Resolution of multiple color GFP variants using two-photon microscopy and imaging spectroscopy

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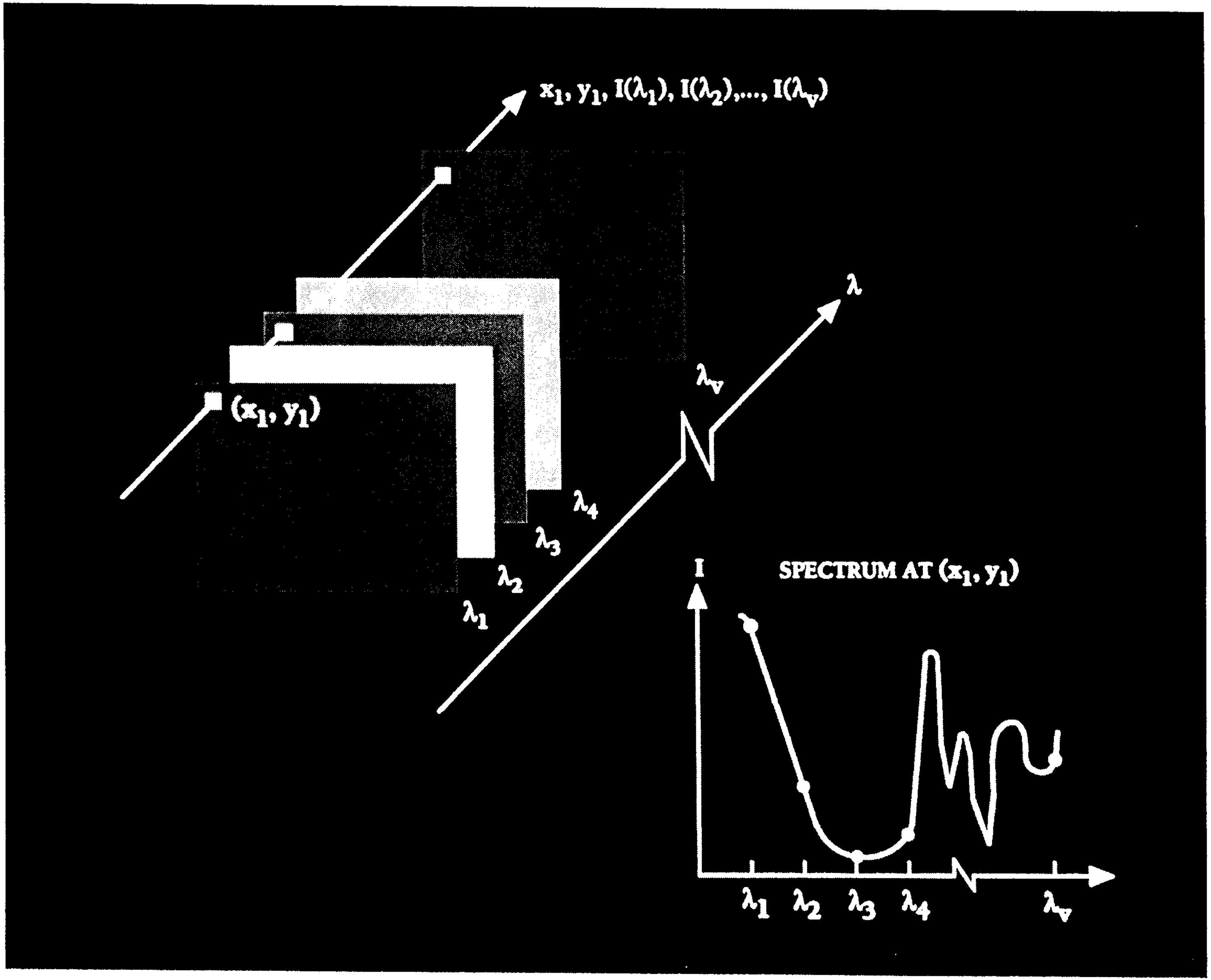
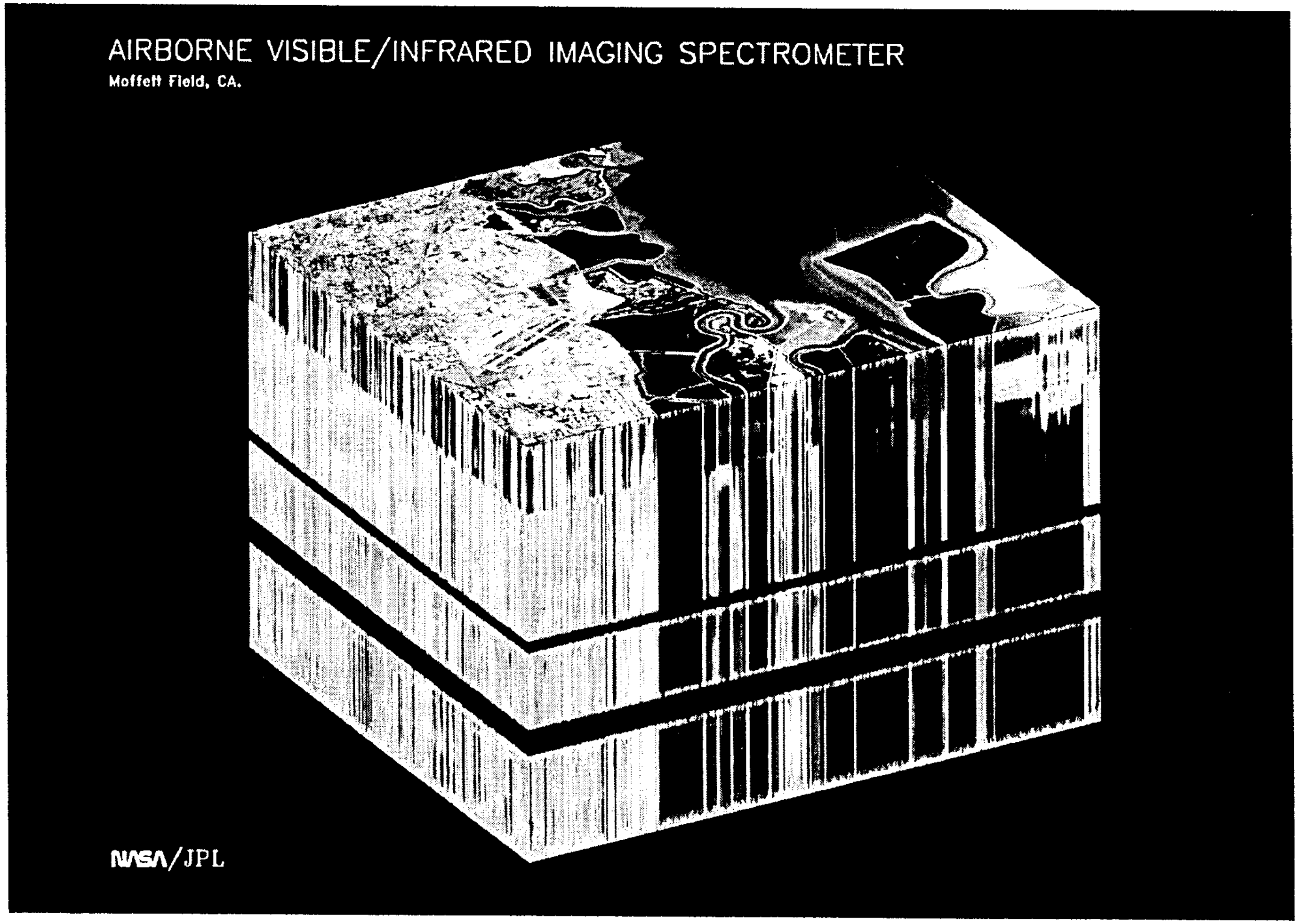
Image cube

