Microtome (Leica SM2010R)

Usage Directions:

1) Take off microtome cover
2) Make sure ice tray is laid flat (red lines showing on setscrews)
3) Retrieve dry ice, crush it, and add crushed dry ice around ice tray
4) Wait until ice tray becomes cold, then add sucrose in middle to make a square base (let it harden/freeze)
5) Pull knife guard back to expose blade
6) Unlock knife sledge
7) Set adjusting knob to desired increment
8) Pull knife sledge back and forth while clicking the feed lever with each full turn (or set onto automatic) to make sucrose base's top flat; push sledge back when finished
9) After cutting brain in desired size, place it onto filter paper and then together onto sucrose base in desired orientation
10) Add some more sucrose using a pipette onto specimen's sides to glue/stabilize it
11) Add more dry ice on specimen and wait until it's frozen to a pink/white color
12) Move ice tray height so specimen's just below blade, then cut a few top slices (by moving the knife sledge and clicking the feed lever) until tissue area's big enough for sampling
13) Retrieve each slice after each cut by using a brush to sweep it from the blade
14) Place each slice into successive wells in a 24-well tray (Go left to right, top to bottom)
15) After all 24 wells are filled, continue to first well (top left well) and place second slices successively into each well, then third slices, etc. until all tissue is cut and placed in wells
16) Cut until filter paper is taken out (don't put into wells) and sucrose base is flat again
17) Push back sledge and roll down ice tray to fix another specimen for cutting
18) When finished, follow clean up instructions

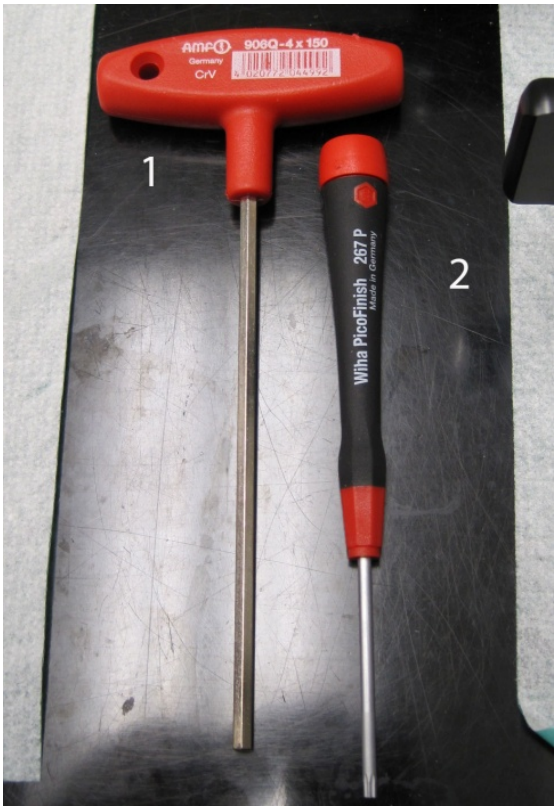
*Add dry ice to sides of ice tray when needed to keep tissue frozen/fixed

Clean up:

-Lower ice tray to bottom using coarse feed wheel & lock it (red on middle/top sign so moving coarse feed wheel will not change ice tray's height)

-Move sledge back & lock knife sledge
-[optional] Remove blade (unscrew with screw driver first), dispose in receptacle or put back into blade holder after cleaning
-Remove dry ice tray (use Allen Key to unscrew below first), including the leftover specimen on it
-Push all debris to waste tray
-Empty, clean and dry waste tray, ice tray, knife holder, & microtome
-Cover microtome
*DO NOT use xylene or acetone to clean instrument, will damage surfaces
*MAKE SURE no liquids enter interior of instrument
*To clean surfaces, use mild cleaner/soap water
*Instrument must be dry before using again
*Add oil to object cylinder or knife holder when needed

Protocol and instructions of Xiangmin Xu's lab at UC Irvine



1: allen key- unscrews screw to remove or fasten ice tray

2: screw driver- loosens and fastens screws for moving the blade

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3: Adjusting knob- set section thickness (up to 60 μm)

4: Coarse feed wheel- changes ice tray/stage height

If red indicator set on clockwise sign: turning clockwise=up, c-clockwise=down

If set on c-clockwise sign: turning clockwise=down, c-clockwise=up

If set on middle sign: moving wheel doesn't change height

5: Manual feed lever- click sideways (either way) to change ice tray height by set increments

