bmseed BioMedical
Sustainable Elastic Electronic Devices

Innovation in Neural Interface Technology

OPERATING INSTRUCTIONS FOR THE IMAGING MODULE

IDT Cameras:
OS7-V3-S1, NXA4-S1, CCM-1510

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1. Overall Imaging Module Setup

The Imaging Module of MEASSuRE systems is located on the top area of the MEASSuRE frame, fixed to the frame's side rails. The overall configuration, shown in **Fig. 1**, consists of the following from top to bottom: camera, speed booster (if any), coupler(s), adapter tube, lens, and any additional components or attachments. An LED light source is used to illuminate the sample.

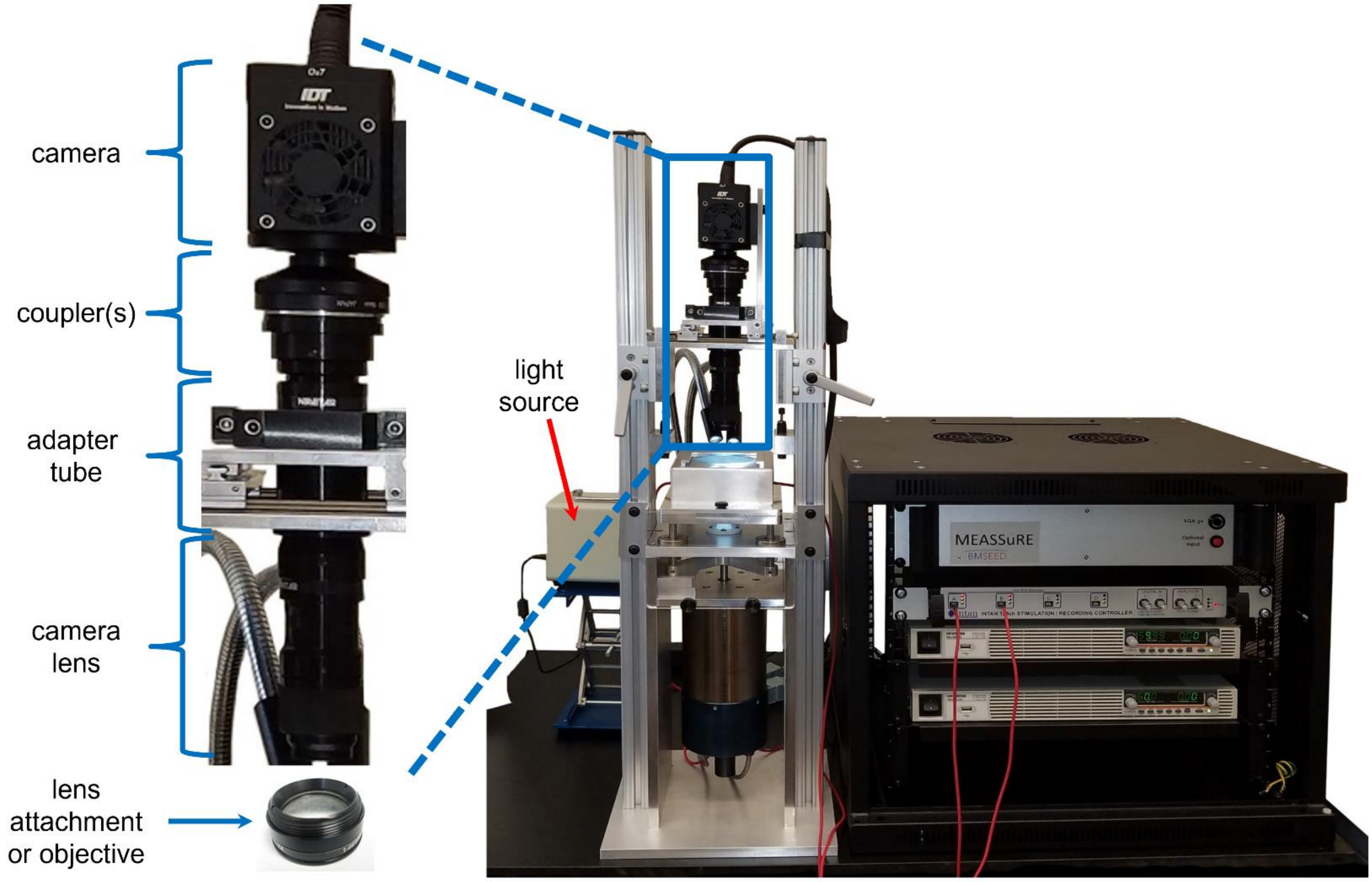


Fig. 1: Overall setup of the Imaging Module (shown here for a MEASSuRE-X system).

Depending on the MEASSuRE model being used and the target application, the Imaging Module will be configured accordingly. **Table 1** shows the typical imaging components for the different models of MEASSuRE.

Upon tool installation, BMSEED will provide and assemble the imaging components based on the user's experimental protocol and target application. If additional or different imaging components are needed, contact BMSEED.

Table 1: Typical imaging components for the various MEASSuRE models.

<table>
<tr><th>Hardware component</th><th>MEASSuRE-Mini</th><th>MEASSuRE-Premium</th><th>MEASSuRE-X</th></tr>
<tr><td>Camera</td><td>Camera from Basler:
• acA2440-75um

Cameras from IDS:
• UI 3370CP-C-HQ
• UI 3060CP-M-GL R2</td><td>Cameras from IDT:
• OS7-V3-S1
• NXA4S1-M
• MotionXtra CCM 1510</td><td>Cameras from IDT:
• OS7-V3-S1
• NXA4S1-M
• MotionXtra CCM 1510</td></tr>
<tr><td>Speed booster (optional)</td><td>Not required</td><td>Not required</td><td>Speed booster from Metabones:
• XL, 0.64×, Nikon G to Micro Four Thirds (MB_SPNFG-M43-BM2)</td></tr>
<tr><td>Coupler</td><td>Coupler from Navitar:
• C-mount coupler (1-6010)

Coupler from Fotodiox:
• Lens C to MFT mount adapter (C-MFT)</td><td>Couplers from Navitar:
• C-mount coupler (1-6010)
• F-mount adapter
• F-C adapter

Coupler from Fotodiox:
• Lens C to MFT mount adapter (C-MFT)</td><td>Couplers from Navitar:
• C-mount coupler (1-6010)
• F-mount adapter
• F-C adapter

Coupler from Fotodiox:
• Lens C to MFT mount adapter (C-MFT)</td></tr>
<tr><td>Adapter tube</td><td colspan="3">Adapters from Navitar:
• 1× standard, tele (1-6218)
• 1× standard, F-mount (1-64299)
• 2× standard (1-6030)
• 2× short (1-6233)</td></tr>
<tr><td>Lens</td><td colspan="3">Lenses from Navitar:
• Zoom 6000 lens, 12mm fine focus (1-60135)
• Zoom 6000 lens w/ aperture, 12 mm fine focus (1-60135A)
• UltraZoom 6000 super high resolution objecting coupling lens, 12 mm fine focus (1-60190)</td></tr>
<tr><td>Lens attachment (optional)</td><td colspan="3">Lens attachments from Navitar, only compatible with Zoom 6000 lenses:
• 2× lens attachment (1-60113)
• 1.5× lens attachment (1-60112)</td></tr>
<tr><td>Objective (optional)</td><td colspan="3">Objectives from Navitar, only compatible with UltraZoom 6000 lenses:
• 10× Motic objective (1-62829)
• Required for any objective: Mitutoyo objective coupler (3-60160)</td></tr>
<tr><td>Light source</td><td colspan="3">Light source from AmScope:
• LED fiber optic illuminator w/ dual gooseneck lights, 50W (LED-50WY)

Light source from Techniquip:
• LED fiber optic illuminator with ring light and diffuser, 80W (Prolux X6K-TQB)</td></tr>
</table>

A brief description of the various imaging components:

- Camera: The IDT cameras listed in **Table 1** are high speed cameras that are well suited for MEASSuRE-X and -Premium systems that will be used in very fast impulse stretches (i.e., that move to the target position up to within a few milliseconds). These three cameras, which will be described in **Section 2** below, are fixed to the MEASSuRE frame via two screws.
  The Basler and IDS cameras are better suited for MEASSuRE-Mini systems that will be mainly used in cyclic or oscillatory stretches that do not require high recording rates. These cameras are explained in a separate user manual.

- Speed booster: A speed booster increases the field of view and brightness. The latter is especially critical when running fast impulse stretches at high magnifications. While speed boosters are not necessary, they are recommended when using MEASSuRE-X system.

- Coupler: Couplers are used to connect the camera (or speed booster if using one) and the adapter tube. The Navitar C-mount coupler is typically used in all MEASSuRE models.

- Adapter tube: The adapter tube (like the coupler) serves as the connection between the camera and lens. A clamp on the imaging stage is used on the adapter tube to fix the imaging assembly to the MEASSuRE frame (see **Fig. 1**). Depending on the target application and other hardware components, the adapter tube could have different magnifications, lengths, and mounts.

- Lens: The lens allows the user to focus on and zoom in and out of the sample (from 0.7× to 4.5×). Typically, the Navitar Zoom 6000 lens is used on MEASSuRE systems. There is one model of the Zoom 6000 lens that allows the user to adjust the aperture to control the depth of field by trading off the amount of light reaching the sample.
  If larger magnifications are required, the Navitar UltraZoom 6000 lens can be used instead. This lens allows the user to attach objectives to the imaging assembly. The main downsides of larger magnifications are a smaller depth of field and light reduction.

- Additional components:
  - Lens attachment: Lens attachments are only compatible with Zoom 6000 lenses (not UltraZoom 6000 lenses). Lens attachments are screwed in at the bottom of the lens. Typically, a 2× lens attachment is provided with MEASSuRE systems as it is a quick and simple way to increase magnification at an increased image resolution but with the tradeoff of a shallower depth of field. Note that adding a lens attachment reduces the working distance.
  - Objectives: Objectives are only compatible with UltraZoom 6000 lenses (not Zoom 6000 lenses). Objectives require a coupler prior to mounting them to the lens and are only recommended if a large magnification is needed. When using an objective, the working distance is reduced even further than when using a lens attachment.

- Light source: An LED light source is required to illuminate the sample as they do not heat up the sample. Dual gooseneck lamps or a ring light can be used with the light source. While the gooseneck lamps can apply more illumination, the ring light is fixed to the bottom of the imaging assembly making it a more convenient and repeatable option.

2 Protocol to Connect the Camera

BMSEED is currently offering the MEASSuRE-X and -Premium systems with three different IDT cameras: the **OS7-V3-S1**, the **NXA4-S1**, and the **CCM-1510 camera**. The OS7 and NXA4 cameras are monochrome systems with 1920×1280 pixels image resolution at 1350 fps (frames per second) at full frame for the OS7, and 1024×1024 pixels image resolution at 1000 fps at full frame for the NXA4 camera. The CCM camera offers a color image with 1440×1024 pixels resolution at 2000 fps at full frame. Higher frame rates can be achieved for smaller frames. From left to right, the images below show the different components of the imaging module of MEASSuRE systems: the OS7-S1 camera, the NXA4 camera, the CCM camera, the breakout cable, and a 24 VDC power supply.

Fig. 2: (a) The OS7-S1 camera, (b) the NXA4 camera, (c) the CCM camera, (d) the breakout cable, and (e) a 24VDC power supply.

To connect the camera, do the following:

1) For the OS7 and NXA4 cameras: Once the camera has been fixed on the MEASSuRE frame, connect the 19-pin LEMO connector end of the breakout cable to the back of camera.

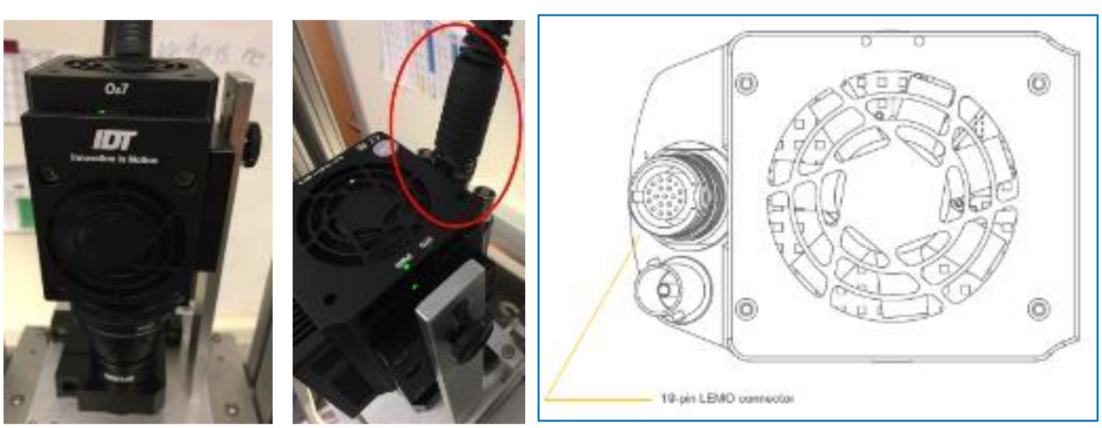


Fig. 3: A connected **OS7** camera and a schematic of the camera connections.

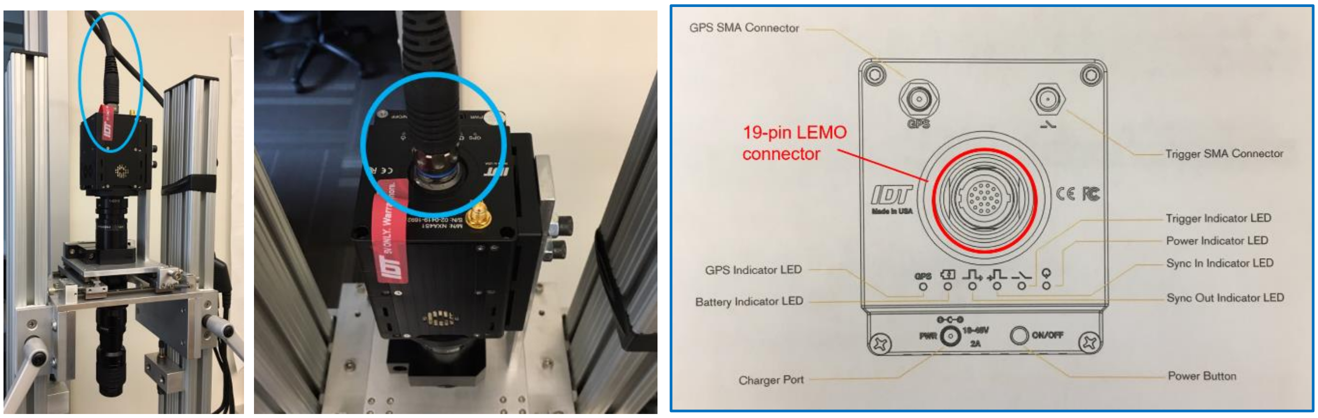


Fig. 4: A connected **NXA4** camera and a schematic of the camera connections.

For the CCM-1510 camera: Once the camera has been fixed on the MEASSuRE frame, use the USB-C to USB-C cable to connect the camera to the USB-C to 19-pin adapter. On the other end of the adapter, connect the 19-pin LEMO connector end of the breakout cable.

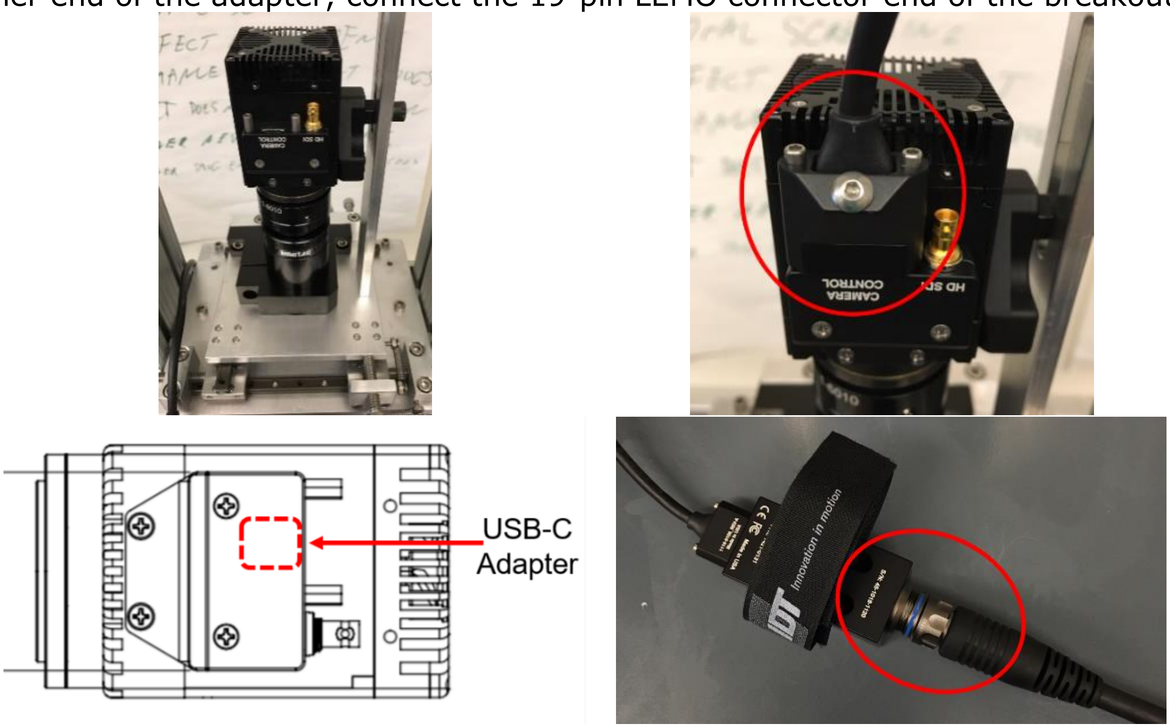


Fig. 5: A connected **CCM** camera and a schematic of the camera connections.

When connecting the LEMO connector, line up the notches on the LEMO connector with the recesses on the camera. Secure the connection by turning the screw nut (**Fig. 5**, bottom right).

2) Connect the Ethernet cable connector end of the breakout cable to the network card on the PC or MAC computer. Shown in a cyan circle in the picture below (**Fig. 6**).

Fig. 6: Ethernet cable connections.

3) Connect the 4-pin DIN connector from the 24V Power Supply to the matching DIN power connector on one end of the breakout cable. Shown in a red circle in the picture above (**Fig. 6**).

4) Leave the SMA cables/connectors (i.e., “Sync-in”, “Sync-out”, or “Trig in”) unconnected as shown below (**Fig. 7**).

Fig. 7: SMA connectors in breakout cable

5) Once confirmed that all previous connections are securely fastened, plug the AC power cable from the power supply to a wall outlet (**Fig. 8**).

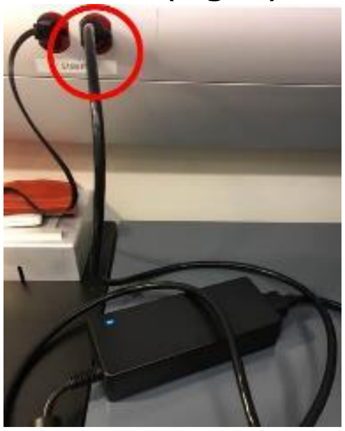


Fig. 8: Power adapter plugged to outlet.

3 Camera Software Setup Instructions

The IDT **Motion Studio x64** software is used to control all three cameras.

3.1 Download and Install the Motion Studio x64 Software

1) Download the Motion Studio x64 software at:
   https://idtvision.com/products/software/motion-studio/?cn-reloaded=1

2) Scroll down until you see the yellow download links. Select the Full Package of the Motion Studio 2 download for your operating system (e.g., "Download Windows 64 bit" in **Fig. 9**).

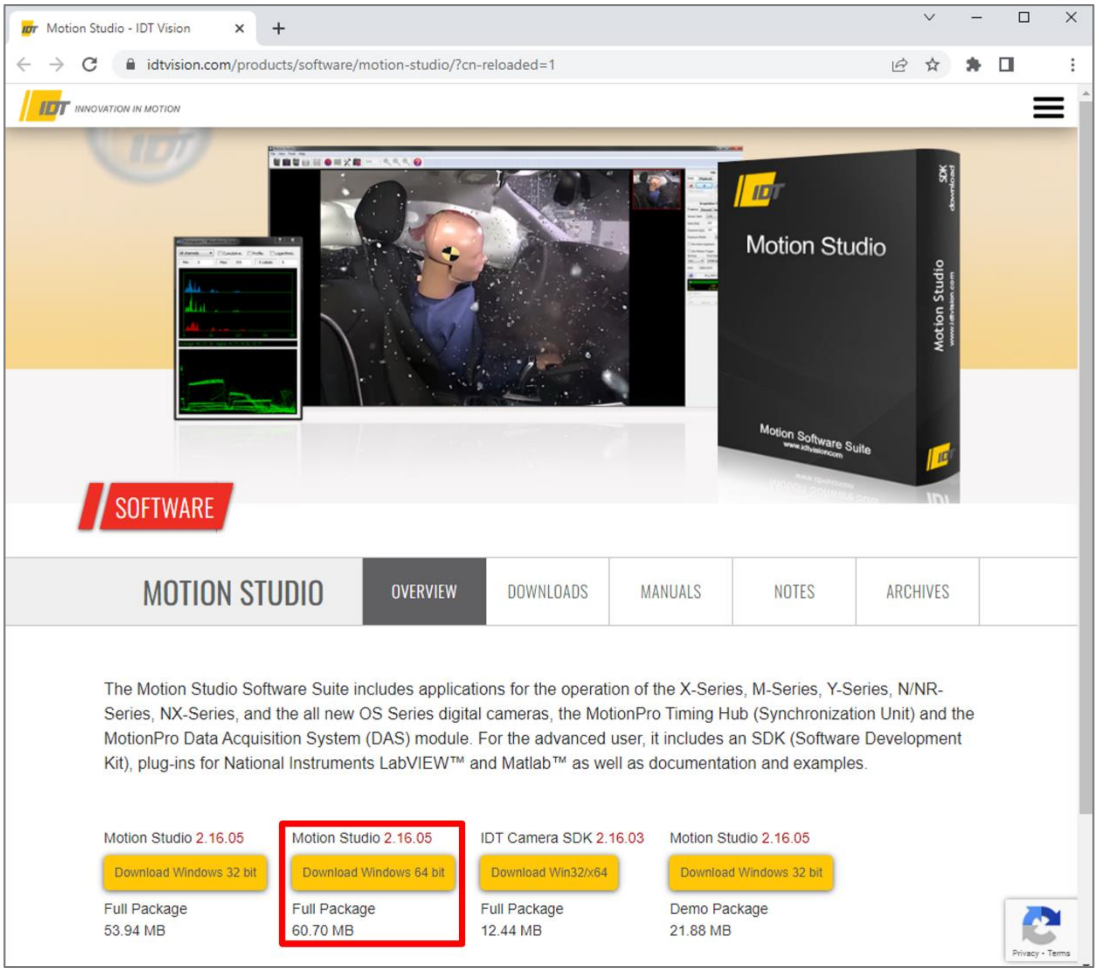


Fig. 9: IDT website with available software downloads (screenshot taken on 04/25/22).

3) Run the downloaded installer file (e.g., "mstudio64_21605") and follow the prompts on the Motion Studio x64 Setup installer.

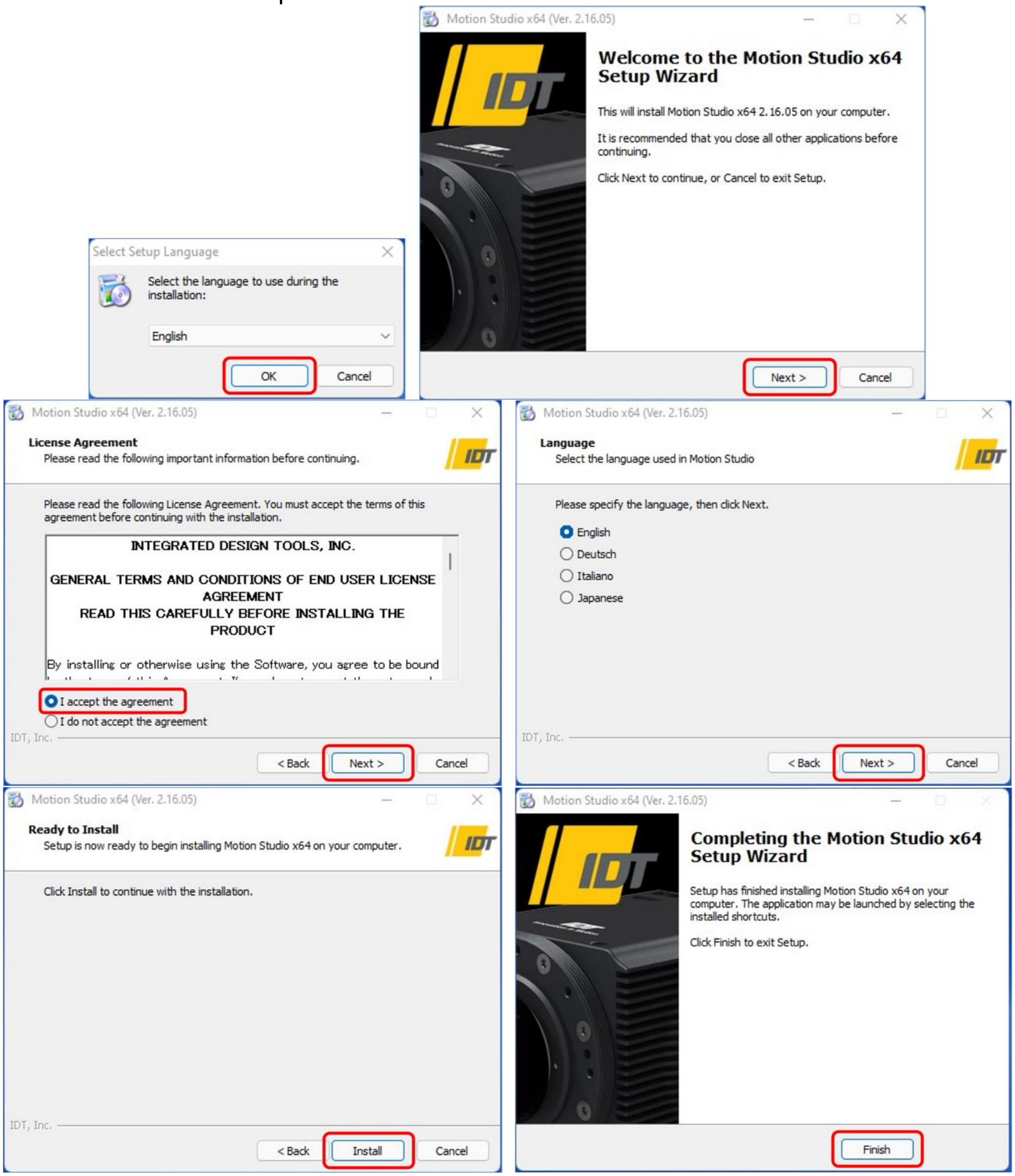


Fig. 10: Motion Studio x64 setup/installer windows.

3.2 Protocol for Software Initial Setup

1) If using the **NXA4** camera, prior to loading the software, turn on the camera by clicking the "On/Off" button, circled in cyan in the picture below. The power LED, circled in red, should lit up.

   If using the **OS7** and **CCM** cameras, simply plugging the power cord should turn them on.

(a) (b)

Fig. 11: The (a) **NXA4** and (b) **CCM** cameras powered on.

2) Open the Motion Studio x64 software.

3) After a few seconds the Main Menu window will pop up. Select "Cameras" and click "OK".

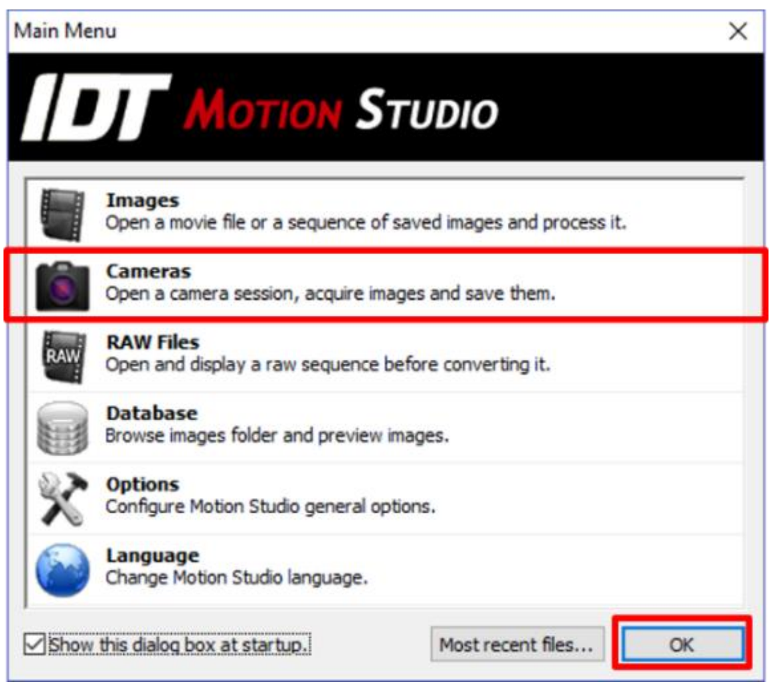


Fig. 12: Motion Studio's Main Menu window.

4) Using the IDT camera OS7-V3-S1, the NXA4-S1, or the CCM-1510, place a checkmark on the option "MotionXtra N/NR/NX/OS/CC on Giga-Ethernet" and click "OK". The computer will then search for all connected cameras.
Note: The other options could either be enabled or disabled.

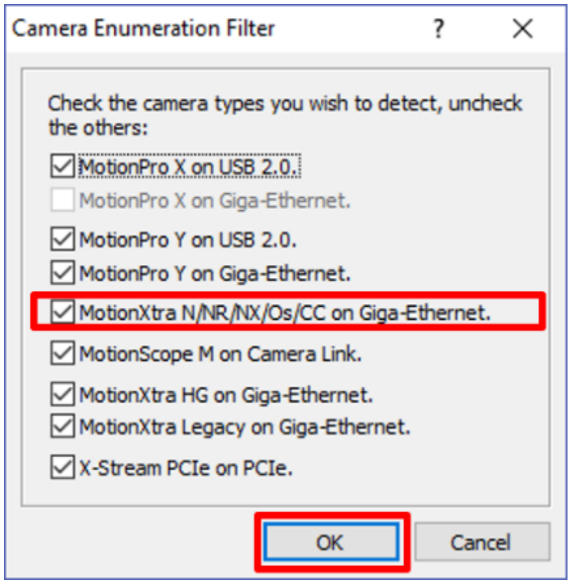


Fig. 13: Selecting the types of cameras to detect.

5) When connecting your computer with the camera for the first time or after the camera has been used on a different computer, the following warning will pop up. Click "OK".

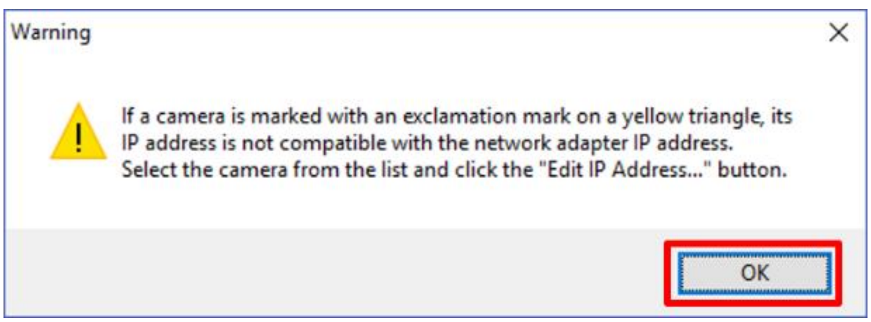


Fig. 14: IP address warning indicating incompatibility between the camera and the computer's network adapter IP address.

6) In the "Open Cameras" window, you will see the "OS-S7 Camera," "NXA4-S1 Camera," or "CC-1510 Camera" listed. If you see the warning symbol (i.e., a yellow triangular sign with an exclamation mark) as in the screenshot below, select the camera in the list and click to the "Edit IP Address" button before proceeding to the next step.

If you do not see the warning symbol, proceed to **step 13)** of this protocol.

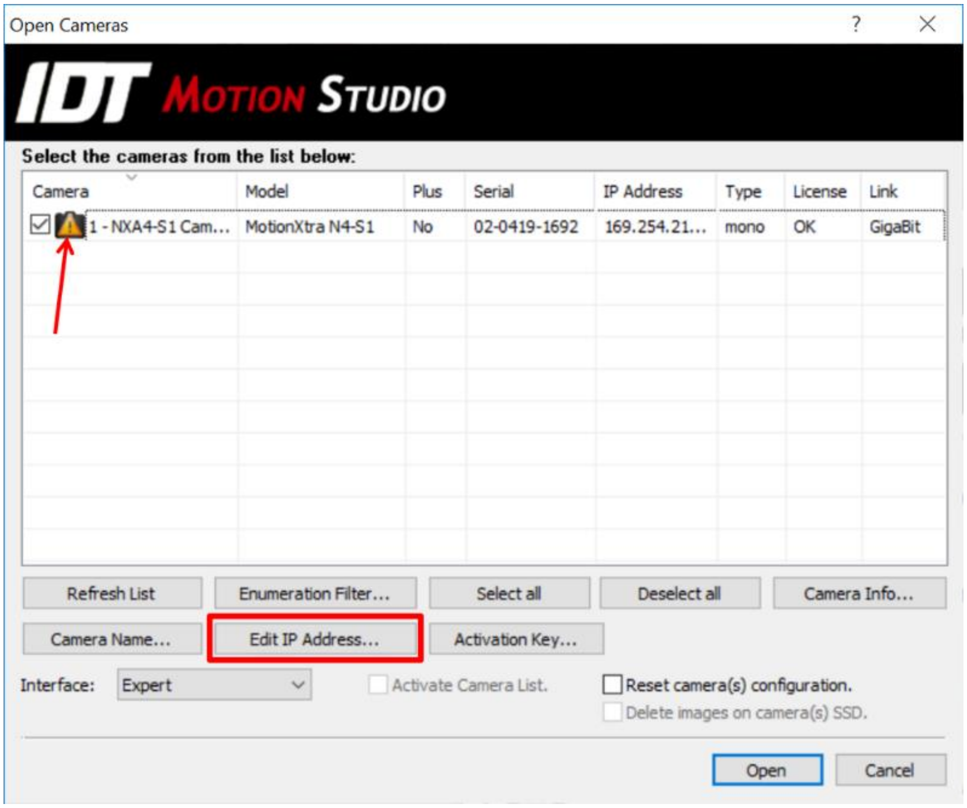
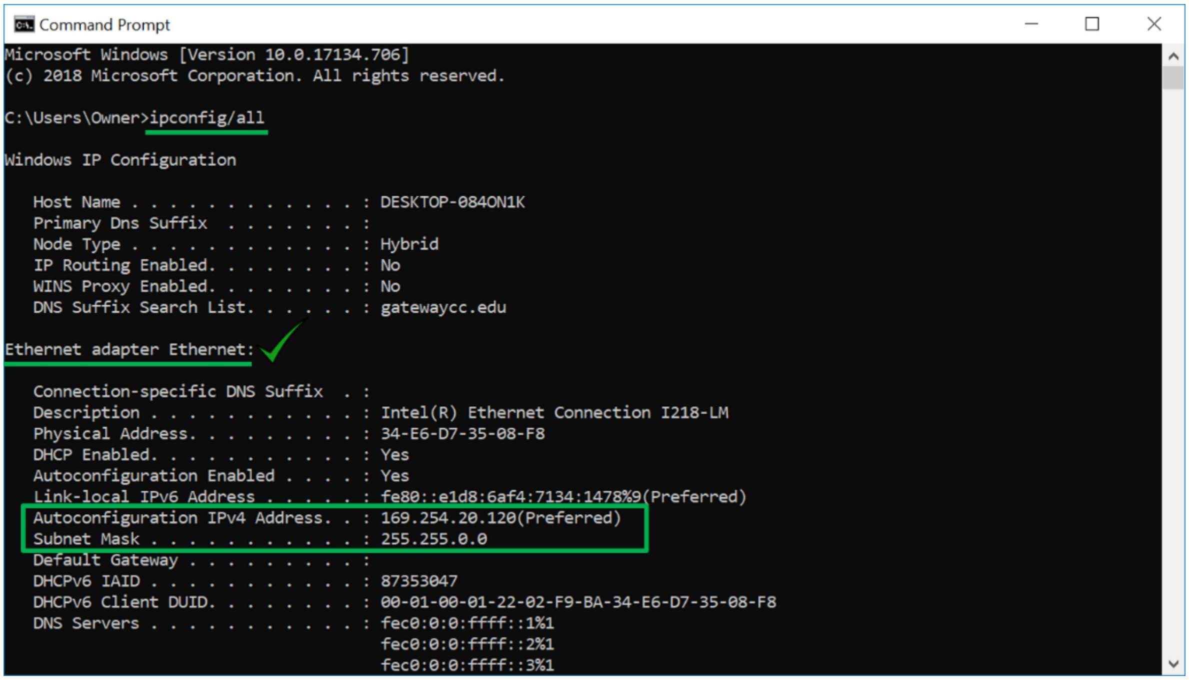


Fig. 15: The "Open Cameras" window showing the detected cameras (e.g., **NXA4**) and yellow warning symbol.

7) The "Edit IP Address" window will pop up. Here you can manually define the camera's IP Address and Subnet Mask as it is connected in the computer's Ethernet port.
You must identify your computer's Ethernet adapter's IP Address and Subnet Mask. If using a PC, type "cmd" in the Start menu. This should open the Command Prompt window. Type "ipconfig/all" and press [Enter]. Info of the different adapters will appear.

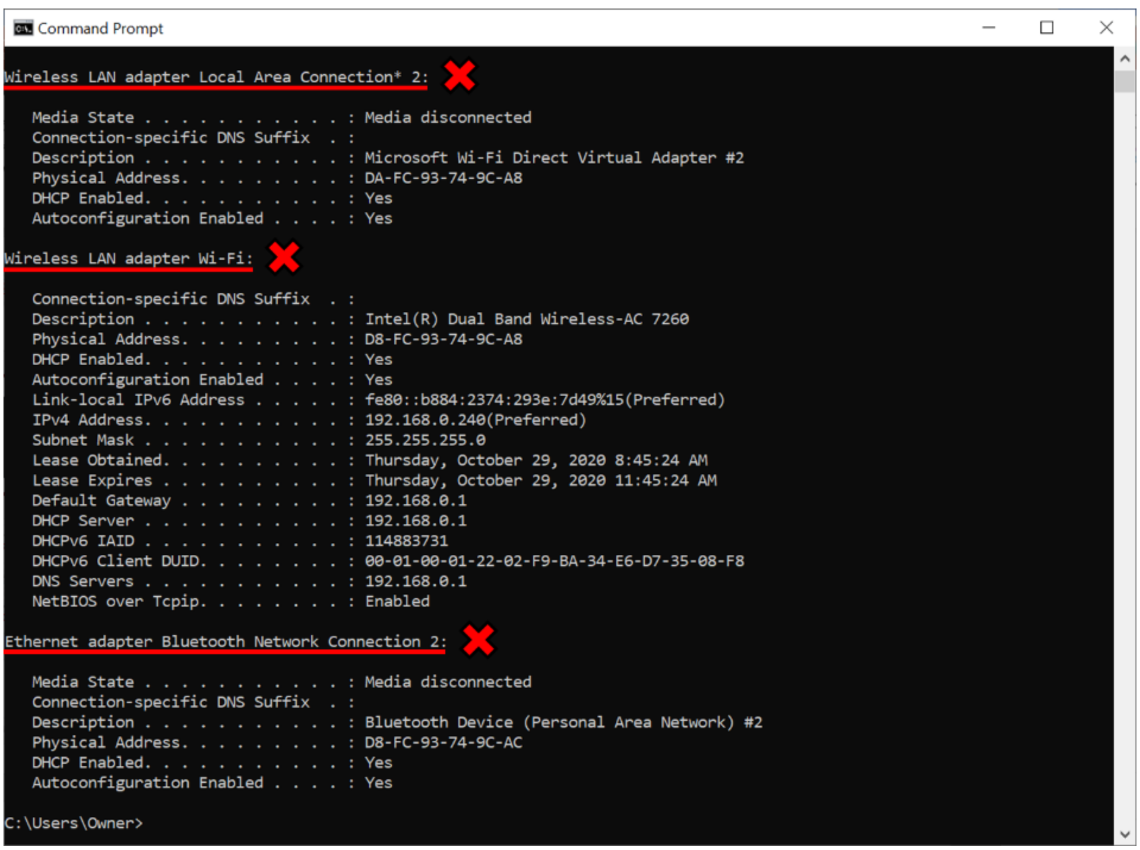
IMPORTANT: The adapter of interest is the one corresponding to your Ethernet port, typically labeled "**Ethernet adapter Ethernet**" as shown by the green underline and checkmark in **Fig. 16** below. Note that this might vary slightly depending on the computer.



```
Command Prompt
Microsoft Windows [Version 10.0.17134.706]
(c) 2018 Microsoft Corporation. All rights reserved.

C:\Users\Owner>ipconfig/all

Windows IP Configuration

   Host Name . . . . . . . . . . . . : DESKTOP-084ON1K
   Primary Dns Suffix  . . . . . . . :
   Node Type . . . . . . . . . . . . : Hybrid
   IP Routing Enabled. . . . . . . . : No
   WINS Proxy Enabled. . . . . . . . : No
   DNS Suffix Search List. . . . . . : gatewaycc.edu

Ethernet adapter Ethernet:

   Connection-specific DNS Suffix  . :
   Description . . . . . . . . . . . : Intel(R) Ethernet Connection I218-LM
   Physical Address. . . . . . . . . : 34-E6-D7-35-08-F8
   DHCP Enabled. . . . . . . . . . . : Yes
   Autoconfiguration Enabled . . . . : Yes
   Link-local IPv6 Address . . . . . : fe80::e1d8:6af4:7134:1478%9(Preferred)
   Autoconfiguration IPv4 Address. . : 169.254.20.120(Preferred)
   Subnet Mask . . . . . . . . . . . : 255.255.0.0
   Default Gateway . . . . . . . . . :
   DHCPv6 IAID . . . . . . . . . . . : 87353047
   DHCPv6 Client DUID. . . . . . . . : 00-01-00-01-22-02-F9-BA-34-E6-D7-35-08-F8
   DNS Servers . . . . . . . . . . . : fec0:0:0:ffff::1%1
                                       fec0:0:0:ffff::2%1
                                       fec0:0:0:ffff::3%1
```

Fig. 16: Finding the computer's Ethernet adapter IP address.