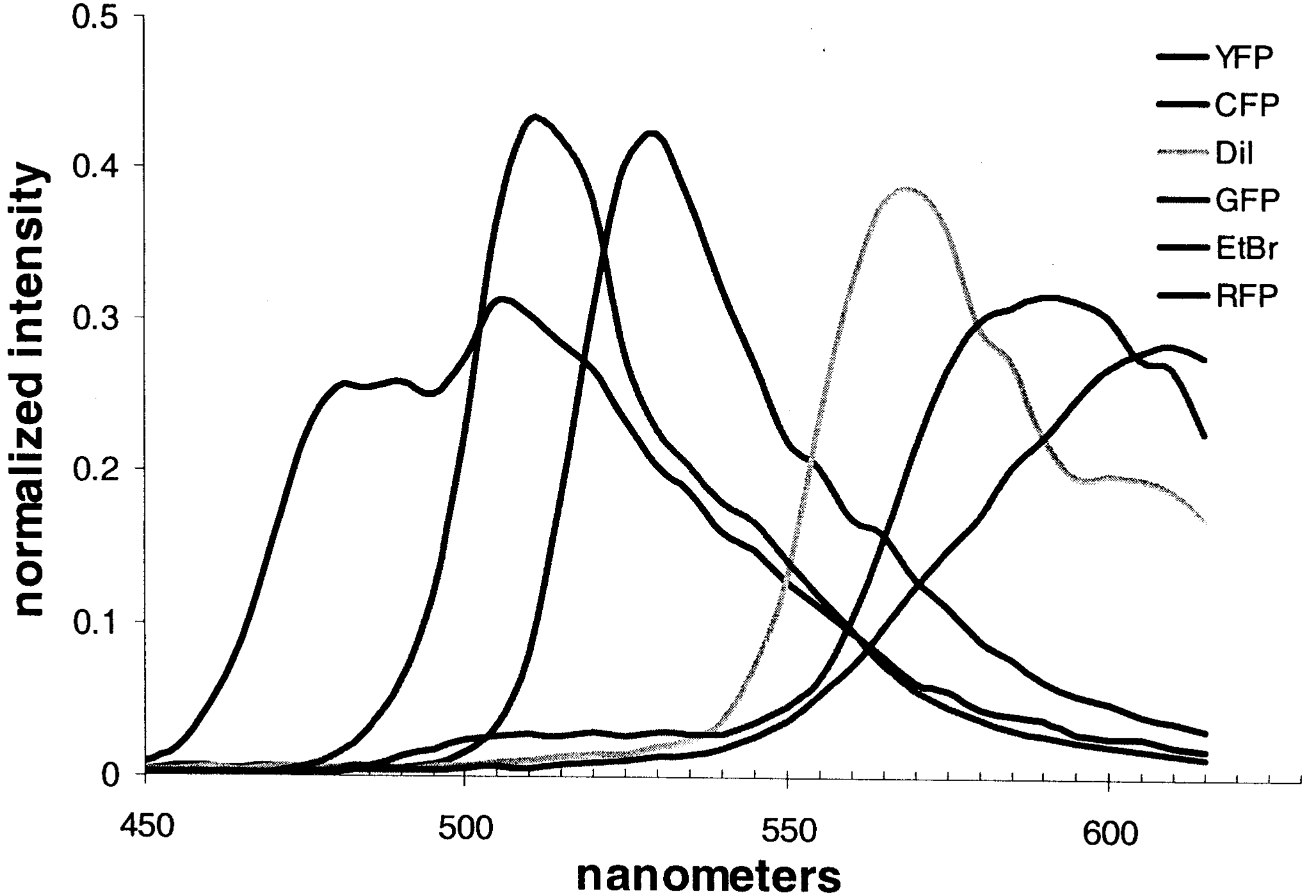


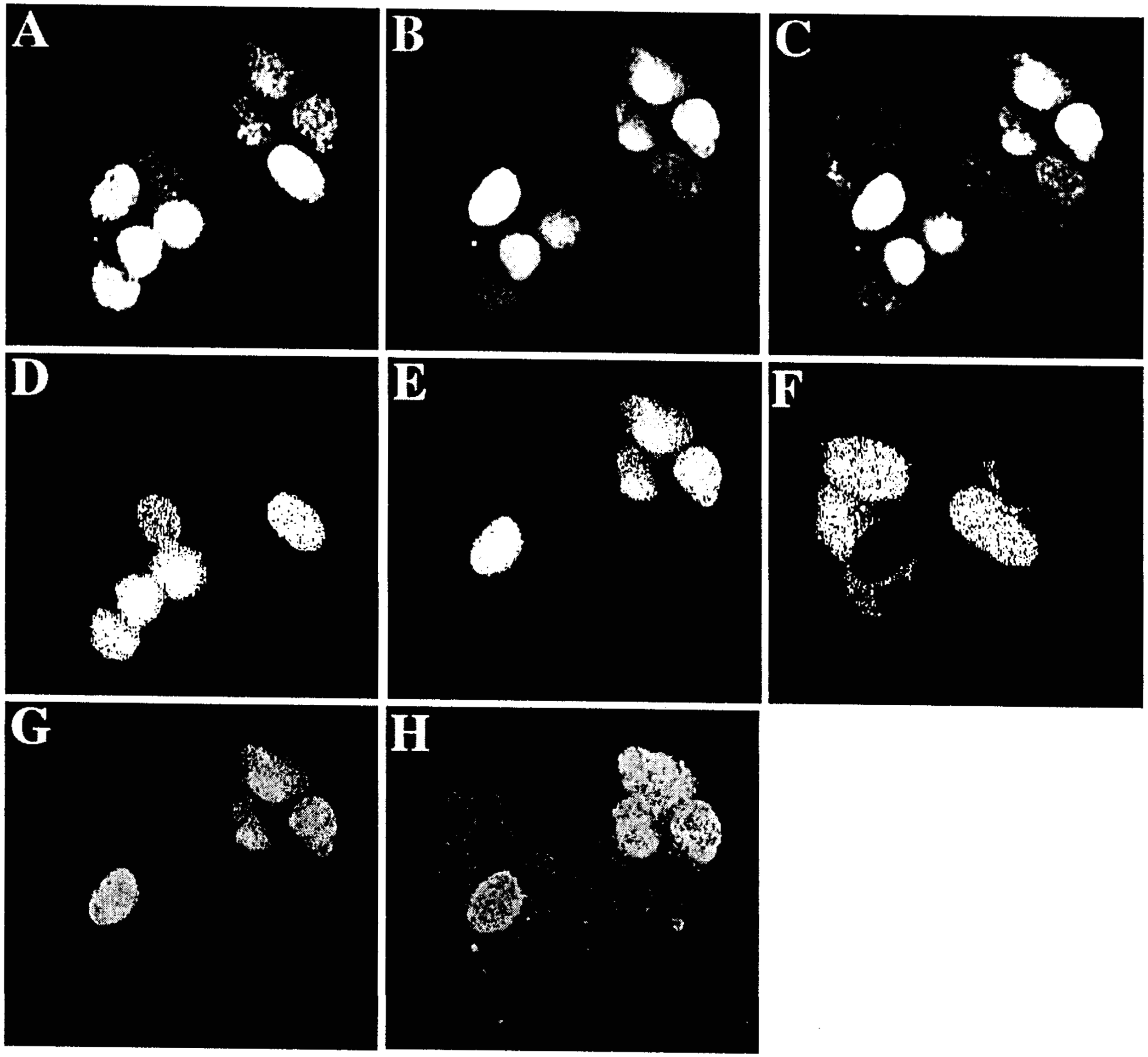
$x_1, y_1, I(\lambda_1), I(\lambda_2),\ldots, I(\lambda_v)$
λ
λ_v
(x_1, y_1)
λ_4
λ_3
λ_2
λ_1
I
SPECTRUM AT (x_1, y_1)
λ_1 λ_2 λ_3 λ_4 λ_v

