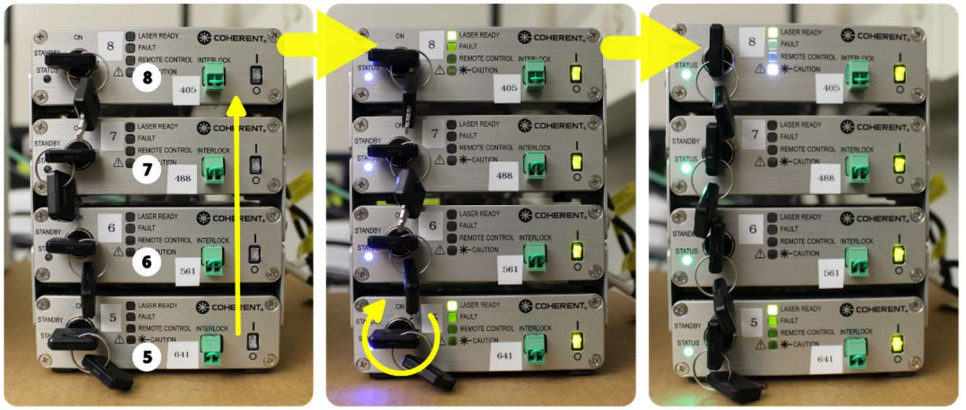
Olympus SpinSR10 Basic Quick Guide

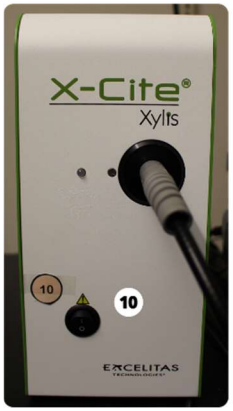
Turning on

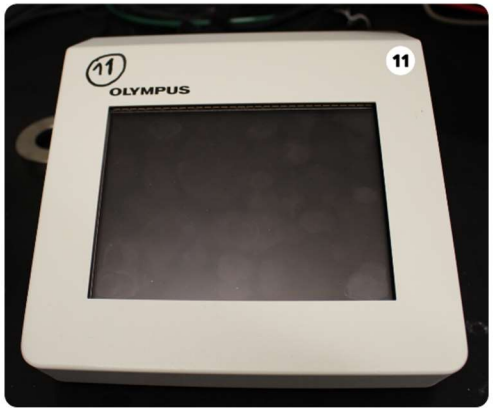
1. Switch on the buttons in the following order: Power supply **(1)**, unit **(2)**, unit **(3)**, unit **(4)**.

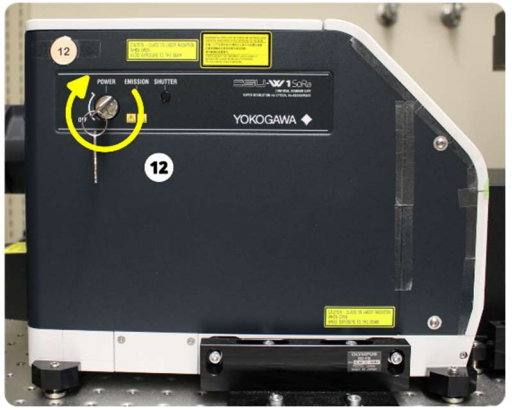
2. To turn on the laser's power supply, first flip up the switches **(5** to **8** – laser lines 641, 561, 488 and 405**)**, wait for all lights to turn blue, then turn the keys clockwise and the lights will turn green.

3. Turn on the unit **(9)**, the epifluorescence light **(10)**, then the touch pad **(11)** and finally turn the Spinning Disk key **(12)**.









Turning on

1. Log in to the PC.

2. Write down the start time of use in the logbook and enter the **FBS** system **(A)**.

3. Start the **cellSens Dimension** software **(B)**.

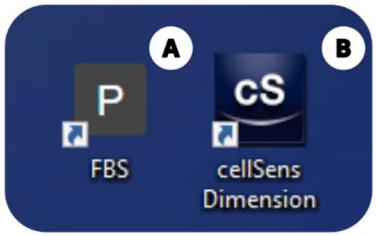
4. As the software starts up **(C)** the touch pad screen will change **(**from **D** to **E)**.

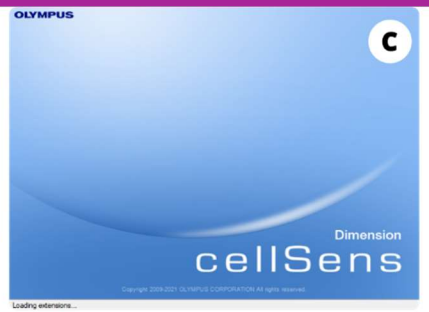
5. Click **NO** when asked to execute the cleaning for IX3-SSU **(F)**.

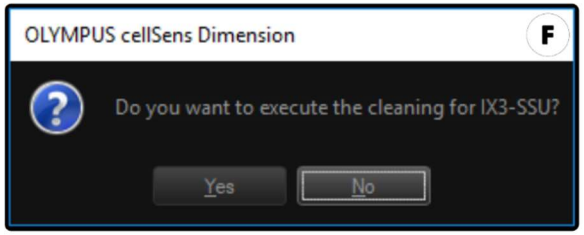
6. On **Camera Control (G)** click on **Acquisition Settings (H)** to set the destination folder where your files will be saved.

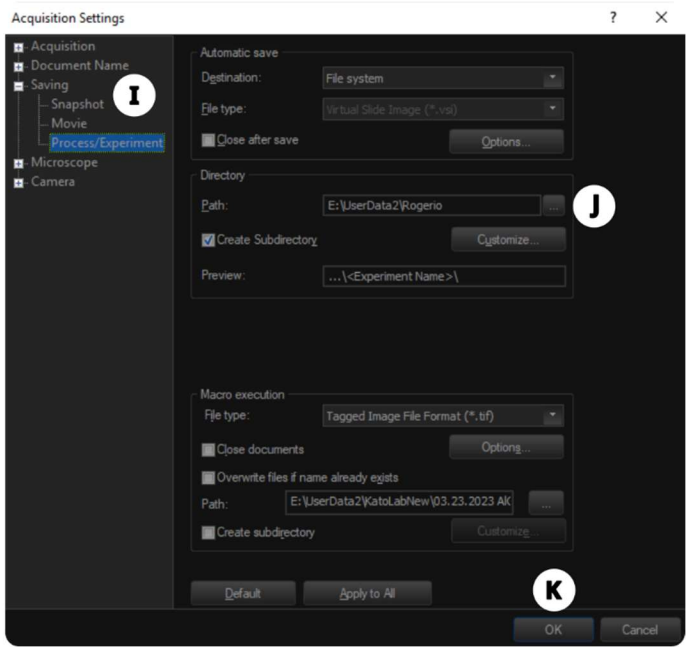
7. On **Saving (I)** define the **Directory (j)** path for **Process/Experiment** the Snapshot and Movie can also be defined here if necessary.

