

Light Sheet Z.1:

REQUIRED CONSUMABLES:

1) Sample Holder

All samples must be mounted via glass capillaries, 1mL syringes, or FEP tubing. The HCBI bought a number of reusable glass capillaries. Each user will be initially issued two capillaries of a size compatible with their experiment until our supply is exhausted. The HCBI **will not** reorder capillaries; this will be the responsibility of individual labs. Capillaries and plunger kits can be ordered directly from Zeiss or home-built. FEP tubing can be ordered from Cole-Parmer.

FEP tubing catalog numbers and approximate price (by inner tube diameter):

800um EW-06406-60 \$15.00 for 25ft

3.2mm EW-06406-64 \$30.50 for 25ft

4.7mm EW-06406-66 \$42.00 for 25ft

Other diameters are available, check the Cole Parmer website for details.

All samples containing carcinogenic dyes (DAPI, PI, Hoescht, etc) **MUST** be imaged in sealed FEP tubing.

A video on sample prep can be viewed here: http://www.youtube.com/watch?v=3KIMDtdq_1c

2) Buffer & Loading Supplies

All samples must be suspended in ~30mL of Millipore water or the user's buffer of choice. Users must bring their own water/buffer as well as a syringe and a 100mm or longer extension line (with Luer Lock). Only water and salt buffers are allowed. Samples that are infused or imbedded with/in reagents that may damage objective seals must be placed in sealed FEP tubing.

Extension lines can be ordered from:

B. Braun medical Inc: 1-800-227-2862 100cm extension lines (100) Product #8255067 9this product is currently only available in Germany, we are attempting to source it in the US. Another option is product #V6203

or

Cole Parmer: WU-30526-18 (female end connects to microscope) (sometimes long back orders)

Syringes should have a capacity >30mL and have a female luer lock compatible with the extension line.

You will also need a 1% low-melting point agarose (w/v) solution made up in the same buffer as you will use to fill the imaging chamber. The HCBI has a heating block available for you to keep this solution warm.

3) Cleared Tissue Supplies

Magnets to attach tissue to capillary or syringe: <http://www.rare-earth-magnets.com/p-6-nsn0566.aspx>

Epoxy to attach magnets to tissue and capillary: <http://www.supergluecorp.com/super-glue/original-super-glue/super-glue>

4) Data Storage

Lightsheet data sets are VERY large. Although our system can store over 8TB of data, we expect we will have to clear the hard drives monthly. Therefore, you will need to download your data after each experiment to your RC account or portable storage drive. We recommend a 1TB or larger USB 3.0 drive. These can now be bought for ~\$100.

5) Multiview 3D restoration

If you will be performing multiview analysis, you will need to purchase fluorescent beads. Please talk to Casey or Doug for further information about this technique. Here is a list of potential beads we order from Life Technologies:

For 20x objective:

100 or 200nm Yellow/green or red beads

For 5x objective:

1um or 2um yellow/green or red beads