AVIRIS Image Cube

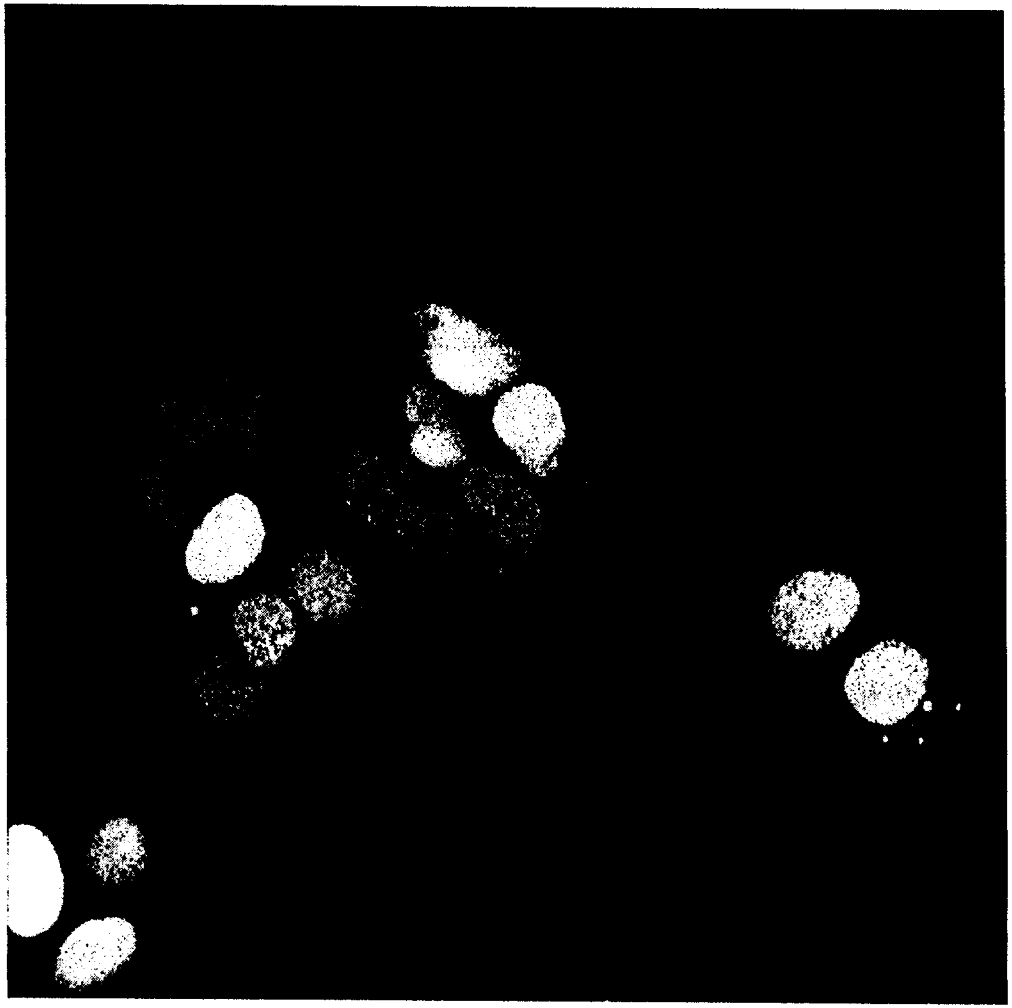


Normalized reference spectra measured from individually transfected cells that expressed only one color GFP targeted to H2B. Data for the two dyes, EtBr and DiI was acquired from cells that had been separately stained.