8. Click **OK (K)**.

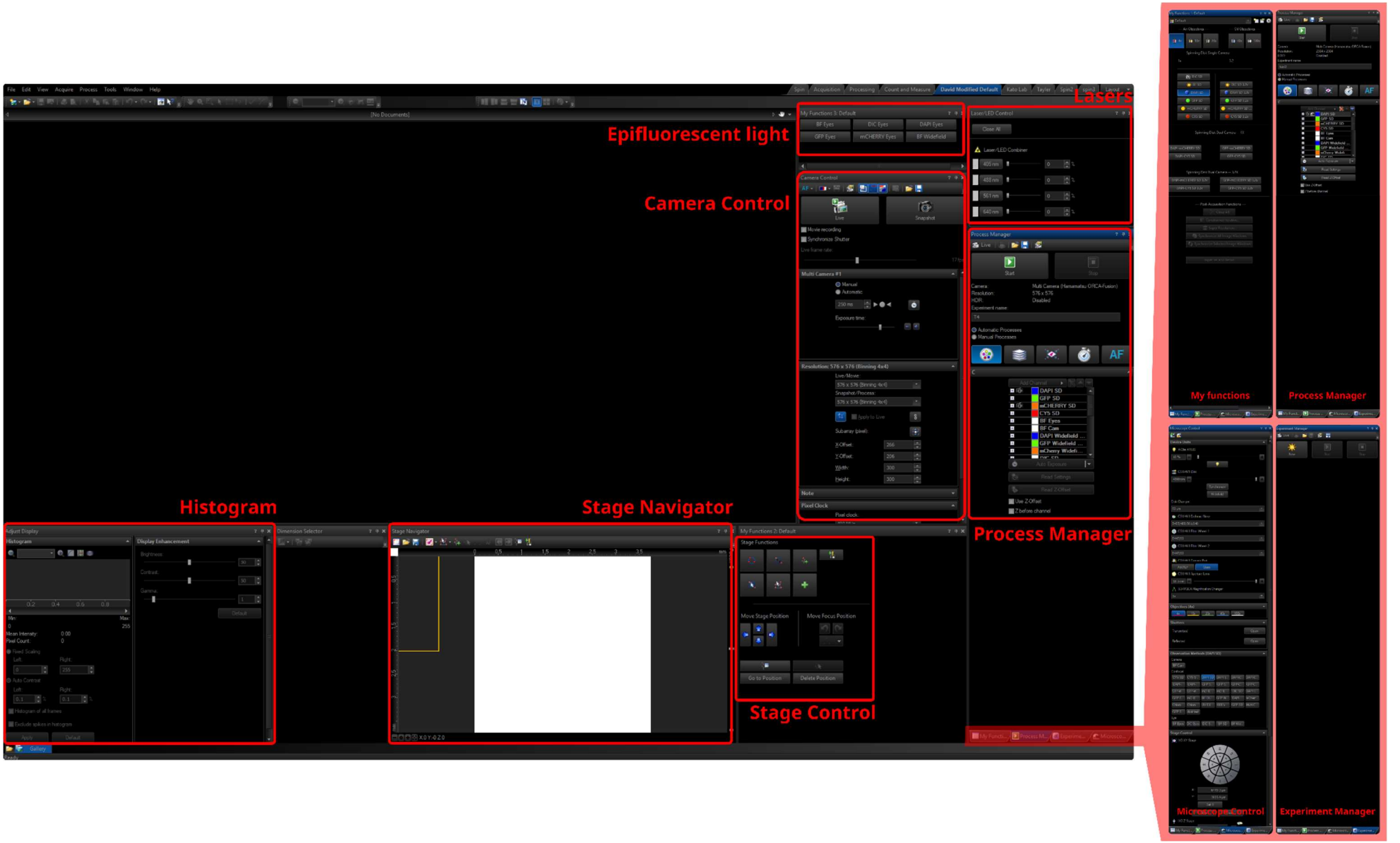








cellSen software layout



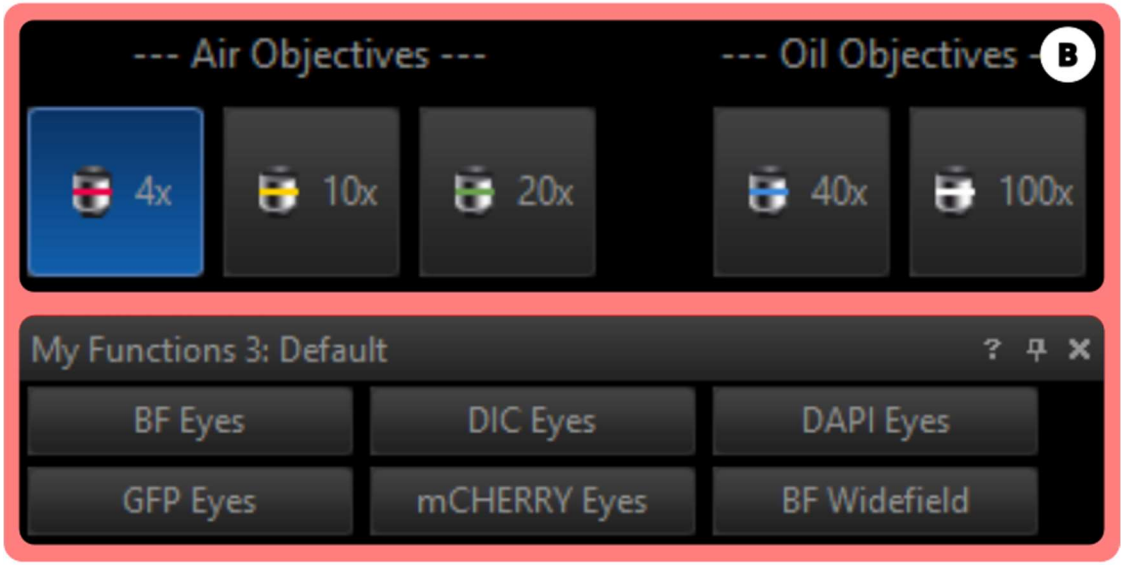
Eyepiece Observation

1. You can select the objectives and filter cubes from the microscope touchpad **(A)** or through the **cellSens** software **(B)**.

