Sample preparation

Learning objectives

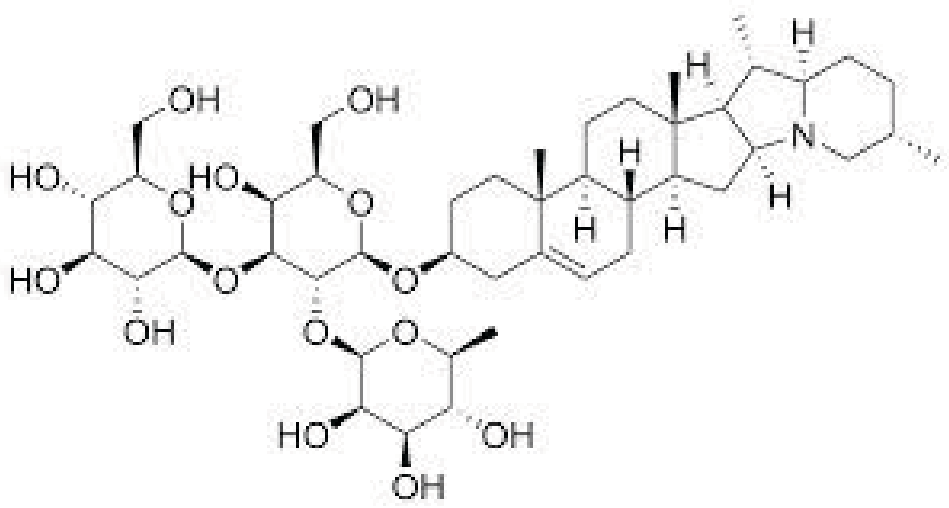
- Understand the differences in sample preparation for different sample formats
- Appreciate the critical steps for embedding and cryosectioning of tissue samples for LCM
- Embed, section, and stain representative tissues and adherent cells

Three main sample preps

- Suspension cells: isolate and lyse
- Cultured adherent cells: fix, stain, and microdissect
- Tissues: embed, cryosection, fix, stain, and microdissect

Suspension preparations

- Add 0.1% (w/v) saponin to the first step of poly(A) amplification
- Permeabilize cells and release mRNAs
- Does not interfere with downstream enzymatic steps

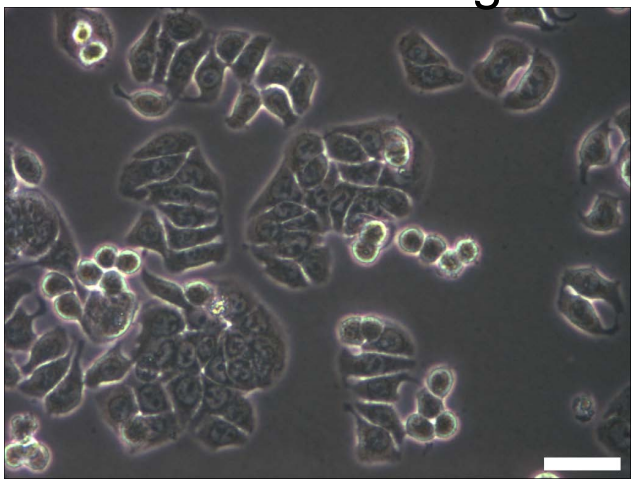


Cultured adherent cells

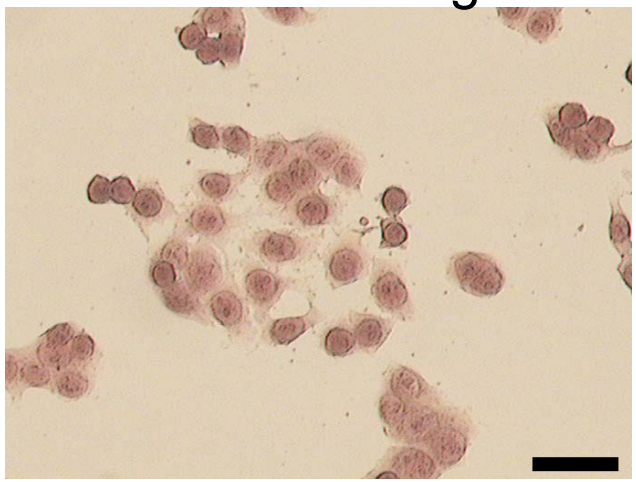
- EtOH fixation on glass
- Histologic staining (nuclear fast red, hematoxylin, toluidine blue) < 2 min aqueous!
- EtOH dehydration and xylene clearing

