Tissue Integrity Testing 11-11-24

**Goal:**

To run many cycles (40) to assess as how much tissue loss there is. We will also use some highly effective stains to demonstrate that the antigens are still available and functional after 40 cycles.

**Marker:**

DAPI will be used as proxy for the tissue and loss of DAPI regions will indicate if tissue lift off has occurred.

**Protocol:**

1. Make fresh mCPBA bleach stock and dilute to 10uM
2. Do one normal cycle with SMA and MUC2 at 25% normal [C], so 1:4k and 1:2k respectively.
3. Do 38 cycles of ‘blanks’. A blank is just 1:25k DAPI in PBS instead of 1:25k DAPI in antibody dilution buffer with antibody cocktail.
4. All steps are normal timings with 10 minute bleach and HDR exposure expect for DAPI which is always predictated. Even taking A555 channel which is nothing
5. Final SMA and MUC2 cycle

**Analysis:**

1. Use cellpose to segment DAPI in images and track cell counts over time
2. Use cycle 40 and cycle 0 DAPI image composites to visually display similarities