M1D7:
Visualization and data analysis of γH2AX-labeled DSBs

10/07/2016

Wrapping up M1!

- Data summary draft
  - due by 5pm on Wed., October 12
  - revision due by 5pm on Mon., October 24

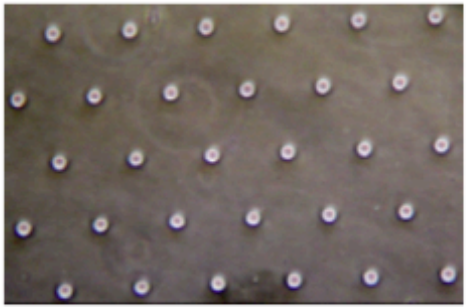
- Lab notebook
  - M1**D2** in Benchling will be graded by Emily at 10pm tonight
  - Check out the 10-point rubric on the wiki (Communication tab)

- Mini presentation due by 10pm on Sat., October 15
- Blog post for M1 due by 5pm on Tue., October 25

Extra office hours

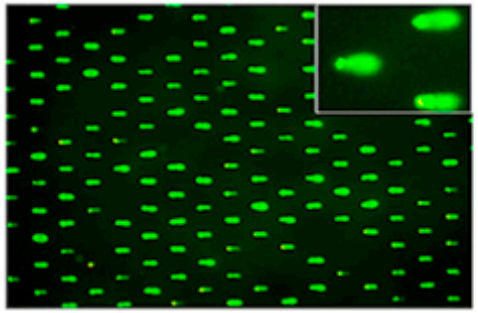
- M1 Data Summary draft
  - due 5pm on Wednesday, October 12
- Office hours:
  - Friday, October 7, 9am-10am: Maxine in 16-336
  - Sunday, October 9, 10am-12pm: Maxine in 56-302
  - Monday, October 10, 3pm-5pm: Leslie in 56-302
  - (regular Monday office hours cancelled)
  - Tuesday, October 11, 1pm-4pm: Noreen in 56-302
  - Wednesday, October 12, 10am-12pm: Leslie in 16-429b
- Come early, even with nothing ;-)
  - ask questions, get feedback on the fly

Only report on M1D1-M1D4
in your Data Summary



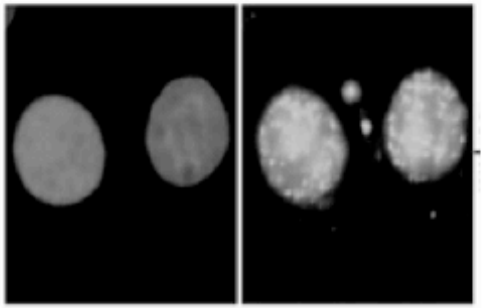
1. Optimize comet chip assay

- 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



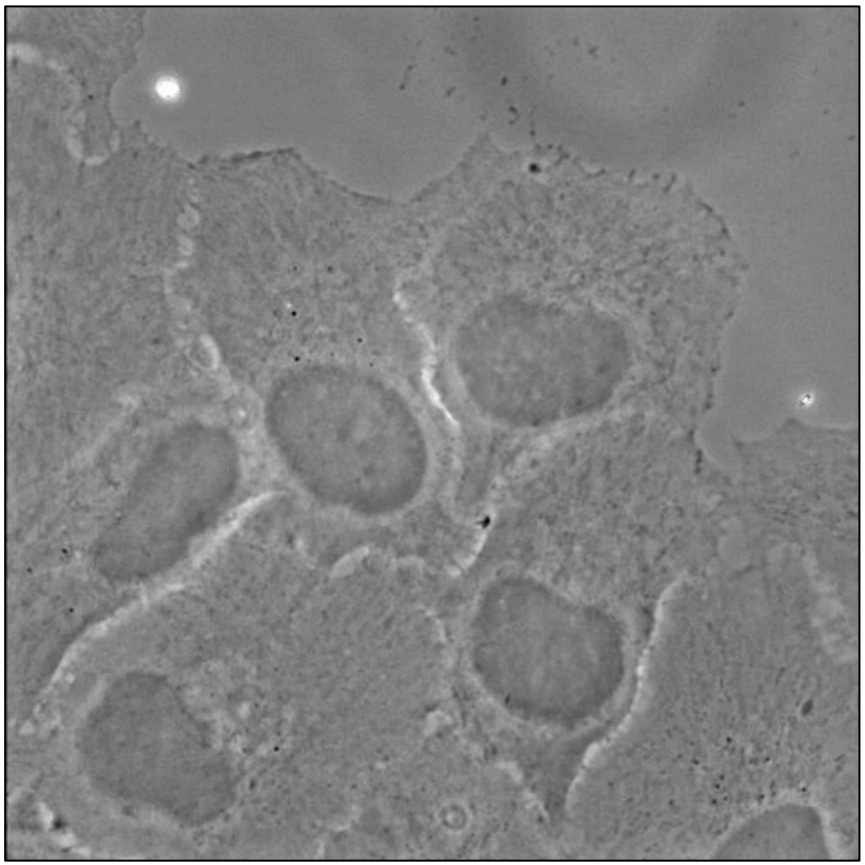
3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of H_2O_2 on DSB abundance

