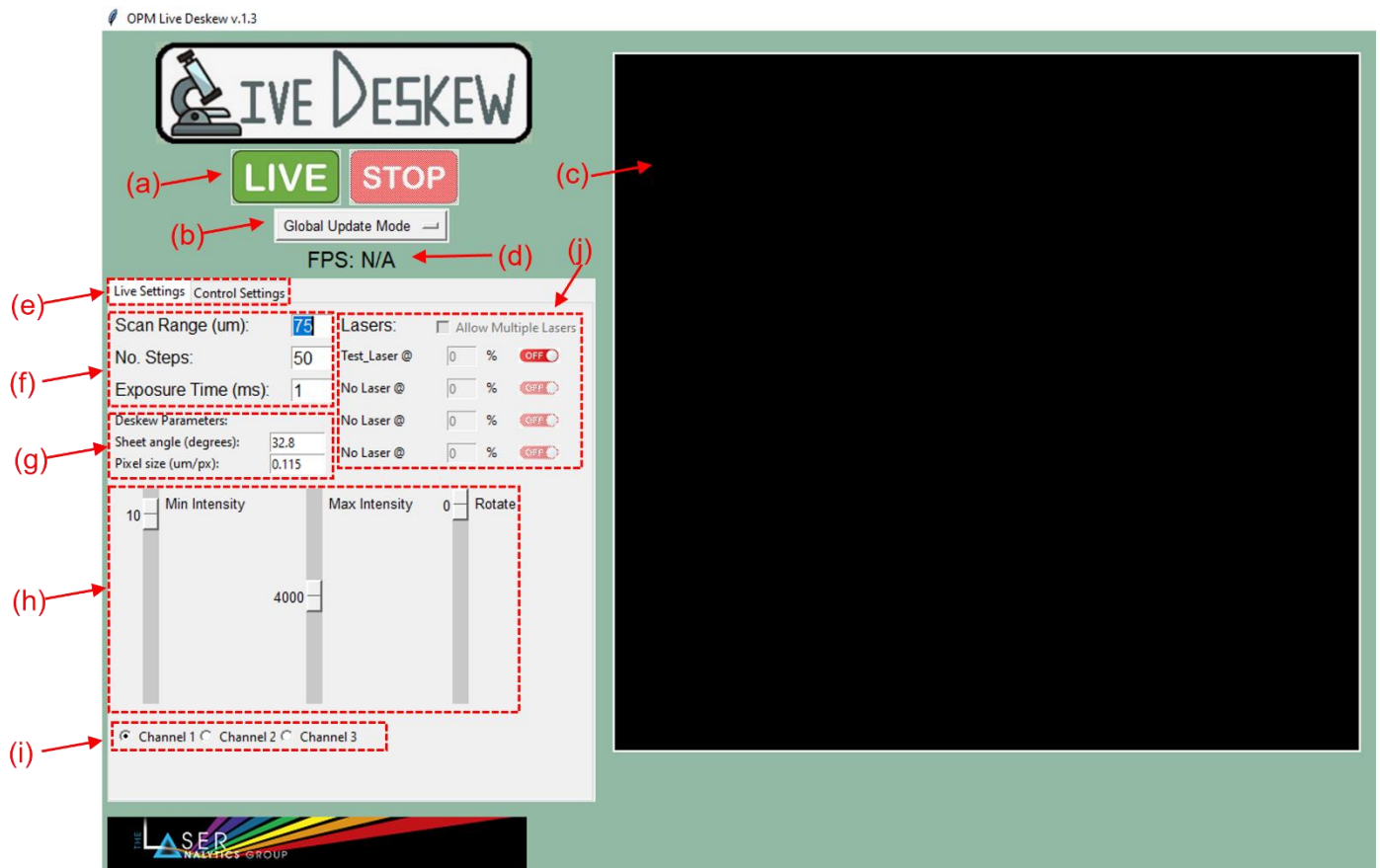


Live Deskewing Software User Manual

GUI Overview:



