# A meandering introduction into the world of light microscopy

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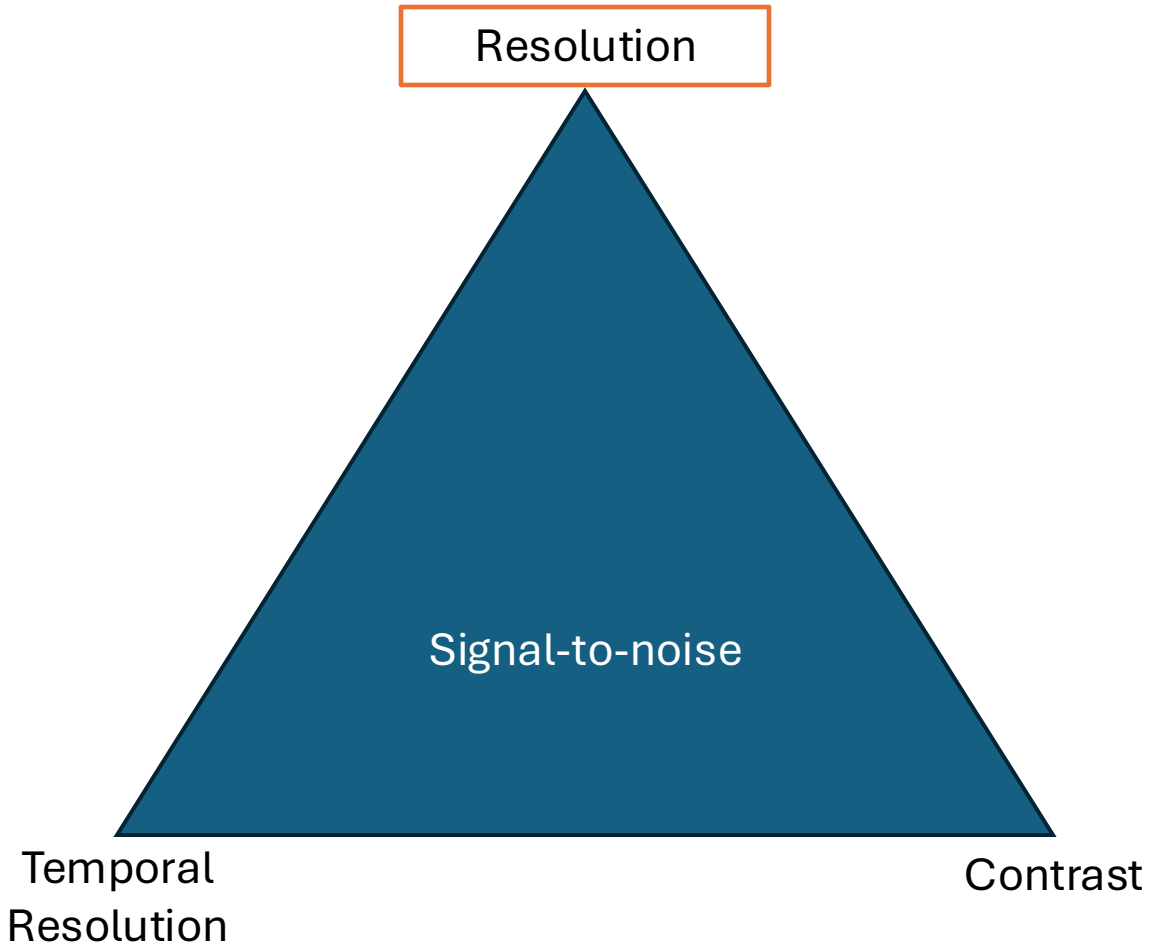
# The trilemma: The challenges of using light



# Magnification: Refraction of light using lenses

- We, particularly Japan and Germany, have gotten really good at making lenses
  - Chromatic aberration corrections
  - Spherical aberration corrections
  - Anti-reflective coatings

