	20×
Working Distance[β] (mm)	**92**	**36**	**92**	**36**	**33.5**	**33.5**
Distance between lowest imaging component and top of interface board holding the sample[γ] (mm)	80	26	80	26	24	24

[α]: Not taking into account the 0.7-4.5× magnification from the Zoom 6000 and UltraZoom 6000 lenses
[β]: As given by Navitar datasheets for various imaging component combinations
[γ]: These distances were measured experimentally and are a bit smaller than the working distance for that specific configuration due to the sample surface being located roughly between 10 and 12mm below the top surface of the interface board.

The table above shows the working distance for different imaging hardware configurations. Overall, the two adapter tubes included in the table do not seem to affect the working distance. However, the presence of a lens attachment or objective lens does seem to determine the working distance.

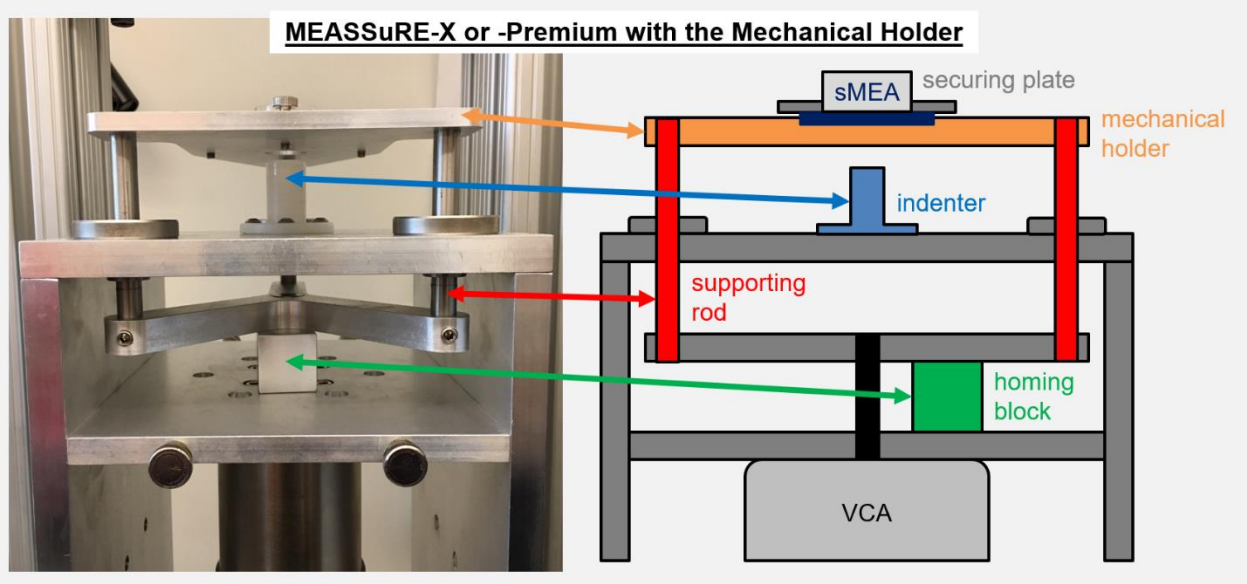
The distance measurements between the lowest imaging component and top of the interface board is illustrated in the image to the right. When setting the vertical position of the camera stage holding all imaging components, this distance measurement can be used as a reference instead of the actual working distance as it is not practical to measure the distance from the surface of the sample. Keep in mind that, in practice, this distance may be a bit larger or smaller as the focus knob of the lens provides a 12mm fine focus.



