

3D glomeruli in a portion of an ECI-cleared kidney scanned by Light Sheet Microscopy. Courtesy of Prof. Norbert Gretz, Medical Faculty Mannheim, University of Heidelberg, Germany

TCS SP8 DLS

LightSheet Microscope Step by Step Guide

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TCS SP8 DLS Light Sheet – Step by Step Guide

Before starting LAS X, make sure that the Hardware components you need for your experiment are taught in. Here is how you can do it by starting with the Hardware Configurator.

Hardware Configurator

1. Teach in the detection objectives and mirror caps in pairs:

- > Open the Leica Hardware Configurator
- > Select Configure -> DLS-Objective-Holder -> DLS-Objective-Turret
- > Teach in the respective DLS detection objective(s) together with the mirror cap(s):

Detection Objective	Article Number	Mirror Cap	Article Number
5x/0.15 IMM	15506534	5 mm	158007011
		7.8 mm W	158007013
		7.8 mm Gly	158007014
		7.8 mm BABB	158007016
		7.8 mm Gly/BABB	158007020
10x/0.30 W	15506524	2.5 mm	158007012
		5 mm	158007011
		7.8 mm W	158007013
		7.8 mm Gly	158007014
		7.8 mm Gly/BABB	158007020
16x/0.6	15506536	2.5 mm	158007012
		5 mm	158007011
		7.8 mm W	158007013
		7.8 mm Gly/BABB	158007020
20x/0.5 W	15506421	2.5 mm	158007012
		5 mm	158007011
		7.8 mm W	158007013
25x/0.95 W	15506399	2.5 mm	158007012
		5 mm	158007011

Table 1: Possible combinations of detection objectives and suitable mirror caps.

Hardware Configurator

File Camera Help

Setup

Hardware Setup Firmware Update Configure Software Updater

Components

DMS CS DLS Objective H4857 Focussative XY Stage

Navigation DIC Tuner E-Tuner Camera

Ports Function Keys

Subcomponents

DLS-Filter-Tuner DLS-Objective-Turret

DLS Objective Turret

Setup Component Data

Position	Article No.	Name
1	11506299-158007011	Z5w039 HC FLUCOR L - DLS HalfFlat Sense
2	11506299-158007012	Z5w040 HC FLUCOR L - DLS HalfFlat 2 Sense
3	11506534-158007013	10u03 HC APO L - DLS TwinFlat 7 Beam W
4	11506532-158007013	5u015 HC PL FLUCOR - DLS TwinFlat 7 Beam W
5	11506532-158007014	5u015 HC PL FLUCOR - DLS TwinFlat 7 Beam Gy
6	11506524-158007011	10u02 HC APO L - DLS TwinFlat Sense

Search filter: ACTIVE entries found(14/14)

ObjectArticleNo	ObjectiveType	Magnification	Aperture	MirrorDeviceArticleNo	MirrorDeviceName	MirrorDeviceFocalPlaneDistance	MirrorDeviceReflectiveIndex	
1	11506299	HC FLUCOR L	25x	0.95	158007011	DLS TwinFlat Sense	354	1.884
2	11506299	HC FLUCOR L	25x	0.95	158007012	DLS TwinFlat 2 Sense	251	1.344
3	11506471	HC APO L	25x	0.5	158007011	DLS TwinFlat Sense	584	1.884
4	11506471	HC APO L	25x	0.5	158007012	DLS TwinFlat 2 Sense	584	1.884
5	11506471	HC APO L	25x	0.5	158007013	DLS TwinFlat 7 Beam W	493	1.624
6	11506524	HC APO L	10x	0.3	158007011	DLS TwinFlat Sense	354	1.884
7	11506524	HC APO L	10x	0.3	158007012	DLS TwinFlat 2 Sense	351	1.334
8	11506524	HC APO L	10x	0.3	158007013	DLS TwinFlat 7 Beam W	531	1.884
9	11506524	HC APO L	10x	0.3	158007014	DLS TwinFlat 7 Beam Gy	531	1.451
10	11506532	HC PL FLUCOR	5x	0.15	158007013	DLS TwinFlat 7 Beam W	531	1.334
11	11506532	HC PL FLUCOR	5x	0.15	158007014	DLS TwinFlat 7 Beam Gy	531	1.451
12	11506534	HC PL FLUCOR	5x	0.15	158007013	DLS TwinFlat 7 Beam W	495	1.334
13	11506534	HC PL FLUCOR	5x	0.15	158007014	DLS TwinFlat 7 Beam Gy	495	1.451
14	11506534	HC PL FLUCOR	5x	0.15	158007016	DLS TwinFlat 7 Beam BAB	495	1.563

Teach in DLS detection objectives with mirror caps.

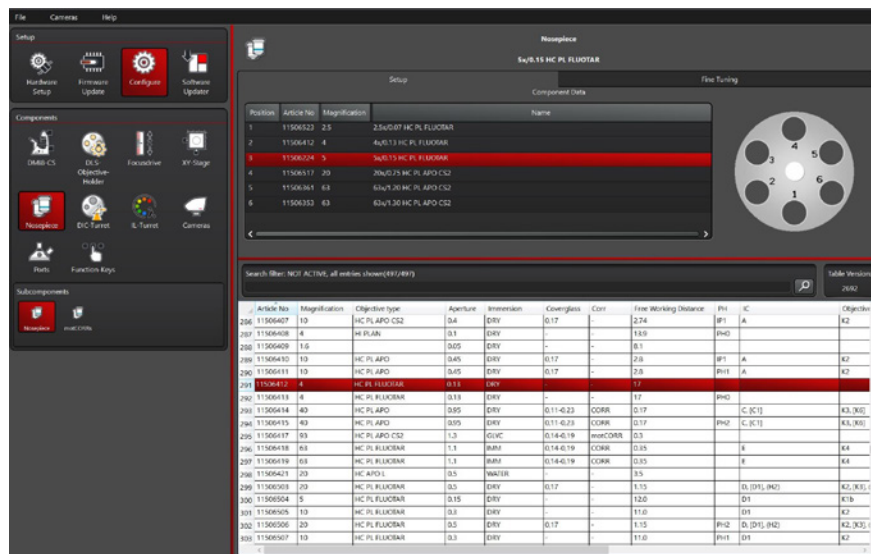
Hardware Configurator

2. Teach in the illumination objectives:

- > Select Configure -> Nosepiece -> Setup
- > Teach in the DLS illumination objective(s)

Illumination Objectives	Article Number
1.6x/0.05	15506409
2.5/0.07	15506523
4x/0.13	15506412
5x/0.15	15506224

Table 2: Available DLS Illumination objectives (see also page 21)



Teach in DLS illumination objectives and, if needed, further objectives.