Disregard the info of the other adapters listed. Examples of other adapters are shown by the red underline and red crosses in the screenshot below.



```
Command Prompt
Wireless LAN adapter Local Area Connection* 2:

   Media State . . . . . . . . . . . : Media disconnected
   Connection-specific DNS Suffix  . :
   Description . . . . . . . . . . . : Microsoft Wi-Fi Direct Virtual Adapter #2
   Physical Address. . . . . . . . . : DA-FC-93-74-9C-A8
   DHCP Enabled. . . . . . . . . . . : Yes
   Autoconfiguration Enabled . . . . : Yes

Wireless LAN adapter Wi-Fi:

   Connection-specific DNS Suffix  . :
   Description . . . . . . . . . . . : Intel(R) Dual Band Wireless-AC 7260
   Physical Address. . . . . . . . . : D8-FC-93-74-9C-A8
   DHCP Enabled. . . . . . . . . . . : Yes
   Autoconfiguration Enabled . . . . : Yes
   Link-local IPv6 Address . . . . . : fe80::b884:2374:293e:7d49%15(Preferred)
   IPv4 Address. . . . . . . . . . . : 192.168.0.240(Preferred)
   Subnet Mask . . . . . . . . . . . : 255.255.255.0
   Lease Obtained. . . . . . . . . . : Thursday, October 29, 2020 8:45:24 AM
   Lease Expires . . . . . . . . . . : Thursday, October 29, 2020 11:45:24 AM
   Default Gateway . . . . . . . . . : 192.168.0.1
   DHCP Server . . . . . . . . . . . : 192.168.0.1
   DHCPv6 IAID . . . . . . . . . . . : 114883731
   DHCPv6 Client DUID. . . . . . . . : 00-01-00-01-22-02-F9-BA-34-E6-D7-35-08-F8
   DNS Servers . . . . . . . . . . . : 192.168.0.1
   NetBIOS over Tcpip. . . . . . . . : Enabled

Ethernet adapter Bluetooth Network Connection 2:

   Media State . . . . . . . . . . . : Media disconnected
   Connection-specific DNS Suffix  . :
   Description . . . . . . . . . . . : Bluetooth Device (Personal Area Network) #2
   Physical Address. . . . . . . . . : D8-FC-93-74-9C-AC
   DHCP Enabled. . . . . . . . . . . : Yes
   Autoconfiguration Enabled . . . . : Yes

C:\Users\Owner>
```

Fig. 17: Other network adapters that can be disregarded.

8) For the camera to be properly configured, the first three sets of numbers of the IP address must match your Ethernet adapter's IP Address (inside the green box in the screenshot above). The last set of numbers can be any number between 1 and 255. In this

example, the Ethernet's IP Address is 169.254.20.120. Thus, we set the Camera's *IP Address* to **169.254.20.100** by typing these numbers in the IP address tab of the "Edit IP Address" window. The *Subnet Mask* of the "Edit IP Address" window must remain at the default value of **255.255.255.0** set by the camera software developer.
After updating the IP Address, click "Test IP Address" to check if the address is available for the camera to use.

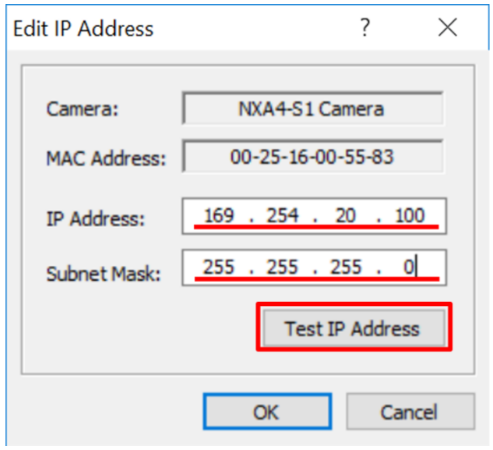


Fig. 18: Updating the IP address and subnet mask of the camera.

9) The "Info" window will pop up. If the IP Address is available, click "OK" and proceed to the next step.

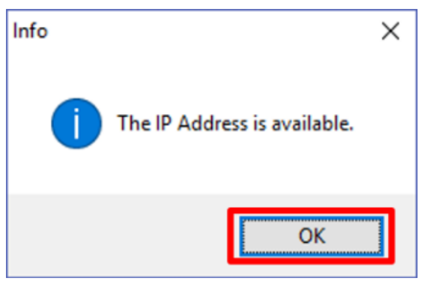


Fig. 19: Window indicating if the newly entered IP address is available.

If the IP Address is already taken, return to the previous step and set the last set of numbers of the IP address to a different value within 1 to 255.

10) In the "Edit IP Address" window, click "OK".

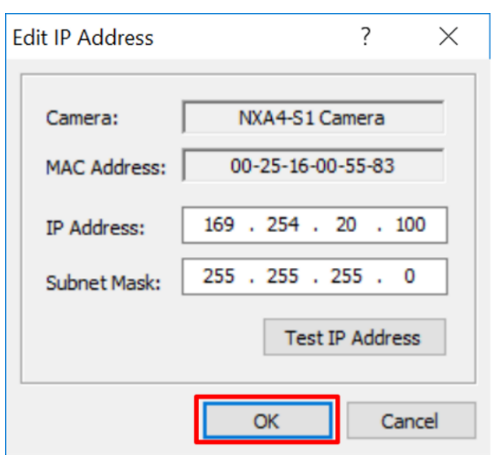


Fig. 20: Clicking "OK" after updating the IP address and subnet mask.

11) The following "Warning" window will appear. Click "OK".

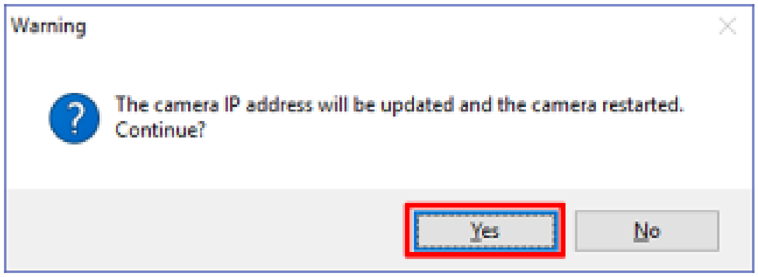


Fig. 21: Warning when the IP address is about to be updated.

12) The same "Warning" message as in **Step 5)** (**Fig. 14**) may appear. If it does, click "OK". After a few seconds of reconfiguring, you will return to the "Open Cameras" window. The warning symbol should no longer be there. If so, click "Open" and a window will indicate that the software is initializing.

If the warning message is still there, return to **Step 7)** and set a new IP Address.

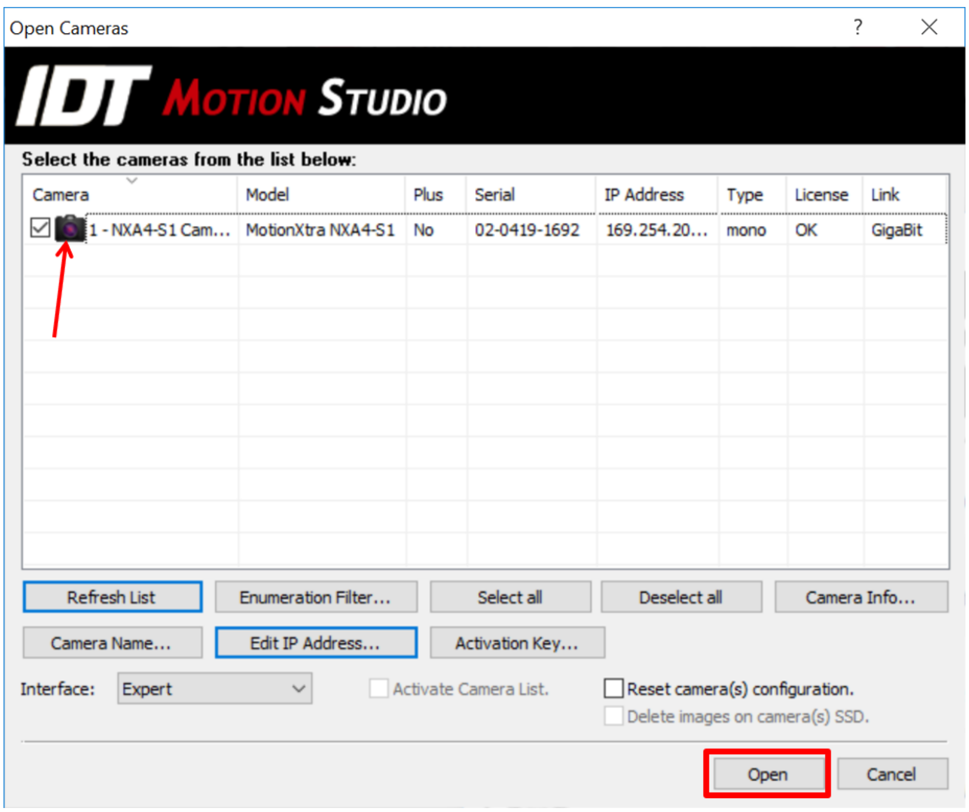


Fig. 22: List showing the detected camera showing no warning icons after the IP address has been updated.

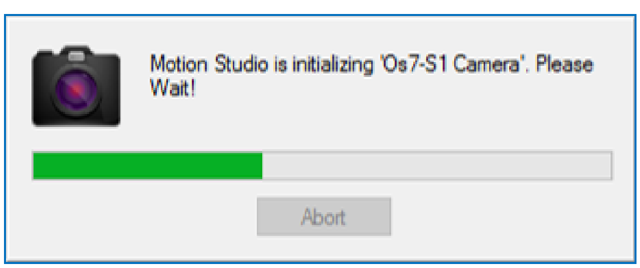


Fig. 23: Window showing the status as the software initializes the camera.

13) The Motion Studio software will open and is ready to use.

Note: The screen may be dark if the light source is not turned on.

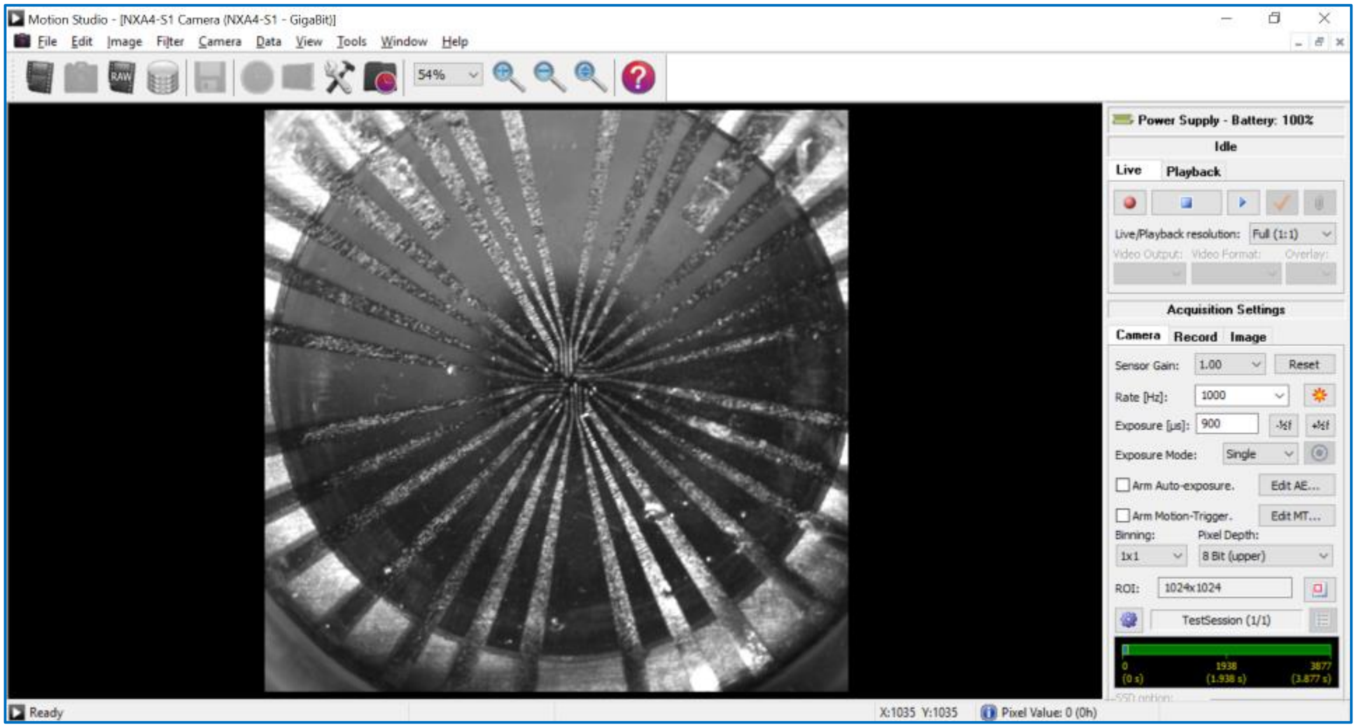


Fig. 24: The Motion Studio main window after connecting to the camera.

14) Click the "Live" button to ensure the image is live.

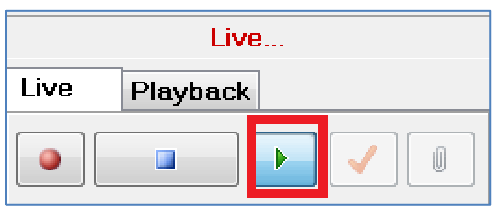


Fig. 25: The "Live" button.

4 Protocol for Software Initial Setup

The camera will be mounted into the frame as shown in **Fig. 1** and **Fig. 26**.

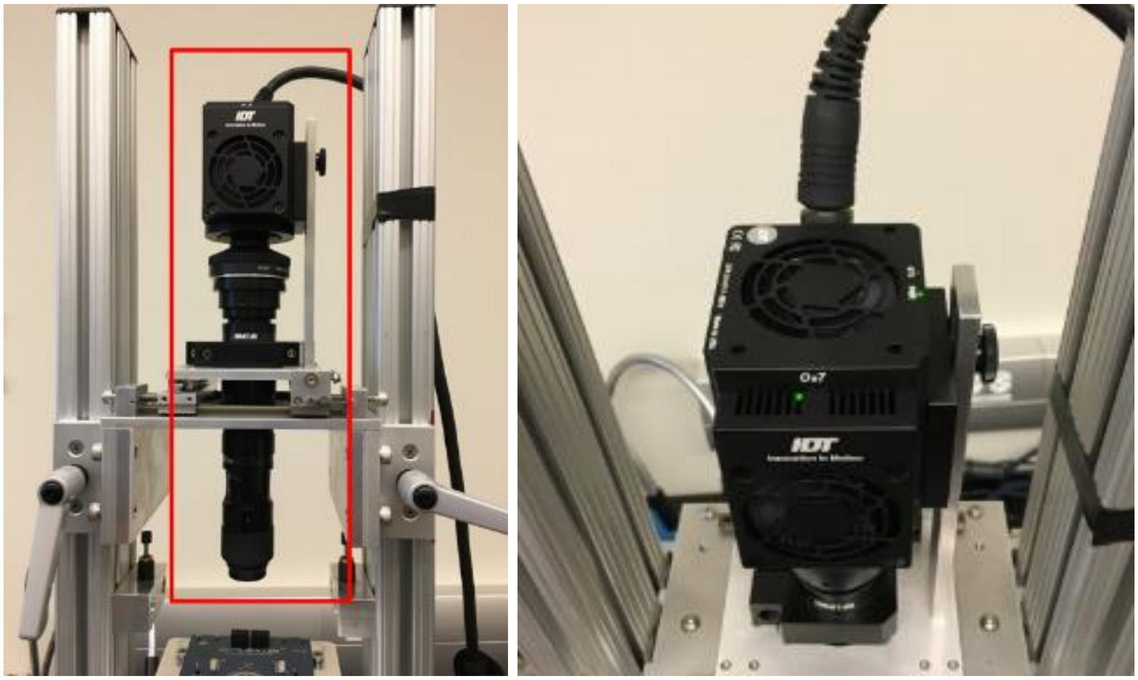


Fig. 26: The camera and other imaging components mounted to the MEASSuRE frame.

<u>Note</u>: See **Appendix A** for the protocol to swap camera lenses and adapter tubes if necessary.

Looking at the camera from the top, we can define the x- and y-coordinates of the camera stage as shown in the following figure.

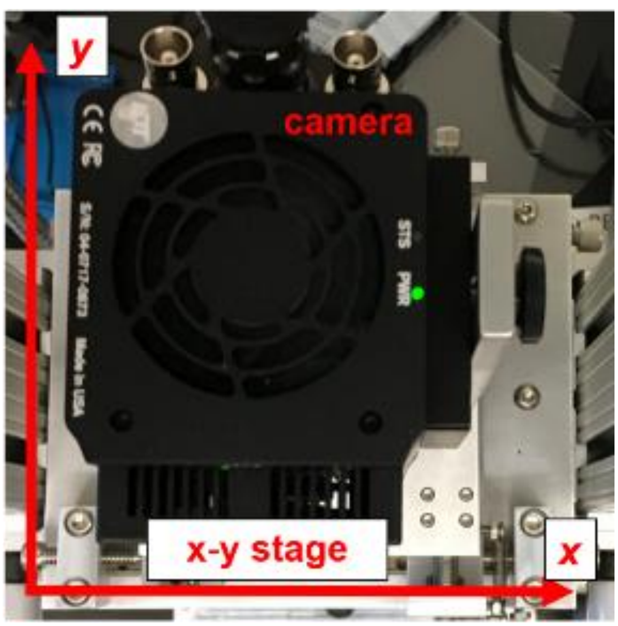


Fig. 27: Top view of the camera with the x- and y-axes displayed.

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

1) The vertical z-position can be adjusted by loosening the two knobs following a counter-clockwise motion (cyan in **Fig. 28**), moving the camera platform up or down, and re-tightening the knobs (applying a clockwise motion). The camera is held in place by an x-y stage which can be used to adjust the camera's position.

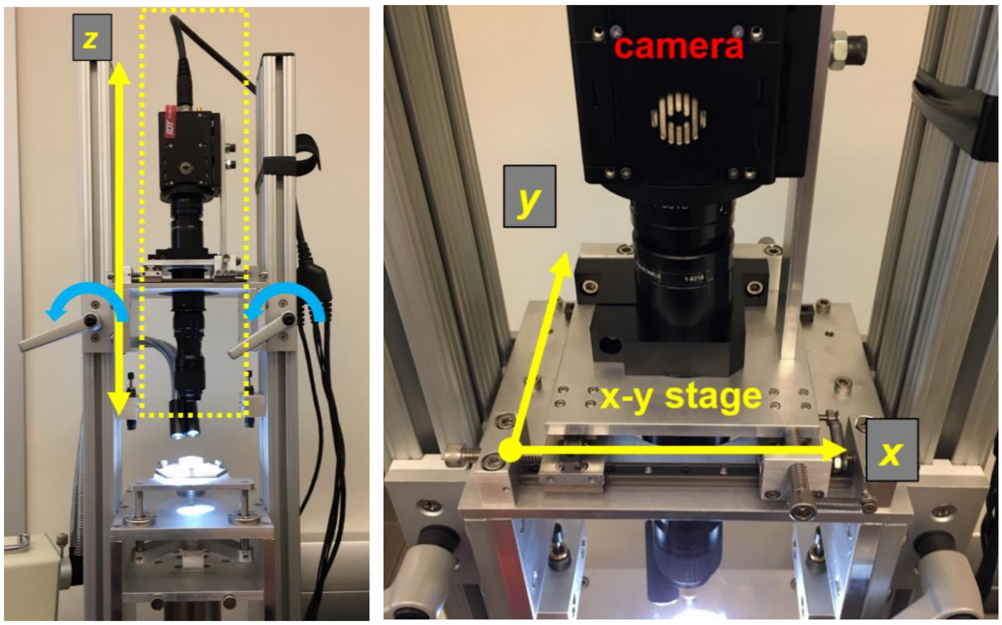


Fig. 28: Adjusting the camera position in all three axes.

<u>Note</u>: See **Appendix B** for a table listing working distances for various imaging hardware configurations, which serve as references when finding an optimal vertical z-position.

2) Note the orientation of the sMEA on the movable platform (simple mechanical holder in **Fig. 29** below) of the MEASSuRE frame.

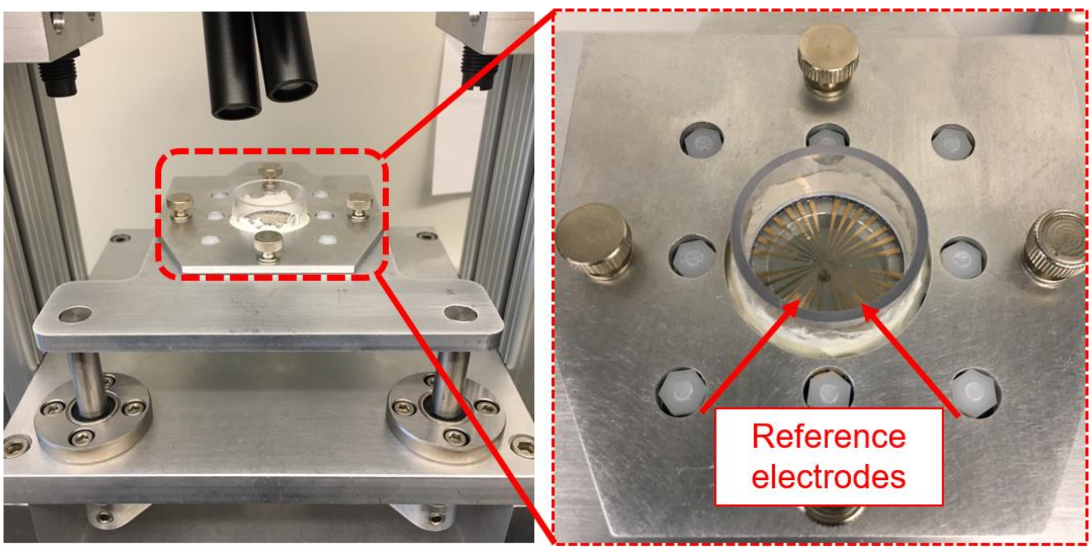


Fig. 29: sMEA in the mechanical holder with the reference electrodes towards the front.

3) Note the orientation of the sMEA as displayed in the camera image.

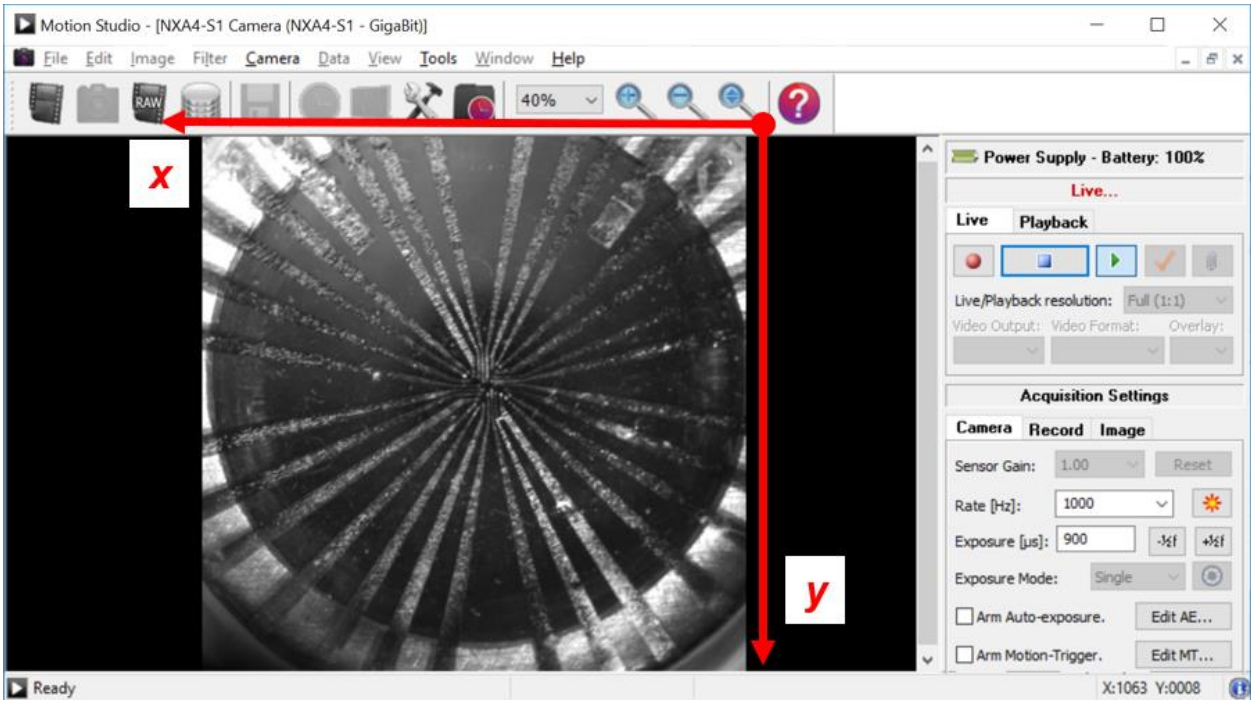


Fig. 30: Live view of the sMEA with Motion Studio with the x- and y-axes labeled. In this example, the reference electrodes are located towards the back.

4) To adjust the orientation of the image so that it matches the physical orientation of the sMEA on the platform: (i) right-click on the image; (ii) go to "Image"; (iii) click on "Orientation"; (iv) select the corresponding rotation (in this example, "180 Degrees CW") or flip; and (v) click "Ok."

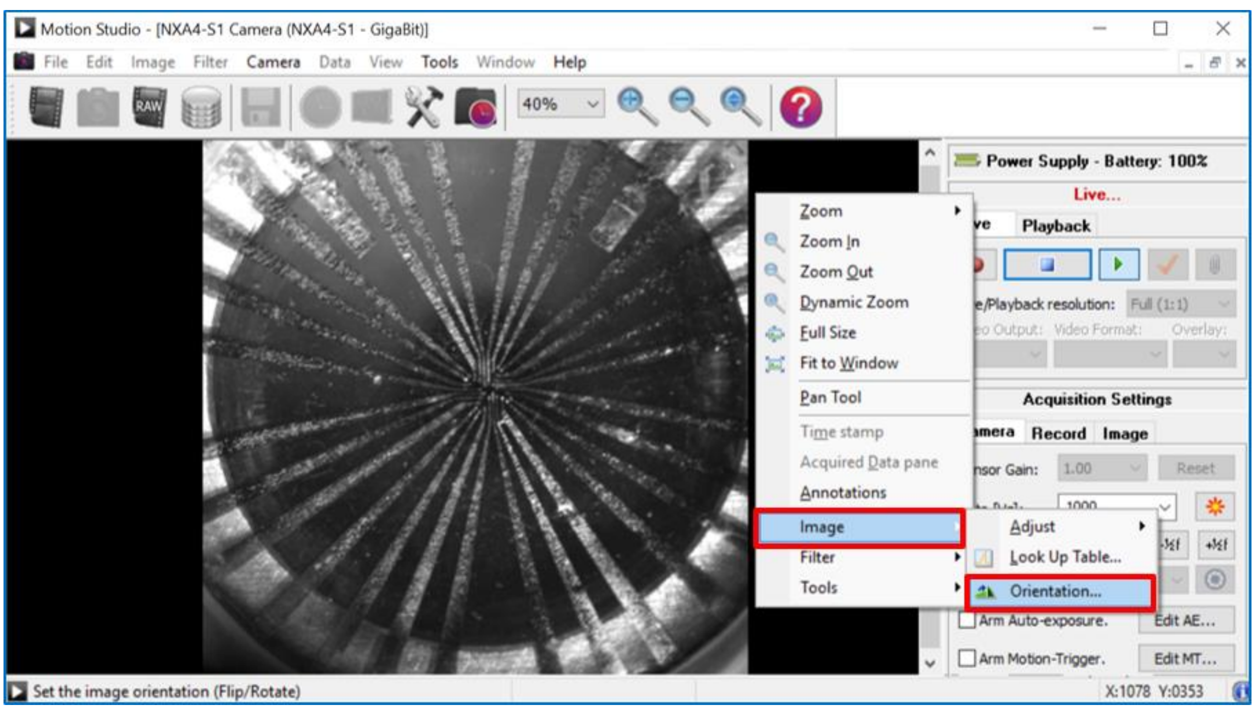


Fig. 31: Adjusting the orientation of the image captured by Motion Studio.

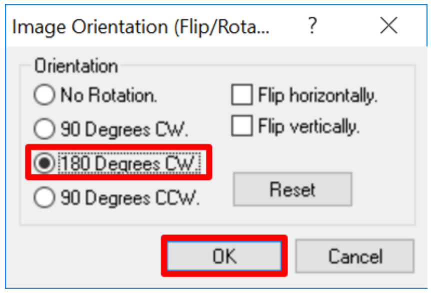


Fig. 32: Options when adjusting the image orientation.

5) The orientation of the displayed image should match the physical orientation of the sMEA.

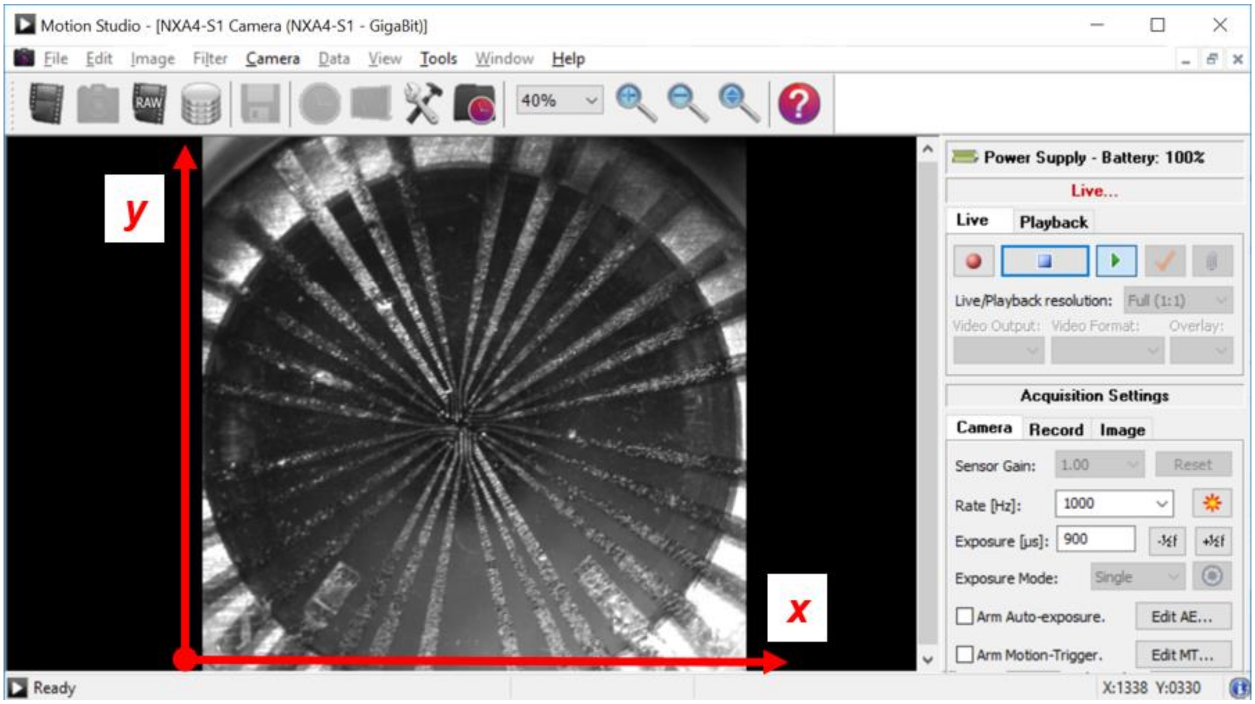


Fig. 33: Updated image with the orientation shown by the software matching the physical orientation of the sMEA when viewed from the top.

6) Adjust the brightness of the light source (e.g., LED-50WY, AmScope) as needed. This is particularly important when recording at a high frame rate since exposure time is limited and at high magnifications when light is limited.

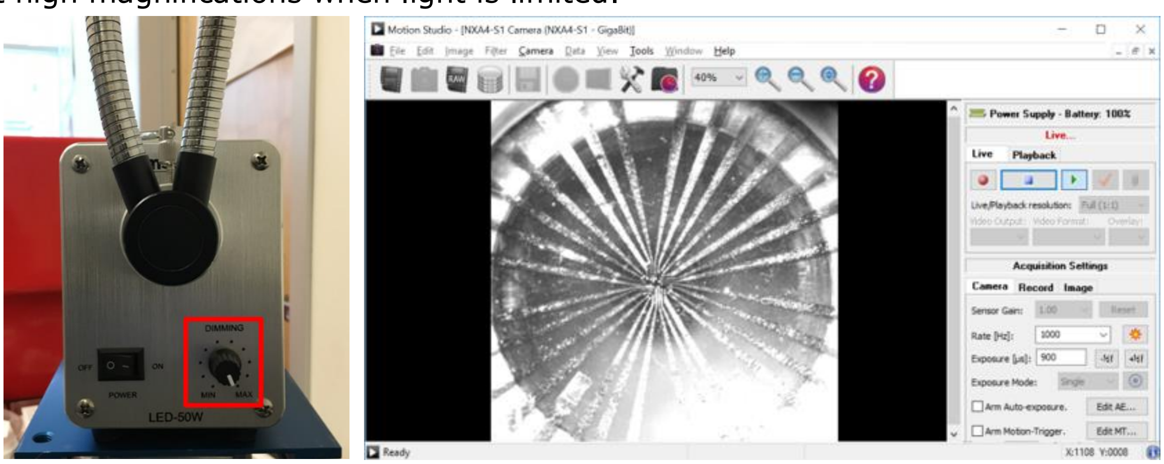


Fig. 34: Adjusting the image brightness using the external light source.

5 Protocol to Record Videos

MEASSuRE-X and Premium systems use **IDT cameras** (model **OS7-V3-S1**, **NXA4-S1**, or **CCM-1510**) to record videos.

5.1 Loading the Motion Studio x64 Software

1) Open the Motion Studio x64 software.
2) In the pop-up Main Menu window, select "Cameras" and then click "OK".

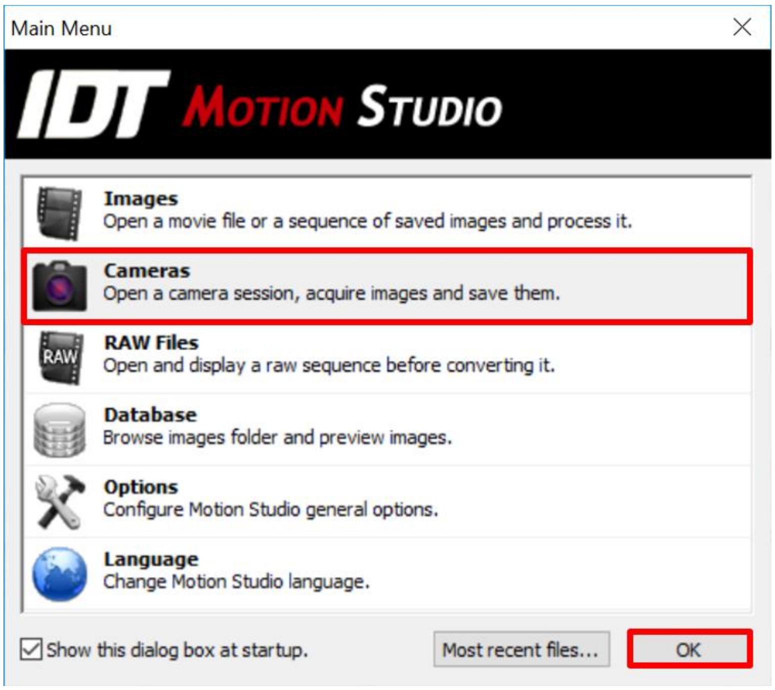


Fig. 35: Selecting "Cameras" to open a camera session.

3) In the Camera Enumeration Filter, check the box "MotionXtra N/NR/NXOs/CC on Giga-Ethernet" and click "Ok."

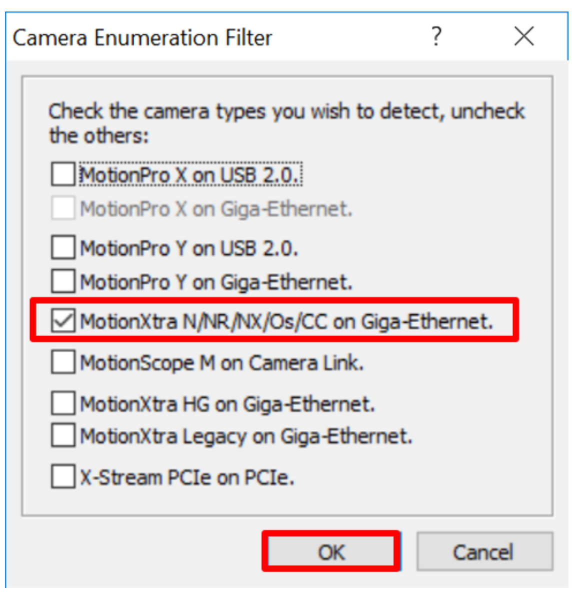


Fig. 36: Selecting the types of cameras for Motion Studio to detect.

4) In the Open Cameras window, check the box for the camera "1-Os7-S1 Camera," "1-NXA4-S1 Camera," or "1-CC-1510 Camera" and click "Open."