HT-29 cells

a Before staining



b After staining

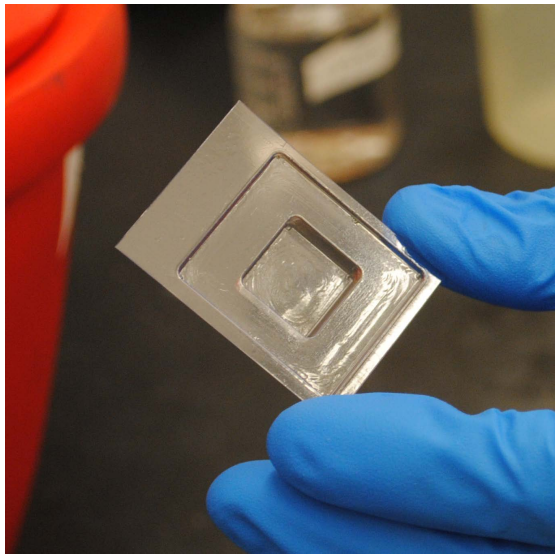
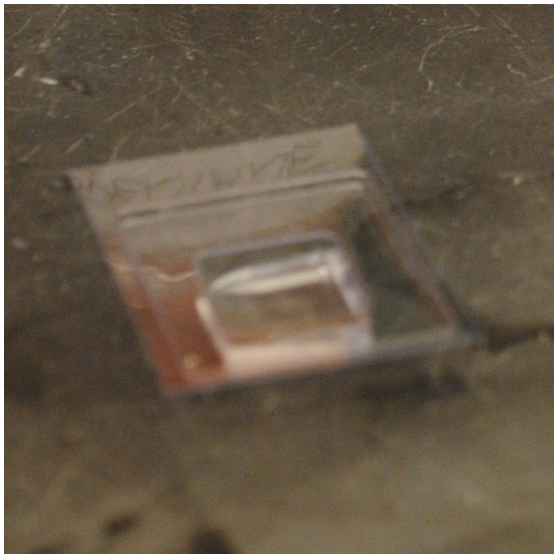
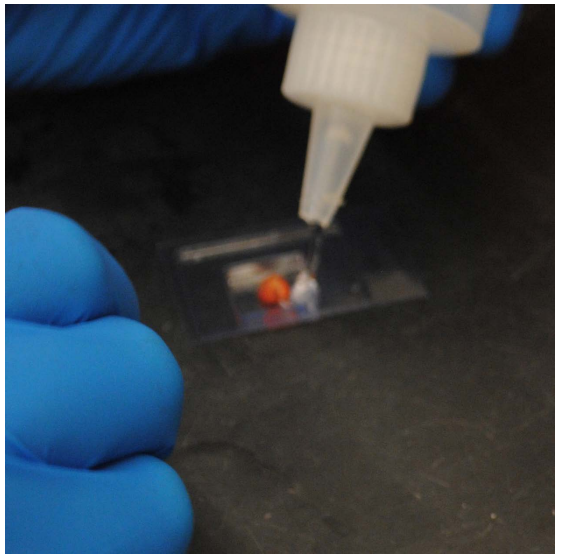