Note: For calibration at least one illumination objective < 5x needs to be installed.

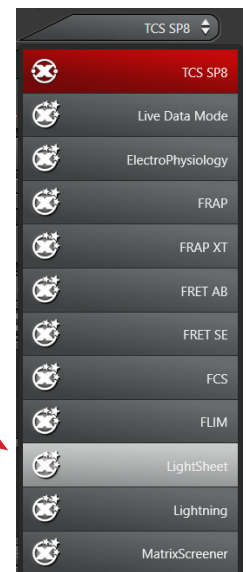
Start your experiment

- > Start LAS X
- > Open the LightSheet wizard in LAS X

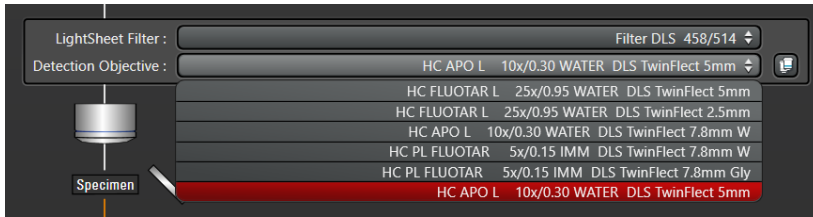
Before you start imaging your sample, you have to calibrate the Light sheet in 3 steps

1. Calibration-Detection
2. Calibration-Illumination
3. Calibration-Optimization

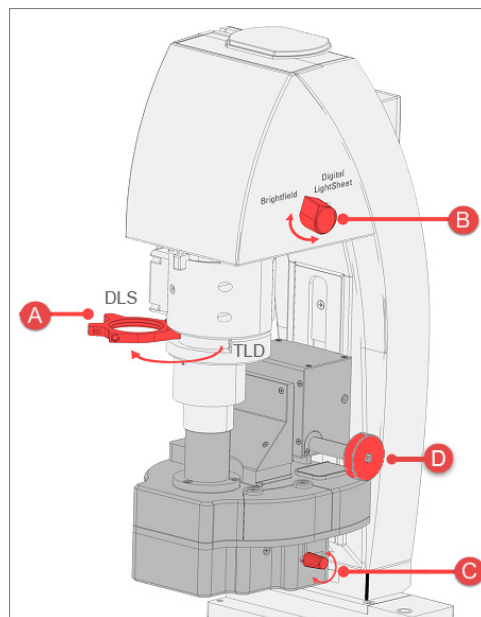
→ The light sheet wizard opens directly in the DLS Calibration Step1 "Calibration-Detection".
If you have not yet switched on the laser(s), the wizard asks you to do so.



Hardware Configurator



- > Select the correct Detection Objective with mirror cap from the list.
- > Check that the mirror cap and illumination objective are compatible with each other (see page 4, Table1. Possible combinations of detection objectives and suitable mirror caps).
- > Orientate the mirrors parallel to your sample by turning the screw set (C). If the objective with the mirrors is very strongly twisted, press the screw set backwards to unlock. Hold it in this position and turn the objective by hand until the mirror and sample are parallel. Exact fine alignment is performed later.

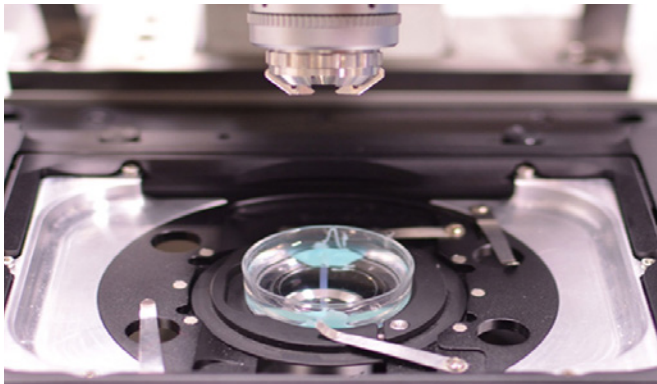


- A: Lens for TLD (transmitted light detector)
- B: Knob to change to BF (Brightfield)
- C: Setscrew to align the mirror caps
- D: Z-actuator of the DLS objective holder

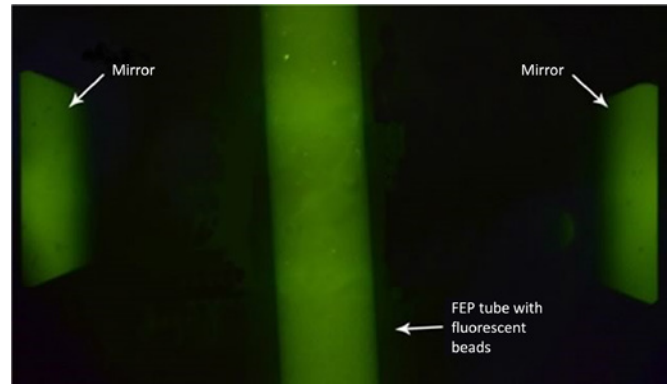
Focus your sample

- > Make sure that the TLD lens (A) is swung out.
- > Move the DLS objective holder (D) upwards to a safe height to prevent a collision with the sample when tilting the transmitted light arm.
- > Fasten the sample on the stage. The sample (e.g. FEP tube with beads) in the Petri dish has to be centered between the two reflecting surfaces of the mirror cap.
- > Fill the Petri dish at least 2/3 full with distilled water/medium. Ultimately, the detection objective needs to be immersed in the medium.
- > Set the EL6000 fluorescence lamp / LED to the lowest level (on the EL6000 intensity regulator and microscope display).

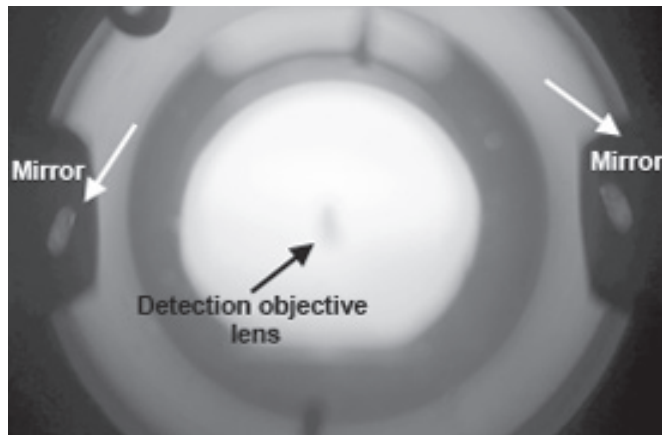
Note: There is no difference between using fluorescence or transmitted light to focus the sample. It is always advisable for beginners to check both.