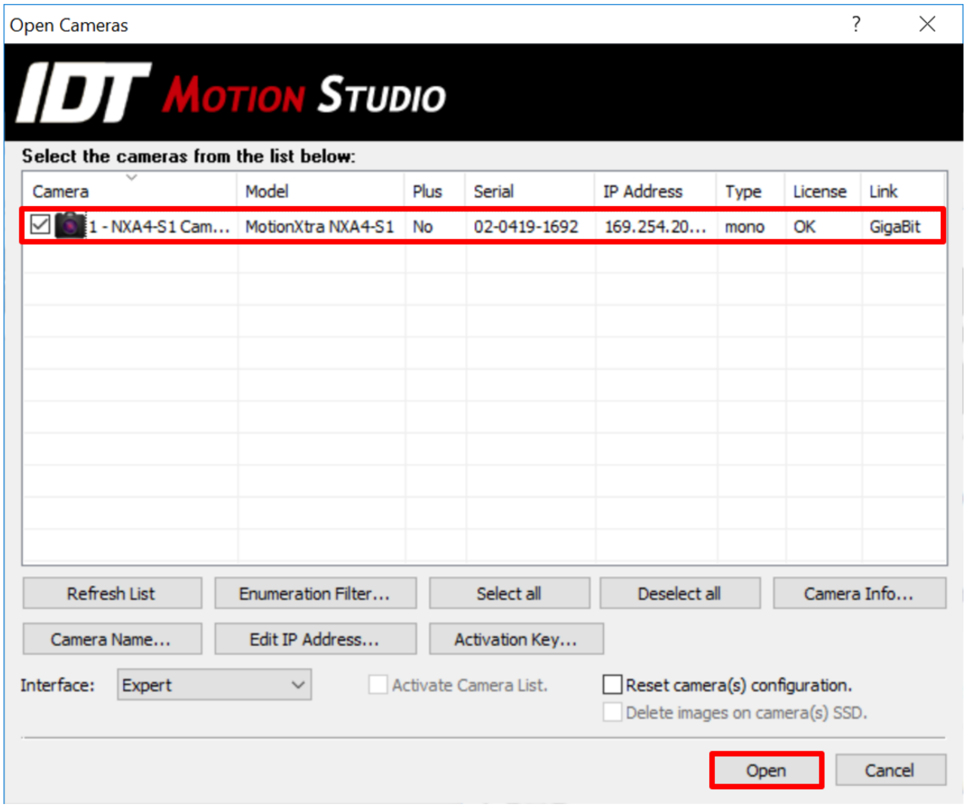


Fig. 37: Opening the camera connected to the computer.

5) The software will load, and a camera snapshot will be displayed.

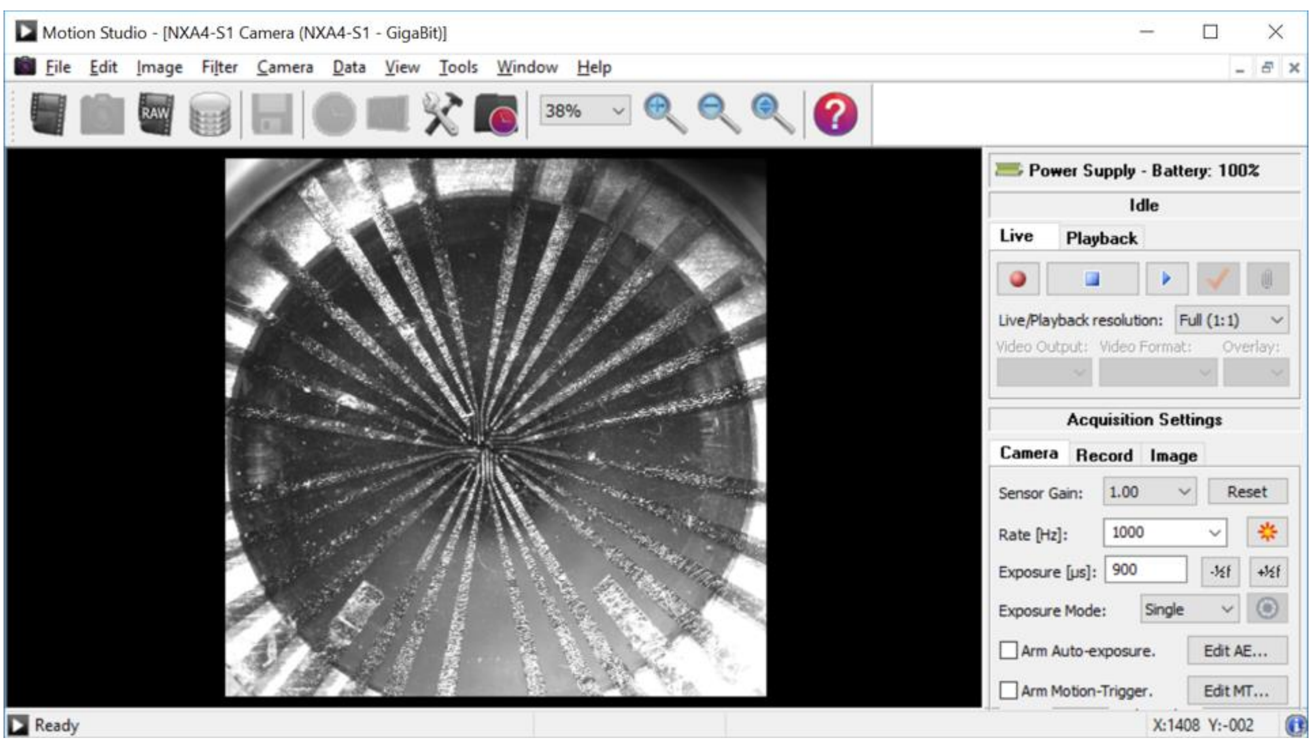


Fig. 38: Motion Studio's main window showing a snapshot of the connected camera.

5.2 Pre-Recording Setup

1) If necessary, adjust the camera's height by loosening the handles (counterclockwise rotation) and moving the camera stage up or down. Once adjusted, re-tighten the knobs (clockwise rotation).

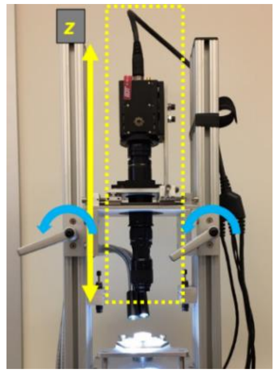
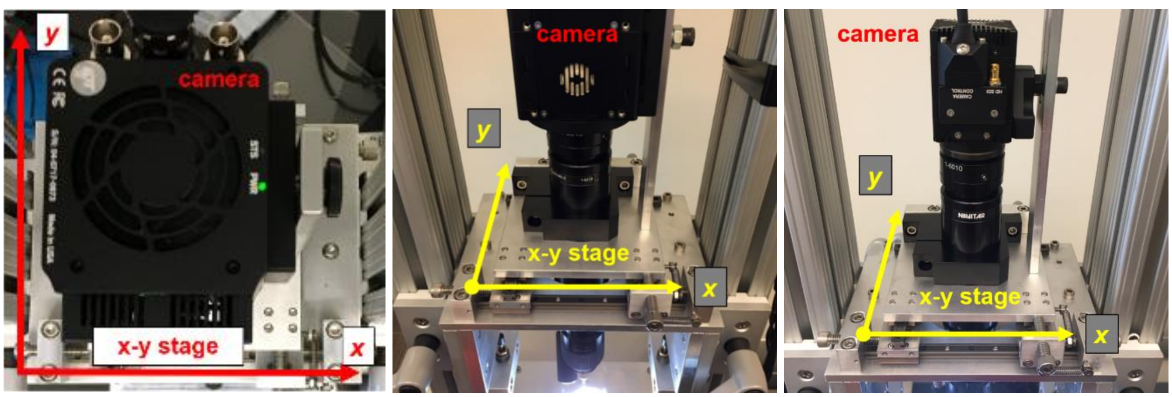


Fig. 39: Adjusting the vertical position of the camera.

2) Adjust the camera's stage by physically moving the camera stage in the x- and y-directions to center on the area of interest.



(a) (b) (c)

Fig. 40: Adjusting the x- and y-positions of the (a) OS7 camera, (b) NXA4 camera, and (c) CCM camera.

3) Adjust the zoom using the zoom control (shown in red in **Fig. 41**) on the camera lens.

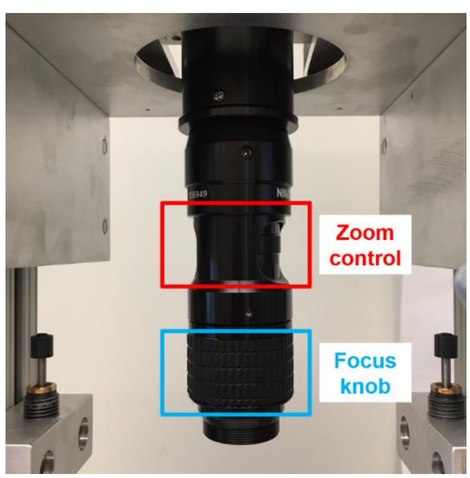


Fig. 41: The Zoom 6000 lens and its zoom control and focus knob.

4) Adjust the lens focus by using the focus knob (shown in cyan in **Fig. 41**).

5.3 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.

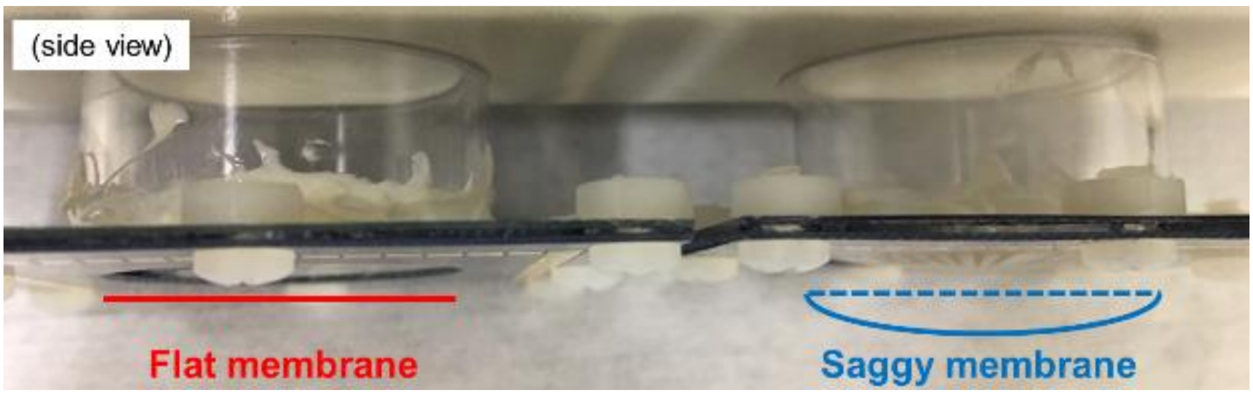


Fig. 42: Side view of sMEAs displaying a flat (taut) and a saggy PDMS membrane.

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 -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 strongly recommended to verify the flush position using the voice coil actuator (VCA) and camera** to avoid any discrepancy between the desired/target strain and the actual measured strain. Furthermore, 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 Motion Studio to monitor the sample.

1) Use the Motion Studio x64 software to adjust the camera position, zoom, focus, and lighting to ensure that a clear image is obtained.
2) 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 you are using.
    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 User Manual** for further details on how to operate the VCA.

3) In MotionLab, click on the "Position" tab and set the manual increment to 100 µm. Note that MotionLab will automatically modify the increment value based on the conversion between "counts" and microns, as set by the "stroke" motor parameter.

4) Click the [Enable Motor] button.

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

6) Remove the two aluminum homing blocks underneath the platform.

7) 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 sMEA silicone membrane.

8) 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.

9) 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).

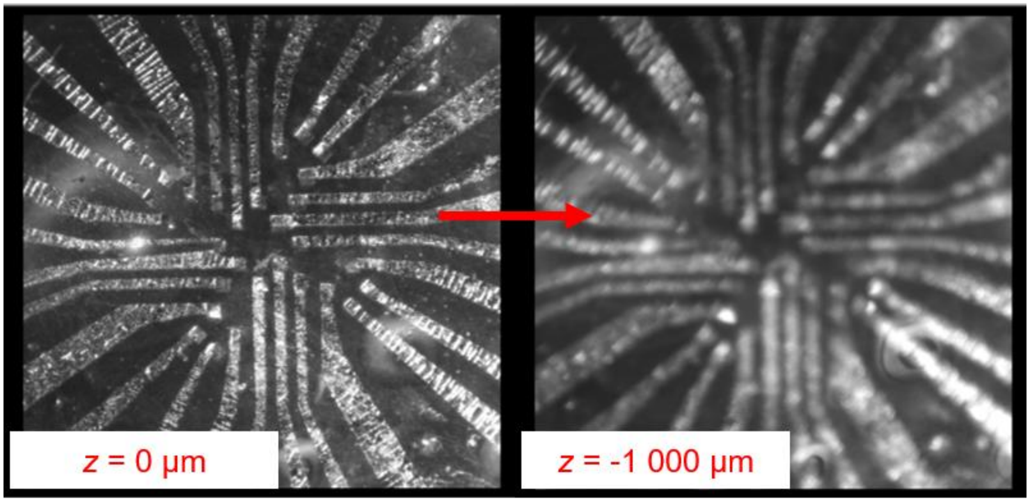


Fig. 43: Images showing loss of focus when a sample is moved down by 1000 µm, indicating that the higher position is **not** the flush position.

10) 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)**.

11) **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.

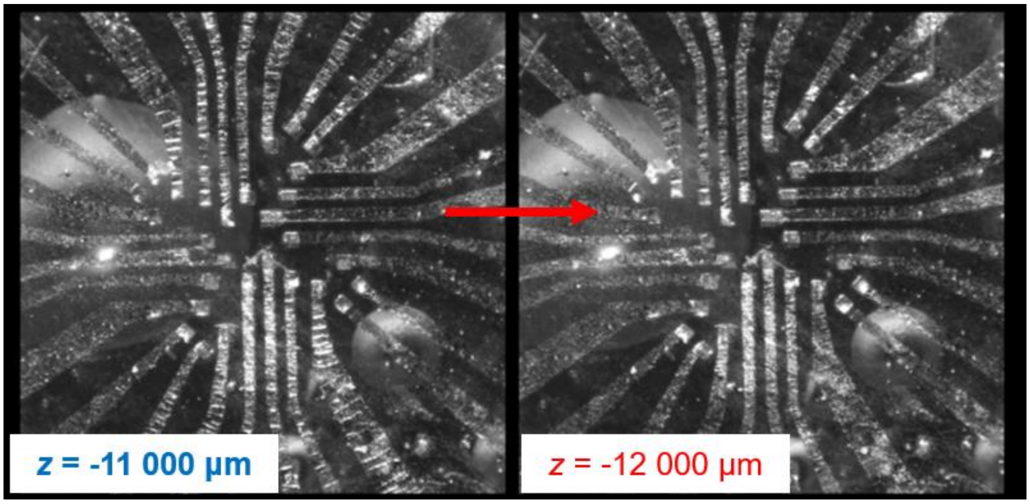


Fig. 44: Images showing that focus remained when a sample is moved down by 1000 μm, indicating that the starting higher position is the **flush position**.

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.

Fig. 45 below shows 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.

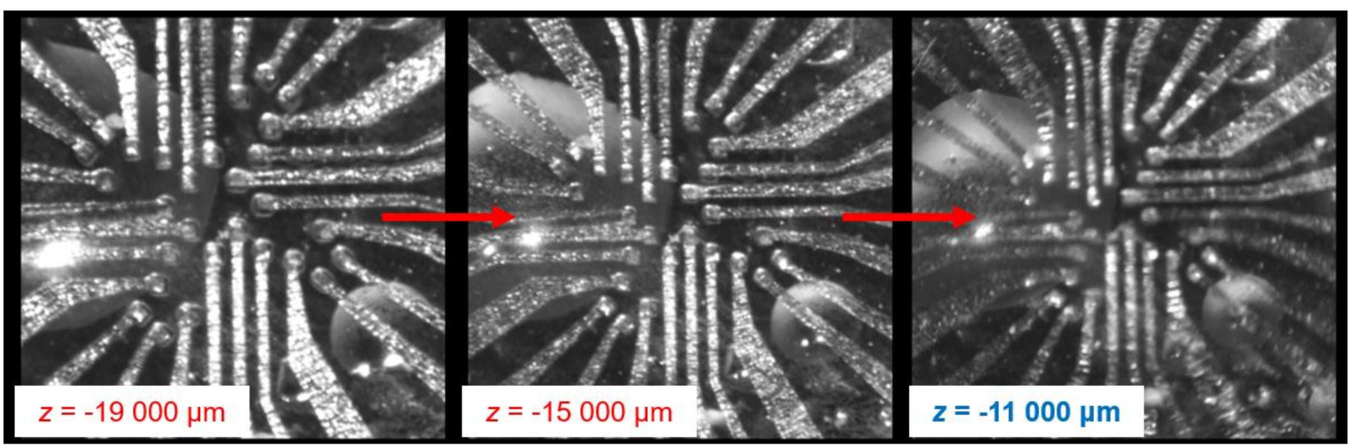


Fig. 45: Verifying the flush position by examining the focus as the VCA is moved.

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

5.4 Setting Up the VCA prior to Video Recording

Using the determined **flush position** and the protocol described in the **Mechanics Module User Manual**, set the VCA's movements. **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:

1. <u>Impulse stimulation</u>: 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 (e.g., <100 ms).
2. <u>Cyclic stimulation</u>: The platform moves following a sinusoidal profile. The user can specify the baseline position, amplitude, and frequency of 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.

5.5 Saving an Image

Motion Studio allows the user to easily save images.

1) Once the software has loaded, click on the "Live" button (i.e., ► icon) in the "Live" tab. When not recording, the status bar will display the camera status as "Idle."

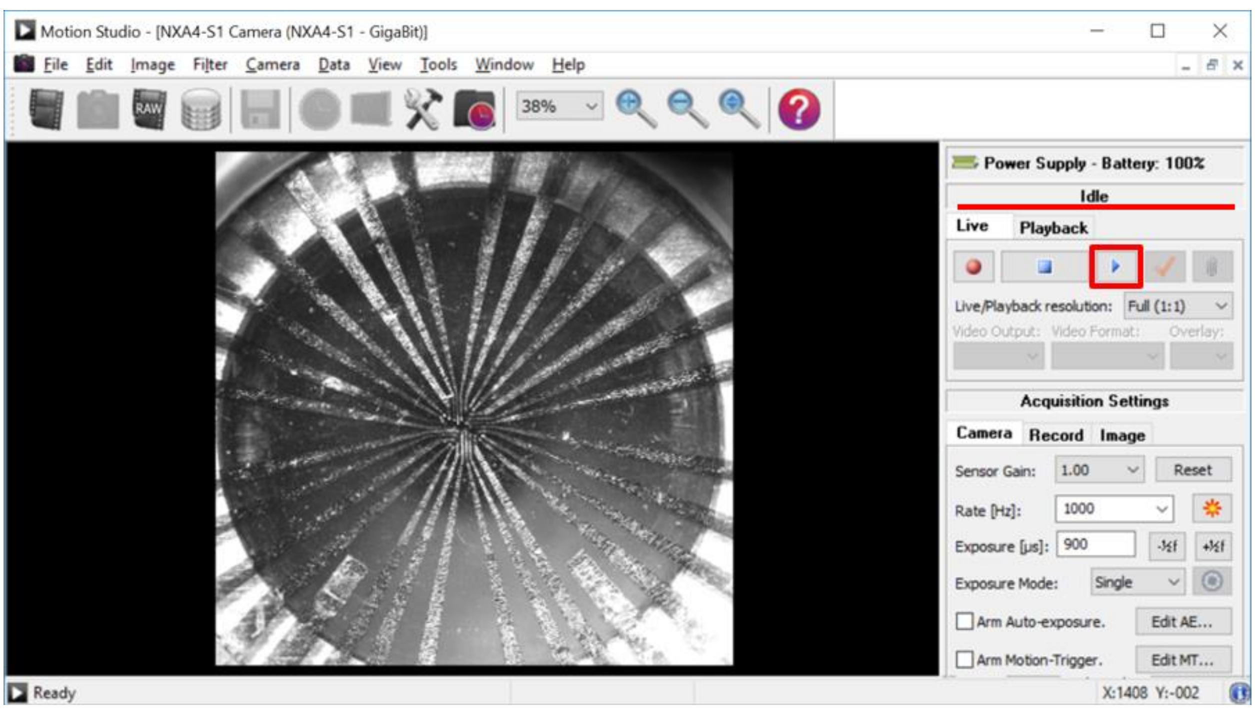


Fig. 46: Enabling the "Live" view.

2) Make sure the area of interest is being displayed and that the image is in focus. If necessary, adjust the settings of the camera and/or those of the VCA.
<u>Note</u>: the status bar will now display "Live…" in red font.

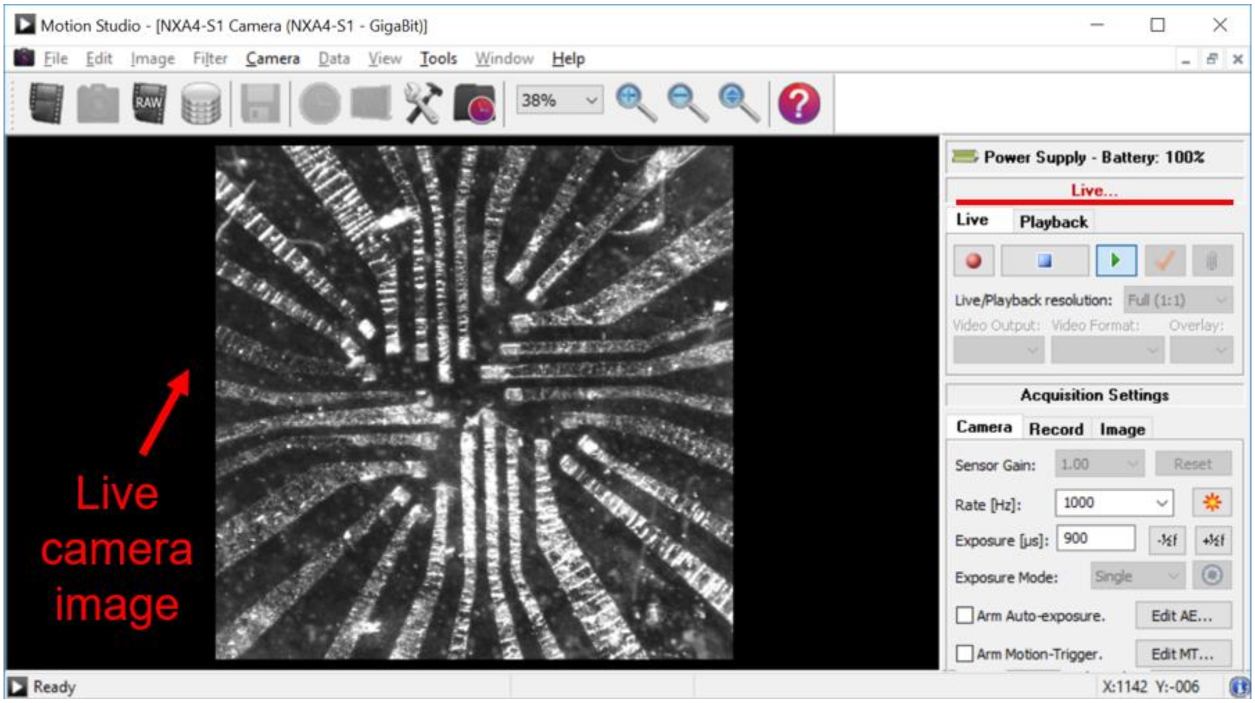


Fig. 47: "Live" view from the connected camera.

3) Once the image is ready to be captured click the "Stop" button ("Live" tab). The last image or frame from the live session will be displayed. Note: status returns to "Idle."

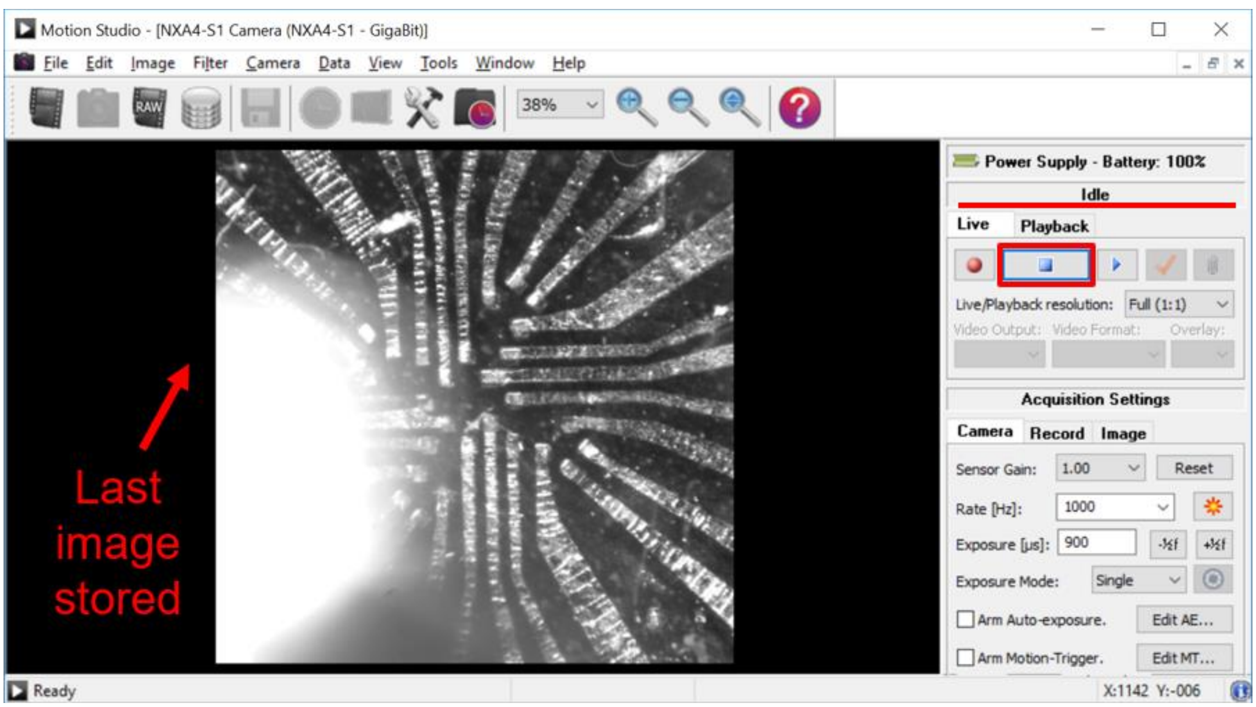


Fig. 48: Last image stored before the camera's live view was stopped.

4) On the main menu, go to "File" and click "Save snapshot." A pop-up message saying "Motion Studio is saving a snapshot image. Please wait." will be displayed.

5) Once the pop-up window closes automatically, the snapshot will be saved and ready to view. To find the location where the images are saved, click on "Tools" and then "Options." The directory is displayed under "Image Download Folder."

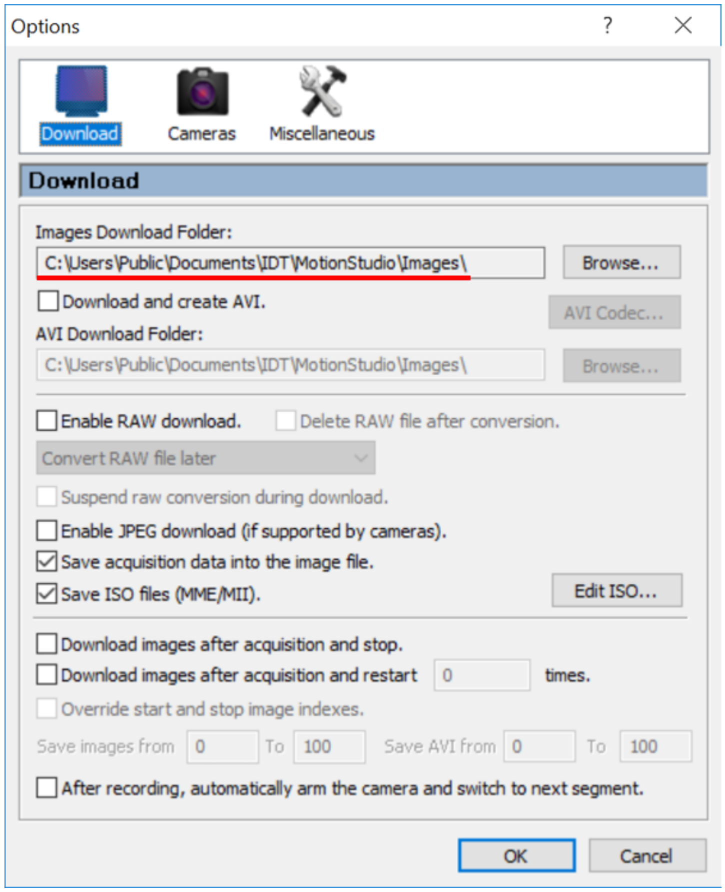


Fig. 49: The Options window showing the directory where files are saved to.

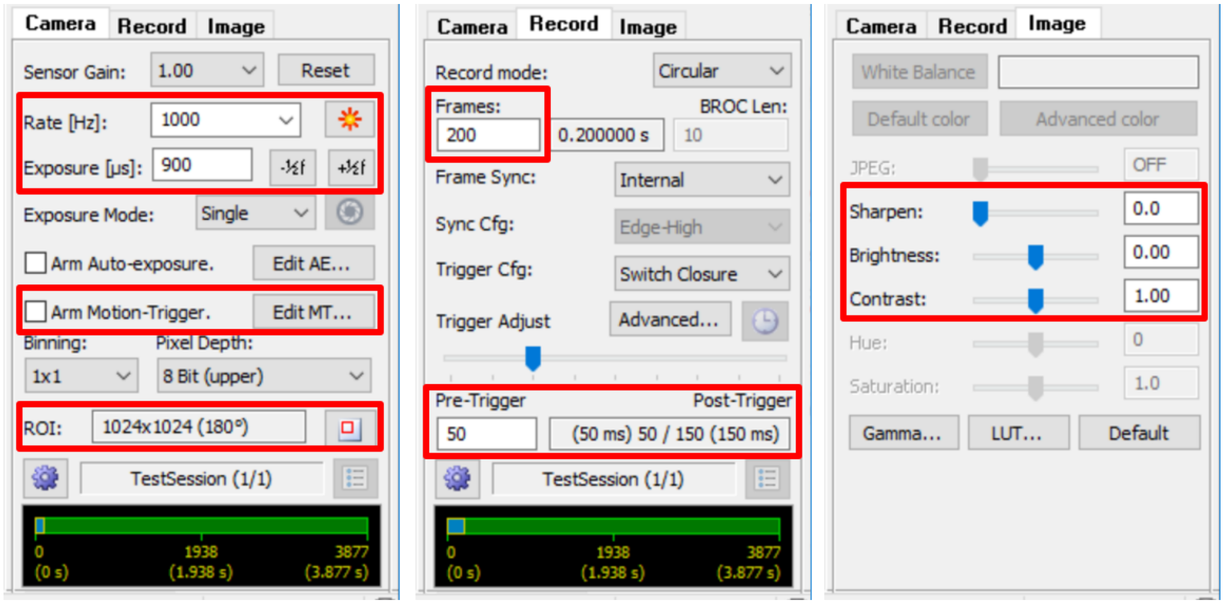
5.6 Recording a Video

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

1) It is highly recommended to perform one or multiple preliminary stretches to ensure the area of interest is always captured by the camera and displayed in the Motion Studio x64 window. If not, make sure to make any corrections with the movable stage and lens zoom control.

2) **Image Optimization:** Under the "Acquisition Settings" menu, there are three tabs with setup parameters for the recording of the video. Set the different parameters accordingly. Here is a brief description of some of these parameters.

   Note: A more detailed image optimization protocol is presented in **Section 6.**



(a) (b) (c)

Fig. 50: The different tabs of the acquisition menu that show camera parameters.

- <u>"Camera" tab</u>:
  - The *recording rate* and *exposure time* are critical to obtain a clear recording. The greater the rate, the darker the acquired image will be. Thus, it is important to have a powerful light source to provide enough illumination. **For lighting purposes, you want to maximize exposure time. However, keep in mind that larger exposure times also increase motion blur. Thus, a good balance is needed.** Rate and exposure time are inversely related. For example, if recording at a rate of 1000 Hz, exposure time cannot be greater than 1 ms (as shown **Fig. 50a**).
  - The *low light* button (the yellow/orange sun button) will change the rate to 30 fps and the exposure time to its maximum.
  - The *Arm Motion-Trigger* checkbox and *Edit MT…* button allow the user to setup a trigger during the mechanical stimulus. The former should be checked. The latter opens the Motion-Trigger window. The trigger mode should be selected to "Motion Detection" and the trigger level should be set appropriately (the more to the left the level is set, the more sensitive the trigger will be).

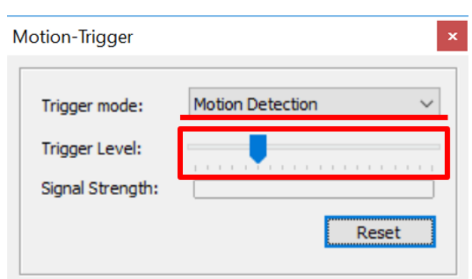


Fig. 51: The "Motion-Trigger" window to set the trigger mode and sensitivity.

  - The region of interest, or *ROI*, can be specified as well to change the resolution of the acquired images/frames. Select the tab with the red square () to make changes to the ROI. The camera needs to be in "Idle" mode to change the ROI.

   - The "Arm Auto-exposure" must remain unchecked as enabling this feature results in the camera automatically adjusting the exposure to match the average value in the specified region of interest.

- "Record" tab:

   - In this tab, the *number of frames* to be recorded can be specified.
     Note: Dividing the number of frames by the recording rate (from the "Camera" tab) determines the total duration of the recording.

   - The *pre-trigger* frames specify the number of frames that will be recorded prior to the trigger being activated. The *post-trigger frames* are calculated and displayed next to the pre-trigger frames.

   - Setting *Record mode* to "Circular" results in the camera waiting for an event trigger to complete the acquisition and restarting when the memory is filled.

   - The parameters *Frame Sync*, *Sync Cfg*, *Trigger Cfg*, and *Trigger Adjust* are currently not used with MEASSuRE systems.

- "Image" tab: Use the sliders to change the *sharpness*, *brightness*, and *contrast* of the acquired frames.

3) Set the VCA so that it is ready to perform the motion of interest.

4) In this step, there are two different ways to start or trigger the recording: i) manual triggering with the "Camera trigger" button, or ii) automatic triggering with the "Motion trigger." Each method is described below. **At the end of this step, the frames will be stored in the camera memory and subsequently be saved to the computer.**

   i. **Manual triggering** using the "Camera trigger" button:

      - In the "Live" tab, click the "Record (Arm)" button. Ensure that "Arm Motion-Trigger" is unchecked.

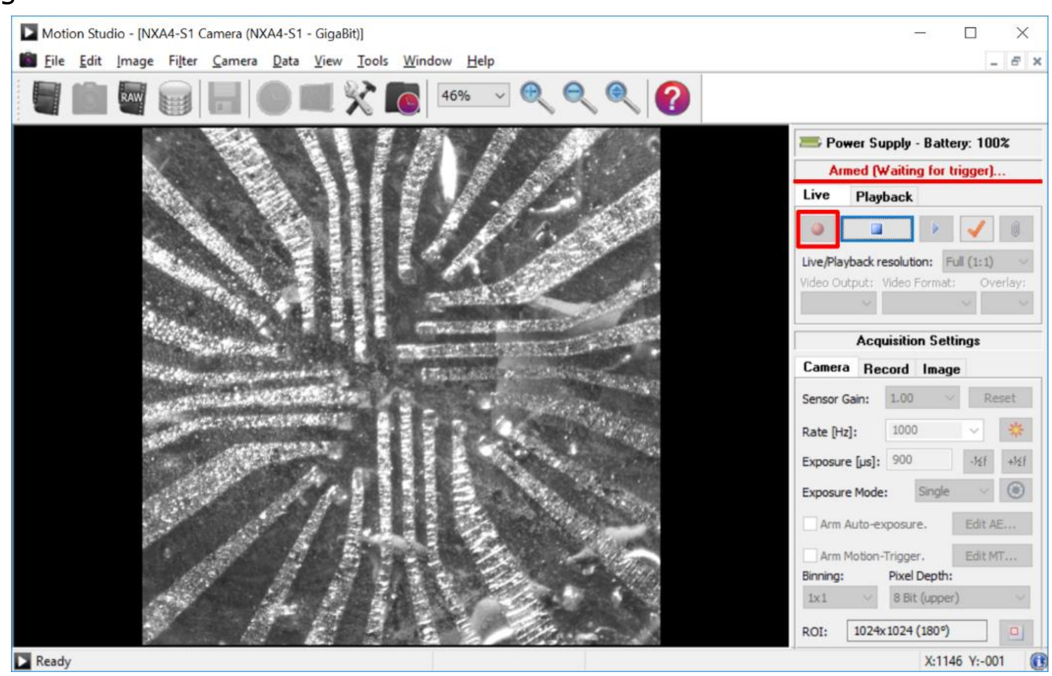


Fig. 52: Arming the camera to wait for a trigger to record.

Note: The status will change to "Armed (Waiting for trigger) …" in red font.

- **Right before executing the VCA motion, click the "Camera trigger" button (orange checkmark button) in the "Live" tab to start the video recording.**
  Note: The motion of the VCA must be completed within the specified camera recording duration.

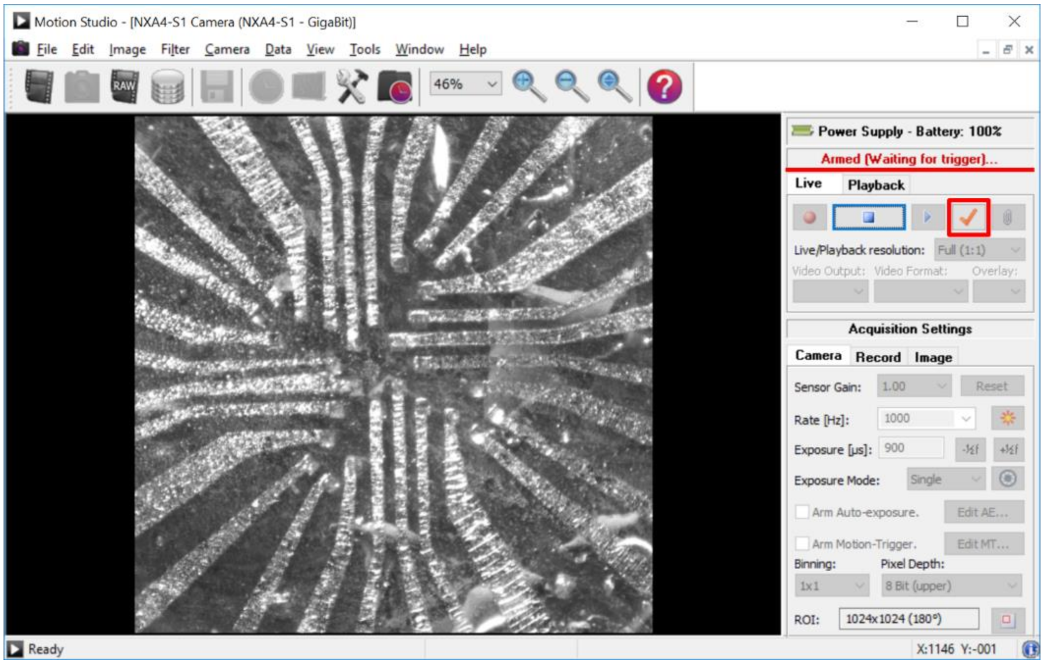


Fig. 53: Clicking the "Camera Trigger" button to manually start video recording.

- In the "Info" pop-up window, click "OK".
  Note: the status will now display "Images in Memory" with a red font.

