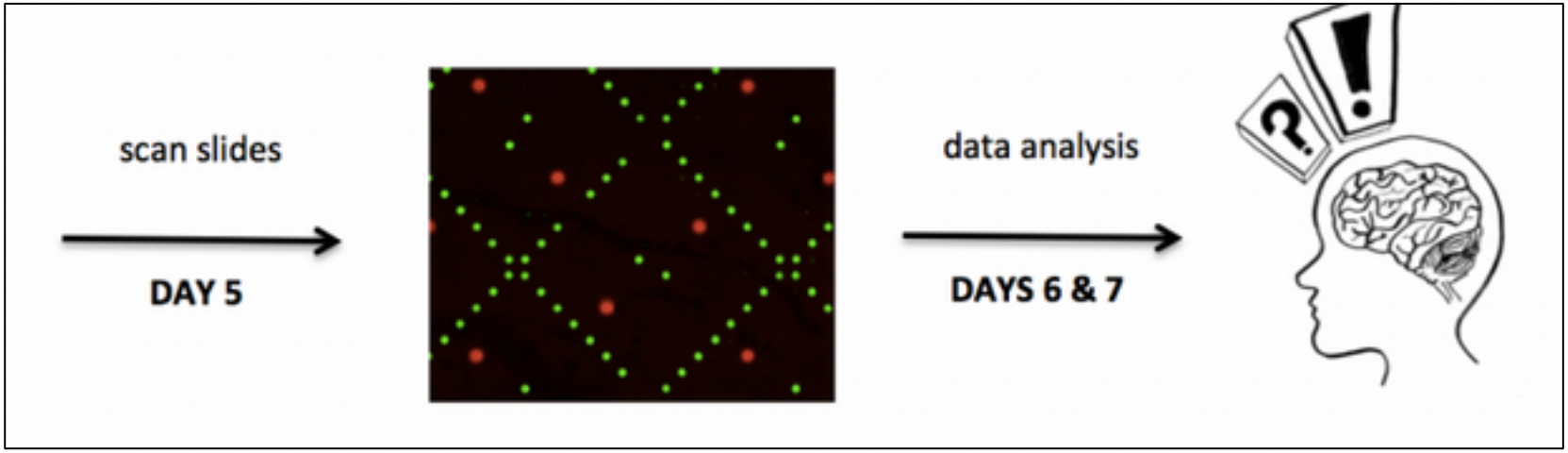
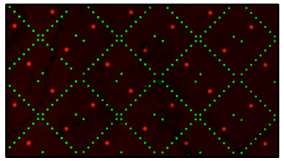
M1D5:
Scan slides to identify FKBP12 binders