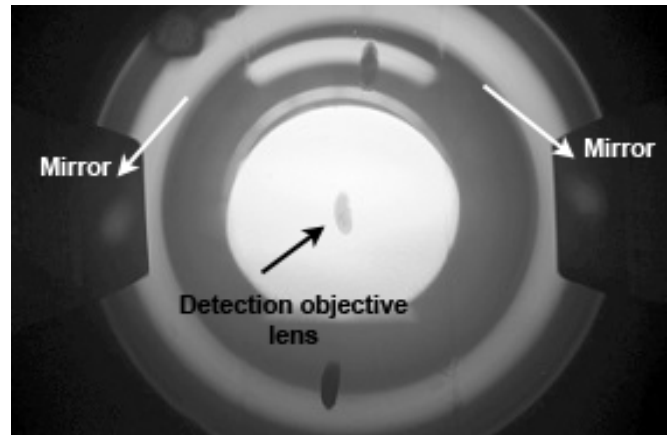
Orientation of the sample (here beads in FEP tube)



Fluorescence image of FEP tube with beads (center) and the two mirrors of the mirror cap (left and right)



Transmitted light image (focus on mirrors)



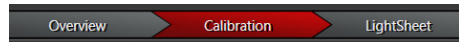
Transmitted light image (focus directly on sample)

- > Tilt the transmitted light arm down.
- > Carefully move the DLS objective holder downwards with the setting wheel (D) until the mirror cap with the 2 mirrors is in the medium.
- > Focus on the sample by using Z-Wide as usual. Check focus via the eyepieces.
- > Do not use Z-Galvo!
- > Move the detection objective with mirror cap further down, check via the eyepieces until you can see both the right and left mirror.
The process is finished once the sample is reflected in the mirror surfaces and either the sample or its reflection in the mirrors can be seen sharply through the eyepieces.
- > Align the mirrors by turning the rotation plate (C).

Note: You cannot focus on both the mirror image and the sample at the same time. It is only possible to see either the reflection or the sample sharply in the mirror. Either one can be used.

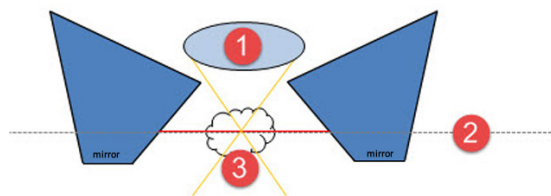
Focus your sample

DLS Calibration



Calibration Step 1: Detection

Aim: Bring your sample into the focal plane of the detection objective by using the camera.



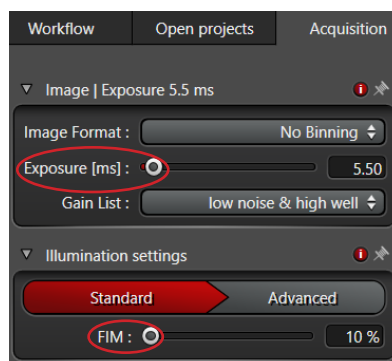
Calibration Step 1: Detection, schematic:

1 = Detection objective

2 = Focal plane of the detection objective

3 = Sample

- > Use the rotary switch (B) to change from the Brightfield to Digital LightSheet mirror position.
- > Click "Detection" in LAS X.
- > Select a suitable filter cube, such as GFP, for "Fluo Filter Cube". For "LightSheet Filter" select a compatible fluorescence filter, e.g. filter cube I3 in combination with a 505–545 band pass filter, or the Empty position. The Empty position on the side of the LightSheet filter is recommended, because this allows you to see the sample in a quasi-brightfield mode.
- > Click the "Live" button.
- > Move the DLS objective holder (D) carefully until the sample is in focus.
 - > If necessary, rescale the image using the slider of the color look-up table.
 - > If necessary, reduce the exposure time ("Exposure" slider) and the illumination of the microscope ("FIM" slider) in LAS X.



LAS X LightSheet Wizard: Camera Settings

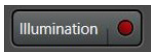
Note: From this point on, the DLS objective holder (D) should no longer be moved.

Calibration Step 2: Illumination

Aim: Measure the Z Position of the detection objective to be able to ultimately place the illumination objective at the optimal position at later steps.

Note: If the TwinFlect 2.5mm is used, the Illumination part of the calibration has to be performed with the illumination objective < 5x. Otherwise, the bottom edges of the mirrors might not be hit by the illumination beam (see below).

> Click the button "Illumination"

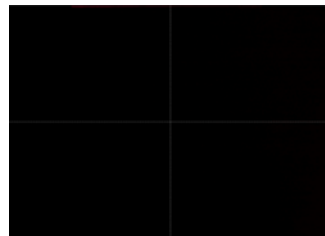


The following measurement is done using the confocal capabilities of the system: PMT is automatically placed under the laser line (488nm) and AOBs/beam splitter (depending on your system configuration) is set to reflection.

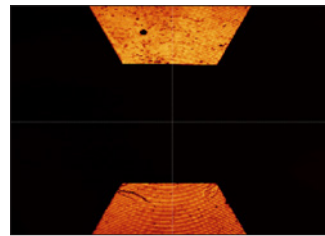
> Click "Live".

> Use Z-Wide to focus on the mirror caps until you see the reflection of the bottom mirror edge of the detection objective. Make sure that you have found the correct maximum during calibration.

Note: The first reflection comes from the coverslip and not from the mirrors. Find the reflection maximum closest to the reflection of the cover slip!



TwinFlect 2.5 mm with 5x illumination objective, 25x detection objective



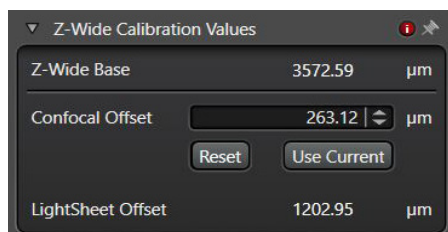
TwinFlect 2.5 mm with 2.5x illumination objective, 25x detection objective

The lower surfaces of the TwinFlect 2.5 mm are only visible by using the 2.5x or 4x illumination objective. If the 5x illumination objective is used, the lower surfaces are outside the field of view and, hence, no base position can be determined.

