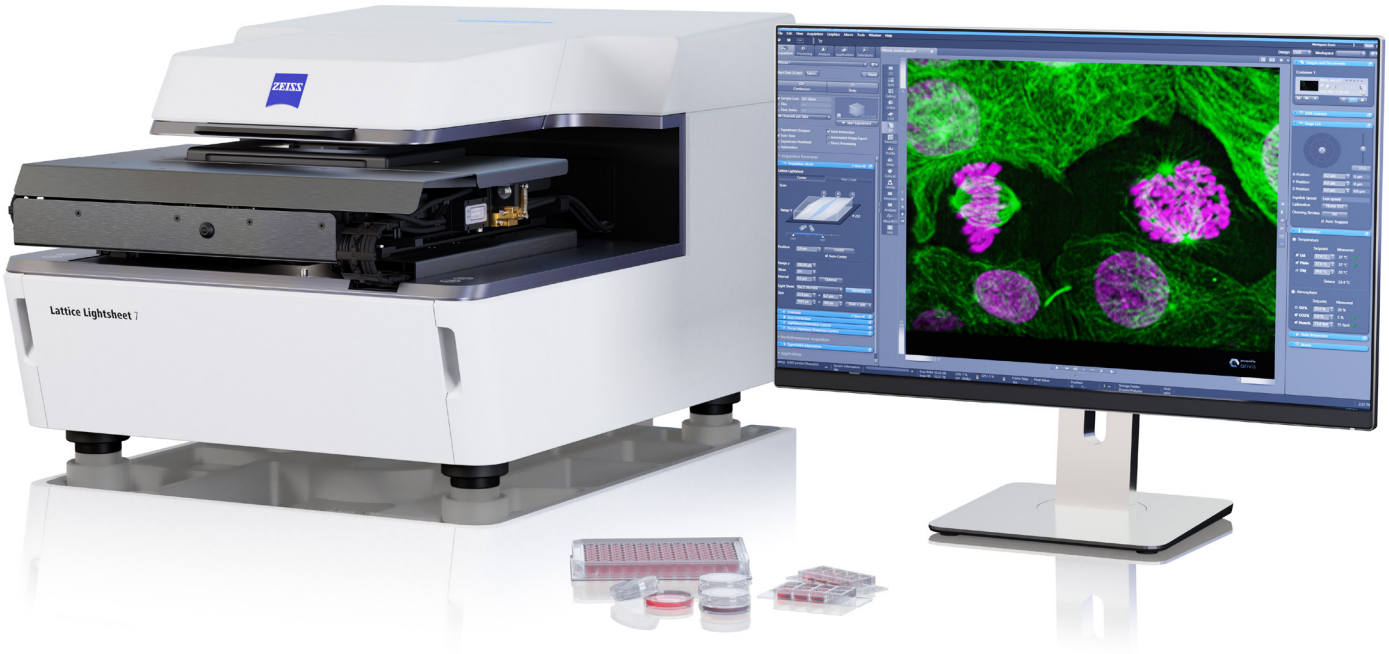
ZEISS Lattice Lightsheet 7

Sample Preparation for Lattice Light Sheet – a Quick Guide



Seeing beyond

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General Guidelines for Lattice Light Sheet Microscopy

Fluorescent Labels for Lattice Lightsheet 7:

All common types of fluorescent proteins or organic dyes usually conjugated to antibodies are suitable to be used for lattice light sheet. Make sure to have a highly specific labeling with low background to obtain a good signal to noise ratio. For multi-color samples the fluorophores should be selected for minimal spectral overlap to avoid crosstalk.

- Excitation Laser Wavelengths (nm): 488 | 561 | 640
- Filters:
  - LBF 405 / 488 / 561 / 642
  - BP 495-550 / BP 570-620 BP 495-550+LP655
  - BP 570-620+LP655 EF LP 570
  - EF LP 488
  - Empty ND filter

Media:

- Ideally the sample should be in a medium that matches the refractive index (RI) of water (1.33).
- Clearing solutions which have a different refractive index will affect the image quality, but some clearing solutions, such as TDE, can be adjusted by % to approach closer to the RI of water:

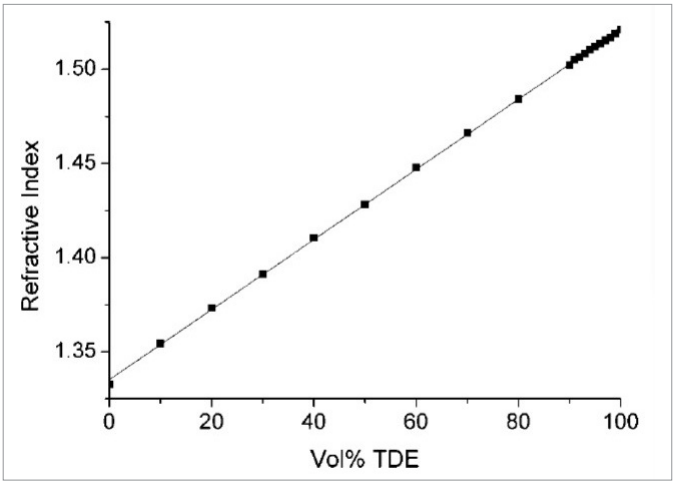


Figure 1 *TDE (2,20'-thiodiethanol) is miscible with water in any proportion. The refractive index of the solution can be precisely tuned to any value between 1.333 (water) and 1.521. The latter is even slightly larger than that of immersion oil. Source: T. Staudt et al 2007; Microscopy Research and Technique DOI 10.1002/jemt*