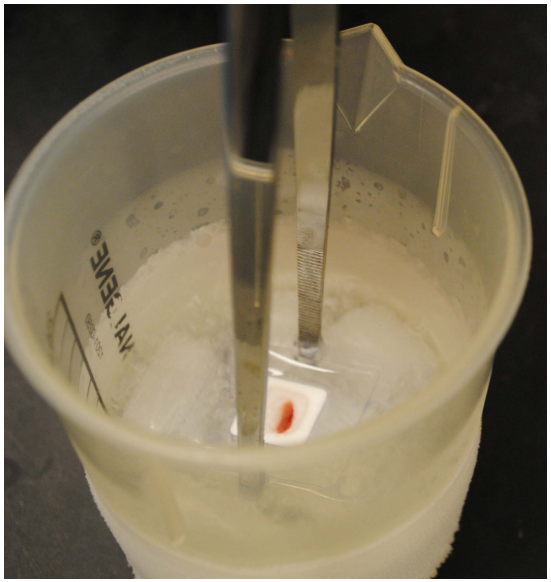
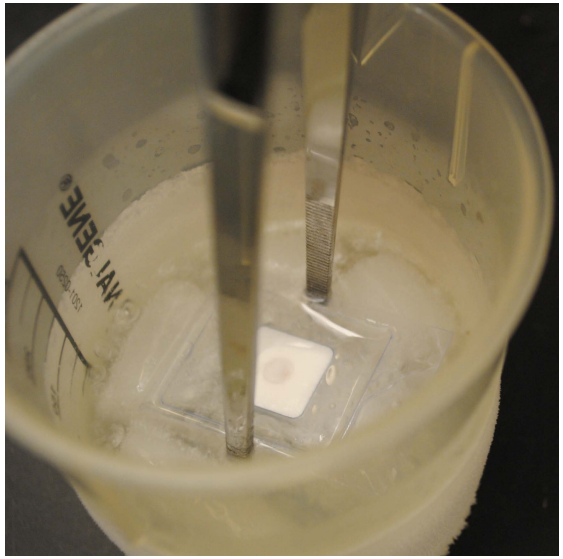
NFR = brownish red