2. To turn on epifluorescent light for eyepiece observation, select among **DAPI Eyes**, **GFP Eyes**, **mCherry Eyes**. For bright field imaging select **BF Eyes** or **DIC Eyes**.

3. Find and focus the samples thought the eyepieces by adjusting the joystick and focus knobs.

4. Press the **Epi shutter (C)** to turn off the epifluorescence light to avoid photo-bleaching your samples.



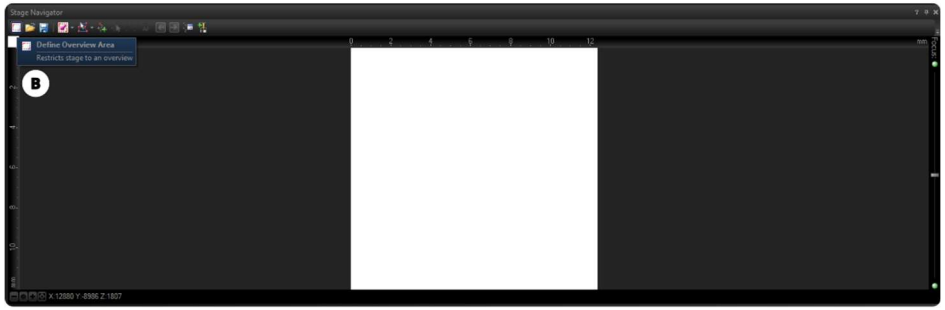
Defining Overview Area

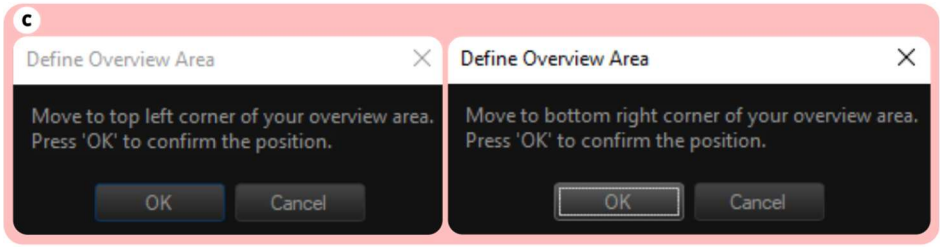
1. On **Stage Navigator (A)** click on **Define Overview Area (B)**.

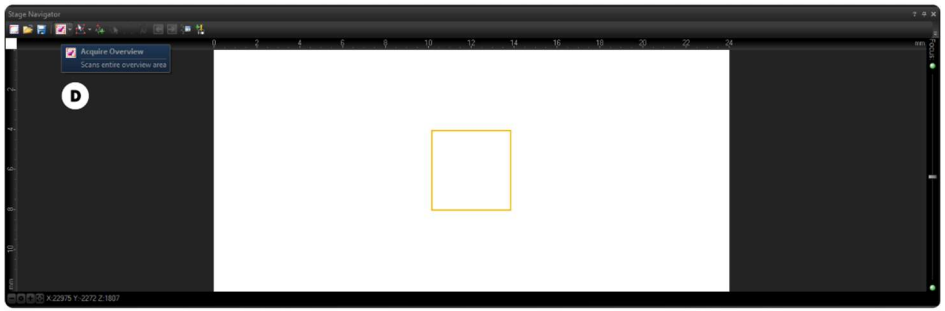
2. Using the joystick move the stage following the instructions on the pop-up windows **(C)** to cover your region of interest.

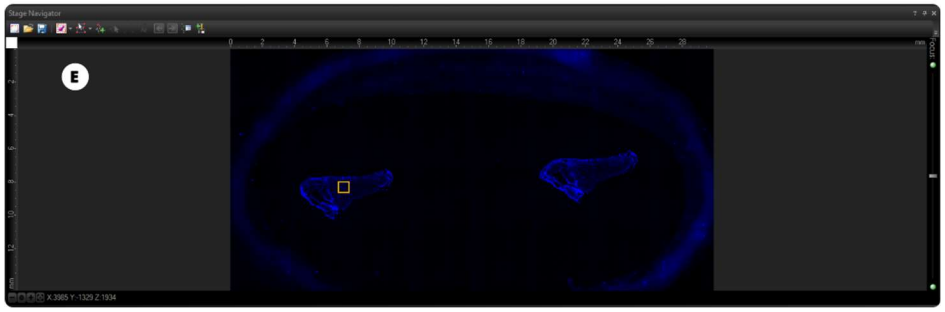
3. To make an overview, choose a channel click on **Live**, define the focus position of your sample using the focus knob and click on **Acquire Overview (D)**. For a fast acquisition use a lower magnification objective (i.e., 4x).

4. You will have an overview image of your slide or region of interest. Doble click on the area of interest to move the stage. Now you can change to a higher magnification to images with higher resolution.









Single/Dual Camera Imaging

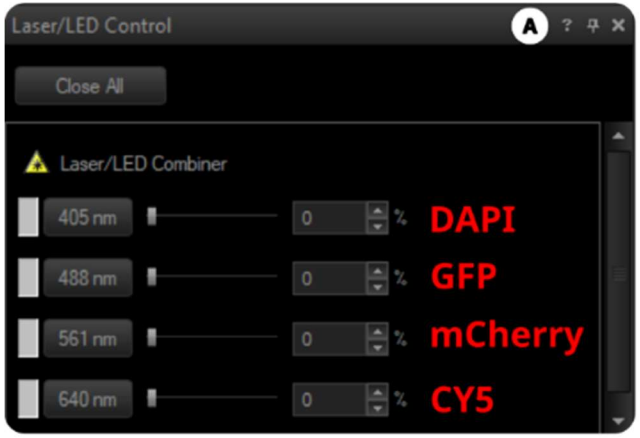
1. Turn on the **Laser** (405, 488, 561 and 640) that will be used by typing in a starting percentage for each laser **(A)**.