- Objectives typically go up to magnification of 100x

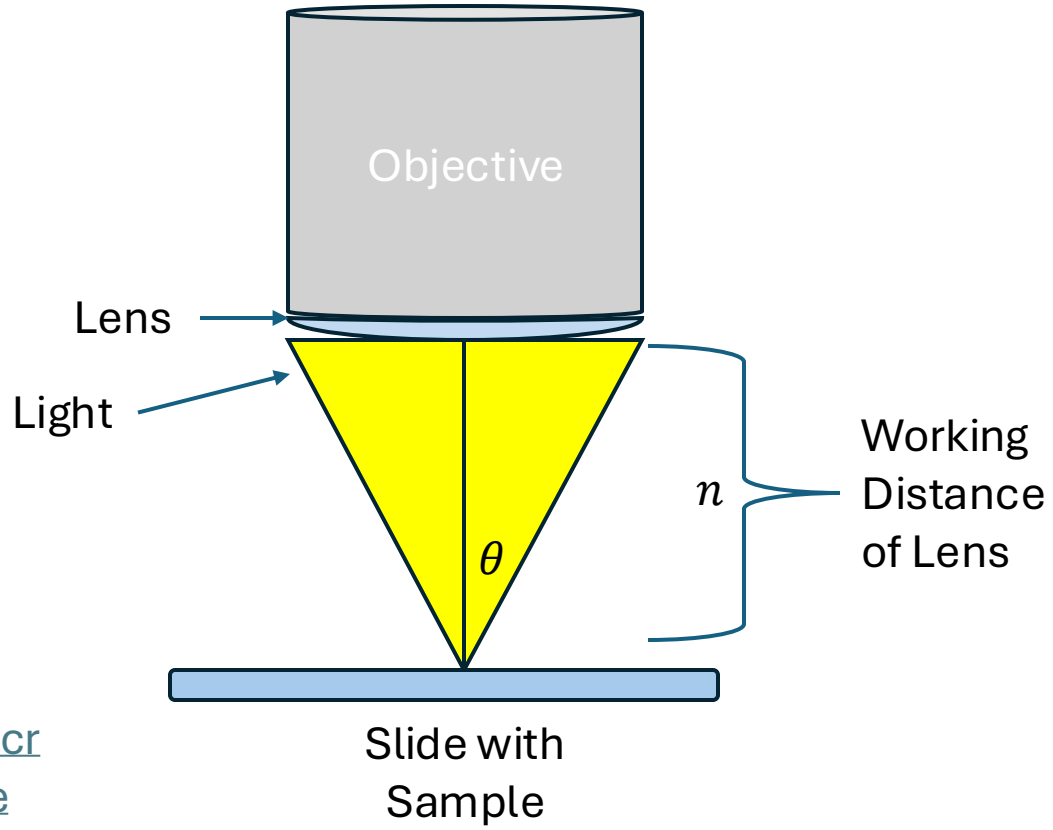
- Not much point in going past 100x when using light due to the diffraction limit

