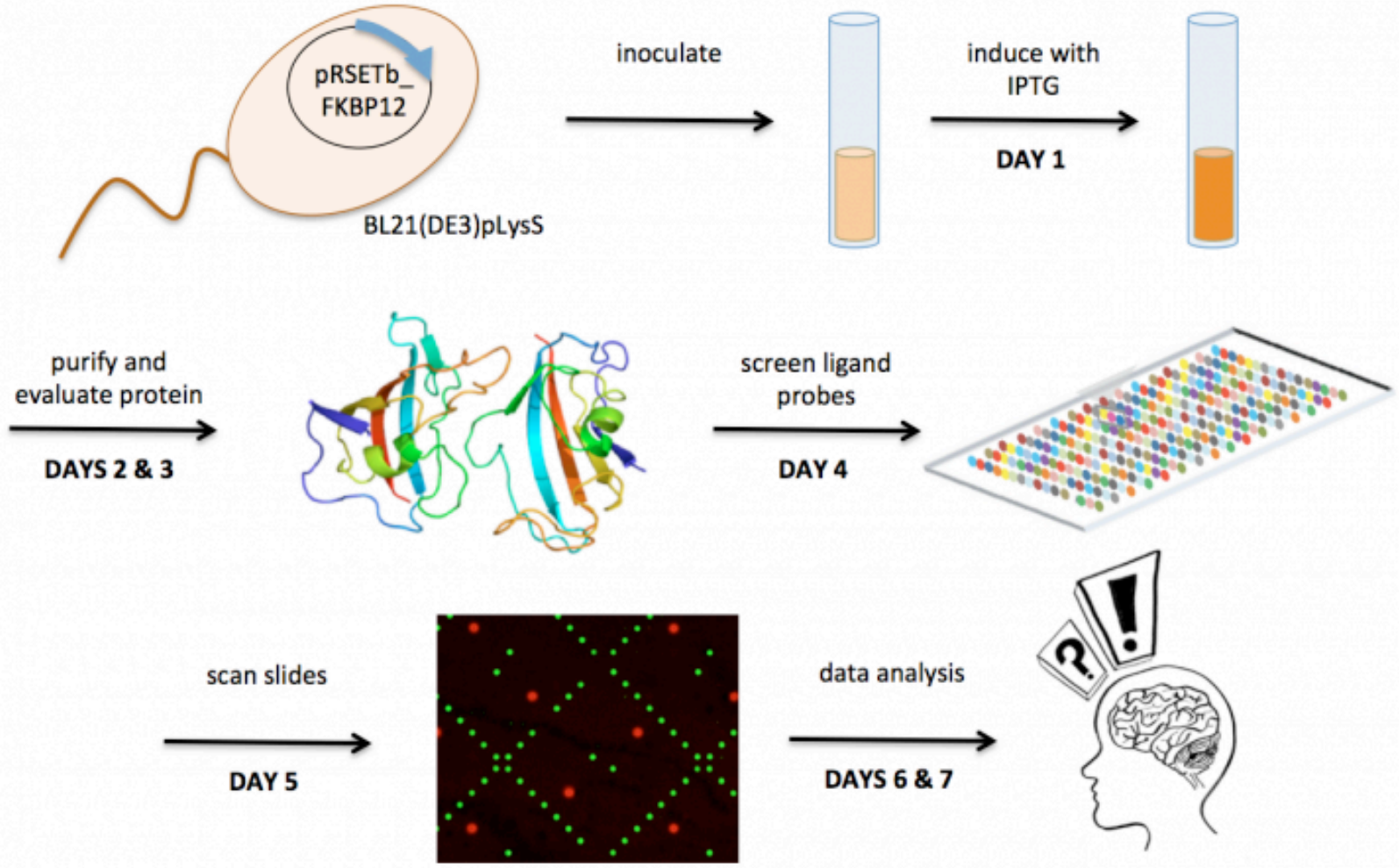
M1D5: Scan slides to identify FKBP12 binders