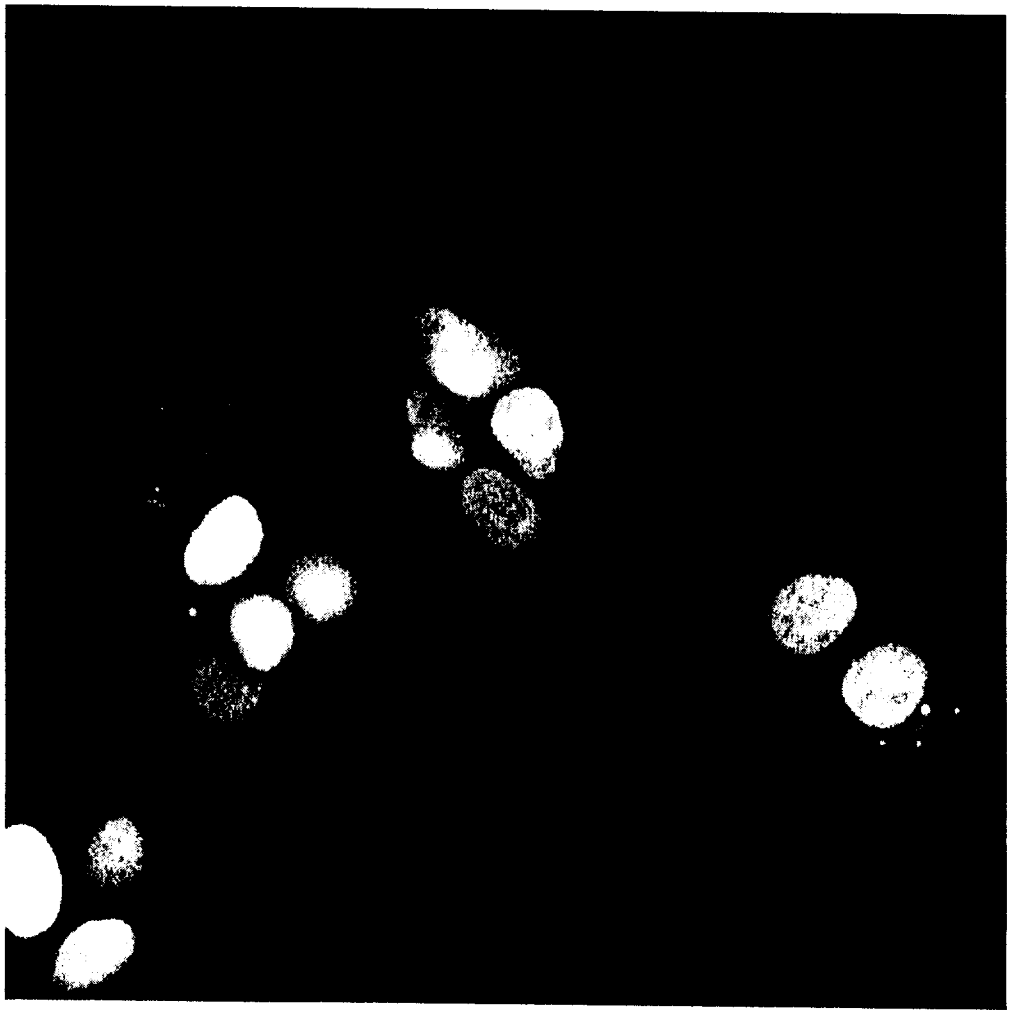



A
B
C
D
E
F
G
H

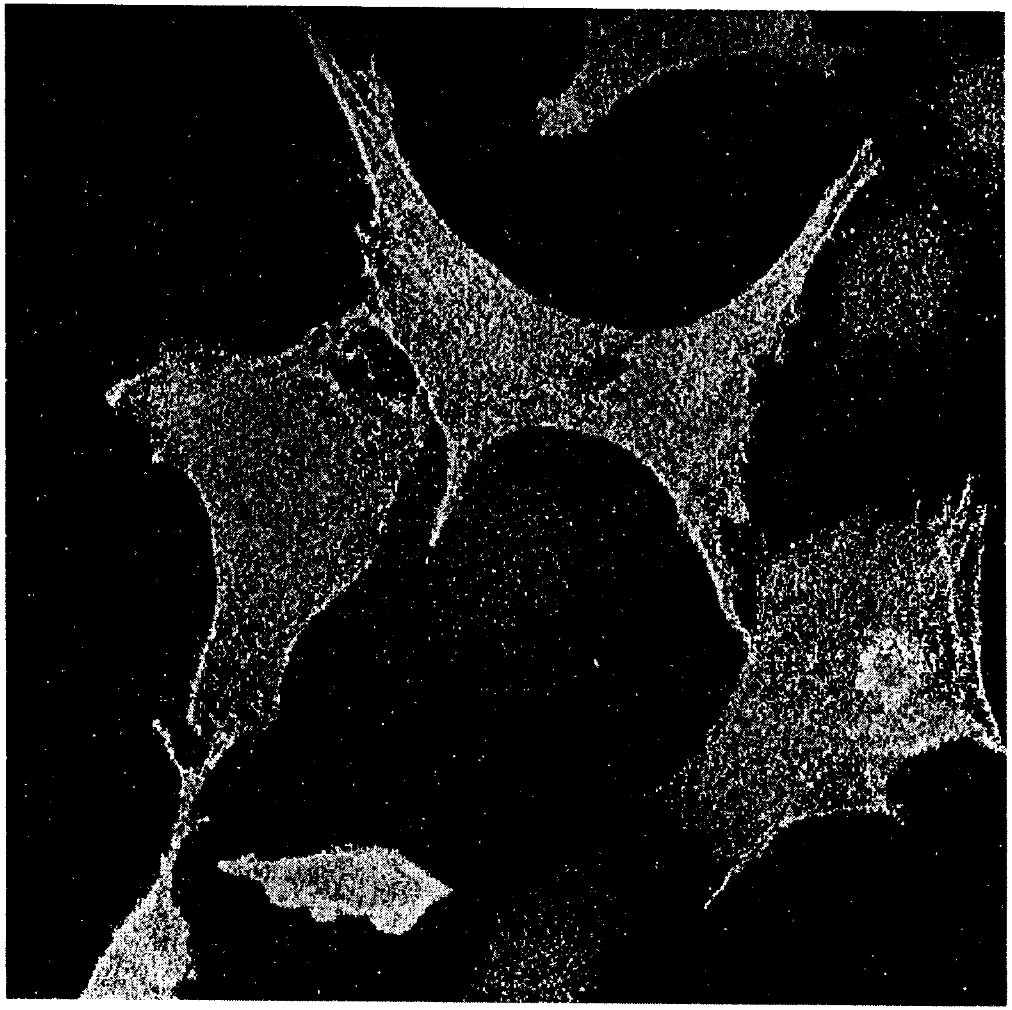
Intensity mapped classified image of 3 GFP color variants in which the color intensity is proportional to the amount of fluorophore present.



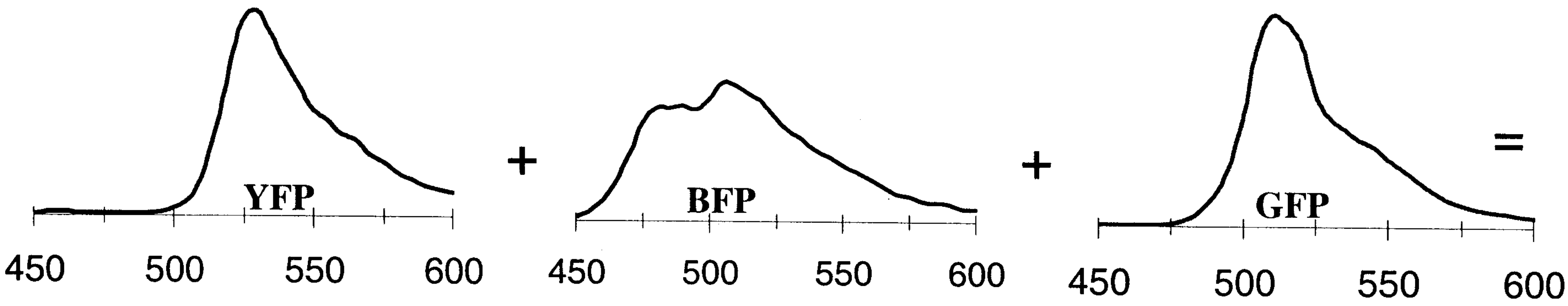
Intensity image summed over spectral range of 450-600 nm for each pixel