02/28/2017

In lab today

- BE Communication Lab workshop



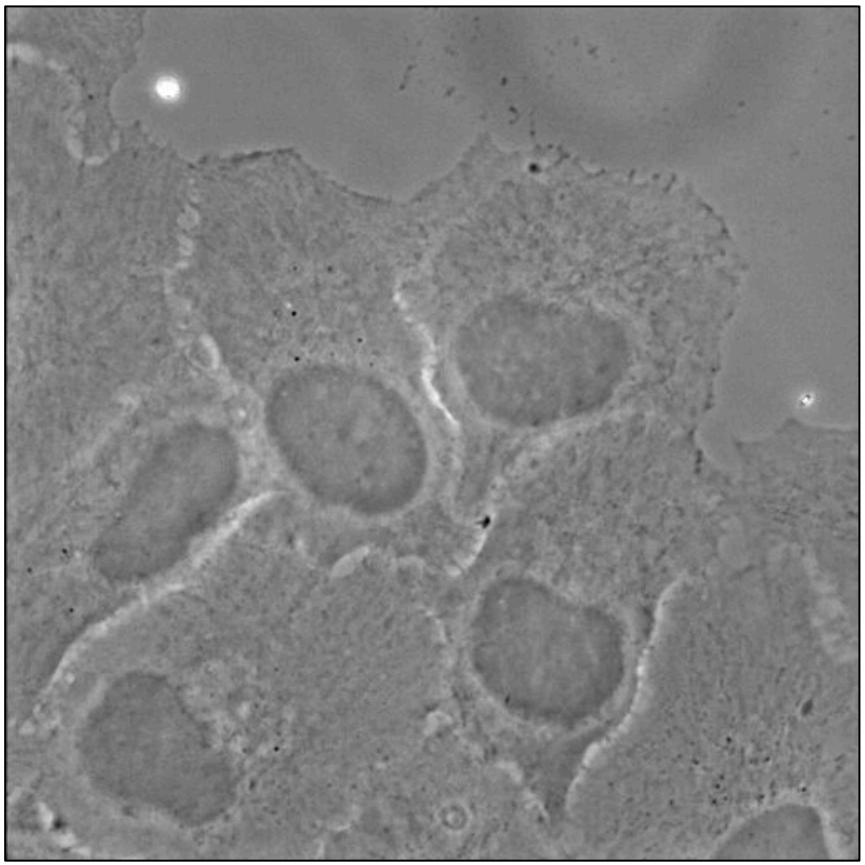
- Demo of SMM scanning by TA Rob Wilson

Skim

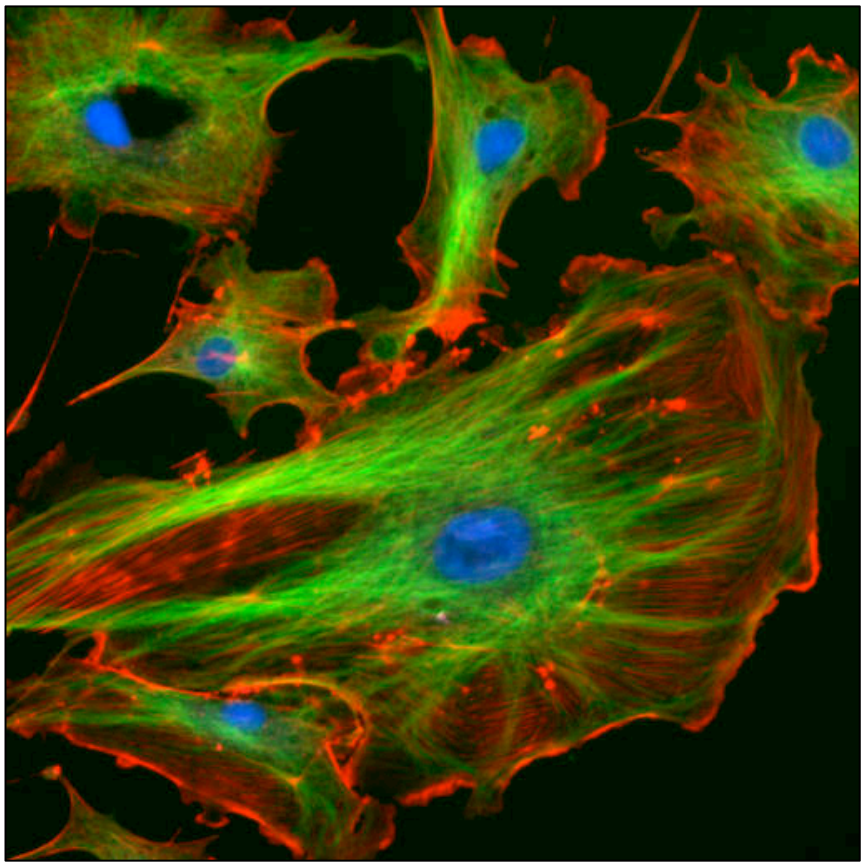
- Read paper to discuss “future directions” ideas

Why is fluorescence imaging so widely used in biology?