03/01/2017

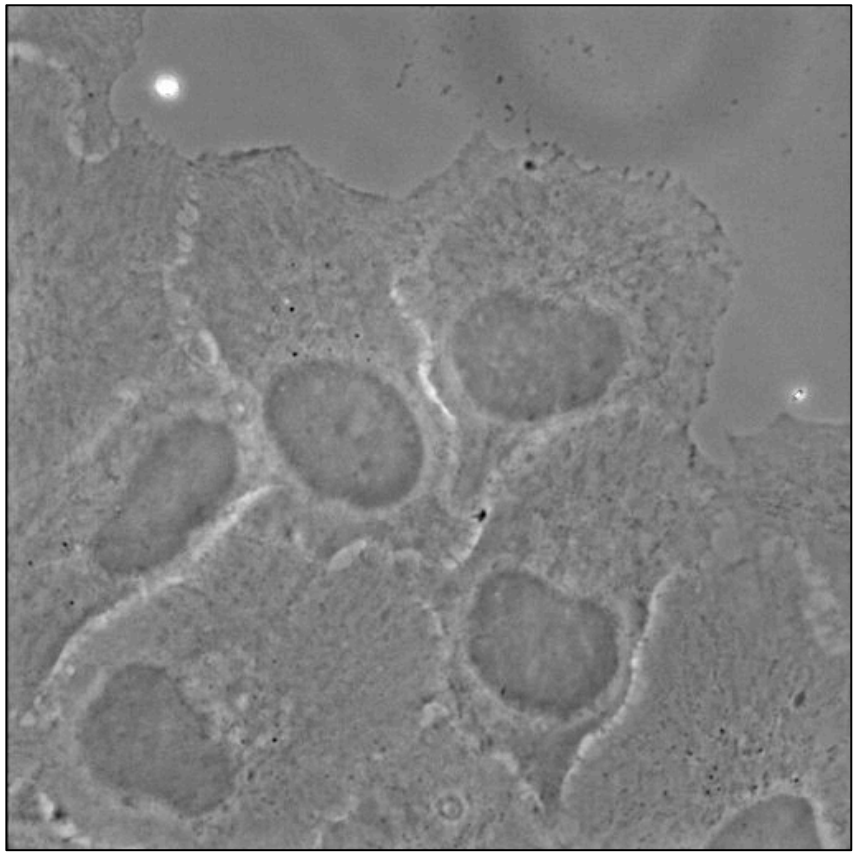
1. Quiz
2. Comm lab workshop- Abstracts
3. Prelab discussion
4. SMM scan in Koehler lab
5. Read Sadaghiani *et al.* in lab during downtime