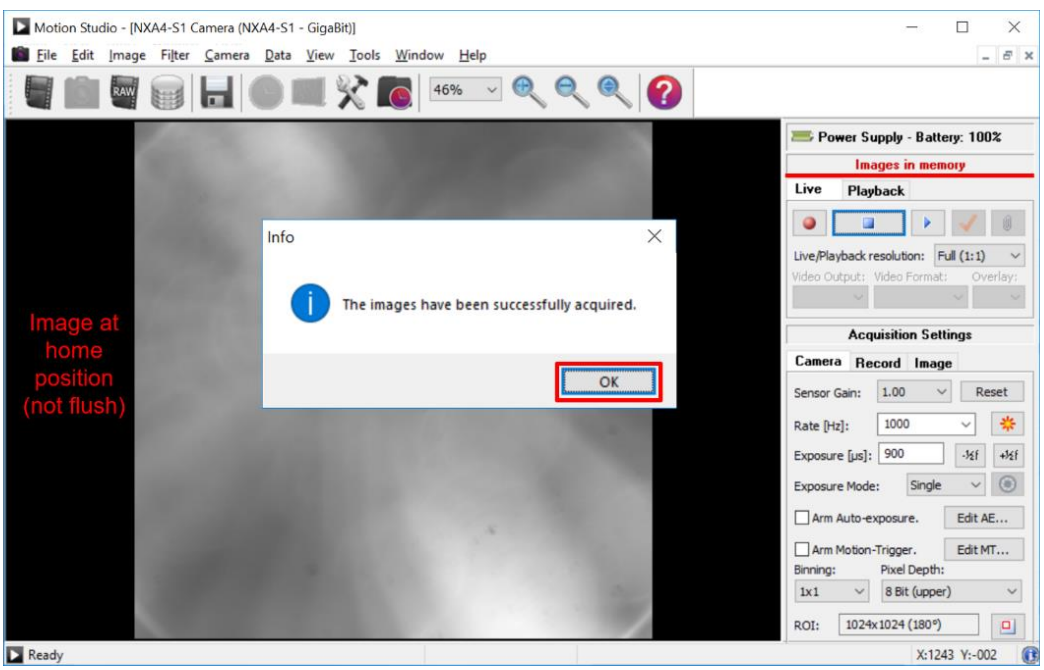


Fig. 54: "Info" window showing that the images were successfully recorded.

ii. **Automatic triggering** using the "Motion triggering" feature – **Recommended**

The Motion Trigger feature allows the user to trigger the camera to record images automatically when the VCA motion starts. This is the recommended procedure for recording images while stretching the sMEA because it does not require the user to manually trigger the camera prior to triggering the VCA motion.

- In the "Camera" tab, click the "Edit MT …" button. MT stands for Motion Trigger.

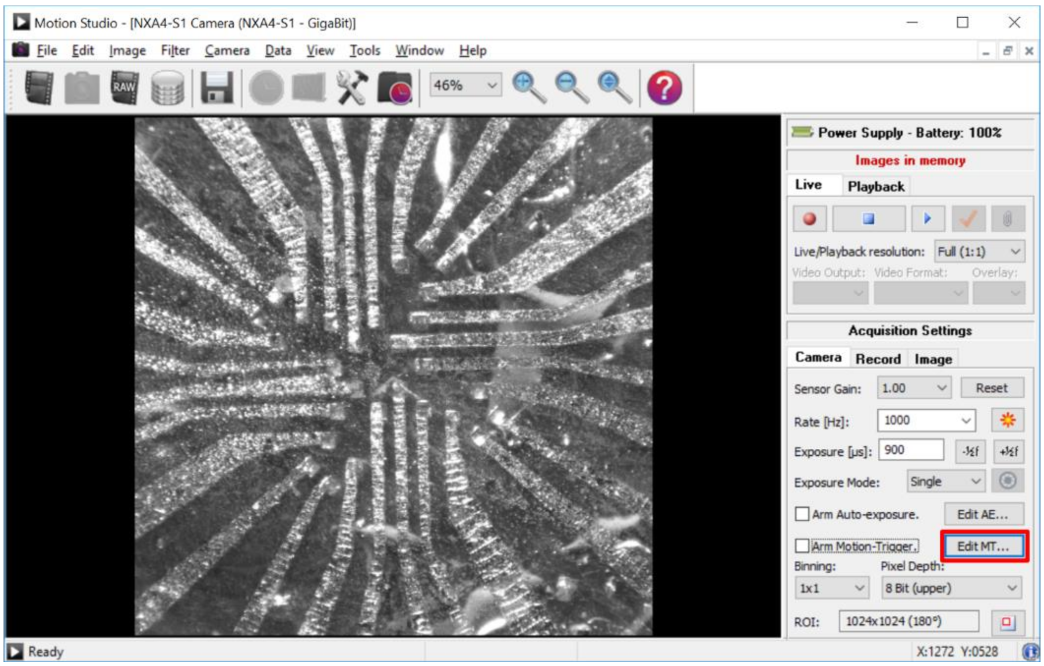


Fig. 55: Accessing the motion trigger parameters by clicking "Edit MT."

- In the "Motion-Trigger" pop-up window, you can change the *Trigger mode* parameter, which sets how the camera is triggered, as well as the *Trigger Level*, which set the sensitivity of the trigger. The latter one refers to the sensitivity of the trigger.

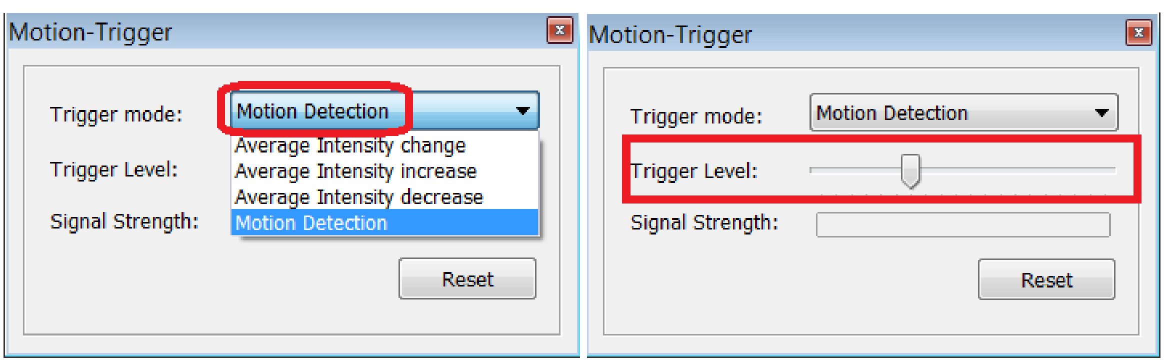


Fig. 56: Setting the trigger mode and trigger level in the "Motion-Trigger" window.

We recommend using the Trigger Mode "Motion Detection" and a low-medium setpoint for the Trigger Level.

Note: While this window is open, the sensitivity can be tested in real-time. For instance, you may perform a slow motion with a short travel in the VCA. If the signal strength is larger than the trigger level, the trigger would have been activated.

- Once you have set these parameters, close the "Motion-Trigger" window. **Note**: the "Arm Motion-Trigger" will now be checked.

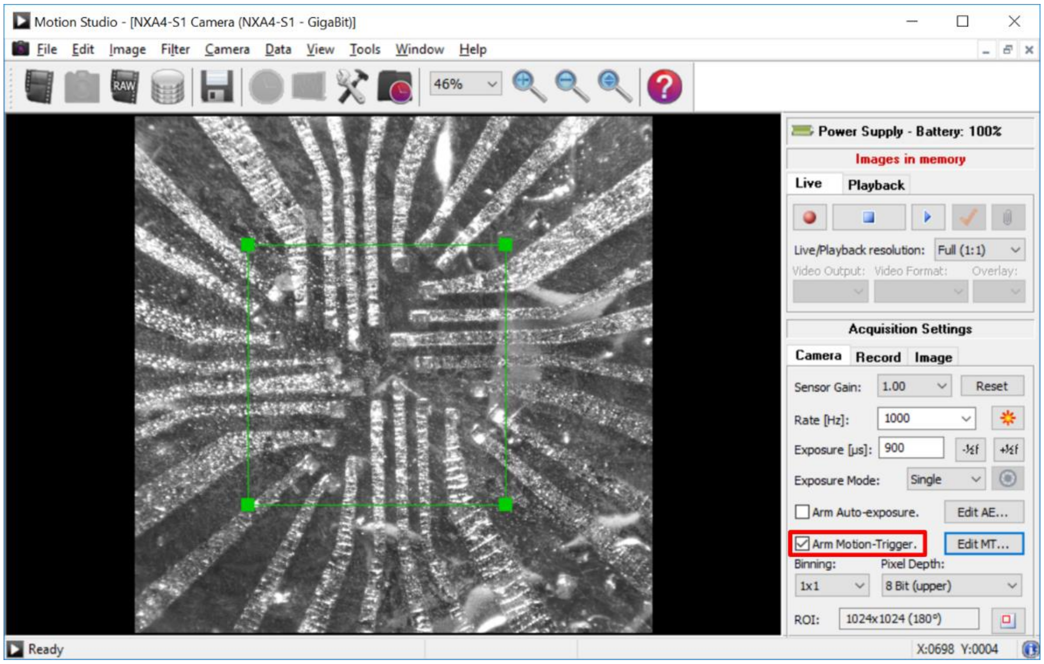


Fig. 57: After setting the motion trigger parameters, the "Arm Motion-Trigger" option will be enabled (checked) indicating the automatic trigger has been set.

- It is recommended to capture images before the VCA motion starts to ensure that pre-stretch images are available for strain analysis. Use the Pre-trigger function in the "Record" tab to capture images pre-trigger.

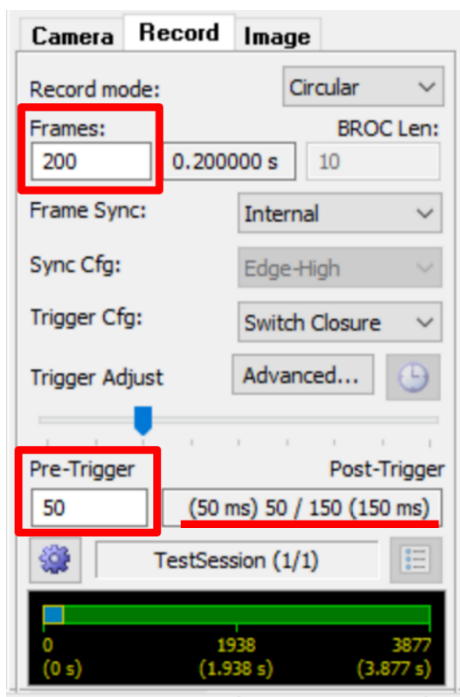


Fig. 58: Recording parameters in the "Record" tab.

Important: The number of frames and pre-trigger frames are set depending on the motion being recorded.

Example #1: If an impulse stretch with a strain of 5% at a rate of 1/s is applied, it takes 50 ms for the VCA to apply the stretch and another 50 ms to return to its starting position. Thus, a recording should be at least 100 ms long. As in **Fig. 58** above, with a rate of 1000Hz, the total number of frames can be set to 200 and the pre-trigger frames to 50.

Example #2: If an impulse stretch with a strain of 50% at a rate of 50/s is applied, it takes 10 ms for the VCA to apply the stretch and another 10 ms to return to its starting position. Thus, a recording should be at least 20 ms long. With a rate of 1000Hz, the total number of frames can be set to 60 and the pre-trigger frames to 20.

Although frames where no stretch is being applied are recorded, these will be trimmed off (or removed) when saving the acquisition later. **Removing unnecessary frames is highly recommended** since video files and image files can be quite large.

- In the "Live" tab, click the "Record (Arm)" button.
  Note: If images are already stored in memory, the following warning window will pop-up. In that case, click the "Yes" button. The status will change to "Armed (Waiting for trigger) …" in red font.

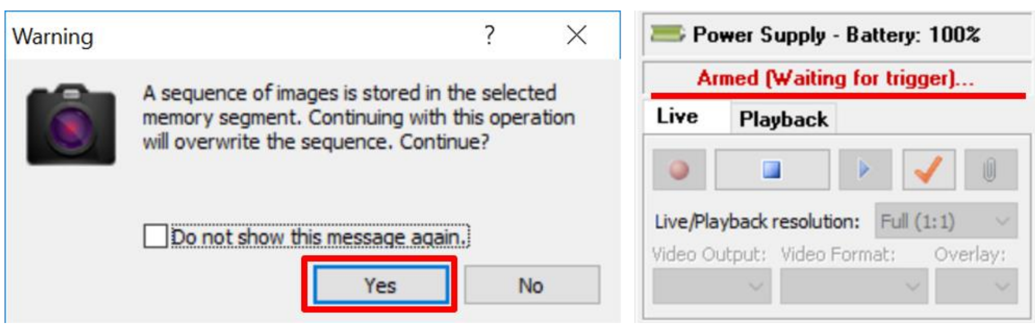


Fig. 59: After clicking the "Record (Arm) button, a warning message will appear and the status changes to "Armed (Waiting for trigger) …".

- **At this point the camera will be waiting for motion to be detected to start recording. When ready, press the "VCA go" button in the electronics enclosure for the VCA to start the desired motion.**
- Once a motion has been detected, the camera will record and store images post trigger. The rate, number of frames, and duration of the recording depends on the specified settings. In the Info pop-up window, click "OK."

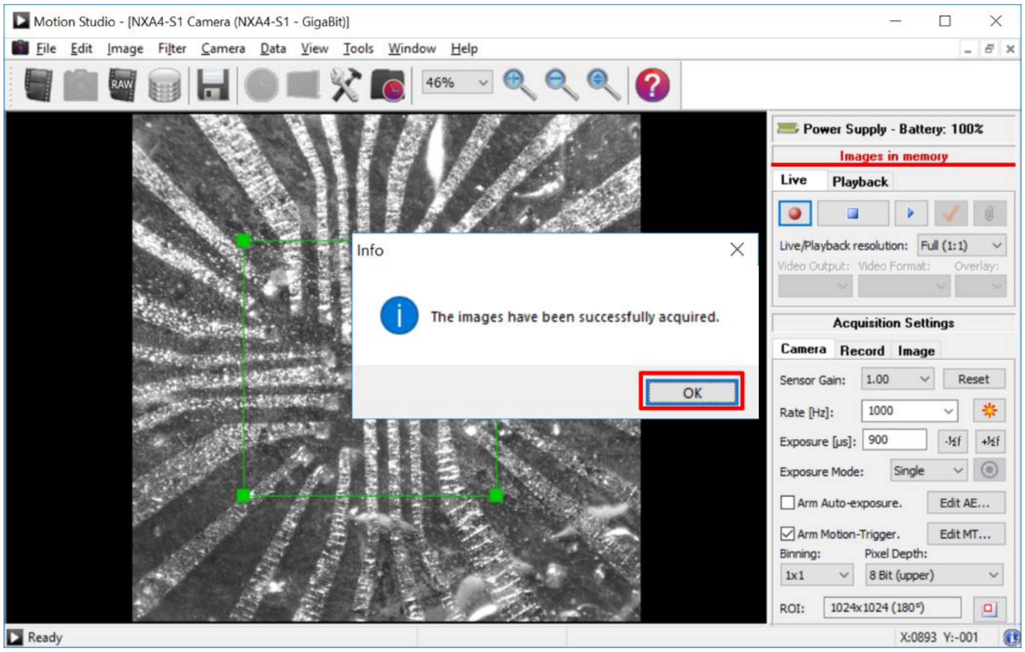


Fig. 60: After a recording is completed, the "Info" window pops up.

The last saved image will now be displayed in the main window.

5) Once a recording has been stored into the camera memory, the recording can be saved to the computer. Click "File" on the main menu and click "Save Acquisitions."

6) In the "Save Acquisitions" pop-up window, settings to the exported video and images/frames can be specified. The *Session name* and *File Type* can be set. Note: To see the *file type* options shown in **Fig. 62**, ensure that "Enable RAW download" remains unchecked in the Options window (**Fig. 49**).

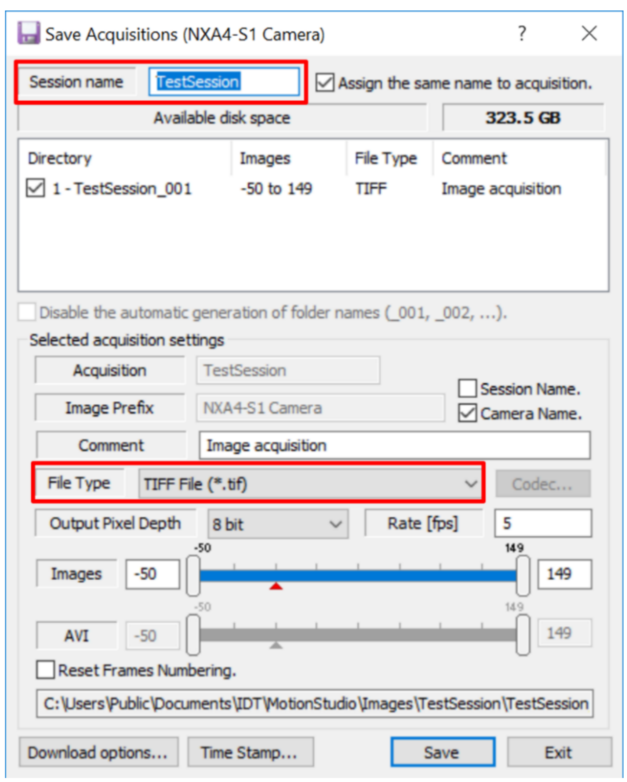


Fig. 61: The "Save Acquisitions" window.

For the *file type*, saving it as a TIFF file or PNG file would only save each individual frame. Selecting an AVI file would only save a video file at the rate specified by “Rate [fps].”

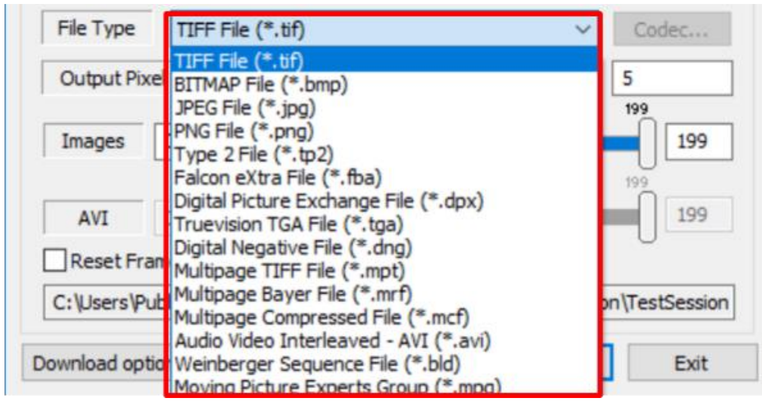


Fig. 62: The different options of output file types.

<u>Important</u>: For most computers, you need to select an “Output Pixel Depth of “8 Bit” even though the images are acquired with 12 Bit. Most computers cannot process 12 Bit pixels and the image files get corrupted.

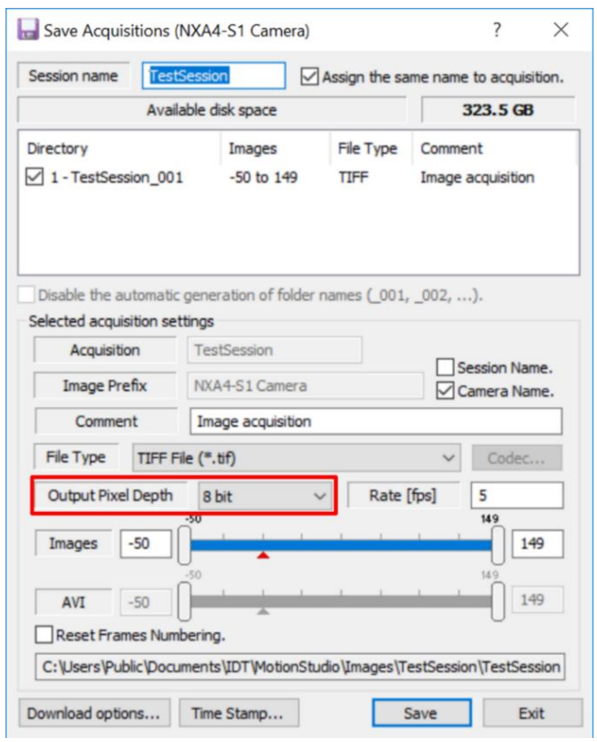


Fig. 63: Output Pixel Depth must be set to “8 bit” to avoid issues when opening the image files.

7) After specifying these parameters, click the “Download options…” button in the “Save Acquisitions” window. The “Options” pop-up window will appear.

8) Make any changes to the download/export setup and click “OK.”
<u>Note</u>: the download folder will be displayed and may be changed by clicking the “Browse…” button.

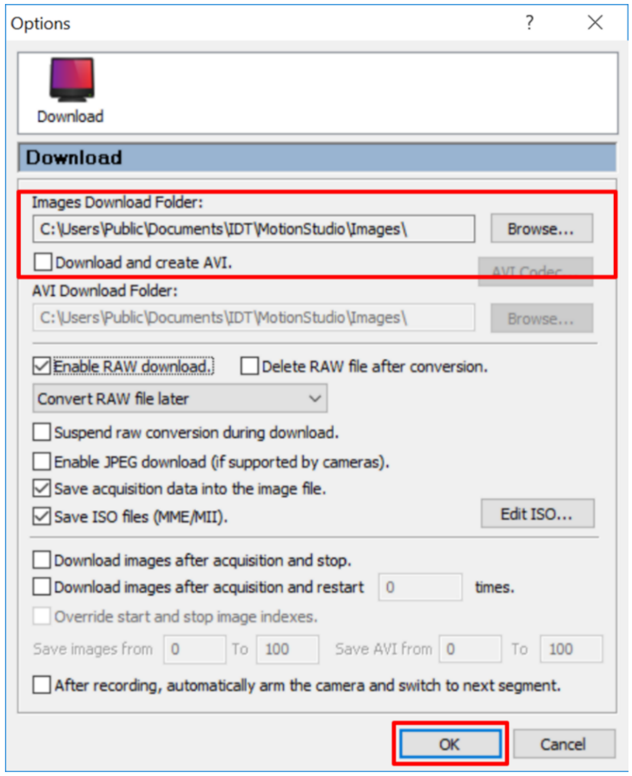


Fig. 64: "Options" window to specify output file parameters.

9) Back in the "Save Acquisitions" window, click the "Time Stamp…" button. The "Time Stamp Configuration" pop-up window will appear.

10) It is highly recommended to add a time stamp to each of the saved frames. A sample time stamp could have the following setup:

- 02 - Frame Number: "Bottom/Left" position
- 08 - Date: "Top/Left" position
- 09 - Time: "Bottom/Left" position
- 10 - Time from Trigger: "Bottom/Left" position
- 12 - Rate: "Top/Left" position
- 13 - Exposure: "Top/Left" position
- 18 - Resolution: "Top/Left" position

For the "Common settings," we suggest the following:

- Font: Arial
- Size: 72
- Color: Black
- Background color: White

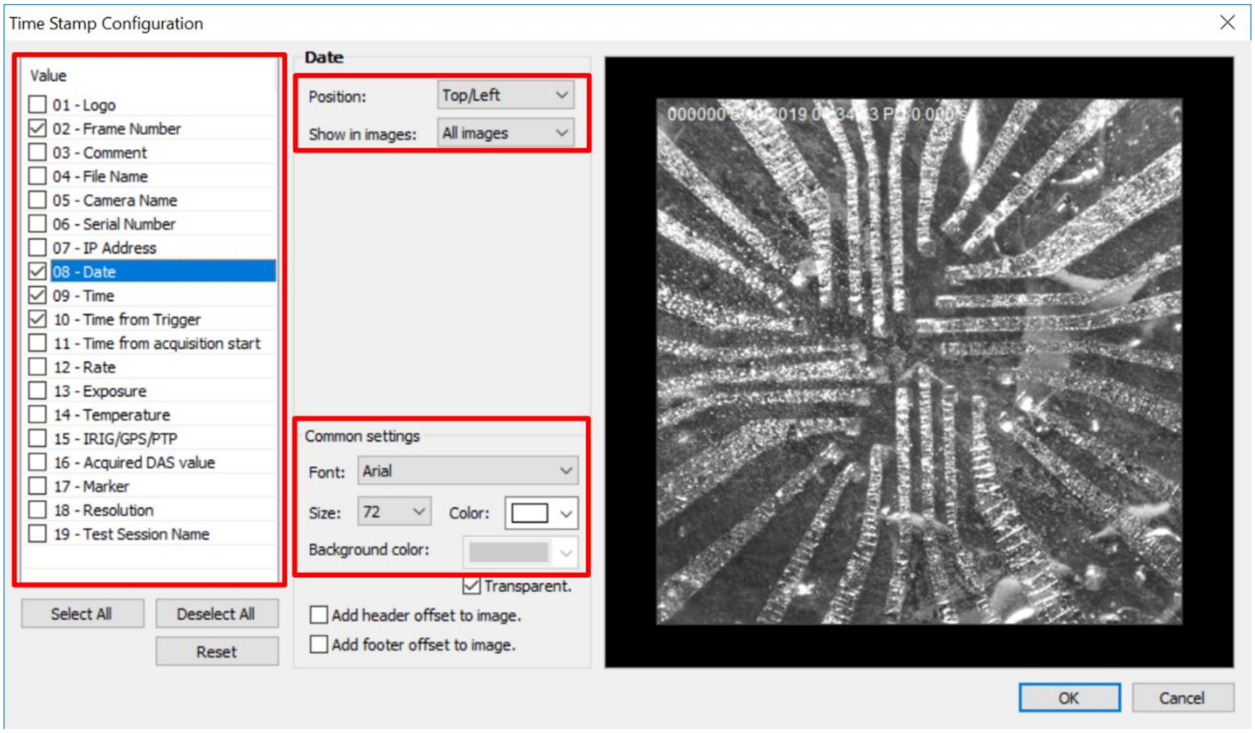


Fig. 65: "Time Stamp Configuration" window to edit each frame's time stamp.

11) After updating the parameters, click "OK."

12) Back in the "Save Acquisitions" window, click the "Save" button. Ensure the File Type is set to "RAW – Convert file later" to save the recording as a RAW file.

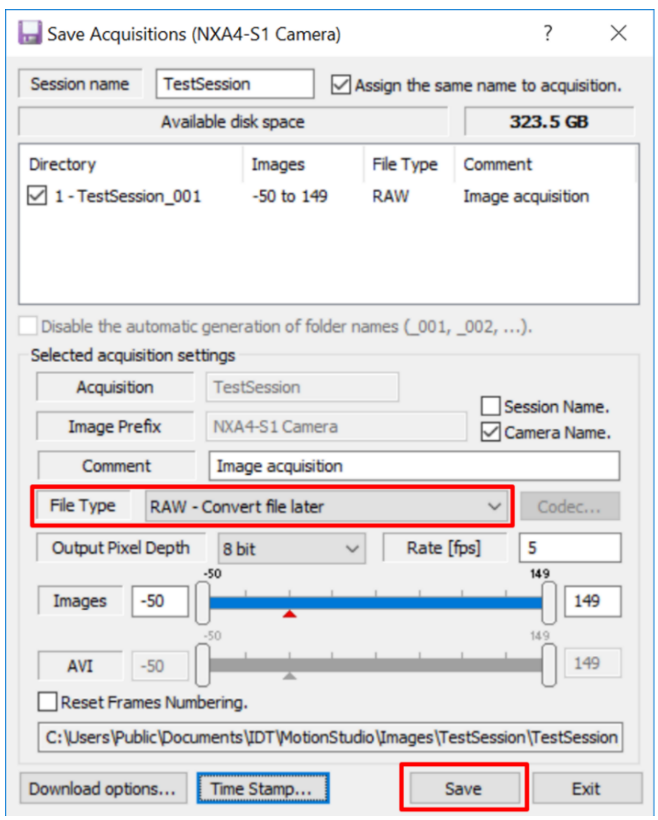


Fig. 66: Setting the output file type to "RAW."

13) The "Download Manager" window will pop-up and display the saving progress bar.

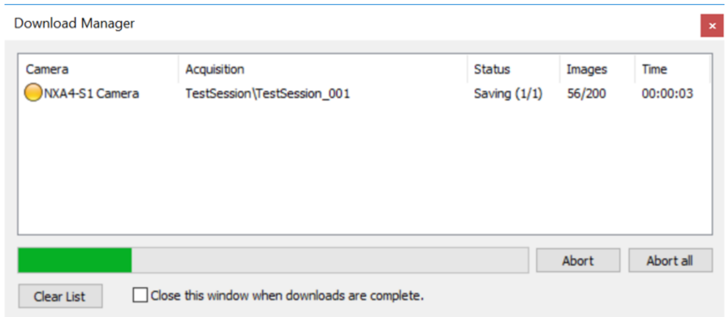


Fig. 67: "Download Manager" with a progress bar as the frames or video are being saved.

14) Once completed the status circle next to the camera will change from yellow to green. Close the "Download Manager" window.

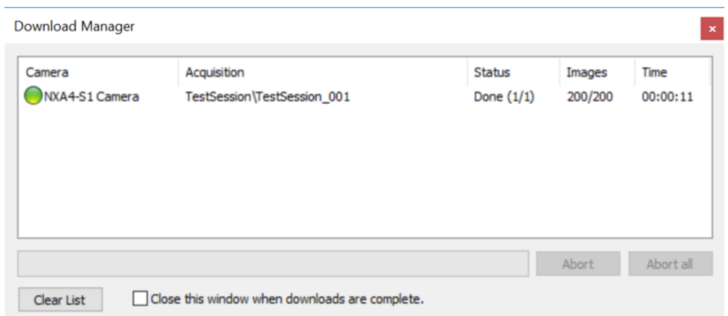


Fig. 68: "Download Manager" after frames or video have been saved.

15) Back in the Motion Studio main window, notice the overly large time stamps. Although they look large in the software, the saved images will have more appropriately sized time stamps.

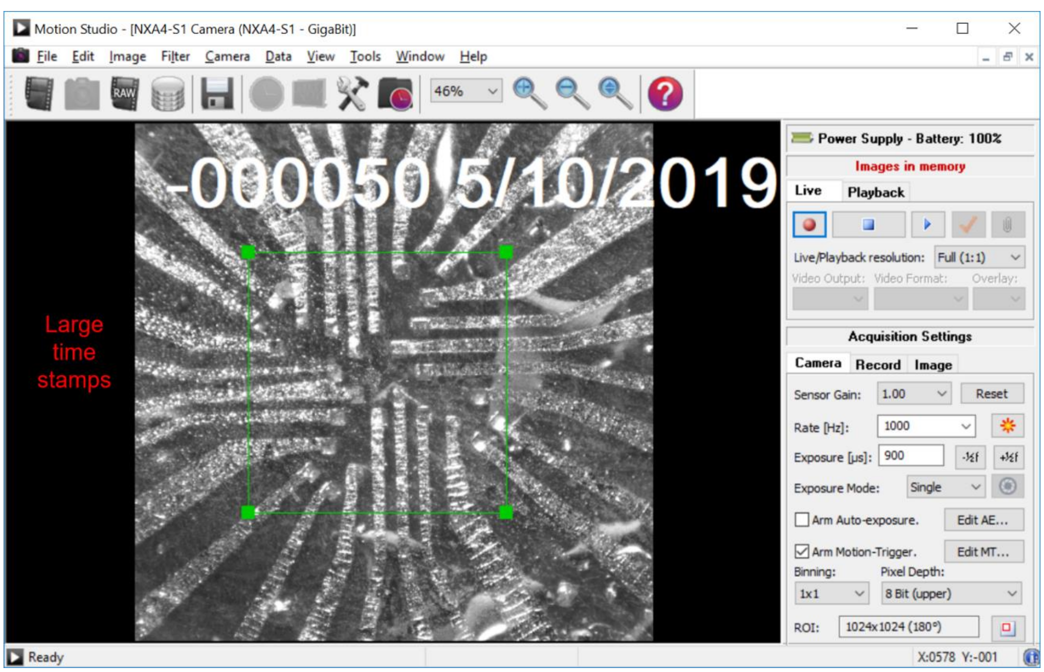


Fig. 69: Time stamps now appear in the main Motion Studio window.

16) The recording has been saved and are ready to be viewed.

6 Optimizing Image Quality for Video Recording

The purpose of this section is to help the user optimize the image quality, with a focus on imaging cells at high frame rates. In optics, there are numerous tradeoffs that affect image quality. This procedure is a guide to produce quality images around these tradeoffs using the Zoom 6000 Navitar lens.

<u>**Note**</u>**:** See **Appendix F** for a brief description of various parameters that affect image quality.

6.1 Parameters to Optimize Image Quality

We first explain below the most critical tradeoffs that affect image quality:

1) <u>Magnification vs. Depth of Field</u>
   There are three ways to increase the magnification:
   i. zoom in using the zoom control (**Fig. 41**) of the Zoom 6000 lens,
   ii. add a 2× lens attachment (Navitar, 1-60113) to the Zoom 6000 lens, and
   iii. swap the 1× adapter (Navitar, 1-64299) with a 2× adapter (Navitar, 1-6030).
   Methods *i* and ii to increase magnification will reduce the depth of field, i.e., the image will get blurry more easily. However, method *ii*. of using a 2× lens attachment has the benefit of improving the image resolution by a factor of 2. Method *iii* of using a 2× adapter tube only doubles the magnification and has no effect on the image resolution or depth of field. For all these three methods to increase magnification, more light is needed.
2) <u>Region of Interest (ROI) vs. Frame Rate</u>
   The region of interest is the area defined for image processing and analysis using the camera. Reducing the ROI reduces the area being processed by the camera, thus, reducing the image size and bandwidth required for each image. While areas of the image are 'lost' when decreasing the ROI, the camera's max attainable frame rate increases as the *max frame rate is inversely proportional to the ROI*.
3) <u>Frame Rate vs. Brightness</u>
   Large frame rates have reduced amount of time for each pixel to collect photons. Thus, *larger frame rate results in images with lower brightness* due to less light per frame. There should be a balance when setting the frame rate so that enough frames are recorded for the motion of interest but not too large to negatively affect the image brightness.
4) <u>Frame Rate vs. Exposure Time</u>
   Setting the camera's frame rate is dictated by the applied stretch to the sMEA. For the faster strain rates (i.e., motions that take a few milliseconds from flush position to target position), a camera frame rate of 1000Hz is required. However, increasing the frame rate decreases the max attainable exposure time, or time light reaches the image sensor. The max exposure is inversely proportional to the frame rate, i.e., $exposure \leq 1/frame\_rate$. For example, the largest possible exposure when frame rate has been set to 1000Hz is 1ms.
5) <u>Exposure Time vs. Motion Blur</u>
   While longer exposure times result in brighter camera images, *increasing the exposure time increases the motion blur* of moving objects. A shorter exposure time (i.e., faster shutter speed) reduces motion blur as the object will move less while the shutter is

open. Thus, exposure time should not simply be maximized when brighter images are desired.

As a general rule, light source intensity should be the main source of illumination and image brightness. After defining the frame rate and ROI, exposure time can be set.

6) Sensor Gain vs. Noise
While increasing the sensor gain results in brighter images with greater contrast, *increasing the sensor gain increases the image noise as well*. In general, sensor gain should only be increased after optimizing the light source illumination and the exposure time for a pre-specified frame rate.

Other camera settings parameters that can be optimized to help improve image quality and do not have a significant tradeoff with other parameters.

1) Gamma correction: controls the overall image brightness by correlating a pixel's numerical value to its actual luminance.
2) Look up table (LUT): allows improving the image contrast and brightness by transforming original pixel values to more desirable output values. Thus, specific areas of the image can be further highlighted at the expense of areas that might not be as relevant.
3) Sharpness: helps increase edge contrast (i.e., produces more distinct contours) to improve the detail in the image. Excessive sharpening could result in too much contrast, sharpening shadows and highlights, and unwanted saturation.
4) Brightness: sets the image pixels' lightness/darkness.
5) Contrast: differentiates the lighter and darker areas to emphasize details in the image.

6.2 Protocol to Optimize Image Quality

For this sub-section, the figures shown were collected with the following imaging hardware configuration:

- OS7-V3-S1 camera
- Metabones speed booster XL 0.64×
- Couplers
- 2x adapter (Navitar 1-6030)
- Zoom 6000 lens (Navitar 1-60135)

Follow the steps below to optimize image quality when preparing to record a video at a high frame rate.

1) Using the MotionLab software, run the VCA and position the sMEA at the flush position or a few hundreds of microns below flush.

   Note: See BMSEED's "Mechanics Module User Manual" for more information on how to run the VCA.
2) Load the Motion Studio software.
3) Open a camera session.
4) Enable the live view by clicking the "**Live**" button (**Fig. 25**).
5) Set the **light source** to the mid intensity of level 5 (i.e., halfway marker between the "Min" and "Max" labels).

6) Ensure the camera parameters have the following starting values:

<u>On the camera (Fig. 70)</u>:

- Zoom 6000 lens magnification: smallest value of 0.7×

Fig. 70: Camera lens magnification set to smallest value of 0.7×.

<u>Motion Studio Camera tab (Fig. 71)</u>:

- **Sensor Gain:** smallest value of **1.00** (also the default value)
- **Rate [Hz]:** **1000** Hz (default value for impulse stretch motions)
- **Exposure [μs]:** **500** μs (half of the max allowed with a rate of 1000 Hz)
- **ROI:** largest value of **1280×1920** pixels (for an OS7 camera)
  <u>Note</u>: To change the ROI, "Live" view must be disabled by clicking the stop button. Then click the button to the right of the ROI values, and a new window called "Edit Region of Interest" will pop up. Here you can change the ROI size.

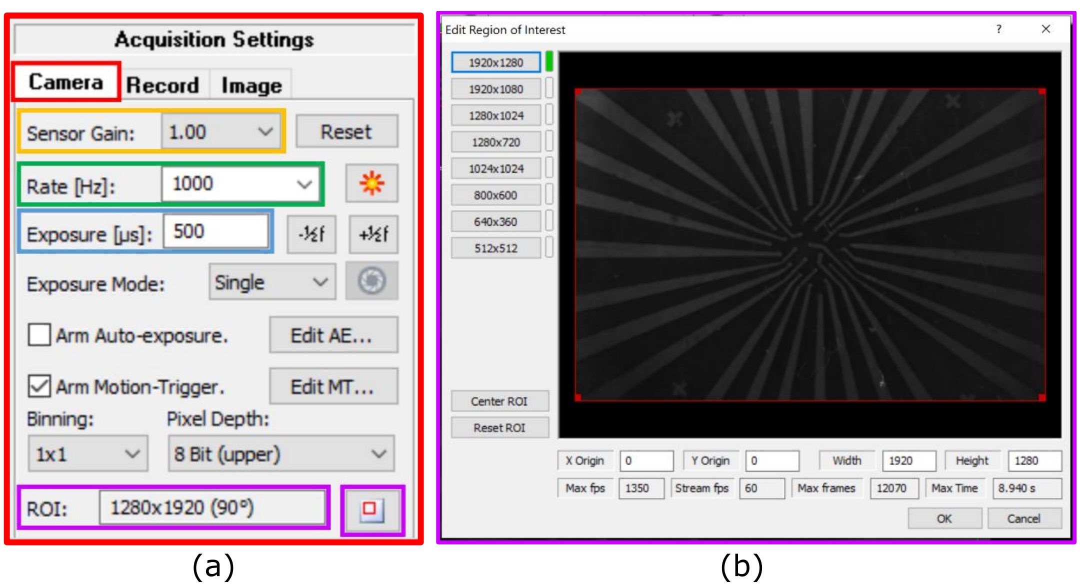


Fig. 71: (a) Camera tab showing the starting values for sensor gain, rate exposure, and ROI. (b) "Edit Region of Interest" window where the ROI can be set.

Motion Studio Image tab (Fig. 72a):

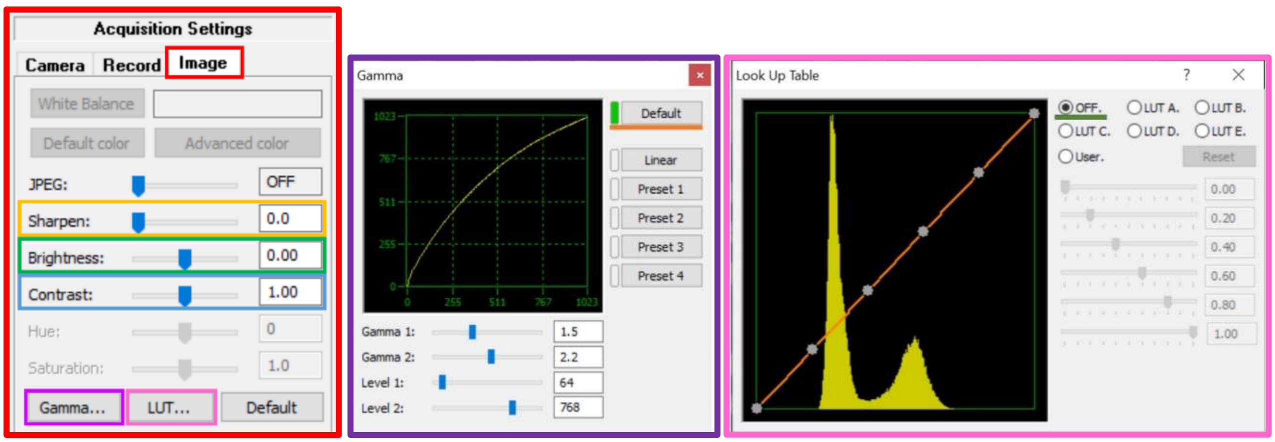
- Sharpen: smallest value of **0.0** (also the default value)
- Brightness: default value of **0.00**
- Contrast: default value of **1.00**

Motion Studio Gamma window (Fig. 72b):

- Gamma properties: default values (Gamma 1 → **1.5**, Gamma 2 → **2.2**, Level 1 → **64**, Level 2 → **768**).