nuclei
microtubules
actin

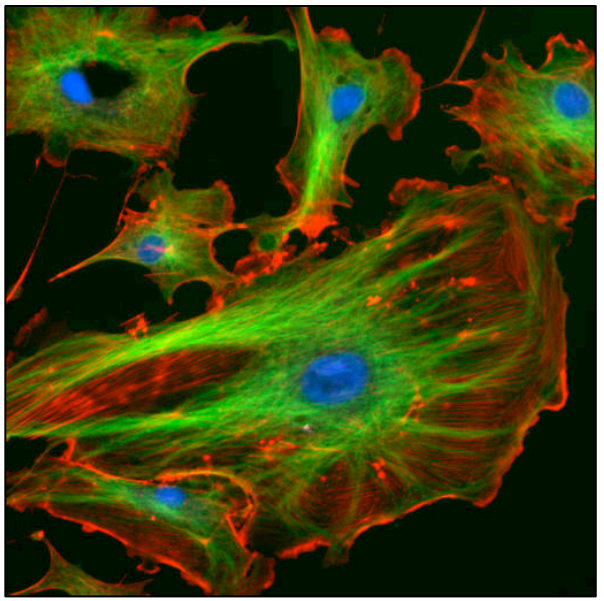


bright-field



fluorescence

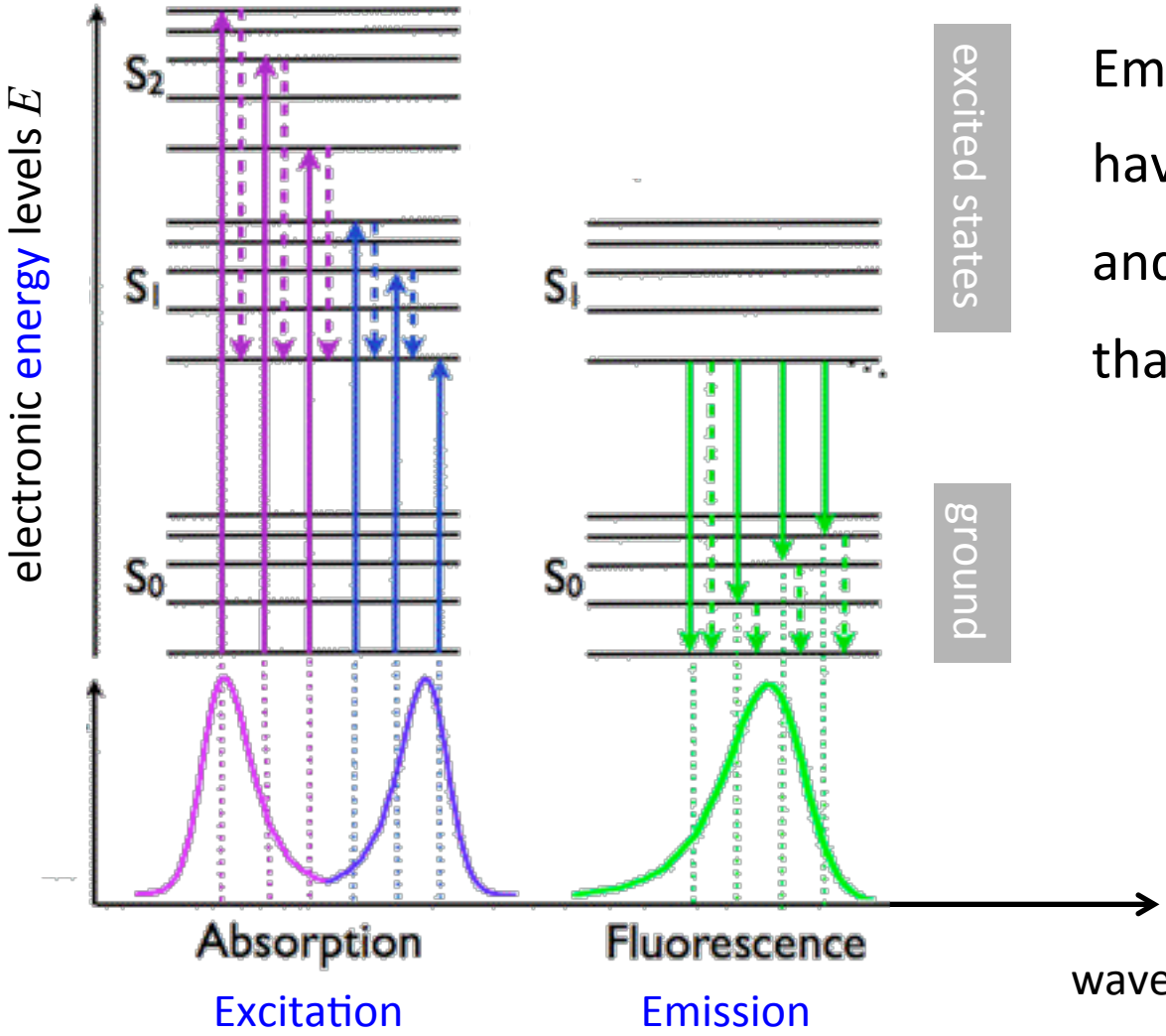
Considerations for fluorescence imaging



