Whole mounting adipose tissue

Adapted from Peirce-Cottler lab, Scott Seaman protocol

Day 1:

* Harvest tissue
* Aliquot small tissue samples (about the size of a hole punch) into 4% PFA for \_\_hrs
* Wash samples 3x with PBS for 5min/wash (on rotator)
* Block and permeabilize by submerging tissue in 1% BSA, 0.3% (v/v) Triton X-100 in PBS for 3h at RT
  + Make up ~10mLs PBS-serum-Triton
* Aspirate blocking/permeabilizing solution
* Add 100uL of BODIPY at 10ug/mL diluted in 5% mouse serum, 0.3% (v/v) Triton X-100 in PBS. Incubate for 20min at 37C in the dark
* Wash 3x with PBS-T
* Add antibodies diluted in 1% BSA, 0.3% (v/v) Triton X-100 in PBS. Incubate 2hrs at RT or O/N at 4C protected from the light.
  + \_\_\_\_ samples \* 1.2 \* 100uL = \_\_\_\_\_ (total V)
  + Ab #1: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
    - total V / \_\_\_\_\_ (dilution factor) = \_\_\_\_\_ (Ab #1 V)
  + Ab #2: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
    - total V / \_\_\_\_\_ (dilution factor) = \_\_\_\_\_ (Ab #2 V)
  + Ab #3: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
    - total V / \_\_\_\_\_ (dilution factor) = \_\_\_\_\_ (Ab #3 V)
  + Ab #4: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
    - total V / \_\_\_\_\_ (dilution factor) = \_\_\_\_\_ (Ab #4 V)

Day 2:

* Wash samples 3x with 0.3% (v/v) Triton X-100 in PBS
* Mount on gelatin coated slides with 50:50 PBS/glycerol solution and seal coverslips with nail polish
* Store at -20C protected from light, but image same day (to avoid fluorophores fading!)

*Notes:*

* *Should image samples within 1-2 days to prevent from fluorophore/stain fade*
* *Avoid have too thick of adipose tissue. If tissue is too thick, mounting with 50:50 PBS/glycerol will be difficult and will leak. Will take some optimization as to how thick the piece of adipose tissue can be.*