

## Photoconversion/Activation with DMD using USB only option

This guide is meant to photo activate **independently** of your acquisition. For example, acquire for 3 min and activate once in a while not precisely timed. This could be used for photoconversion, if the conversion timing is not so important or in an exploration phase. If you want to make a precisely timed light train (regular excitation and activation), then use the TTL option in combination with “Illumination sequence” module (of NIS-Elements).

With the digital mirror device (DMD) you can illuminate a specific ROI in your total field of view with one of the LEDs of the Aurall/SpectraX (395 nm, 470 nm, 555 nm, or 640 nm) while simultaneous imaging with your regular setting. The ROI is created by the DMD and the light is directed to the sample through a dichroic mirror (DM) in Turret-up (see also Light path with activation sections). The dichroic mirror will also cut off the emission of your sample that is of a shorter wavelength than the cut off value of DM placed in the Turret-up. With our current set-up we advise you to use the DM 405 at position 1. This DM reflects light the 405 nm very well, and reflects the 470, 555 and 640 moderately, but importantly without compromising your detected emission intensity. The light output of the DMD in combination with Aura/SpectraX will be sufficient for activation, but probably too low for photo bleaching. The DMD needs to be aligned with the chosen DM in the Turret-up on the selected camera. See Excitation and emission options section for wavelength details.

### Excitation DMD (activation) options

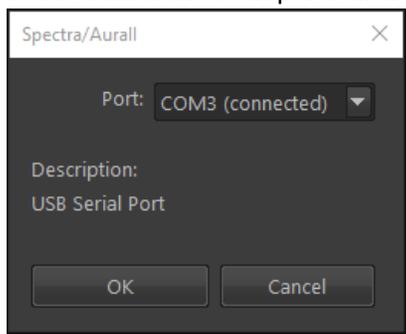
Aurall/SpectraX contains 4 LEDs with excitation filter:

1. 395 nm (395/25)
2. 470 nm (470/24)
3. 555 nm (555/28)
4. 640 nm (640/30)

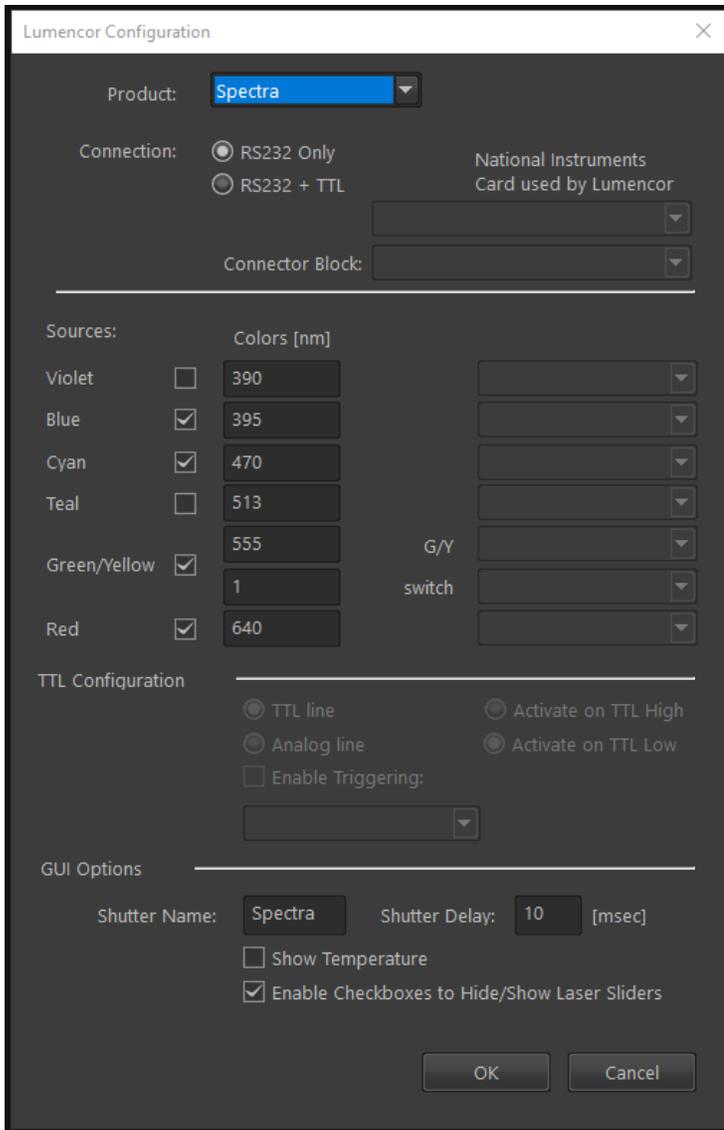
### Before starting

make sure the Aura II is correctly connected.