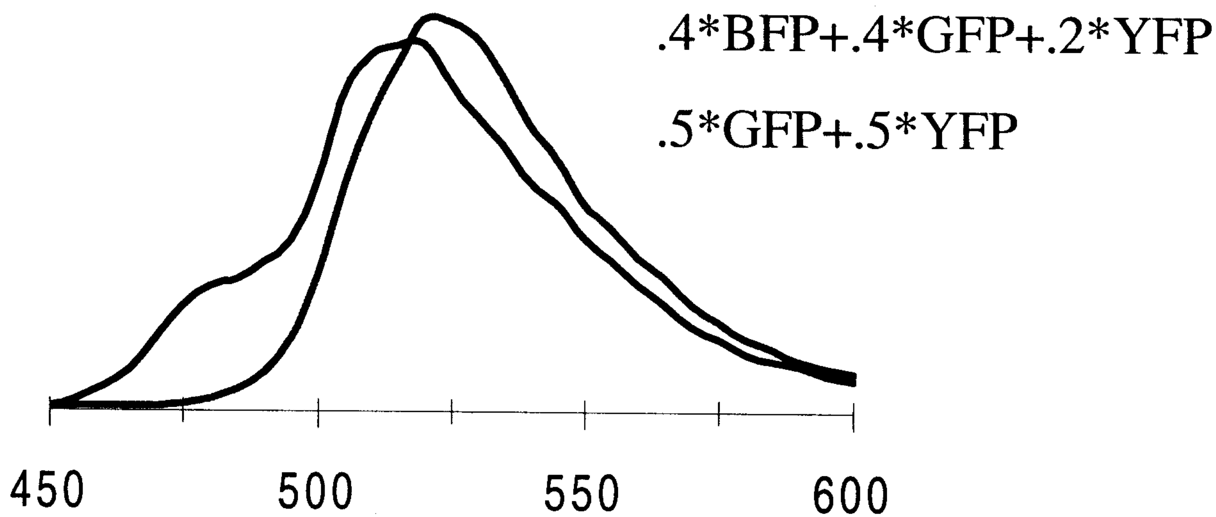


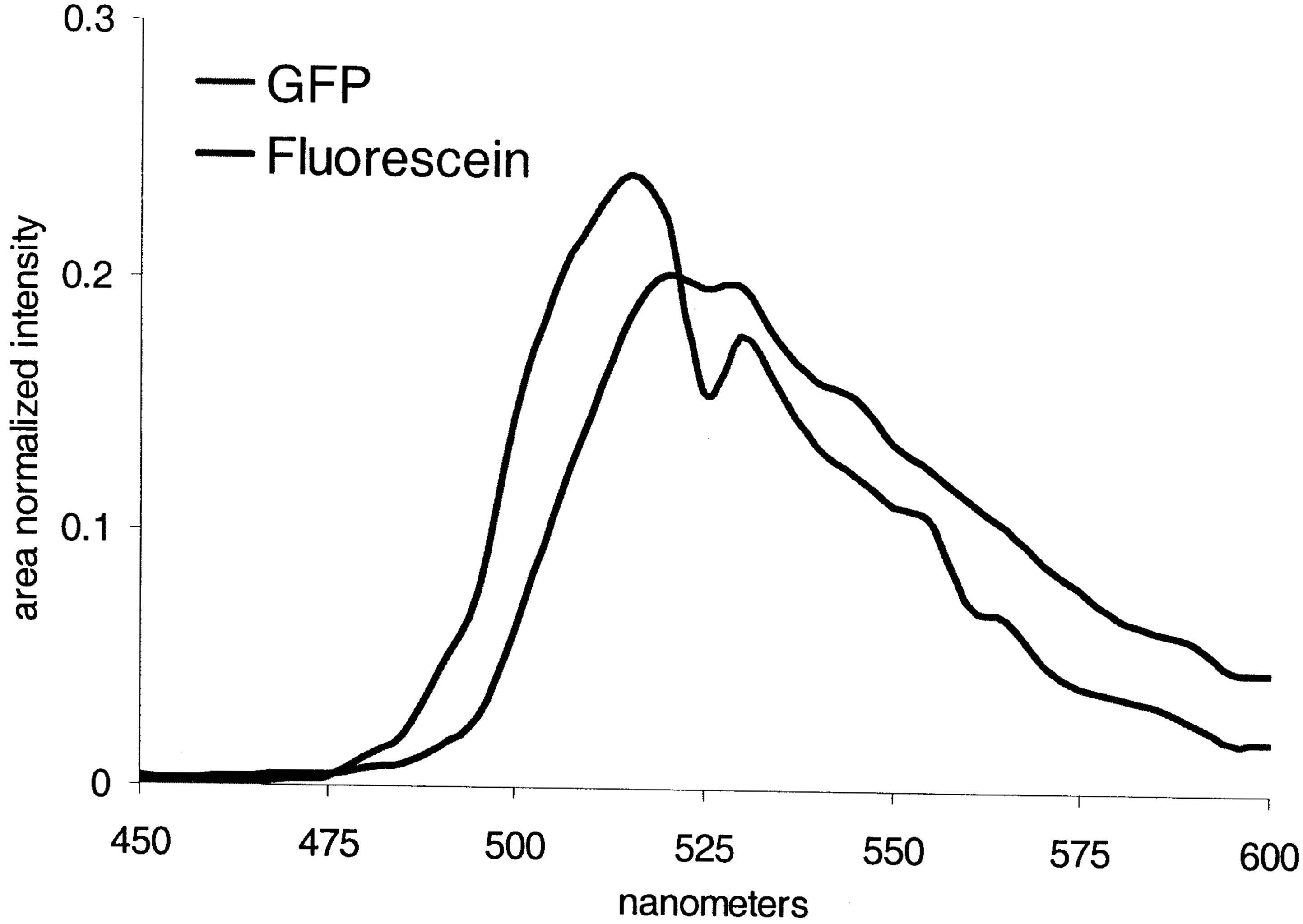
A triply labeled classified image obtained by spectral unmixing of an image cube. The data has been intensity mapped so that pixel brightness is proportional to the amount of fluorescent probe. The nuclei (blue) were targeted with BFP, the actin with GFP (green) and the mitochondria (red) with YFP.



Linear unmixing can determine the relative weights (abundance) of component spectra of a composite spectrum even when the individual spectra overlap.







GPF and fluorescein spectra measured with 2-photon confocal imaging spectrometer

GFP labeled nuclei (red) and fluorescein labeled actin (green) spectrally unmixed image obtain with 2-photon confocal microscopy. The images planes for each color probe have been intensity mapped so that pixel brightness is proportional to the amount of fluorophore.

Computed-Tomography Imaging Spectrometer

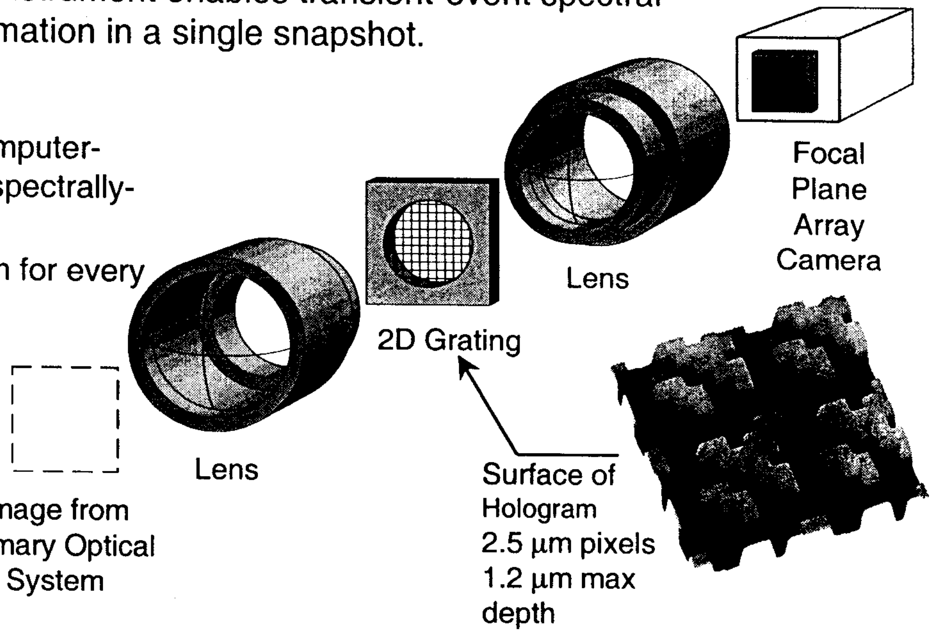
A new concept in imaging spectrometers, this instrument enables transient-event spectral imaging by capturing spatial and spectral information in a single snapshot.

Principle of Operation

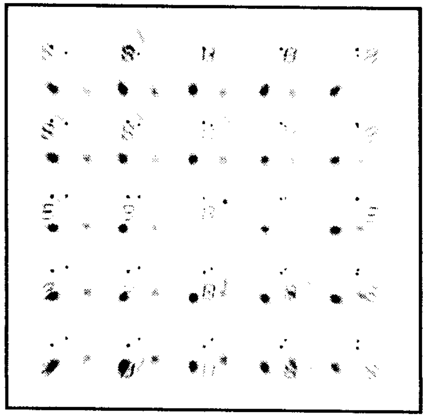
- JPL designed and electron-beam fabricated computer-generated hologram splits scene into multiple, spectrally-dispersed images
- Tomographic reconstruction yields the spectrum for every pixel in the scene

