Protocol 5: **Preparation of segmented microtubules.**

All solution volumes below are for chamber volume 15-20 µL; increase proportionally if larger chamber is used.

5.1. Prewarm unlabeled tubulin mix (45 µL motility buffer supplemented with 1 mM Mg-GTP and with 10-15 µM unlabeled tubulin) for 30 s at 35°C. Perfuse at 30 µL/min.

5.2. Monitor microtubule growth with DIC optics. During 5-7 min incubation the microtubules usually grow ~ 10 µm long.

5.3. Prepare Rhodamine-tubulin mix (65 µL motility buffer supplemented with 0.5 mM GMPCPP and 2-5 µM of Rhodamine-labeled tubulin with 0.5-1 molar ratio of Rhodamine to tubulin) and warm the solution at 35°C for 30 s.

5.4. Perfuse immediately at 30 µL/min. Incubate for 8-10 min to promote formation of stable fluorescent caps at the microtubule tips. Stable microtubule segments will also nucleate spontaneously and will be visible with DIC optics.

5.5. Wash the chamber well with 100 µL of motility buffer at 20 µL/min to remove tubulins and nucleotides, as well as soluble microtubule fragments.

5.6. With DIC, confirm that the microtubules are visible; their number, however, should decrease because many microtubules disassemble during capping.

Note 1: Segmented microtubules are very stable and can be used for at least 2 hrs. However, the lifetime of these microtubules decreases with excessive solution exchange, or if 2-mercaptoethanol is used in the imaging buffer.