- 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



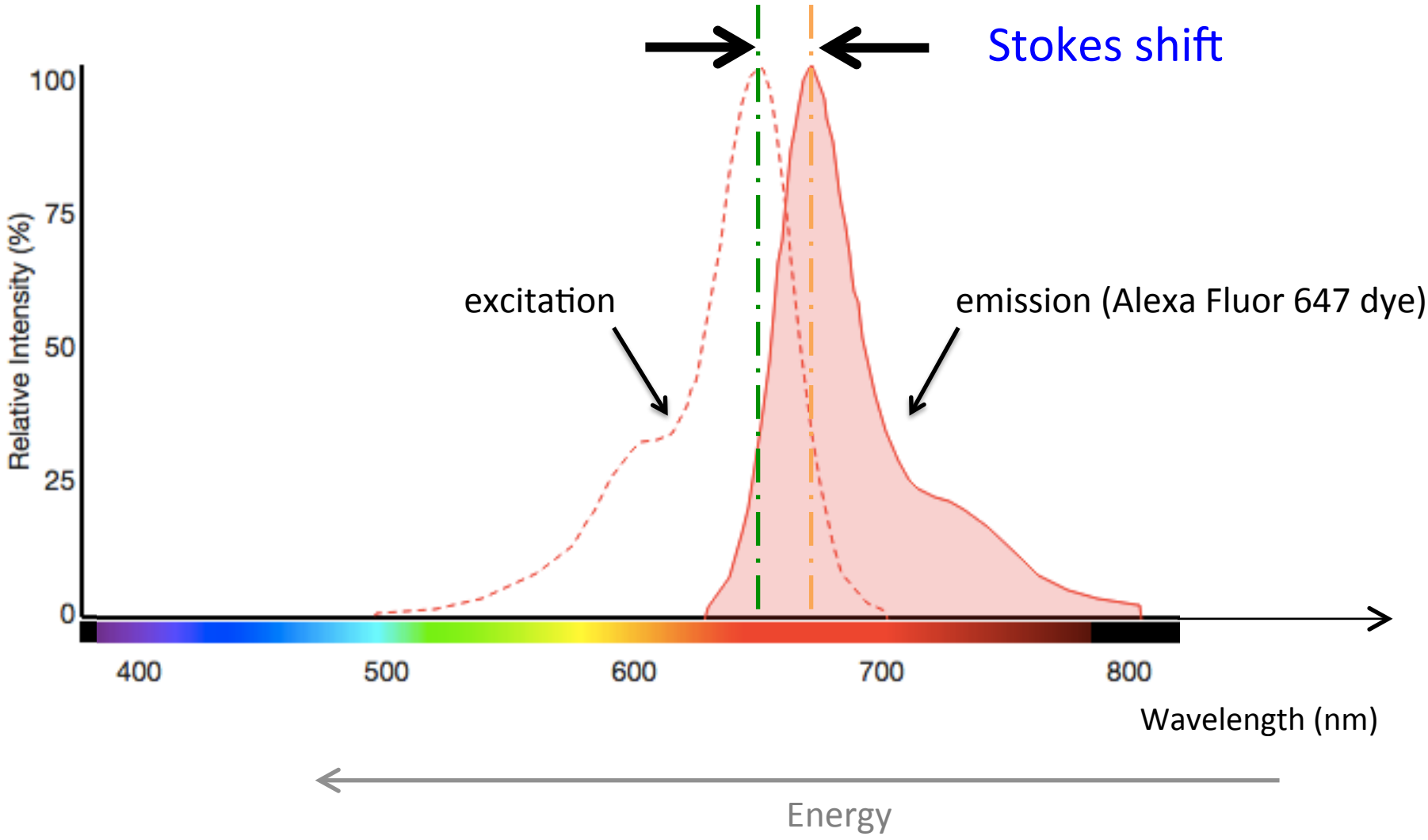
Emitted fluorescence photons have lower energy and longer wavelength than excitation photons

