M1D7: Analysis of sub-nuclear foci

10/06/16

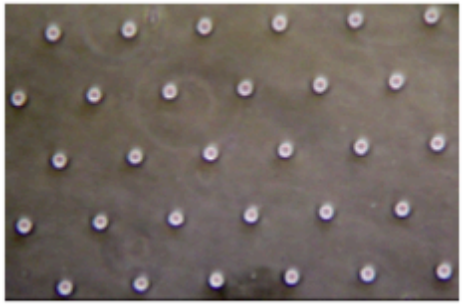
1. Quick prelab
2. Mount coverslips and image on microscope
3. Finish M1D7 Results assignment

Mod1 Day2 Benchling page will be graded for Mod1 notebook.

Extra office hours

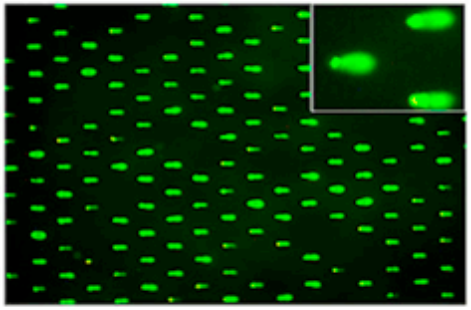
- M1 Data Summary draft
  - due 5pm on Wednesday, October 12
- Office hours:
  - Friday, October 7, 9am-10am: Maxine in 16-336
  - Sunday, October 9, 10am-12pm: Maxine in 56-302
  - Monday, October 10, 3pm-5pm: Leslie in 56-302
  - (regular Monday office hours cancelled)
  - Tuesday, October 11, 1pm-4pm: Noreen in 56-302
  - Wednesday, October 12, 10am-12pm: Leslie in 16-429b
- Work at Office Hours even if you don't have specific questions!

Only report on M1D1-M1D4 in your Data Summary



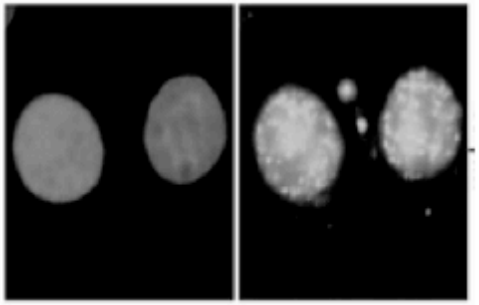
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals



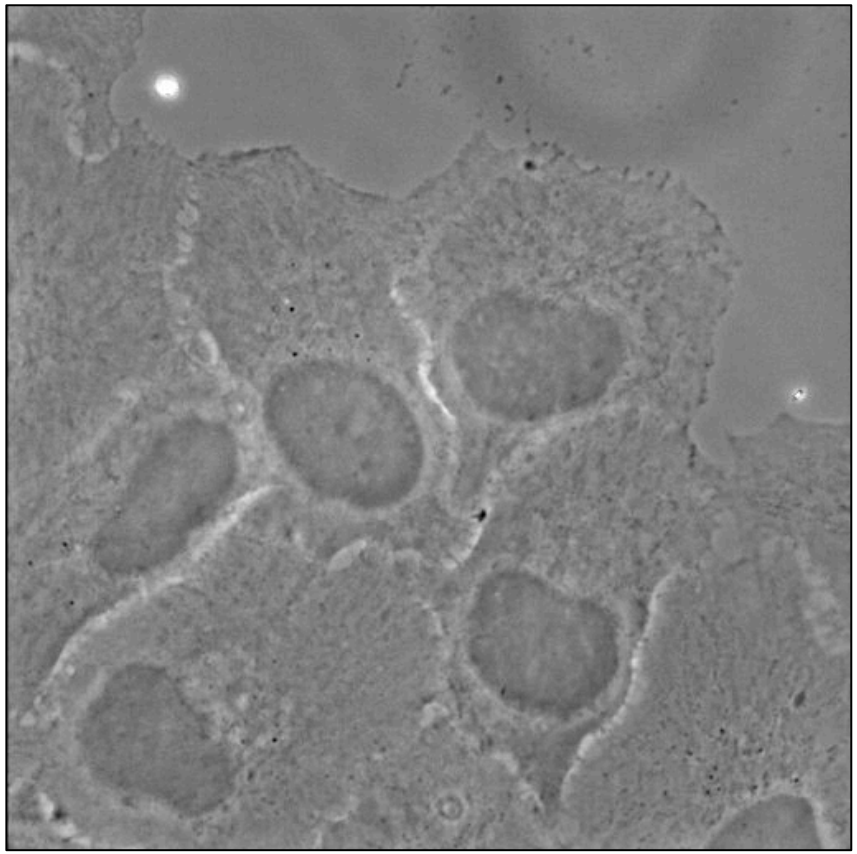
3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of H_2O_2 on DSB abundance

