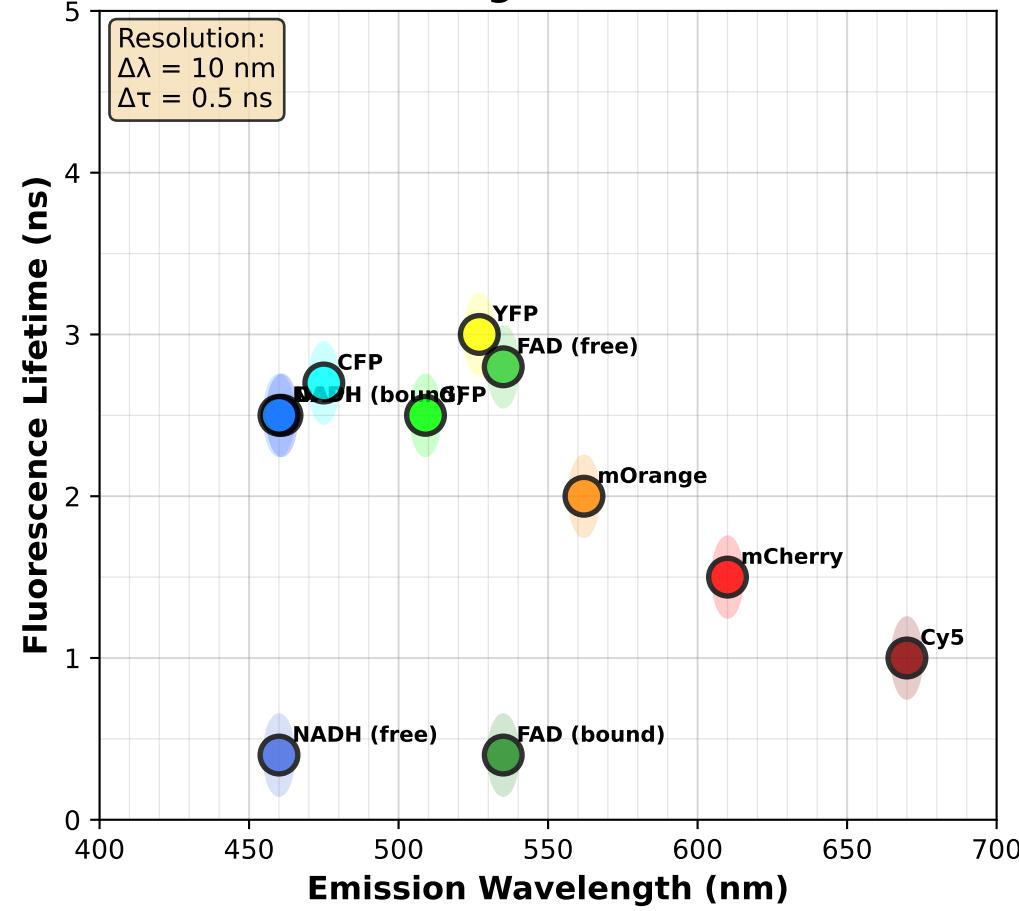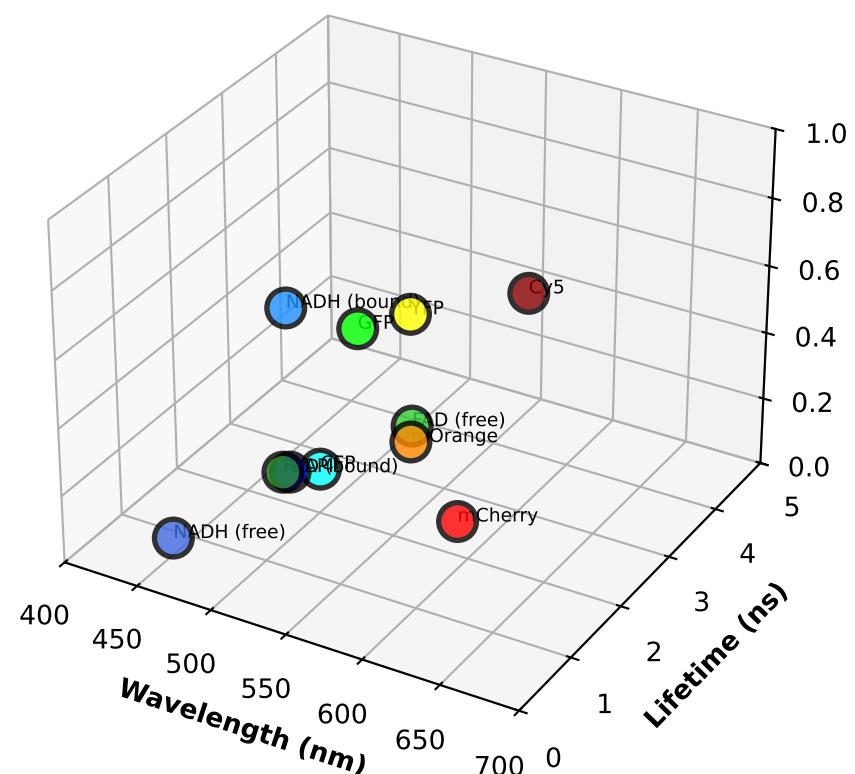# Fluorophore Separation Capacity Analysis
Resolution: λ=10nm, τ=0.5ns, Pol=2 states