- a) **Live/Stop Buttons:** These buttons start and stop the live deskewing. 'Live' starts the software and 'stop' stops it. Note that the scan parameters are disabled whilst the software is running.
- b) **Update Mode Selection:** Here the user can choose between the global and rolling update modes. Global mode updates the output image after each complete stack and is suitable for experiments with low exposure times. Rolling mode updates the output images after each camera frame and is suitable for long exposure time experiments. (We would consider a 'long' exposure to be $> \sim 20\text{ms}$)
- c) **Image display:** This is where the output deskewed image will be displayed. Note that the size of this window will vary depending on the camera region of interest and the sample scan range used.
- d) **Frame rate indicator:** Here the output frame rate of the image display will be plotted. This is how quickly the output image in the GUI is updated thus will be a lot higher in rolling mode.
- e) **Tab selector:** The 'Live settings' tab contains all the controls needed to operate the live deskewing software and changing the imaging characteristics. The 'Control settings' tab is where hardware can be initialised.

- f) **Scan parameters:** Here the scan range, number of steps per volume and camera exposure time can be set. These can only be varied whilst the deskewing is not running.
- g) **Deskewing parameters:** Here the sheet angle and pixel size can be set. These must be accurately set in order for the software to correctly deskew the raw images. Sheet angle is the angle between the sheet and the axis of sample scanning. Pixel size is the size of each camera pixel in the sample.
- h) **Live image control:** Here the contrast of the image can be adjusted whilst the software is running by changing the minimum and maximum pixel intensity values. The rotate slider dynamically adjusts the skew factor to give a rotated perspective of the sample. 0 corresponds to a top down view and 90 gives a side on view.
- i) **Channel selection (Only for Optosplit):** When a triple colour optosplit is used the live image controls (h) will control the image of the channel selected by these radio buttons. E.g., if channel 1 is selected the controls will change the displayed output of channel 1.
- j) **Laser control (Only when lasers are initialised):** Here the lasers can be turned on and off. Power is controlled by changing the value in the edit box and then they can be turned on and off using the buttons to the right. Unless the 'Allow Multiple Lasers' button is checked (which can only happen if an optosplit is installed) then turning a laser on will turn all other lasers off.

Running the software:

1. Open the camera in micromanager and select the ROI where the sheet is formed.
NB: Smaller ROIs will have a higher final framerate.
2. Run the script Live_Deskew_GUI.py
3. Enter the correct scanning and deskewing parameters. (If using your own hardware control make sure the scan range and No. steps match the values set in your code)
4. Press the start button to begin displaying a live deskewed output

Hardware control modes:

Note that when the software is first opened no hardware is initialised and the software will only control the camera in an internal triggering mode.

Your own software:

The live deskewing software can be used in conjunction with your own hardware control software. In this case, your software will have to handle synchronisation of sample scanning with the camera exposures. The live deskewing software will continually take images from the camera and then process and plot them. Your software will have to move to the next sample position (either move the stage or move the sheet) after each camera image. To do this we recommend setting your system to be externally triggered to move to the next scan position by a TTL output line from the camera. We use an exposure output line on the camera which is high whilst the camera pixels are exposing and low otherwise. The falling edge, when no pixels are exposing, is used as the trigger for our NI DAQ card to then move to the next galvo voltage. We have this set up in LabView software

which is running whilst the live deskewing software is running. We use a Photometrics Kinetix 22 so you will need to check the operating manual of the camera you use to find the appropriate triggering line.

Note that your software will have to be initialised with the same parameters as the live deskewing GUI. This means using the same scan range and number of steps, set in box (f), in both the live deskewing software and your hardware control. E.g., if the live deskewing software has Scan range = 75um and No. steps = 50, your software will have to scan a range of 75um in 50 steps.