Advantages

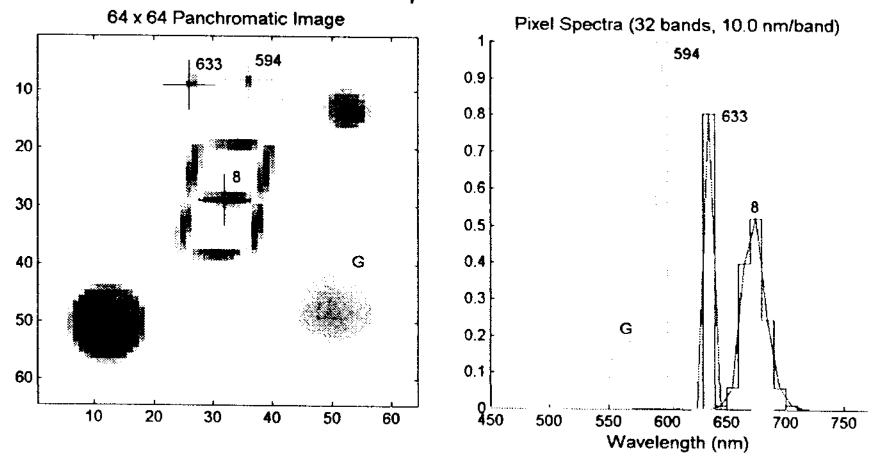
- Does not employ scanning of any type
- Multiple spatial-spectral data cubes having different dimensionality can be reconstructed from the same frame



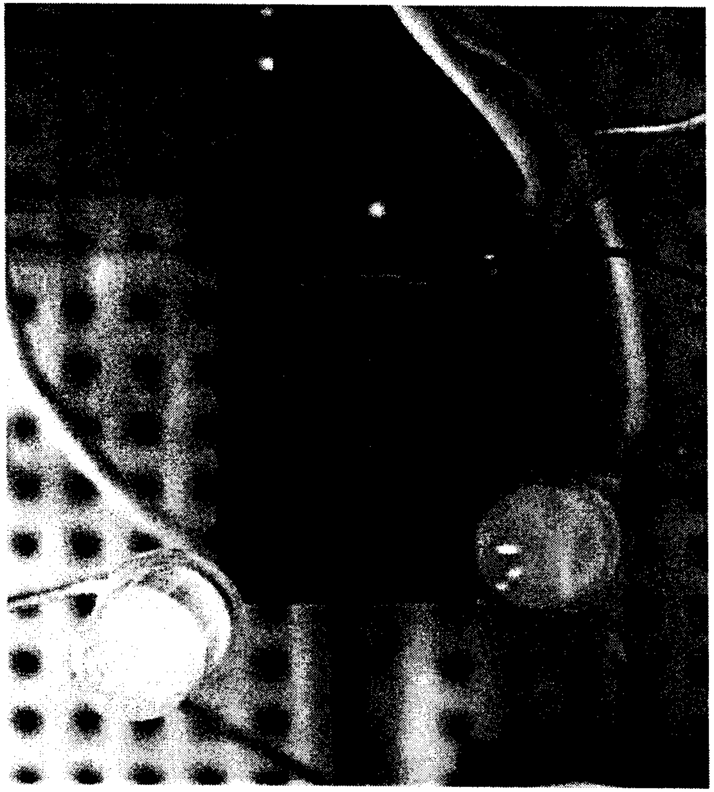
Experimental Scene



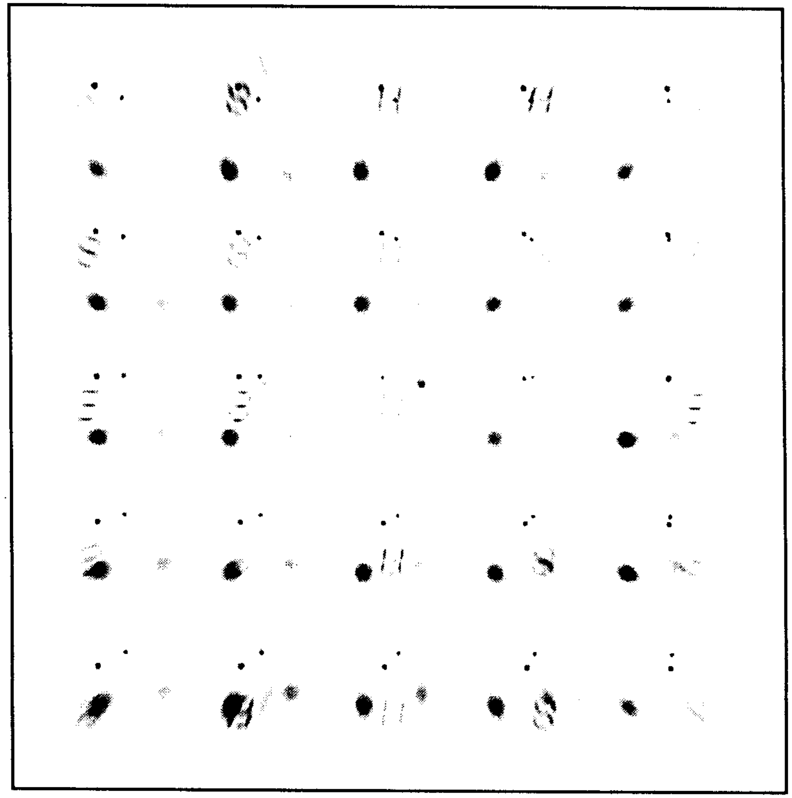
Intensity on Focal Plane Array
(Image taken in dark ambient)



