



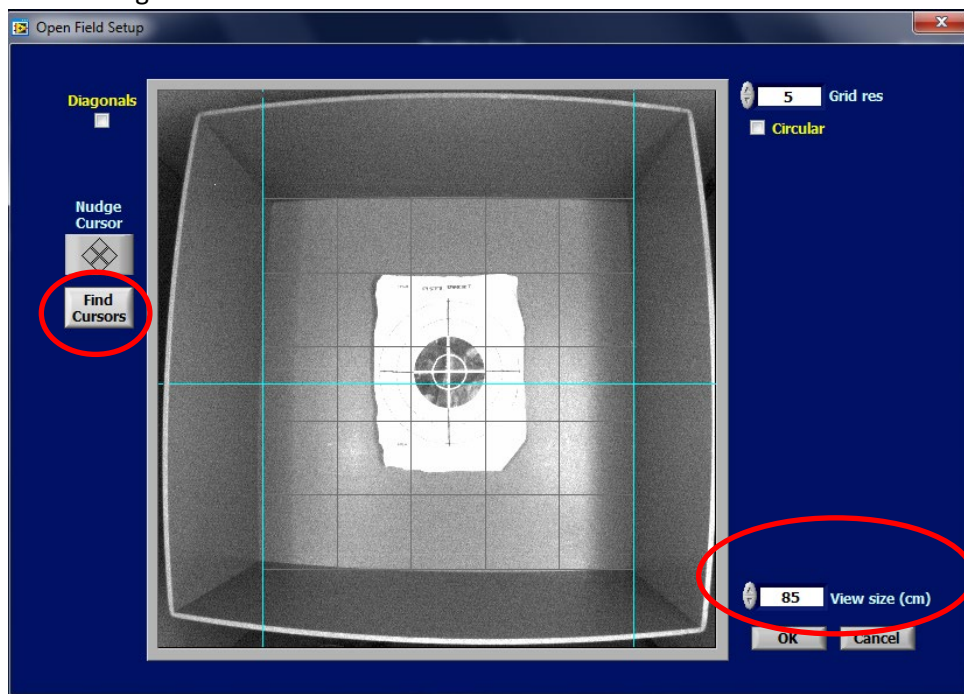
### Open Field Protocol

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 red light 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: OF\_Date
- 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
  - Set your grid resolution to 5x5 (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
- Make sure you zoom in enough where you can see the outline of the OF box and the target is in focus. See image below:





- Click OK and move onto the next chamber until you are set up to begin your experiments
- Under duration enter: 300
- Enter each animal ID
- Take a reference with no animal inside the chamber box
- Use a camera cap to find the tracker
- Grab your animal and place in the center of the box while grabbing the camera cap at the same time
- 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
  - Count the droppings if you want to use it as a measure of anxiety
- 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

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Behavioral Phenotyping Core

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