Motion Studio LUT window (Fig. 72c):

- LUT: default value of "**OFF**" (values of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0)

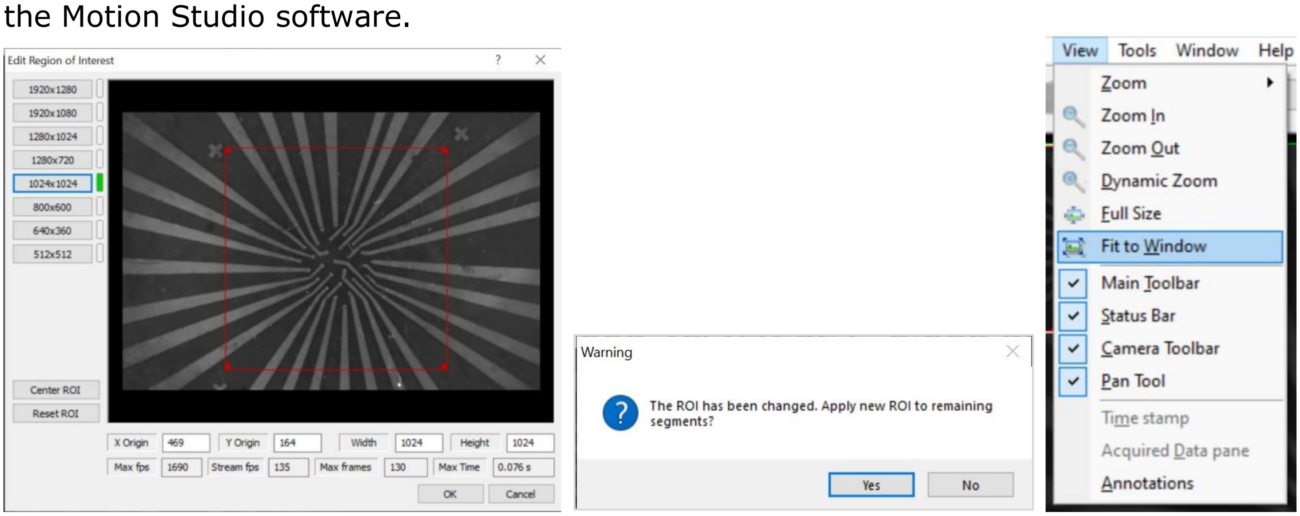


(a) (b) (c)

Fig. 72: (a) Image tab showing the starting values for sharpen, brightness, and contrast. The **(b)** "Gamma" window and **(c)** "Look Up Table" window where these parameters can be updated.

7) Decrease the **region of interest (ROI)** to 1024 pixels × 1024 pixels (**Fig. 73**). A smaller ROI 'trims off' areas of the image, reducing the size of each frame and allowing larger attainable frame rates.

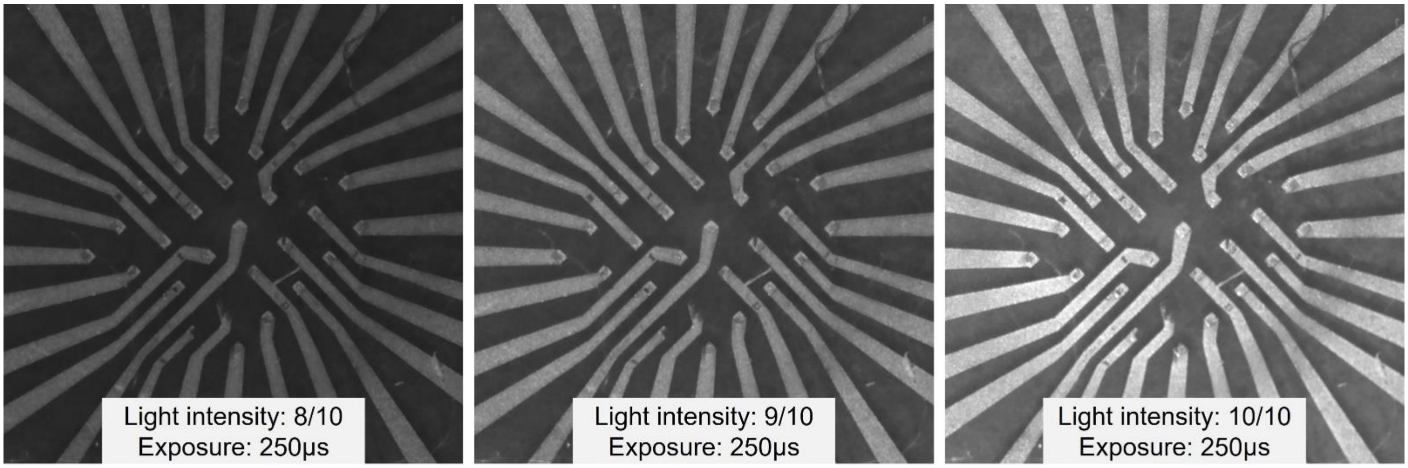
Note: After updating the ROI, click on "View" and "Fit to Window" in top menu bar of the Motion Studio software.



(a) (b) (c)

Fig. 73: (a) "Edit Region of Interest" window where the ROI can be updated. (b) "Warning" window after updating the ROI. (c) "Fit to Window" in top menu bar.

8) Set the **light source** to the max intensity of level 10 (i.e., set the dial to the "MAX" marker).
    - If using gooseneck lamps, position them to light up the center of the sMEA.



(a) (b) (c)

Fig. 74: Camera images brightness as the light source intensity is increased to (a) level 8, (b) level 9, and (c) level 10.

9) Starting with the Zoom 6000 lens at the lowest magnification, use the zoom control (**Fig. 41**) to increase the **magnification** using the following recommendations for each imaging hardware configuration AND cell type:
    - **Recommendation when looking at dissociated cells:**
      1× adapter tube with a 2× lens attachment → 3.0× magnification
    - **Recommendation when looking at tissue slices:**
      2× adapter tube with no lens attachment → 1.25× mag. (**Fig. 75a**)
    - Alternatives when looking at tissue slices:
        - 1× adapter tube with no lens attachment → 3.5× magnification
        - 1× adapter tube with a 2× lens attachment → 2.0× magnification
        - 2× adapter tube with a 2× lens attachment → 0.7× magnification

   Note: The larger the lens magnification (i.e., zoom level), the darker the image will get.

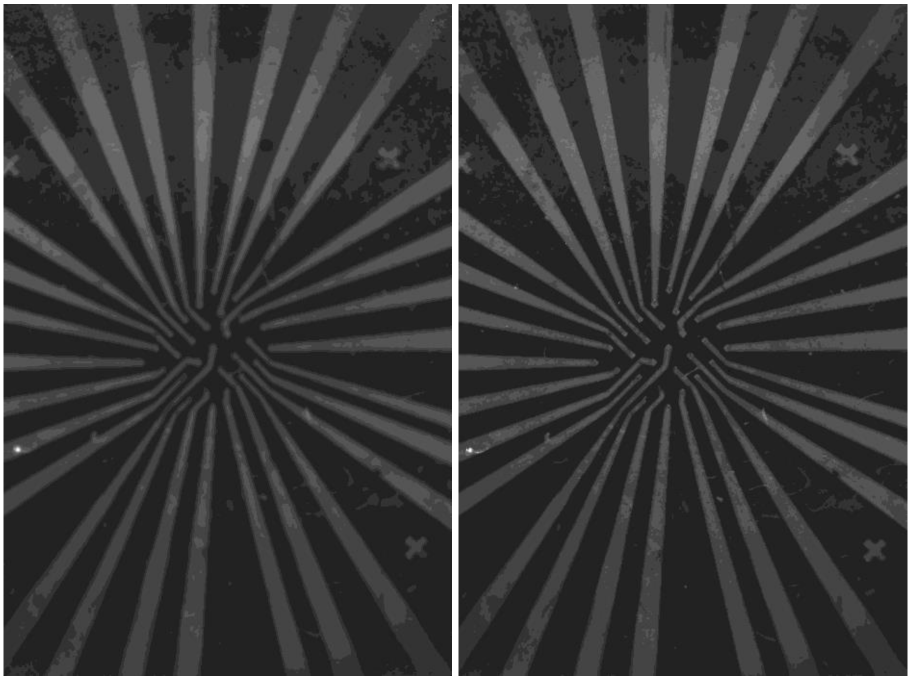
(a) (b)

Fig. 75: (a) Camera lens magnification set to 1.25× (i.e., midway point between the "1.0" and "|" markers). (b) Threaded hole that can be used to identify the focus midpoint.

10) Set the focus to the mid-point value within the focus knob (**Fig. 75b**). At this step, the image is not expected to appear in focus.

11) Adjust the camera assembly position (as described in **Fig. 28**) to obtain an image like the one shown in **Fig. 76a** below:
    - z-position by moving the assembly up and down
        → to achieve an acceptable degree of focus on the sMEA top surface
    - x- and y-positions by adjusting the camera stage
        → to center the camera on the sMEA recording sites.

12) Make fine adjustments to the focus (**Fig. 76b**) using the focus knob.



(a) (b)

Fig. 76: Camera image at the lowest magnification with (a) an acceptable focus after adjusting the camera stage z-position and (b) a finely adjusted focus. These images were taken with the OS7 camera max ROI of 1280 pixels × 1920 pixels and light source mid-level intensity (i.e., level 5).

13) While the focus does not need to be re-adjusted, the camera x-y stage might need to be re-adjusted to re-center the camera on the sMEA recording sites.

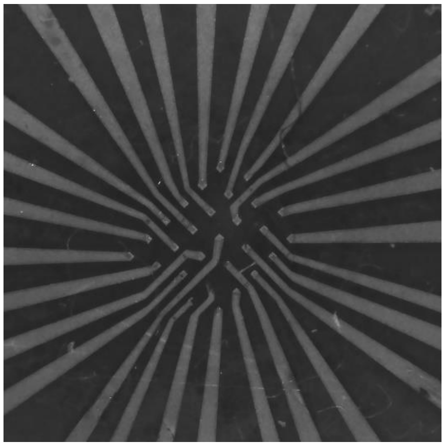
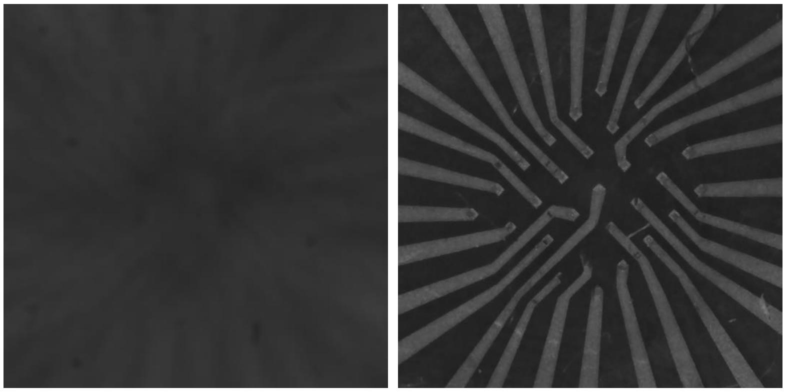


Fig. 77: Re-centered image after decreasing the ROI.

14) If the gooseneck lamps shifted during any of the prior steps, re-adjust the gooseneck lamps position to light up the center of the sMEA and improve the illumination on the image.



(a) (b)

Fig. 78: (a) Image zoomed in to a level in which the recording sites occupy at least half the area of the display window. (b) Image after the focus has been readjusted and camera stage re-centered on the recording sites.

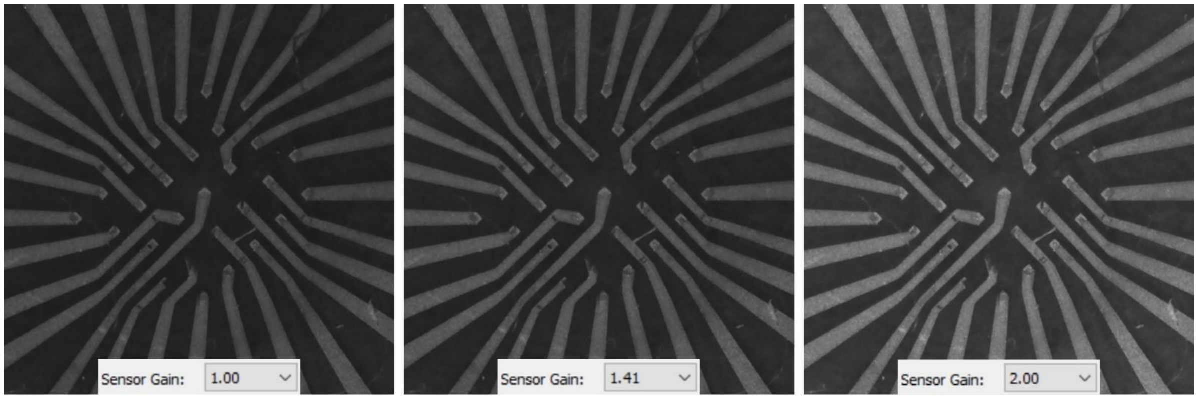
15) Ensure that the image orientation displayed in the Motion Studio window matches the sMEA when viewed from the top. **Fig. 31** shows how the image orientation can be updated.

16) Under the "Camera" tab (**Fig. 71**), ensure the **frame rate** is set to 1000 Hz, which is BMSEED's recommended rate for fast impulse motions.

17) Under the "Camera" tab (**Fig. 71**), decrease the **exposure** to 250μs, which is one quarter of its max attainable value for a rate of 1000Hz.
    Exposure is decreased to this extent to minimize motion blur as the sMEA and cells are stretched during the fast VCA motions. Image brightness will be decreased.

18) If the light source is at its max intensity and the image is still too dark, increase the **sensor gain** to 1.41 or 2.0 under the "Camera" tab to increase the image brightness as shown in **Fig. 79**.

    Note: To change the sensor gain value, live view must be disabled by clicking the stop button.



(a) (b) (c)

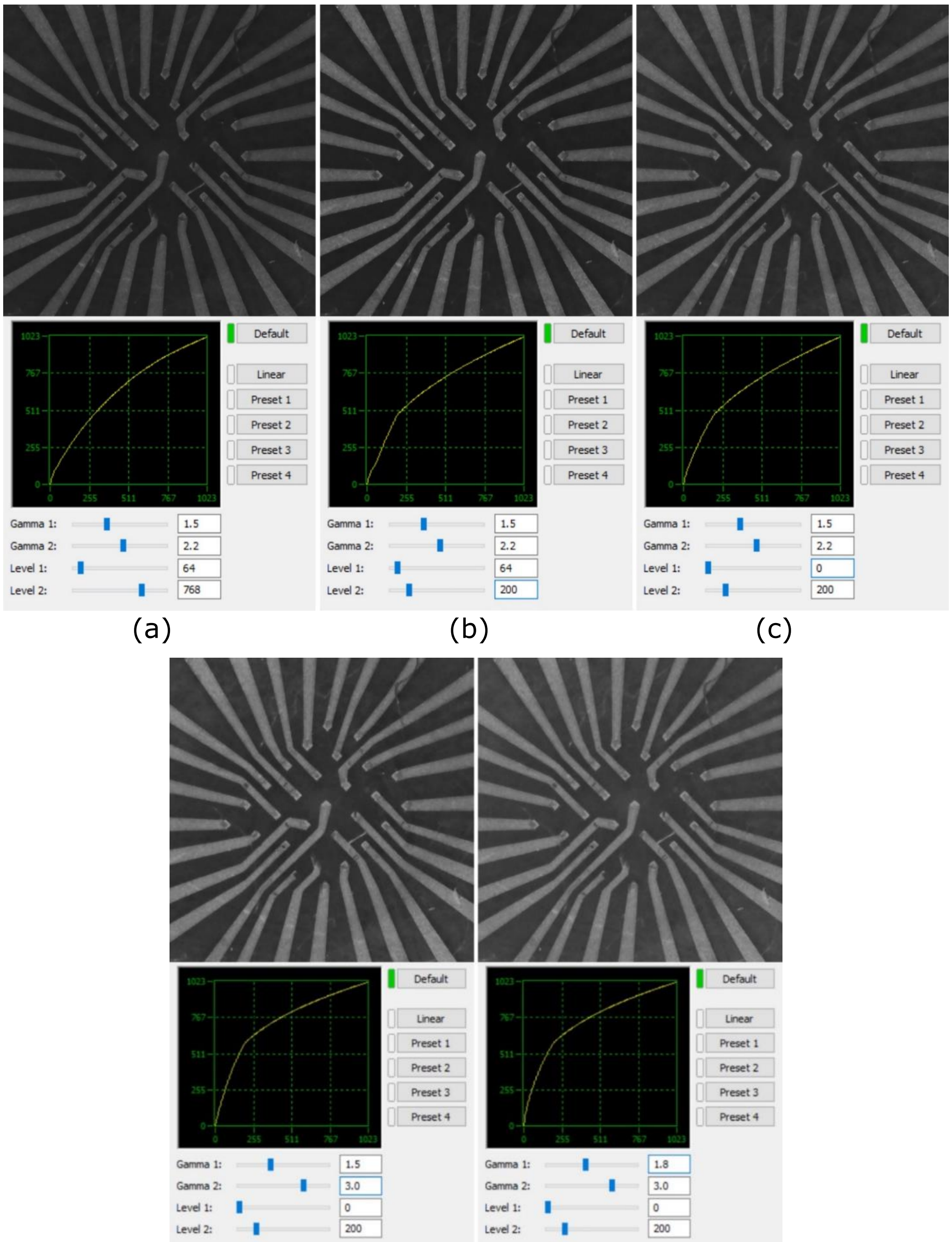
Fig. 79: Effect of increasing the sensor gain on the image brightness. Sensor gain was (a) maintained at the default value off 1.0, (b) increased to 1.41, and (c) increased to 2.0.

Note: For these images, light source intensity was set to level 8, and exposure was set to 250μs.

19) If the image is still too dark, update the **gamma** correction parameters of the image under the "Image" tab. One of the preset values can be selected or each of the four parameters can be updated manually. **Fig. 80** show how image brightness was improved by manually changing the parameters one by one from the bottom up.

Use the following values as starting points to improve the image brightness:

- Gamma 1: 1.8 (>default)
- Gamma 2: 3.0 (>default)
- Level 1: 0 (<default)
- Level 2: 200 (<default)



(a) (b) (c)

(d) (e)

Fig. 80: Image brightness as the gamma parameters are successively updated one by one: (a) default values, (b) smaller Level 2, (c) smaller Level 1, (d) larger Gamma 2, and (e) larger Gamma 1. Note: For these images, light source intensity was set to level 8; exposure was set to 250µs; and sensor gain was set to 1.0.

20) If the image is still too dark, update the **look up table** under the “Image” tab. By default, the LUT is turned off. Similar to the gamma parameters, one of the preset options (labeled A through E) can be selected or each of the six parameters can be updated manually. **Fig. 81** shows how the image brightness changes with the different look up tables.

    Select “LUT B” (**Fig. 81c**) or “LUT C” (**Fig. 81d**).

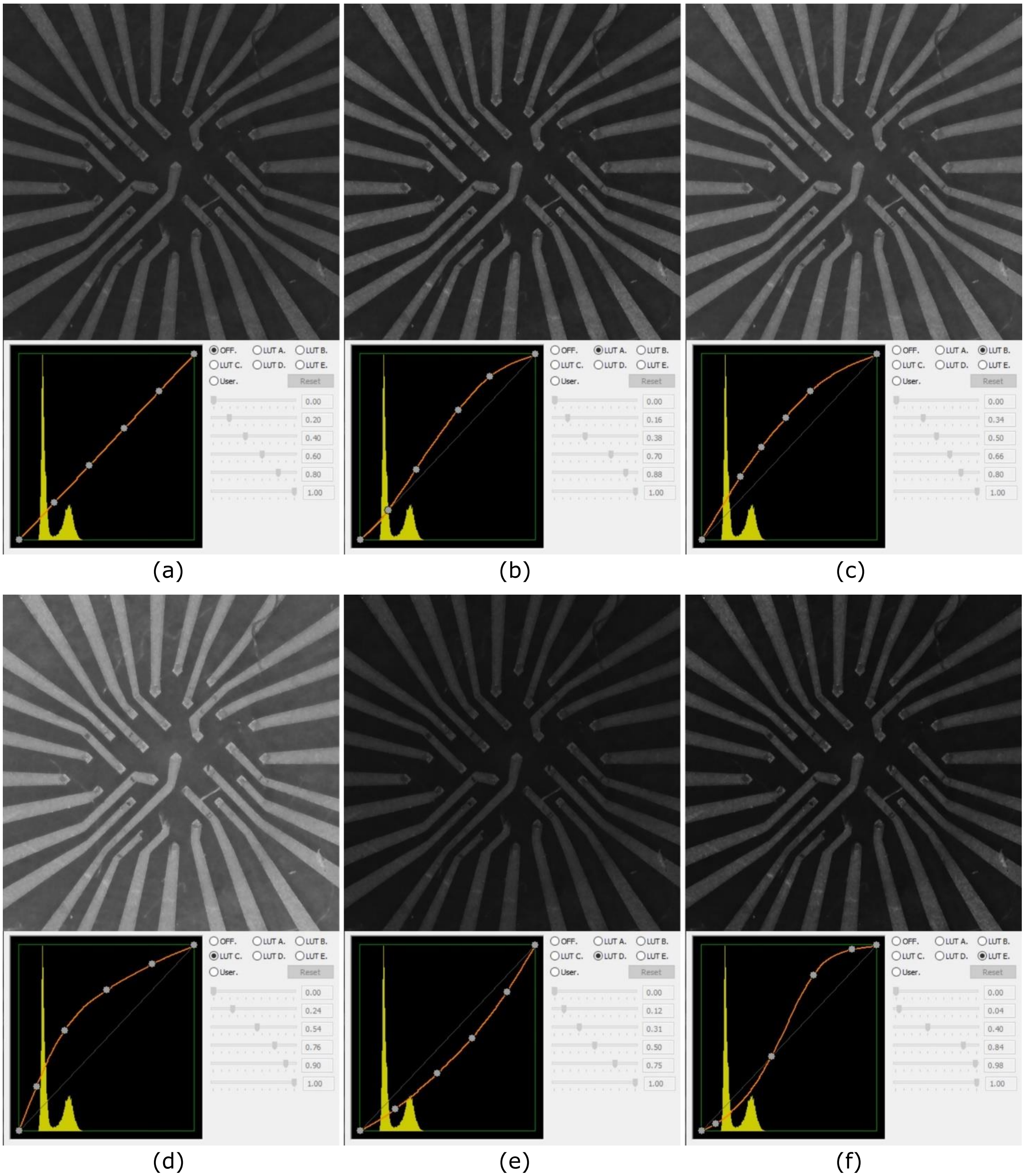
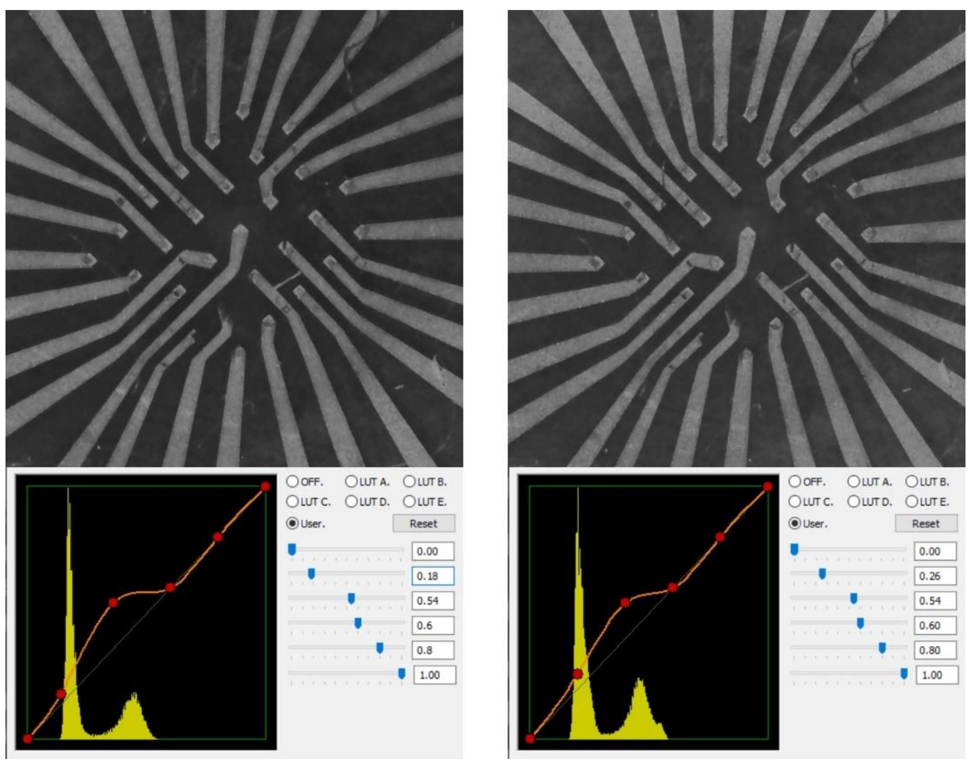


Fig. 81: Image brightness as the various look up table preset options are selected: (a) off or linear (default), (b) LUT A, (c) LUT B, (d) LUT C, (e) LUT D, and (f) LUT E. Note: For these images, light source intensity was set to level 8; exposure was set to 250μs; sensor gain was set to 1.0; and gamma parameters were set to default values.

21) Make fine adjustments to the **LUT** by selecting the "User" option and manually editing the six parameters. Try the following parameters from top to bottom: 0.0, 0.26, 0.54, 0.60, 0.80, and 1.0. If one of the preset options selected in the previous step resulted in brighter images, use that preset option instead.

A few remarks when manually updating the LUT parameters:

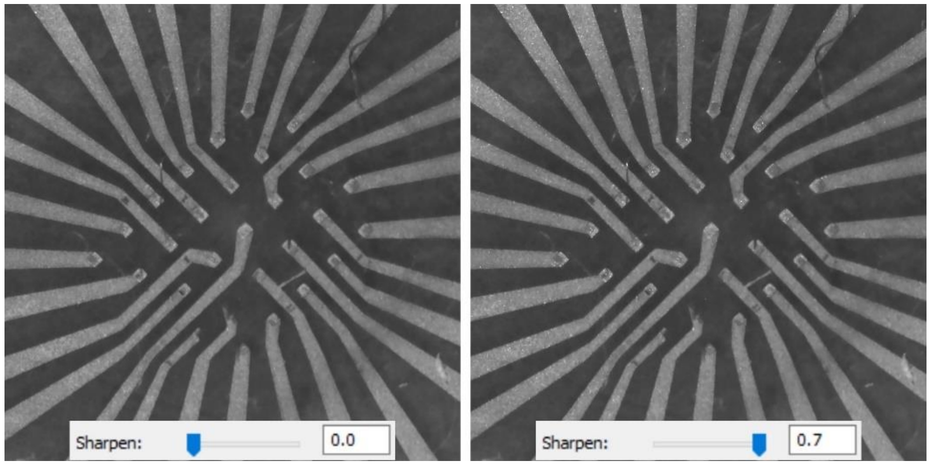
- Changes made to the parameters corresponding to markers above the yellow histogram affects the image brightness the most. For instance, looking at the histograms in **Fig. 81** above, the second and third parameters from the top would have the largest effect on the image.
- It is *recommended to use parameter values that raise the markers above the histogram* as this results in brighter images. For instance, **Fig. 82** shows bright images obtained with two different sets of LUT parameters. While the second parameter value from the top is different (0.18 vs. 0.26) in each instance, markers were raised above the linear, light gray curve for both instances.
- Another recommendation is to use one of the following as the starting points for the six parameters: i) the "OFF" or linear preset option, or ii) the preset option or the two preset options that resulted in the image(s) with the best quality.



(a) (b)

Fig. 82: Images with two different sets of 'User' parameters (a) and (b). Although the second parameter values are different, in both instances the markers are raise above the linear, gray curve resulting in bright images. Note: For these images, light source intensity was set to level 8; exposure was set to 250μs; sensor gain was set to 1.0; and gamma parameters were set to default values.

22) Increase the image **sharpness** (i.e., labeled as "sharpen") to 0.7 to improve edge contrast of the Au traces and recording sites.

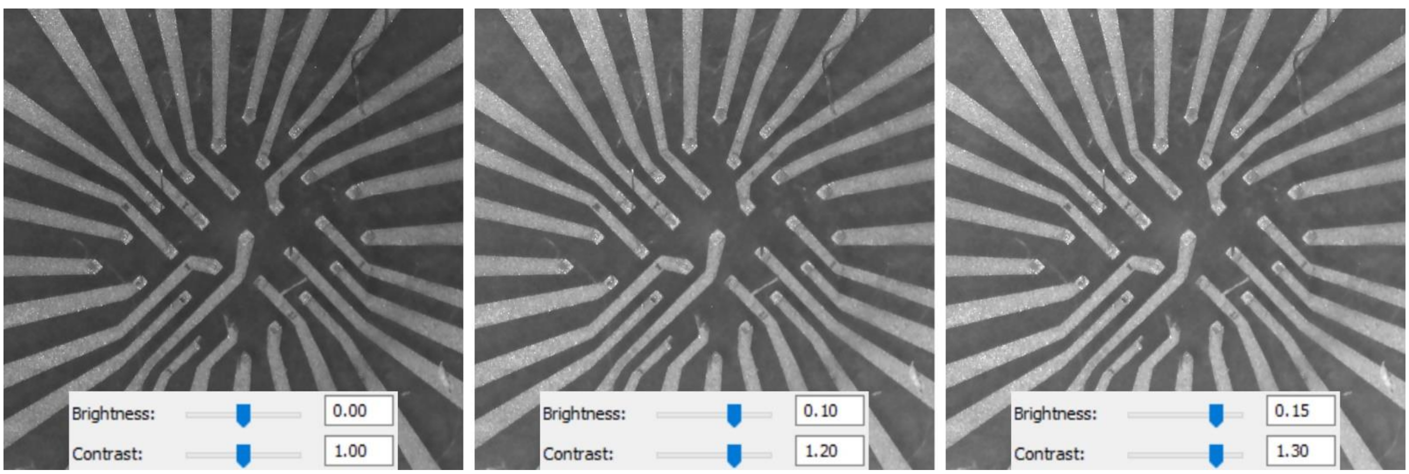


(a) (b)

Fig. 83: Camera images with a sharpness of (a) 0.0 and (b) 0.7. <u>Note</u>: For these images, light source intensity was set to level 9; exposure was set to 250µs; sensor gain was set to 1.0; gamma was set to default; and LUT was set to "OFF."

23) Increase **brightness** and **contrast** to improve the image quality. We recommend increasing both parameters concurrently relative to each other: for every increase of 0.01 units of brightness, increase 0.02 units of contrast as shown in **Fig. 84**.

Set brightness to 0.15 and contrast to 1.30.



(a) (b) (c)

Fig. 84: Camera images as brightness and contrast are increased from (a) 0.00 and 1.00 to (b) 0.10 and 1.20, and (b) 0.15 and 1.30, respectively.

24) If the camera image is still too dark, increase **exposure** by 150µs. **Repeat steps 21) through 26)**. Otherwise, go to the next step.

If you reach this step again, the image is still too dark, and exposure is ≥700µs, **contact BMSEED as components of the imaging hardware might need to be upgraded** to meet your requirements.

25) Once you get a bright and clear image, the **imaging parameters are set**. Motion Studio is ready for video recording of an sMEA stretching.

<u>Note</u>: See **Appendix G** for a table comparing capabilities and limitations when using different adapter and lens attachment pairs with a Navitar Zoom 6000 lens (1-60135).

6.3 Flow Chart of the Image Optimization Protocol

Fig. 85 and **Fig. 86** show a flow chart, split into two parts, summarizing the optimization protocol described in **Section 6.2** after the sMEA has been brought in contact with the indenter using MotionLab and the camera session has been initiated with Motion Studio.

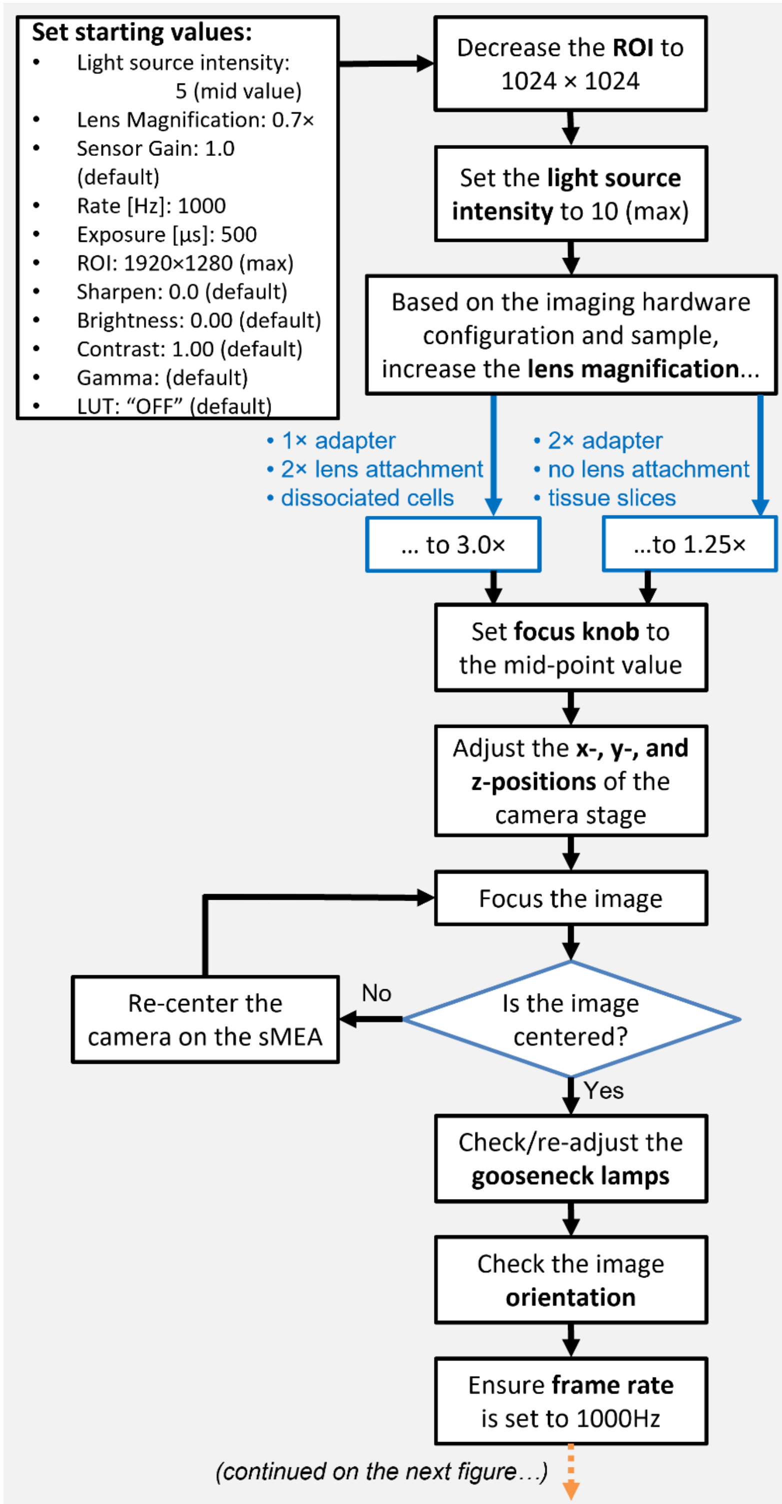


Fig. 85: First part of the flow chart summarizing the image quality optimization protocol.

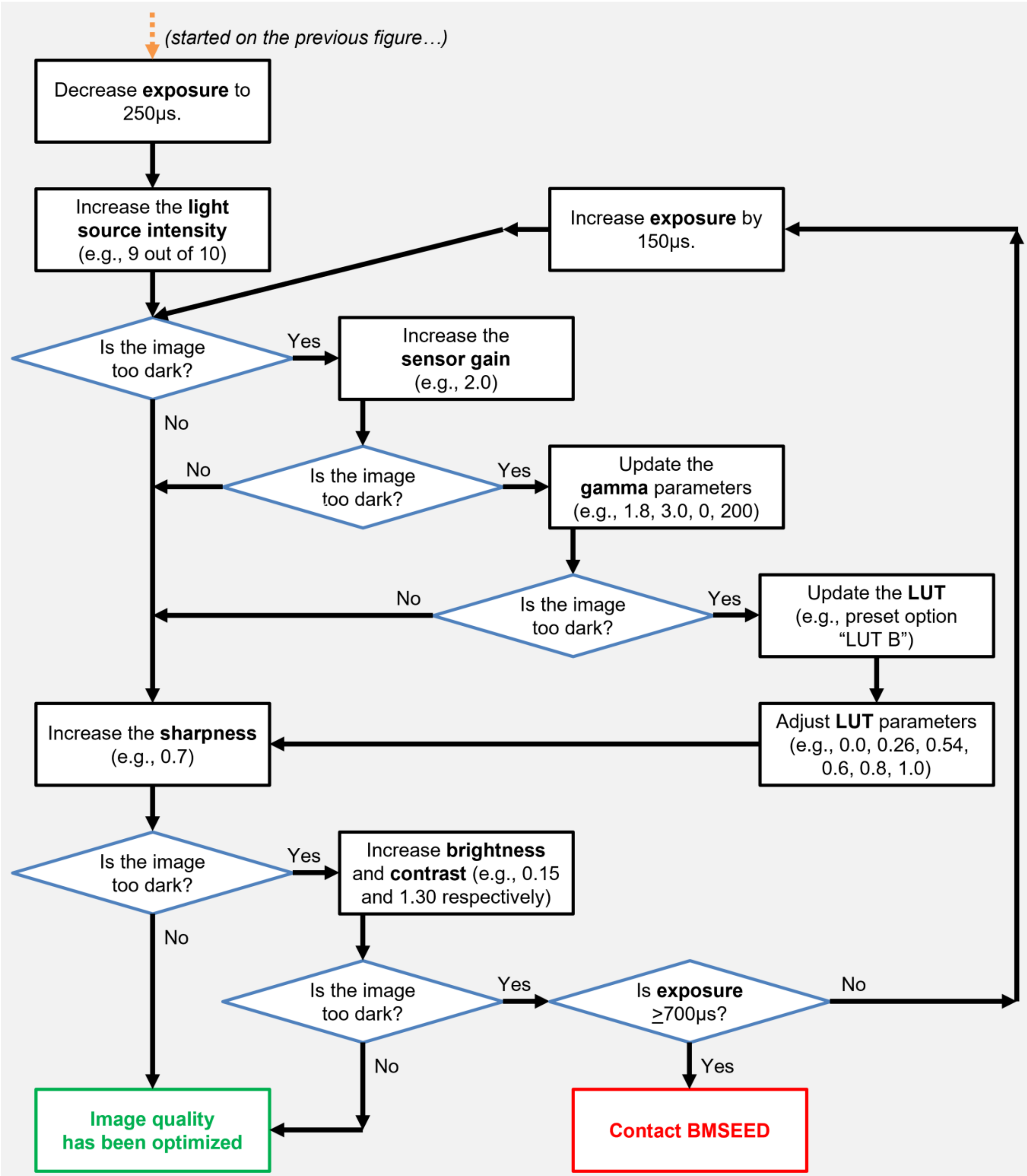


Fig. 86: Second part of the flow chart summarizing the image quality optimization protocol.