# Diffraction Limit

⚠ Equations on this page! ⚠

$$Resolution = \frac{0.61\lambda}{NA}$$

$$NA = n \sin\theta$$



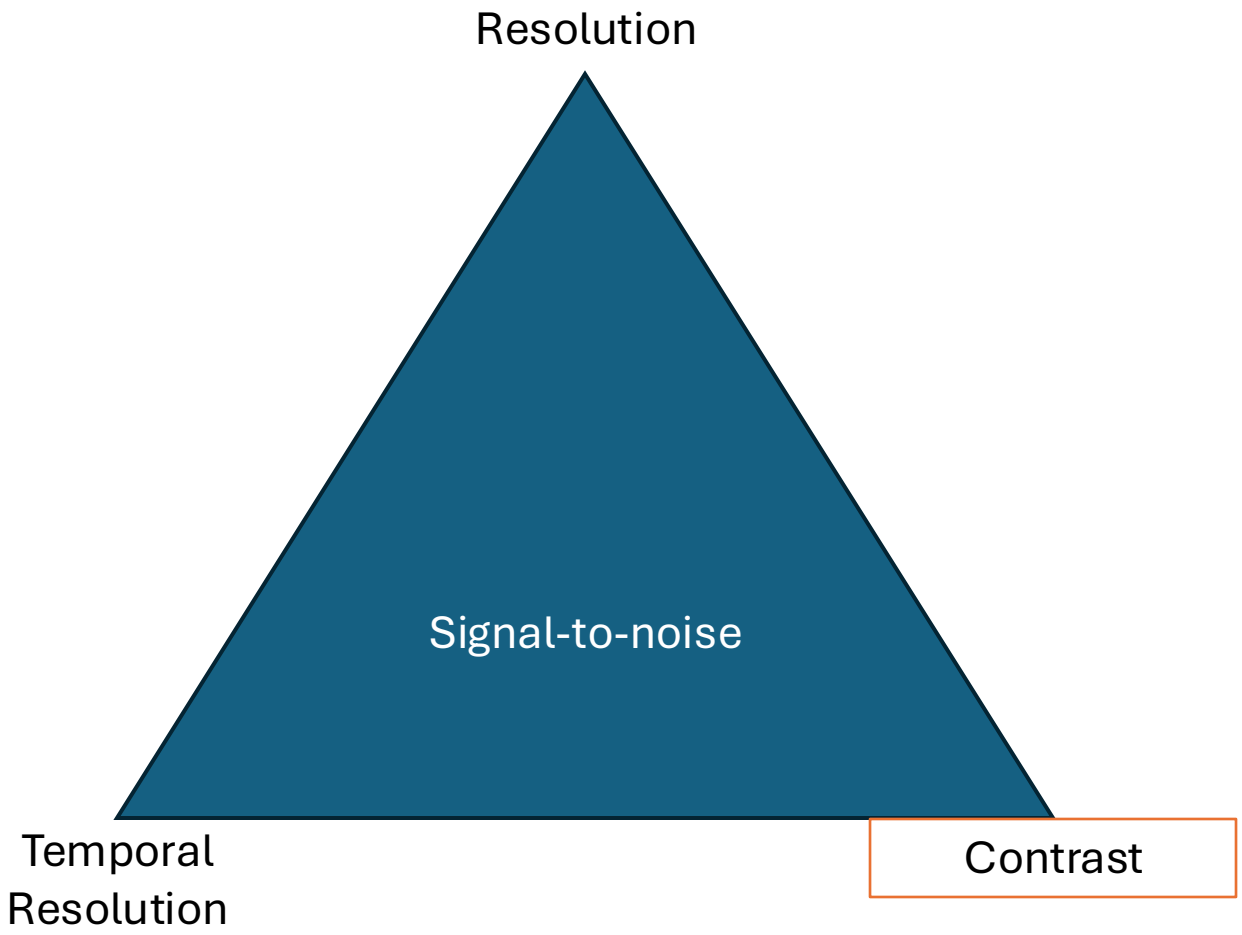

- $n$= refractive index of media (air 1.0, water 1.33, oil 1.515)
- $\theta$ = half-angle of the maximum cone of light that the lens can capture
- $\lambda$ = Wavelength of the light
- NA = Numerical Aperture of objective

https://www.microscopyu.com/microscopy-basics/numerical-aperture

https://www.microscopyu.com/microscopy-basics/refractive-index-index-of-refraction

