Summary

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When to use which microscope

- Always think about the application
- Resolution - spatial and temporal
- Sample mounting
- Sample thickness

Widefield

- Highest sensitivity
- Fast - full frame at once
- High out of focus light contribution - reduced a lot by deconvolution
- Good for routine fixed cell imaging or any live cell imaging, especially weak fluorescence.

Laser Scanning Confocal Microscope (LSM)

- Optical sectioning
- Thicker samples
- Samples with high background
- Steerable laser for FRAP/FLIP and photo activation
- Spectral detection and linear unmixing
- Relatively slow - point scans
- About 10x LESS sensitive than widefield
- Good for fixed samples, or thicker, but bright live samples or dynamics (eg FRAP)

Spinning disk confocal

- Optical sectioning - not as good as LSM
- Fast acquisition
- Steerable laser for FRAP/FLIP and photo activation
- Compromise in sensitivity, about 4x LESS than widefield, but at lest 2x better than LSM
- Good for live cell imaging, especially in medium thickness samples. Dynamics via FRAP etc…

Lightsheet microsocope

- Larger samples up to > 1 mm
- Sensitive and low light exposure
- Fast acquisition
- Very large data sets
- Good for large live samples, or very long acquisition

2-photon confocal microscope

- Allows imaging at roughly 2x depth
- Gentle to live samples as IR photons have low absorption and only excite in focus plane
- Second harmonic generation, label free imaging of some samples
- Slow as a point scanning method
- Expensive laser needed
- Lower resolution as longer wavelength used
- Good for live imaging at high depths (>50 um) or live animal imaging

Super resolution imaging

- Increased resolution
- Slow - SIM 15 images per plane, SMLM 1000-100,000 images per plane, STED small pixels, so relatively slow
- High light doses
- At least 10x as much work as conventional imaging
- Good if you really need that extra resolution.

Survey

surveymonkey.com/r/R7HWPGW