6.4 Protocol to Evaluate and Further Improve a Recording's Image Quality

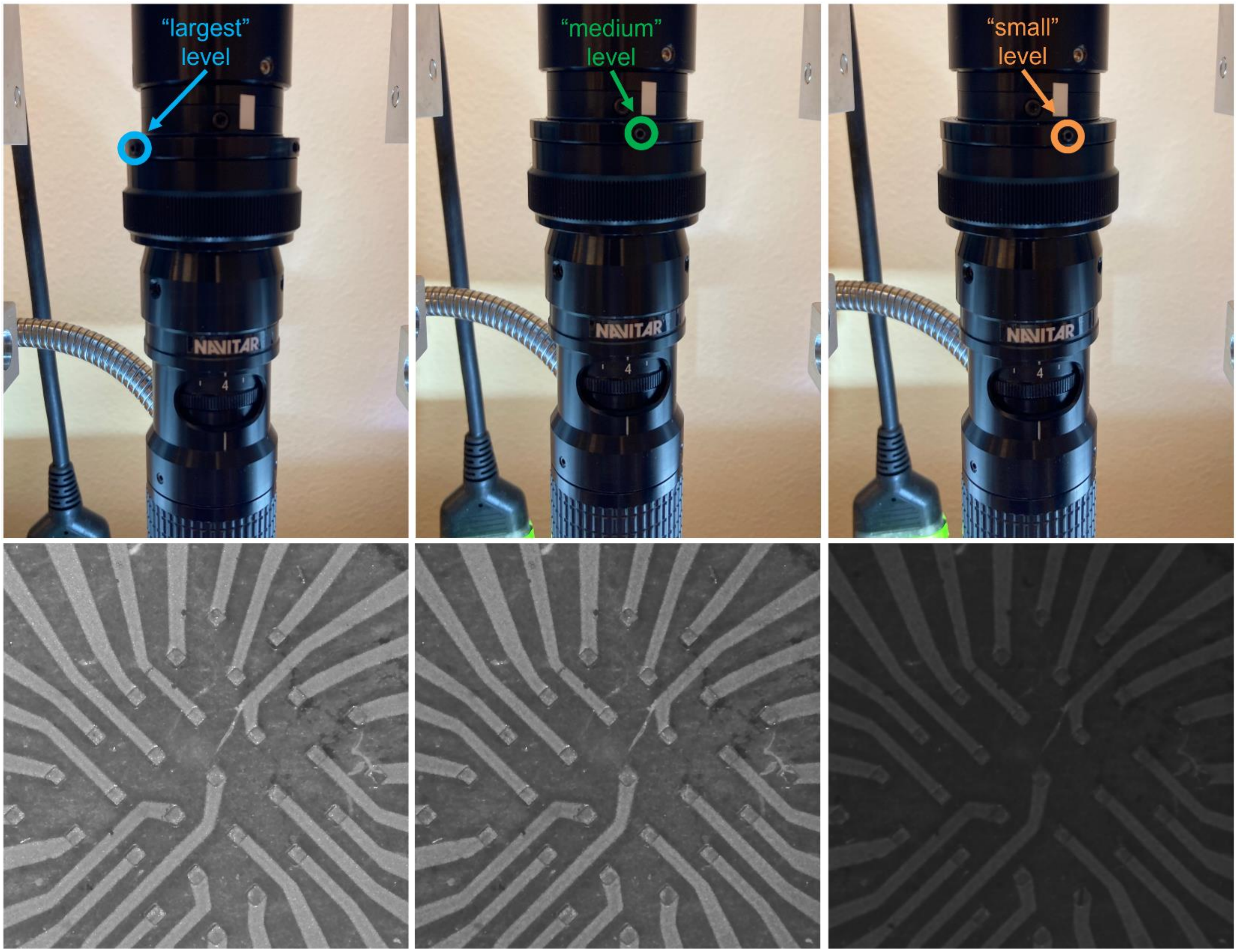
In terms of video recordings, very fast impulse stretches are the most challenging motions to record due to the limited number of frames that can be recorded and motion blur. Depending on the set magnification, light can be limited as well. This sub-section provides a set of parameters that can be used as a starting point when running these motions and how to make adjustments to improve the overall quality of the video recording.

The **light source should be set to its maximum intensity**, and the gooseneck lamps (if applicable) should be positioned as close as possible to the sample without interfering with its stretch motion. The following camera software settings may be used as a starting point:

- Region of interest (ROI): 1024 × 1024
- Lens magnification: *variable depending on target magnification*
- Rate: 1500 Hz
- Aperture (if applicable): *medium* (see **Fig. 88**)
- Exposure: 250 μs
- Sensor gain: 2.0
- Gamma: 1.8, 3.0, 0, 200
- LUT: LUT E
- Sharpness: 0.7
- Brightness: 0.25
- Contrast: 1.50

(a) (b) (c)

Fig. 87: (a) "Largest" or fully open, (b) "medium", and (c) "small" aperture levels of a Zoom 6000 lens with aperture (Navitar 1-60135A).



(a) (b) (c)

Fig. 88: Setting the aperture of a Zoom 6000 lens with aperture (Navitar 1-60135A) to the (a) "largest" or fully open, (b) "medium", and (c) "small" levels using the highlighted set screw as an indicator. Tradeoff of image brightness as the aperture is closed is illustrated.

While the set of parameter values listed above serve as a starting point when trying to optimize the video recordings, they can be updated based on the user needs. **Table 2** lists typical issues with image quality (i.e., brightness and resolution) that may be encountered while running stretch motions and steps to address them. Similarly, motion blur issues (i.e., focus consistency, intermittent blurring, and planar displacement) and corresponding troubleshooting steps are listed in **Table 3**.

Note: Keep in mind that the aperture can only be adjusted if a Zoom 6000 lens with aperture (Navitar 1-60135A) is being used.

Table 2: Troubleshooting steps for the different image quality issues you may encounter when setting/testing the imaging module.

Image Quality Issue	Troubleshooting Suggestions
1) The recorded video/images are not bright enough.	Source of the problem: not enough light is reaching the sample. Adjustments to the imaging hardware components setup*: • Light source: increase the light intensity (if possible), and if using gooseneck lamps, reposition them to better illuminate the sample. • Aperture (if applicable): increase by the minimal amount needed to increase brightness to an acceptable level. Adjustments to the camera software settings*: • Sensor gain: increase to 2.0 if currently set to a lower value. • Brightness and contrast: increase proportionally to each other. • Gamma: adjust the values to improve brightness. • LUT: select a different LUT set (e.g., LUT B or LUT C) to further increase the brightness. Manual adjustments could also be made. • Exposure: increase by the minimal amount needed to increase brightness.
2) The image resolution is not optimal and could be improved. The images, that are in focus, are not crisp.	Source of the problem: focus is sub-optimal. Adjustments to the imaging hardware components setup: • Lens: after setting the VCA to the flush position or slightly below it (e.g., 500μm below it), adjust the focus. A test sMEA might be used. • Aperture (if applicable): increase by the minimal amount needed. • Lens attachment: if not using one and target total magnification allows for it, add a lens attachment as this improves resolution but decreases the depth of field. Adjustments to the camera software settings: • Sharpness: increase to its max value (if possible). • Follow the camera software settings adjustments for Issue 1) above.

*Listed in the preferred order (i.e., from least to most detrimental to image focus).

As mentioned in **Section 6.1**, to obtain video recordings with good image quality throughout, there must be a balance between different parameters. For instance, during very fast impulse stretches at magnification $\geq$8×, balancing parameters that affect image brightness and parameters that affect blurring is critical.

Table 3: Troubleshooting steps for the different motion blur issues you may encounter when setting/testing the imaging module.

Motion Blur Issue	Troubleshooting Suggestions
1) Focus is inconsistent (i.e., focus is lost) during the stretch motion.	Source of the problem: focus quality cannot be maintained during a stretch. Adjustments to the imaging hardware components setup: • Lens: after setting the VCA to the flush position or slightly below it (e.g., 500μm below it), adjust the focus. If the issue persists, set the VCA to an even lower position and re-adjust the focus. In some cases, you might need to **use a test sMEA** and adjust focus while stretching it to the target strain. • Aperture (if applicable): increase by the minimal amount needed. It is also possible, that the membrane of the sMEA being stretched is no longer taut. If this is the case, the current sMEA will need to be replaced with a "new" sMEA with a taut, flat membrane.
2) There is intermittent blurring between video frames / images (i.e., every other frame is out-of-focus).	Source of the problem: motion blur is affecting the video recording, and/or the depth of field might be too shallow. To address motion blur, adjust the camera software setting: • Exposure: decrease by the minimal amount needed to reduce motion blur without sacrificing too much light. To address a shallow depth of field, adjust the imaging hardware setup: • Aperture (if applicable): decrease by the minimal amount needed not to sacrifice the amount of light reaching the sample. • Magnification: decrease the magnification if your sample/application allows for it. If applicable, replace the lens attachment with an adapter of the same magnification. The corrective measures listed above could negatively impact image brightness. Follow the camera software settings adjustments for Image Quality Issue 1) from **Table 2** to address light reduction.
3) The displacement of the sample in the x-y plane seems too large.	Source of the problem: frame and/or sample might not be fully secured. Adjustments to the MEASSuRE frame setup: • sMEA: ensure that the sMEA is fixed in place by checking: i) the interface board (IB) locking pins or mechanical holder screws ii) if using the IB, the clamps holding the IB to the movable platform iii) set screws fixing the movable platform to the Y-adapter. • Top cross plate: if the imaging assembly allows for it, attach the top cross plate above the side rails to further stabilize the frame. • Camera stage: verify all screws to position the camera stage are tightly secured. • Frame base plate: further secure the base plate of the MEASSuRE system frame by: i) placing a dampening material (1/4"-thick wooden board) beneath it ii) clamping (e.g., using C-clamps) it to the workbench.

If video recording issues persist while running stretch motions with the VCA, contact BMSEED.

7 Protocol to Extract Frames from Video

To extract frames from our recorded image files or video file, the Motion Studio software will be used.

1) Open Motion Studio x64, select "Images" in the Main Menu window, and click "OK."

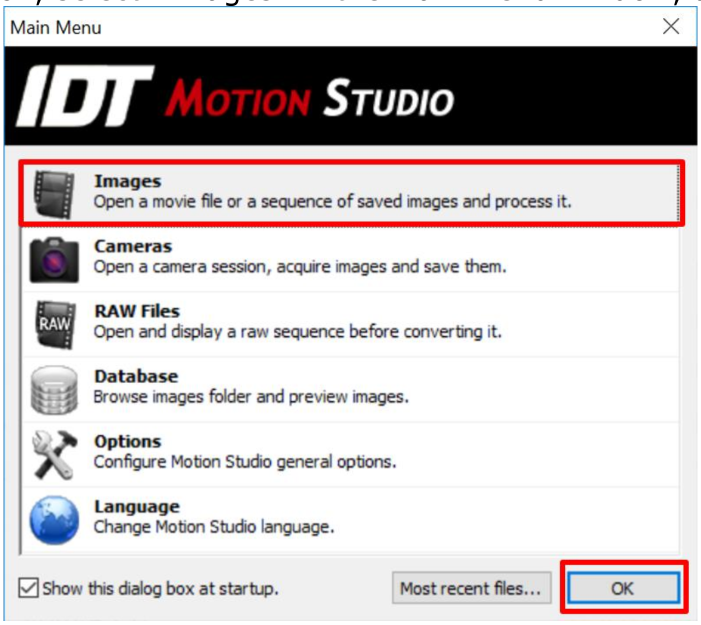


Fig. 89: Selecting "Images" to open recorded images or videos for processing.

Alternatively, if Motion Studio is already open, click the "Open Image" button (film icon).

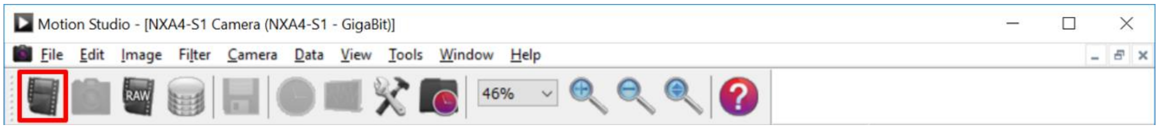


Fig. 90: Clicking the "Open Image" button to access the "Open Image File."

2) In the "Open Image File" window, go to the directory where the snapshots and videos from Motion Studio are saved. Here, you will find any saved snapshots and saved acquisition sessions (in folders).

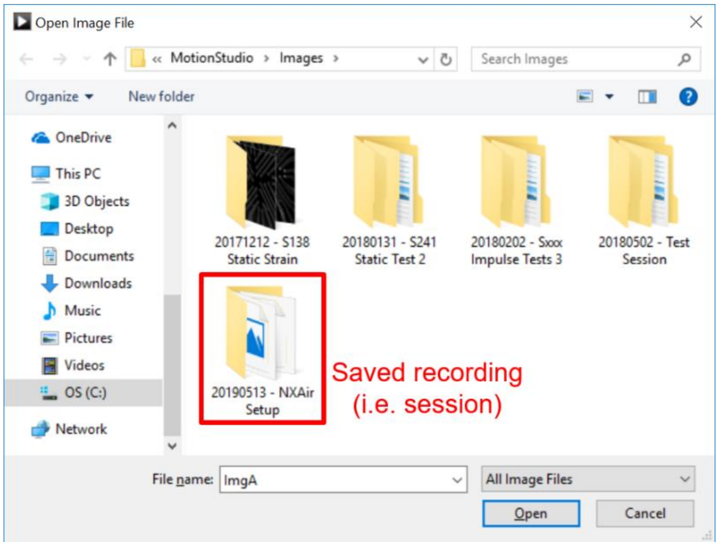


Fig. 91: Selecting the directory where the file(s) of interest are stored.

3) Double-click on the saved recording of interest.

4) In the "Open Image File" window, select the video file (e.g., raw files, avi files) or first image (e.g., tiff, png, jpeg) of the recording sequence and click "Open."

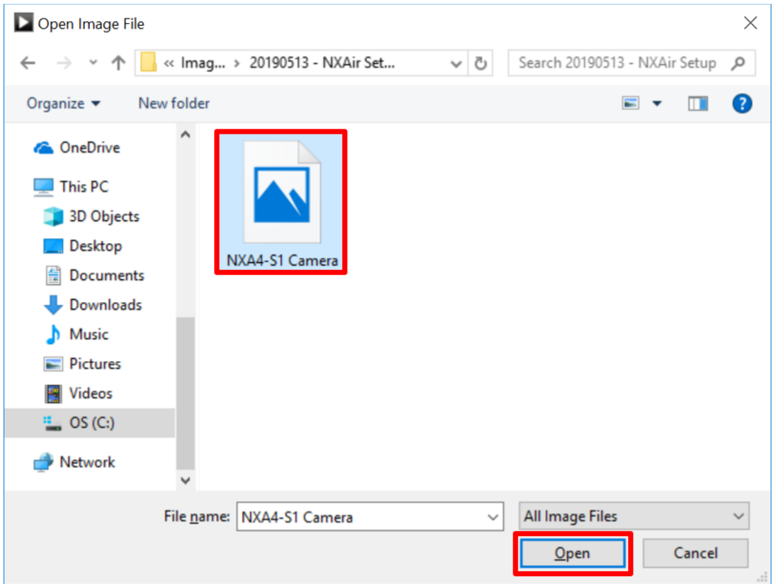


Fig. 92: Opening the file of interest.

5) The Motion Studio software will now display the current frame and will allow you to navigate through the different frames using the menu on the right. Click on the "Playback" tab to navigate between frames.

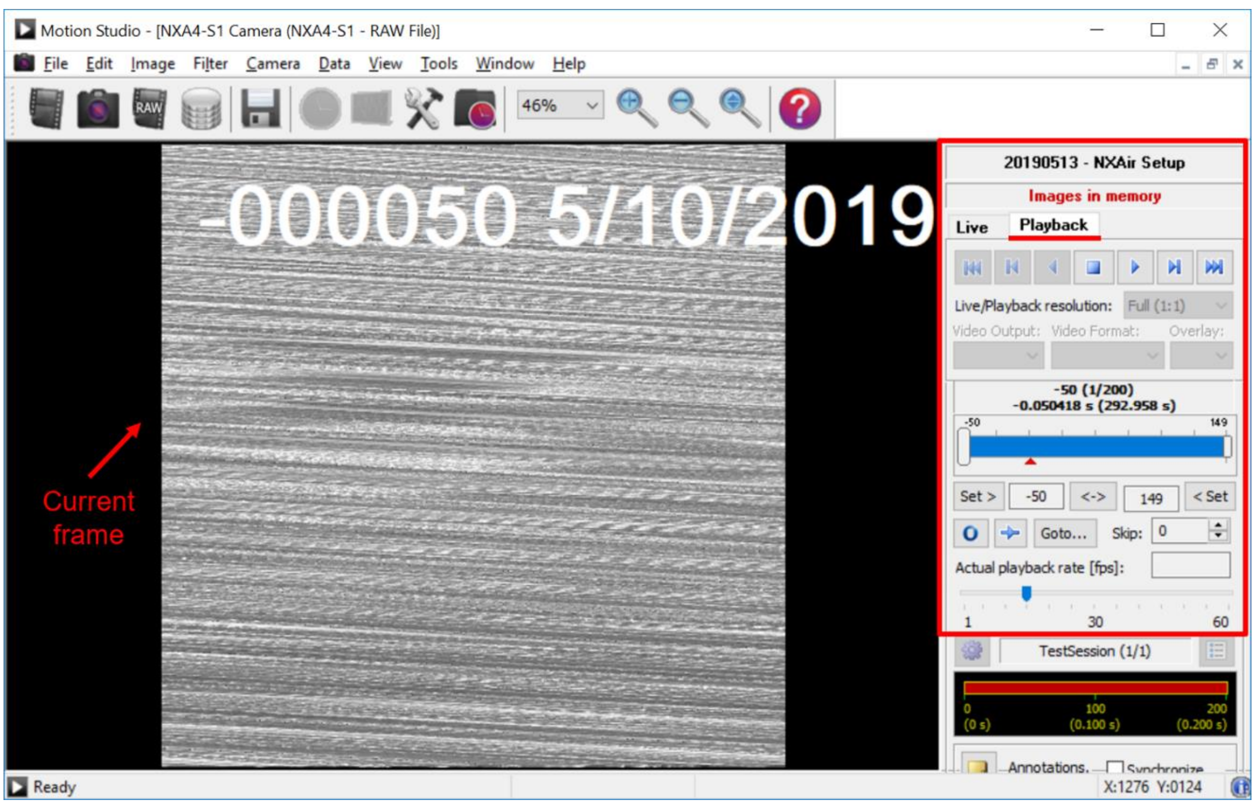


Fig. 93: The "Playback" tab when processing a saved video or image.

Note: The first frame from the video may appear noisy. All other frames will NOT have this noisy appearance.

6) Find the frame where the strain seems to be at its minimum. Use the forward [►|] and backward [|◄] buttons to advance or back up by one frame, respectively to identify this frame.

7) Once the frame of the minimum strain has been found, make sure to keep track of this image file.

> **Alternatively, it might be beneficial to, prior to performing the motion, save a snapshot of the sMEA when the VCA is on the flush position.** This might be especially important when the trigger is set to Motion-Trigger because the initial frame when the motion is starting might not be recorded, and thus, the strain calculations would be inaccurate.

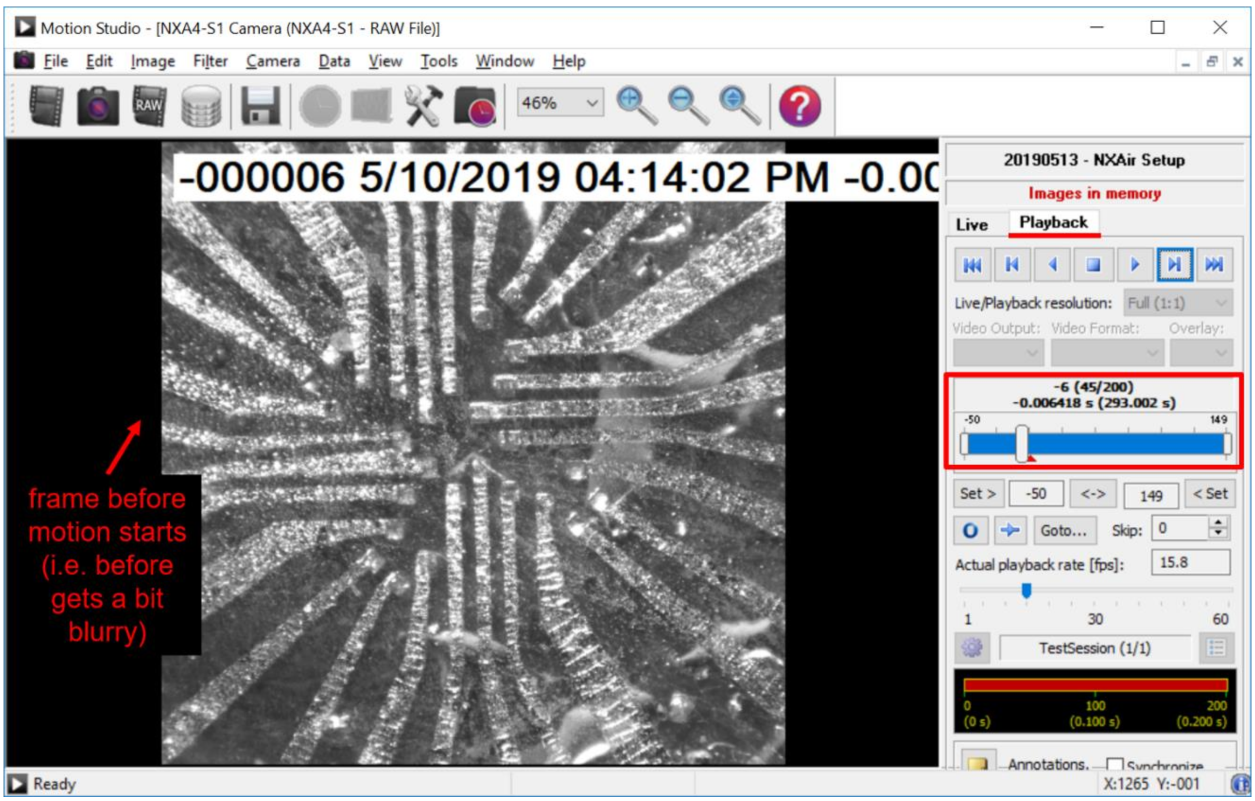


Fig. 94: Using the slider to navigate through the different images or frames to find the frame right before the motion starts.

8) Repeat **steps 6) and 7)** for the frame where the **strain seems to be at its maximum**.

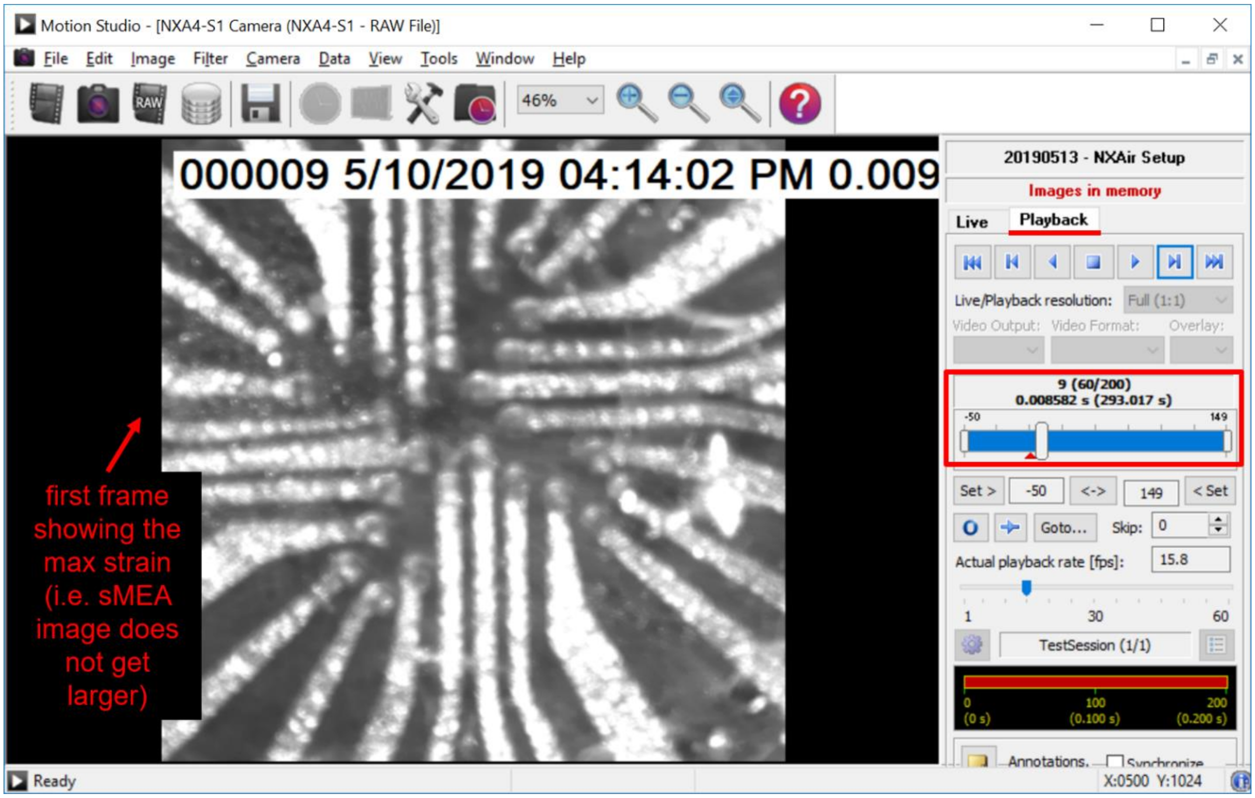


Fig. 95: Finding the first frame at max strain.

9) Repeat **steps 6) and 7)** for the **frame where the strain seems to be returning to the flush position** after applying the target strain.

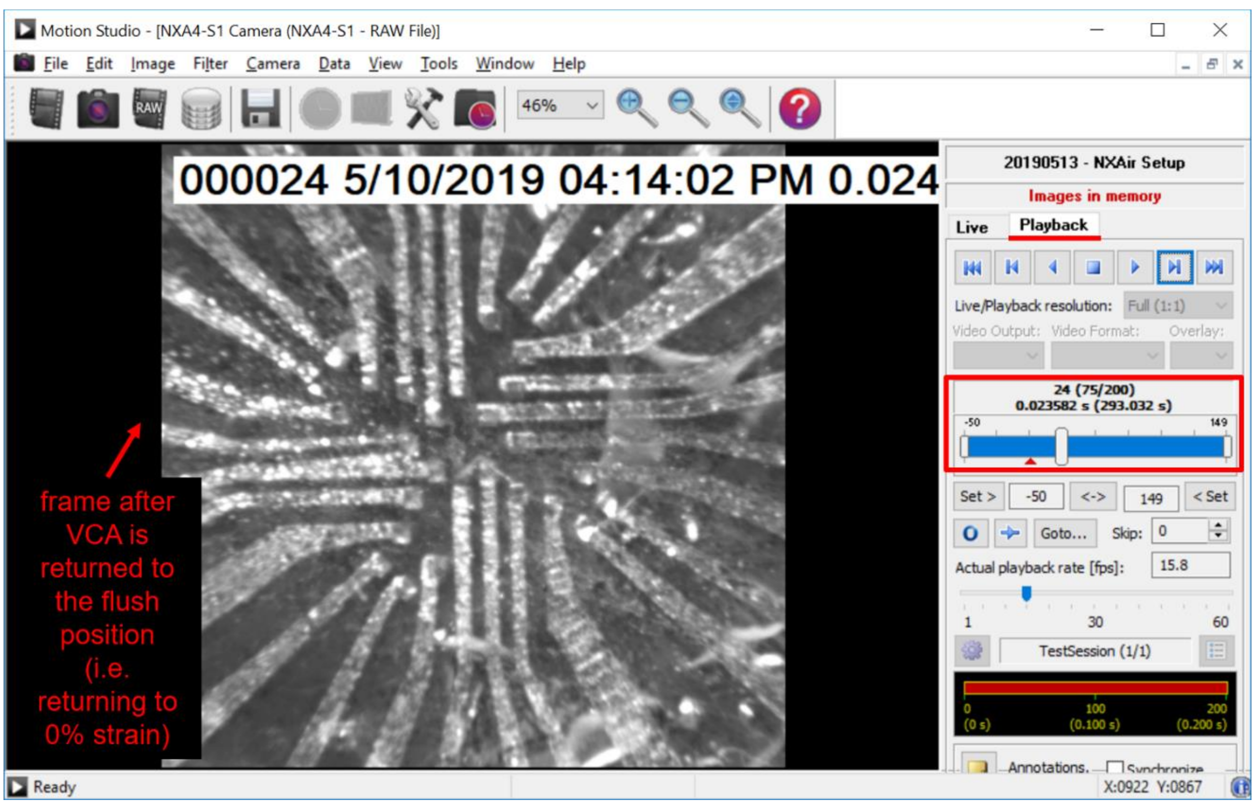


Fig. 96: Finding the frame after the VCA returns to the flush position.

10) If you saved the recording as a sequence of images, skip to **step 14)** to relabel the files of interest for use in Matlab.

11) If you opened a raw video file, click on "File" and "Save Acquisitions…". Select the range of images that include the last frame before the motion starts and the frame where the strain seems to be returning to the flush position and click "Save."

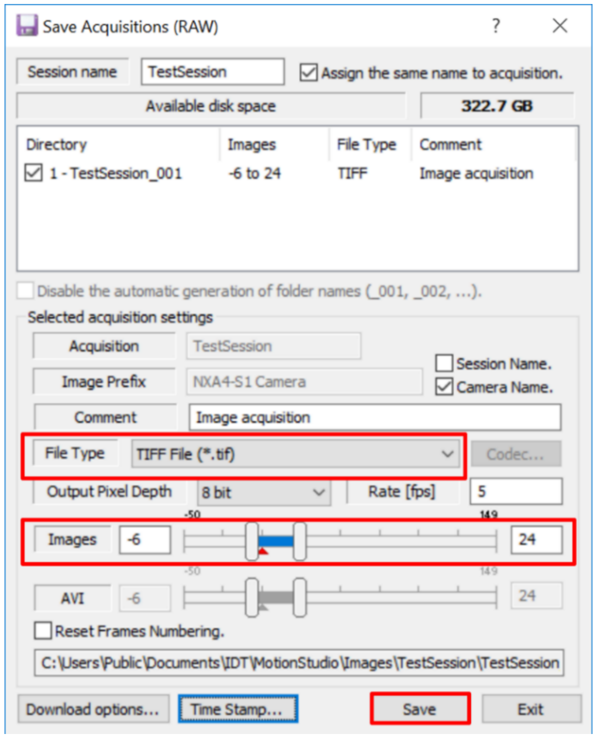


Fig. 97: Defining the type of the output files and range of images to be saved.

Note: At this point you may make changes to the time stamps for the saved images. For instance, the font size could be reduced to 32 and colors could be updated.

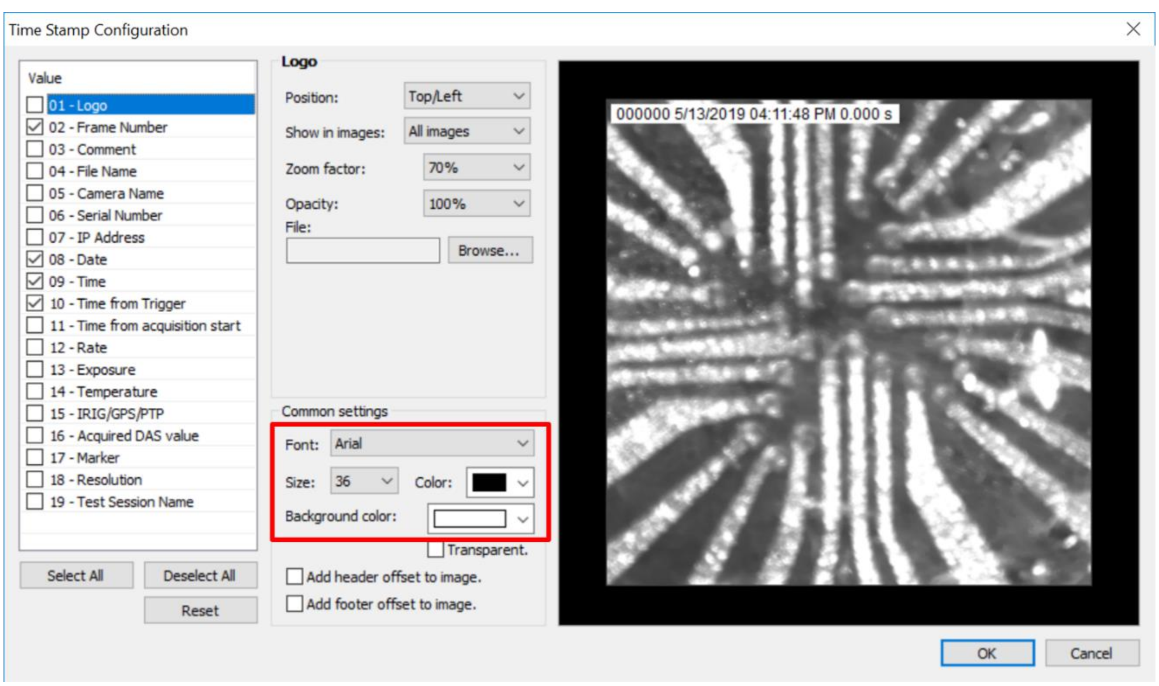


Fig. 98: Defining the font type, size, and color.

12) Once the images are saved, the "Download Manager" will show the status as completed. The number of saved images will be displayed here.

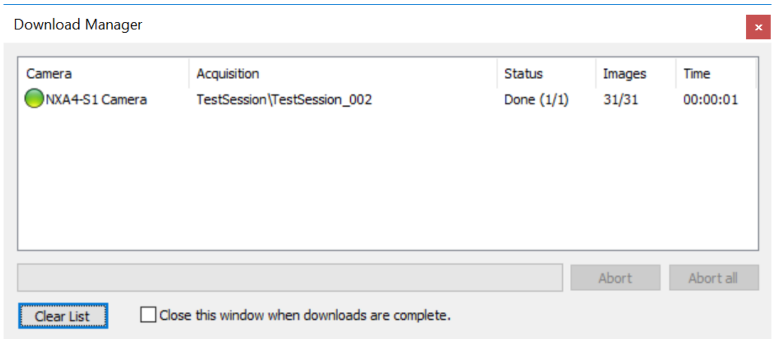


Fig. 99: The "Download Manager" after the download has completed.

<u>Note</u>: We recommend saving a range of images, as opposed to only at the flush position and target strain, for flexibility in choosing the frames to be used in the strain calculation.

13) The images will be saved in the image directory found in "Options…" under the "Tools" menu (**Fig. 64**). It is recommended to sort the images by *date* so that the image files are displayed in chronological order. Sorting the files by *name* would result in file being ordered alternating between negative and positive frames.

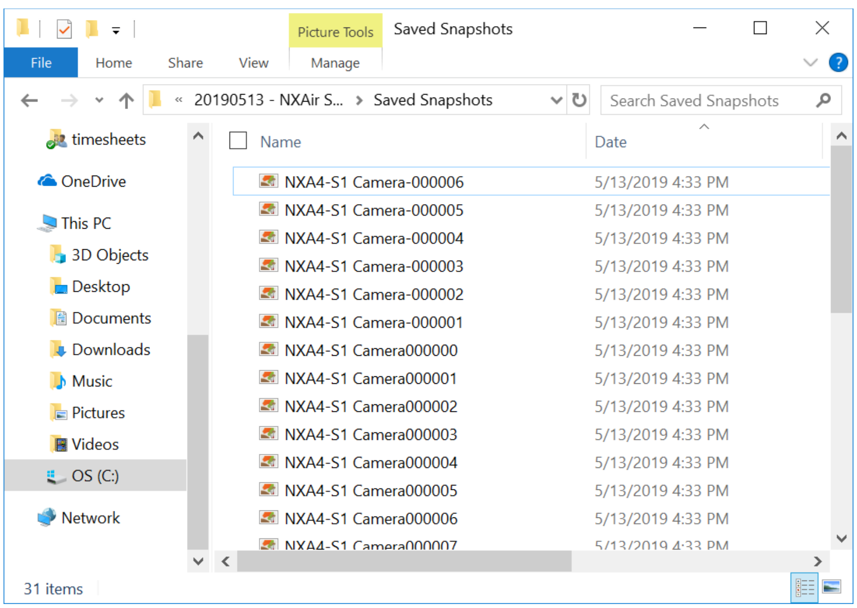


Fig. 100: Saved files in the specified directory in the "Options" window (**Fig. 64**).

14) Re-label the of files of interest the following way

- At flush position just before the motion started:
  <u>syntax</u>: -*sample_frame#_time_fps_date*
  for example, **-S449_frame-003_-003ms_1000fps_20190513**
- At the target (max) strain of this stretch
  <u>syntax</u>: -*sample_frame#_time_fps_date*
  for example, **-S449_frame+009_+009ms_1000fps_20190513**

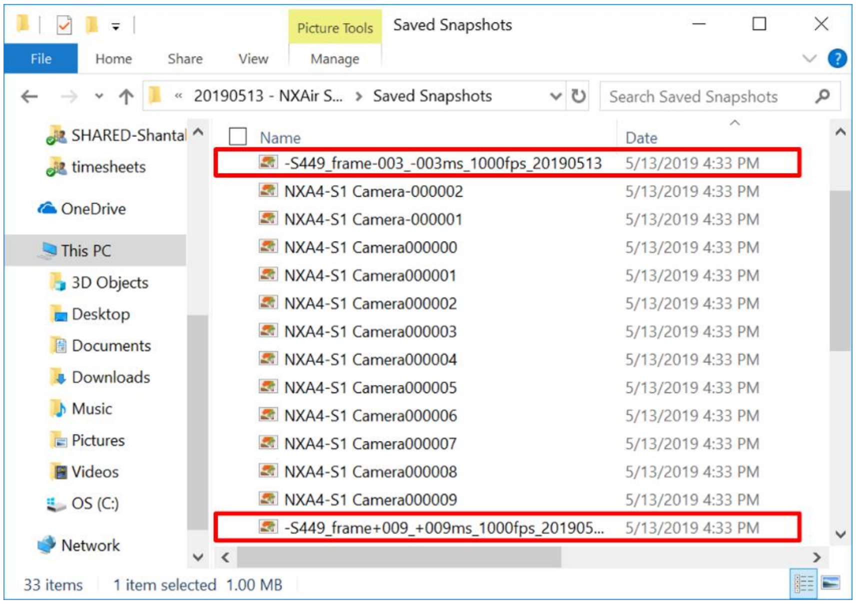


Fig. 101: Renaming the images files when the sample is at flush position (0% strain) and at the max strain.

Alternative to Motion Studio

An alternative to Motion Studio (for extracting frames from video) is the free software **VirtualDub**, which can be downloaded at https://sourceforge.net/projects/virtualdub/.
Refer to the **Imaging Module User Manual using IDS Cameras** for detailed instructions on how to use VirtualDub software.

8 Protocol to Measure Strain from Images

To measure strain from images, we use **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*.

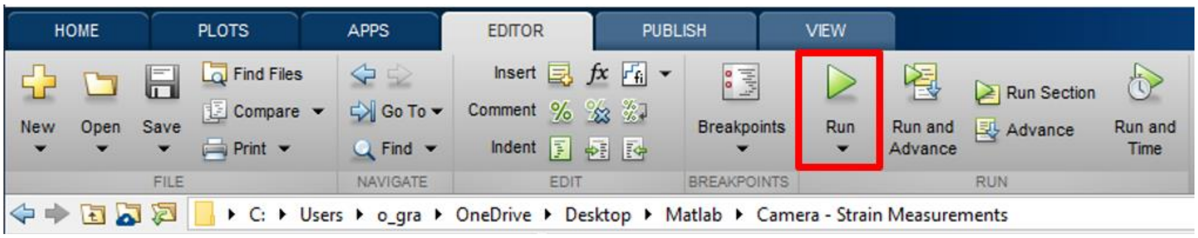


Fig. 102: The "Run" button in the Editor top menu bar.

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**".

