Things we tried with the hydrogels

1. Varying chemistry
   1. PEG-ene with dithiol crosslinker – pores were too small and we were working right at the edge of the gelling range
   2. acrylamide with bis crosslinker
2. Varying composition of crosslinker, etc, within one system
3. Varying photoinitiator (LAP/light vs APS/chemical)
4. Porogens (alginate nanospheres and dextran)
   1. Alginate (couldn’t make nanospheres reliably)
   2. Dextran/dextranase (dextranase did chew up the dextran but it still didn’t escape the gel well enough) (dextranase also chewed up FSFG)
5. Writing thin gels with confocal microscope
   1. 50 um minimum line thickness – good
   2. Wrote thin lines and donuts
   3. Tried using photoinhibitors to reduce stray crosslinking, but didn’t work well enough
   4. Swelling/buckling was a problem
6. Constructs
   1. SSSG as a negative control for FSFG gels (this actually works)
   2. Covalently-tethered NTF2 (didn’t work)
   3. FSFG cct2 (works) and cct3 (doesn’t express)
   4. Kap121 with GFP-NLS (only tested once, didn’t enter gel)
7. Fixed free dye problem
   1. Rigorous labeling protocol and testing, keep frozen until use
   2. Tried a few different labeling chemistries (maleimide, NHS and SDP esters)
   3. Made GFP-NTF2 (didn’t bind to FSFG)
8. Trying to incorporate more FSFG using different reaction groups
   1. Longer/shorter PEGDA
   2. Multi-armed PEGDA
   3. Acrylate-PEG-maleimide
9. Various chamber compositions
   1. Sticky tape chambers
   2. NOA chambers with or without port tubing
   3. Silanated PDMS chambers
   4. PDMS gasket chambers
   5. Membrane as an additional scaffold for gel
   6. VALAP-sealed 6um-spacer chambers for thin FRAP
10. Various chamber geometries
    1. X-chambers
    2. Small-outlet chambers
    3. Ring chambers (with Katie’s molds or confocal)
    4. Drop gels (‘semi-infinite slabs’)
    5. ~~Taylor’s attempts at large-volume wells (not my work, didn’t turn out anyway)~~
11. Other small things
    1. Using masks vs. crosslinking everything
    2. Troubleshooting the “edge dip” from masks
    3. Attempts at experiment-theory comparison with Mike’s code
    4. Attempt to calculate pore size
    5. Effect of presoaking (haven’t tested with a reliable system)
    6. Making molds with the craft cutter
12. FCS attempts – never were able to get a good signal or fit. I don’t have any of this data.
13. Varying crosslinker
    1. DNA (wouldn’t gel)
    2. Coiled-coil proteins (wouldn’t express)
    3. 1kD vs 8kD dithiol linkers for PEG-ene gels (8kD makes gels with larger pores, more swelling, less mechanical stability)