2. In the **My Functions 4: Spin Functions (B)**, there are **Single Camera (C)** settings and **Dual Camera (D)** settings.

3. Select the corresponding channel (i.e., **DAPI SD** for DAPI Spinning Disk).

4. DAPI SD 3.2x stands for a 3.2x zoom in the DAPI channel while imaging. This **3.2x (E)** is a zoom factor that's built in the camera.

5. Under Dual camera options you can select a combination of two channels. The longer wavelength light always goes to Camera #1, the shorter wavelength light goes to camera #2 **(Important when adjusting exposure time for individual cameras)**.



Adjust image brightness

1. In **Camera Control (A)**, click **Live (B)** to see a live view of your sample.

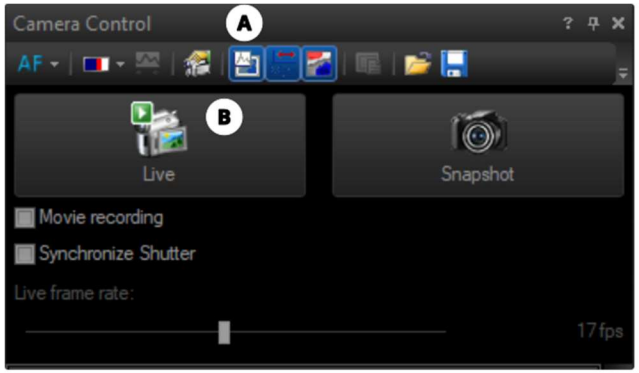
2. For fluorescent images select on **Adjust Display (C)** select **Auto Contrast (D)**.

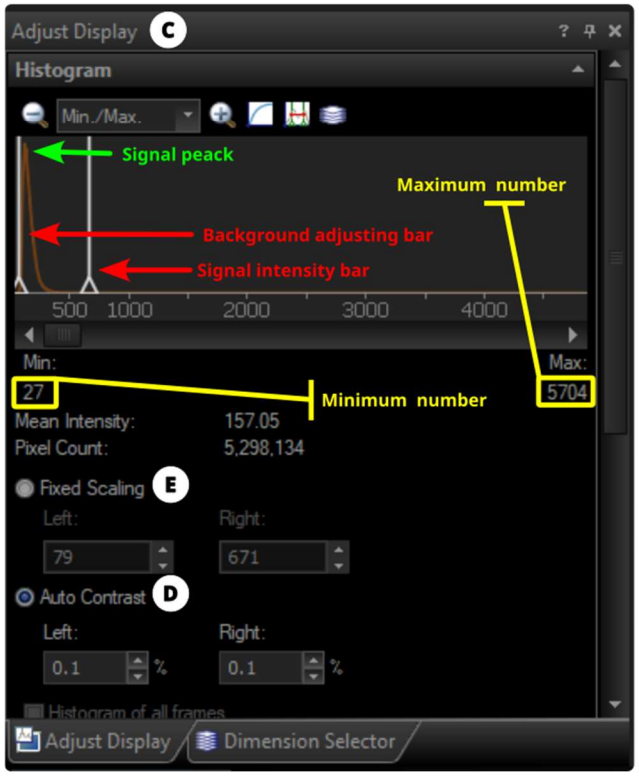
3. Once o see an image, click on **Fixed Scaling (E)**.

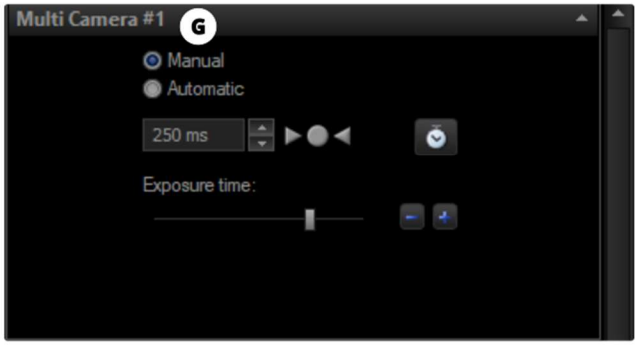
4. Adjust the focus by using the focus knob or by simultaneously pressing the Ctrl key and use the roller on the mouse.

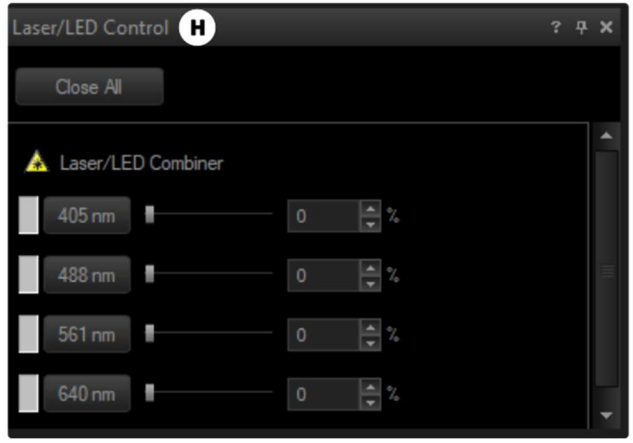
5. Once on the focal plane, adjust the brightness of the image through three parameters: **Histogram (E)**, **Exposure Time (F),** and **Laser Power (G)**.

- **Histogram:** In the **Histogram**, the white vertical bar on the left adjust he background, the white vertical bar on the right adjusts the signal intensity. For the **signal intensity bar**, the more to left (smaller maximum number), the stronger the signal the more to the right (bigger maximum number), dimmer the signal. For the background adjusting bar the more to he left (smaller maximum number), stronger the background, the more to the right, or closer the signal peak, the blacker the background. In most cases, the background bar should not go pass the signal peak.
- **Exposure Time:** Adjust the camera time manual in the Camera Control. Good numbers to start are 100ms or 200ms. The longer the exposer time, the stronger the signal.
- **Laser Power:** Adjust the laser power by either typing in the number or drag the bar to change it. The stronger the laser power, the brighter the signal, and more the photobleaching. Start with 10-30%.