M1: High-throughput ligand screening

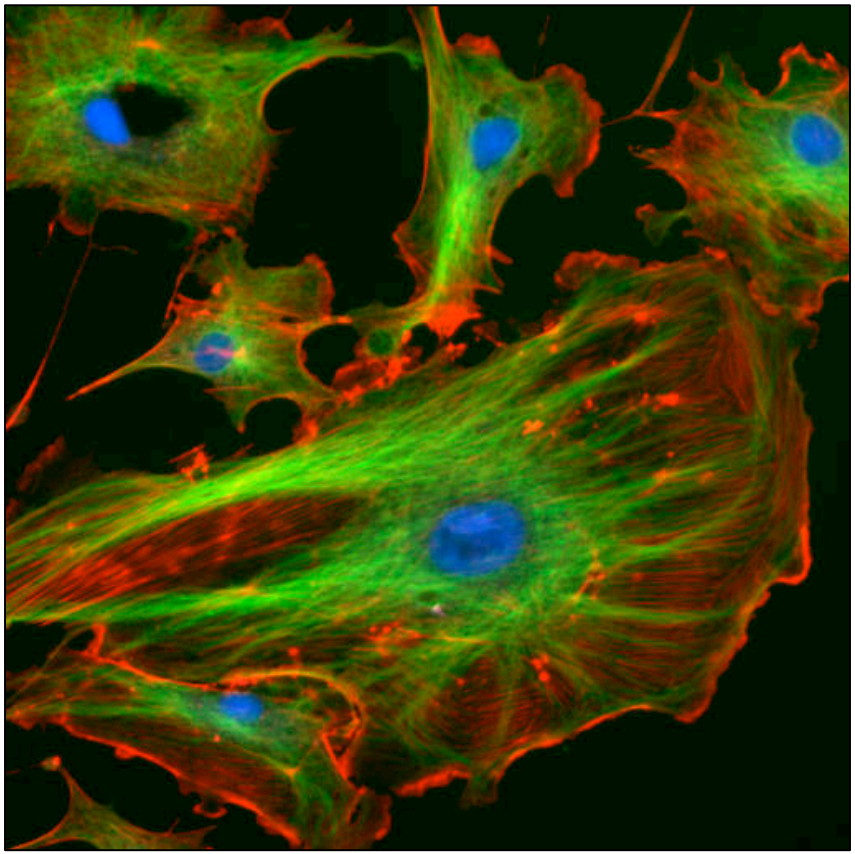


Why is fluorescence imaging so widely used in biology?

nuclei
microtubules
actin

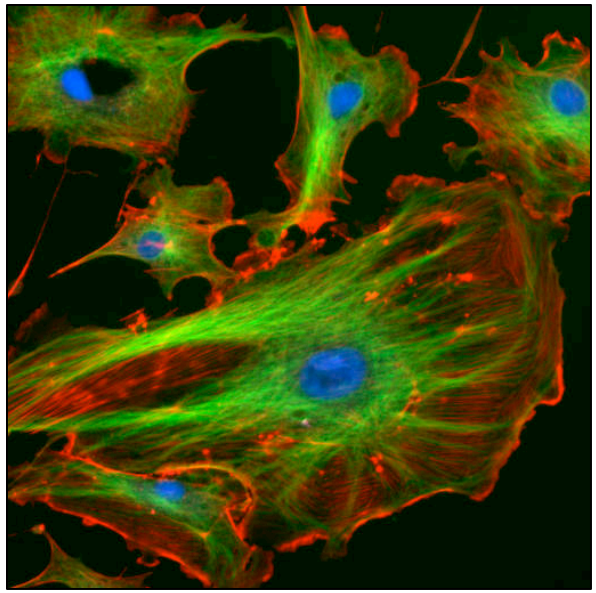


bright-field



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:

Stokes observation

300-400 nm
invisible

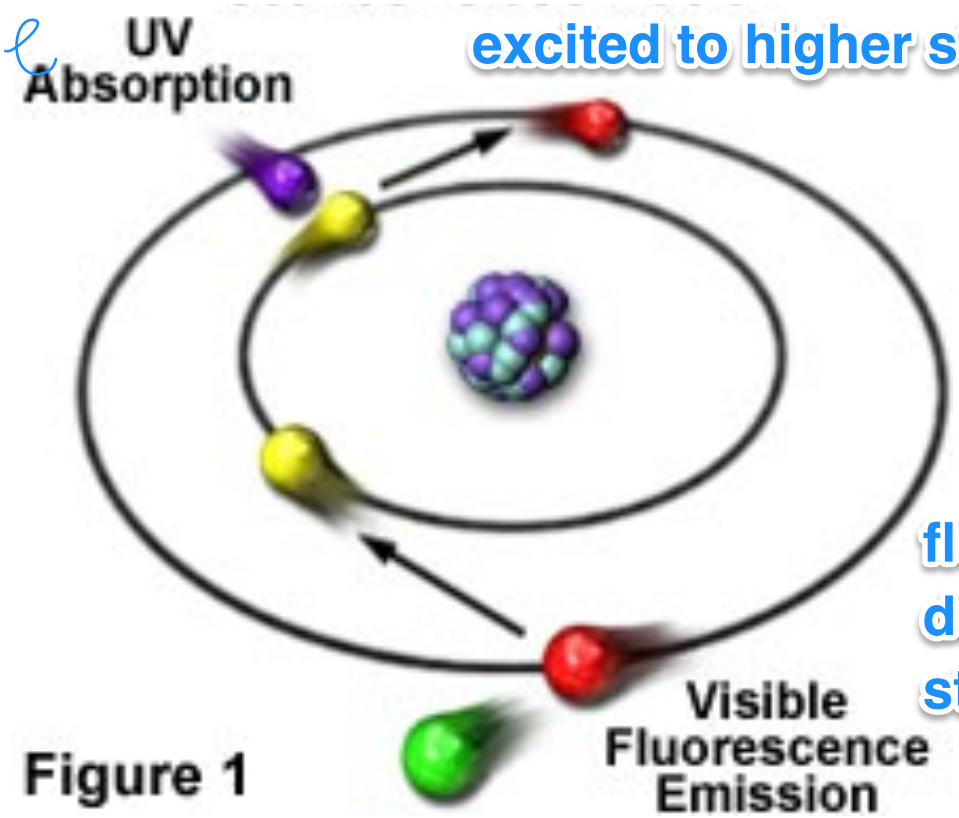


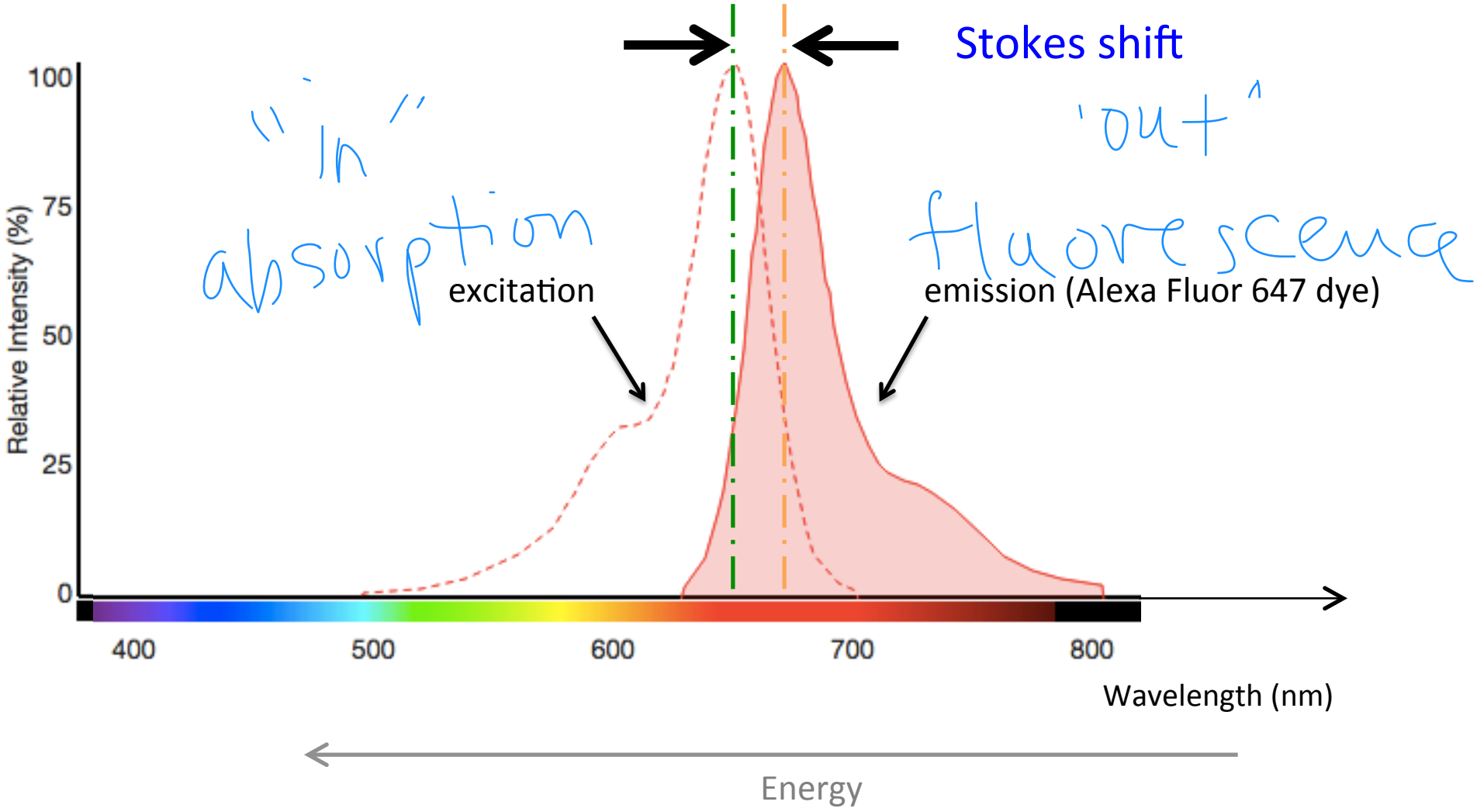