1. In Nikon software (top bar), click on: Devices > Device manager, this gives a new window “Manage devices”.
2. Select Spectra/Aurall from the list
3. Click on Connection Parameter, check if COM3 (connected) is selected, or change to COM3 if it reads a different COM port. Click OK

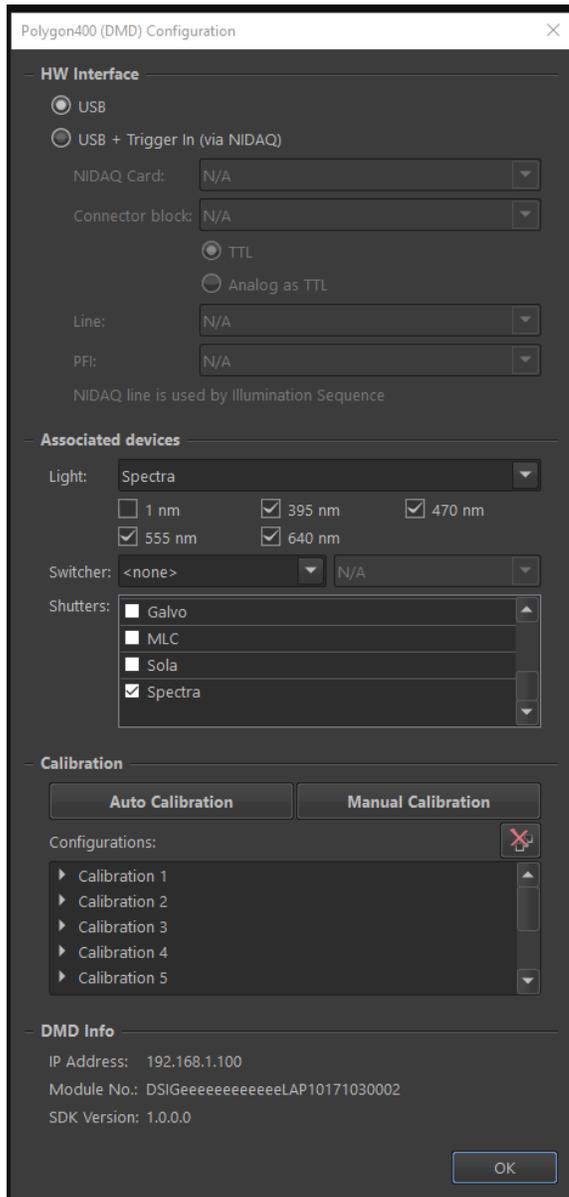


4. Back in the “Manage devices” window (still with Spectra/Aurall selected from the list), click on configure device.

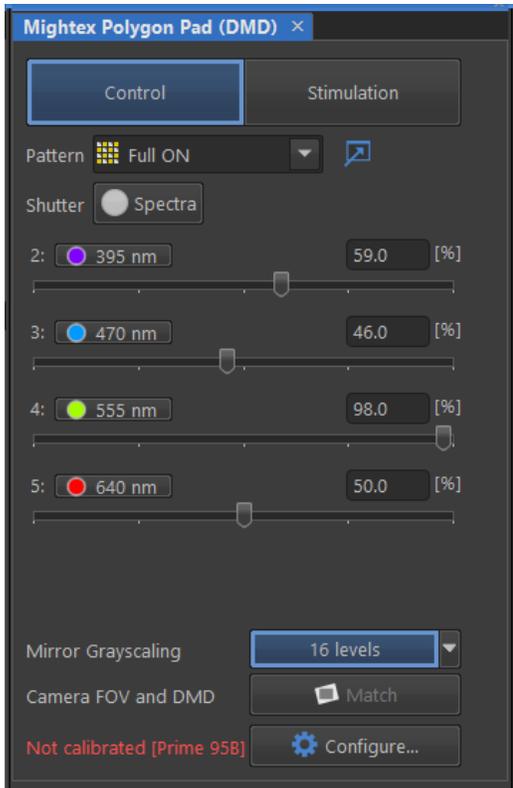


5. Make sure the settings are the same as in the screen shot above. Especially pay attention to the connection type "RS232 Only" and the checked boxes with the corresponding wavelength.