Tissue embedding

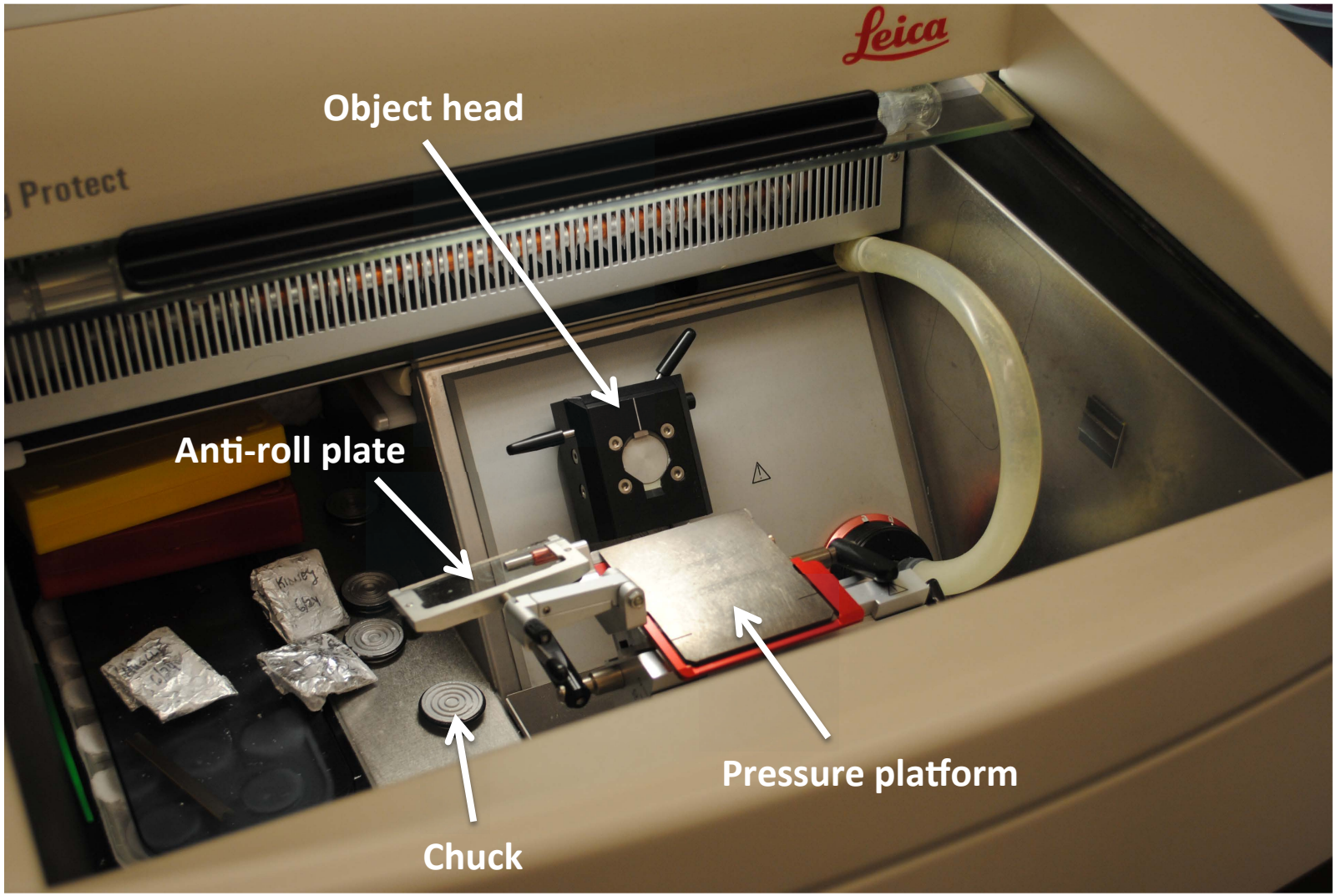
- Snap freeze tissue pieces immediately in liquid nitrogen or dry ice-isopentane (store at –80ºC)
- Embed in a small cryomold with NEG50 (like OCT but less flaky at low temps)—make sure that the tissue piece is completely covered
- Work quickly and accept some thawing on the exterior of the tissue piece (do not sample on the edge)
- Blocks can be stored long term, but sectioned ONCE