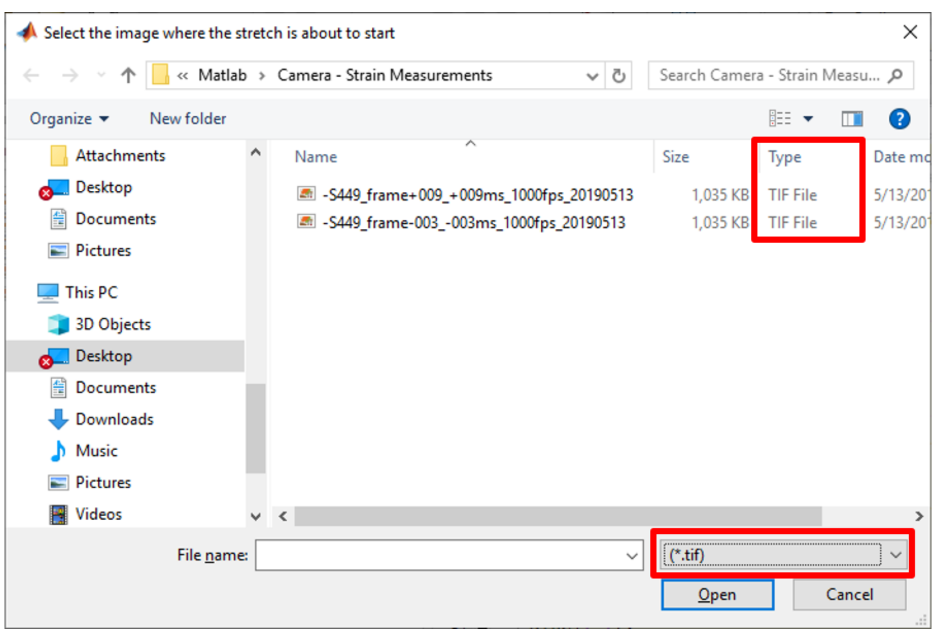


Fig. 103: Selecting the image files for strain measurements.

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**".

<u>Note</u>: 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."

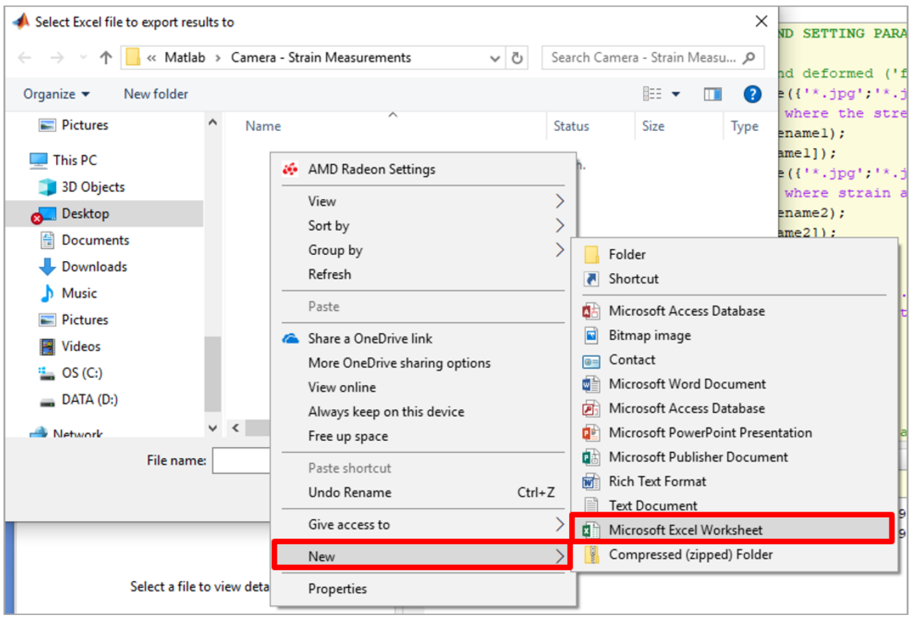


Fig. 104: Creating a blank Excel workbook to export results to.

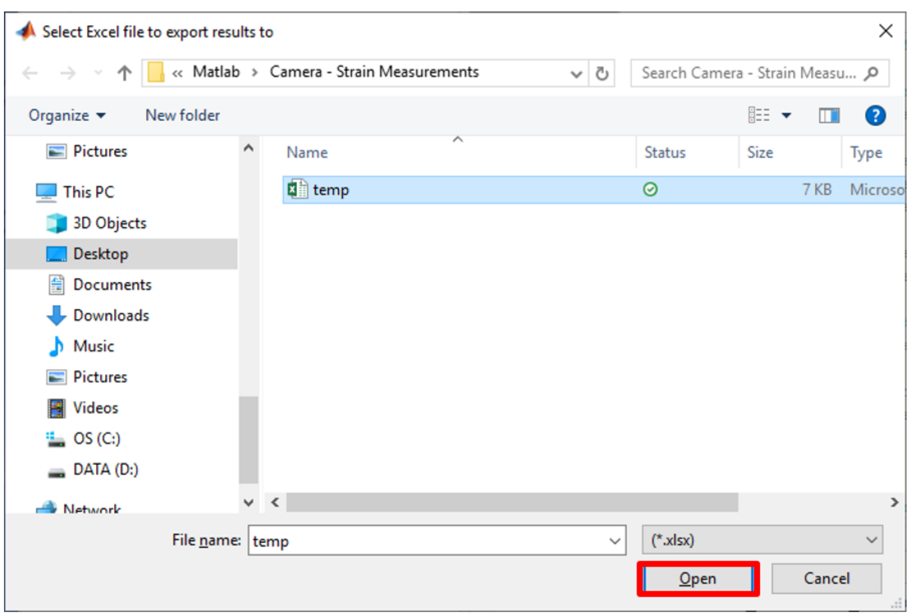


Fig. 105: Selecting the blank Excel workbook.

6) The program will ask you for the <u>number of slices or areas of interest</u> in the image. Type a number and press "Enter."

   For example, input "**2**".

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

8) The program will ask you about the <u>frame rate</u> 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 <u>frame # of the first image</u> (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 <u>frame # of the second image</u> (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 and the time elapsed.
    For example, input "**9**" as specified by the filename of the second image.

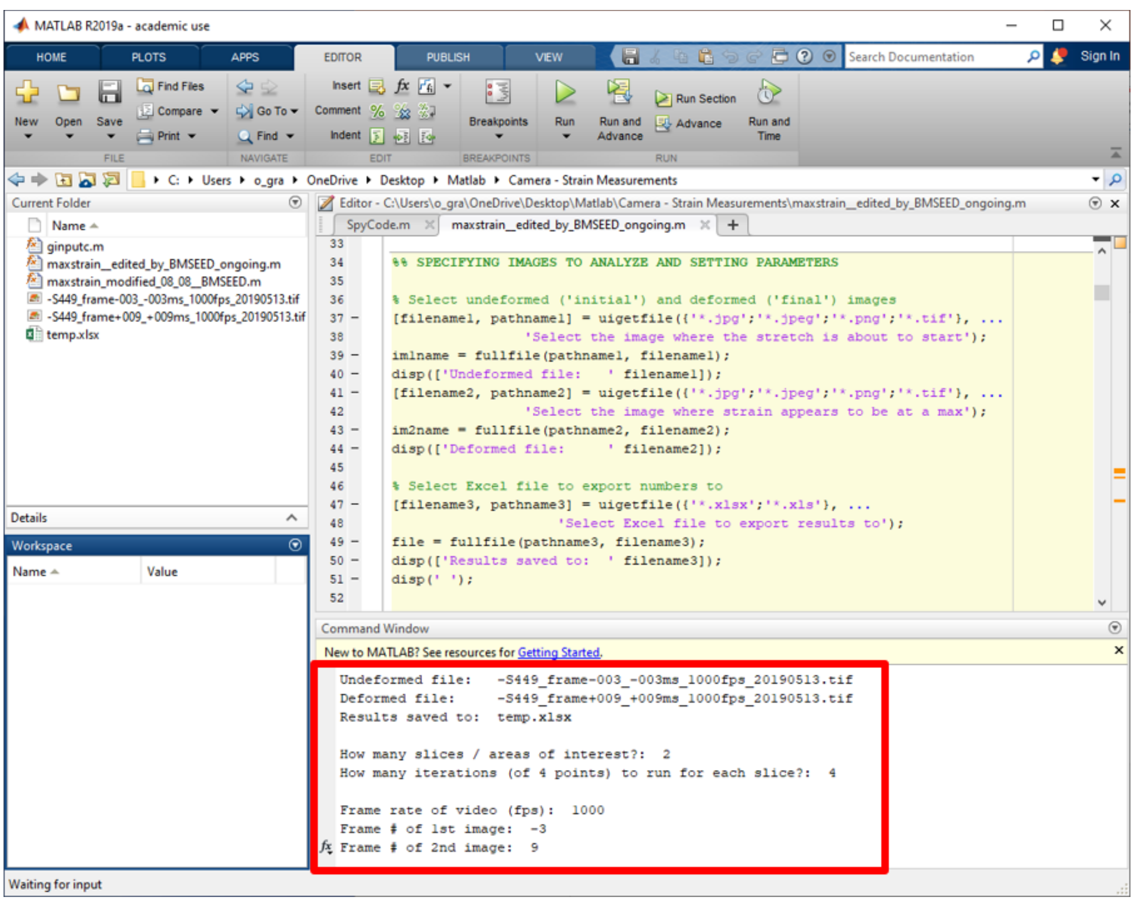


Fig. 106: Prompts and sample user input in Matlab's Command Window.

11) Matlab will now open the two images: undeformed image in Matlab Figure 1 and deformed image in Matlab Figure 2. Starting with Matlab 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.

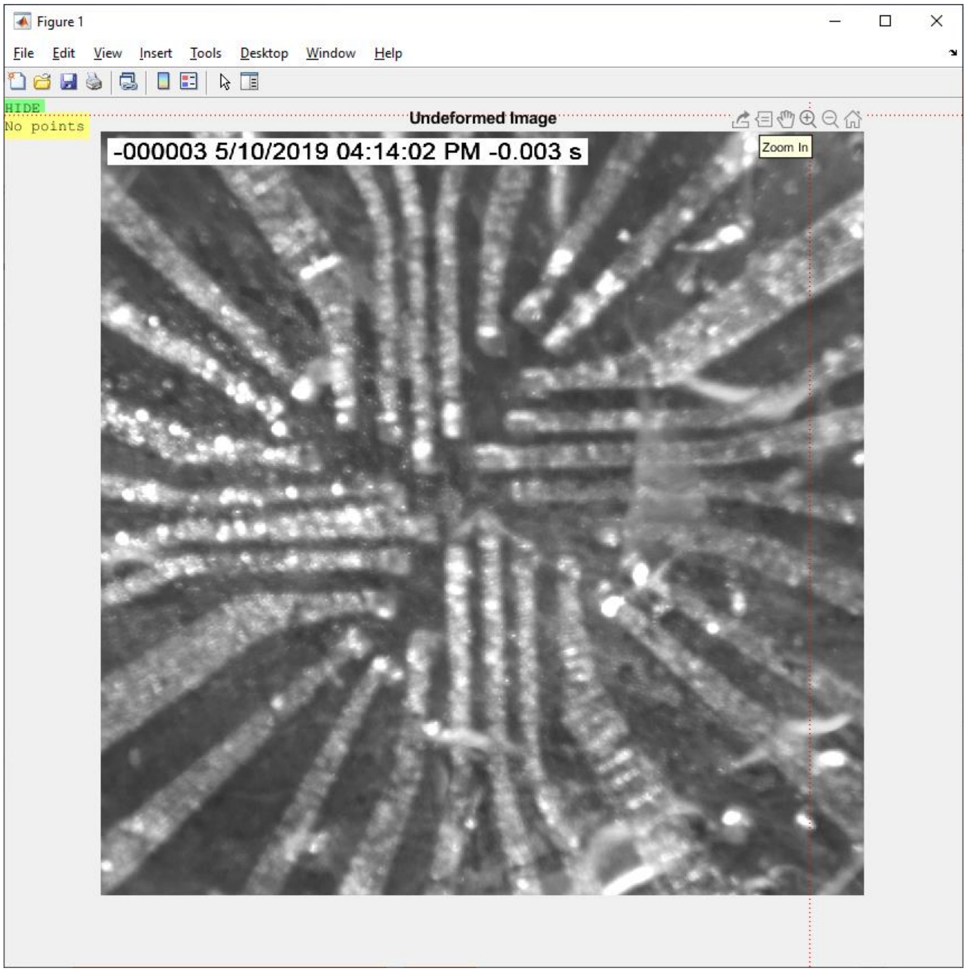


Fig. 107: Saved image file displayed in Matlab for strain measurements.

12) Locate the exact same feature in Matlab 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 has completed 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.

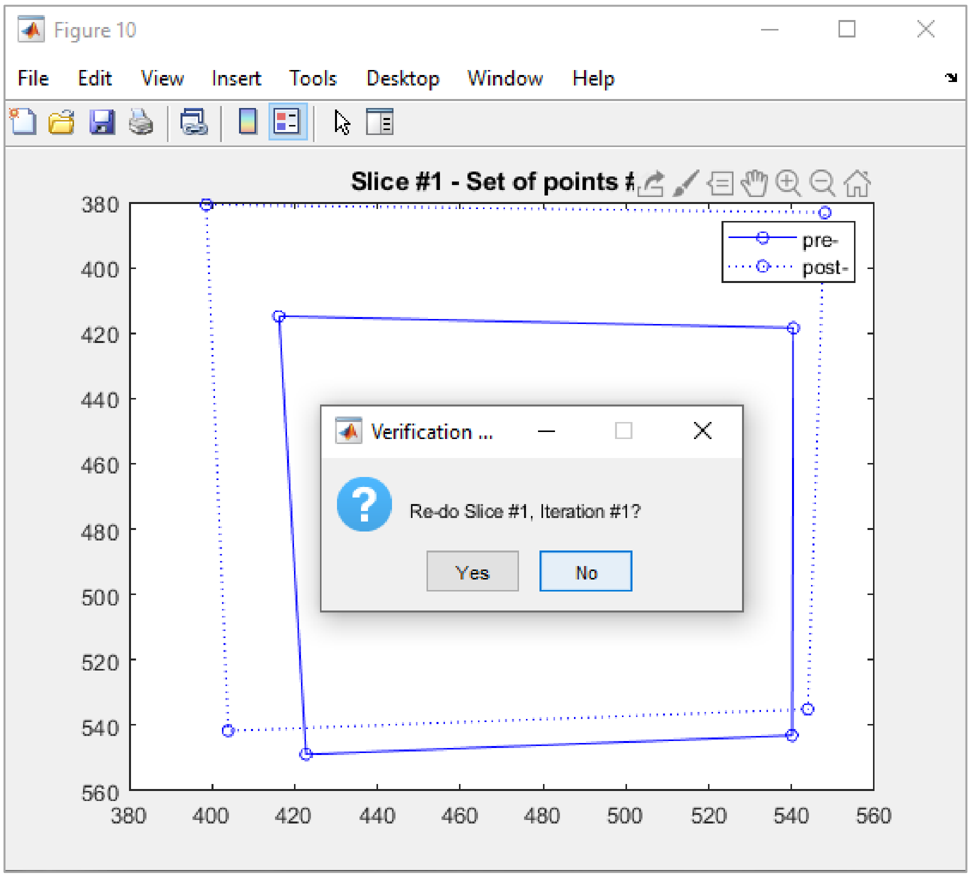


Fig. 108: Verifying the current iteration of markers selection.

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.

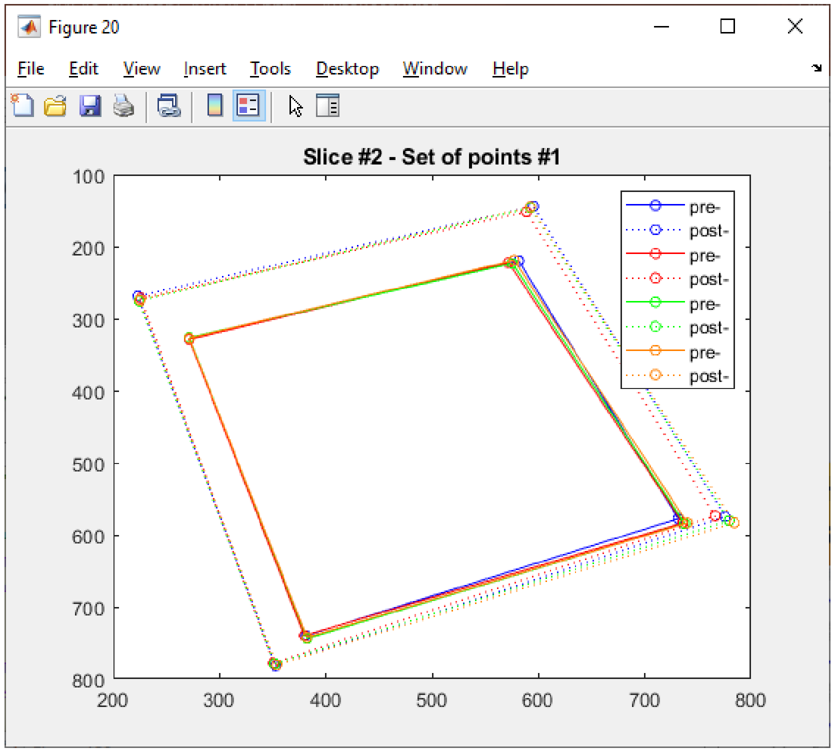


Fig. 109: Matlab figure overlaying the selected markers on the un-stretched image (i.e., at the flush position, or 0% strain) and stretched image.

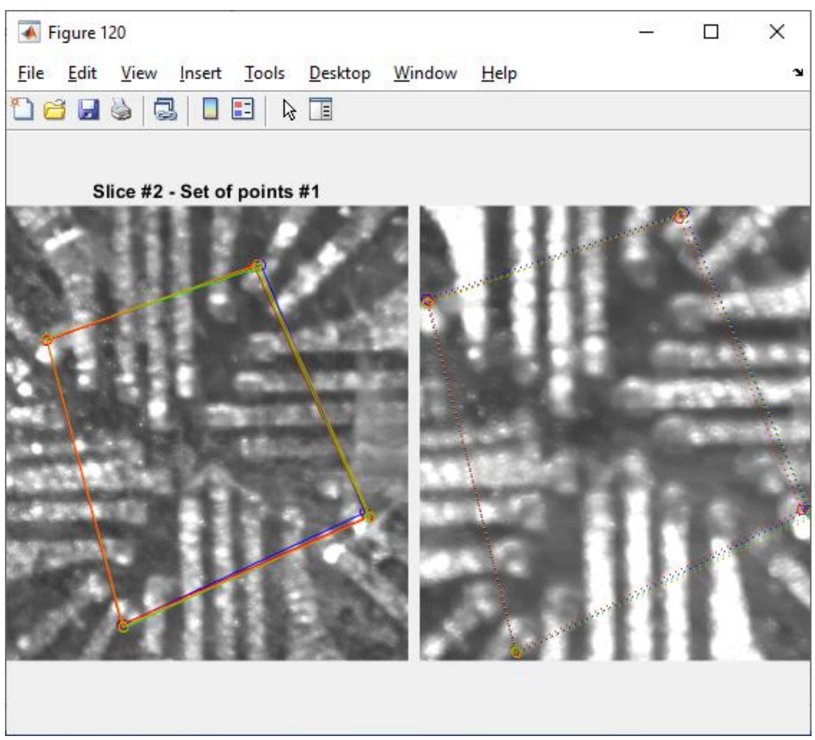


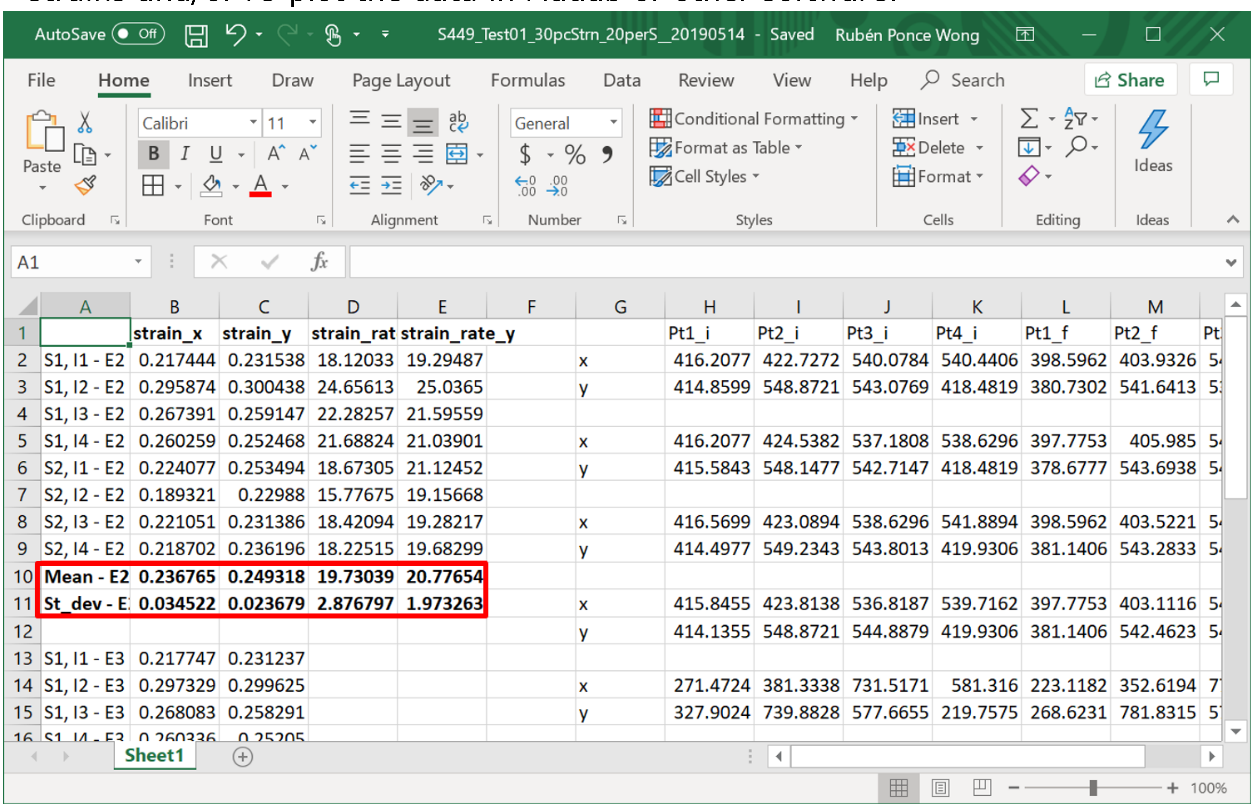
Fig. 110: Matlab figure showing the markers on the un-stretched and stretched images side-by-side overlaid on their respective camera images.

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

8.2 Output from Matlab Code

1) **<u>Excel spreadsheet</u>:** The main output from running the Matlab code can be found in the specified spreadsheet (specified in **step 5) from Section 8.1** 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 points 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	N
1		strain_x	strain_y	strain_rat	strain_rate_y			Pt1_i	Pt2_i	Pt3_i	Pt4_i	Pt1_f	Pt2_f	Pt
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	5
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	5
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	5
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	5
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	5
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	5
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	5
12							y	414.1355	548.8721	544.8879	419.9306	381.1406	542.4623	5
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	7
15	S1, I3 - E3	0.268083	0.258291				y	327.9024	739.8828	577.6655	219.7575	268.6231	781.8315	5
16	S1, I4 - E3	0.260336	0.25205											

Fig. 111: Screenshot of a workbook showing an example of the strain measurement results after running the Matlab script.

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) **Result figures:** 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 (as shown in **Fig. 109** of this manual) 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 (as shown in **Fig. 110** of this manual) 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) **Matlab workspace variables:** The Matlab (.mat) file allows the user to revisit results or perform further analysis using Matlab at a later time.

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

IMPORTANT: 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.

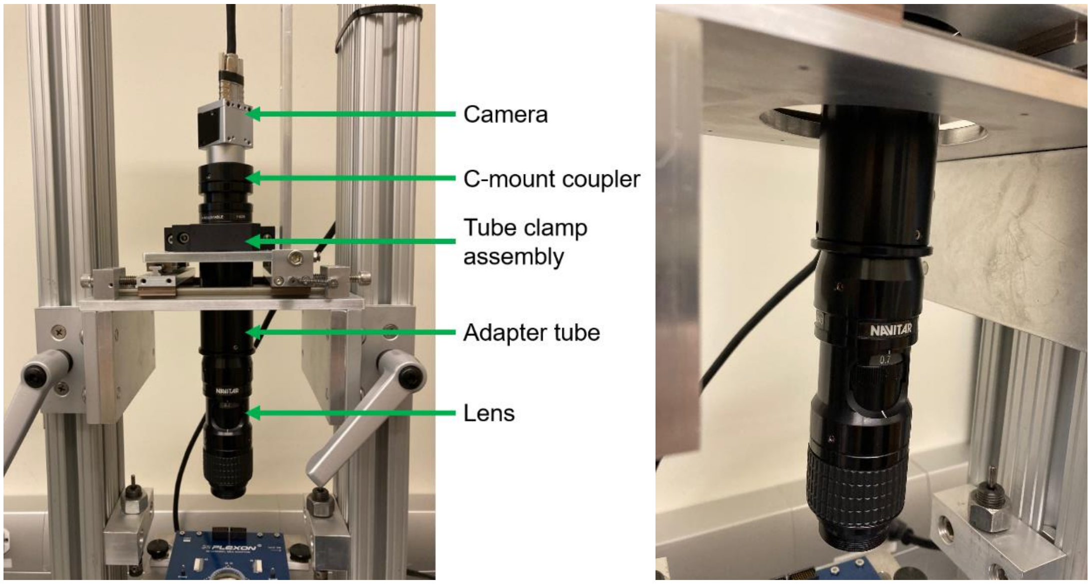


Fig. 112: Typical imaging hardware configuration: camera, C-mount coupler, adapter tube, and zoom lens.

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 **Fig. 113** below.

Fig. 113: UltraZoom 6000 lens, Mitutoyo objective coupler, and Motic objective.

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

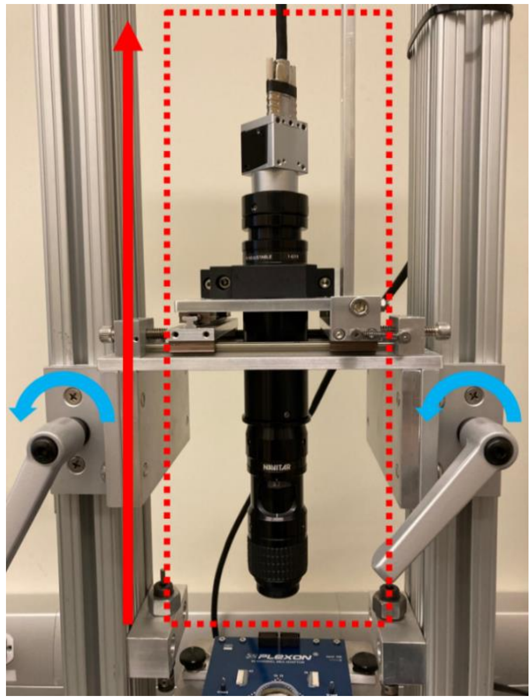


Fig. 114: Raising or lowering the vertical (z-) position of the camera assembly.

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.

   <u>IMPORTANT</u>: Make sure to always hold the lens with one of your hands while loosening the set screws.

Fig. 115: Removing the zoom lens from the adapter tube.

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.

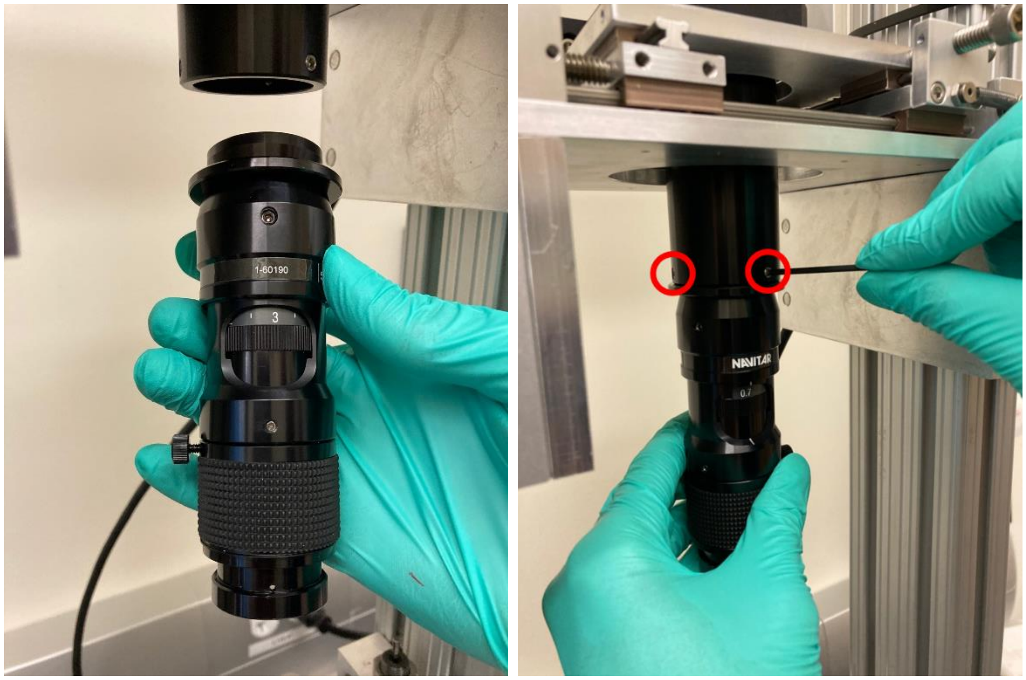


Fig. 116: Replacing the zoom lens into the adapter tube.

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

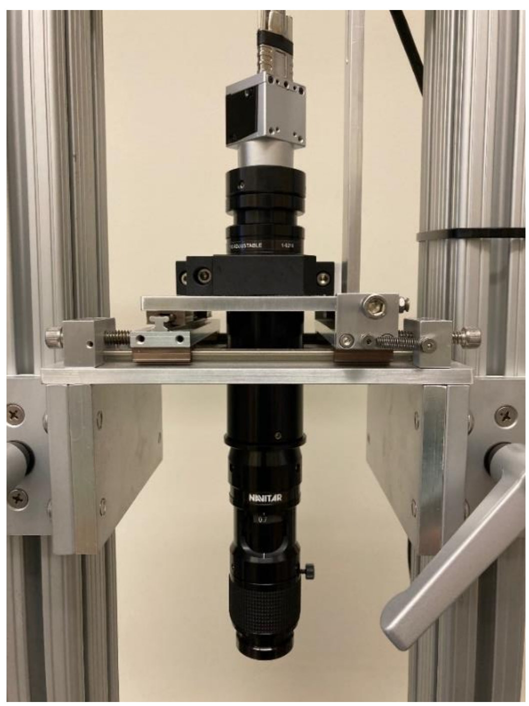


Fig. 117: Updated imaging assembly with the 'new' zoom lens.

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).

Fig. 118: Attaching the objective coupler to the 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.

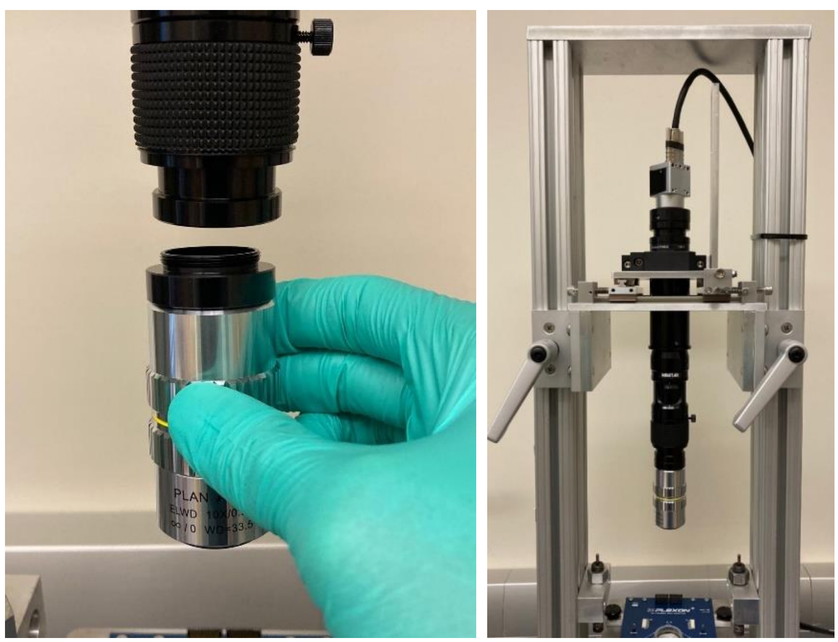


Fig. 119: Attaching the objective to the imaging assembly.

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.

Fig. 120: The 1 and 2 adapter tubes side by side.

1) Raise the camera stage assembly on the MEASSuRE frame by loosening the two knobs following a counter-clockwise motion (cyan in **Fig. 114**), moving the camera platform up (red in **Fig. 114**), 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.

   <u>IMPORTANT</u>: Make sure to always hold the lens with one of your hands while loosening the set screws.

(a) (b) (c)

Fig. 121: (a)-(b) Removing the zoom lens. **(c)** Imaging assembly with the zoom lens removed.

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.

   <u>IMPORTANT</u>: Make sure to always hold the camera with one of your hands while loosening the set screws.

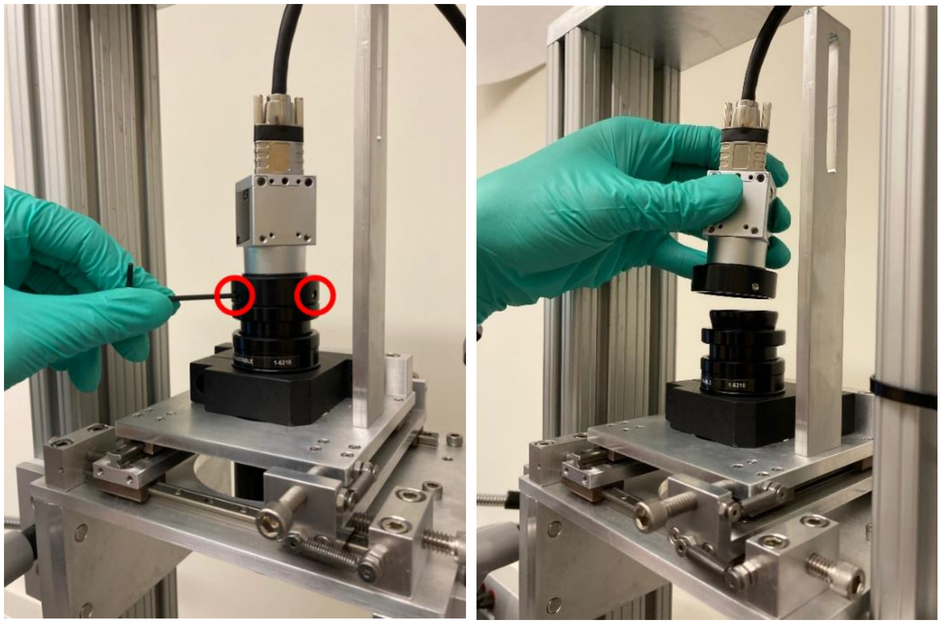


Fig. 122: Removing the camera and C-mount coupler from the imaging assembly.

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.

   <u>IMPORTANT</u>: Make sure to always hold the adapter with one of your hands to avoid damaging it.

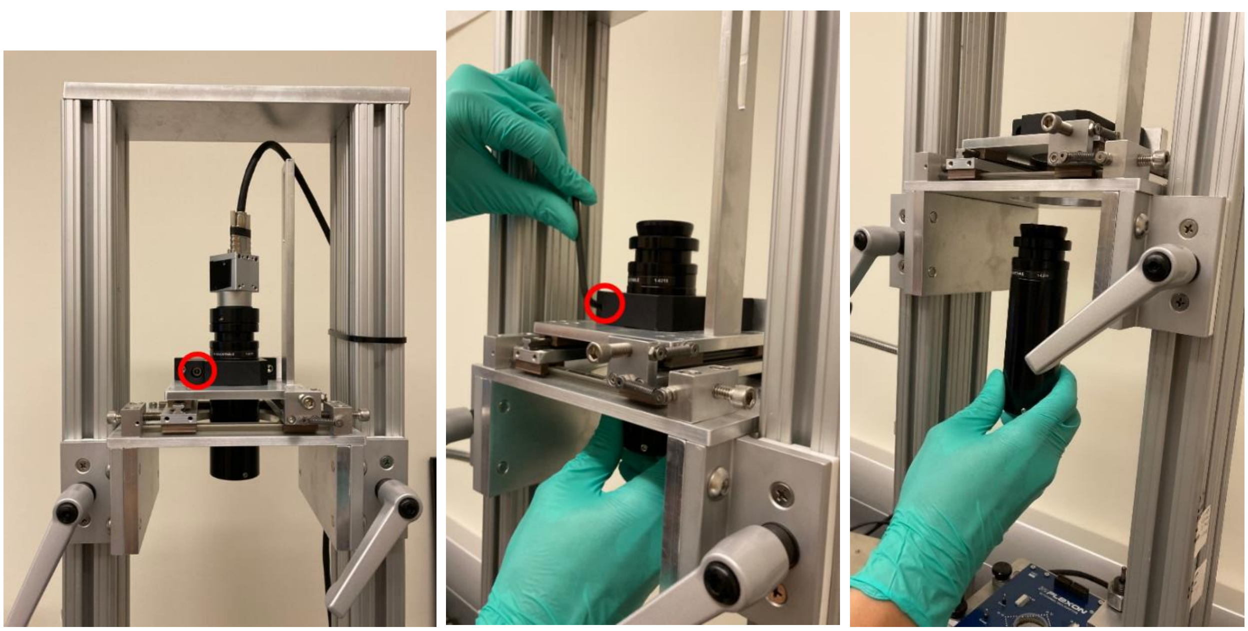


Fig. 123: Removing the adapter tube from the MEASSuRE frame.

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.

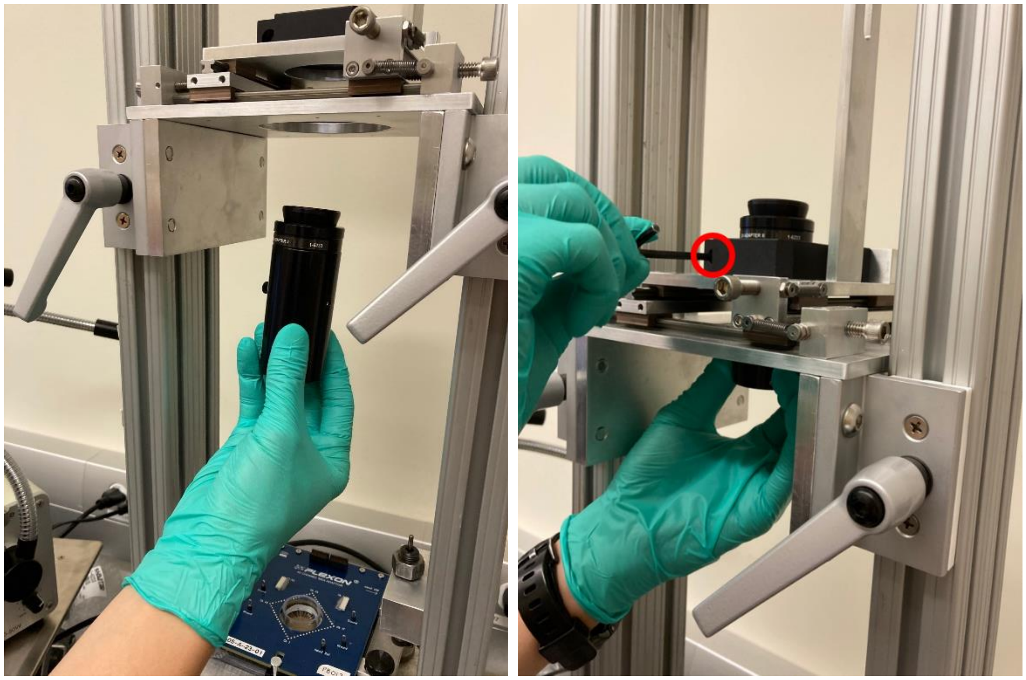


Fig. 124: Attach the new adapter tube to the MEASSuRE frame.

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.

