Protocol 4: **Attachment of microtubule seeds to the coverslips.**

4.1. Assemble flow chamber as per manufacturer's instructions using silanized coverslips and proceed to step 4.2. If using custom-made coverslips (Protocol 1), follow the steps below.

4.1.1. Attach two pieces of double-sided tape (5 x 30 mm) along the central ~ 5mm wide area, put silanized coverslip atop the tape, press firmly.

4.1.2. Fill the chamber with BRB-80 through one of the tubes and plug both tubes with round toothpicks.

4.1.3. Squeeze a small drop of two colored Kwik Cast sealant on top of a small plastic Petri dish, and mix quickly but thoroughly using a toothpick. The sealant will turn green; apply immediately, carefully sealing all edges of the coverslip. If the sealant penetrates too deeply under the coverslip, open one of the tubes by removing the toothpick plug and apply gentle pressure to prevent sealant from leaking inside the tubes.

4.1.4. Let the chamber dry for 10 min and confirm that the flow is not restricted before proceeding further.

4.2. Place the chamber on a microscope stage prewarmed to 32°C and attach one of the tubes to a pump, which will pump the liquid out. The length of the inlet tube should be minimized to avoid the unnecessary loss of reagents: the recommended length is 5-7 cm. Immerse this end in a 0.5 ml tube with BRB-80 buffer. This and all solutions below should be prewarmed to 32-35^{o}C.

4.3. Apply a gentle pressure with a pump or simply lift the end of the outlet tube to squeeze out the air bubbles, which may form occasionally when the inlet tube plug is removed.

4.4. Set the pump rate at 100 µL/min. Wash in 2 chamber volumes of 1:30 dilution of anti-digoxigenin antibodies in BRB-80, incubate 15 min to allow antibody adsorption.

4.5. Wash with 5-10 chamber volumes of warm BRB-80, incubate 10 min with 1% Pluronic F-127 in BRB-80 to block the hydrophobic surface of silanized coverslip.

4.6. Wash with 5-10 chamber volumes of motility buffer (BRB-80 supplemented with 0.4 mg/ml of casein).

4.7. Reduce the pump speed to 10 µL/min and perfuse microtubule seeds diluted 1:200-1:600 in 30-40 µL motility buffer. Incubate 15 min to promote binding of the seeds to the coverslip-adsorbed antibodies.

4.8. Wash the chamber at 100 µL/min with 400 µL of motility buffer to remove any unbound material.

Note 1: The resulting density of seeds should be 10-30 per microscope field. To troubleshoot, use fluorescently labeled tubulin during polymerization (step 3.1) for easier detection of the coverslip-attached seeds.

Note 2: Axonemes prepared from Chlamydomonas or other biological sources, as well as the pellicles of lysed and deciliated Tetrahymena cells can also be used as microtubule nucleators. These biological are useful for creating small microtubule arrays, and are preferred when microtubules with specific number of protofilaments are desired (GMPCPP seed nucleates one microtubule that contains ≥14 protofilaments). These structures can be attached to the cleaned coverslips by non-specific absorption, but the attachment is usually less stable compared with antibody-based attachment, especially when using the silanized coverslips.