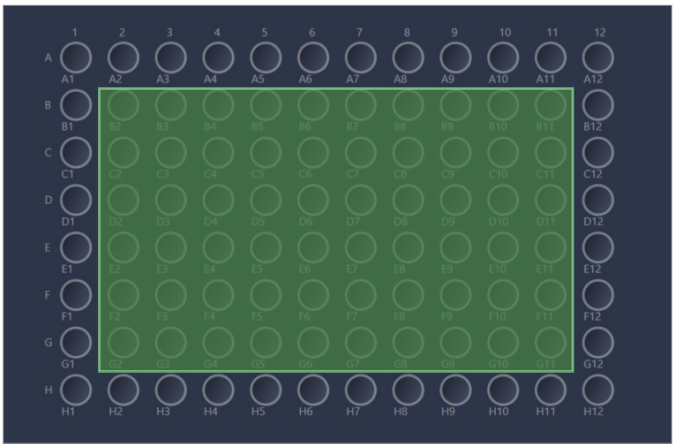
Shipping live samples:

- If shipping live cells for the demo center (Dublin, CA) we request overnight shipping the Monday a week prior to a scheduled demo to give us enough time to ensure samples are healthy and survived shipping
  - E.g. if a demo is Wednesday, Feb 24; samples should arrive no later than Tues, Feb 16
- All live samples for the demo center must be BSL1 compatible

Sample carriers and compatible plates:

- All samples must have a no.1.5 cover glass bottom
- For fixed samples that might typically be imaged on a chamber slide or petri dish, we recommend shipping in aqueous media in a centrifuge tube and allowing us to transfer to the appropriate dish for the demo
- Glass bottomed multiwell plates must have a skirt <0.5mm – below are tested and recommended glass bottom plates:
  - Greiner, CELLviewTM96-Well (Cat No 655891) https://shop.gbo.com/en/germany/products/bioscience/cell-culture-products/microscopy/cellview-plates/
  - Eppendorf, CellImaging Plates 96-Well (Cat No0030741030) https://online-shop.eppendorf.com/OC-en/Cell-Culture-and-Imaging-Consumables-110320/Cell-Imaging-Consumables-120210/Eppendorf-Cell-Imaging-Plates-PF-19458.html
  - Perkin Elmer, Viewplate-96 Black (Cat No 6005430) https://www.perkinelmer.com/de/product/glass-bottom-viewplate-96-f-40x1b-6005430

We recommend limiting experiments to wells A2 -G11 (green shaded area below) for the best demo experience.



- Glass bottomed 35 mm petri dishes that are known to be compatible:

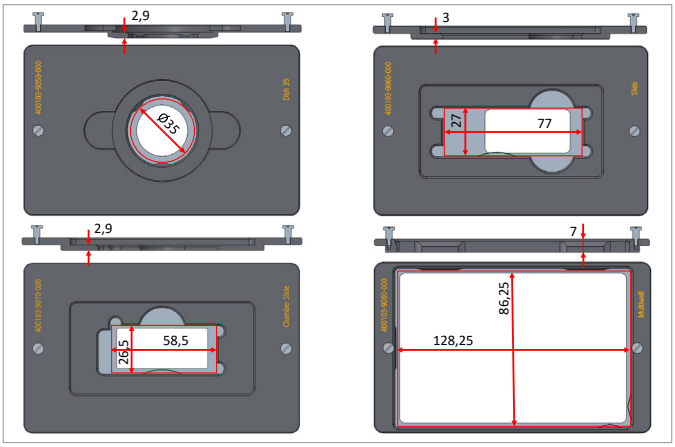
  **Not all ‘labeled 35 mm dishes’ are actually 35 mm – larger ones may not fit the sample carrier

  - MatTek 35 mm Petri dish (Cat No P35G-1.5-14-C) https://www.mattek.com/store/p35g-1-5-14-c-case/

- Glass bottomed chamber slides known to be compatible:

  - Ibidi 8 well #1.5 coverglass bottom (Cat No 80807) https://ibidi.com/glass-bottom/252--slide-8-well-high-glass-bottom.html

- Sample carrier dimensions (mm):



- Tested products that have no 1.5 cover glass bottom but are not compatible with the above sample holders:

  - Cellvis ‘35mm dish’ (Cat No D35-14-1.5-N) – actual outer diameter is 39 mm https://www.cellvis.com/_35-mm-glass-bottom-dish-with-14-mm-micro-well- number-1.5-cover-glass_/product_detail.php?product_id=31

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