

## Perfusion Set-up

### Materials

- \*bottle of refrigerated AMES
- \*cap w/ 4 white holes from oven
- \*bubbler from oven (puffy white end)
- \*tubing
- \*inflow/outflow plastic containers
- \*white "L" tube

### Procedure

- 1) fill large water bath  $\frac{1}{2}$  way w/ tap water
- 2) Switch caps on AMES bottle (if dissection, pour AMES into small bottle)
- 3) insert bubbler w/ tube attached
- 4) insert "L" tube (white)
- 5) attach inflow/outflow tubes
  - ↳ don't forget to tighten screws!
- 6) connect AMES bottle to tubing above water bath
  - ↳ gas connect to bubbler tube
  - ↳ inflow place in AMES (fig A has 2 inflow, use the one coming out of the metal bar)
  - ↳ outflow drip in water bath, then rest above AMES
- 7) turn on water bath (button @ back) in bottle.
- 8) set  $37.0^{\circ}\text{C}$
- 9) turn on gas (20 psi... 800 psi)
  - ↳ or 1500?
  - ↳ turn on backup vac (next to incubator) → Make sure waste bin (below inflow/outflow flask is connected)
- 10) Make sure AMES is bubbling
- 11) clean perfusion dish w/ kim wipe
- 12) turn on perfusion mechanism (button @ back of inflow/outflow tubes)
- 13) Refer to computer set-up

## Electrode Set-up

### Materials

- \*electrode
- \*pipette
- \*electrode filler
- \*1 mL syringe
- \*3M NaCl w/ generator

### Procedure

- 1) remove electrode from rig
  - ↳ pull off tubing & unscrew white knob
- 2) sandpaper electrode
- 3) attach red wire to back (NOT top)
- 4) dip electrode in NaCl w/ black electrode
- 5) turn on power & allow 10 bursts
- 6) check for black tip
- 7) reattach electrode into rig (is it swingable?)
  - ↳ reattach tubing
- 8) Fill syringe w/ AMES
- 9) attach electrode filler
- 10) Fill a P52. w/ AMES
- 11) attach to electrode (push all the way to the back)
- 12) is it swingable?
- 13) put 10x @  $z=7000$  (in RigB, max z)
- 14) find pipette & focus in z
  - ↳ electrode  $x=0, y=0, z=0$
  - ↳ move 10x to  $z=3000$ 
    - ↳ adjust pipette focus as necessary
- 15) switch to 60x
- 16) adjust z to 3000 & fix pipette focus as necessary
- 17) set home in (press & hold)
- 18) flip to approach & scroll out in x
- 19) is it swingable?
- 20) set home out
- 21) test home in & out
- 22) move groundwire in until touching AMES (use black knob @ back)
- 23) pipette offset \*in Y-clamp!
- 24) pipette offset 25) IRMS  $\sim 5$  ideal
- 25) turn off IRMS
- 26) run light step to check groundwire
- 27) prop rig for mounting

