**Frozen sectioning of cryofixed 3D structures** Entered by Kevin Janes
Janes Lab Protocols 1/5/07

**When referring to this protocol, please cite:** Janes KA, Wang CC, Holmberg KJ, Cabral K, Brugge JS. (2010) Identifying single-cell molecular programs by stochastic profiling. *Nat Methods*, 7, 311-7.

1. Transport embedded samples on dry ice to the cryomicrotome
2. Place sample in the cryomicrotome box and equilibrate the box temperature to –24°C
   - *Higher box temperatures (e.g., –20°C) are acceptable but can cause distortion of the acini at the periphery of the Matrigel during sectioning*
   - *Keep the slides at room temperature*
3. Replace microtome blade (if disposable)
4. Spray microtome blade and anti-roll bar with a Kimwipe moistened with ethanol
   - *Be careful not to cut yourself when cleaning the blade*
5. Pop the embedded sample out of the cryomold, mount on the sectioning platform with Neg-50, and freeze until the sample has solidified on the sectioning platform
6. Mount the sectioning platform on the cryomicrotome and cut trim sections until ~3 mm of Matrigel is visible on the cutting surface
   - *Matrigel will appear as off-white against the white Neg-50; test slides can be prepared and checked under an inverted microscope to confirm that acini are on the slides*
7. Cut 5 μm sections, adjusting the anti-roll bar to prevent the section from accordioning
   - *Do not graze sections (subsequent section will be 10 μm instead of 5 μm)*
   - *Alternatively, one can omit the anti-roll bar and use the brush technique*
   - *5 μm sections are thin enough to image by widefield microscopy*
8. Wick sections onto Superfrost plus slides (VWR #48311-703) and move the slide immediately to a slide rack at room temperature
   - *Keep the slide rack at room temperature so that the section can adhere strongly to the slide*
   - *Two sections per slide can be wicked*
   - *Superfrost Plus slides have low autofluorescence in the FITC channel and allow the sections to adhere better*
9. Continue cutting and wicking sections until good cuts are no longer possible
   - *One embedded sample typically yields 50-100 slides with two sections per slide*
   - *Do not return a half-sectioned sample to –80°C (the exposed sample will dehydrate and become damaged)*
10. Dry the slides in the slide box at room temperature for at least 30 min
    - *Slide mailers can be labeled during this time*
11. Transfer the slides to five-slide mailers and store at –80°C for several months or more