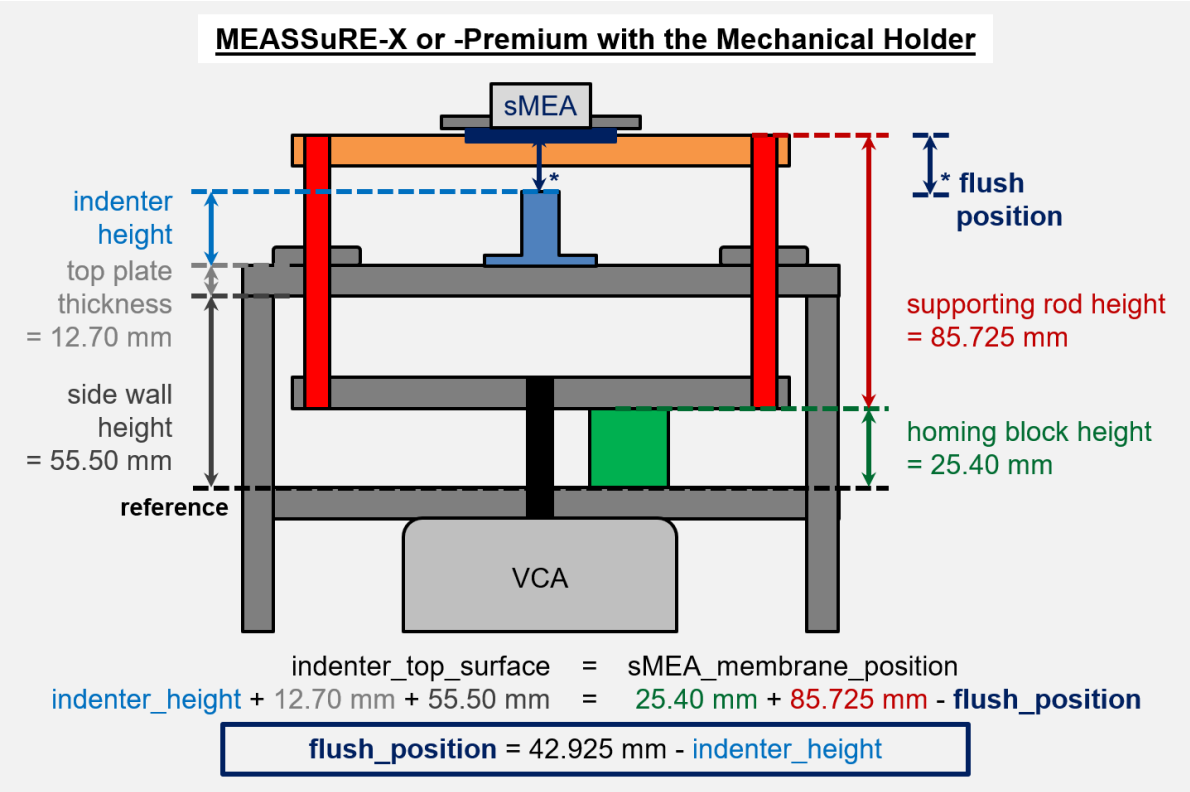
Appendix C: Finding the Flush Position

The different MEASSuRE configurations with their respective schematics and calculations are presented here:

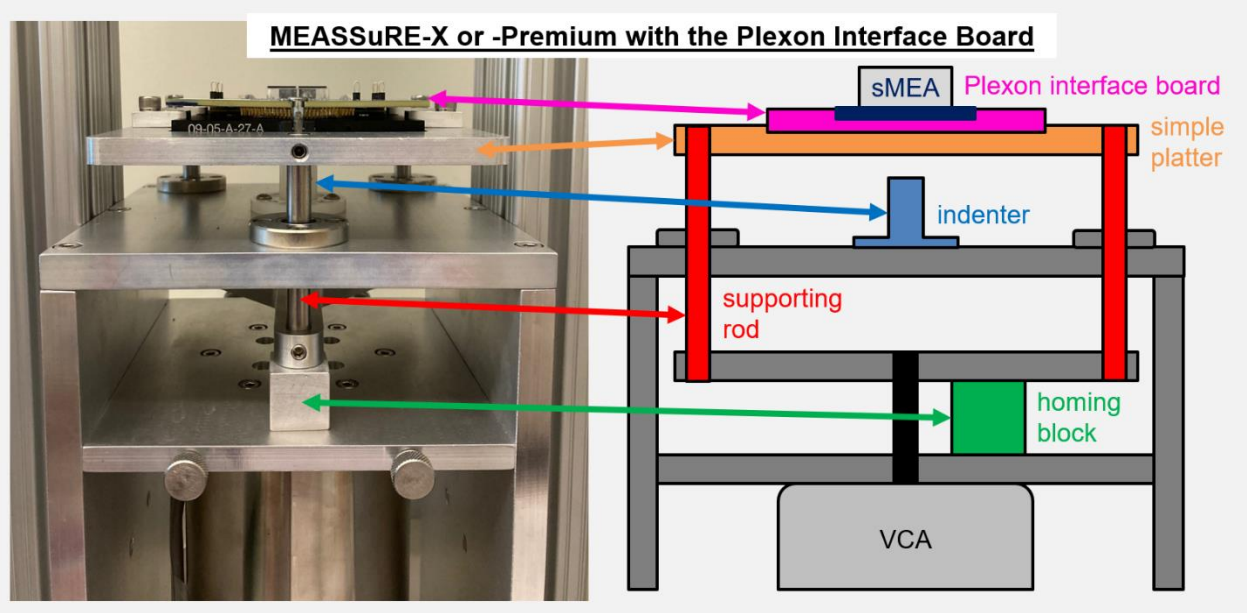
1. **MEASSuRE-X or -Premium with the mechanical holder:** The flush position depends on the height of the homing block, the height of the indenter, and the length of the supporting rods. The following schematic shows the different components of the MEASSuRE system involved in this calculation.