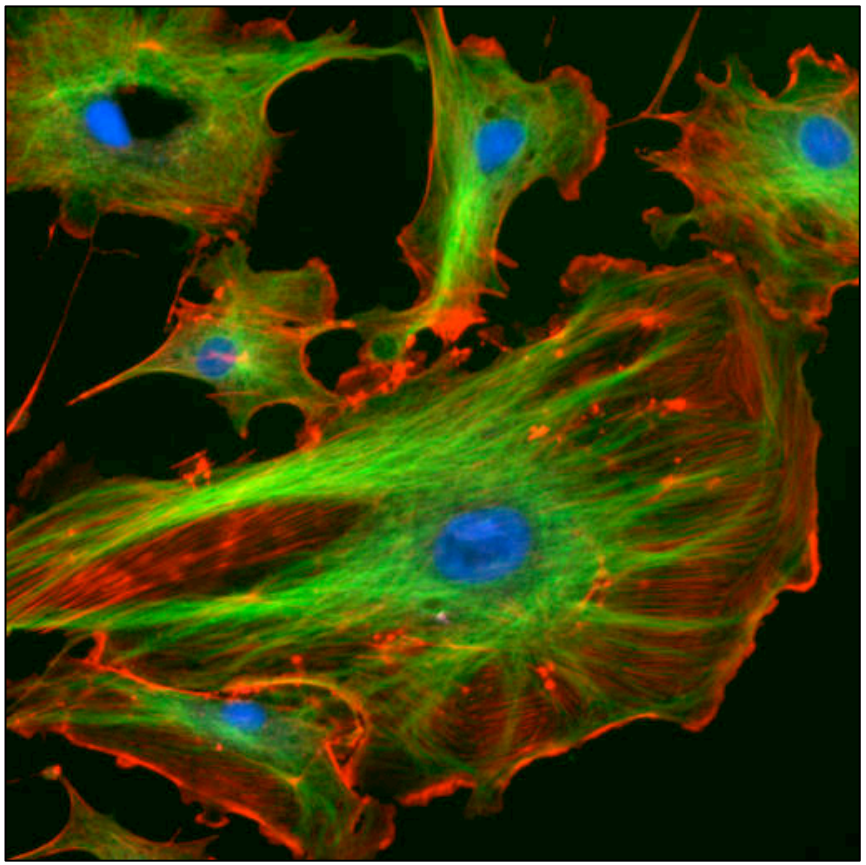
TODAY

Why is fluorescence imaging so widely used in biology?