> Adjust the two mirrors of the cap to be as parallel and straight as possible by turning the setscrew on the objective holder (C).

> Click "Calibrate Z-Wide Position".

The automatic calibration is carried out by means of a z-stack. The Z-Wide position of the illumination objective, where the highest reflection is measured, is automatically stored as the "Z-Wide Base" value. The values are displayed in the "Z-Wide Calibration Values".



For additional information see page 18.

Calibration Step 3: Optimization

Aim: Focus the “beam waist” of the light sheet in the focal plane of the detection objective.

Now we start to work with the light sheet. As this is the first time, the next steps are calibration of some parameters that depend on each other. The most important parameters are „LS Position Z” and „LS Position X”.

- > LS Position Z
- > LS Position X
- > LS Position Y
- > LS Width
- > Mirror Cap Orientation
- > (Z Pos Sample)

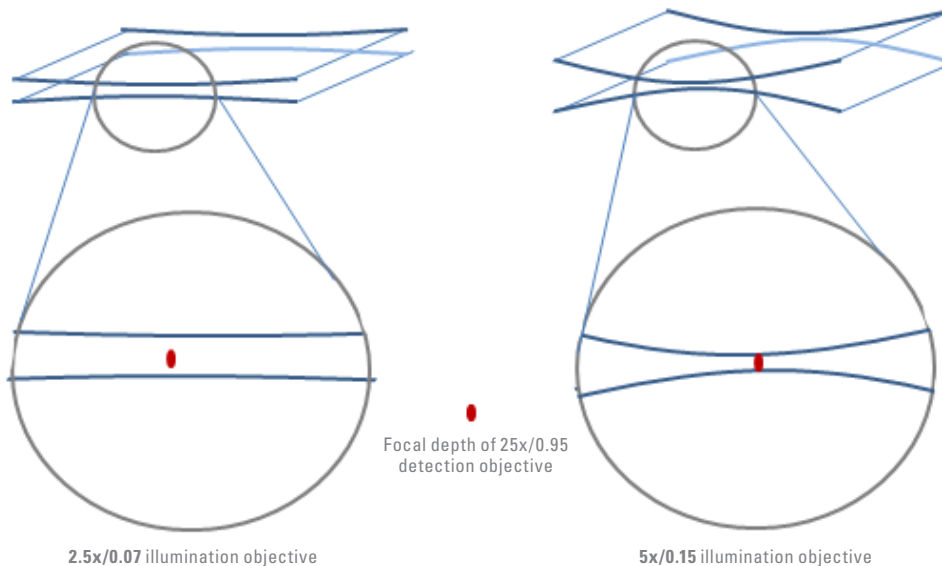
For the next steps, use the control panel. This figure shows you the drives which are in the LightSheet system (for detailed information see page 19).

LS Position Z 6359.02 μm	LS Position Y	LS Position X	LS Width	Z Pos Sample 0 μm
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Why do we call it the “beam waist”?

The light sheet is created by a line scan of the illumination objective (=virtual light sheet). Therefore, it has the shape of the point spread function of this objective.

An illumination objective with a higher NA creates a thinner and shorter light sheet than an objective with a lower NA (see page 22, table showing the light sheet properties). For the recommended combinations, the geometry of the light sheet fits to the z-resolution and field of view of the detection objective. The thinnest part of the virtual light sheet is the “beam waist”.

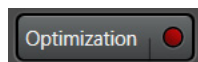


The light sheet is generated with two different illumination objectives and detected with the 25x detection objective. The shape of the light sheet (focus = “beam waist”) is determined by the illumination objective. The higher NA of the 5x/0.15 illumination objective compared to the 2.5x/0.07 illumination objective makes it possible to generate a shorter, but also thinner light sheet. In this example, 5x illumination with a 25x detection objective gives you much better contrast and signal-to-noise ratio (SNR).

Overview of the next steps

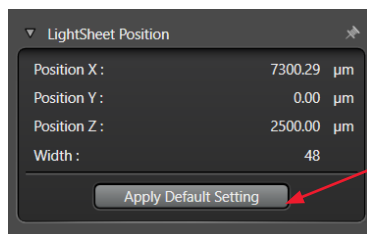
- A. Preparation – First steps
- B. Focus the beads – Adjust LS Position Z
- C. "Center the light sheet in vertical direction" – Adjust LS Position Y
- D. Fine Adjustment of the mirror orientation – Turn Objective holder manually
- E. "Center the light sheet in horizontal direction" – Adjust „LS Position X“

A. Preparation – First steps



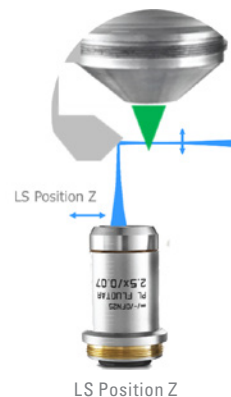
- > Click "Optimization". The mode is changed to LightSheet.
- > For "LightSheet Filter" select the empty position (this is suitable for samples in agarose which generate scattered light).
- > Set "LS Width" to 48 to make the light sheet narrower and brighter.
- > Use the LUT (look up table) slider to scale the image (pull it down to make the image in LAS X brighter).
- > Click "Live" to focus the beads via "LS Position Z" on the control panel.

Note: If you don't see any light sheet at this point, click "Apply Default Settings". This button only works correctly, if the current detection objective/TwinFlect combination has been selected from the drop-down menu.



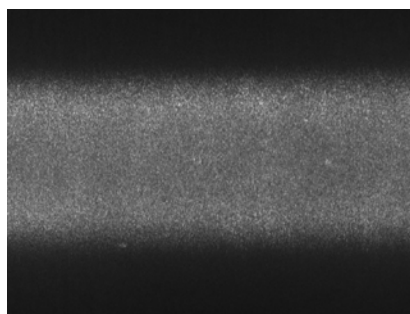
B. Focus on Beads – Adjust LS Position Z

Depending on your sample, there are two alternatives to adjust "LS Position Z": a manual and an automatic calibration.

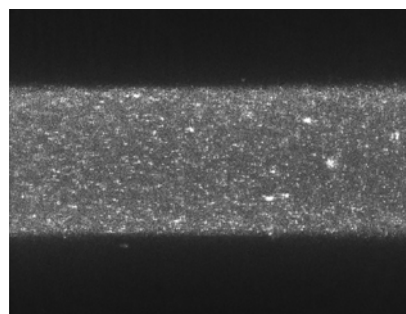


Manual adjustment of LS Position Z:

- > Click "Calibrate" to open the "Calibrate Mirror Position" dialog.
- > Select any mirror (left or right): Focus the beads / find the highest contrast via "LS Position Z" on the control panel.
- > Click "Define Mirror Position".
- > Enable the other mirror: Focus the beads like before.
- > Click "Define Mirror Position".
- > If necessary, repeat the steps.

