24-3-23 AutoCyPlex Full Auto Test

Cycles (3x)

1. Cycle 1
   1. Villin-488 1:100
   2. PDGF-555 1:100
   3. Gamma-actin-647 1:100
2. Cycle 2
   1. PCNA—488 1:100
   2. ECAD-555 1:50
   3. Glut2 -647 1:100
3. Cycle 3
   1. Ezrin-488 1:100
   2. Chromogranin A-594 1:100
   3. Na/K ATPase-647 1:100

Notes

* Tiling is snake style that starts in upper left corner and goes right, down, left down, etc. and is always rectilinear
* All antibodies are used in 1:100 [C] and are prepared with 350uL
* mCPBA is used in [C] of 5mM for 5 minutes. Prepared from 1M stock in ethanol made fresh on 12-15-22
* All antibodies diluted in blocking buffer that is odyssey blocking with 0.1% triton x-100

Steps to Take

1. Mount to slide and install pressure plate.
2. Mount to tilt stage
3. Tighten coupler set screws
4. Flow fluid until certain it is good and flowing well and not leaking
5. Find tissue
6. Adjust to kinda kohler excitation
7. Tweak tilt to get pretty flat
8. Define MM surface
9. Tweak code to make sense with flow
10. Run

Notes

* No bleach effect with m-CPBA with new stock or old stock.
* Did by hand with m-CPBA and nothing. Did H2O2 by hand and bleached
* Testing m-CPBA with alexa-488 secondary dye to see if chemical is expired.
* Dye with m-CPBA had almost no effect. M-CPBA is non reactive now. Need fresh