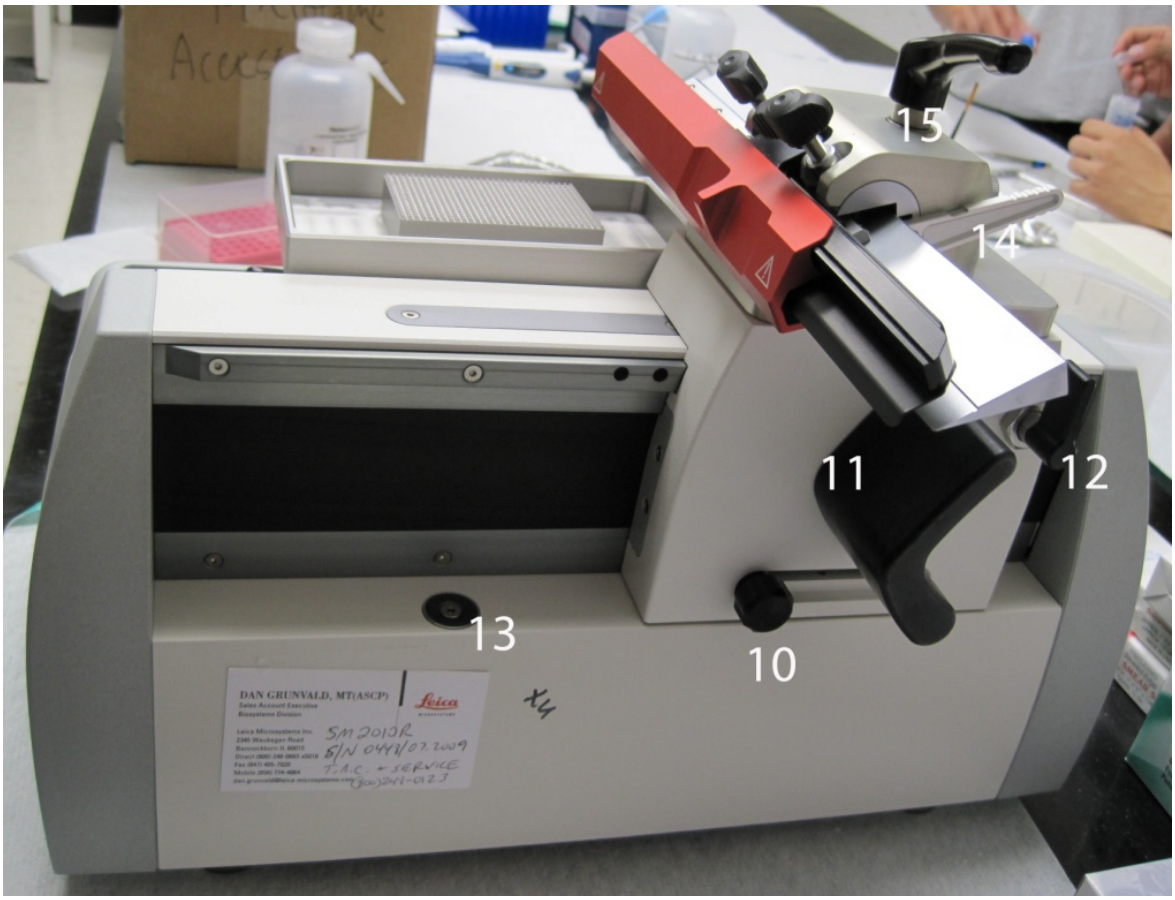
6: Setscrews- orient angle of ice tray; red lines showing indicate 0°

7: Specimen orientation lever- loosen (turn c-clockwise) to move setscrews, fasten (turn clockwise) to set in place

8: Dry ice tray- fill with dry ice on sides to freeze tissue; specimen placed in middle