Embedding workflow

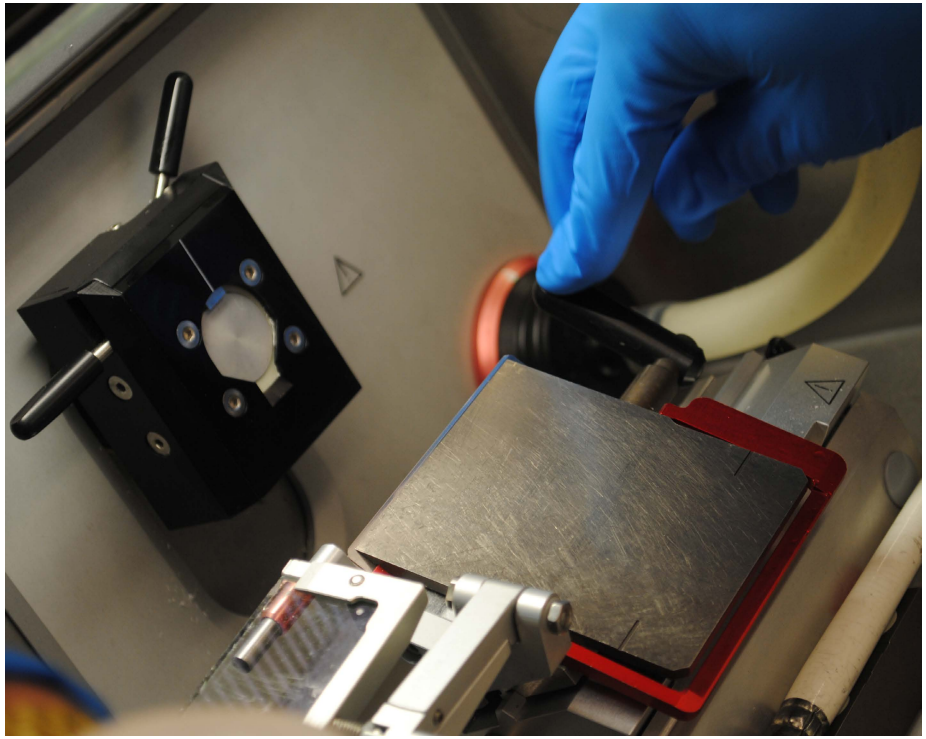



Cryostat nomenclature

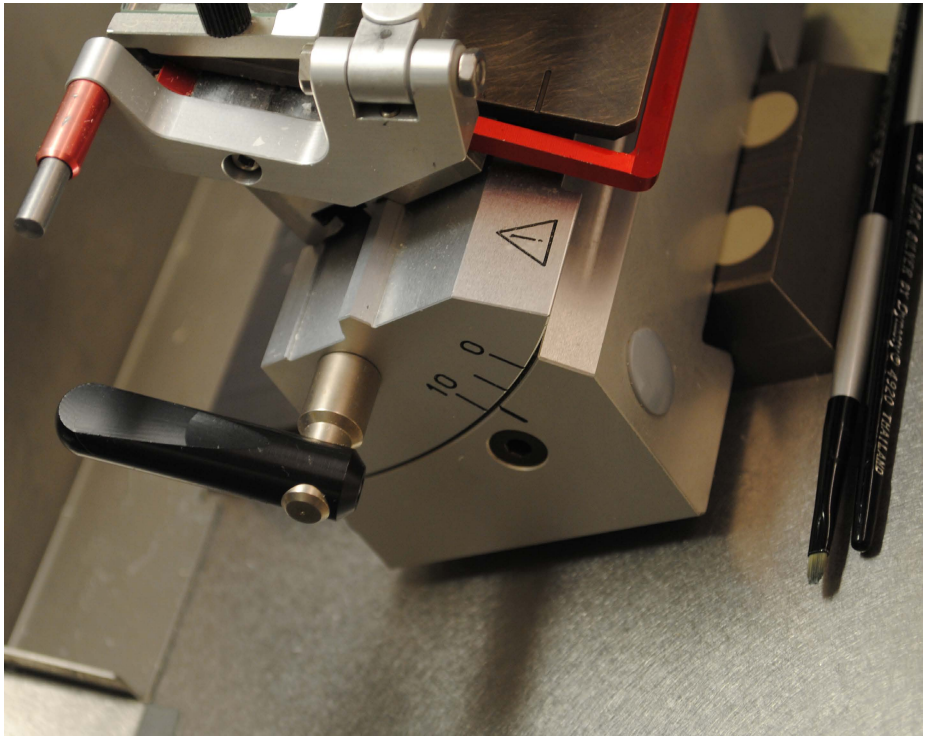


Before starting

- Equilibrate at a cold temperature (< –20ºC)

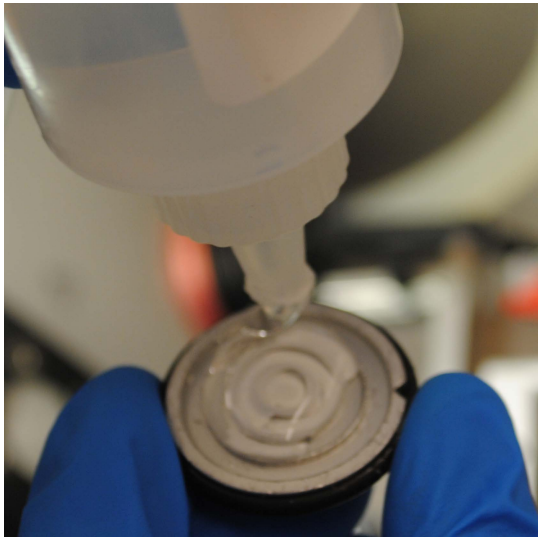
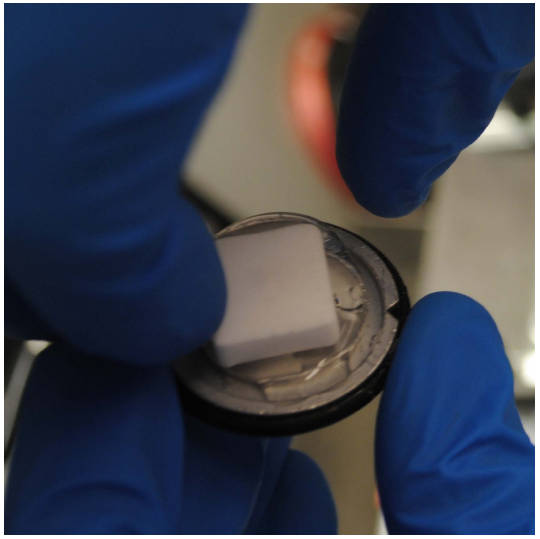