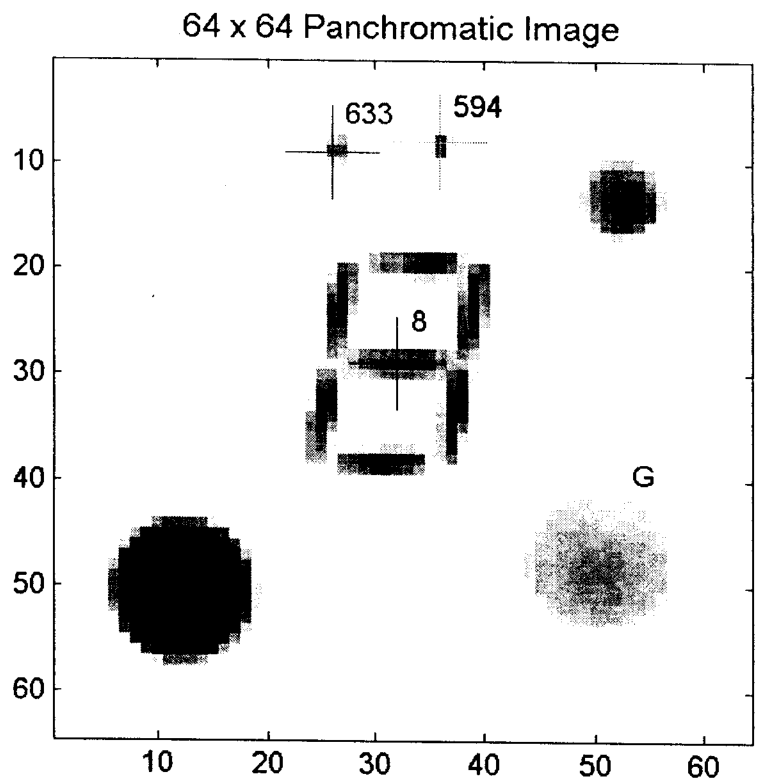
Reconstructed Spatial-Spectral Scene

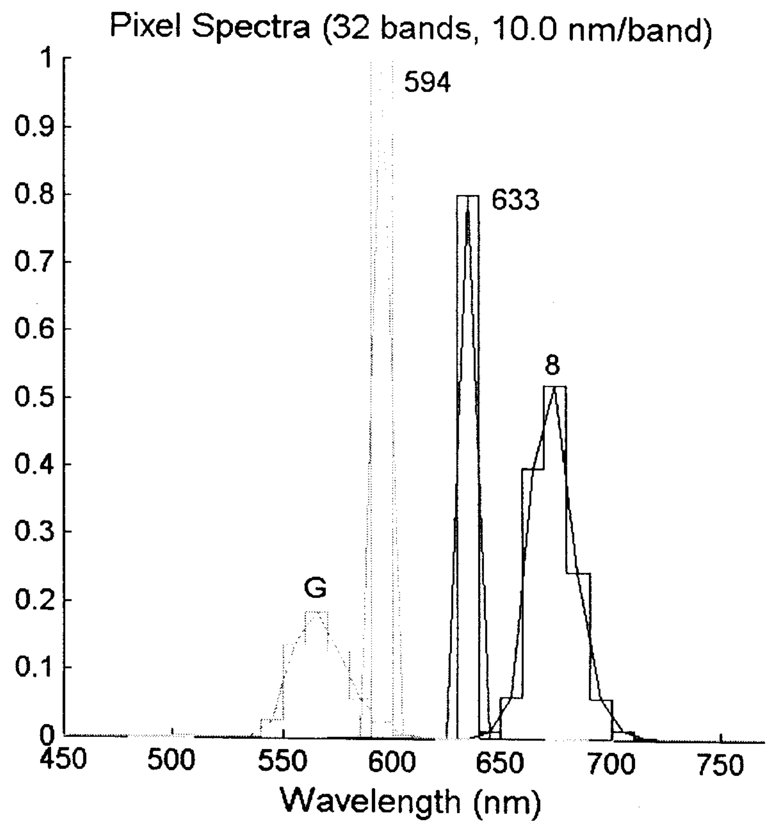


Experimental Scene



Intensity on Focal Plane Array (Image taken in dark ambient)





Reconstructed Spatial-Spectral Scene

Features of snap-shot imaging spectrometer

Fast data acquisition--limited only by signal

Can take image cubes at video rates if adequate signal

Compact device that uses mostly COTS components--lenses/cameras/computers

Data can be "squeezed" during reconstruction to decrease spectral bands and increase spatial information or *vice versa*

Spectroscopy without a spectrometer

Gregory Bearman† and Peter Miller‡

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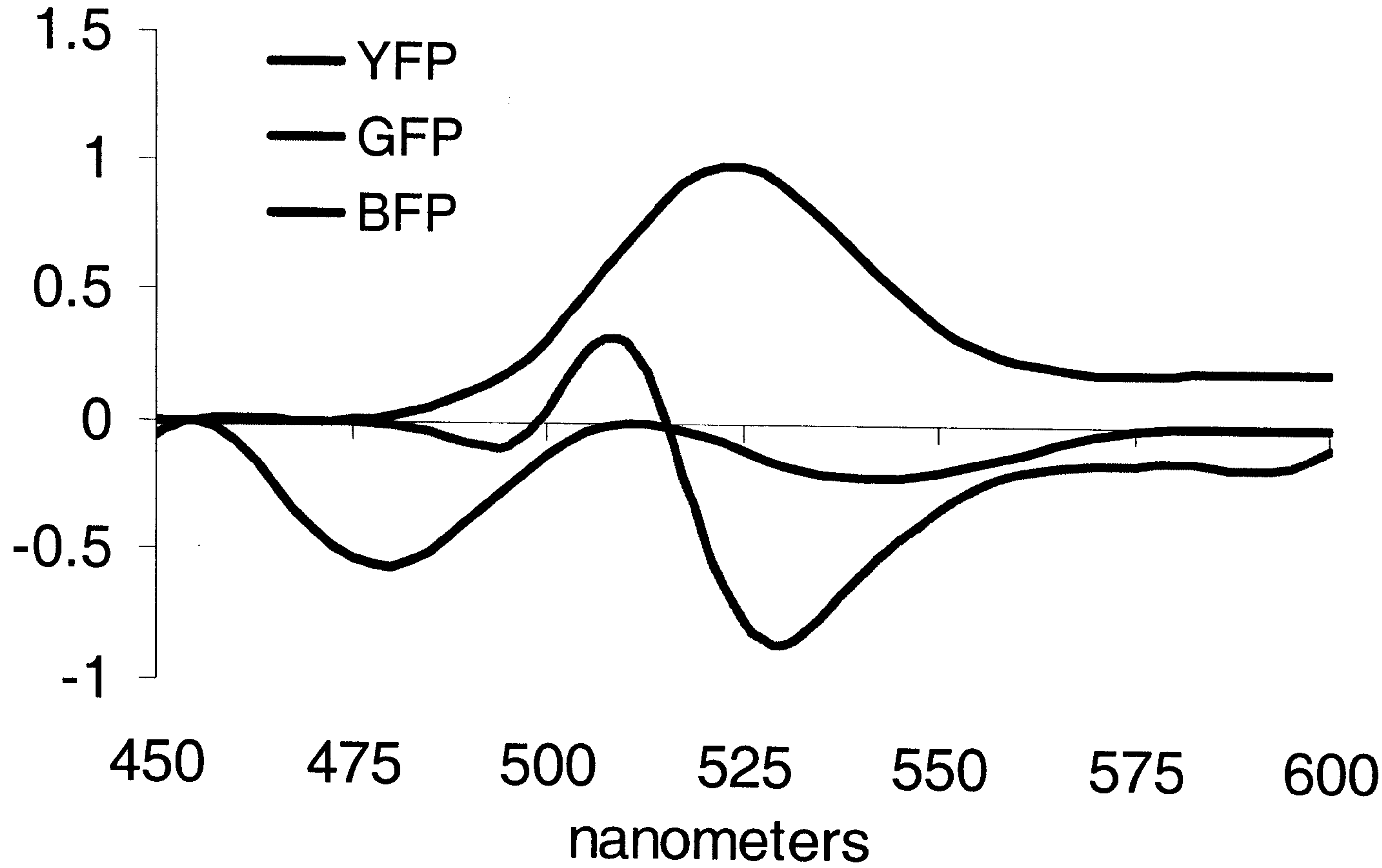
California Institute of Technology

‡Cambridge Research and Instrumentation

Matched filters for 3 GFP color variants calculated from measured spectra



- Note that some of the filter values are negative, similar to the xyz tri-stimulus curves
- Similar to xyz color scales, if one could make filter whose transmission that matched these eigenvectors, then one could image directly in a color space or, in our case, a classified space.
- The negative values are eliminated by making filters that are appropriate linear combinations of the eigenvectors which are positive definite.