nuclei
microtubules
actin

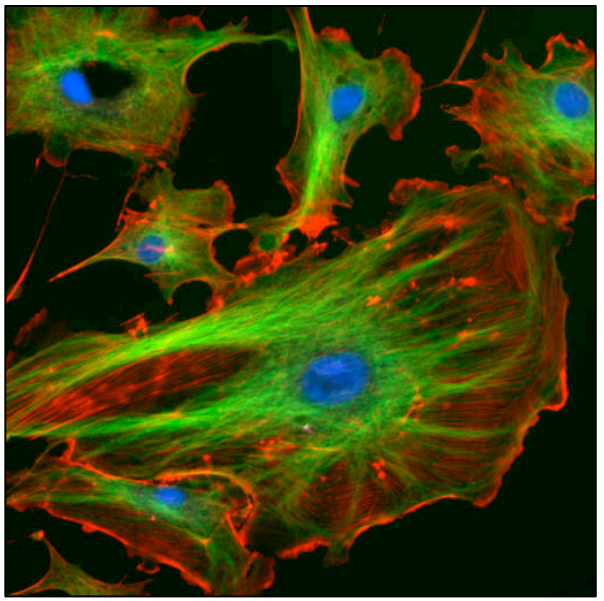


bright-field



fluorescence

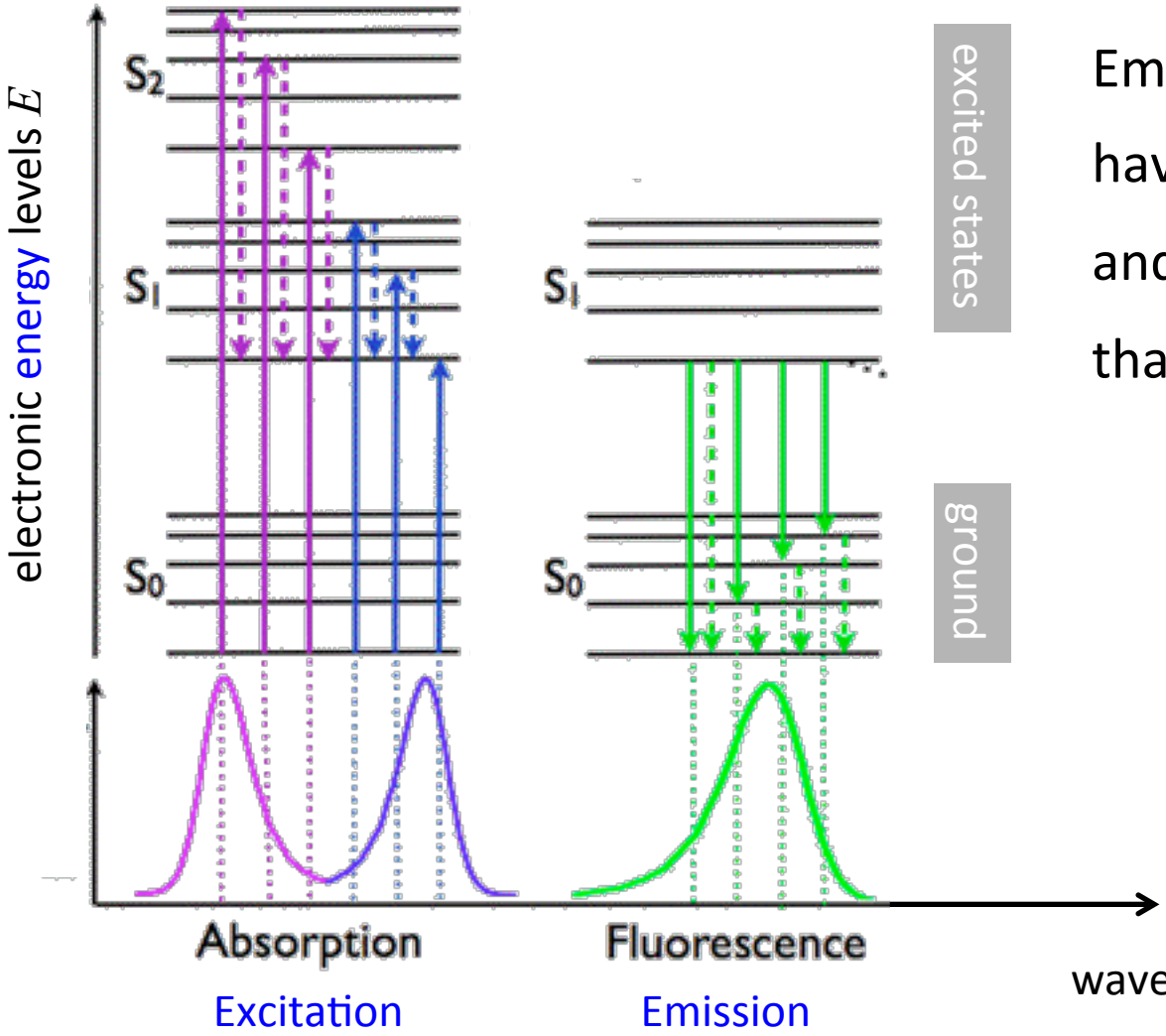
Considerations for fluorescence imaging