Adjust image brightness

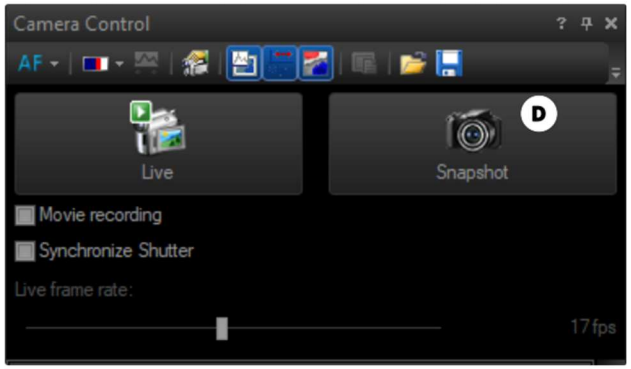
6. Once you are at your best focal plane switch to **Auto Contrast (A)** in the **Histogram**, pay attention to the **maximum number (B)**. The overall range of the camera is up to 60,000. If the **maximum number** is over 50,000 is considered too high. If your maximum number is less than 1,000 that may be too low.

7. If the **maximum number** is too low, it means the auto contrast is automatically dragging the signal adjusting bar to the left (smaller max number) to increase the brightness of the image. In this case, increase the **laser power** or the **exposure time**, until the max number is at least above 1,000 (anywhere from 5,000 is good).

8. If the **maximum number** is too high, that means the auto contrast is automatically dragging the adjusting bar to the right (bigger max number) to decrease the brightness. In this case, you need to decrease the **laser power** or the **exposure time** until the max number is at least below 50,000 (anywhere around 5,000 is good).

9. Once you are happy with the outcome, switch back to **Fixed Scaling (C)** so the histogram keeps the same throughout your imaging.

10. Click **Snapshot (D)** to capture the image.



Process Manager (Multichannel imaging)

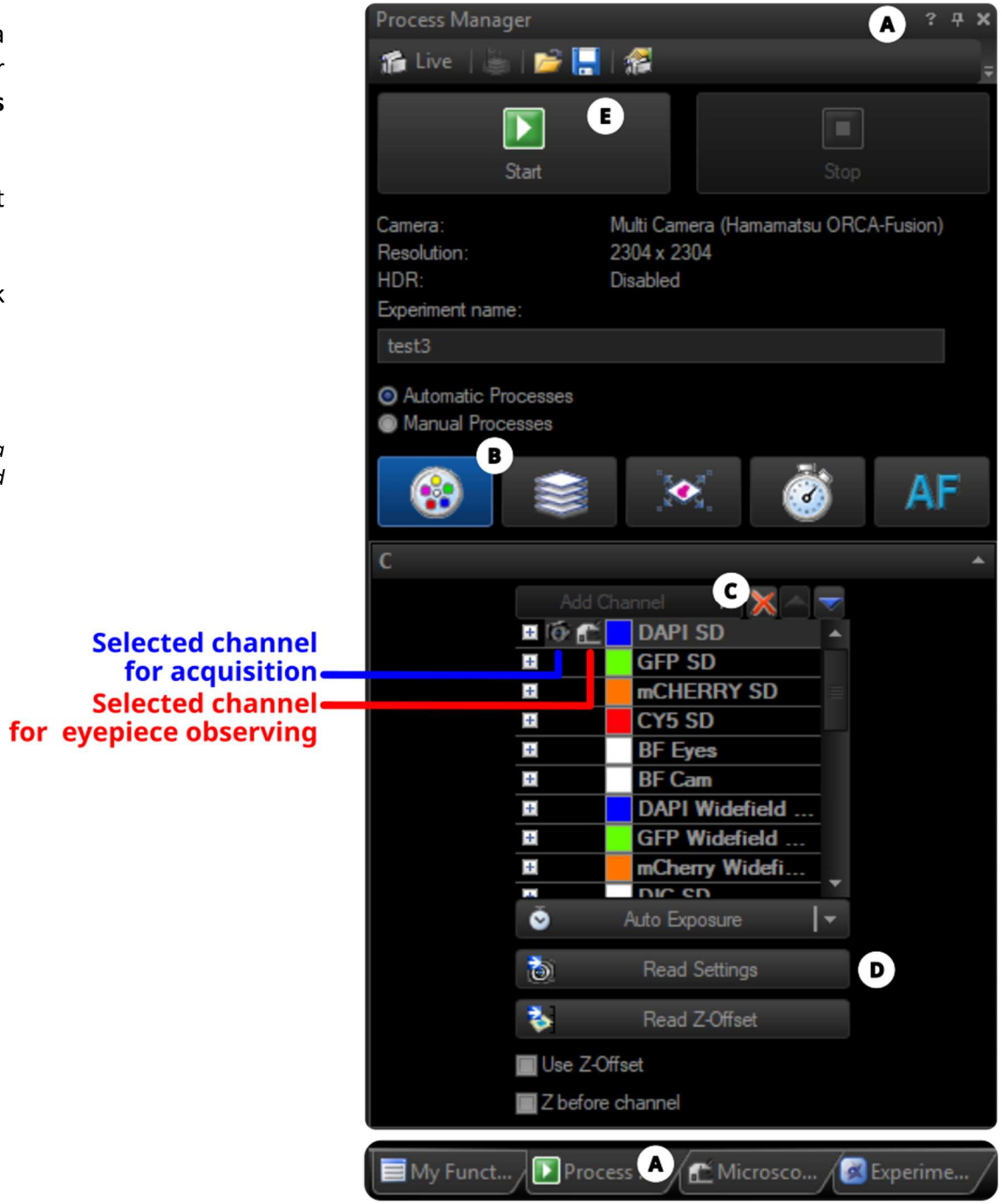
1. If you have more than 2 fluorescent channels (or a two-channel camera setting does not meet your requirements), you can set up the experiment in **Process Manager-Multichannel imaging (A** and **B)**.

2. Select a channel by clicking on it. If channel is not listed, click on **Add Channel (C)**.

3. After adjusting exposure time and laser power click **Read Settings (D)** store the settings for each channel.

4. Click **Start (E)** to image after experiment is set up.

A single camera setting cannot be combined with a dual camera setting in this section. You can only set up a combination of single and dual camera in he Experiment Manager.