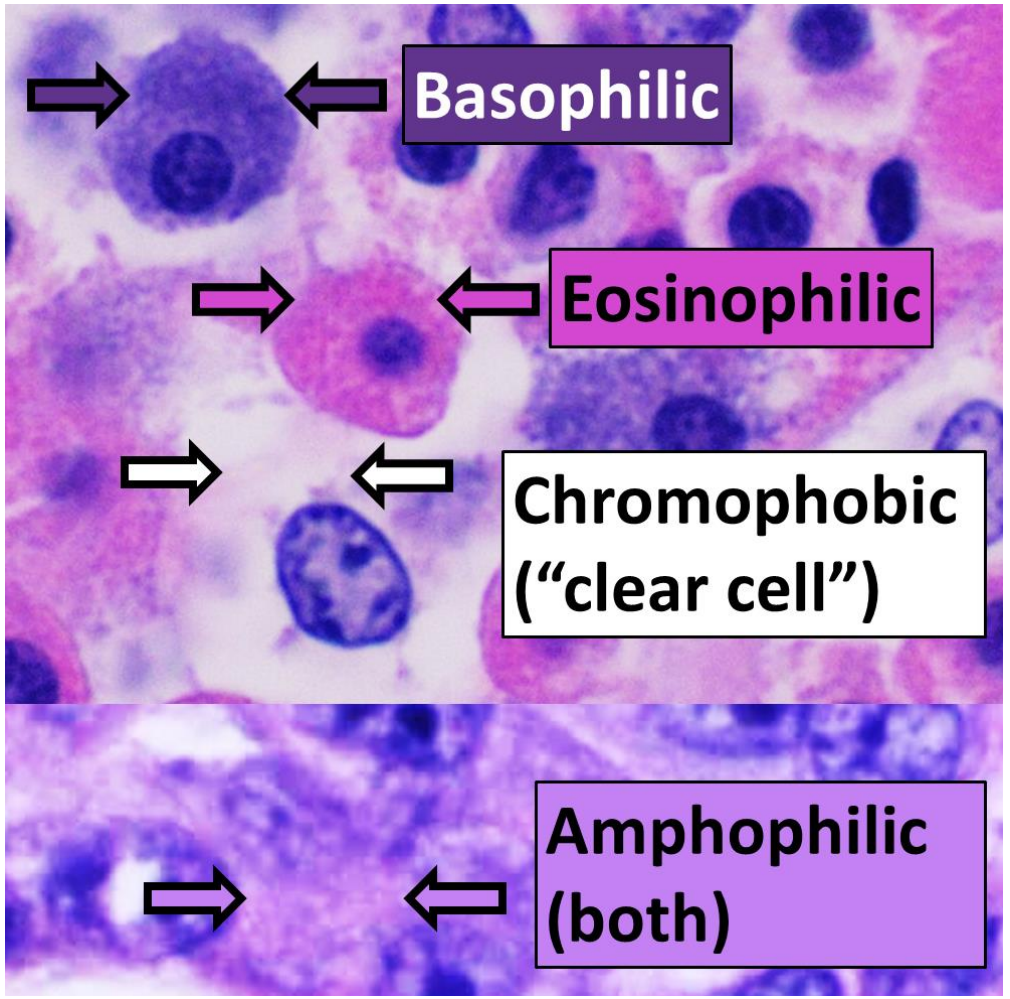
https://www.microscopyu.com/microscopy-basics/resolution

# The trilemma: The challenges of using light



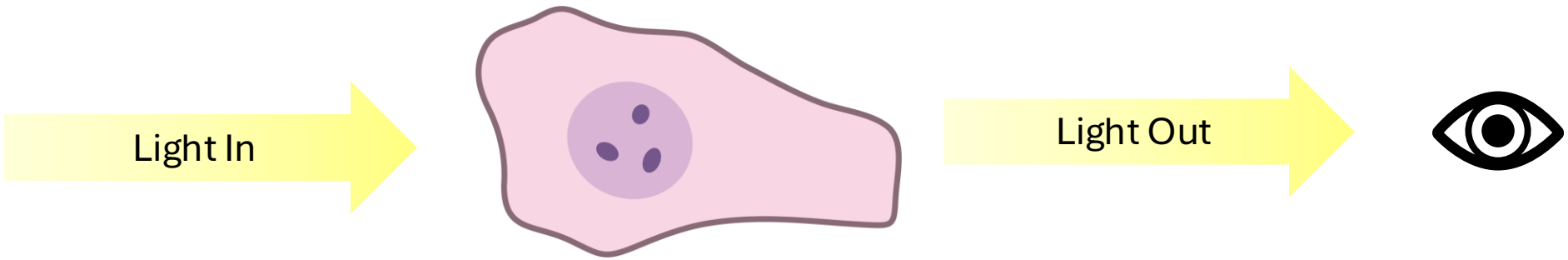
# A brief introduction to contrast

- Classic microscopy techniques typically use stains to provide contrast
- For example: Hematoxylin and Eosin (H&E)