- 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



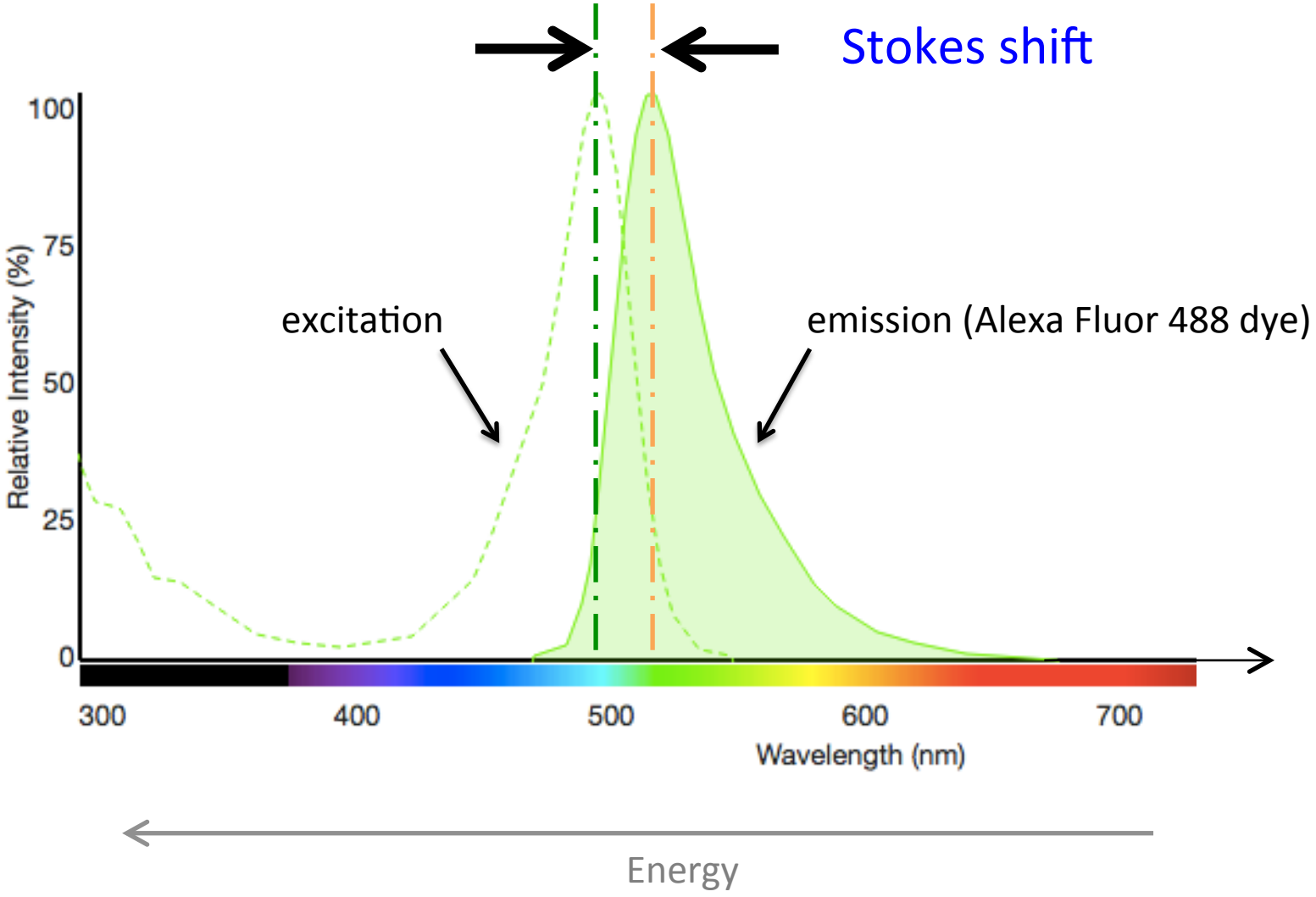
Emitted fluorescence photons have _lower_ energy and _longer_ wavelength than excitation photons