Process Manager (Z-stacks)

1. On **Process Manager** click on the **Z-Stack** button **(B)**.

2. On **Camera Control** Click on **Live (B)**

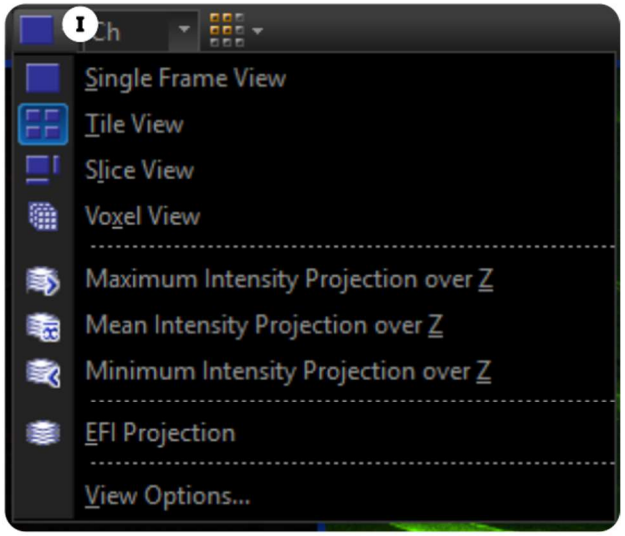
3. Move the focus knob or use the Ctrl+mouse to the bottom of the interested Z-range as the **Start** point, then click **Set (C)**.

4. Mode the focus knob to the top of the interested Z-range as the **End** point, then click **Set (D)**.

5. Apply the **Recommended Step Size (D)** or a desired **Step Size (E)**. If you do not know what step size to use, you can also directly type in the desired **Z-slices (F)** number.

6. Click **Start (G)** to capture the Z-stack image.

*Click on **option button (H)** to have different options to view the Z-stack.*
*Use the **Z slices bar (I)** to move through the Z-stack.*



Process Manager (Multi-points imaging)

1. Click on the **Multi-points (A)** button to activate.

2. Click on **Live (B)** and move around the joystick to find field of views of interest.

3. Click on **Add Position (C)** to add area to position list.

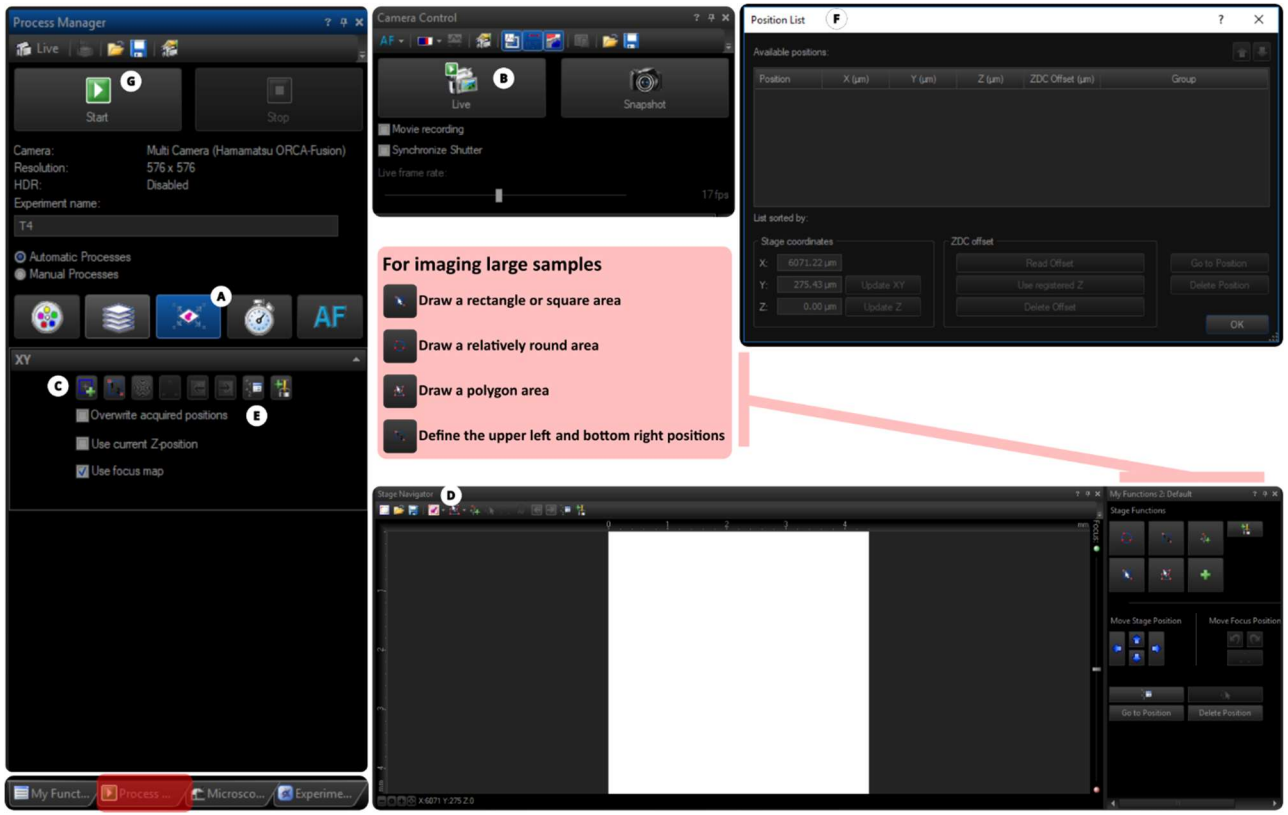
4. You can also use the **Stage Navigator (D)** window to add a selected position.