6. On *Mightex polygon pad (DMD)* pad, click “Configure”. (If the Mightex polygon (DMD) pad is not open, then: right click in the background > acquisition panels > Mightex polygon (DMD) pad)
7. The “*Polygon400 (DMD) Configuration*” window appeared and check with the screenshot if all settings are correct.
8. Especially pay attention to
  - a. ‘USB’ interface
  - b. Light = Spectra
  - c. Only 395, 470, 555 and 640 nm checked
  - d. No switchers
  - e. At Shutters, only the Spectra should be checked.
  - f. Click Ok



- 9.
10. The *Mightex polygon pad (DMD)* pad should look like:



Shutter reads “Spectra” and 4 LED options. If this doesn’t look correct go back to step 7/8 and toggle around with the Light option (click on the arrow down next to the light option, select something else, click again on the arrow down of the light option and select Spectra, check if all other settings are correct, then click on OK. Repeat step 10)

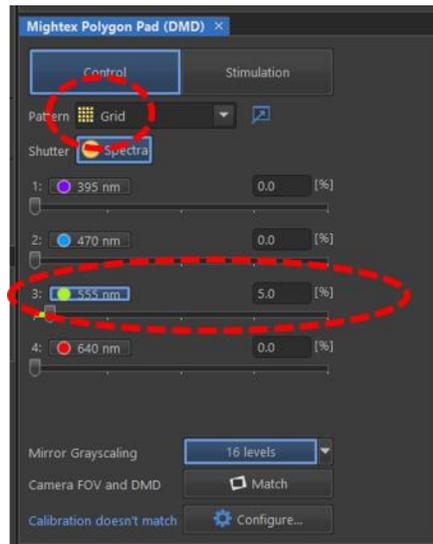
11. Continue with “in combination with CSU”

In combination with CSU spinning disk and photometrics camera

DMD alignment

1. Place a mirror slide as sample (you’ll find one in the transparent drawer unit on the microscope table). Place the mirror side towards the objective. The mirror side is the side where a tool does touch its reflectance, there is also something written on the mirror side.
2. Use the same objective you want to use for your experiment.
3. In the *Ti2 pad*, in the Turret-Up, select “405 DM” (position 1).
4. In the *Filters, Shutters and Switchers* pad switch “MLC” shutter off (meaning no laser light).

- In the *Mightex Polygon Pad (DMD)* set the DMD on grid only. Select one of the 470/555/640 LEDs, set the light intensity low about 5 %. (Don't use 395nm, that is will harm your eyes!!!!)

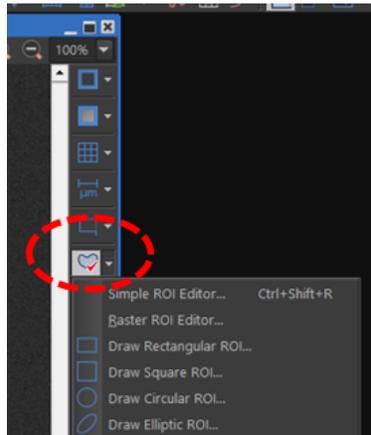


- In the software in the *Ti2 pad* or on the microscope stand. Set the light path to the eye piece (Middle up).
- Focus on the mirror slide, the light will get brighter when your closer to the slide. You will see a rectangular grid when you're in focus.
- In the *Prime 95B settings pad*, set the camera at 50 ms exposure time.
- In the *CSU pad* select an empty filter position (position 5).
- Light path the right (photometrics camera)
- Select the LED color, that you will use for activation (*if you use the 395nm for activation, then change here*).
- Go live (But make sure there is **no** excitation laser on! MLC shutter off or no lasers activated) and refocus on the camera
- Click on the *Mightex Polygon Pad (DMD) pad* > configure > auto calibration
- Follow the wizard. You're already focused, so click next, it asks to click "Stimulate S1 group", now only the ROI in your live image should lit up. This is just to verify the calibration. If that looks good, click "OK" in calibration window, click "OK" again in the configuration window.

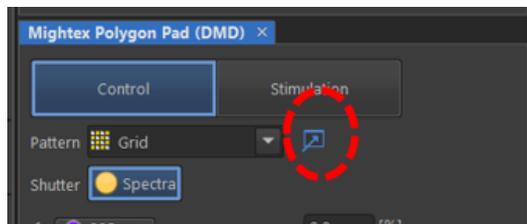
#### Making ROIs for activation

- Open a live window or make a snap shot

- In your live view or snapshot, right-side column, click on the little white arrow next to the ROI icon and choose your favorite ROI shape to make an ROI.