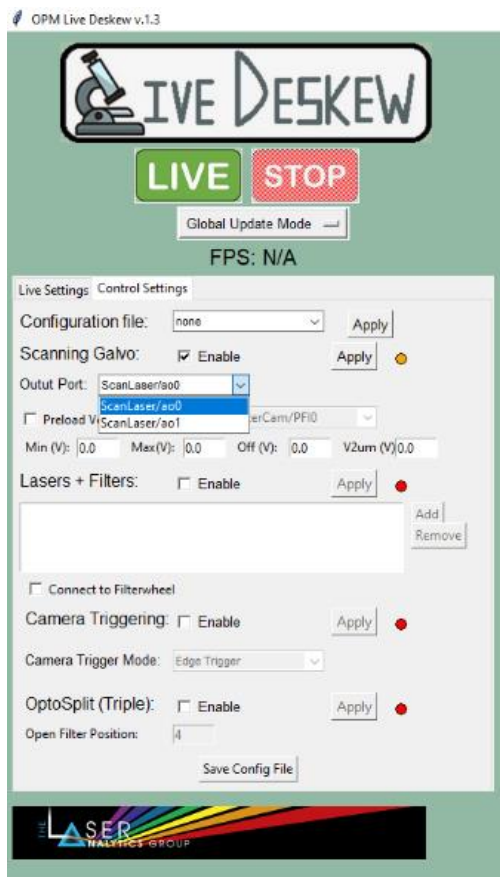
Using our software to control hardware:

Our software can handle the lasers and hardware synchronisation, or any subset of the hardware you wish to use.

Scanning Galvo control: (Galvo Scanning OPM):

The movement of the scan galvo can be controlled by an analogue output of a NIDAQ card. The voltage can either be written before each camera frame or can be preloaded into the DAQ card and externally triggered by an output line from the camera. The latter will increase the imaging rate.

1. To initialise the galvo first check the enable button. The status indicator (right of the apply button) will now change orange to indicate the settings have been change but not applied.
2. Select the output NIDAQ card port from the drop-down list. This is the voltage port that controls the galvo position. The software will automatically scan the computer and find the available ports.
3. If you want to externally trigger the voltages, select the 'Preload Voltages' check box and choose the NIDAQ card line which will receive the trigger from the camera. If this is not selected the code will default to writing the voltages before each camera exposure.
4. Input the minimum allowed galvo voltage 'Min (V)', maximum allowed galvo voltage 'Max (V)', galvo offset voltage 'Off (V)' (this is the voltage the software will scan around) and the volts to um conversion 'V2um'.
5. Click the apply button. The status indicator should now be green showing that the galvo is initialised and ready to use.