# Fluorescence: A New Kind of Contrast

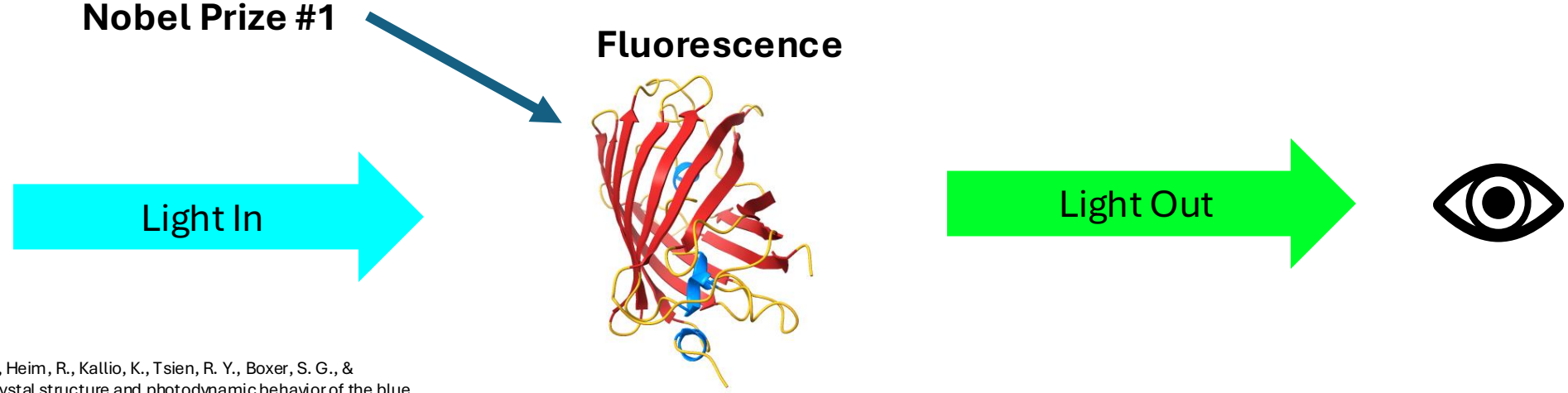
**Transmitted Light**




Very Thin, Semi-transparent Stained Sample

**Nobel Prize #1**

**Fluorescence**

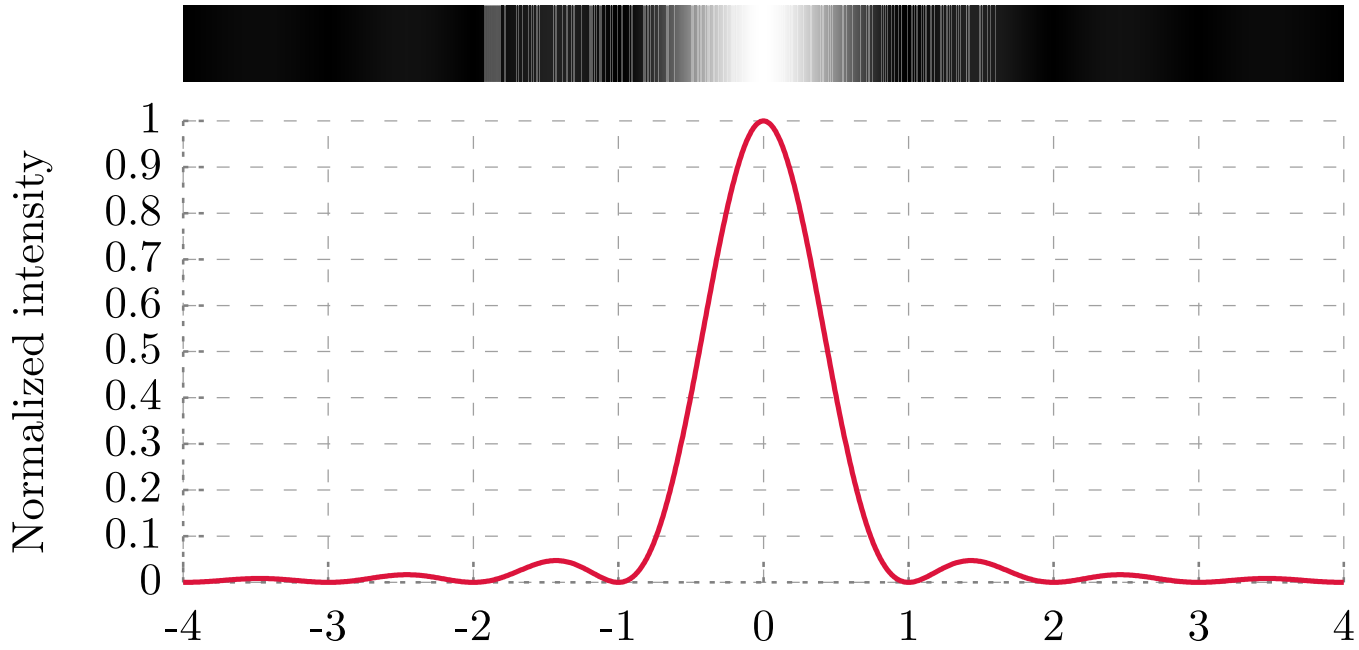



Fluorescent Protein

Wachter, R. M., King, B. A., Heim, R., Kallio, K., Tsien, R. Y., Boxer, S. G., & Remington, S. J. (1997). Crystal structure and photodynamic behavior of the blue emission variant Y66H/Y145F of green fluorescent protein. Biochemistry, 36(32), 9759–9765. https://doi.org/10.1021/bi970563w

NIAID Visual & Medical Arts. (10/7/2024). Vero Cell. NIAID NIH BIOART Source. bioart.niaid.nih.gov/bioart/540

# Diffraction Pattern



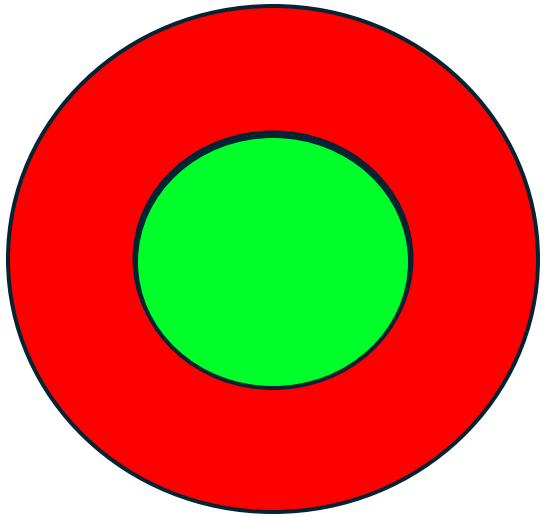

This center peak looks like a Guassian distribution

# Breaking the diffraction limit: 2014 Nobel Prize in Chemistry

- Fluorophores are point sources of light that will create a a diffraction pattern

