**Automate ImageJ Protocol for BgIG-Halo Xist Clouds**

**Original Protocol**

Step 1: create modulation contrast map

* Run macro 16ThrMCNR\_XIST.imj

Step 2: create image with background signal removed

* Run macro ModConFilter11.imj with sigma = 0.8, threshold = 4
* Check intensity of resulting image 🡪 want max intensity ~100 NOT 60,000 (may need to change script from 255 to 65355)
* Split resulting image into 3 separate channels
* Duplicate C2/3 channels
* Manually threshold C2,3 MCF-1 images
* Divide C2,3 MCF-1 by 255
* Create Results of CX by multiplying CX-MCF by CX-MCF-1
* Merge C1-MCF, Results of C2 and Results of C3

Step 3: Align Channels using Chromogen

* Find alignment parameters: compare FUS\_SIR.dv with itself (suffix=\_ALN)
* Change wavelength to 0/1/2 in notepad of resulting Chromogen file
* Align all images taken on same day: Reference = Chromogen file (Edu); Target Files = FUS\_THR\_MCF (data) (suffix=\_ALN\_full)

Step 4: Crop Xist Clouds

* Assign channels: C1= C3 old, C2= C2 old, delete old C1
* Crop image so rectangle boarders signal and only contains stacks with signal present

**Aims of Automation**

Automation of Step 1:

* reduce time spent pressing ok while macro is running

Automation of Step 2:

* make ModConFilter11 automatically: set sigma = 0.8, threshold = 4; determine whether to divide by 255 or 65355; spilt channels and duplicate C2 and C3
* make new macro to automate: dividing thresholded MCF-1 by 255; multiplying MCF by MCF-1; merging C1-MCF, Result of C2, Result of C3

Automation of Step 4:

* make new macro to automate channel reassignment

**Automated Protocol**

* Step 1: run 16ThrMCNR\_Xist\_Step\_1.imj (change Bio-Formats Configuration: Deltavision and BD\_Pathways should be windowless)
* Step 2a: run Updated\_ModConFilter11.imj
* Step 2b: manually threshold C2/3-MCF-1 files (make sure you have all 3 channel images open)
* Step 2c: run Background\_Removal.imj
* Step 3: align channels using Chromogen
* Step 4a: run Channel\_Reassignment.imj
* Step 4b: manually crop Xist Clouds