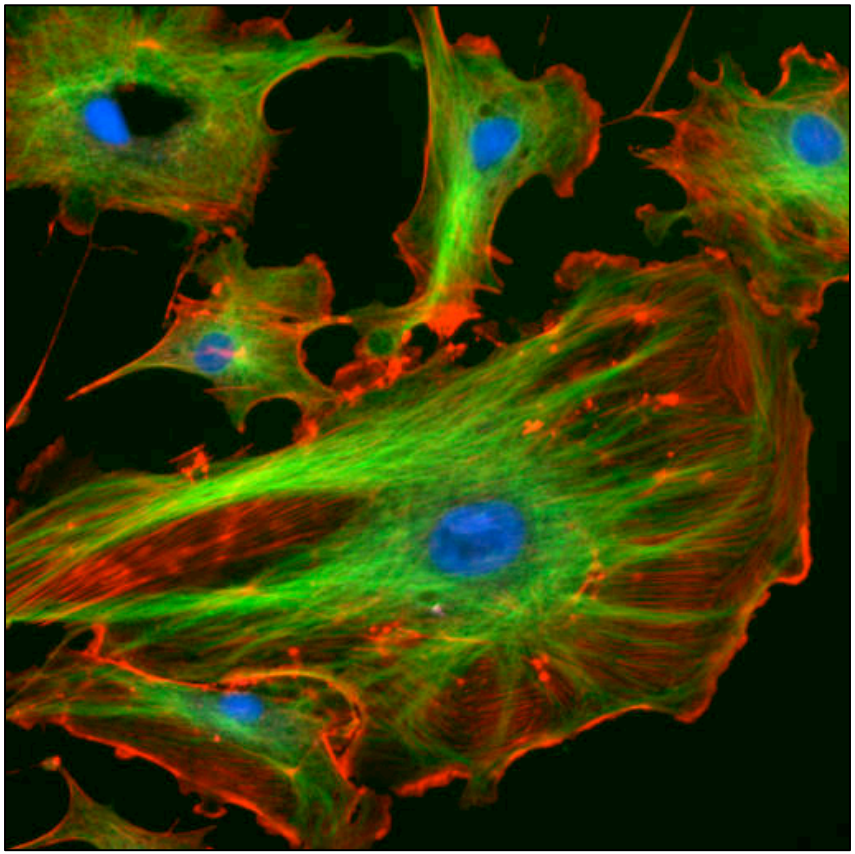
TODAY

Why is fluorescence imaging so widely used in biology?

