**Trypan Blue Exclusion**

* Put in overnight culture.
* The next morning create three tubes, 1 for each condition, by diluting 1:10 and incubating for 3 hours. This allows cells to enter log phase again. So 0.1ml overnight culture and 0.9ml media.
* After 2 hours, spin down and remove the supernatant.
* Make 1ml of 1.11mM Thiacloprid from 500mM stock solution.
  + 2.22µl stock + 995.56µl media + 2.22µl MeOH.
  + Do the same for malathion but DMSO instead of MeOH.
  + Control (995.56µl media + 2.22µl DMSO + 2.22µl MeOH).
  + Final solvent concentration will be 0.2%.
* Resuspend in 0.9ml of 1.11mM Thiacloprid or Malathion or Control and 0.1ml Trypan 0.4%. Incubate for 30 minutes then spin down and sample 2.5µl from pellet and create slides and image.
* To make the slides: add 2.5µl cell suspension to slide. Heat up YPD agar stock at 70°C, allow to cool slightly and take up 2.5µl. Mix this on the slide with the cell suspension. Once thoroughly mixed remove 2.5µl and place on cover slip.
* Resuspend pellet.
* Repeat at t = 1h, 2h, 4h and overnight (16-24h).
* For each slide take 3 images in different places and count number of viable and non-viable cells.
* Repeat the whole experiment twice.

**Using the Microscope**

* ABCDE switches to turn on.
* Zen Pro.
* Put stand down and insert slide after place a drop of oil on it.
* Put stand back up.
* Acquisition tab, skip calibration.
* Go onto my profile.
* make sure red channel and DIC are ticked. Click on DIC bar and click live and focus.
* Make sure the autosave names are sensible.
* Click start experiment.
* Click live again to move frame.
* Click start experiment again.
* Once finished remove slide by putting stand down.
* Put stand back up and EDCBA to turn off.
* Check the channel exposures of RFP 1-2s (??) and DIC 50-100ms.

**Timing**

1000 – Dilute cells.

1200 – Heat up stocks.

1230 – Make 1.11mM Thia and Mala.

1250-1300 – Spin down cells and add insecticide and trypan blue.

1330 – Image (t=30m)

1400 - Image (t=1h)

1500 – Image (t=2h)

1700 - Image (t=4h)

**Statistical Analysis**

For each timepoint of each tube I will have a proportion of nonviable/total cells. take cells as the unit of replication and perform a fisher’s exact test (like a Chi-squared test). Repeat this on two different days to see if effect holds.

What is the question I am trying to answer? I want to know when there is statistically significant effect of treatment on cell viability in comparison to control. Therefore, compare between treatment and control separately for each time point?