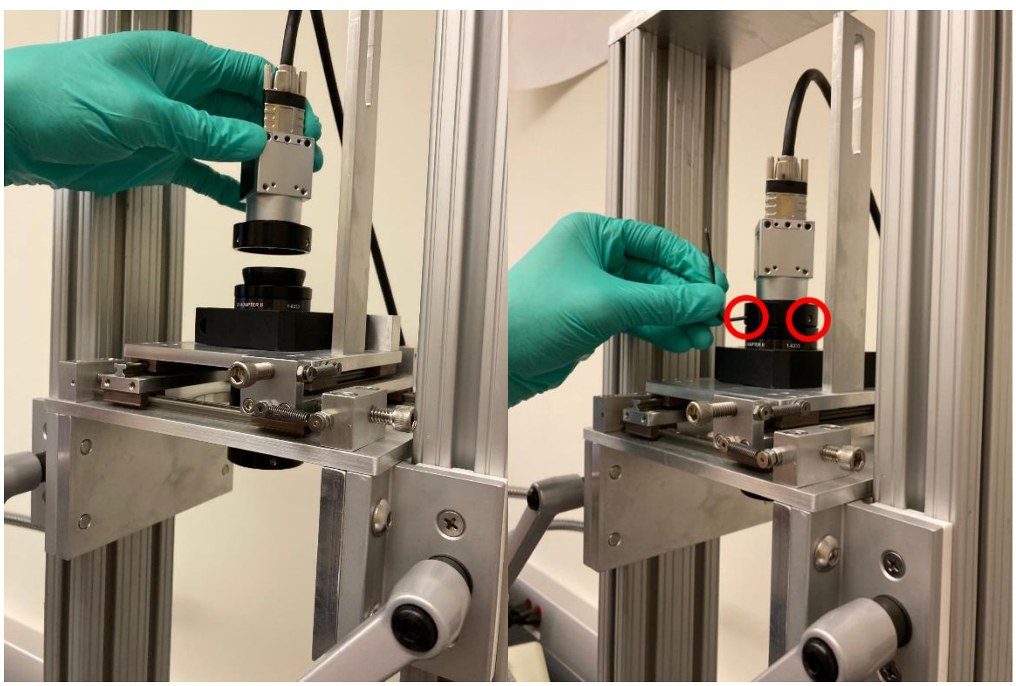


Fig. 125: Reattaching the camera and C-mount coupler to the newly inserted adapter tube

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.

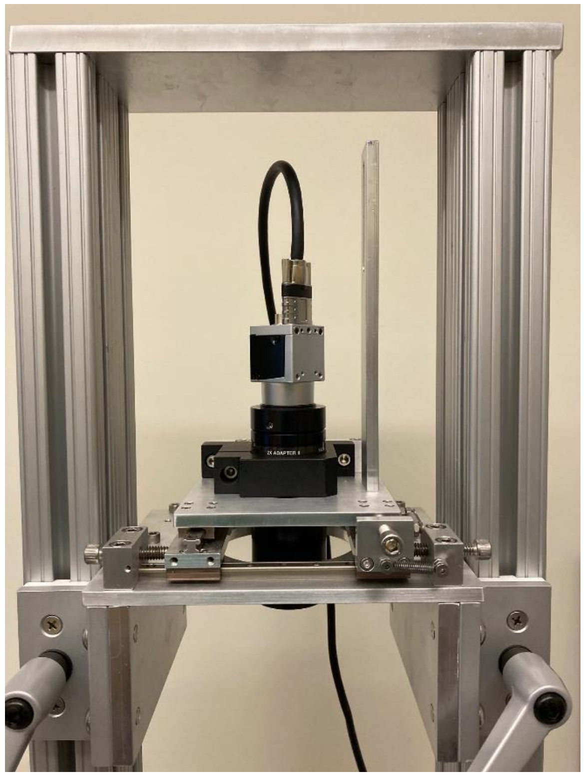


Fig. 126: Camera, C-mount coupler, and adapter tube mounted to MEASSuRE.

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

Table 4: Working distances for various 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 the Plexon interface board holding the sample[γ] (mm)	80	26	80	26	24	24

α: Not considering 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.

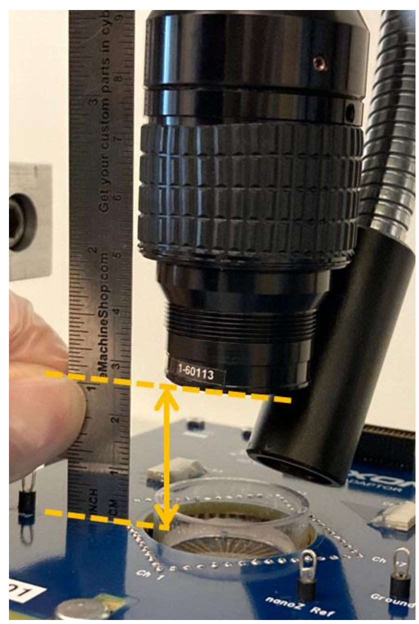


Fig. 127: Measuring the working distance.

Appendix C: Finding the Flush Position

The configurations for the different MEASSuRE systems 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.

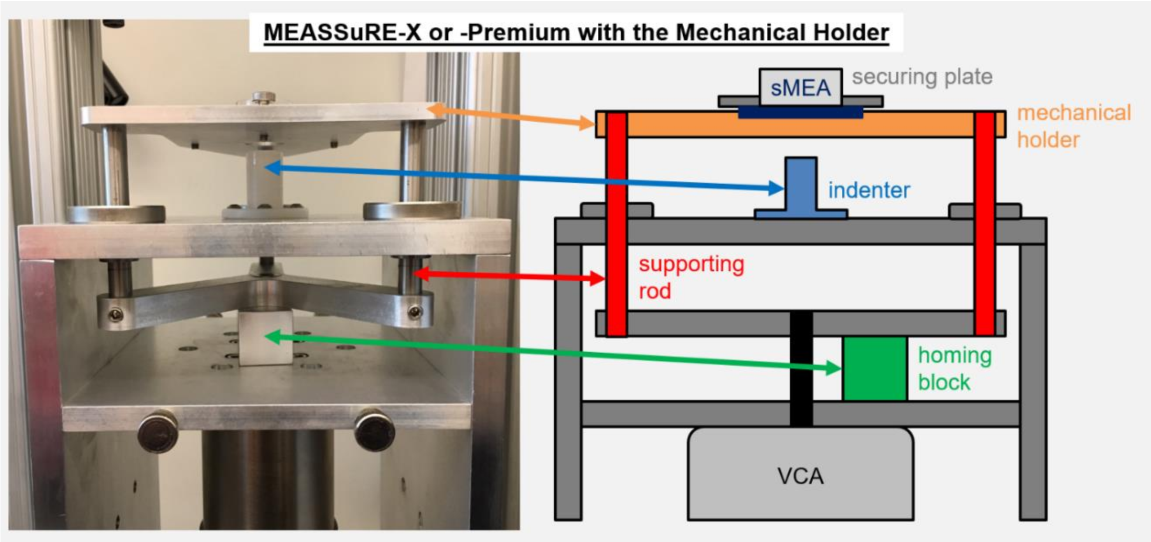


Fig. 128: Schematic of components when using a MEASSuRE-X or -Premium with the mechanical holder.

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

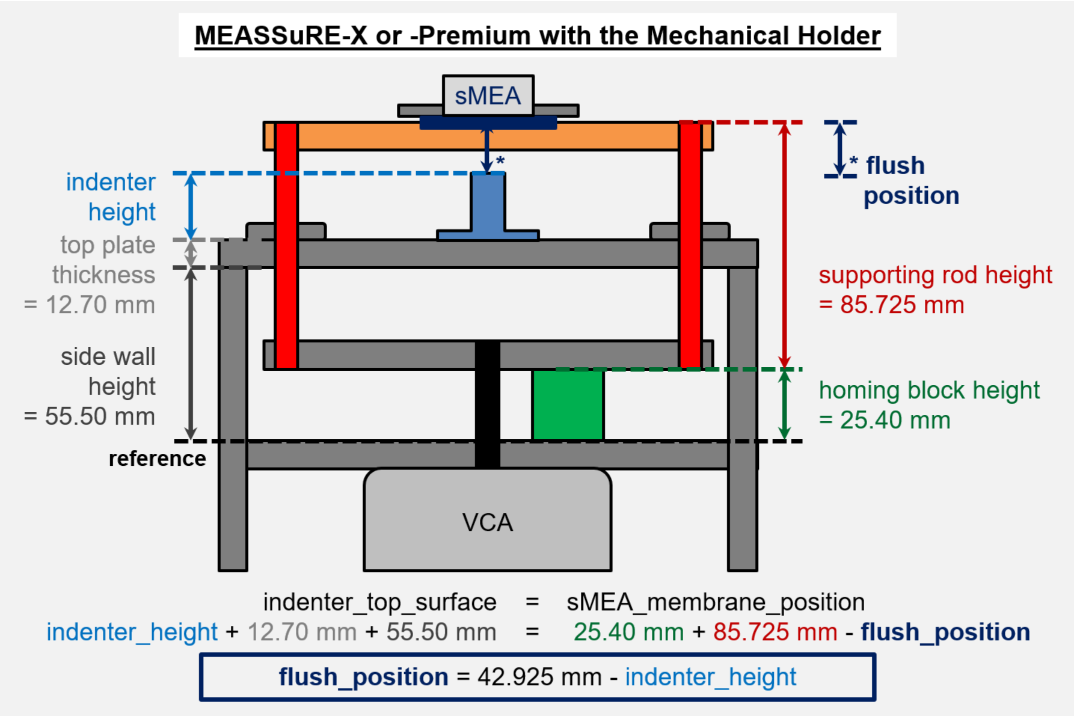


Fig. 129: Schematic for flush calculations when using a MEASSuRE-X or -Premium with the mechanical holder.

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.

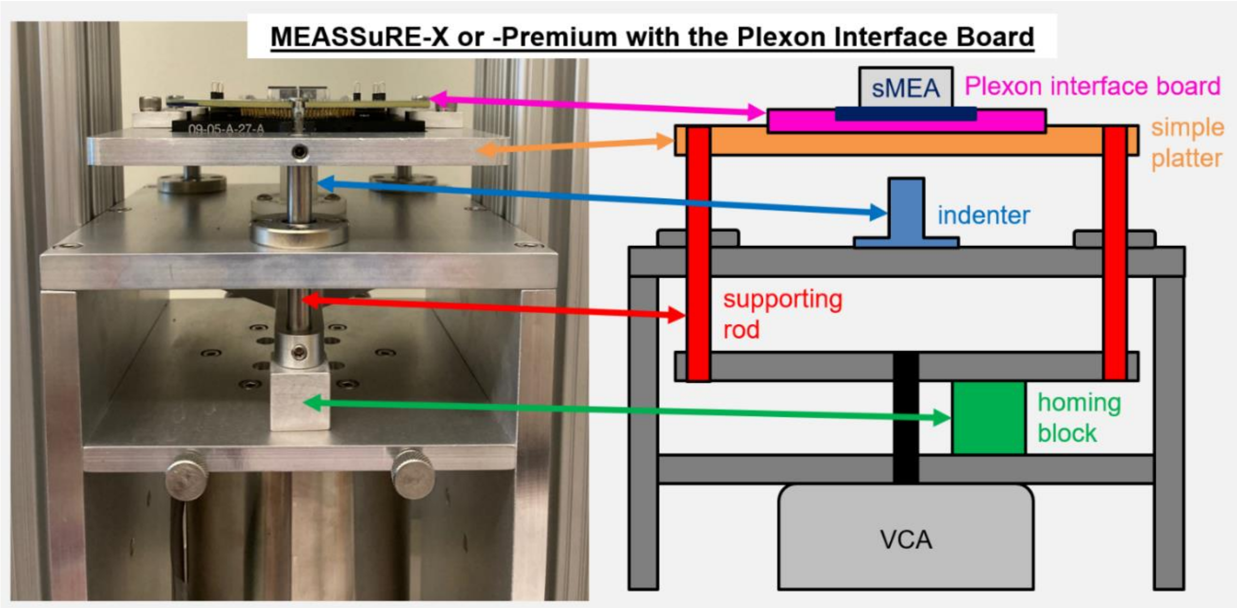


Fig. 130: Schematic of components when using a MEASSuRE-X or -Premium with the Plexon interface board.

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

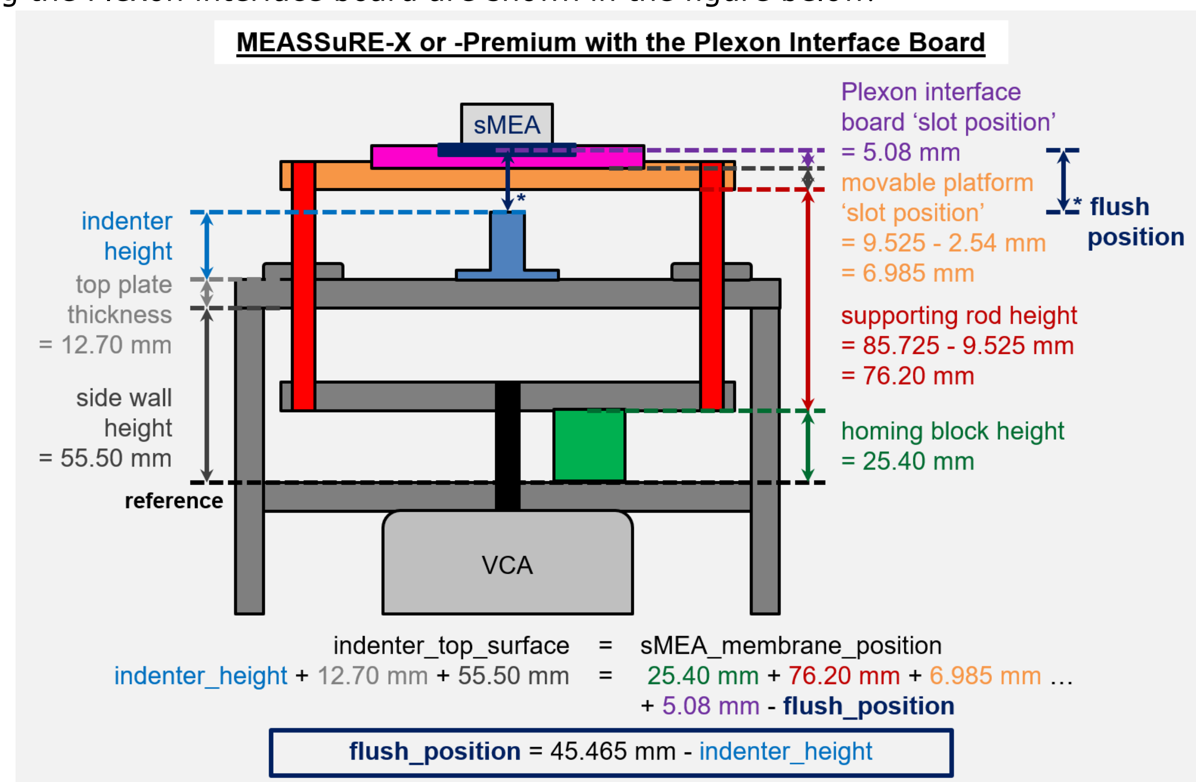


Fig. 131: Schematic for flush calculations when using a MEASSuRE-X or -Premium with the Plexon interface board.

3. **<u>MEASSuRE-Mini with the Plexon interface board</u>:** 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.

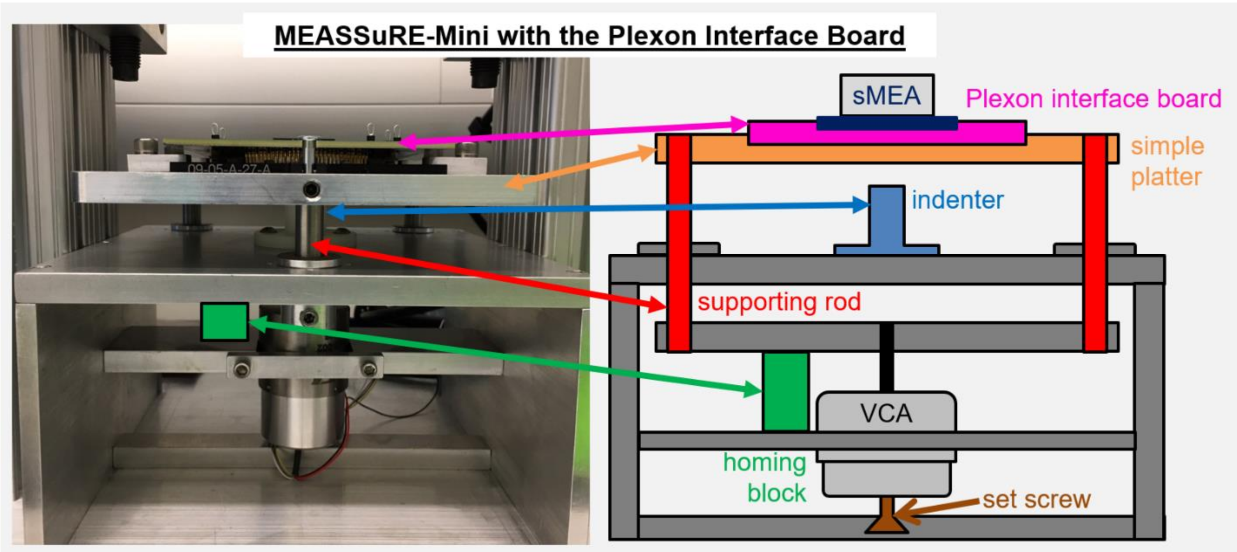


Fig. 132: Schematic of components when using a MEASSuRE-Mini with the Plexon interface board

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

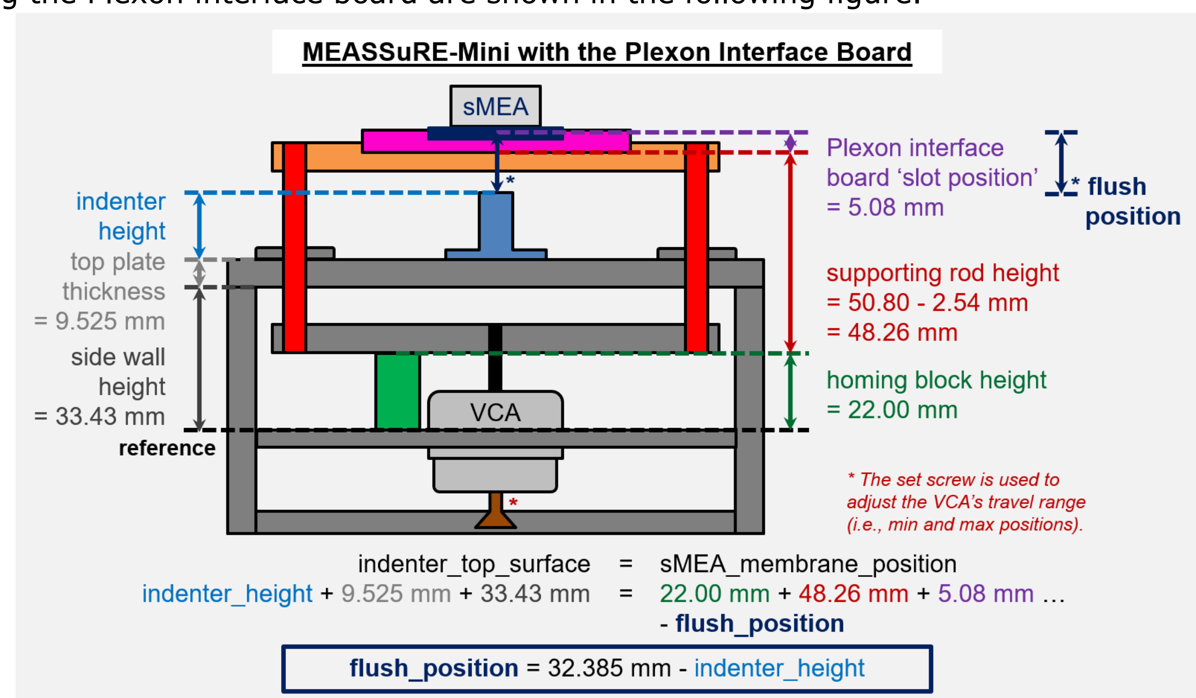


Fig. 133: Schematic for flush calculations when using a MEASSuRE-Mini with the Plexon interface board.

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**.

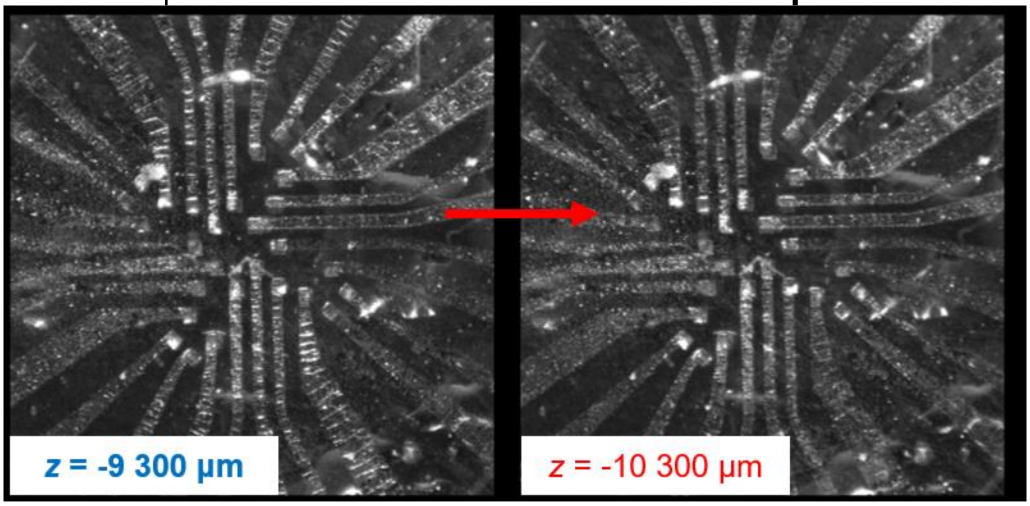


Fig. 134: Example of a flush position being identified at *z* = -9300µm using the NXA4 camera.

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.

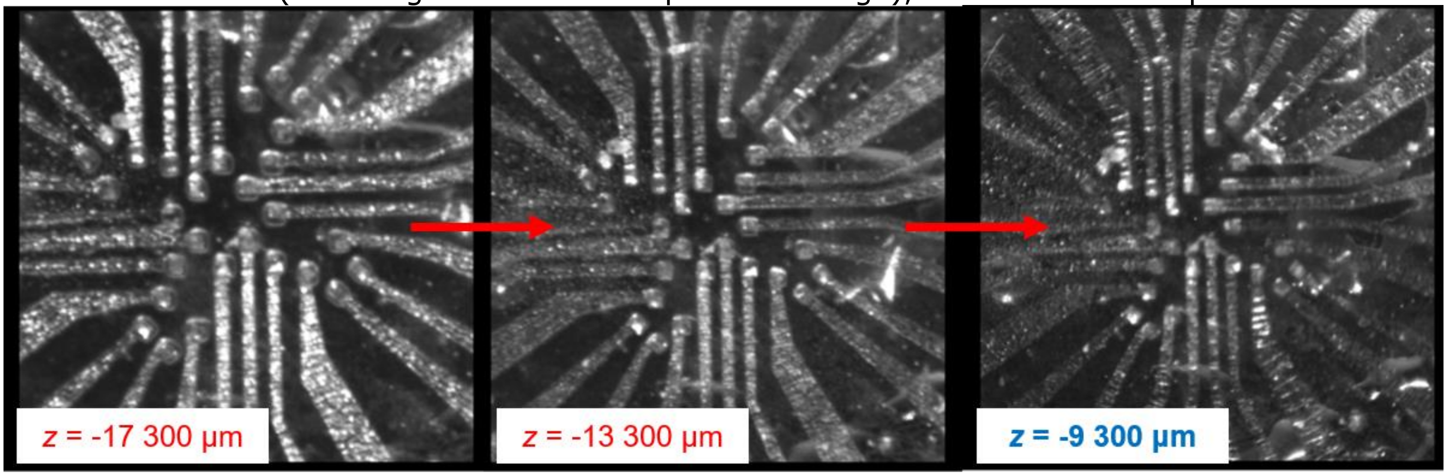
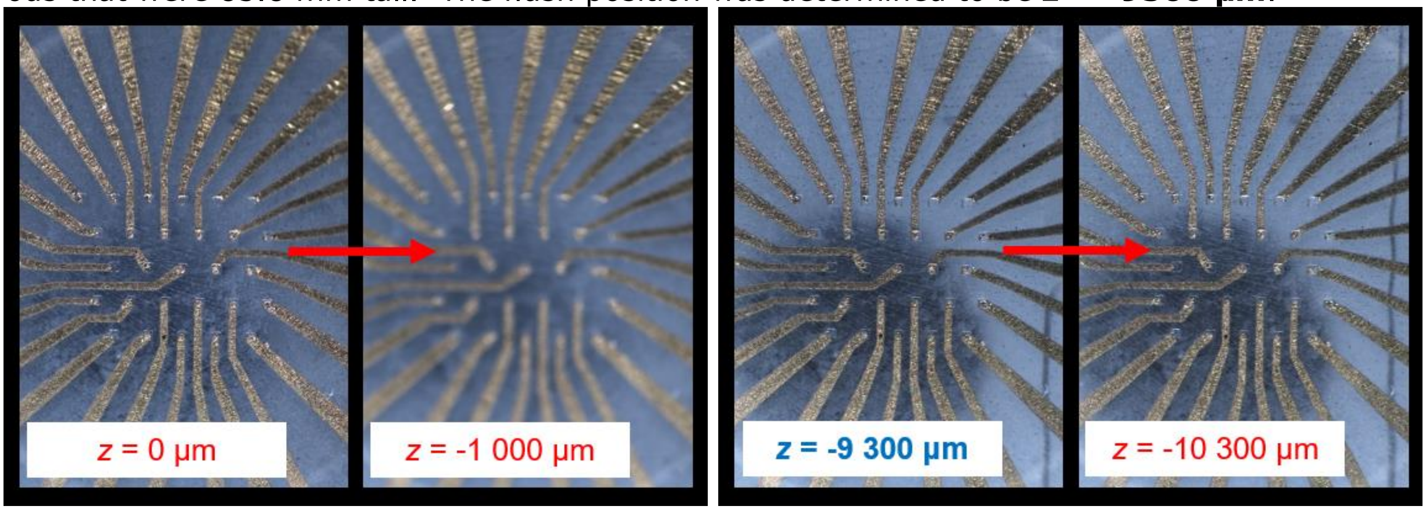


Fig. 135: Testing the flush position identified earlier at *z* = -9300µm using the NXA4 camera.

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**.



(a) (b)

Fig. 136: (a) Example of starting to search for the flush position using a CCM camera. (b) Example of a flush position being identified at *z* = -9300µm using the CCM camera.

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.

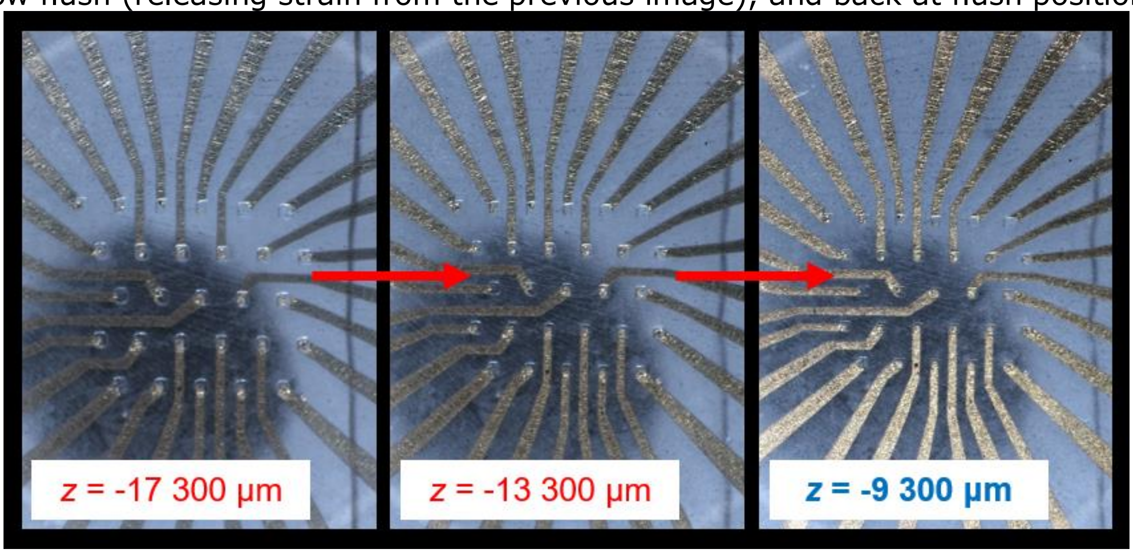


Fig. 137: Testing the flush position identified earlier at *z* = -9300µm using the CCM camera.

Appendix F: List of Parameters to Optimize Image Quality

- **<u>Light source</u>:** the main source of illumination for the camera image. Some parameters of the light source that affects the image quality include:
  - Power (wattage) of the LED
  - Intensity setting
  - Type of lamp: gooseneck vs. ring light
  - If using a gooseneck lamp, proximity to sMEA and angle of light on the sMEA
  - If using a ring light, the presence or absence of a light diffuser.

- **<u>Aperture</u>:** the opening of the diaphragm of the lens through which light passes. A smaller aperture results in a greater depth of field but with reduced light. Conversely, a larger aperture results in more light but with a smaller depth of field, making the image more prone to blurring.
  Note: In MEASSuRE systems, this parameter can only be adjusted if using a Zoom 6000 lens with aperture (i.e., Navitar 1-60135A).

- **<u>Region of interest (ROI)</u>:** the camera setting that allows only a subset of the camera sensor array to be read (i.e., is less than the total available area of the camera sensor). Using the setting to reduce the field of view helps increase the maximum frame rate, as frame rate is inversely proportional to the number of rows in the ROI.

- **<u>Exposure (a.k.a. shutter speed)</u>:** the amount of time that light reaches the image sensor. Exposure is limited by the sampling rate as its maximum value is inversely proportional to the frame rate (i.e., exposure $\leq$ 1/rate). Note that long exposure times result in more susceptibility to motion blur, thus, it is not recommended to maximize the exposure.

- **<u>Sensor gain</u>:** the analog signal amplification from the camera's sensor. Analog gain is gain before the analog-to-digital (ADC) converter and can be useful in low light conditions. In general, the sensor gain should be increased only after optimizing the lighting settings (e.g., light source settings) and after exposure time has been set. Digital gain, which is gain after the ADC, is not true gain as some information is lost in the process.

- **<u>Gamma</u>**: the grayscale reproduced on the image. Gamma can be used to increase the signal to noise ratio and can be thought of as the ability to stretch either side (i.e., black or white) of the pixels' dynamic range. A gamma of one indicates that the camera sensor is precisely reproducing the object grayscale (linear response). In Motion Studio, the gamma correction to the image ranges from 0.1 to 4.0 (default of 1.0).

- **<u>Look up table or LUT (a.k.a. colormap)</u>:** camera setting that can improve the contrast and brightness of an image by modifying the dynamic intensity of regions with poor contrast. LUT transformations can highlight details in areas containing significant information at the expense of other areas that are not as relevant.

- **<u>Sharpness</u>:** the clarity of detail in the camera image. Higher sharpness result in images with more distinct contours. In Motion Studio, the "sharpen" parameter can be set between 0.0 to 0.7 (default of 0.0).

- **<u>Brightness</u>:** the value of a pixel in the camera image, representing the lightness value from black to white. In Motion Studio the brightness can be set between -0.25 to 0.25 (default of 0.0).

- **<u>Contrast</u>:** the measure of the difference in the bright and dark areas of an image. High contrast results in more lively images, and low contrast results in flatter and more monotonous images. In Motion Studio the contrast can be set between 0.5 and 1.5 (default of 1.0).

Appendix G: Tradeoffs in Magnification and Depth of Field

Zoom 6000 Field of View Matrix (in mm at nominal W.D.)

Lens Attach-ment	Working Distance (mm)	Camera Format/ Parameters	.5X Adapter Low - High	.67X Adapter Low - High	1X Adapter Low - High	1.33X Adapter Low - High	2X Adapter Low - High	3.3X Adapter Low - High	5X Adapter Low - High (2)	Resolve Limit (µm) Low-High	Depth of Field (mm) Low-High
0.25X 0.006 - 0.018 NA 1-6044	300 (nominal) 180-334 (1) W.D. range	Mag.	0.09X - 0.56X	0.12X - 0.75X	0.18X - 1.13X	0.24X - 1.50X	0.35X - 2.25X	0.59X - 3.73X	0.88X - 5.62X	55.56 - 18.52	13.89 - 1.54
		1/3" Sensor	68.64 - 10.64	51.12 - 8.04	34.32 - 5.32	25.80 - 4.00	17.16 - 2.66	10.40 - 1.61	6.88 - 1.08	55.56 - 18.52	13.89 - 1.54
		1/2" Sensor	91.36 - 14.16	68.06 - 10.66	45.68 - 7.08	34.34 - 5.32	22.84 - 3.54	13.84 - 2.14	9.12 - 1.44	55.56 - 18.52	13.89 - 1.54
		2/3" Sensor	91.40 - 19.52	93.62 - 14.66	62.84 - 9.76	47.25 - 7.34	31.42 - 4.88	19.04 - 2.96	12.56 - 1.96	55.56 - 18.52	13.89 - 1.54
		1" Sensor (3)	182.72 - 28.32	136.12 - 21.32	91.36 - 14.16	68.68 - 10.64	45.68 - 7.08	27.68 - 4.28	18.24 - 2.88	55.56 - 18.52	13.89 - 1.54
0.5X 0.011 - 0.035 NA 1-60110	175 (nominal) 132-180 (1) W.D. range	Mag.	0.18X - 1.13X	0.24X - 1.50X	0.35X - 2.25X	0.46X - 2.99X	0.70X - 4.50X	1.16X - 7.40X	1.75X - 11.25X	30.30 - 9.52	4.13 - 0.41
		1/3" Sensor	34.32 - 5.32	25.56 - 4.00	17.16 - 2.67	12.90 - 2.01	8.58 - 1.33	5.20 - 0.81	3.43 - 0.53	30.30 - 9.52	4.13 - 0.41
		1/2" Sensor	45.68 - 7.08	34.03 - 5.33	22.85 - 3.56	17.18 - 2.68	11.42 - 1.77	6.92 - 1.08	4.57 - 0.71	30.30 - 9.52	4.13 - 0.41
		2/3" Sensor (3)	45.70 - 9.76	46.81 - 7.33	31.43 - 4.89	23.63 - 3.68	15.71 - 2.44	9.52 - 1.48	6.29 - 0.98	30.30 - 9.52	4.13 - 0.41
		-	-	68.06 - 10.66	45.70 - 7.12	34.36 - 5.36	22.84 - 3.54	13.84 - 2.16	9.14 - 1.42	30.30 - 9.52	4.13 - 0.41
0.75X 0.017 - 0.053 NA 1-60111	113 (nominal) 95-116 (1) W.D. range	Mag.	0.27X - 1.69X	0.35X - 2.25X	0.53X - 3.38X	0.70X - 4.49X	1.05X - 6.75X	1.75X - 11.15X	2.63X - 16.88X	19.60 - 6.28	1.73-0.18
		1/3" Sensor	22.86 - 3.56	17.04 - 2.67	11.43 - 1.78	8.59 - 1.34	5.72 - 0.89	3.46 - 0.54	2.29 - 0.35	19.60 - 6.28	1.73-0.18
		1/2" Sensor	30.46 - 4.74	22.69 - 3.56	15.23 - 2.37	11.45 - 1.78	7.62 - 1.19	4.62 - 0.72	3.05 - 0.47	19.60 - 6.28	1.73-0.18
		2/3" Sensor	30.50 - 6.52	31.21 - 4.89	20.95 - 3.26	15.75 - 2.45	10.48 - 1.63	6.35 - 0.99	4.19 - 0.65	19.60 - 6.28	1.73-0.18
		1" Sensor (3)	60.92 - 9.48	45.38 - 7.12	30.46 - 4.74	22.90 - 3.56	15.24 - 2.38	9.24 - 1.44	6.10 - 0.94	19.60 - 6.28	1.73-0.18
None 0.023 - 0.071 NA	92 (nominal) 80-92 (1) W.D. range	Mag.	0.35X - 2.25X	0.47X - 3.00X	0.70X - 4.50X	0.93X - 5.89X	1.40X - 9.00X	2.31X - 14.85X	3.50X - 22.50X	14.5 - 4.70	0.95 - 0.10
		1/3" Sensor	17.16 - 2.67	12.77 - 2.01	8.58 - 1.33	6.45 - 1.00	4.29 - 0.67	2.60 - 0.40	1.72 - 0.27	14.5 - 4.70	0.95 - 0.10
		1/2" Sensor	22.85 - 3.56	17.01 - 2.67	11.42 - 1.77	8.59 - 1.33	5.71 - 0.89	3.46 - 0.54	2.28 - 0.36	14.5 - 4.70	0.95 - 0.10
		2/3" Sensor	22.90 - 4.89	23.40 - 3.65	15.71 - 2.44	11.81 - 1.83	7.86 - 1.22	4.76 - 0.74	3.14 - 0.49	14.5 - 4.70	0.95 - 0.10
		1" Sensor (3)	45.70 - 7.12	34.02 - 5.34	22.84 - 3.54	17.18 - 2.66	11.42 - 1.78	6.92 - 1.08	4.56 - 0.72	14.5 - 4.70	0.95 - 0.10
1.5X 0.034 - 0.106 NA 1-60112	51 (nominal) 45-52 (1) W.D. range	Mag.	0.53X - 3.38X	0.71X - 4.50X	1.05X - 6.75X	1.40X - 8.98X	2.10X - 13.50X	3.47X - 22.28X	5.25X - 33.75X	9.80 - 3.14	0.43 - 0.04
		1/3" Sensor	11.43 - 1.78	8.52 - 1.33	5.72 - 0.89	4.3 - 0.67	2.86 - 0.44	1.73 - 0.27	1.14 - 0.18	9.80 - 3.14	0.43 - 0.04
		1/2" Sensor	15.23 - 2.37	11.34 - 1.77	7.62 - 1.19	5.73 - 0.89	3.81 - 0.59	2.31 - 0.36	1.52 - 0.24	9.80 - 3.14	0.43 - 0.04
		2/3" Sensor	15.00 - 3.26	15.60 - 2.44	10.48 - 1.63	7.88 - 1.22	5.24 - 0.81	3.18 - 0.49	2.10 - 0.33	9.80 - 3.14	0.43 - 0.04
		1" Sensor (3)	30.46 - 4.74	22.68 - 3.54	15.24 - 2.38	11.46 - 1.78	7.62 - 1.18	4.62 - 0.72	3.04 - 0.48	9.80 - 3.14	0.43 - 0.04
2.0X 0.040 - 0.142 NA 1-60113	36 (nominal) 34-39 (1) W.D. range	Mag.	0.70X - 4.50X	0.94X - 6.00X	1.40X - 9.00X	1.86X - 11.97X	2.80X - 18.00X	4.62X - 29.70X	7.00X - 45.00X	7.24 - 2.34	0.24 - 0.02
		1/3" Sensor	8.58 - 1.33	6.39 - 1.00	4.29 - 0.67	3.22 - 0.50	2.15 - 0.33	1.30 - 0.14	0.86 - 0.13	7.24 - 2.34	0.24 - 0.02
		1/2" Sensor	11.42 - 1.77	8.51 - 1.33	5.71 - 0.89	4.29 - 0.67	2.86 - 0.44	1.73 - 0.27	1.14 - 0.18	7.24 - 2.34	0.24 - 0.02
		2/3" Sensor	11.40 - 2.44	11.70 - 1.83	7.86 - 1.22	5.91 - 0.92	3.93 - 0.61	2.38 - 0.37	1.57 - 0.24	7.24 - 2.34	0.24 - 0.02
		1" Sensor (3)	22.84 - 3.54	17.02 - 2.66	11.42 - 1.78	8.58 - 1.34	5.72 - 0.88	3.46 - 0.54	2.24 - 0.36	7.24 - 2.34	0.24 - 0.02

The above fields of view are measured diagonally in millimeters (Horizontal = Diagonal x 0.8 and Vertical = Diagonal x 0.6).
(1) Working distance range when using 12 mm fine focus. Field of view will change with shorter or longer working distances.
(2) When using 5X Adapter image quality is greatly reduced. Contact your Navitar sales representative for detailed specifications.
(3) All systems using a 1" sensor should be discussed with a Navitar applications expert.
NA varies depending on system magnification.

Fig. 138: Snapshot of the Zoom 6000 Lens Field of View Matrix with various configurations highlighted with different colors.

The resolve limit (second right-most column) refers to the size of the smallest detail that the camera can image to cover two pixels on the sensor. Smaller values are desirable.

The depth of field (right-most column) refers to the range in which the object appears in focus. Objects closer to the camera or beyond the far end of the depth of field will appear blurry and out of focus. A small depth of field means that there is only a narrow range in which the object will appear in focus. A large depth of field is preferred as the object will appear in focus over a larger position range.