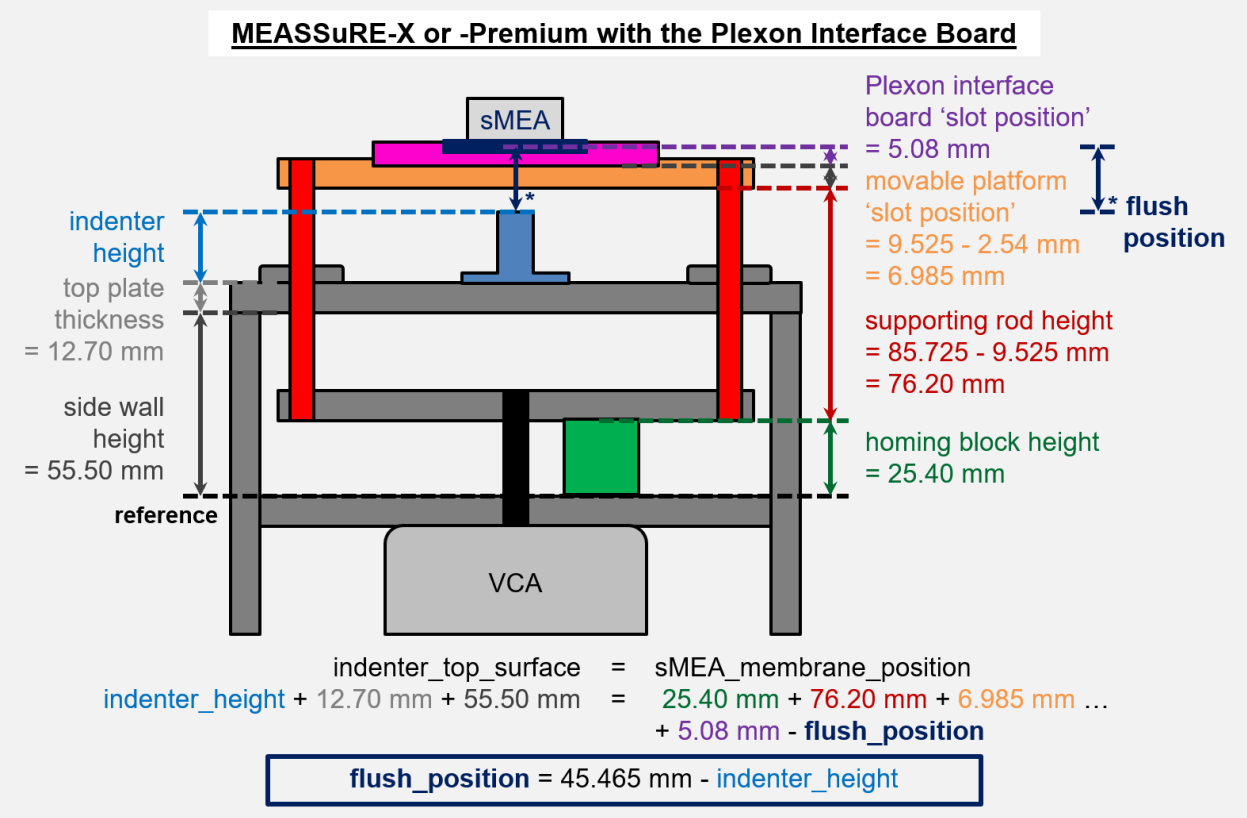
The following figure shows the calculations to determine the flush position with the -X or -Premium configuration without an electrophysiology interface board.



