**TILL**

**mple preparation:**

For 15 ng/ul, use 1-2 ul for 50-80 ul of HEPES-DTT, 0.7 ul yoyo.

**Turning on:**

1. Turn on order:

Machine generated alternative text:


 Turn on computer, Screen.

\*\*\* make sure there is no glass in the TILL before opening the software

1. Open the software, press continue.

**Focusing the sample:**

1. We use oil 100 objective.
2. Right click on the screen to choose zoom resolution.
3. **Filters**: There are 2 sets of filters- penta & quad. Normally we will use the Penta.
4. After choosing the penta, choose the emission (for yoyo).
5. Go to "BIN" and set EM gain to 300 (detection limit).
6. In the left bottom corner of the screen, a small arrow opens the excitation filters- choose the correct one and set it to 100 (normally we will choose the yoyo) .
7. Set exposure time to 100-200 (for yoyo).
8. To begin the scan, press the video camera icon.
9. Right side up- auto- press it to adjust automatic brightness.
10. Start with the max zoom (10 uM)- once you get closer the screen will become brighter, then choose a more gentle zoom.

**Using an automate protocol:**

\*\* it is advisable to view the sample as near as possible to the loading position.

1. Open your protocol (Amit Multi channel) : Acquire -> Protocol editor -> file load
2. After focusing:
   1. Open the grid- mark the area you would like to photo (each square is a shot), click "delete selection" to remove last selection (make sure that the "move to selection" in unchecked).
   2. Press "tile" in the protocol, and then on the record button.
   3. Save the images in a dedicated folder, press save.
   4. Once the pop-up of "please refocus the sample" appears, click quickly "save", to avoid the sample from getting out of focus.
3. The TILL will now commence the shooting, you can choose to view both channels (using the icon).
4. The protocol window shows the estimated amount of time left for the experiment.

**Manual shooting:**

\*\* it is advisable to view the sample as near as possible to the loading position.

1. Open your protocol(Zirkin Multi channel) : Acquire -> Protocol editor -> file load
2. After focusing:
   1. Click on the record button
   2. Save the images in a dedicated folder, press save.
   3. The software will take a photo, then, by using the grid move to a new location, again click record (ctrl+R) then "save" .