2D Fluorophore Separation Space (Wavelength × Lifetime)
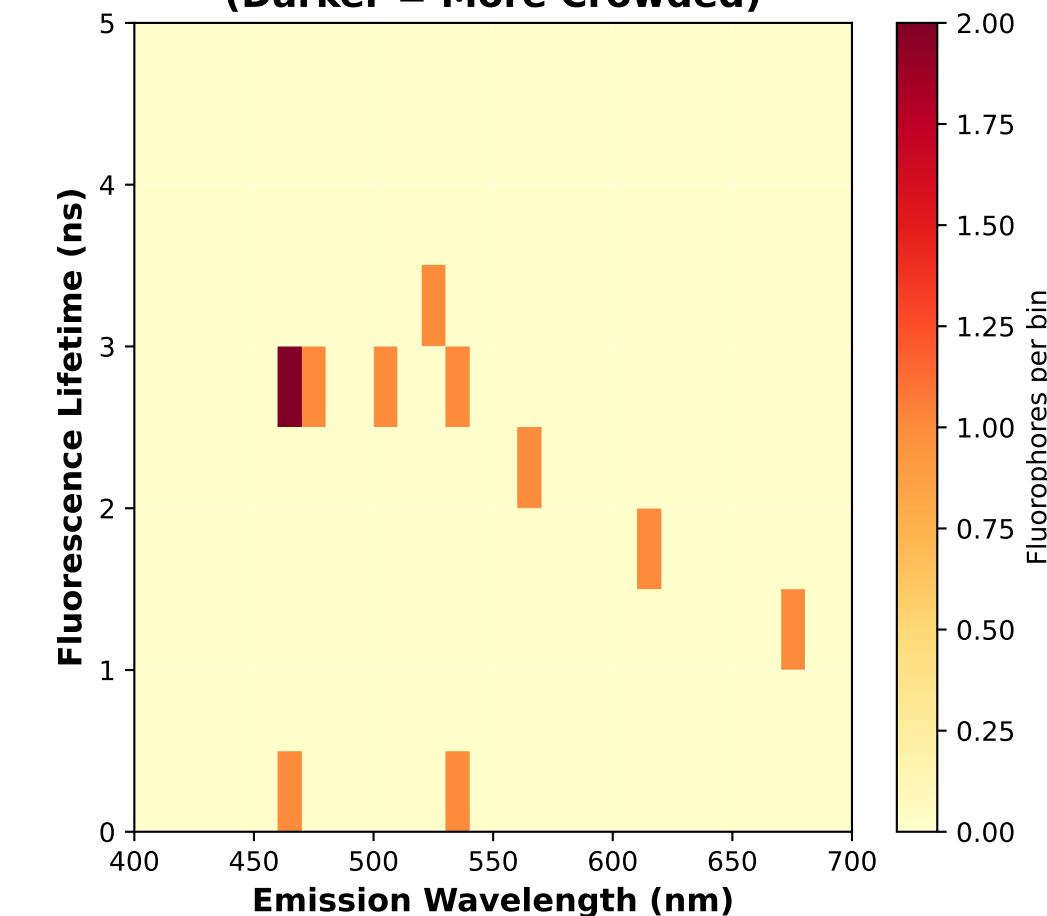