Miltenyi Biotec Ultramicroscope Blaze Light Sheet

Turning ON

LASER

- turn the key ON + Push Emission Button
- power between 40% and 70% (40-50% should be enough to find the sample)

MICROSCOPE

- switch ON the button at the back of the microscope (right)

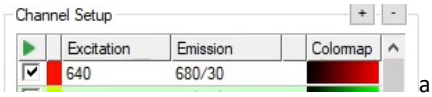
PC

- turn ON and start the acquisition software *Inspector Pro*

Preparing sample

- Glue your sample to one of the sample holders (super glue, bondic UV glue, silicon) or clamp it (using agarose cubes as spacers if needed)
- In the software, click  to lift the lens
- Pull cuvette drawer out : the cuvette containing DBE (or water) will be automatically lowered
- Place sample holder on top of the cuvette arm (it fits in groves)
- Push the front button to lift the cuvette
- Close the front drawer

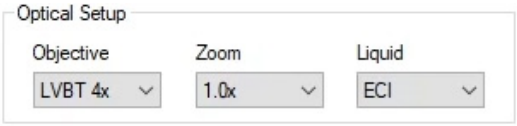
Positioning the sample in the cuvette

- With the lens still UP, choose a channel 

and click live 
- Sheet width 70% (Lightsheet properties) is a good value to start
- Use the joystick to move the sample in the right position in the cuvette : you should see the lightsheet illuminating a plane in your sample. You can change the Joystick speed with the red button

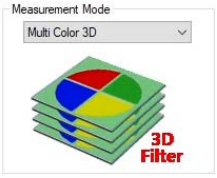
Acquisition settings

- Choose objective (1X / 4X / 12X) and zoom (0.6X to 2.5X) : total range 0.6- 30X

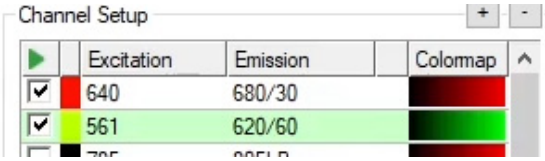


- In *Objective Lens Focus* use  to lower the lens all the way (keep pressing): the lens should dip in the liquid (otherwise you should add some)

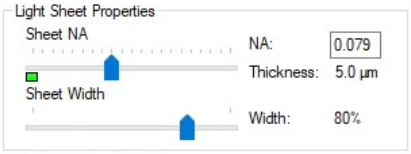
- If you plan to image multiple dyes, select **Multi Color 3D** in *Measurement Mode*

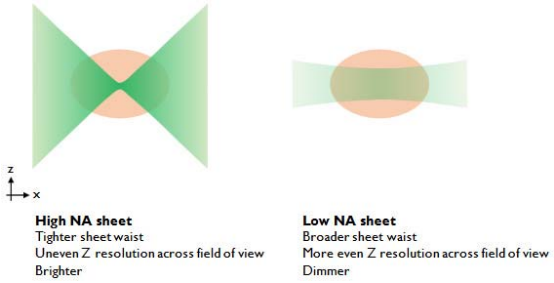


- Choose your imaging channels in channel setup



- In Light Sheet Properties choose Sheet NA to adjust the thickness of the illumination (higher NA generates a thinner beam waist but a shorter field of view, requiring more Horizontal focus to cover the whole sample width)





- Select your channels one at a time (highlighted in green) and adjust focus **manually** or using AutoFocus. Alternatively, you can use multicolor AF (it will focus all checked channels sequentially).

- By right clicking on AF buttons, you can switch to ROI AF 

. You can then define a ROI over a high detail area.
- Adjust **Laser transmission Control** (Attenuator) : Start with high value (80%) and lower if too bright
- Adjust **Exposure Time** for each individual Channel (100ms is a good start value)
- On the display you can use F9 then F10 → to adapt visual range and check the histogram (16bit camera : >65000 grey values)
- For wide samples you will need to use Dynamic Horizontal Focus otherwise the sides will be unsharp
- *Light Sheet selection* 

Left / **Right** / **Both**
If one side is darker, use **Both**. With **Both**, you will need to choose the blending algorithm (**Fixed** or **Adaptive**) in the *Advanced* Tab (**Adaptive** works usually fine)

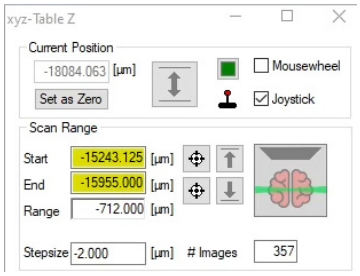
- *1, 2 or 3 beams* : **3 beams** will show more even illumination with less lines (behind obstacles) but Z resolution is better with **1 beam**

For optimal 3 beams imaging a correct ***beam alignment procedure*** need to be done on a regular basis

- Enable *Dynamic Horizontal Focus* to get nice focus along X axis (focus area with thinnest sheet moves in steps)
- Choose the **blending** of the different Horizontal Focus bands: *Focus processing* **Fixed** (physical, light sheet focus needs to be correct) or **Adaptive** (image-based composition)

Autosave settings : Before starting Acquisition, check the path to the directory for the *autosave* **Template** : Saving of settings that you can then re-use

- Define **Z-stack** in *Table-Z*



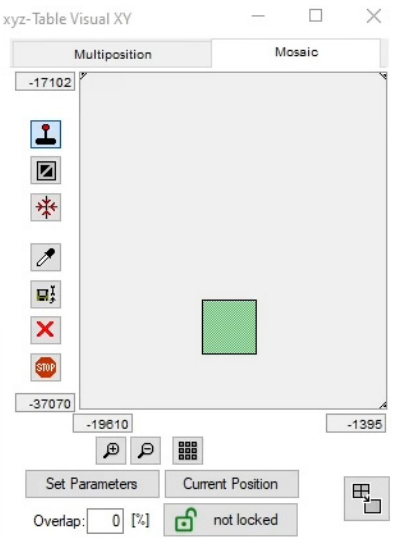
Use the mousewheel or the joystick and define **Start** and **End** positions
Choose Stepsize (3um is a good start value for 4um sheet thickness)

Start Acquisition

- Click Start 

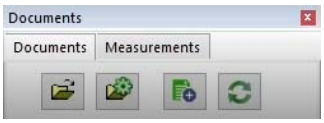
Mosaic (Tile) imaging

- Select **Mosaic** in *Measurement Mode* and **Mosaic** in *Table Visual XY*



- move to starting position (using joystick) → add squares (choose 20% overlap), define order of acquisition: x should be before y
- **Beam settings** in mosaic mode :
  In the *advanced* tab you can check:
  blend over mosaic width This will use left or right excitation on the two sides of the mosaic
  Skip unused exposures Doesn't acquire darkest exposures on each side to **gain time**

Batch Acquisition



- Select ***Measurement*** tab (bottom left) instead of ***Document***
- Define one measurement
- Open another line (+)
- Define next measurement
- Start Batch Acquisition by clicking