## Computer/Rig Set-up

### Procedure

- 1) turn on 4 red switches (sometimes have to plug keyboard in to stage)
- 2) turn on stage computer → shows light projections computer
- 3) open
  - a) LinkLab
  - b) MultiClamp
    - ↳ click restart
    - ↳ open folder button
    - ↳ select standard config
  - c) PPS2
  - d) Symphony (rig our projector)  $\text{ndf} \rightarrow 0$ ,  $\text{ndf}$  should be  $1$  in  $10x$ ?
  - e) PPS2
    - ↳ make sure Waste > perfusion
    - ↳ turn on both (flow into water bath first to clean system)
    - ↳ watch AMES flow in & make sure it's picked up by outflow
  - f) turn on LED & adjust
  - g) in objective mode
    - ↳ adjust X & Y by looking @ Middle of dish
    - ↳ in  $10x$ , find bottom of dish using Z
    - ↳ wiggle X & Y, adjust LED as you go
    - ↳ if spots move, good sign (try to find debris)
  - h) switch to  $60x$ , find bottom of dish
    - ↳  $60x$  can touch AMES? ( $10x$  cannot)
  - i) in LinkLab
    - ↳  $x=0, y=0, z=0$
  - j) in Symphony
    - ↳ set alignment cross ( $\text{ndf} \neq 1$  if  $10x$ ,  $0$  if  $60x$ )
    - ↳ length  $\approx 800$
    - ↳ switch to condenser mode
    - ↳ rotate black knob below dish & Z to find contrast line
    - ↳ scroll in Z to focus contrast
  - k) in UniLab
    - ↳ C=0
    - ↳ spin black knob back open all the way to the right
    - l) turn off LED
    - m) in Symphony ( $60x$ !  $\text{ndf}=0$ )
      - ↳ click play for alignment cross
    - n) move silver knobs until find cross
    - o) scroll in Z to sharpen cross → write down what C= at p.
    - p) switch to objective mode
    - q) test light step Rstar  $\approx 200$ ish (darkness standard)
      - ↳ Intensity: 0.3 in high cross is gone
    - r) stop once alignment cross is gone
    - s) switch to  $10x$  and raise it up to max Z (or  $7000 \pm$  in RigA)
    - t) prepare for electrode set up

### Prepare Rig for mounting

- 1) remove glass pipette from electrode
- 2) shut off perfusion
- 3) move inflow & outflow away
- 4) retract groundwire
- 5) vacuum AMES off dish
  - ↳ backup vac yellow  $\text{Y} \rightarrow 0$
- 6) in objective mode\*
- 7) max Z in  $10x$
- 8) shut off all monitors
- 9) pull down black curtains
  - ↳ click on side magnets
  - ↳ except cage curtain
- 10) make sure oxygen is on

### Disssection Set-up

#### Materials

- \* 2 fine forces
- \* 4 napkins
- \* 4 petri dish lids
- \* Superglue
- \* petri dish filter papers (2)
- \* tape
- \* 1 scissors
- \* 1 scalpel (blade + blade holder)

\* mounting dish

\* grease

\* poly-D lysine / coverslips

\* plastic harp (place in extra petri dish)

#### \* 2 coarse forceps

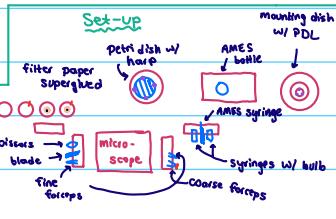
- \* AMES in a small bottle (take from large AMES before setting up perfusion)

- \* glass pipettes w/ bulbs



#### Procedure

- 1) check microscope batteries
- 2) tape 4 paper towels to dissection table
- 3) prepare instruments
- 4) clean mounting dish w/ lens cleaner and Kim wipe (delicately)
- 5) 3 grease dots
- 6) attach poly-D lysine from fridge
  - ↳ use coarse forceps
- 7) 4 grease dots on harp (be cautious)



↳ strings should be as close to tissue as possible

### Making AMES

#### Materials

Ames' Solution 1L - with Ames from US Biologicals  
 945 mL Water → 600 mL ultra pure, milli-Q water  
 1.9G Sodium Bicarbonate (Sigma Cat# S55761)  
 8.8G Ames' Medium (US Biologicals Cat# A1372-25)  
 1% Pen Strep (10 mL / liter)  
 Osmolarity should be bang on without testing (282 – 286 mOsm)  
 CO<sub>2</sub> for 20 minutes 2-3 psi  
 Filter Sterilize

With CO<sub>2</sub> for 20 minutes, pH should be ~7.2

\* 2 mL bottle(s)

\* 9900 plastic tub (measure to black line)  
 ↳ attach pipette bulb

**BEWARE OF CONTAMINATION:** must minimize!!

#### Procedure TAKE Pen strip out of freezer!!

- 1) Fill plastic tub w/ ultrapure water (let out some into sink before filling)
- 2) weigh out Sodium bicarbonate (red lid chemical) and add
  - ↳ if only making 2L, use more precise scale

↳ if only making 2L, do it in a separate AMES 2L bottle, then transfer

- 3) weigh out AMES medium & add
  - ↳ mini fridge door

- 4) add Pen strip (liquid) → must thaw from freezer
  - ↳ whole bottle if making 10L

↳ technique tip: first take 1L of water, add additional materials, THEN add the rest of the water

- 5) mix solution using stir bar for ~10 min
  - ↳ if still more, maybe add stir stick?

