



### **Novel Object Location and Recognition Protocol**

*Objects: 50 mL conical tubes, pyramids and spice jars, Blocks with Plastic Hats, OF arena, Blue Strip of plastic*

*Protocol: Use blue strip standing vertical on one side of arena for days 1-4*

*Day 1-3: 1-hour empty arena with no cue - all mice from same cage in one arena*

*Day 4a: Test one mouse per arena for ten minutes each. Use 2 identical objects: A, A'*

*Day 4b: 2.5 hour intersession delay to test Object Location Memory (OLM), i.e. move one of the objects to a new location. Preferably, move the object investigated for less time*

*Day 5a: Novel Object Recognition (NOR) with ten-minute trial: 24-hour testing for NOR (A vs B). Day*

*5b: Then, 1 hour after testing, (optional) using with B vs C, where B is the object less investigated for the 24-hour test, 10 minutes per trial.*

Set up all 4 chambers with appropriate zoom/focus on the cameras.

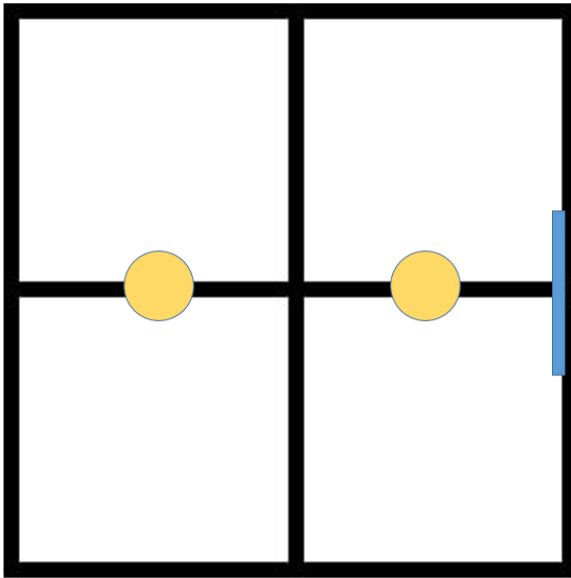
- Wipe down each chamber before and after each animal with 70% ethanol
- Use white light plus extra LED light bulb and keep the shades up
- Allow 15-60 minutes for animal room acclimation

### **Open Limelight 4 Software**

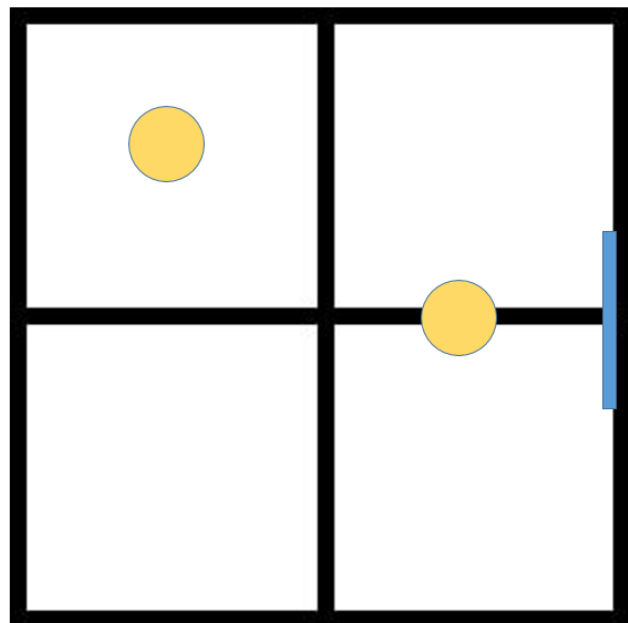
- File -> Open data file
  - Create a new file
    - Make sure there is space available on the desktop. See bottom right corner for GB available
- Double click on the chamber you want to set up
  - Make sure you zoom in enough where you can see the outline of the OF box and the target is in focus.
  - Set your grid resolution to a 2x2 grid (can be changed during analysis)
  - Click on the find cursors button and set up the **blue grid lines**
  - Set the view size:
    - Chamber 1: 85 cm
    - Chamber 2: 84 cm
    - Chamber 3: 80 cm
    - Chamber 4: 81 cm
- Click OK and move onto the next chamber until you are set up to begin your experiments
- Under duration enter appropriate duration for test day
- Enter each animal ID
- Take a reference with no animal inside the chamber box
- Grab your animal and place in the top left corner of the box
- Press Start; Do the same for all chambers

- When the trial is over, the animal ID changes to yellow. Grab the mouse and wait for the rest of the animals to finish their session
- Wipe down the boxes with ethanol. Reset the boxes in case they moved during cleanup. Enter new animal IDs and repeat for the steps for the set of animals
- When done with experiment, wipe down all the OF boxes and return in proper place
- Transfer your data off of the computer and into your BPC server folder

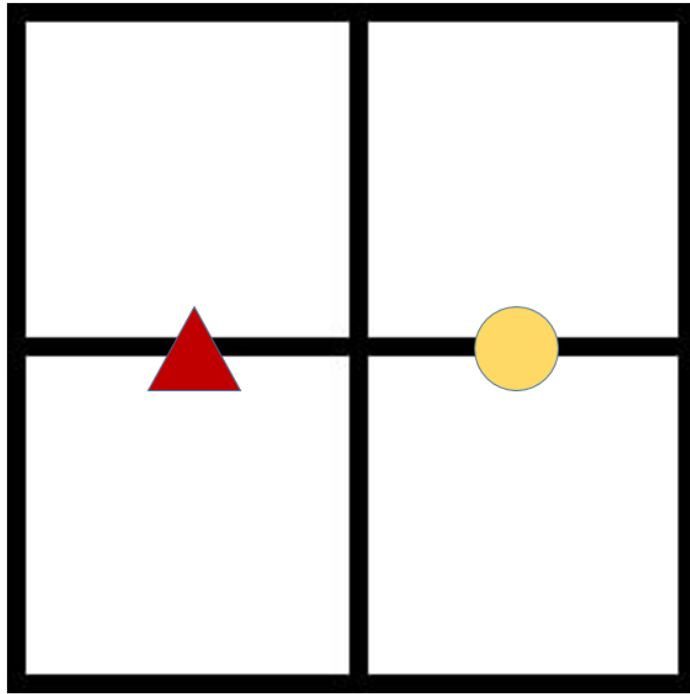
Day 4a



Day 4b



Day 5a



Day 5b