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

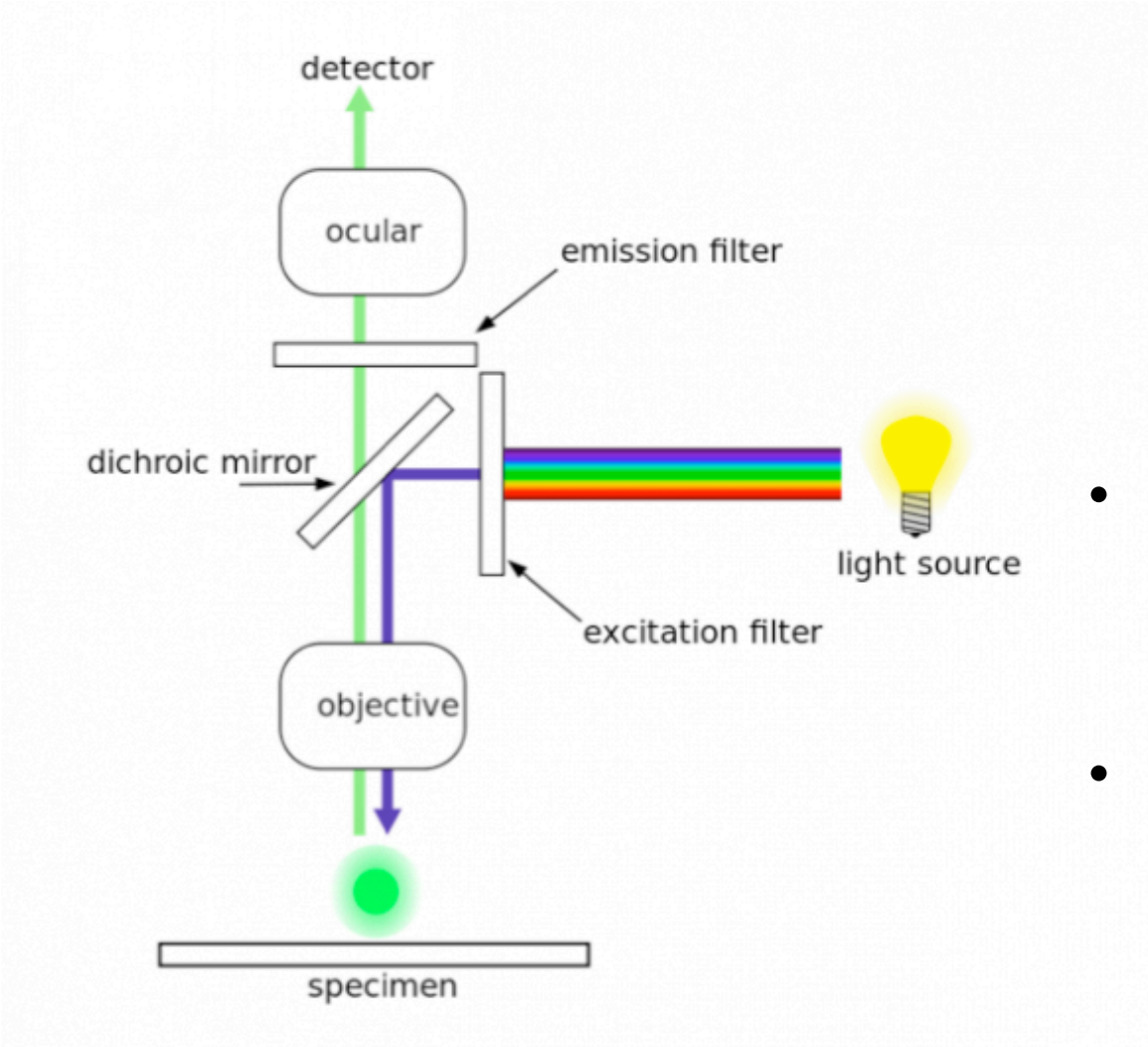
http://photobiology.info/Visser-Rolinski.html