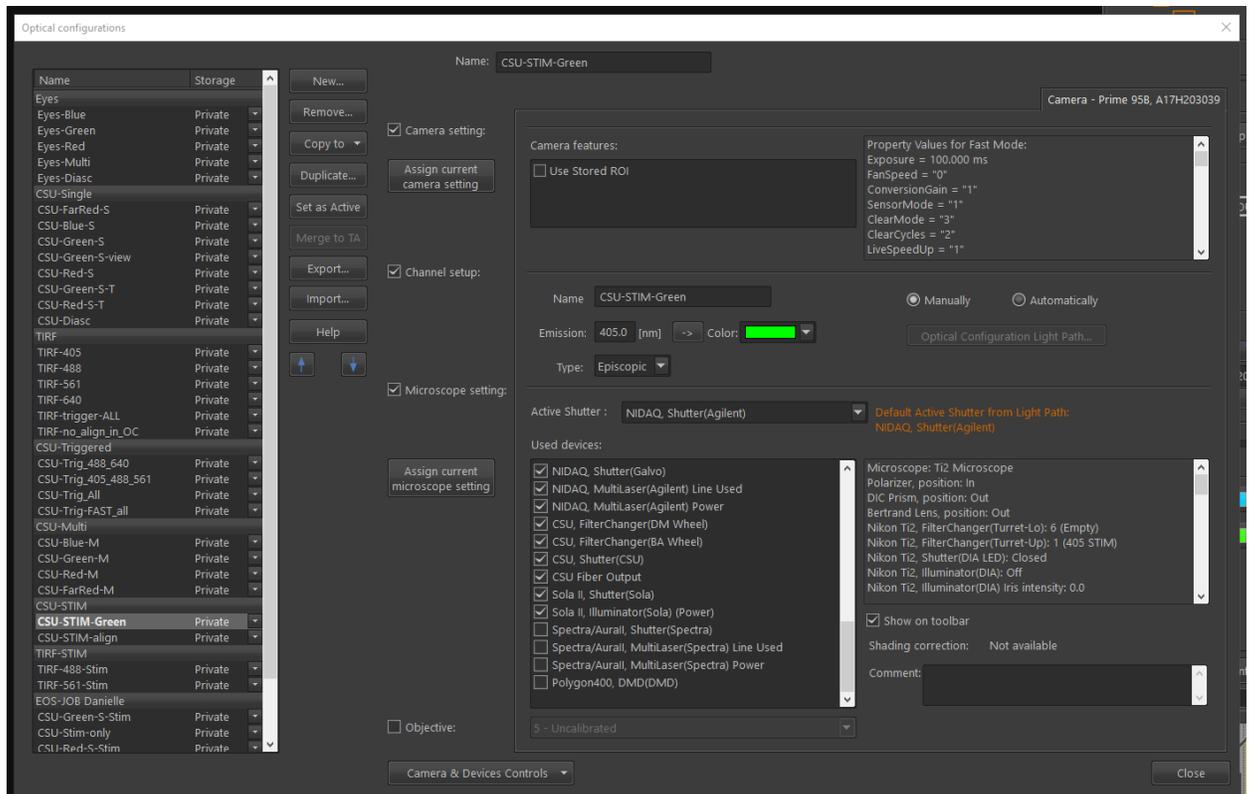
- For ease of use, right-click on an ROI > ROI properties > set transparency at 100 % (hit enter) > close. That way you see better where you put your ROI in your image. You can check or uncheck the box “show labels”.
- You can make multiple ROIs and assign them individually to (S1 – S3) or all together (Ctrl+A) by right-click on an ROI and select “Use as stimulation ROI: S1” (top of your new menu you see how many ROIs you have selected). You can also add ROIs to group S1-S3 later on.
- Or create multiple ROIs, select them, right-click on an ROI and choose “export as DMD properties”, save the pattern. The location defaults to: C:\ProgramData\Laboratory Imaging\Platform\yourname\Mightex (change the location, if you want).
- Exported patterns can be reopened. On *Mightex polygon pad (DMD)* pad, click on the blue-arrow-in-in-the-box (see red highlight in figure below). Click on the plus sign, browse for the right pattern. You most likely find your pattern in: C:\ProgramData\Laboratory Imaging\Platform\yourname\Mightex (unless you changed the location when you exported the pattern). Select new pattern, choose DMD, Click OK.



## OC setup

To operate the DMD independently of your OC you should have the Spectra/Aura LED and DMD unchecked in your OC.

- In the OC panel, right click on the OC you use > edit > under microscope settings (scroll down in the list) and uncheck the parameters: “Spectra/Aurall, shutter”, “Spectra/Aurall, MultiLaser(Spectra) Line Used”, “Spectra/Aurall, MultiLaser(Spectra) Power”, Polygon400, DMD(DMD)”
- Click close, when done



## Acquisition

1. Use ND acquisition – timelaps
2. At the Lamda tab – select the OCs you want
3. Make sure the saving options are correct
4. Check if your ROIs are positioned correct and assigned as Stimulation ROIs (S1) or use a pattern.
5. In the DMD pad, at pattern select S1 (or a saved pattern)
6. Make sure the correct LED is activated with corresponding power.
7. Run the ND acquisition
8. Use the Spectra shutter on the DMD pad when you want to activate/photoconvert during the ND acquisition.