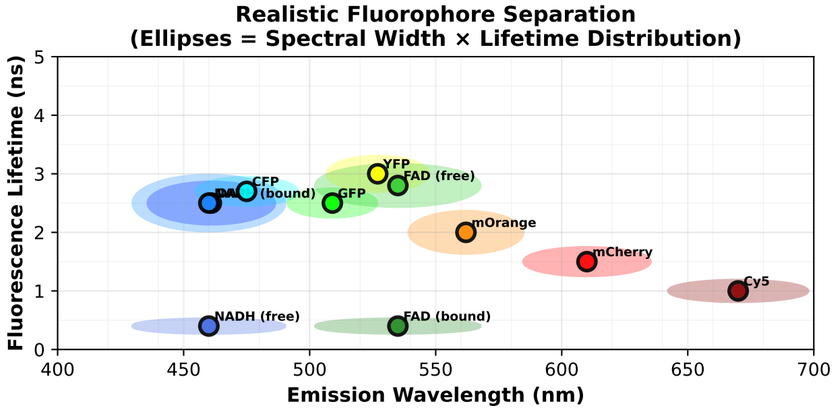
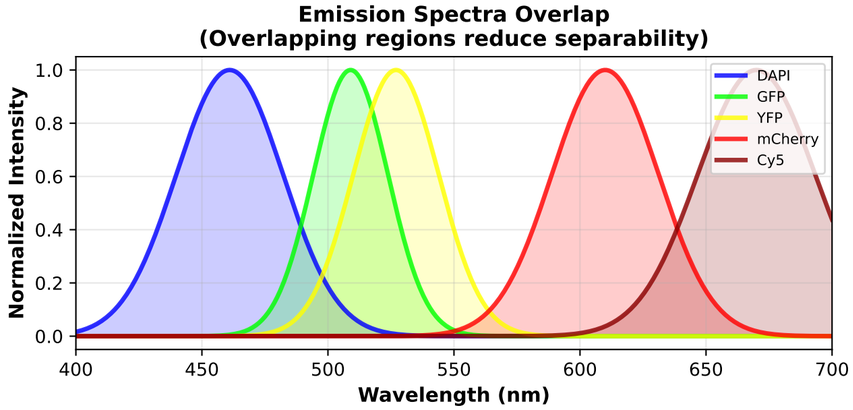
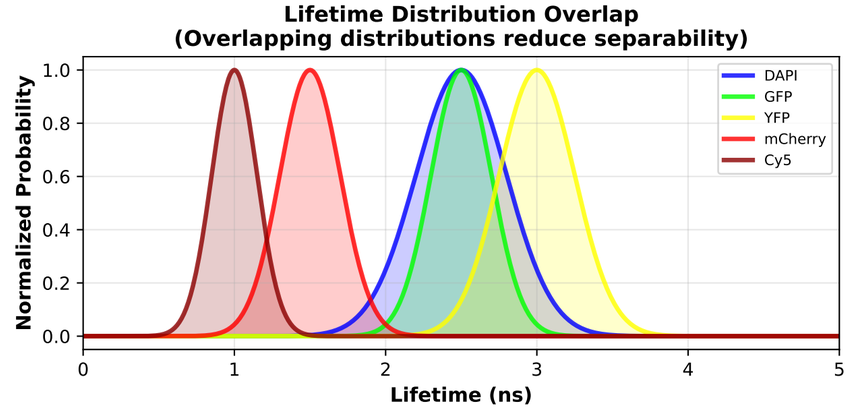
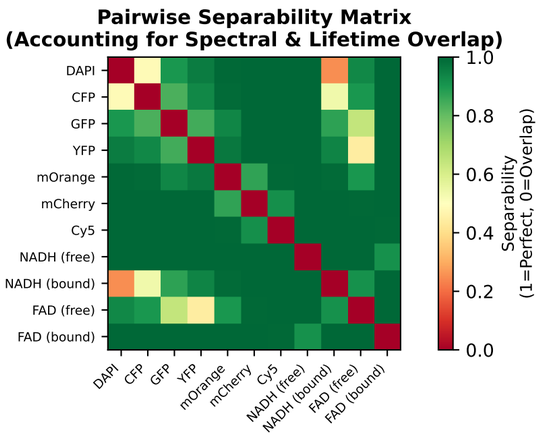
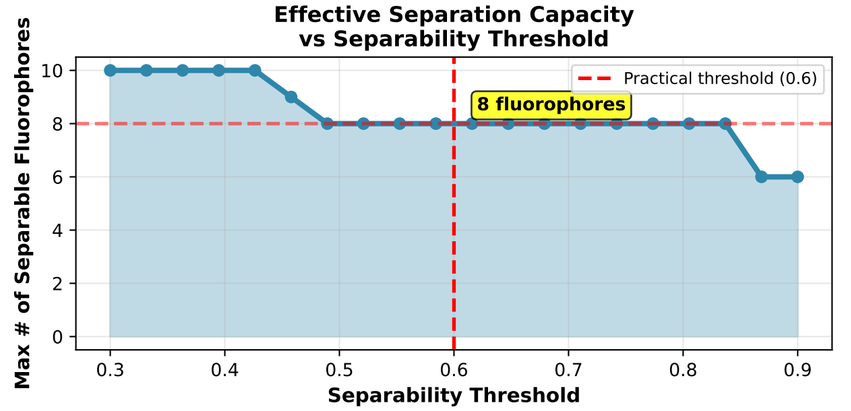
Realistic Fluorophore Separation Capacity Analysis
Accounting for Spectral Overlap and Lifetime Distributions

