Lab exercise (exercise number from 2016 lab manual, note that mitosis lab and inhibitor lab at end were not done in 2016)

cells needed BASED ON 8 GROUPS

**Lab 1**

learn about scope; phase & fl 10 Cvs fixed LLCPK1; phalloiden stained

**Lab 2**

image formation

Kohler illum

Phase constrast use same slides as lab #1

**Lab 3**

Numerical aperture

Oil immersion 10 Cvs fixed LLCPK1; phalloiden, DAPI, microtubules

**Lab 4**

Resolution same slides as lab 3; also fluorescent beads

Camera binning

**Lab 5**

Photobleaching **10 fresh slides**

LLCPk1 fixed stained for microtubules, actin, DAPI

For this lab, need medium to dense plating with bright staining.

**Lab 6**- no cells; discuss software, superresolution, etc

**Lab 7**

Immunofluorescence 1. 30 Cvs fixed, unstained LLCPk1 cells; 10 3T3, breakdown:

(Tubulin staining: 10 to stain with 1o,2 abs; 10 for 20 only)

(actin staining: 10 LLCpk and 10 3T3)

2. 10 Cvs live LLCPk1 cells (to fix and stain)

**Lab 8**

**Identification of cellular organelles**

**Need fixed and pre-stained slides; 3 of each structure (see below)**

**Pre stained cells:**

Antigen fixation method Primary dilution Primary host

Gamma tubulin MeOH or Para/glut 1:100 mouse

Alpha actinin formaldehyde only 1:200 mouse (**IgM)**

LAP2 MeOH 1:100 Mouse

Golgi 58K Formaldehyde only 1:100 Mouse

Hec1 MeOH 1:200 Mouse

Have used ER ab from Hebert lab in past; have also used ZO1, but it is no longer working.

**Lab 9**

Imaging living cells

1 dish LLCPk cells expressing GFP-tubulin per group

2 dishes of live LLCPk to stain with mitotracker or ceremide

LLCPk1 cells previously transfected with plasmids expressing GFP-tagged unknowns. 1 dish per group, two unknowns (or cells from Pat’s lab)

**Lab 10**

Imaging cells to organisms

Need to have or obtain:

Bacteria expressing a GFP fluorescent tag

Protists from Caroline (or similar), good results with Hydra, volvox, amoeba. Obtain zebrafish from Karlstrom or Downes lab; brain slices might still be OK; we don’t have moss unless a lab other than Bezanilla uses it.

**Lab 11**

Cell Motility (NOTE; other years this has been a three week lab; in 2018 it will be two lab periods and the use of an inhibitor is moved to the end)

Part: one control cells task one: 10 dishes live 3T3; 10 dishes live LLCPk1

Task two: 10 dishes melanoma

Task three: 10 dishes live LLCPk1 expressing GFP-actin

Future: fish epith cells from scales

Part two use of inhibitors; not doing this in 2018

*Part three: cell motility, experiments designed by students; need to query students for type of cells and number needed.*

**Lab 12**

Endocytosis: Part I 3 coverslips of live 3T3 cells per group. Few extra just in case.

Endocytosis: Part II fixed cells: 2 coverslips per group of live 3T3 fibroblasts

Live cells: 3 coverslips LLCPk 1 per group; one expressing GFP-tubulin, two control

**Lab 13**

Signaling: Part I 3T3 fibroblasts, live, in mattek dishes, 3 per group and extras. Dense plating is best for this.

Signaling: Part II 3T3 fibroblasts, live, 3 mattek dishes per group and extras. Dense plating is best for this.

**Lab 14:** Mitosis and Cytokinesis

1 dish LLCPk cells expressing GFP-tubulin per group

2 dish LLCPK cells with myosin and tubulin per group

**Lab 15:** using an inhibitor

live LLCPK1 parental cells, on coverslips for treatment and fixation; 8 coverslips

Live llcpk1 cells expressing GFP tubulin (or tubulin and myosin) in dishes to live observation with inhibitors added. 8 dishes.