nuclei
microtubules
actin

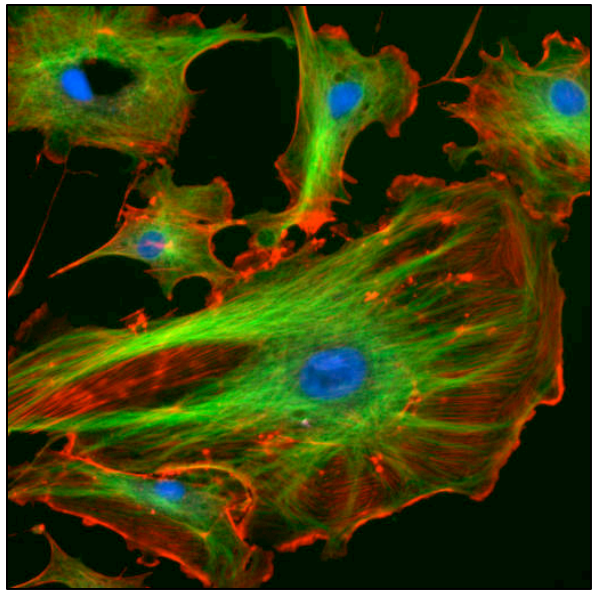


bright-field



fluorescence

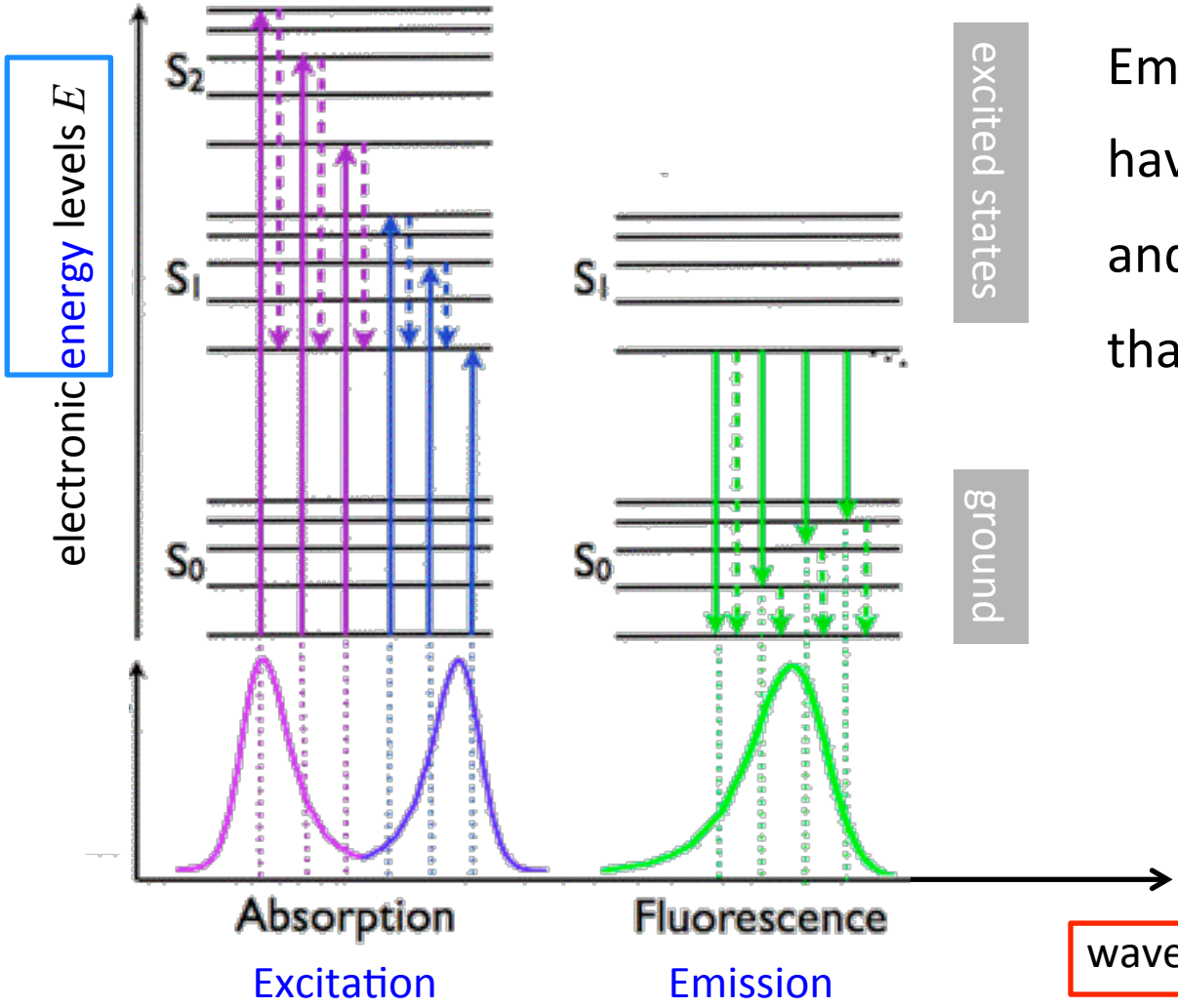
Considerations for fluorescence imaging



- pros:
  - low background
  - excellent contrast
  - multiple colors
  - molecular and structural specificity
  - biochemical sensitivity for functional imaging (Ca^{2+}, pH)
  - genetic expression
  - specialized techniques for 3D and high-resolution imaging