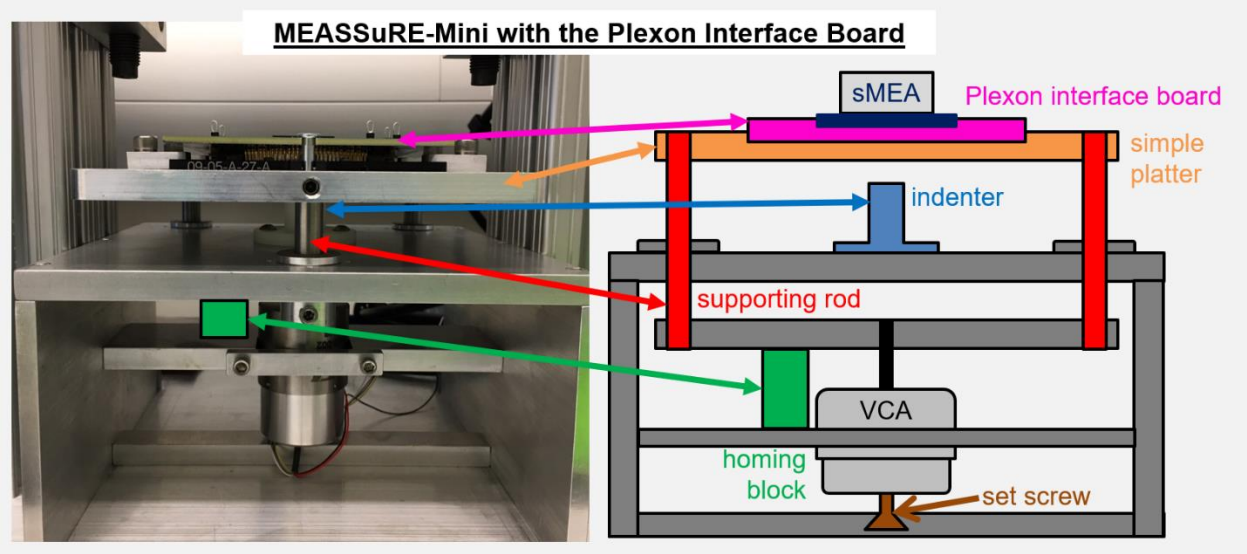
2. **MEASSuRE-X or -Premium with the Plexon interface board:** When using a simple movable plate and Plexon board to hold the sMEA, the heights of this plate and interface board must also be taken into consideration as shown in the figure below.



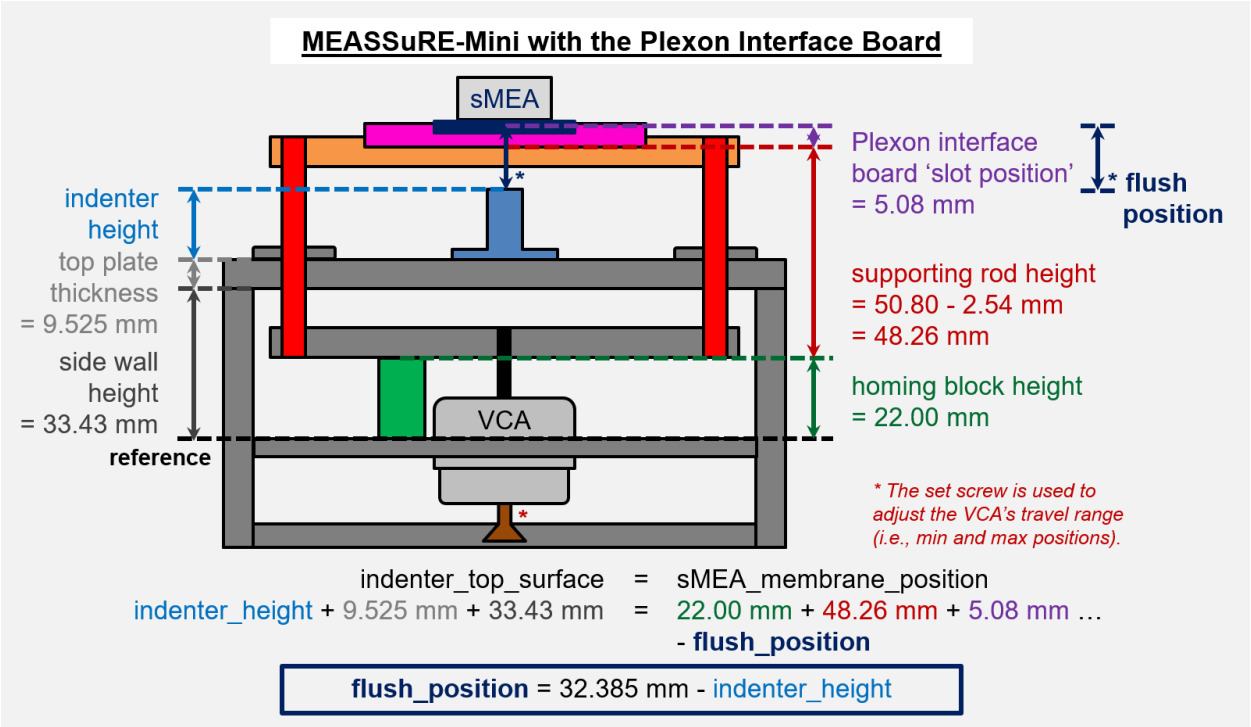
Calculations to determine the flush position with the -X or -Premium configuration when using the Plexon interface board are shown in the figure below



