**Confocal imaging of cilia under fluid flow**

Materials:

Media (Media composition depends on cells you are working with)

SC-22/40 Coverslip

Petri Dish

Fibronectin (if needed to coat coverslip)

Collagen (if needed to coat coverslip)

RC-31 Flow Chamber

Stage Adaptor

CS-22/30 Coverslip

GS-30S/15 0.15 375um Gasket

Genie Plus Syringe Pump

1ml Syringe

15ml vial

Sterile grease

Cell Plating:
1: If the cell type you are working with requires the SC-22/40 Coverslips to be coated before plating, do so following either the fibronectin or collagen coating protocols.

2: Follow replating protocols and plate cells at ~100,000 cells/ml on two coverslips in one petri dish.

3: Allow to incubate until desired confluence (80% for IMCD).

Flow Chamber Preparation:

1: Create a small line of grease around the edges of where the SC-22/40 coverslip will sit on the bottom plate.

2: Create a small line of grease around the edges of where the SC-22/30 coverslip will sit on the glass-midlayer.

3: Place a SC-22/30 coverslip in its slot on the glass-midlayer.

4: Place the GS-30S/15 0.15 375um Gasket over the SC-22/30 coverslip on the glass-midlayer. (Note: Make sure to not block the input/output holes or else you will block flow.)

5: Fill the 1ml syringe with media and flow into the intake and output tubes attached to the glass-midlayer to flush air from the system.

6: Place the cell seeded SC-22/40 coverslip face up in the gasket on the bottom plate.

7: Cover the cell seeded SC-22/40 coverslip with media (usually 5-6 drops from the 500µl syringe).

8: Place the glass-midlayer gasket side down on top of the SC-22/40 cell seeded coverslip.

9: Screw on the top plate, tightening it down completely.

10: Place flow chamber in stage adaptor and attach 50µl syringe to the input tube.

11: Flush any bubbles from the viewing area gently, too strong of a flow will cause primary cilia and cells to shear off.

Imaging:

1: For computer/microscope startup, follow computer/microscope startup protocols

2: Remove the preset stage in the Lecia Multi-photon Confocal Microscope and install stage adaptor and flow chamber. (Note: if you are using an oil objective, add oil before this step as it is hard to get to the objective once the chamber is installed)

3: Tape a 15ml vial to the side of the microscope and submerge the exit flow tube into the medium

4: Place the 1ml syringe into the Genie Plus Syringe pump and set the pump to an flow rate of 150 µl/min.

5: Set the Lecia software to GFP settings and acquire desired images.(Note: Pump can go both forward and backwards, thus you do not need to continually refill the syringe, just alternate directions after a set of images is recorded.)