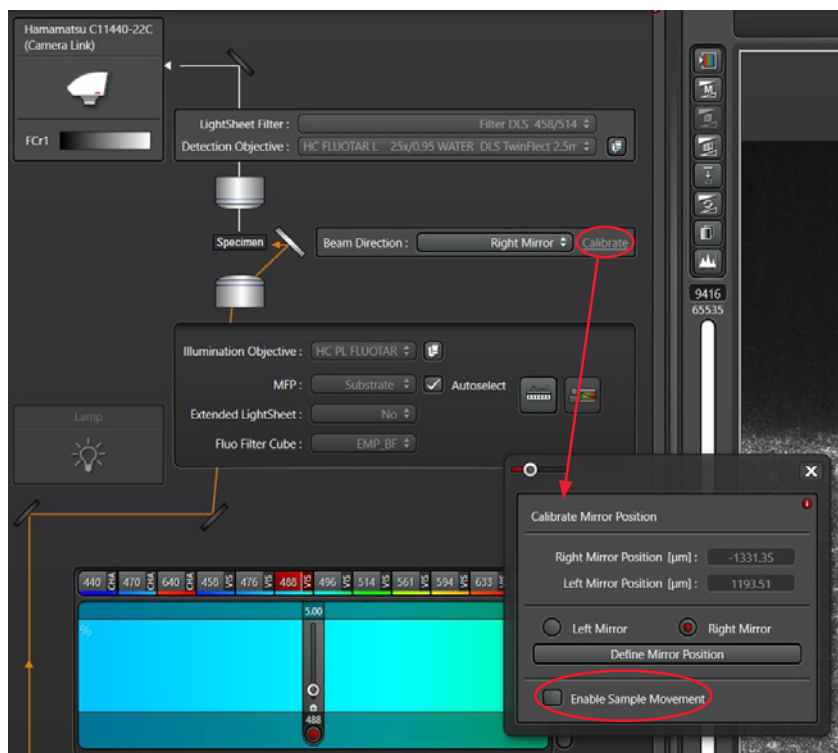


Beads out of focus



Beads in focus

Note: Z-Galvo (sample movement) is blocked in the Calibration tab by default. On the control panel, Z-Galvo is called "Z Pos Sample". It can be activated in the "Calibrate Mirror Position" dialog. Don't touch Z-Wide at this point during the calibration.



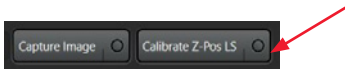
Adjust „LS Position Z“ in the "Calibrate Mirror Position Dialog"

Alternatively, an automatic adjustment of LS Position Z can be done:

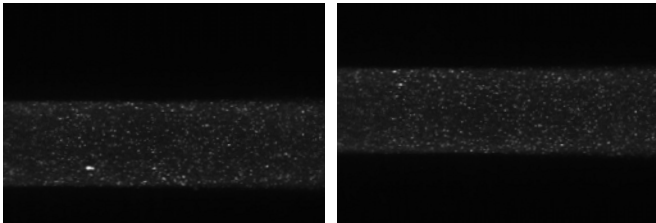
Go to a position of your sample, where you can either get a homogenous fluorescent signal or a homogenous signal in reflection (the latter is shown in our beads sample, reflection of agarose used for embedding).

> Click "Calibrate Z-Pos LS" for automatic calibration.

Note: For each combination of detection objective, TwinFlect, and illumination objective, you also need to find the proper „LS Position Z” (see also page 20).



C. "Center the light sheet in vertical direction"– Adjust LS Position Y

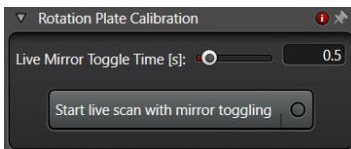


> To center the light sheet in the middle of the viewer, use the „LS Position Y” on the control panel.



D. Fine Adjustment of the mirror orientation

Use the "Rotation Plate Calibration" dialog to align the mirror surfaces with the direction of the line scanner. This is a pure manual adjustment on the DLS objective holder.



Fine adjustment of the mirror orientation

- > Click "Start live scan with mirror toggling" (light flickering is normal).
- > During the "Toggle" Scan, use the setscrew on the DLS objective holder (see page 7, C) to turn the rotation plate far enough until the overlap in the horizontal direction is complete.
- > The adjustment is finished once the images from both light sheets are completely superimposed.

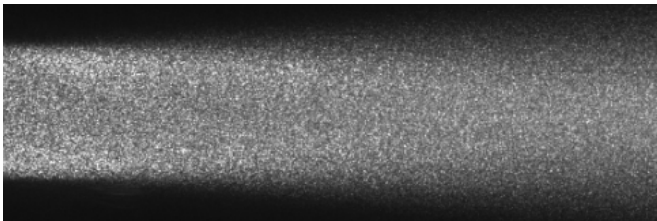


Fine adjustment of the mirror cap

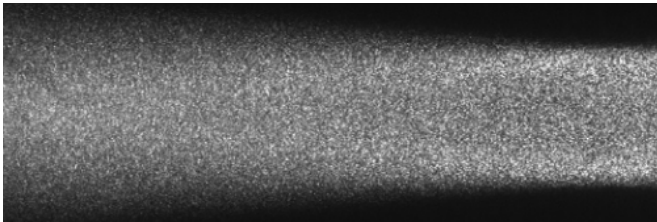
E. "Center the light sheet in horizontal direction" – Adjust LS Position X

Adjust the focus position of the light sheet (= the "waist" of the illumination beam) in the center by using „LS Position X“.

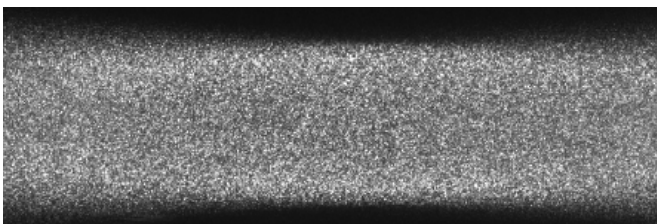
- > Click "Live".
- > The „LS Position X“ value is displayed on the control panel.
- > If now the „LS Position X“ is changed during Live Scan, you will see how the focus of the illumination objective moves either to the left or to the right.