3. **MEASSuRE-Mini with the Plexon interface board:** Compared to the -X and -Premium systems, MEASSuRE-Mini systems have shorter supporting rods and a set screw underneath the VCA to help adjust the travel range. A photo of this configuration and its schematic are shown in the figure below.

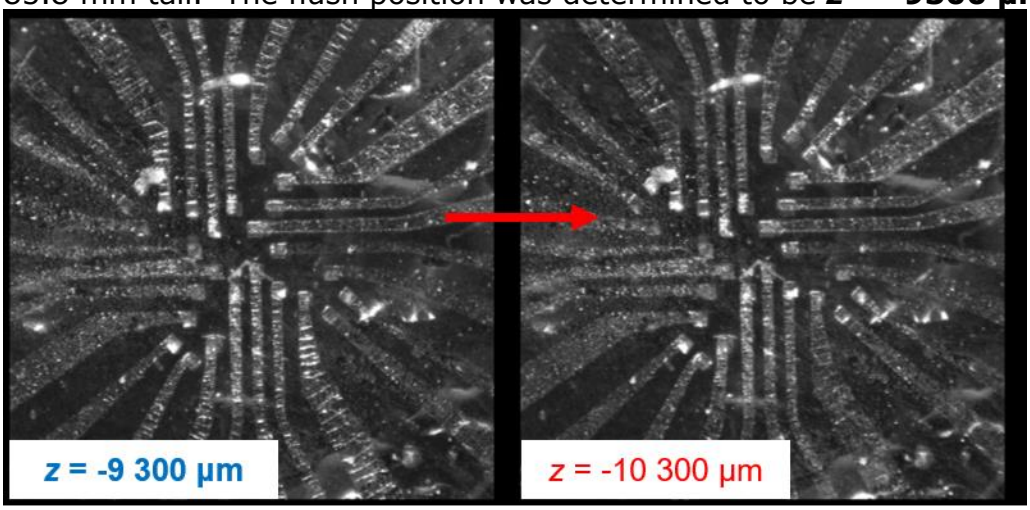


Calculations to determine the flush position with the MEASSuRE-Mini configuration when using the Plexon interface board are shown in the following figure.