5. Click on the **Position List (E)** button to open and use it to review, add or delete positions **(F)**.

6. For imaging large samples, on live mode move around with the joystick do define areas and under **Stage Functions**, use one of the different ways to define your large field of view:
   - *Draw a rectangle or square area.*
   - *Draw a relatively round area.*
   - *Draw a polygon area* with your mouse.
   - *Define the upper left and bottom right positions.*

*Your defined area will show in the Stage Navigator. Pay attention to the number of areas created. In this illustrated figure, there are 2 areas created. So, both areas will be capture during acquisition. To remove any position, click on **Select Position** and delete it.*

7. Click **Start (G)** to capture **images**.



Process Manager (Time lapse)

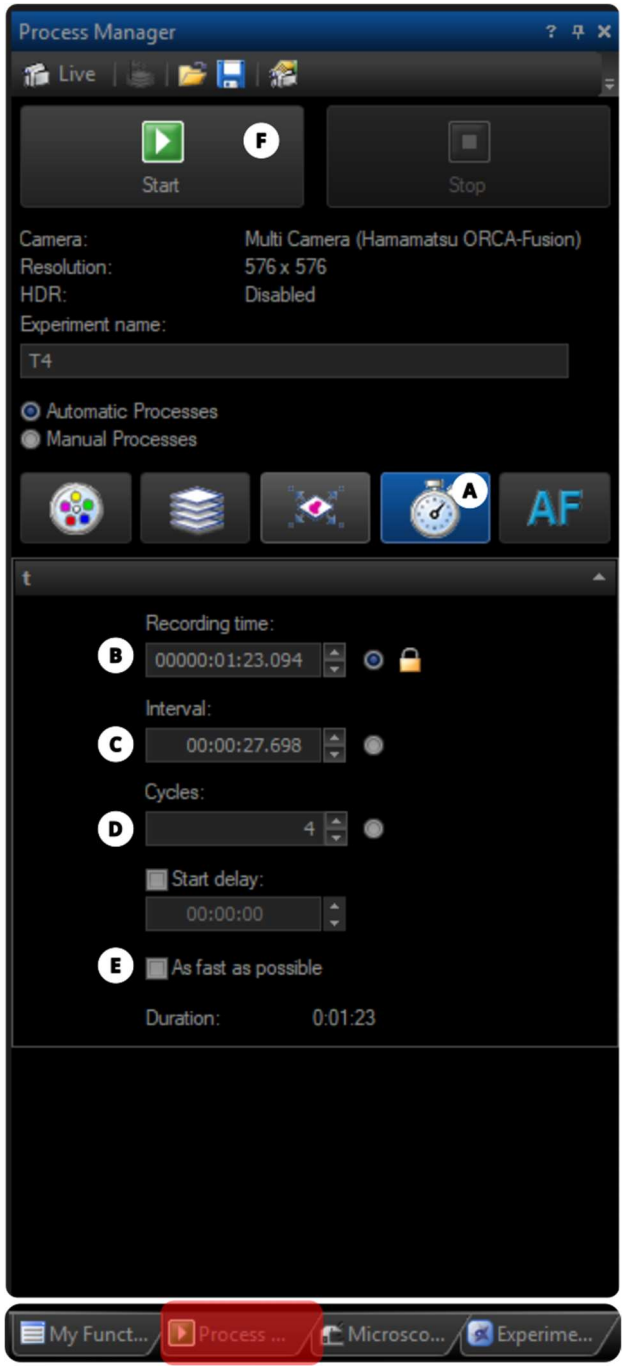
1. Click on **Time-lapse (A)** to activate the time-lapse imaging window.

2. Configure the desired settings:

- **Recording time (B)**: the total duration time to be set.
- **Interval (C)**: the time between each image
- **Cycles (D)**: how many images will be captured within the total duration time.
- Check **"As fast as possible" (E)** and the time to capture one image will be applied to the interval.

3. Click **Star (F)t** to begin the experiment.

*All functions in the **Process Manager** can be combined as needed. Click Start and all selected functions will be engaged. Uncheck the ones not needed.*



Experiment Manager

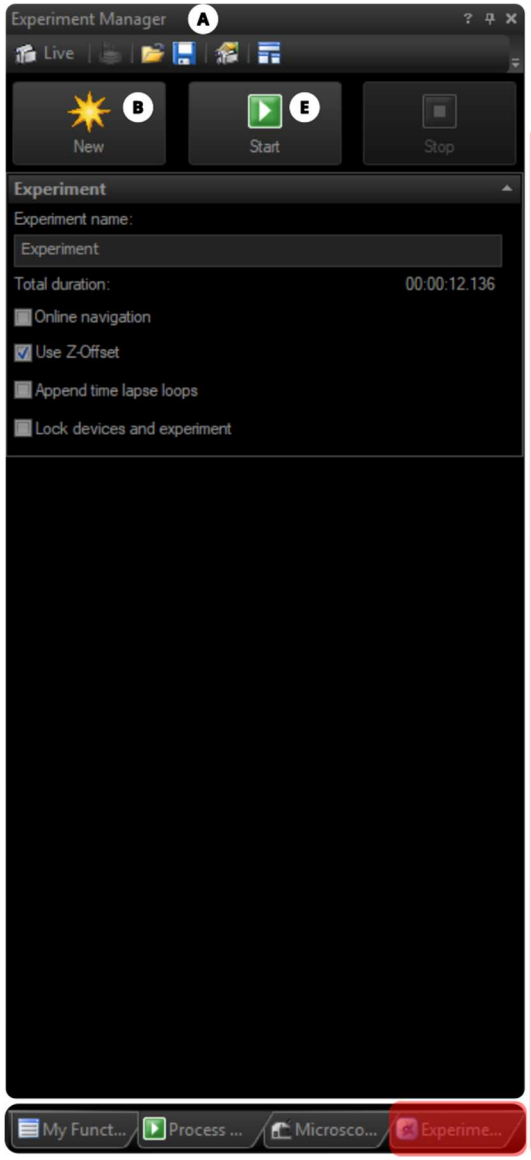
At the **Experiment manager (A)** it is possible to customize your experiment.