Figure 1

500-600nm
visible

www.olympusmicro.com/

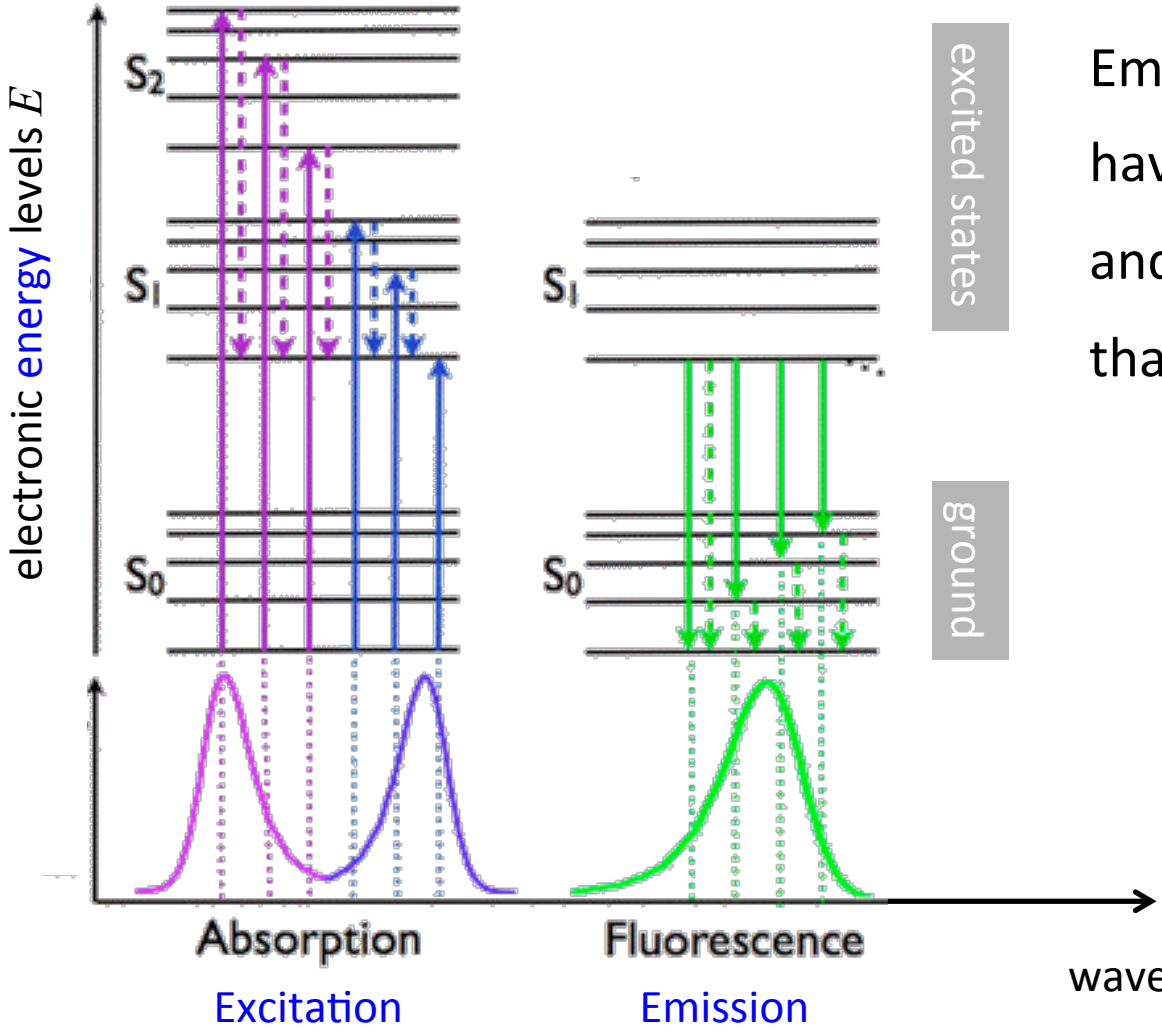
Physical principles of fluorescence:

Stokes (red) shift of emission wavelength



Physical principles of fluorescence:

Jablonski diagram

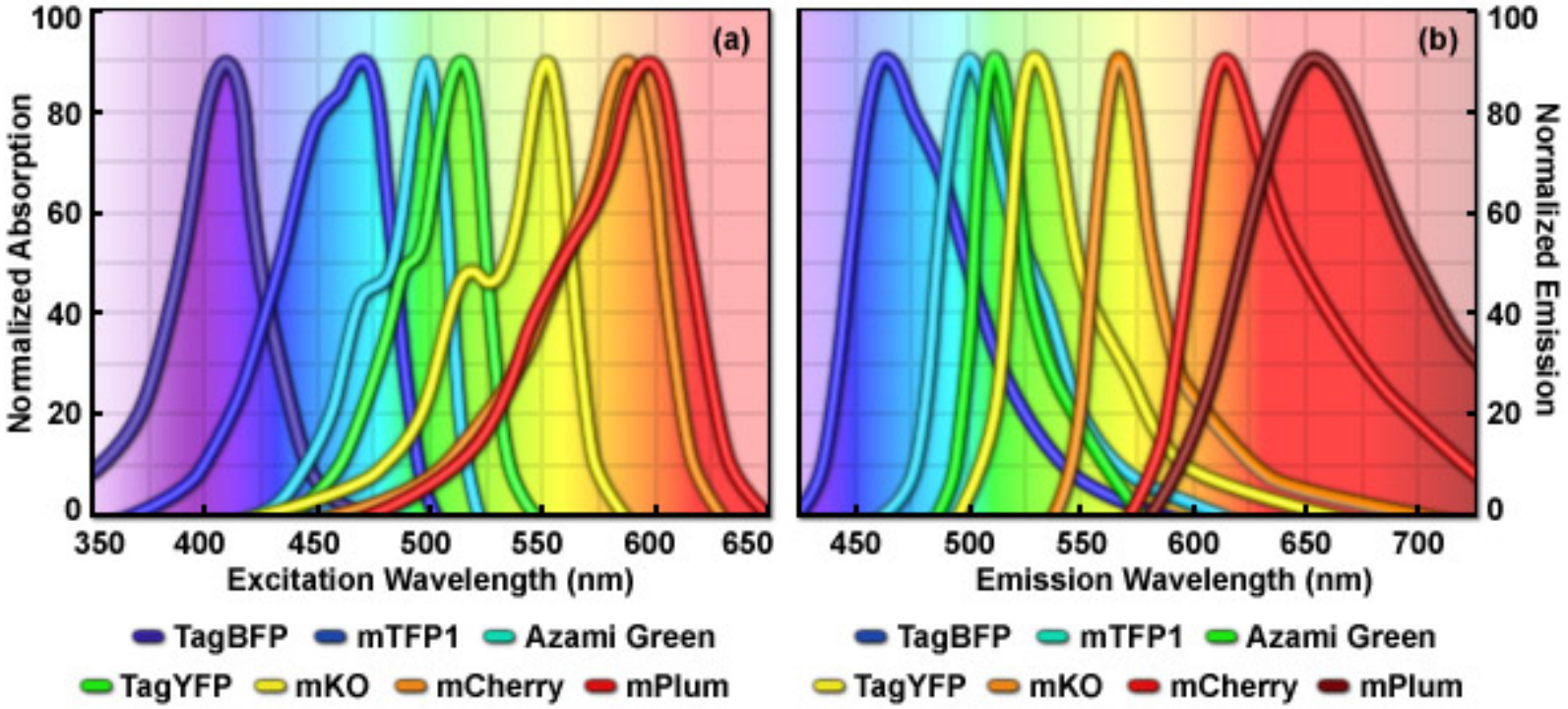


Emitted fluorescence photons have lower energy and longer wavelength (higher) than excitation photons