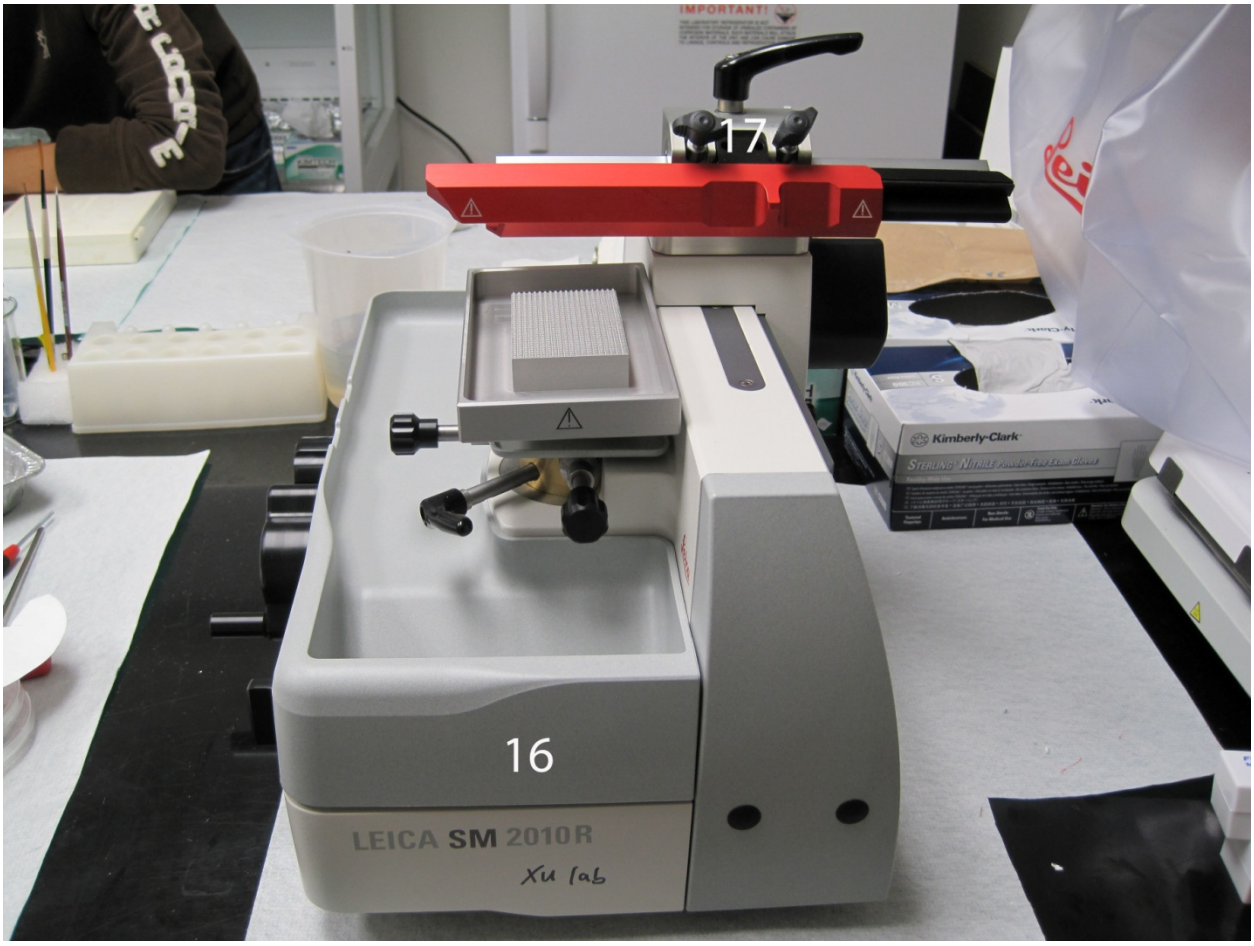
9: Knife guard- guards blade (sliding sideways to left covers the blade); blade should be covered when not using blade but doing other things around microtome to prevent any accidents

Protocol and instructions of Xiangmin Xu's lab at UC Irvine



10: Automatic feed lever- Only effective up to 30 um thick sections; to lock sledge at desired position for automatic height change—unscrew, push back, and rescrew lever at desired position
11: Knife sledge & grip- move knife sledge back & forth against ice tray's specimen for blade to cut tissue slices
12: Knife sledge lock- push back: sledge free to move; at middle/top: sledge locked/no movement
13: Magnetic sledge immobilizer- immobilizes sledge when on contact
14: Clearance angle scale- adjusts angle the blade cuts at
15: Clamping lever- loosen (c-clockwise) and fasten (clockwise) to move knife holder orientation

Protocol and instructions of Xiangmin Xu's lab at UC Irvine



16: Removable waste tray- pull up & out to remove tray for easy cleaning

17: Knife holder- clamps blade; loosen & fasten both knobs to remove blade, move blade sideways, or set blade in place