**Trypan Blue Exclusion**

* Prep overnight cultures. 8 for both malathion, thiacloprid and control. Perform 4 of each on each day.

OR

* One tube for each treatment or calculating z scores.
* The next morning dilute 1:10 and incubate for 2 hours. This allows cells to enter log phase again. At this point add trypan blue 0.04%. 1:10 dilution. So 0.1ml 0.4% trypan blue, 0.1ml overnight culture and 0.8ml media.
* Spin down and remove the supernatant. Create a slide for each tube and image. This is t = 0.
* To make the slides: add 2.5µl cell suspension to slide. Heat up YPD agar stock at 70°C 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.
* Make 5ml of 1mM Thiacloprid from 500mM stock solution.
  + 10µl stock + 4990µl media.
  + Do the same for malathion.
* Resuspend in 1ml of 1mM Thiacloprid or Malathion. Incubate for 30 minutes then spin down and sample 2.5µl from pellet and create slides and image.
* Resuspend pellet.
* Repeat at t = 1h, 2h, 4h and overnight (16-24h).
* Repeat the whole experiment twice. So n=8 for each treatment. Although these as usual with yeast are only technical replicates.
* To make the slides: add 2.5µl cell suspension to slide. Heat up YPD agar stock at 70°C 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.
* For each slide take 3 images in different places and calculate the proportion of dead cells over total cells.

**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.
* Click start experiment.
* Click live again to move frame.
* Click start experiment again.
* Make sure the autosave names are sensible.
* 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.

**Statistical Analysis**

For each timepoint of each tube I will have a proportion of nonviable/total cells. As this is between 0-1 values won’t be normally distributed. Think I have to perform beta regression.

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?

Or I could take cells as the unit of replication and calculate a z score. Proportion of dead/total for control and treatment. Then calculate total proportion of dead/total. Then plug them into z statistic. Repeat this on three different days to see if effect holds.

Or could I do a Chi-squared test?