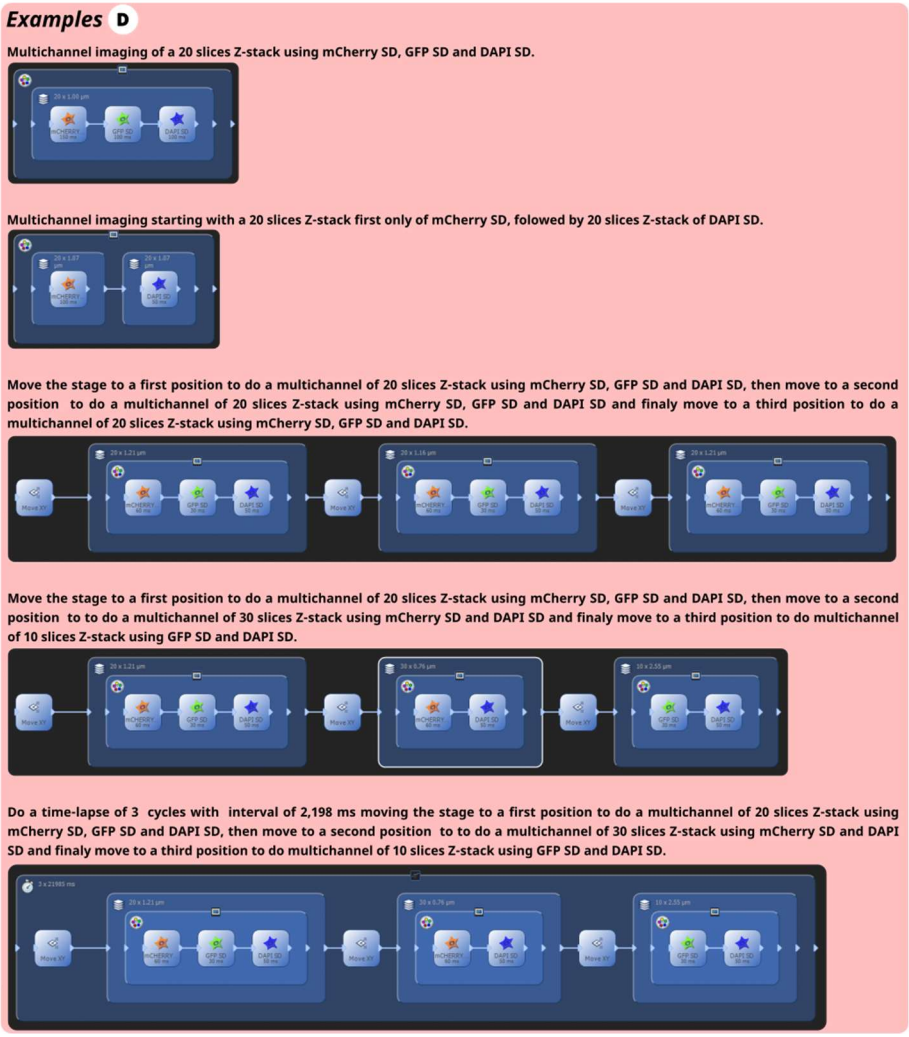
1. Click on **New (B)** to open a new Experiment Manager window.

2. In **Experiment (C)** window you can add different elements to set up customized experiments.

3. Here are some **Examples (D)** to have an idea on how to set up your experiment.

4. Once you are set click on **Start (E)** to begin the image acquisition.

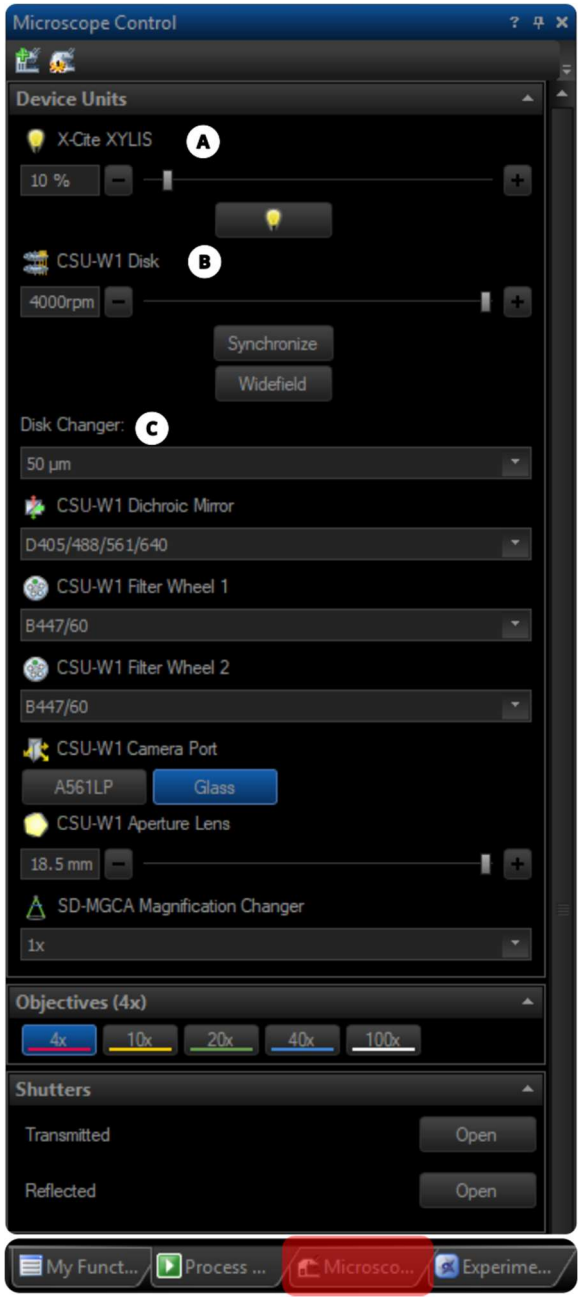




Microscope Control

Three commonly used functions in the **Microscope Control** window.

- **Halogen Lamp (bright field imaging) (A)**: You can control the halogen lamp through the microscope touch pad, or through the halogen lamp controller in the **Microscope Control** window.
- **Synchronize (B)**: If you see pinhole track patterns in your image that do not disappear after adjusting camera exposure time, you can click Synchronize to synchronize the spinning disk rotating speed and the camera expose time to eliminate the pinhole tracks. If you keep it at 4,000 rpm just adjust the camera exposure time to multiples of 2.5 ms. This will prevent the tracks.
- **Disk Changer (B)**: Our spinning disk unit is loaded with two disks: 50 µm pinhole disk and SoRa disk. The default disk is the 50 µm disk.



Turning OFF

1. Take out your sample.
2. If using an immersion objective clean it.
3. Return to the 5x objective.
4. Exit **cellSens**.
5. Write down the time of use in the logbook (report any error/problem) and log out the iLab system.
6. Shut down the computer.
7. Switch off the main switches in the inverse order (from buttons 12 to 1)
8. Cover the microscope with the protective cover.