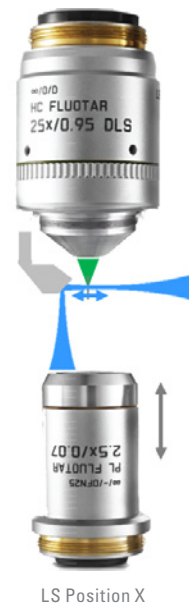
... to the left or ...

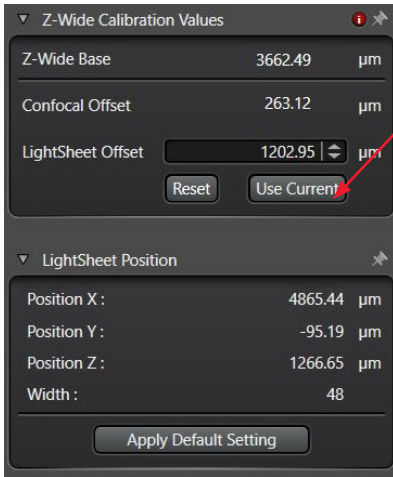


... to the right.



Goal: The "waist of the beam" is in the center.



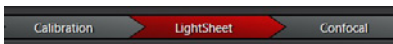


Click "Use Current" to confirm the position of LS Pos X.

> Click "Use Current" under "LightSheet Offset" to confirm the position of "LS Position X". Otherwise, the changes will not be applied for LightSheet imaging.

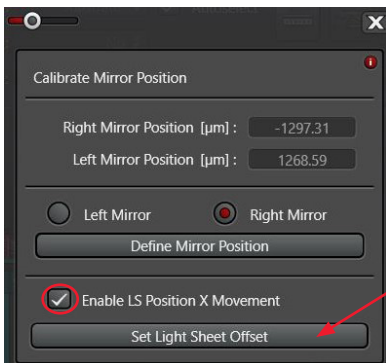
Note: Your current „LS Position X“ is now stored for this combination of detection objective, TwinFlect mirror, and illumination objective. Only by clicking “Use Current”, will changes to the focus of the illumination objective be taken into account once you switch to the LightSheet tab!

DLS Light Sheet



When the calibration is finished and the light sheet image looks good in terms of focus and contrast, change the LS Width to use the complete light sheet for imaging. Now you can run your experiments.

If you recognize that a correction of the light sheet parameters might be necessary, you can perform the mirror calibration (see page 12, Step B, Adjust „LS Position Z“) once again directly in the LightSheet Tab. Even a correction in “LS Pos X” is possible (red circle). Both can be done in the dialog “Calibrate Mirror Position”.



Note: Moving „LS Position X“ directly changes the Z-Wide position of the illumination objective. So any correction of the

„LS Position X“ leads to a change of the „LS Offset“ and must be confirmed by clicking "Set Light Sheet Offset" (red arrow). If not, the new „LS Position X“ (= new adjusted focus of the light sheet) will not be taken into account.

It is the same process as in the Calibration Step (see page 15, step E, Adjust „LS Position X“), where changes of the „LS Position X“ has to be confirmed by clicking "Use current".

What about moving the sample in the z-direction?

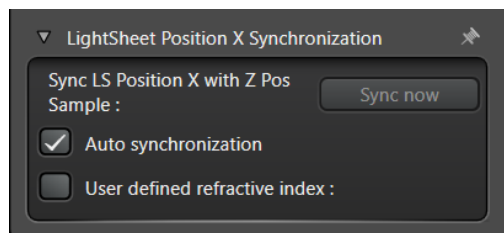
With the control panel drive "Z Pos Sample", you can move your sample in the z-direction using the super z galvo as usual. In contrast, the Z-wide drive is blocked by default to prevent unintended changes of the light sheet focus which may interfere with your previous calibration. Z-Wide is blocked both with SmartMove and the control panel (here Z-Wide is called „LS Position X").

If you want to reposition your sample in the z-direction, but you have reached the end of the Z-Galvo travel range, you can manually readjust the height of the objective holder. In that case, you have to move the detection lens first and then readjust ideally only Z-Wide („LS Position X").

As you already know, Z-Wide can be activated in the dialog "Calibrate Mirror Position" by checking "Enable „LS Position X" Movement" (red circle). This allows you to do fine adjustments without having to go back to the Calibration Tab.

Something to keep in mind:

If you move the sample through the light sheet using Z-Galvo, the relative lengths of the illumination beam inside and outside of the medium differ. As a result, the focus position of the light sheet in the x-direction („LS Position X") also changes, because the lateral focus position of the light sheet is determined by the combination of the two refractive indices (inside and outside of the medium). This lateral focal displacement can be compensated for by a continuous adjustment of Z-Wide during the image acquisition.



This is done with the "LightSheet Position X Synchronization" function. As a result, the lateral x-position of the LightSheet is readjusted depending on the axial z-position via the Z-Wide actuator (*focus tracking*).

For detailed information, see the LAS X Online Help.

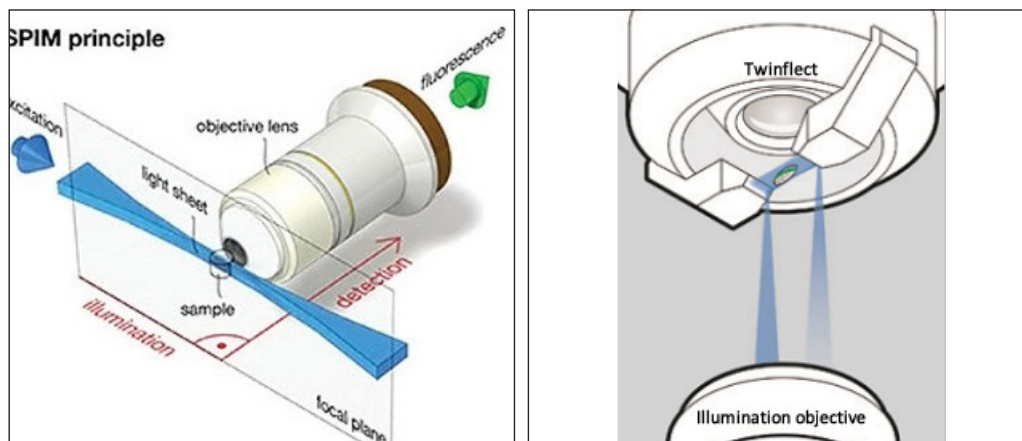
Should I change the illumination objective during the session?