↳ can forget the 10 min mixing step as long as the powder is all dissolved.

- 6) turn on gas to 5 psi (might drop to 3)

- 7) attach bubbler to CO<sub>2</sub> in fume hood

- 8) only allow bubbler to touch soln

- 9) bubble for ~20 min

- 10) retrieve filter from below sink

- 11) attach to vacuum (yellow knob) → vacuum turn on all the way

- 12) screw filter onto bottle & fill 2L in each

↳ be sure to filter well!

- 13) Date & label with tape

- 14) refrigerate

## Looking for Amacrine

### Procedure

- 1) open ScanImage in Matlab
  - 2) rotate both knobs to the right (flip mirrors)
  - 3) turn off LED
  - 4) click upper switch safe → enable
  - 5) set to 256 pixels/line
  - 6) set to 1.0 (can zoom more later)
  - 7) Focus
  - 8) Abort
- ↳ change zoom if necessary  
↳ do not enable stack  
↳ do not enable MROI
- 9) Channel 1,2,3 = red, green, blue (Rig-specific)
  - 10) do not use channel 4

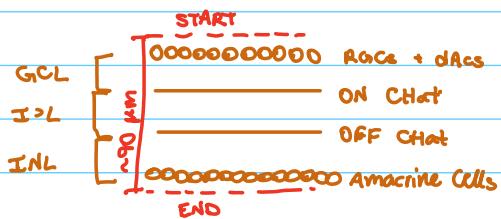
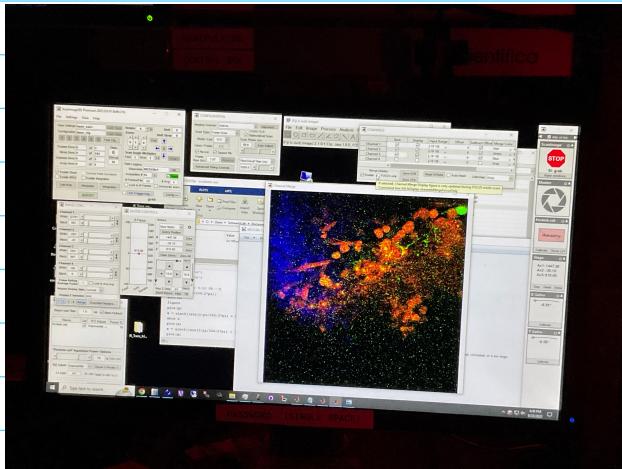
Not  
both  
knobs  
of problem

## Imaging the z-stack \*on channel merge\* Rig B

(after running programs on the cell & removing electrode)

### Procedure under ScanImage

- 1) Data logging Basename date 09/13/2013/4/5 then click DR..  
↳ go to Images Folder  
↳ create new folder w/ date
- 2) View  
↳ Stack controls → bounded
- 3) View  
↳ motor controls  
↳ motors: raw motor
- 4) enable stack
- 5) flip mirrors, turn off LED, remove electrode, etc.
- 6) click focus
- 7) scroll up & down in z on stage
- 8) adjust Image Con in red channel if necessary
- 9) Start low enough so cannot see chat band
- 10) go all the way to other side by scrolling in z.  
↳ check for 2 chat bands  
↳ scroll until red is on top
- 11) Stop focusing
- 12) Step size: 0.5 in stack controls (# of slices should be 143-ish)
- 13) switch config. to S1/2
- 14) click grab
- 15) wait for it to go through
- 17) check in Fiji if it looks good, brightness-wise