Physical principles of fluorescence:

Stokes (red) shift of emission wavelength



Now in practice: epi-fluorescence microscope

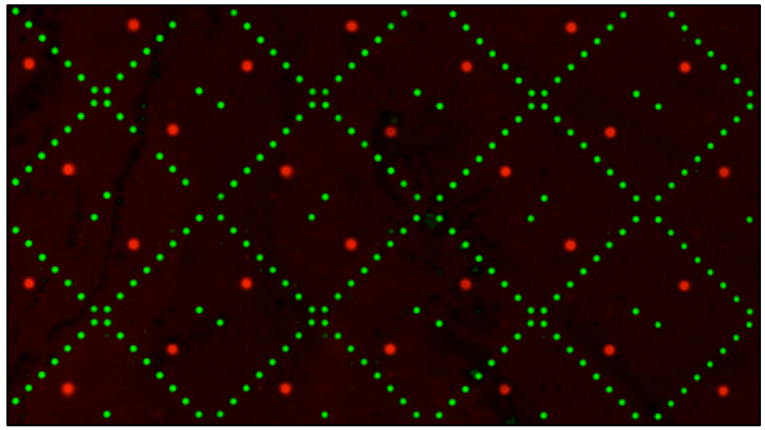


- dichroic mirror
  - reflects excitation light
  - transmits emission light
- barrier / emission filter
  - selects for green light
  - emission ~ 10^{-5} excitation

Today you'll scan your SMM

- Small molecule microarray was incubated
  - with His-tagged FKBP12
  - with anti-His antibody, labeled with Alexa Fluor 647

- Among its ~ 12,000 spots
  - ~ 4,200 small molecules (x2)
  - 4 x 48 positive control spots: rapamycin (known binder of FKBP12)
  - "X" pattern of fluorescein spots