- cons:
  - expensive equipment: laser, filters, sensitive cameras, …
  - toxicity to cells?
  - need for fixing or gene manipulation?
  - does the added fluorophore moiety impair biological function?

Physical principles of fluorescence:

Jablonski diagram



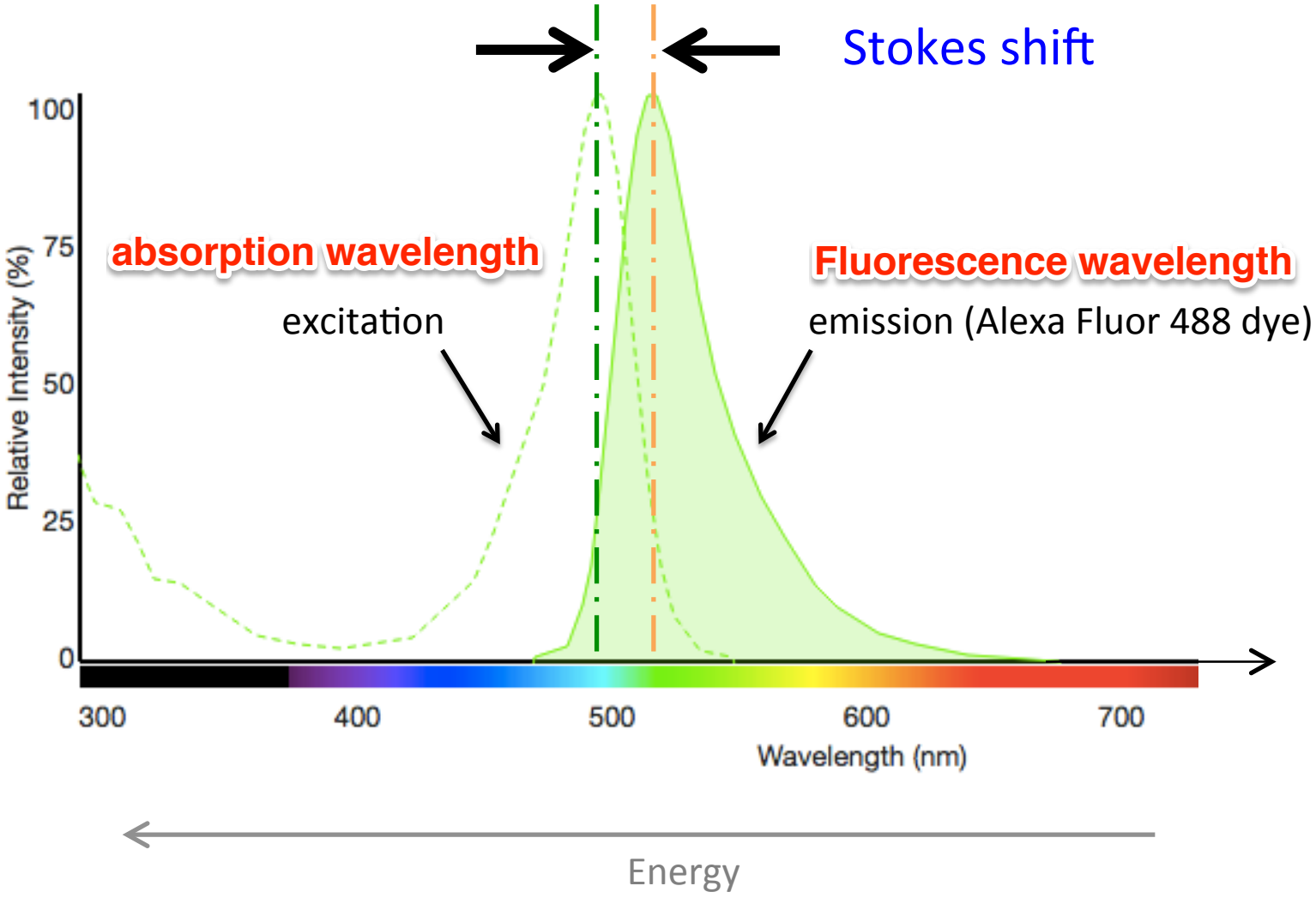
Emitted fluorescence photons have **lower** energy and **higher/longer** wavelength than excitation photons