If you want to switch between different illumination objectives within the LightSheet tab, you need to calibrate these different combinations (for example 25x detection objective/TwinFlex 2.5mm with 5x illumination objective and 25x detection objective/TwinFlex 2.5mm with 2.5x illumination objective) separately in the "Calibration - Optimization" step.

Additional Information

Principle

In Light Sheet Microscopy, the sample is illuminated from the side in a single plane. Conventionally, the generation of a light sheet is made possible by a special optical setup in which the illumination and detection objectives are arranged perpendicularly to each other. Objectives with a low numerical aperture (NA) - which produce a flat focus – are used as illumination objectives.



Conventional generation of a light sheet (Huisken and Stainier, 2009)

Generation of a light sheet with DLS module

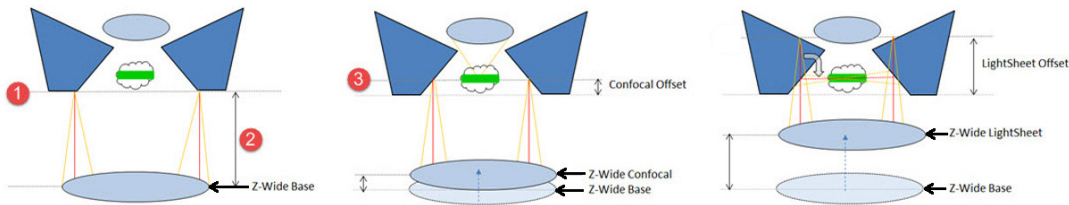
Digital LightSheet Module (DLS Module)

The DLS module consists of an illumination objective and a detection objective along a vertical axis. The two mirrors of the Twinflect deflect the illumination beam at a 90° angle and the sample is positioned between the two mirrors. The light sheet is generated by a laser and a camera records images of the sample area illuminated by this light sheet. To record a 3D- volume, the sample is moved through the light sheet.

The distance between the two objectives has to be calibrated so that the focus of the illumination objective coincides with the focus of the detection objective.

For detailed information see the LAS X Online Help “What is LightSheet Microscopy?”

Overview



Calibration Step 2: Illumination, schematic:

1 = Bottom edge of mirror

2 = Distance between illumination objective and bottom edge of mirror

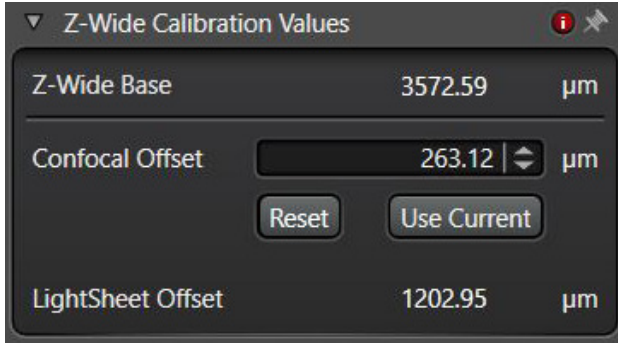
3 = Focal plane of the illumination objective

Term	Explanation
Z-Wide Base*	Z-Position at which the reflection of the mirror's bottom edge is maximum.
Confocal Offset	Distance between the mirror's bottom edge and the focal plane of the detection objective It is a default value, a property of the mirror cap stored in a calibration file.
Z-Wide Confocal*	Z-Wide Base + Confocal Offset If you leave the Calibration tab and go to the Confocal tab, the illumination objective will automatically move to this Z-Wide (Confocal) position.
LightSheet Offset	Confocal Offset + 1/2 Mirror Offset The distance is required to determine, that the light sheet "beam waist" lies centrally between the two mirrors. Note: A default value for each TwinFlect mirror is stored and can be recalled pressing the "Apply default settings" button.
Z-Wide LightSheet*	Z-Wide Base + LightSheet Offset If you go to "Calibration Step 3: Optimization", or to the LightSheet tab, the illumination objective will automatically move to this Z-Wide (LightSheet) position.

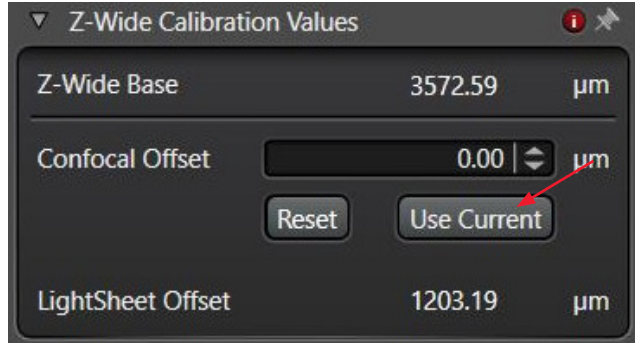
*These z-positions are absolute Z-Wide positions.

Additional Information

Calibration Step 2: Illumination



After calibration of the Z-Wide Position (Z-Wide Base)

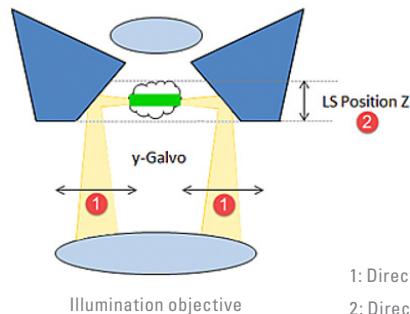


By clicking "Use Current", the Confocal Offset is set to zero. This is only relevant if you switch between LightSheet and Confocal Mode.

LS Position Z 6359.02 μm	LS Position Y	LS Position X	LS Width		Z Pos Sample 0 μm
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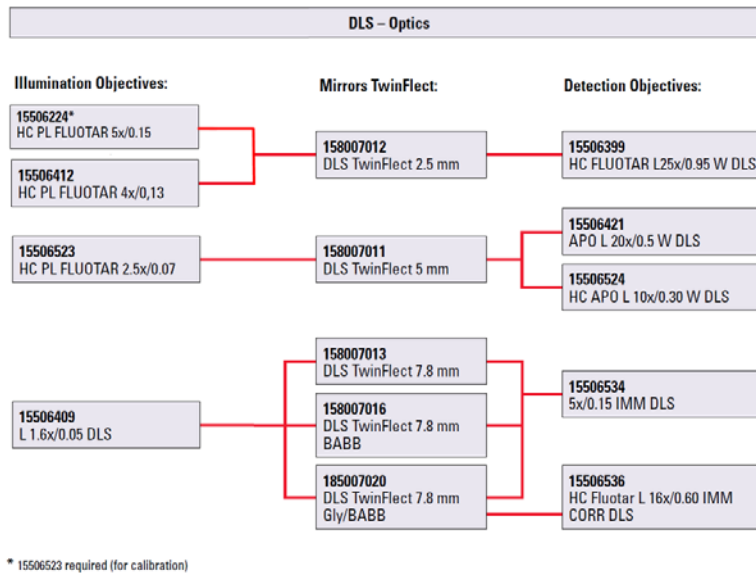
LightSheet system	Confocal system
LS Position Z	Y-Galvo
LS Position X	Z-Wide
LS Position Y	X-Panning (horizontal panning)
LS Width	Zoom
Z Pos Sample	SuperZ-Stage (set to "0" during the calibration)