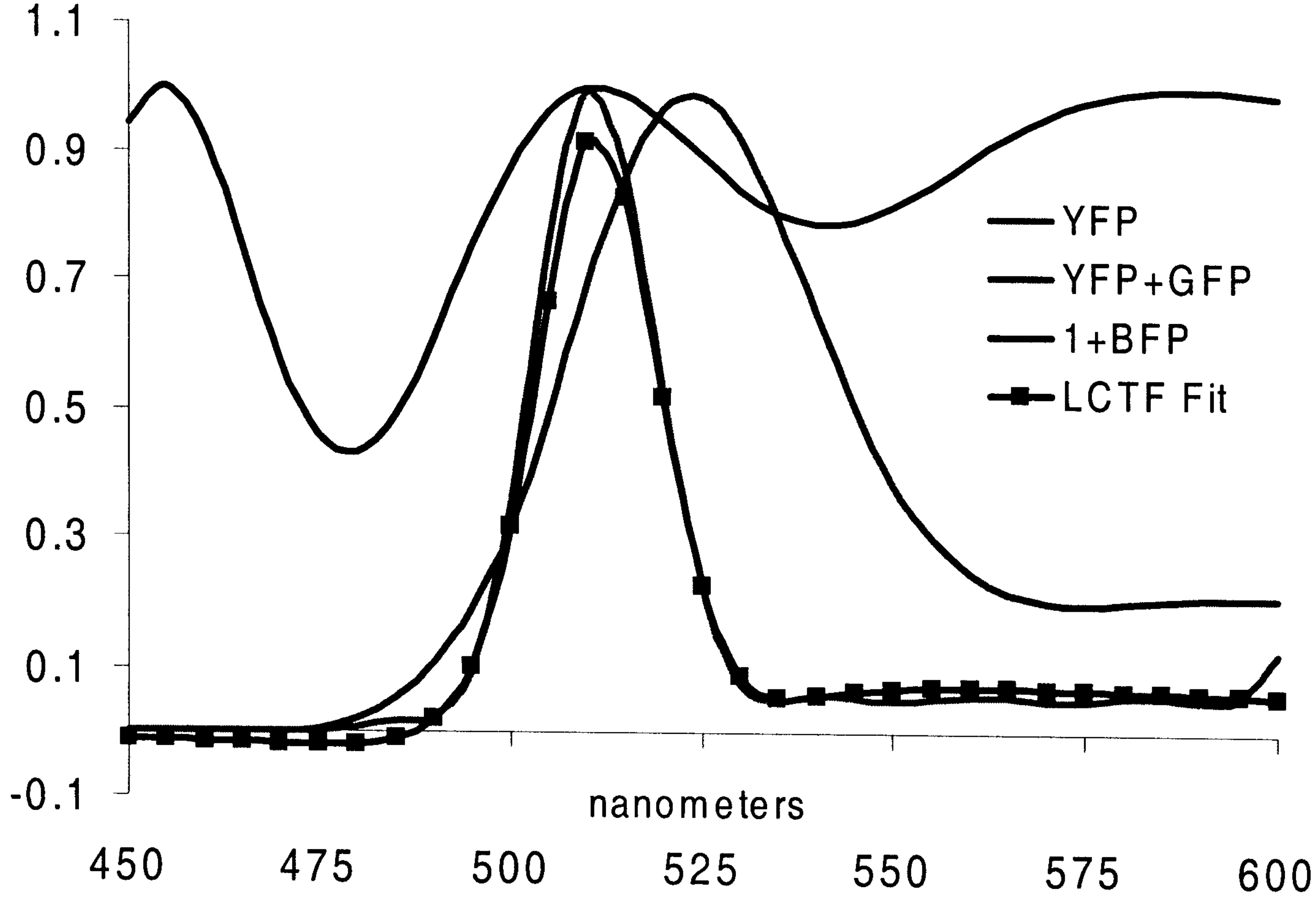
How do we make these filters?

- Liquid crystal tunable filter can be designed to match any n-order polynomial transmission curve
- Diffractive optics

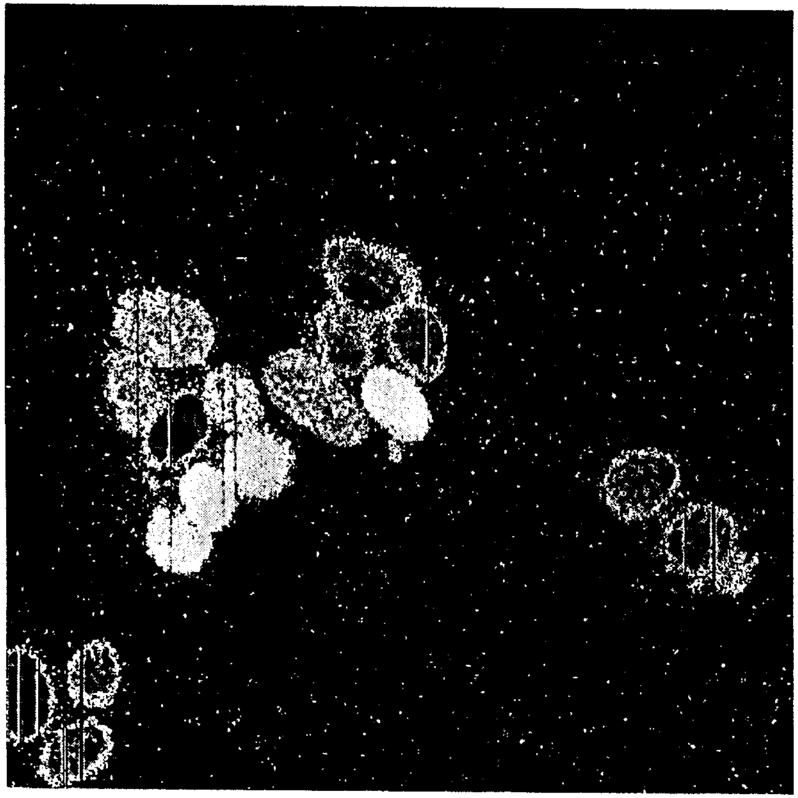
  2D diffractive grating can create any desired spectra profile at a single pixel.

  Whiskbroom imaging with point detector

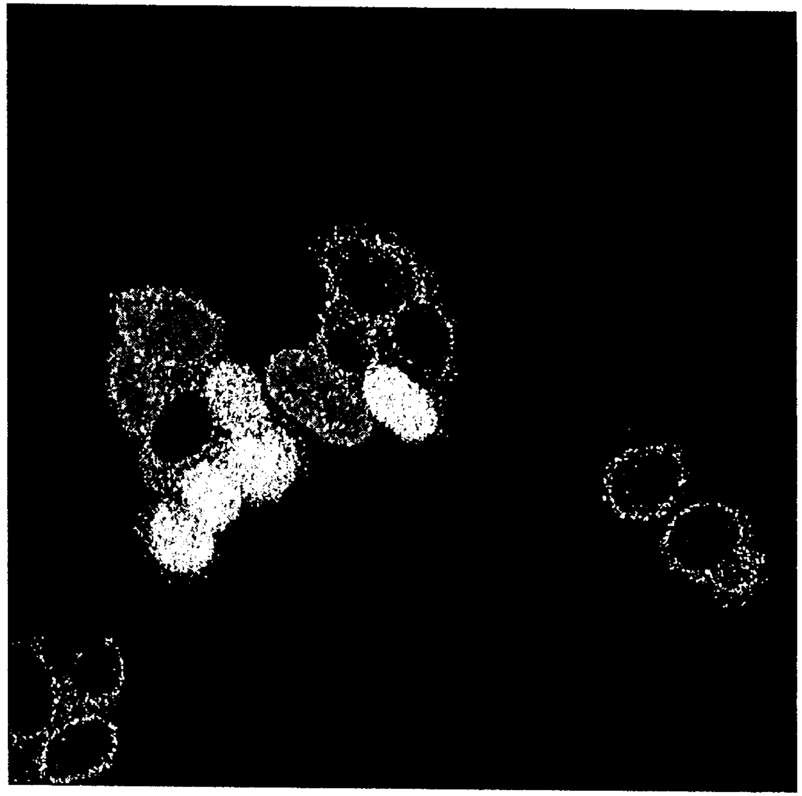
Positive matched filters generated from linear combinations of the eigenvectors. These are transmission curves that can be matched by a realistic LCTF device.



- In practice, then, one takes 4 images
  - YFP, YFP+GFP, 1+BFP and a clear
- The clear image (no filter) provides the normalization image to obtain correct intensity for each fluorophore



Classified matched filter result



Classified result from image cube

Ideal for fluorescent imaging

the fluorophore spectra are known

filters can be designed and built in advance

Large signal as integrating over entire spectrum at once

Reduce data acquisition time and subsequent processing