

# Leica SP8 inverse (Schlieren) - Part 3: Scan settings and z-stack recording

How to modify scan settings and define a z-stack recording.

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▼ XY: 512x512   400 Hz	1.00   1.00 AU 🛛 🚺 🖈
Format :	512 x 512 🗘 🕑
Speed :	400 🗘 🕀
Bidirectional X :	OFF
Zoom Factor : O	1.00
Zoom in	OFF
Image Size :	184.52 μm * 184.52 μm
Pixel Size :	361.1 nm * 361.1 nm
Optical Section :	0.896 µm 🕒
Pixel Dwell Time : 1.2 µs	Frame Rate: 0.773/s
Line Average : 1	•
Line Accu : 1	
Frame Average : 1	¢ (O) ↔
Frame Accu : 1	\$ \$ \$ \$
Auto Gain	
Rotation :	0.00
Pinhole	▼]

# INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis we show how to modify scan settings at microscope located in Schlieren.

Please find detailed information about the system setup here.

#### Step 1 — Scan Parameters - Format and Zoom



- "Format" defines the number of pixels in one scan area.
  - One can choose preset formats via the drop down menu.
  - By clicking the "+" every other format can be chosen.
- Change your field of view by using the "Zoom". You can also use the knob in the external control panel.
- Note: Format and Zoom work together in defining the pixel size in your recording. Proper setting of the xy sampling (pixel size) is crucial for acquiring optimal resolved images.
- To adjust for the correct pixel size you can either use the online calculator such as the <u>SVI Nyquist</u> <u>Calculator</u> or the auto button for an estimate.

#### Step 2 — Scan Parameters - Speed

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	Format : 512 x 512 ¢ • • • • • • • • • • • • • • • • • •		▼ Acquisition Mode 0 ★	Load/Save single setting : 🔶
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Speed: 400 T	Zoom in		^	0.00
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Paul Dawil Time 7/4 Objective : HCPL APO CS2 63//140 OL 3 0 552 Fill CPL APO CS2	Pixel Dwell Time : 1.2 µs Frame Rate: 0.773/s		Bidirectional X : ON	405
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Rotation: 0 0,71 Prihole A	Frame Average : 1 후 않고, 외	Fluo Turret : Scan-	Image Size : 61.51 μm * 61.51 μm	
Unit: AU 7 Aug 7 Pentole: 0 1.30 499 69 29 29 999 699 59 70	Frame Accu : 1 🗘	Specimen	Pixel Size : 120.37 nm * 120.37 nm Optical Section : 0.964 um	
Emulsion & Junel:         580         105.06 µm + 1.000         100	Rotation : 0.71 Pinhole	▼ Internal	Pixel Dwell Time : 800 ns Frame Rate: 0.764/s	Objective : HC PL APO CS2 6

- Change the **scanning speed** either:
  - via the drop down menu (presets)
  - via "+" for every other scan speed.
  - *i* Increased scan speed leads to faster imaging, lower photo-damage and bleaching, but decreases signal to noise and often requires a smaller field of view.
- Use slower scan speeds to increase the **pixel dwell time** and thus collect more light.
- You can also activate "bi-directional" scanning to speed up acquisition (useful e.g. for live imaging).
  - A Ensure, the **phase** is **properly adjusted**.

### Step 3 — Scan Parameters - Averaging/Accumulation



- If you are limited by the laser power but still need to increase the signal (or reduce noise) use:
  - Accumulation (by line or by frame): Useful when using HyDs in counting mode or for very weak signals.
  - Averaging (by line or by frame): may be used to remove noise while using a PMT (e.g. in resonant scanning).

## Step 4 — Scan Parameter - Pinhole

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Frame Average : 1 🗘 🗇 🔘 🕫	
Frame Accu : 1 🗘 🖉 🕂 🎽	
Auto Gain	
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Pinhole	
Unit : AU 🗘 Airy 1	
Pinhole :	
Emission λ [nm] : 580 95.56 μm = 1.00AU	
▼ Z-Stack:	

- The Pinhole settings become available after clicking on the Pinhole drop down button.
- (i) Increasing the pinhole will enlarge the "thickness" of the z-section. In consequence you will get more light on the detector (and one can reduce laser power) while there is a **loss of resolution** in x, y and z dimensions.
- We recommend not to change the pinhole size as long you are not having a good reason to do so!

#### Step 5 — Define a Z-Stack



- Ensure the "xyz" scan mode is selected.
- Use the z-drive controller ("Z-Position") on the "control panel" to define the limits with "Begin" and the "End" of your z-stack.
- **Define** the appropriate **"Z-Step Size"** or go for optimal z sampling by choosing "System Optimized". The "Number of Steps" will be automatically calculated.
- You should refer to the <u>SVI Nyquist Calculator</u> if you plan to deconvolve your image as a postprocessing step.
- "Start" your experiment. The estimated time will be indicated here.