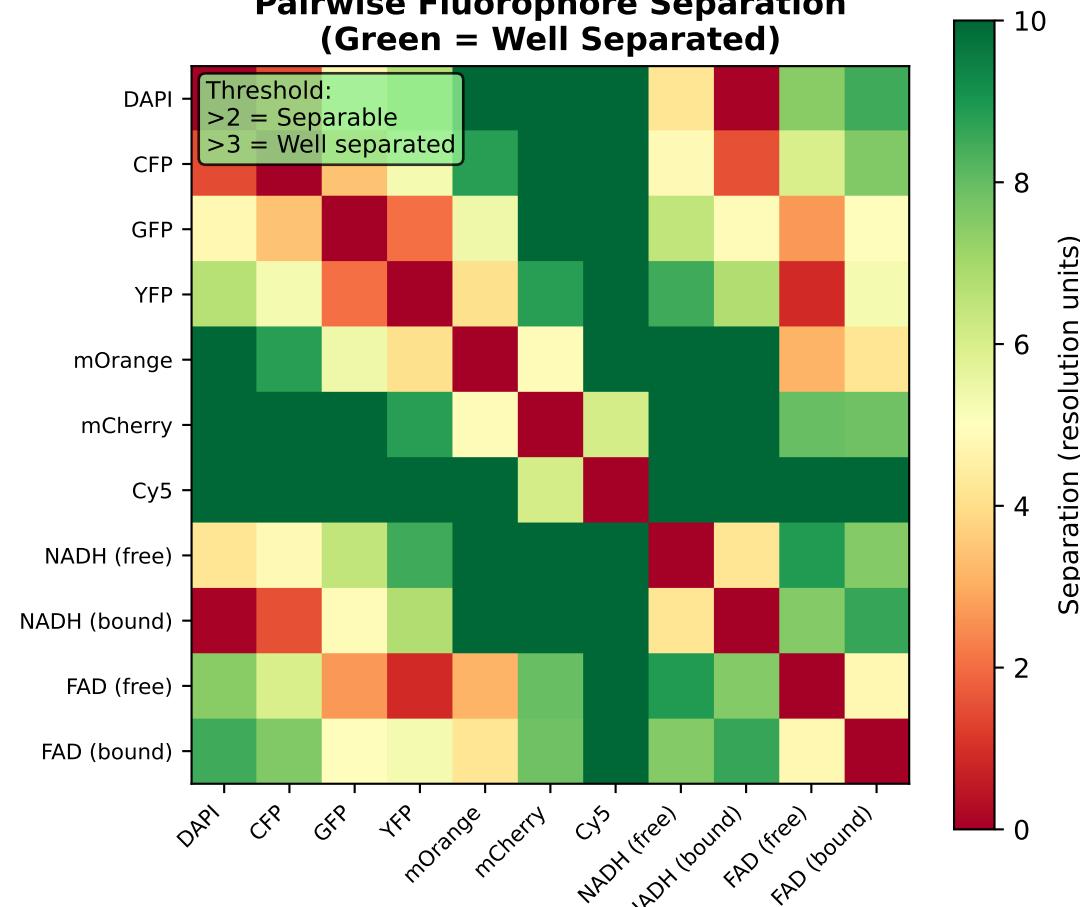
Resolution:
Δλ = 10 nm
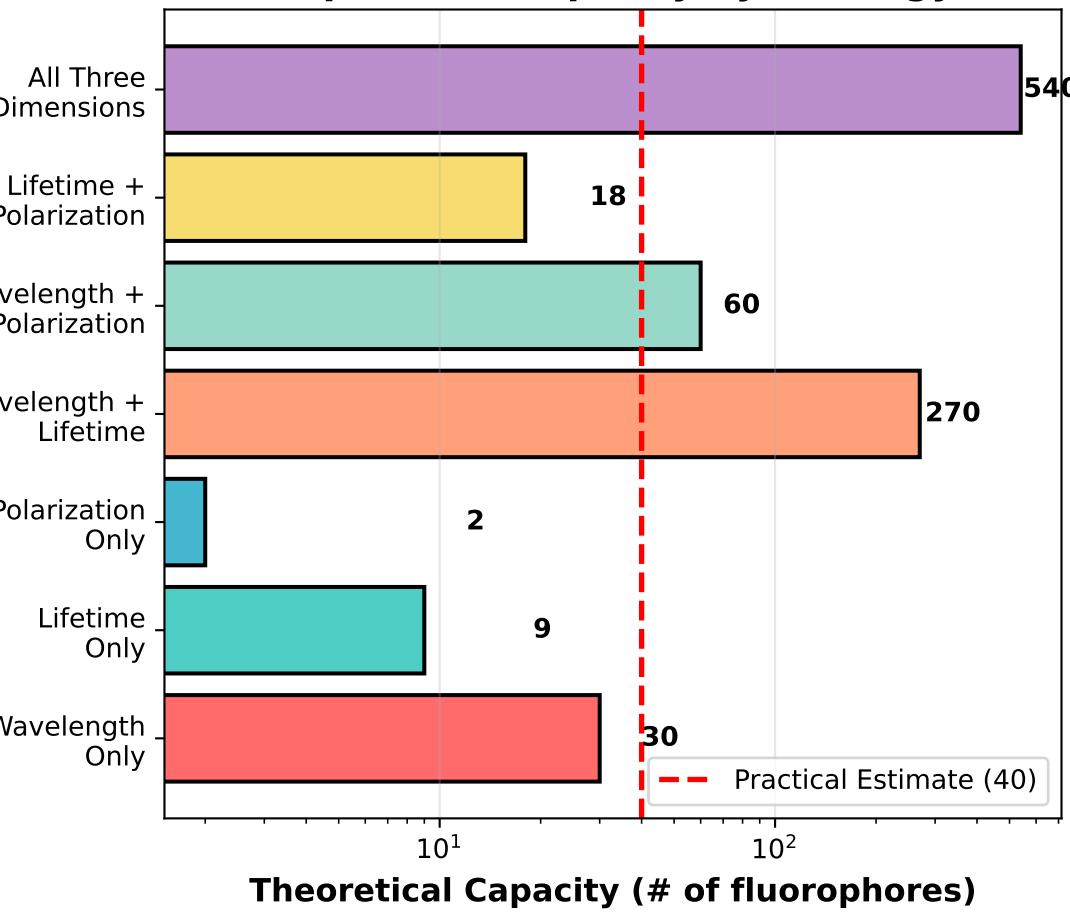Δτ = 0.5 ns