$$E = h\frac{c}{\lambda}$$

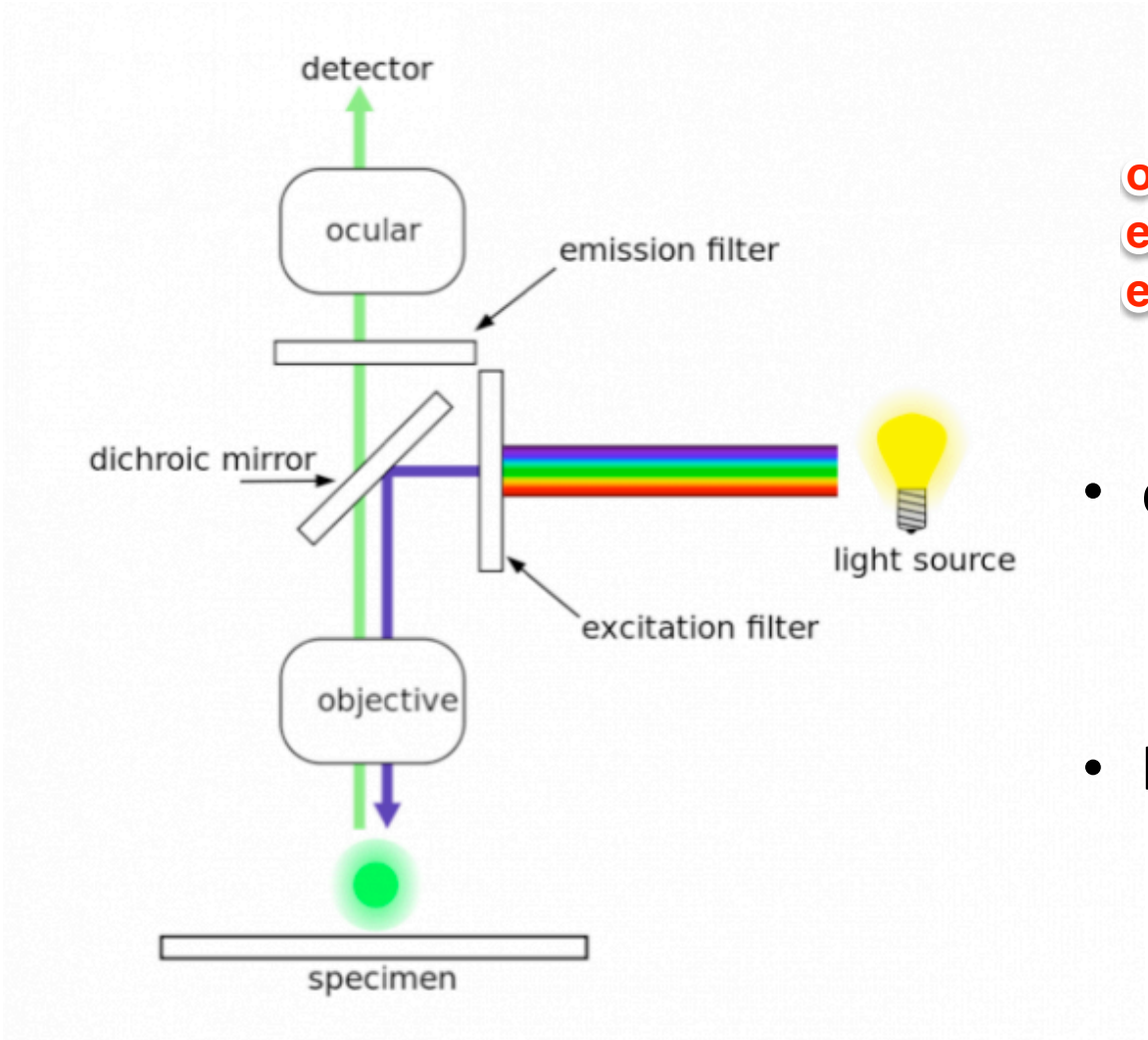
http://photobiology.info/Visser-Rolinski.html

