**LCM Protocol for Collecting Cells**

**Turning System on**

1. Turn ON LCM microscrope
2. Make sure that the control box is ON (Check the key)
3. Turn on Computer
4. Log on to your user (make sure that is not a temp profile)
5. Turn ON / Log in to PALM System
6. Turn ON / Log in to PALM Error Recording

**Setting up for collection**

1. Put microscope side on Slide 2 spot
2. Select section of intrest
3. Add 10uL of EtOH or Xylene to section (NOTE: Xylene will dry out section over a few uses which causes tissue to curl and fall off tissue. The more fat in the tissue the faster this will happen.)
4. Scan Section 10X and save in the Project/Tumor folder. (Mark as Before somewhere in the name, so you know this was before collection.)
5. Scan new collector type
6. Check centering of the caps (NOTE: If using the 8-strip collector, make sure to check the centering using the middle position. For example if you are going to collect into 3 strips, check the center on strip 2D or 2E. I suggest not collecting in more than 3 strips at a time.)
7. Test that the laser is cutting on 10x, 40x and 63x. (NOTE: You should not need to change the settings.)

**Collecting Single Cells (x63)**

1. Find cells of interest. (NOTE: Cells of interest should be at least 1.5 microns from any other cell. Tumor cells will be about 2.5 times larger than red blood cells about 2 time larger than lymphocytes.)
2. Use free hand or circle to draw around cell. (NOTE: I prefer to set 2-3 cycles to cut around the cells, and use the setting for center LPC. For single cells do not set catapult above 25. Preferred setting is 15-20.)
3. In element list: (1) Label cell, (2) change from manual to location of 8-strip caps you want to catapult into (e.g. 1A, 1B, ect.). Therefore, collect only the number of cells you have in a strip. If you want to mark more than you need to only select 1 at a time when collecting.
4. Take a picture of the cell before you collect it with the element marker. Save the image in the folder for that project/tumor and with a unique name. This picture is usually at 63x.
5. Collect cells. Mark down notes of how many time you catapulted and if the capture was clean (Meaning did the section all go up as one piece.) NOTE: It is good to have some pictures of the cells right after capture but it is not required.

**Images and Element Files**

1. After cells have been collected, move to the 10x magnification. You will need to adjust where the elements are so that the collection spots and the elements line up.
2. After lining up the collection spots and elements, take pictures at the 10x magnification so that we have all the cells locations recorded.
3. Save the elements. Make sure to save them in the project/tumor folder and have a unique name.
4. Export the text file of these element to the same location, you will need to alter the name slightly.
5. Rescan rescan Section 10X and save in the Project/Tumor folder. (Mark as after somewhere in the name, so you know this was after collection.)

**Turning System off**

1. IF there are no errors turn off PALM Error Recording without saving. IF there is an error, save the PALM Error Recording with the date and issue.
2. Turn off PALM interface
3. Log out and shut down the computer.
4. Turn off LCM microscrope
5. Cover the microscope.