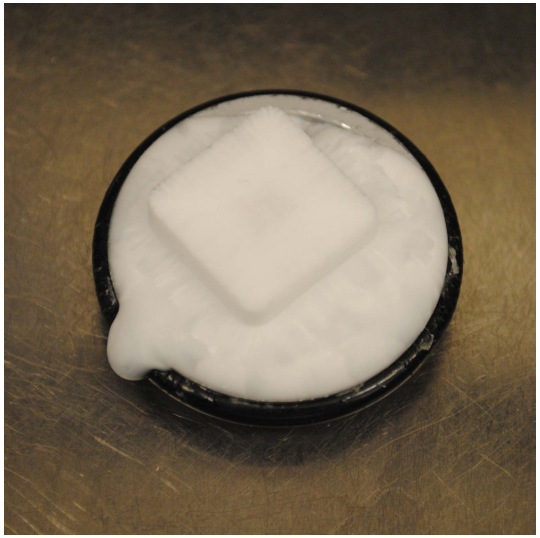
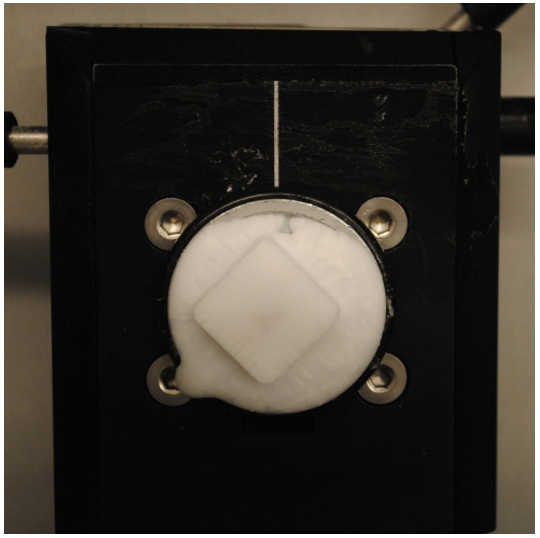
Fresh blade