3D Fluorophore Separation Space (Wavelength × Lifetime × Polarization)

Resolution Bin Occupancy (Darker = More Crowded)

Pairwise Fluorophore Separation (Green = Well Separated)

Threshold:
>2 = Separable
>3 = Well separated

Separation Capacity by Strategy

- All Three Dimensions: **540**
- Lifetime + Polarization: **18**
- Wavelength + Polarization: **60**
- Wavelength + Lifetime: **270**
- Polarization Only: **2**
- Lifetime Only: **9**
- Wavelength Only: **30**

Practical Estimate (40)

Recommended Fluorophore Panels

3-Color Panel (Conservative)
- DAPI: 461nm, 2.5ns
- GFP: 509nm, 2.5ns
- mCherry: 610nm, 1.5ns

5-Color Panel (Moderate)
- DAPI: 461nm, 2.5ns
- CFP: 475nm, 2.7ns
- YFP: 527nm, 3.0ns
- mOrange: 562nm, 2.0ns
- Cy5: 670nm, 1.0ns

Label-Free (Metabolic)
- NADH (free): 460nm, 0.4ns
- NADH (bound): 460nm, 2.4ns
- FAD (free): 525nm, 2.3ns
- FAD (bound): 535nm, 0.4ns

INSTRUMENT SPECS:
- Wavelength: 10 nm resolution
- Lifetime: 0.5 ns resolution
- Polarization: 2 states

CAPACITY:
- Theoretical: 540 fluorophores
- Practical: ~40 fluorophores
- Recommended: 3-10 fluorophores