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

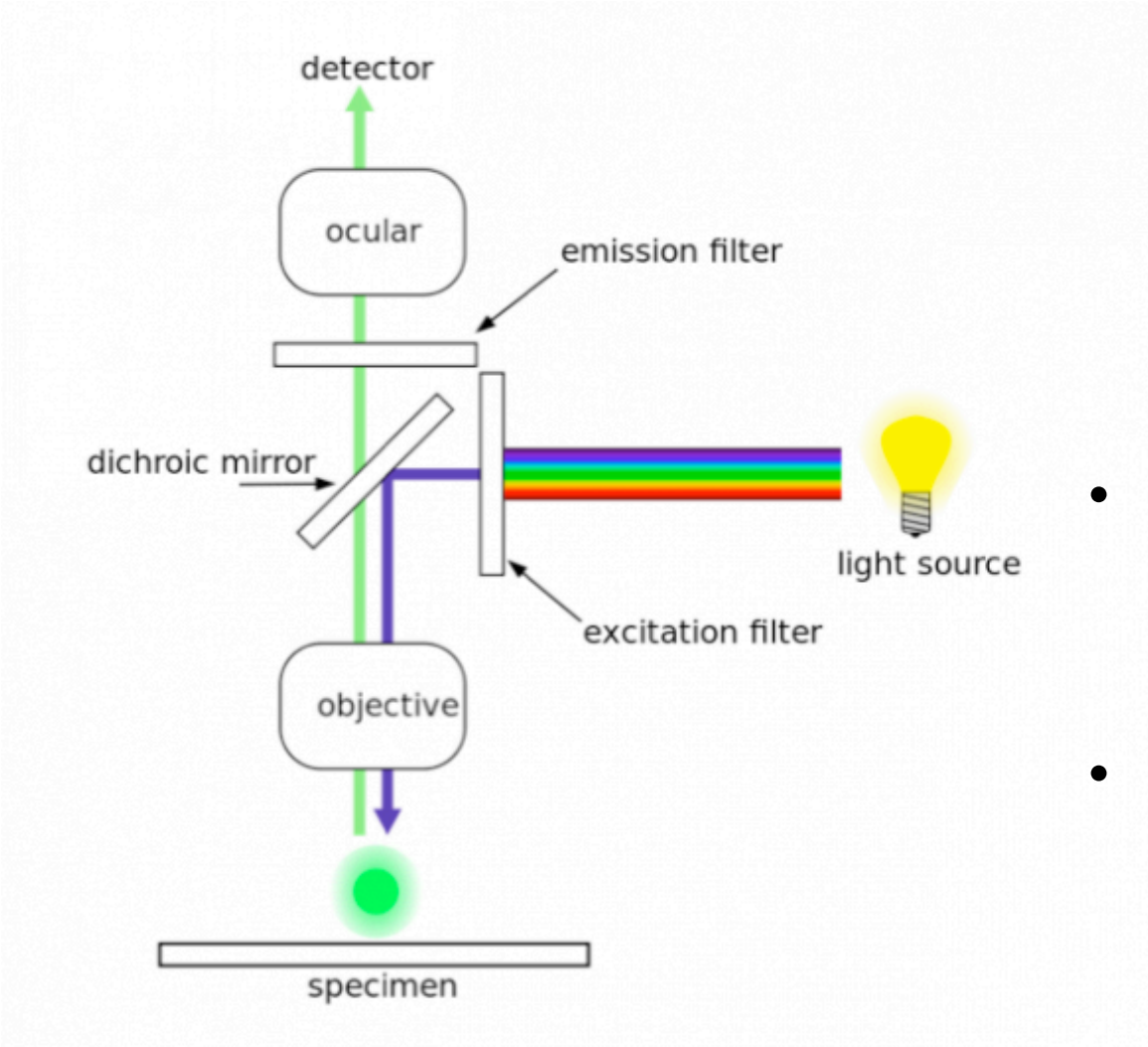
http://photobiology.info/Visser-Rolinski.html