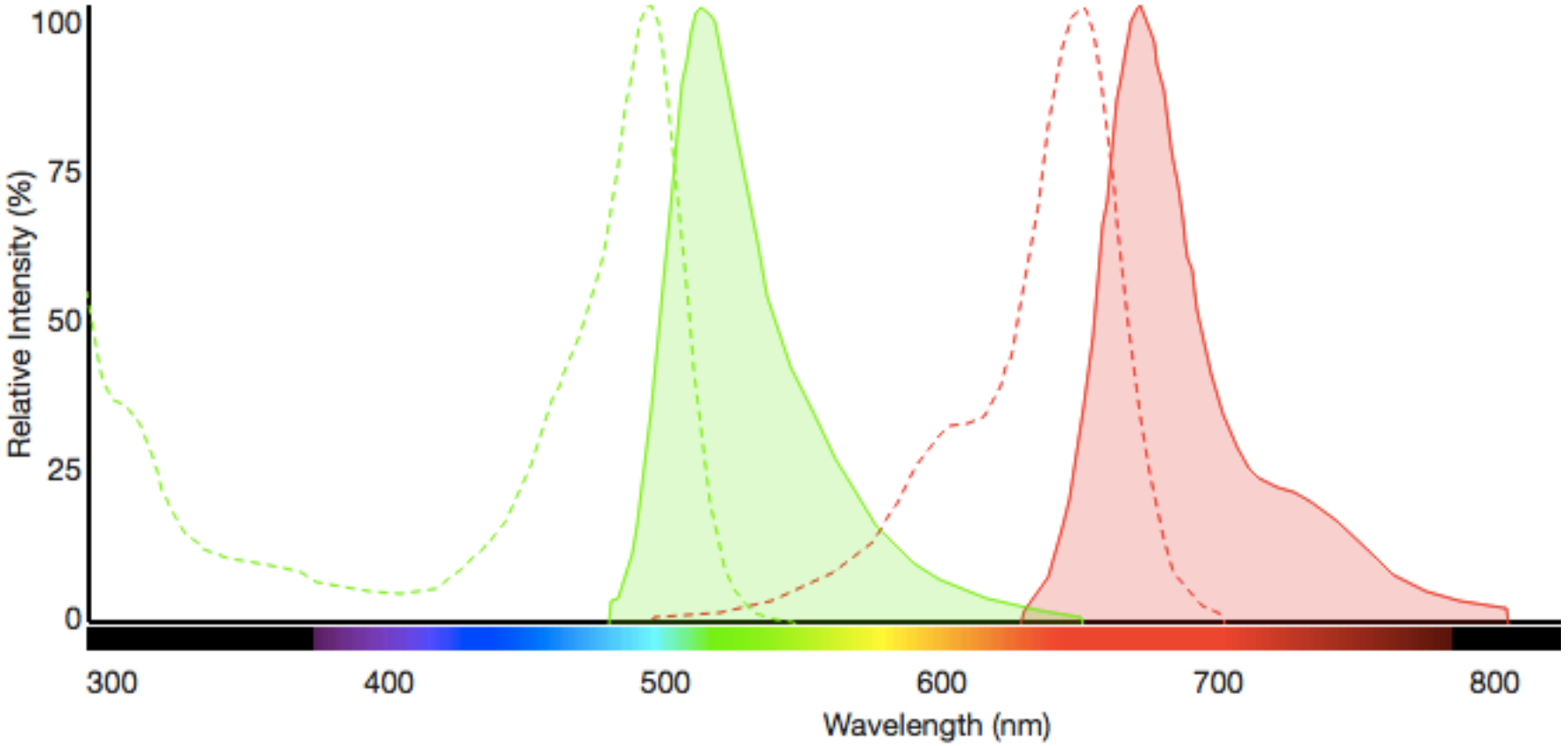


GenePix scanner

2 channels in ~~microscope~~ to visualize 2 fluorescent dyes

2 sets of excitation filter + dichroic mirror + emission filter

	excitation (nm)	emission (nm)
fluorescein	490	525
Alexa Fluor 647	647	665



What to include in your mini-presentation

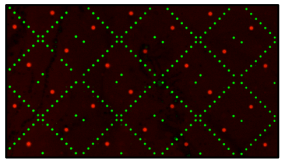
- 3-minute video due at 10pm on Saturday, March 18th
- Homework due M1D6: bulleted outline
  - Introduction: importance of project and info to understand data
  - Results: key findings (state the methods used, but only briefly)
    - quantitative (Z scores, p values)
    - interpretation
  - Conclusion: take-home message, how did your project advance field

- Get an early start on homework due M1D7
  - revised Methods (M1D1 – M1D4)
  - Implication and Future Directions

In lab today

- Update your lab notebook with new barcodes
  - M1D4 repeated with commercial FKBP12 (Abcam)
  - ???

acknowledge the issues / surprises you ran into
explain how you proceeded, propose alternative approaches (cell lysate), speculate on the reasons for the failed experiment



- Demo of scanning by TA Rob Wilson

- Read paper to discuss "future directions" ideas

Also notice Introduction:

(brief) general background
specific background on SOC, CRAC, STIM, Orai, drug discovery
knowledge gap: serious side effects, unknown mechanisms, high IC50
hypothesis: Orai1 inhibitors might have fewer side effects
here we report, we screened, we characterized
"this platform could dramatically increase the speed..."