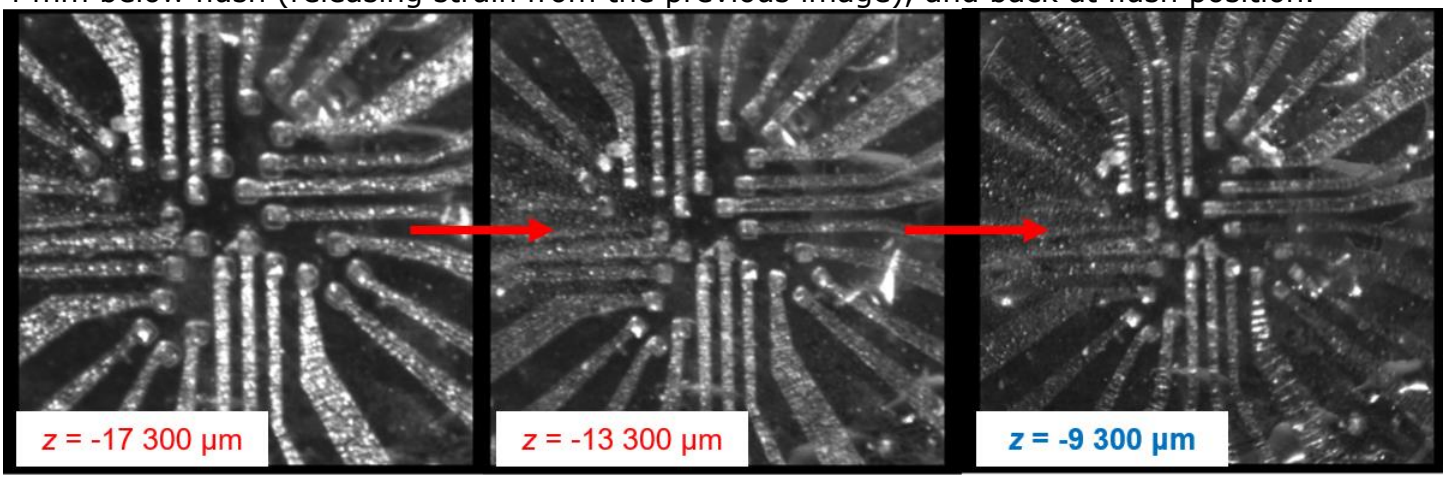


Appendix D: Flush Position Sample Calculation #1

In this alternative example of identifying the flush position, we used the "no Ephys module" configuration with the **NXA4 camera**, a 34.8 mm tall indenter, and supporting rods that were 85.8 mm tall. The flush position was determined to be ***z* = -9300 µm**.

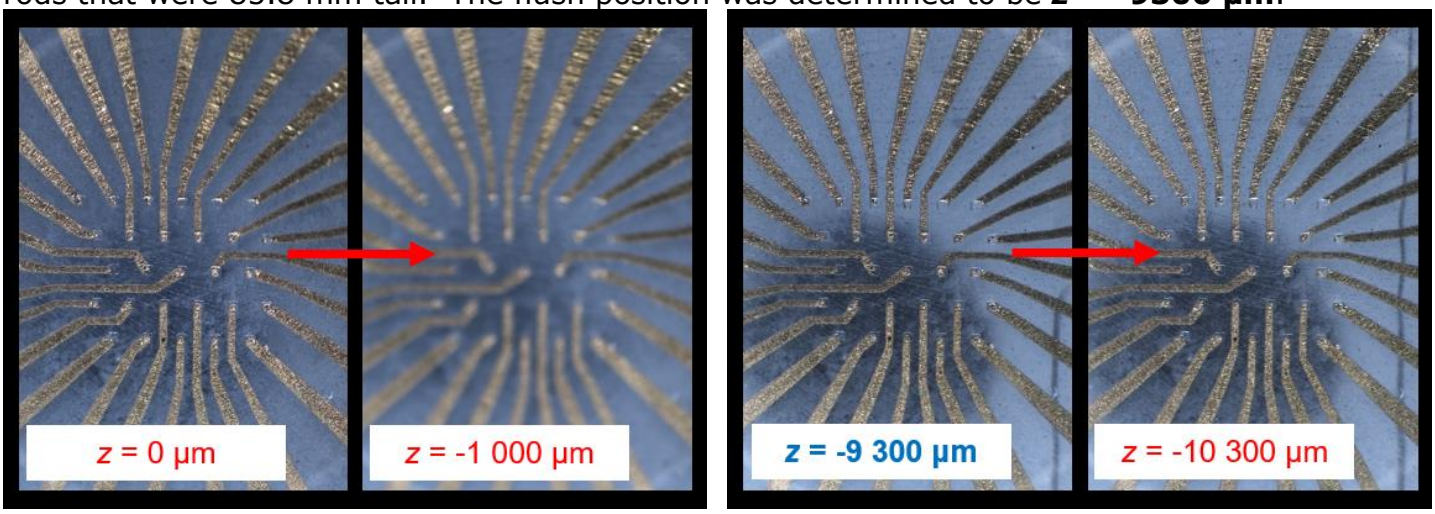


The following images show the camera image with the sequence: 8 mm below the flush position, 4 mm below flush (releasing strain from the previous image), and back at flush position.