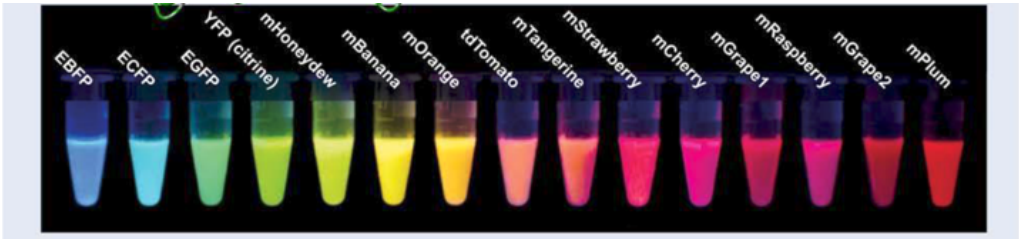
$$E = h\frac{c}{\lambda}$$

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

In practice: engineered fluorescent proteins

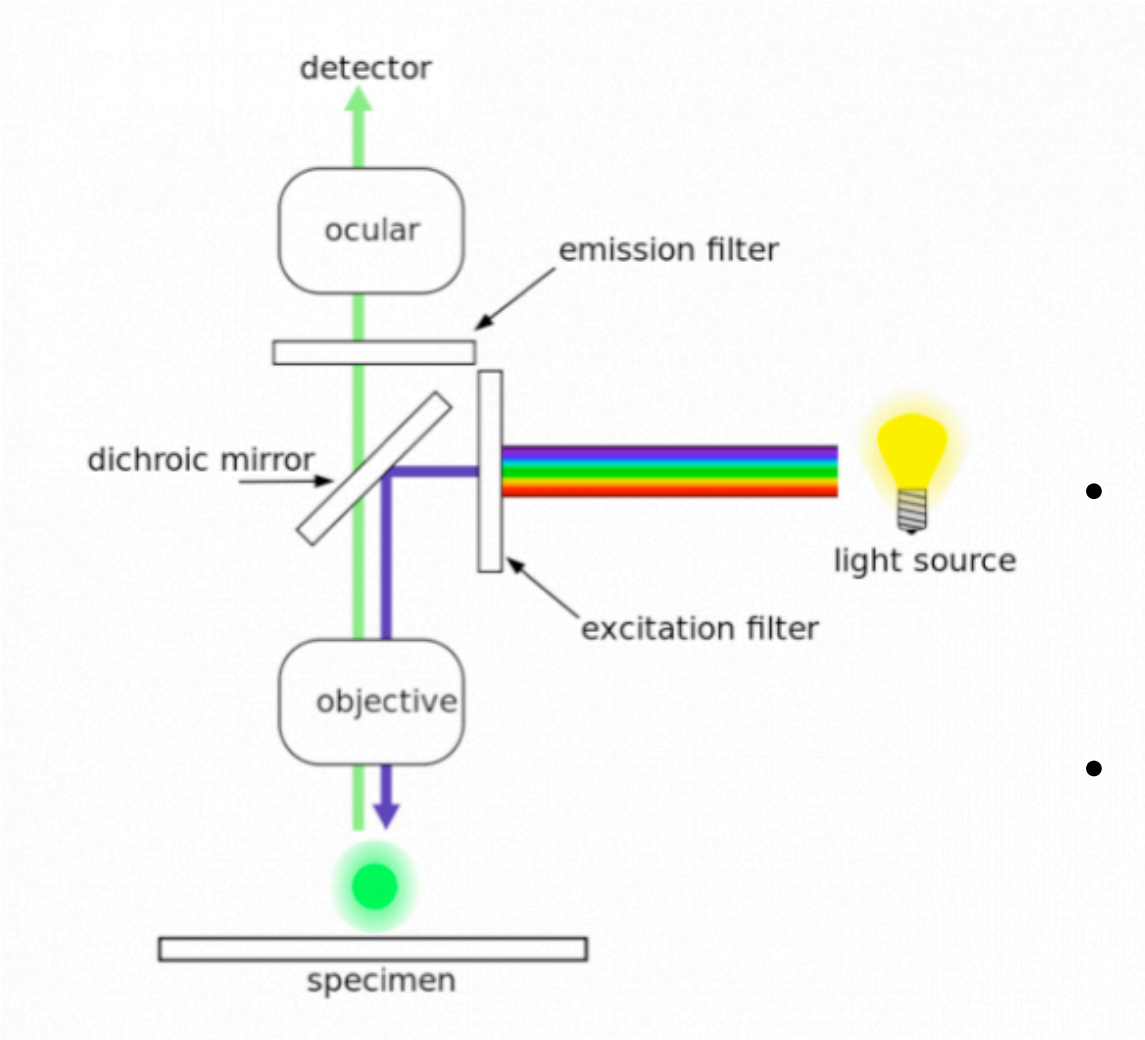


http://zeiss-campus.magnet.fsu.edu



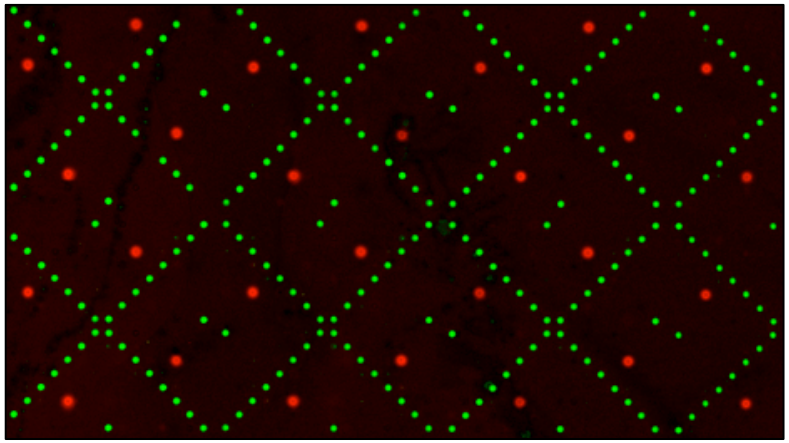
H.C. Ishikawa-Ankerhold et al.

In practice: epi-fluorescence microscope



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

- 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
- 2 channels in scanner to visualize 2 fluorescent dyes



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

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
- Check the wiki assignment page for detailed guidelines, grading rubric and submission details!!

Today in lab:

1. Scan SMM slide at the Koch
2. Read Sadaghiani *et al*

- Homework due Friday, M1D6
  - Bulleted outline of mini presentation
  - Use placeholders for data and conclusions

- Get an early start on homework due M1D7
  - revised Methods and include M1D1 – M1D4 (not *in silico* cloning)
  - Draft Implication and Future Directions

FKBP12 purification figure + results

-discuss the issues/evidence that FKBP12 was not purified
-speculate why the experiment failed
-explain your conclusions
-propose alternative approaches, example using cell lysate or buying commercially available protein

while reading paper in lab today notice:
-brief general background
-specific background: 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..."