Physical principles of fluorescence:

Stokes (red) shift of emission wavelength



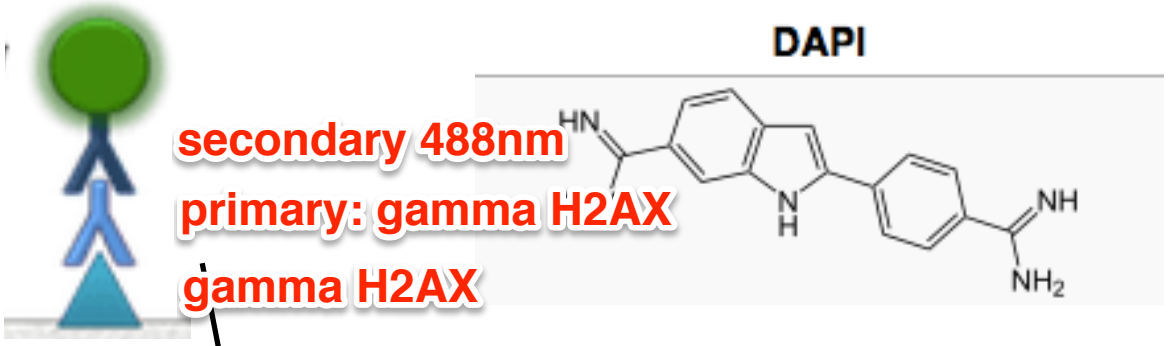
Now in practice: epi-fluorescence microscope



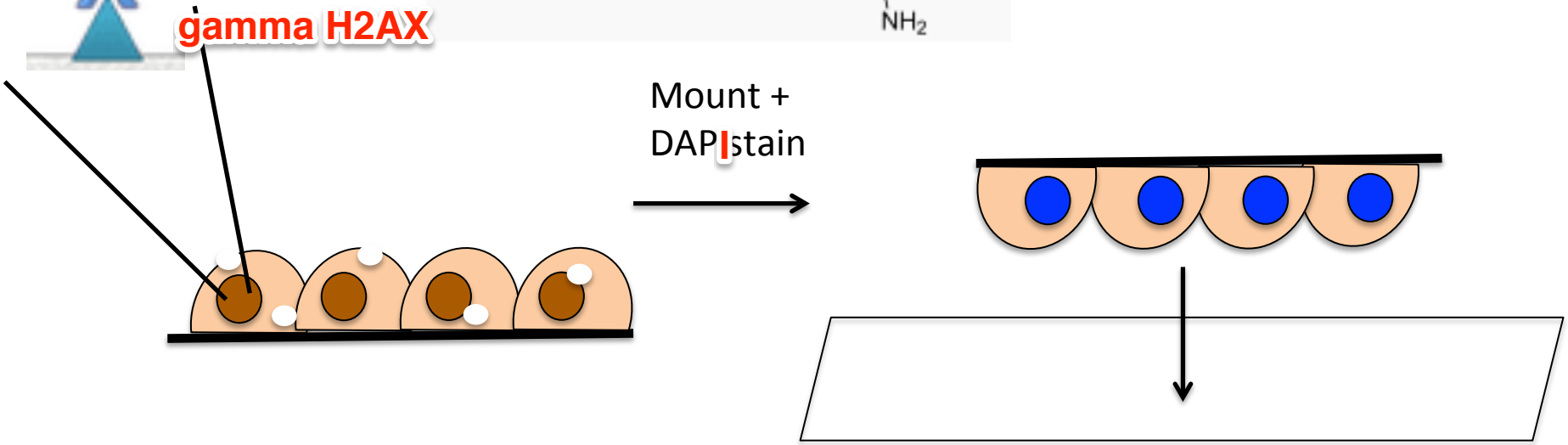
our secondary
excites at 488nm
emits at 520nm