### Preparing Interventions

#### Materials

- \* K Asp (frozen)
- \* 488 Aliquoted fluorescent dye (freezer)
- \* 1ml syringe
- \* hypodermic dispensing needle
- \* blue tip thing
- \* electrode filler

### Imaging

- 1) run light step to reset
- 2) In ScanImage:  
↳ view → motor controls → reinit motors → set to raw motor
- 3) pull off the cell gently  
↳ do NOT blow out!
- 4) flip switches & set for imaging
- 5) be in 1.0 x
- 6) turn on channels 1,2,3
- 7) in laser 980 → 960
- 8) 40-50% power in beam controls
- 9) recalibrate pixels cell/5
- 10) click focus & make sure laser +20
- 11) focus to take image (warning: number above computer)
- 12) scroll in z to see both chat bands
- 13) turn off Matlab
- 14) In ScanImage:  
↳ click enable stack  
↳ step size 0.5 microns  
↳ scroll through both chat bands  
↳ stop to bottom  
↳ start needs to be on top of somas
- 15) Abort (set start to end)
- 16) enable stack (should all turn blue) # of slices should be ~80-150-ish
- 17) edit basename, click DR, save in D: → Images
- 18) set pixels to 512, make sure Line Rate < 1000 (in configuration)
- 19) click grab.
- 20) when done, check in Fiji & Undo everything

config: should be @ 256 normally,  
but when imaging stack, put @ 512.

### Whole cell Patching

#### Procedure

- 1) Locate cell & center it
- 2) clean up area around
- 3) Home out
- 4) Z=3000 for stage
- 5) Fill glass pipette w/ internal
- 6) attach to electrode
- 7) add + pressure 1-1.3
- 8) swing in & home in (make sure @ 3000)
- 9) adjust towards cell
- 10) pipette offset
- 11) add source on computer
- 12) seal & leak program
- 13) Mode: seal
- 14) run seal
- 15) Create dimple → square pulse should start oscillating
- 16) release pressure & suck lightly
- 17) watch for flat line
- 18) don't rush, lightly quickly  suck to create this
- 19) watch it so it's stable
- 20) click I=0
- 21) click IC
- 22) run spots multisize
  - ↳ check your offsets (run ref to get that)
  - ↳ chan 1 more whole cell  on an ROC in advance!
  - ↳ sample rate 50,000
  - ↳ Intensity ≈ 0.3
- 23) run multipulse
  - ↳ check your offsets

#### 24) Ramp

- ↳ Tail time: 1,000
- ↳ Spike threshold: -6
- ↳ Sample rate: 50,000

#### 25) Drifting gratings

- ↳ DG\_currentclamp preset
- ↳ 2 cycles
- ↳ check offsets!
- ↳ NDF = 4 on right, 3 on right
- ↳ contrast 0.3
- ↳ uv led = 150
- ↳ grating speed = 1200-ish, then 2000, then 600
- ↳ run & then pause for a little bit to show light (optional)
- ↳ after finishing all 3 grating spreads

### Closing Dyon Systems

- 0.5) End epoch group!!
- 1) make all images of the day to Ophthalmology → Research → Schmittz Lab → Data → Preana Data
- 2) get h5 file from Data D: → Symphony
  - ↳ move to data folder
- 3) call data in matlab code & click enter to save data as data\_insetion
- 4) while waiting, turn off gas
- 5) put laser in safe
- 6) flip mirrors
- 7) turn on LED
- 8) turn off temperature regulator
- 9) remove dish carefully
- 10) cycle water, ethanol, water
- 11) clean up
- 12) duster through perfusion pipes

### Ripping membranes

#### Procedure

- 1) use larger size pipette
- 2) add ④ pressure 4-5
- 3) clean carefully

### Cleaning Up

### Logging an mouse sac

1) Animal interface

2) User, pass (figure this out)

3) Fill DTO upper left

4) click search

    click  
5) Tag ID

6) deceased & time

    ↳ submit event entry