

## Light Sheet Z.1:

### REQUIRED CONSUMABLES (Non-cleared samples):

#### 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.

A video on sample prep can be viewed here: [http://www.youtube.com/watch?v=3KIMDtdq\\_1c](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) Multiview 3D restoration

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

For 20x objective:

200nm Yellow/green or red beads

For 5x objective:

1um yellow/green or red beads

REQUIRED CONSUMABLES (cleared samples):

**4) Cleared Tissue Supplies**

Sample will be mounted on a 1mL syringe – not all 1mL syringes fit. BD Biosciences REF 309659 (available in the VWR stock room) will work.

Krazy Glue is used to mount sample on syringe

A 30-50mL syringe will be needed to load 25-30mL of clearing solution into the sample chamber (sample is suspended in this solution during imaging). This also requires an IV extension line (see above)

REQUIRED FOR ALL SMAPLES:

**5) Data Storage**

Lightsheet data sets are VERY large. Although our system can store over 30TB of data, we clear the hard drives monthly. Therefore, you will need to download your data after each experiment to your RC account, the HCBI server or a portable storage drive. We recommend a 4TB or larger USB 3.0 drive. These can now be bought for ~\$150.