- dichroic mirror
  - reflects **blue** light
  - transmits **green** light
- barrier / emission filter
  - selects for green light
  - emission ~ 10^{-5} excitation

Completing γH2AX assay



DAPI

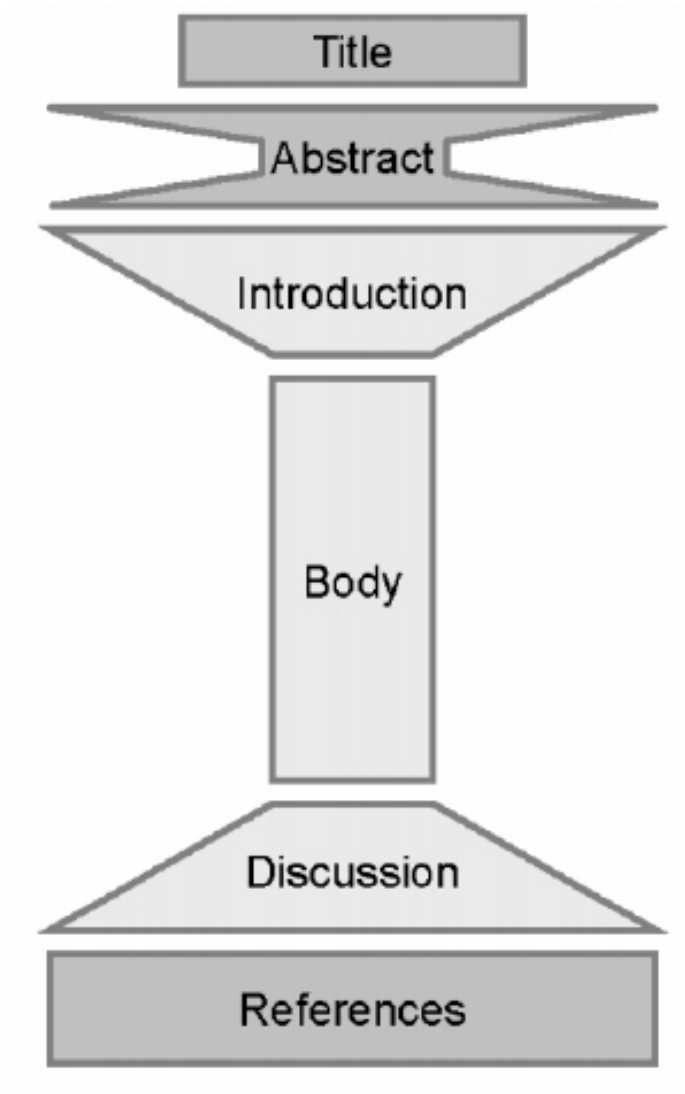


small molecule, fluorescent
binds tightly to DNA
easily passes through membranes
Fluorescent at ABS 358, EM 461