LS Position Z



Additional Information

Recommended (application driven) combinations of illumination and detection objectives



Possible combinations of Illumination objectives and mirror caps

Illumination Objective	Article Number	Mirror Cap	Article Number
1.6x/0.05	15506409	2.5 mm	158007012
		5 mm	158007011
		7.8 mm W	158007013
		7.8 mm Gly*	158007014
		7.8 mm BABB	158007016
		7.8 mm Gly/BABB	158007020
2.5x/0.07	15506523	2.5 mm	158007012
		5 mm	158007011
4x/0.13	15506412	2.5 mm	158007012
5x/0.15	15506224	2.5 mm	158007012

*legacy

Additional Information

Axial and Lateral Resolutions & Light Sheet (LS) properties

		Illumination objectives				Widefield Axial Resolution (in comparison)	Lateral Resolution @530	FOV (full chip)
Axial Resolution		1.6x / 0.05	2.5x / 0.07	4x / 0.13	5x / 0.15			
Detection objectives	5x / 0.15	5.8µm / 13µm	4.0µm / 8.9µm	2.3µm / 5.7µm	1.9µm / 4.5µm	37µm	2.2µm	1470 x 1470µm
	10x / 0.3	4.9µm / 7.8µm	3.7µm / 6.5µm	2.2µm / 4.9µm	1.9µm / 4.1µm	9.3µm	1.1µm	735 x 735µm
	16x / 0.6 (in water)	2.2µm / 2.4µm	2.1µm / 2.3µm	1.7µm / 2.2µm	1.5µm / 2.1µm	2.4µm	540nm	460x460µm
	16x / 0.6 (in glycerol)	3.1µm / 3.5µm				2.6µm	540nm	460x460µm
	16x / 0.6 (in BABB)	2.5µm / 2.8µm				2.8µm	540nm	460x460µm
	20x / 0.5	2.8µm / 3.2µm	2.5µm / 3.1µm	1.9µm / 2.8µm	1.7µm / 2.7µm	3.3µm	650nm	368 x 368µm
	25x / 0.95	1.0µm / 1.0µm	0.9µm / 1.0µm	0.9µm / 1.0µm	0.9µm / 0.9µm	1.0µm	340nm	295 x 295µm
Lightsheet Properties		1.6x / 0.05	2.5x / 0.07	4x / 0.13	5x / 0.15			
LS Thickness @ 488nm		5.8µm / 14µm	4.1µm / 9.2µm	2.3µm / 5.7µm	1.9µm / 4.6µm			
LS Length @ 488nm		~500µm / 3mm	~250µm / 1.2mm	~75µm / 500µm	~60µm / 300µm			
TwinFlect		7.8mm 5mm 2.5mm	5mm 2.5mm	2.5mm	2.5mm			
		with / wo BE*	with / wo BE*	with / wo BE*	with / wo BE*			

*BE=beamexpander

Table 3: Light sheet properties

Overview of lateral and axial resolutions

that can be obtained with the different combinations of detection and illumination objectives

Axial Resolution:	Depends on combination of illumination and detection objective
Axial Resolution for Widefield:	Gives the axial resolution of the detection objective without influence of light sheet illumination
Lateral Resolution:	Depends on NA of detection objective
Field of View (FOV):	Depends on detection objective
Lightsheet Properties:	Depend on NA of illumination objective.
	Lightsheet Thickness = xy resolution of illumination objective (FWHM) Lightsheet Length = z resolution of illumination objective (focal length -> homogenous illumination)
Appropriate TwinFlect:	Depends on FOV of illumination objective

Additional Information

Troubleshooting

Step 1: Detection:

A. After clicking “Live”, there is a black image in the viewer

- > Make sure that the knob on the microscope transmission arm is turned from “Brightfield” to “Digital LightSheet”.
- > Make sure that the shutter of the halogen lamp / EL6000 is open (can you see light on the sample?).
- > Make sure, that the TLD lens (A) is swung out
- > Check that an appropriate “LightSheet Filter” is selected:
 - > “Block Filter” does not transmit any light to the camera
 - > Bandpass filter might not transmit enough light to the camera (e.g. Filter DLS 575- 615 might block all light if Fluo Filter Cube FI/TRITC is selected)
 - > Use the “Empty” position or a Notchfilter (e.g. DLS 405/488/561/633)

B. After clicking “Live”, there is a white (saturated) image in the viewer

- > Turn down the intensity of the EL6000 and FIM of the microscope.
- > Reduce the exposure time of the camera (hidden in “Acquisition”).
- > Move to a “LightSheet Filter” with less transmission (see above).

Step 2: Illumination

A. It is not possible to see the reflection of the lower surfaces when the TwinFlect 2.5 mm is in use.

- > Make sure that the 2.5x/0.07 objective is used as “Illumination Objective” when doing the calibration with the TwinFlect 2.5 mm.

B. Advice: Find the reflection maximum closest to the reflection of the cover slip!

- > Move to a “LightSheet Filter” with less transmission (see above).

Step 3: Optimization

A. It is not possible to see a light sheet

- > Rescale the LUT of the Image Viewer. For a standard light sheet image, you normally don’t need more than 5000 gray values for the 16-bit image.
- > Use the “Usual suspect” values for the Calibration values („LS Position Z”: half the mirror distance; „LS Position Y” = 0; LS Width = 48).
- > Make sure that the detection objective is fully immersed (add more medium if needed).

B. “LightSheet Offset” is completely out of range, which you are used to seeing, once you have positioned the focus to the center and confirmed with “Use current”.

- > Make sure that you have found the correct maximum (the first comes from the coverslip reflection) during the Calibration.

Leica Microsystems CMS GmbH | Am Friedensplatz 3 | D-68165 Mannheim (Germany)
Tel. +49 (0) 6441 29-0 | F +49 (0) 6441 29-2599

www.leica-microsystems.com

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