Moderate platform angle and move platform to object head

Setting up the cryoblock

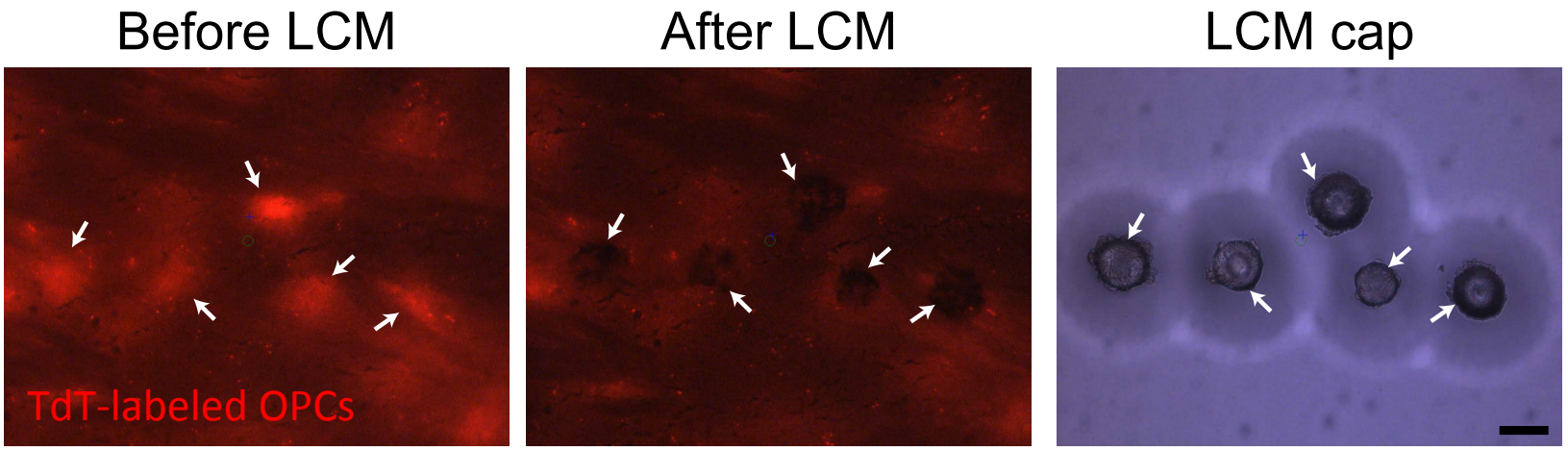




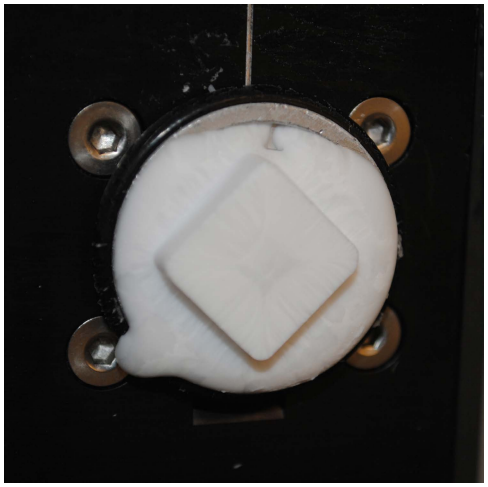
Diagonal (to reduce friction)

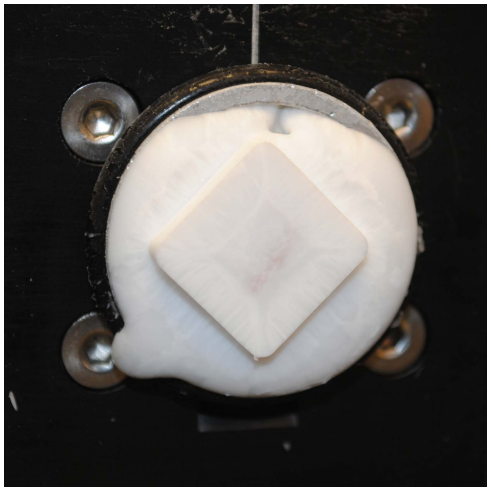
Preserving genetically encoded fluorescence

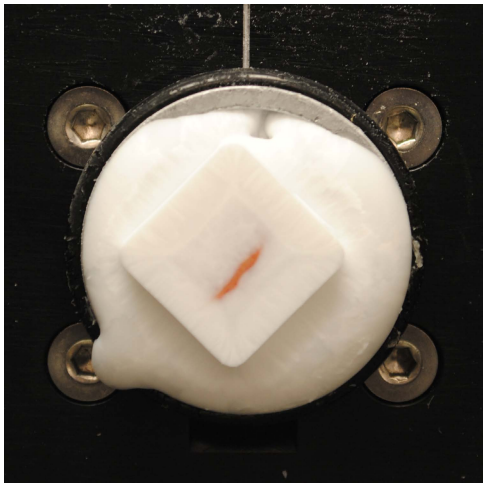
- Proteins diffuse much more rapidly than RNAs upon sectioning
- Soak snap-frozen tissue in 100% EtOH at –80ºC for 24 hrs
- Transfer to a fresh conical tube at –80ºC and vent daily for ~1 week
- Embed and section as for any other tissue