Today in lab…

- Complete the final wash of your coverslips and mount coverslips on slides
  - slides and DAPI mounting media on front bench
- Groups will be taken to the microscope as they finish (~10-15min to image)
- Complete your “in class” γH2AX homework assignment and continue data analysis

Your M1 Data Summary



Title: take-home message

Abstract: the only page *not* in bullet points

In bullet points:
Introduction: background and motivation

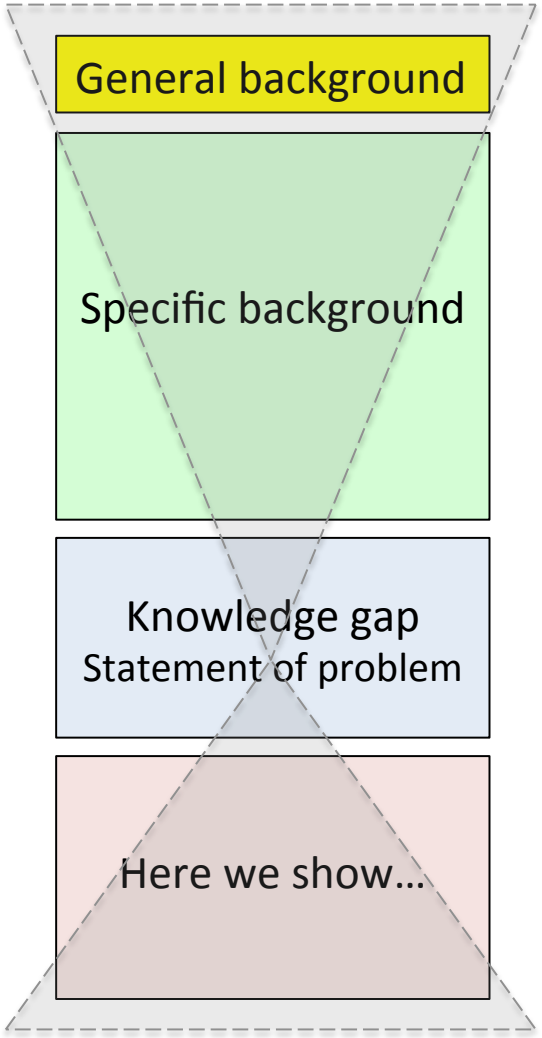
Results and
interpretation / discussion
bundled together

Both MMS and H_2O_2 dose response curves
Your data elsewhere (you may use others' too)

transitions from one page to the next

Implications and future work
References (*not* in bullet points)

What goes into an introduction?



- Your research is anchored in a general topic that your audience cares about.
  - focus on outsiders
  - include references
- All information connects your project with the general topic.
  - minimum essential information
  - accurately represents the field
  - correctly referenced, give credit
- The question you address is clearly articulated, connected to the background, and appears meaningful.
  - give evidence of incompleteness of current understanding, of value of investigation
  - CLEARLY state your hypothesis
- A preview of your findings and their implications fills the demonstrated gap.
  - light on Methods

The meat of your paper

- Figures and captions
  - Decide on these first
  - Use subpanels
  - Text: limited on figure, explicit in caption
  - reasonable size
  - descriptive title
  - caption purely descriptive of image
  - intro sentence in caption
- Results
  - goal / intent / purpose of experiment = intro topic sentence
  - What you did: experiments and expectations, including controls
  - What you found:

What goes into the Discussion / Interpretation?

- Put in context: how does this fit with other studies?
- Highlight significance: how might this impact this/other field?
- Discuss controversial or surprising results
- transition

What goes into Implications & Future Work?

- Start with a very similar paragraph to the last paragraph in your Background/Motivation (major results and broad implications)
- Follow same order as in Figures/Results
  - Describes caveats and suggest remedy
  - Conjecture (one layer only!) implications
- Propose future work, identify new questions that arise
- Make sure you come back to big picture introduced in intro
- Don't overreach / overpromise!