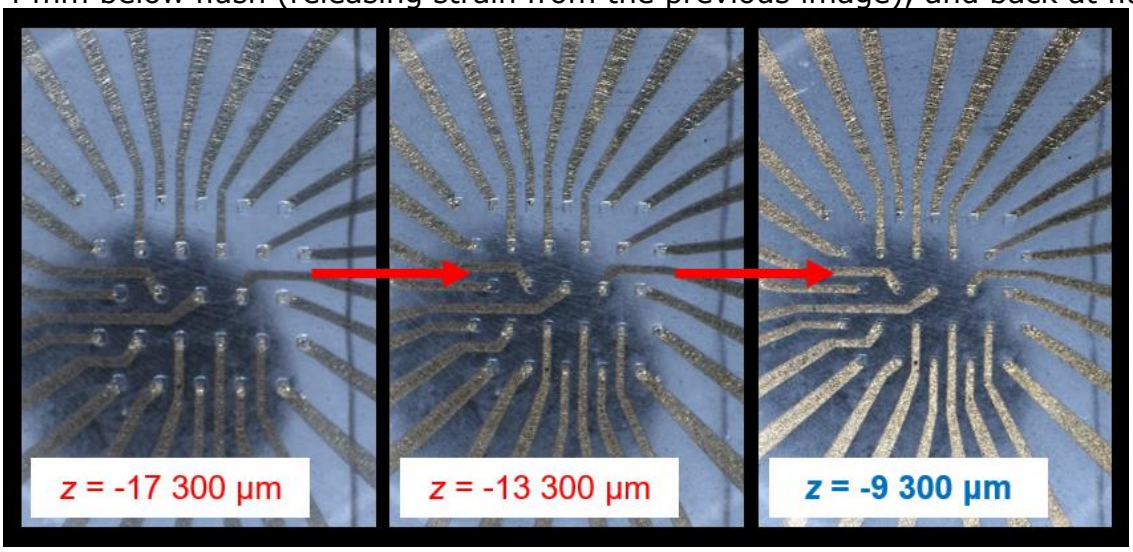


Appendix E: Flush Position Sample Calculation #2

In this alternative example of identifying the flush position, we used the "no Ephys module" configuration with the **CCM-1510 camera**, a 34.96 mm tall (uniaxial) indenter, and supporting rods that were 85.8 mm tall. The flush position was determined to be ***z* = -9300 µm**.



The following images show the camera image with the sequence: 8 mm below the flush position, 4 mm below flush (releasing strain from the previous image), and back at flush position.



Appendix F: Adjusting parfocal and parcentration of the imaging system

The lens must first be adjusted so that it is parfocal. (See Below)

Parfocal

The imaging system is designed to stay in focus when adjusted from one magnification to another. The procedure below describes how to adjust the components.

1. Position fine focus mechanism at the middle point of travel. (If the lens has fine focus.)
2. Adjust the magnification to its highest setting.
3. Adjust focus by either moving entire optical system in its z-axis or using the fine focus provided on the lens. Adjust until you have a sharp image.
4. Adjust the magnification to its lowest setting. At this point do not adjust z-axis height or the fine focus on the lens.
5. Adjust the back focus of the lens. This is done by adjusting the glass in the adapter tube. Most adapter tubes with glass have a locking screw and an adjustment screw. Release the locking screw and then use the adjustment screw to bring the image into focus. If using adapters without glass they can be adjusted using the telescoping feature or the CCD in the camera can be adjusted.
6. Lock the back-focus adjustment.
7. At this point the lens should produce sharp images from low to high magnification.

Parcentration

The imaging system is designed to zoom on center. Due to manufacturing tolerances in different cameras, you may need to adjust the position of your CCD with respect to the optical axis of the lens.

1. Choose a target has many small features.
2. Mark the center point of your monitor.
3. Change the zoom from low to high magnification many times and locate a reference that does not shift laterally as you zoom.
4. Adjust to lowest magnification setting.
5. Adjust the three setscrews on the c-mount coupler to bring that reference point to the center of your monitor. (Use three Allen wrenches at the same time to make this adjustment.)
6. The system should now stay on center when the magnification is adjusted.