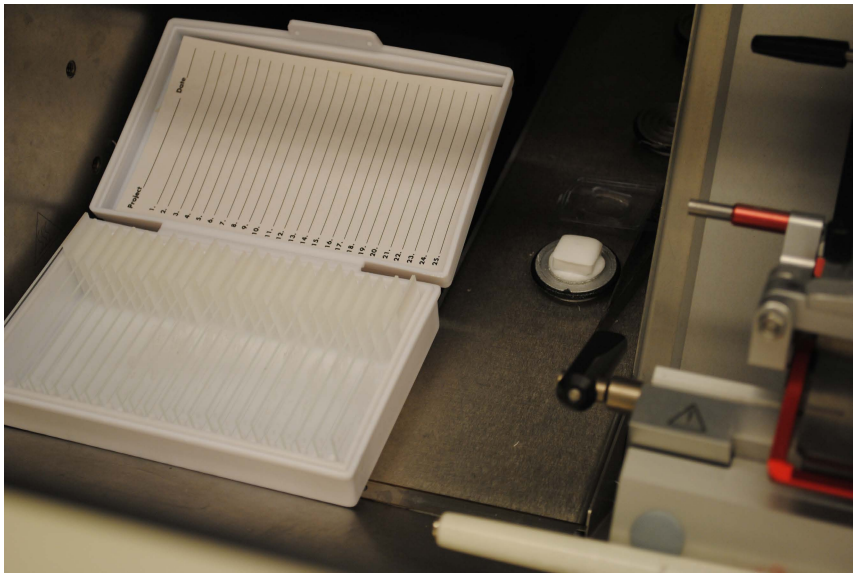


Trim, trim, trim… section









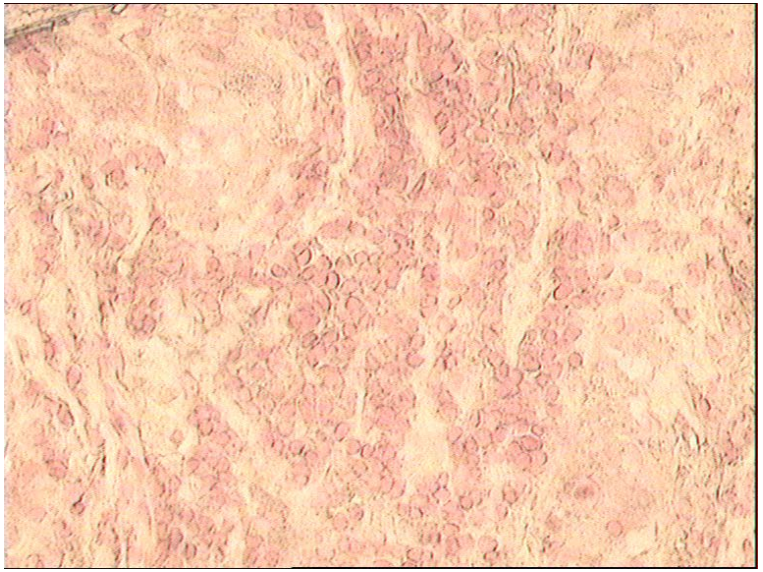
Refreeze sections quickly inside the cryostat box! (Precool slides if needed.)

Try untreated slides and “Plus” slides depending on the application

Cryosection storage and staining

- Transport slides to –80ºC on dry ice
- EtOH fix slides at the –80ºC (no thawing)
- Histologic staining (nuclear fast red, hematoxylin, toluidine blue) < 2 min aqueous!
- EtOH dehydration and xylene clearing

Human melanoma



Questions?