Calcium imaging protocol (for cells)

1. Wash cells 3 times with 2ml of medium without serum
2. Medium without serum supplemented with 0.2%BSA and filter sterilized
3. Eqvoldye+pluronic (final conc of dye=2.5uM) mixed together and added to the above medium. This mix is added to the cells.
4. Cover the plate with al foil and keep at 16C incubator for 30 mins
5. Wash the plate with HBSS 3 times and incubate for 20 mins in HBSS+Ca+Mg at RT
6. Pre-clean tubing and apparatus with water and then run HBSS+Ca+MG thru tubing and ring prior to mounting (to prevent osmotic shock).
7. Prepare 20uM ionomycinsoln in HBSS+Ca+Mg
8. Mount the coverslip in 1ml of HBSS+Ca+Mg
9. Tape the tubing to the mic setup to minimize movement while pushing the plunger
10. Start imaging and add 1ml of the ionomycinsoln after the second image has been captured