Physical principles of fluorescence:

Stokes (red) shift of emission wavelength



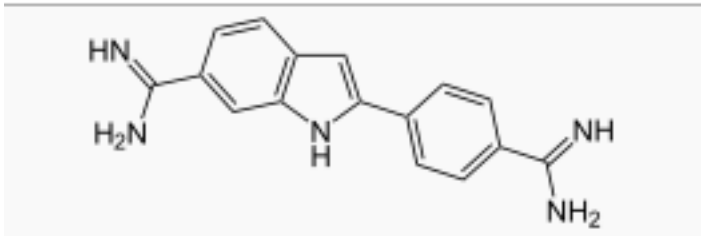
Now in practice: epi-fluorescence microscope

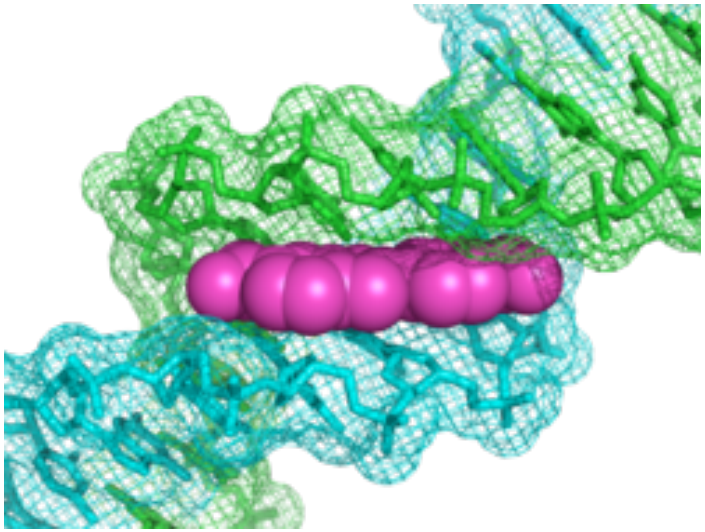


- dichroic mirror
  - reflects blue light
  - transmits green light
- barrier / emission filter
  - selects for green light
  - emission ~ 10^{-5} excitation