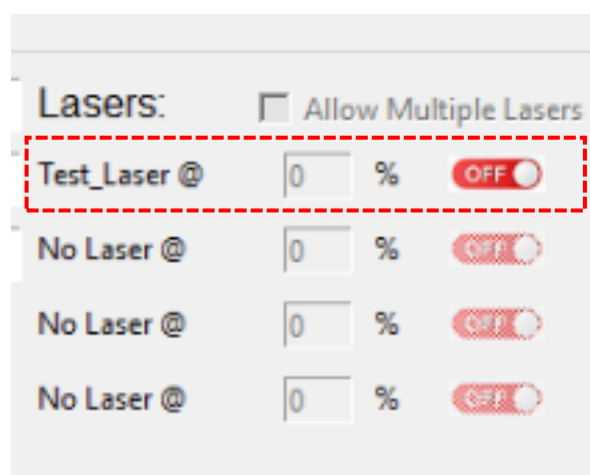
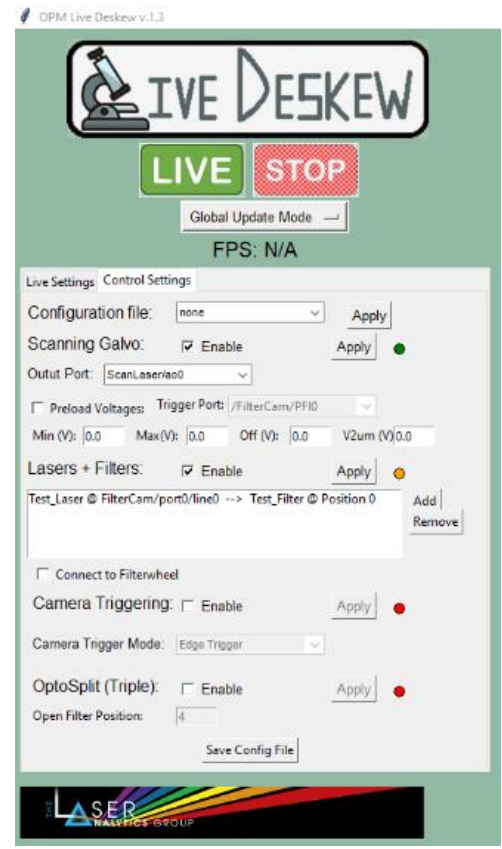
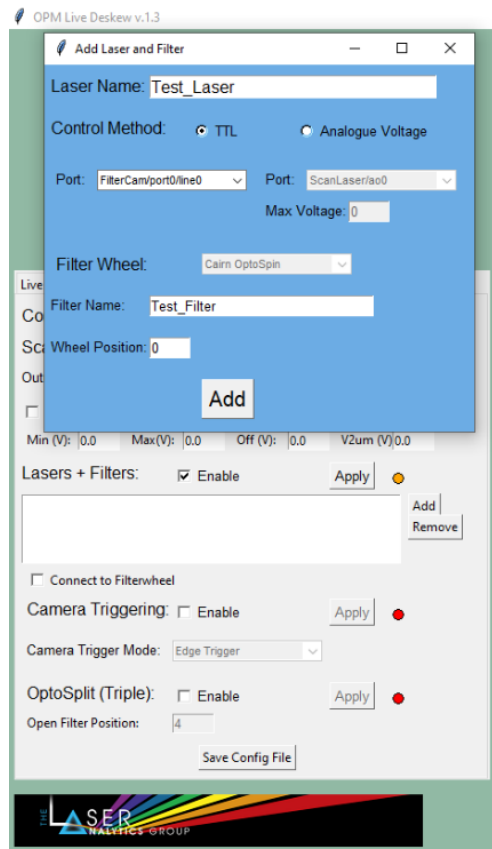
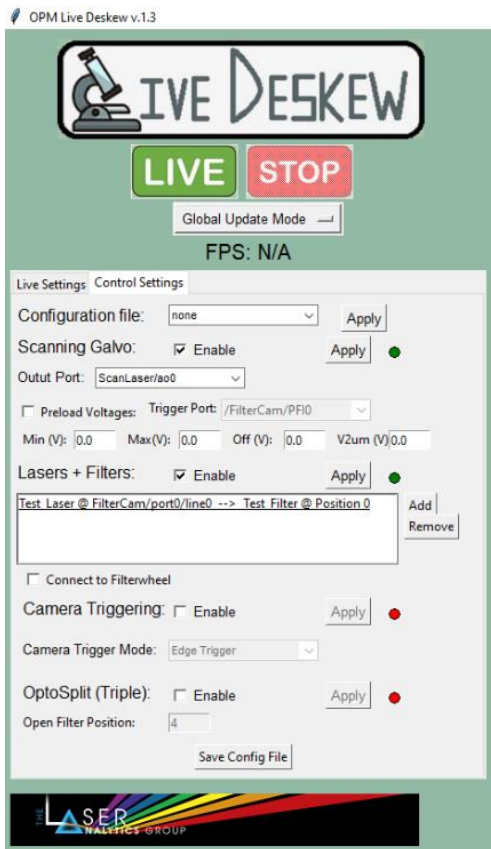


Laser Control:

Lasers can be controlled from within the GUI via digital (on or off) or analogue (voltage) signals from NIDAQ cards.

1. To initialise the lasers first check the enable button. The status indicator (right of the apply button) will now change orange to indicate the settings have been change but not applied.
2. To add a laser, click the 'add' button and a blue window will pop up to allow a laser to be added.
3. Fill in the form. 'TTL' means that the lasers will be turned on when the output is high and off when the output is low (we use this for a shutter in front of our 561nm laser which doesn't have any power modulation). 'Analogue voltage' is where the laser is controlled by an analogue voltage. The max voltage edit box is the voltage at which the laser is at full power. Each laser will need a corresponding filter wheel position. Currently only the Cairn OptoSpin is supported but it should be fairly easy to extend the code to support other filter wheels.
4. Once the form is complete select add and the laser will be added.