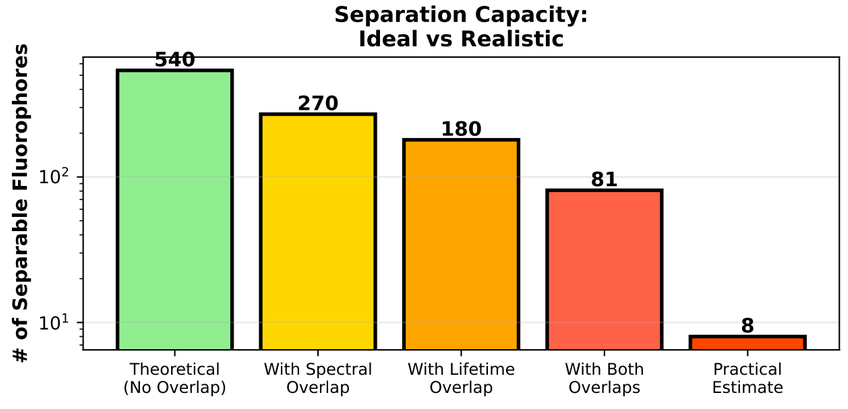


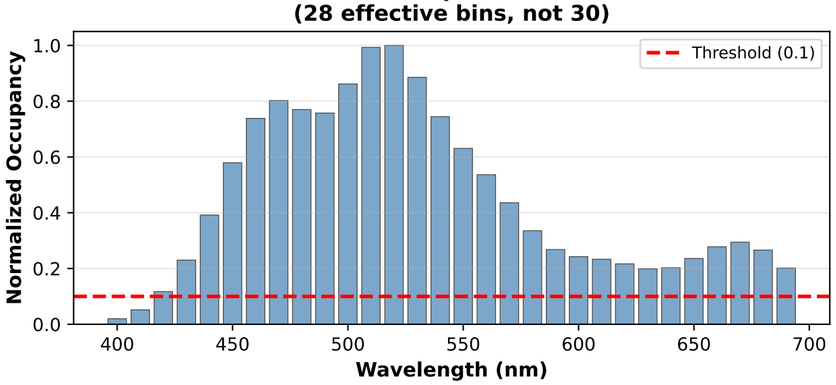


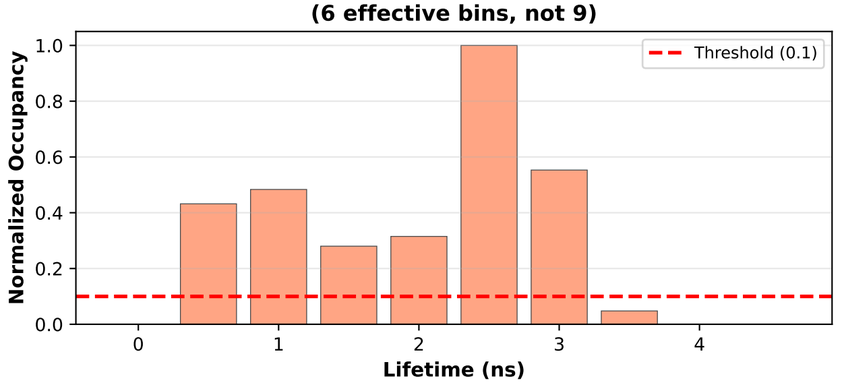










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REALISTIC SEPARATION CAPACITY ANALYSIS

Instrument Specs:
• Wavelength resolution: 10 nm
• Lifetime resolution: 0.5 ns
• Polarization states: 2

Theoretical vs Realistic:
• Theoretical bins: 30 (λ) × 9 (τ) × 2 (pol) = 540
• Effective spectral bins: 28 (not 30!)
• Effective lifetime bins: 6 (not 9!)
• Realistic capacity: 8 fluorophores

Key Limitations:
1. Spectral overlap: Emission spectra are broad
   (FWHM = 35-65 nm, not single wavelength)

2. Lifetime distributions: Not single values
   (σ = 0.1-0.4 ns variation)

3. Crosstalk: Overlapping signals require unmixing

Practical Recommendations:
• Conservative panel: 3-5 fluorophores
• Moderate panel: 5-8 fluorophores
• Aggressive panel: 8-8 fluorophores
  (requires advanced unmixing)

Best Strategy:
• Maximize wavelength separation (most powerful)
• Use lifetime for same-wavelength species
  (e.g., NADH free/bound)
• Polarization adds modest 2× benefit

Example Well-Separated Panel (5 fluorophores):
1. DAPI (461 nm, 2.5 ns)
2. GFP (509 nm, 2.5 ns) - 48 nm separation
3. YFP (527 nm, 3.0 ns) - 18 nm + lifetime
4. mCherry (610 nm, 1.5 ns) - 83 nm separation
5. Cy5 (670 nm, 1.0 ns) - 60 nm separation
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