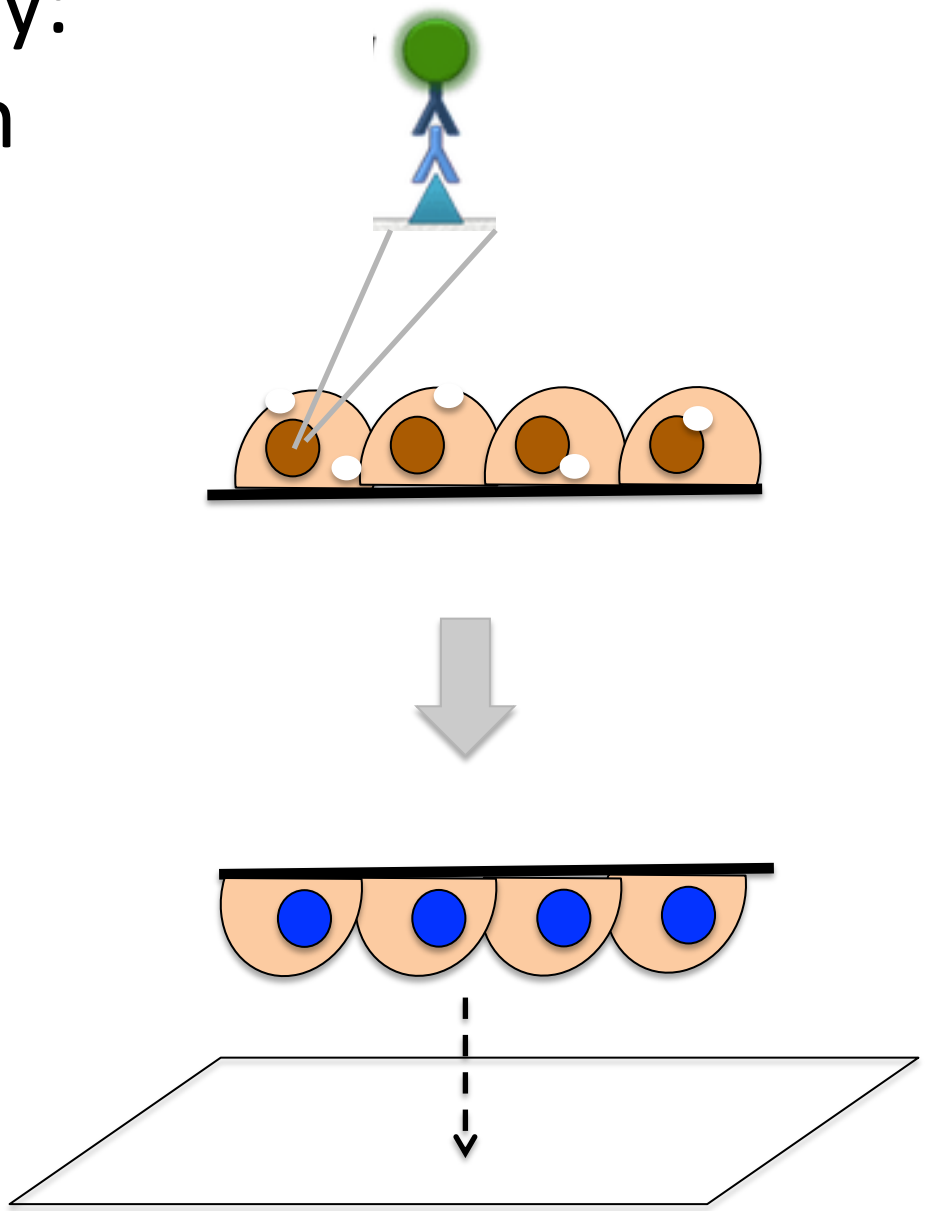
Complete γH2AX assay: Mount and DAPI-stain

DAPI (4',6-diamidino-2-phenylindole)