- If you could **isolate single fluorophores in time**, you could fit the diffraction pattern with a 2D Gaussian

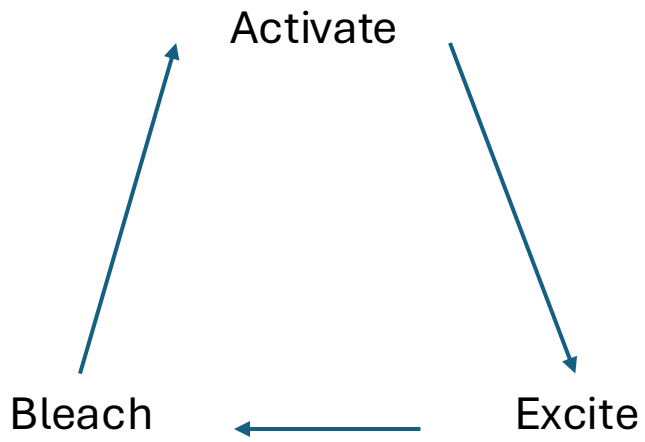
# Stimulated Emission Depletion Microscopy



- **Creates a sub-diffraction-limited excitation zone**
- **Repress the surrounding emission by creating a depletion zone**
- **Scan the region to reconstruct a super resolution image**

https://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/stedconcept/indexflash.html

# PALM: Photoactivated Localization Microscopy




- Use a special PA-GFP that uses UV light to convert inert fluorophores to an active one
- Only use small amount of UV light to convert a small sample
- Intentionally bleach active PA-GFPs
- Then start new activation cycle

https://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/introduction.html

# Cost-Benefit of Enhanced Resolution