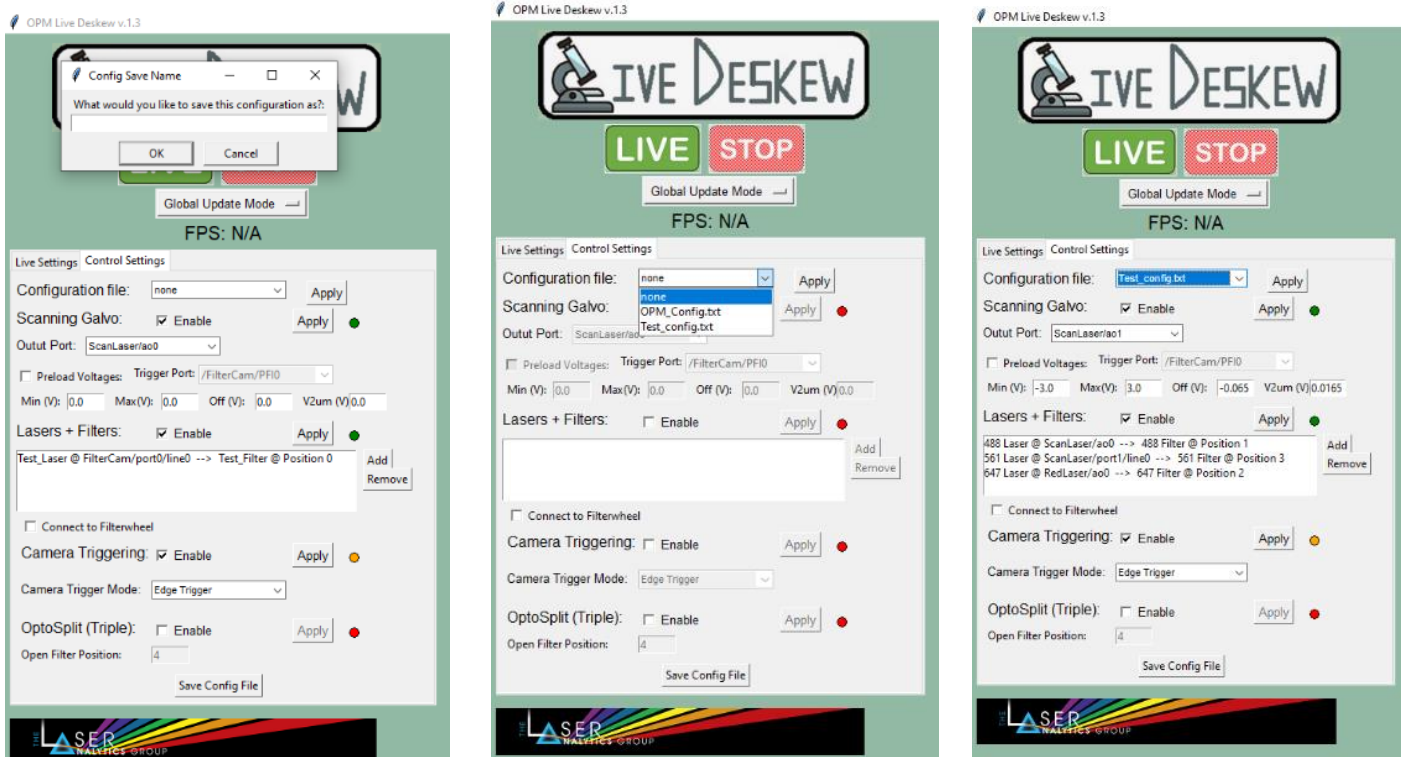
- Once all the lasers you want are added then select apply and the lasers will be initialised in the software. At this point the lasers names in the Live settings tab will be updated.



Saving configuration files:

In order to speed up initialising all the hardware that you want to use you can save configuration files which can then be used to rapidly initialise all the hardware you want at once.

1. Install all the hardware that you want to use.
2. Click on the 'Save Config File' button
3. Insert the name that you want to use and click ok one happy.
4. The configuration file dropdown box will contain all the .txt files in the directory where the *Live_Deskew_GUI.py* file is run.
5. Select the appropriate file from the dropdown box and select apply to initialise all the hardware.



```
Test_config - Notepad
File Edit Format View Help
Galvo: True
ScanLaser/ao1:False:False:-3:3:-0.065:0.0165
Lasers: True
0:488 Laser:ScanLaser/ao0:2.3:488 Filter:1
1:561 Laser:ScanLaser/port1/line0:0:561 Filter:3
0:647 Laser:RedLaser/ao0:2.3:647 Filter:2
end
Camera: False
OptoSplit: True
4
```