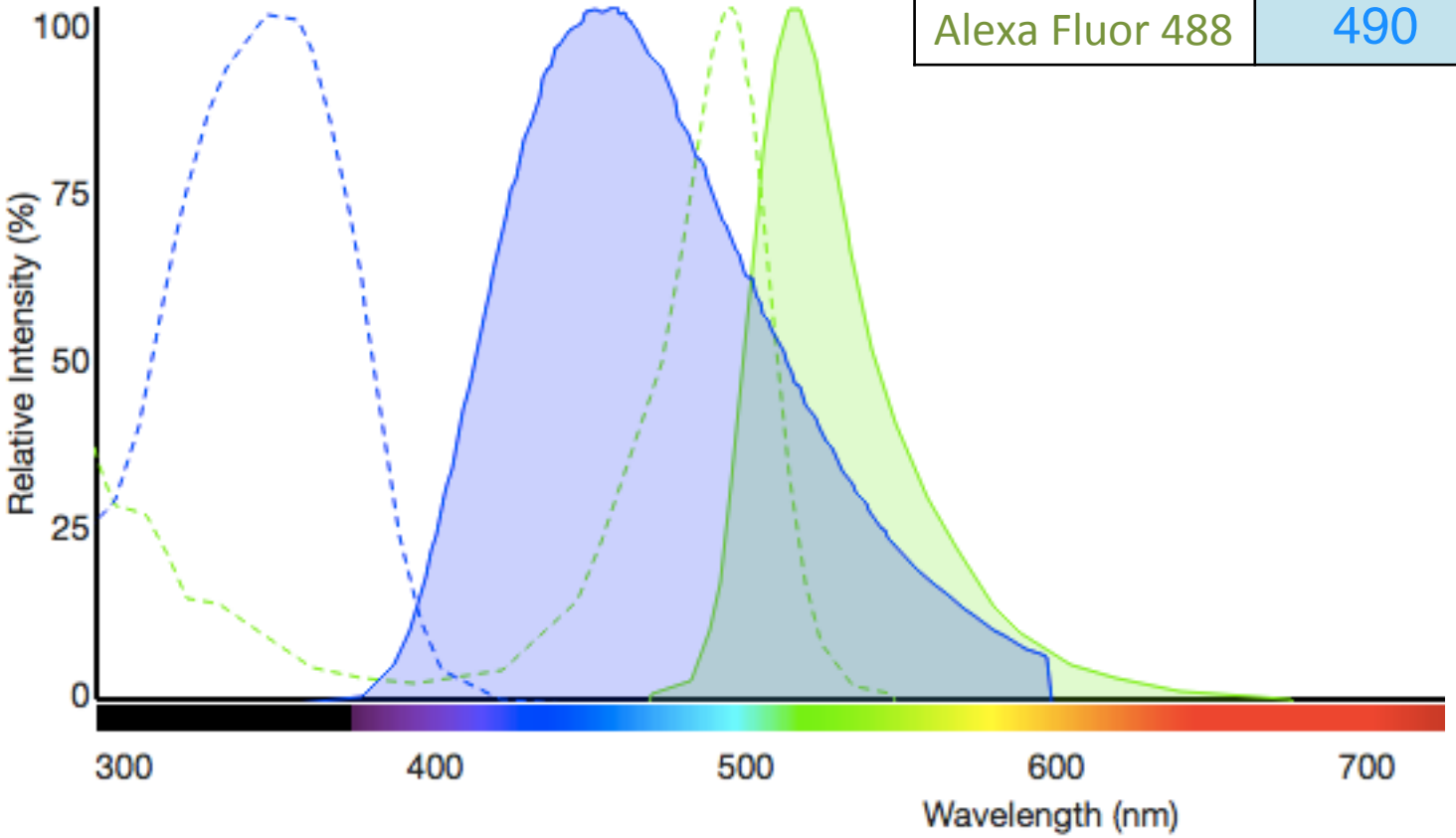


DAPI intercalates the minor groove of DNA

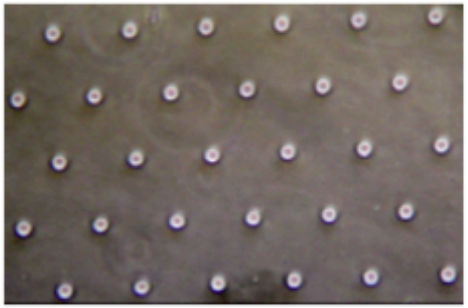


2 channels in microscope
to visualize 2 fluorescent dyes

	excit. (nm)	emiss. (nm)
DAPI	358	431
Alexa Fluor 488	490	520

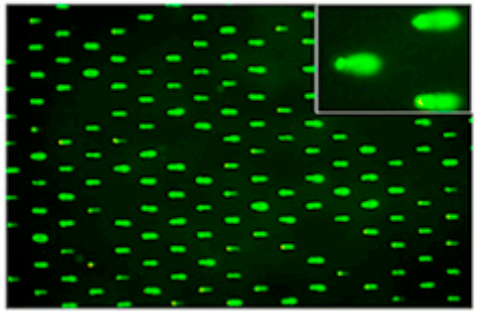


Only report on M1D1-M1D4
in your Data Summary



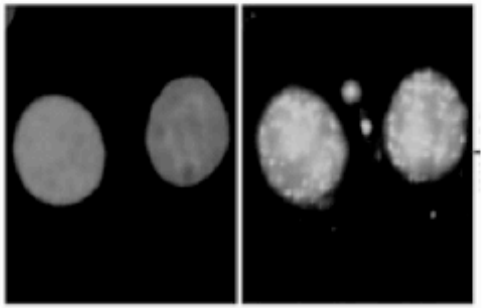
1. Optimize comet chip assay

- 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