SUN LAB - SECTIONING TISSUE ON LECIA CM 1860

PROCEDURE:

1. On day of cutting tissue, turn cryostat to -20˚C (once all sectioning complete, turn up to -15˚C).
2. Make sure there are 1-2 chucks in the machine (chilling).
3. Change blade and glass insert (if not currently chilling).
4. Go back to lab and gather supplies/sample.

Materials:

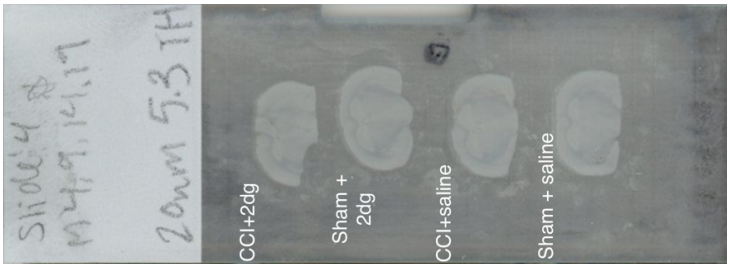
- Uncharged glass slide
- Blade
- OCT
- Pencil
- Slide holder (preferably a smaller one)
- Samples on dry ice
- Paintbrushes
- Tweezers
- Optional: Headphones

5. Ensure all slides are always kept in the cryostat, cold (label them accordingly prior).
6. Adjust thickness to 10 µm, turn on the light, and check temperature is set to -20˚C.
7. Mount tissue on chuck using OCT.

IMPORTANT:

Only put a dot of OCT on the chuck and adhere tissue ensuring no OCT is touching the tissue that will be cut on the slide, OCT is bad for the MALDI. If you know you will be cutting the entire tissue, you can alternatively mount with water (see below for more information).

8. Wait ~2-3 minutes for OCT to solidify by placing the chuck upright in the cryostat.
9. Tighten tissue into mount and adjust the mount so the tissue is at a ~120˚ angle from the blade.
10. Use buttons on the right-hand side that look like arrows to adjust the mount, so the tissue is near the blade. At this time, you may need to adjust the stage's position closer to the tissue or left-to-right so there is a sharp spot of the blade and non-chipped glass mount is available for a clean cut of the tissue.
11. Once the tissue is close to the blade but not yet touching, use the arm on the right of the cryostat to move mount 10 µm at a time closer towards the stage.
12. As you begin to have tissue shave away, test the depth of glass insert and adjust the spring at base of the glass insert if tissue is not entirely beneath glass after rotating arm.
13. Once tissue is cutting flat underneath the glass, lift the insert and use paintbrushes to move tissue away from blade.
14. Take your time and use the paintbrushes to completely flatten the tissue in a spot that won't compromise the integrity of tissue when on the slide.
15. Grab a cold slide and label-side-down, delicately touch your flattened tissue.
16. Try to cut 2-3 replicate slides per tissue, as extras, in case your experiment fails.
17. To prevent wasting machine time and resources down the line, try to cut 2-3 groups on one slide.
    For example, see below:



18. Once all groups have been cut on a slide, this slide can now be placed in slide holder (chilling).
19. After completion of tissue set for the day, follow clean up procedures – remove blade and dispose properly, bring cryostat back up to -15˚C, clean inside of machine to rid of tissue waste and OCT shavings, remove and replace glass insert, lock the arm into place, clean the chucks with 70% EtOH, turn off the light.

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TROUBLESHOOTING:

- Tissue has “ruffles” – Temperature might be too cold, turn up the temperature of cryostat 1 or 2 degrees at a time.
- Tissue has streaks – Glass insert may have a chip, adjust stage left to right to cut tissue in a clean spot.
- Tissue has bubbles – Try to smooth out tissue or lay glass slide completely flat on stage.
- Tissue folds on itself – Use paintbrushes to unroll the tissue before placing slide down.
- Tissue cracks when it strikes the blade – if the tissue has been drop fixed in LN, instead try slow freezing methods during the next tissue collection.

ALTERATIVE – MOUNTING WITH WATER (NO OCT)

PROCEDURE:

1. Turn cryostat to -20˚C.
2. Set the chuck inside of the cryostat on the chiller/tissue holder.
3. At least 1 pair of Fine Tipped Forceps inside the cryostat (make cold enough).
4. Hold a dropper with Deionized (DI) Water on your Left Hand and with your Right Hand you hold the tissue with the Fine Tipped Forceps (or vice versa).
5. Add a small amount of water (about 0.5 mL) or a few drops on the chuck and as the water begins to solidify, set the tissue sample over that drop of water that is starting to become ice.
6. Once the skin is in place on the water/ice interface, you keep adding water and building a small and round ice block over it (not advised to cover entire tissue).
7. Afterwards, let it rest for ~30 seconds on the chiller/tissue holder to fully firm ice block, then start sectioning/cutting.