Hyperspectral Imaging: Bead Identification and Future Directions?

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Presentation 8/28/2017

Overall Goal: Simultaneously identify multiple fluorescent molecules across visible spectrum
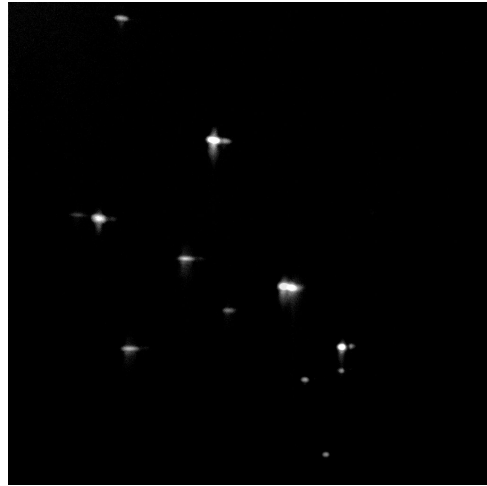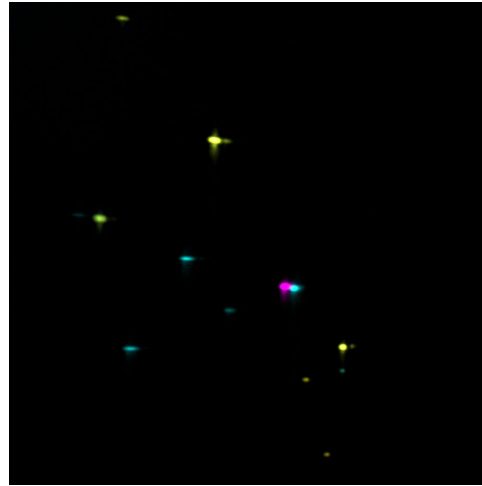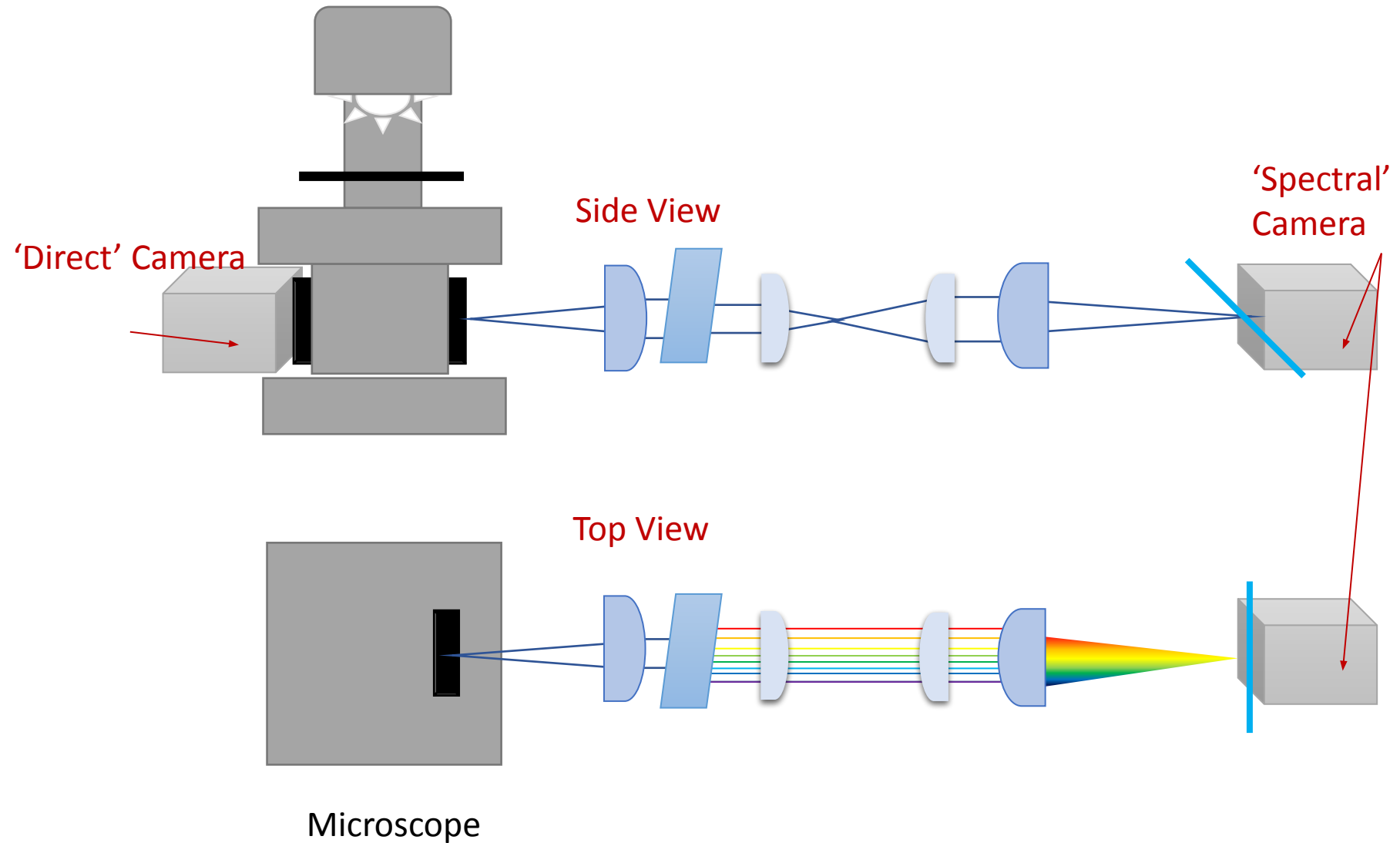

Image of fluorescent beads

Image with spectral data

Uses a dispersive element (prism) in a secondary imaging pathway to disperse light
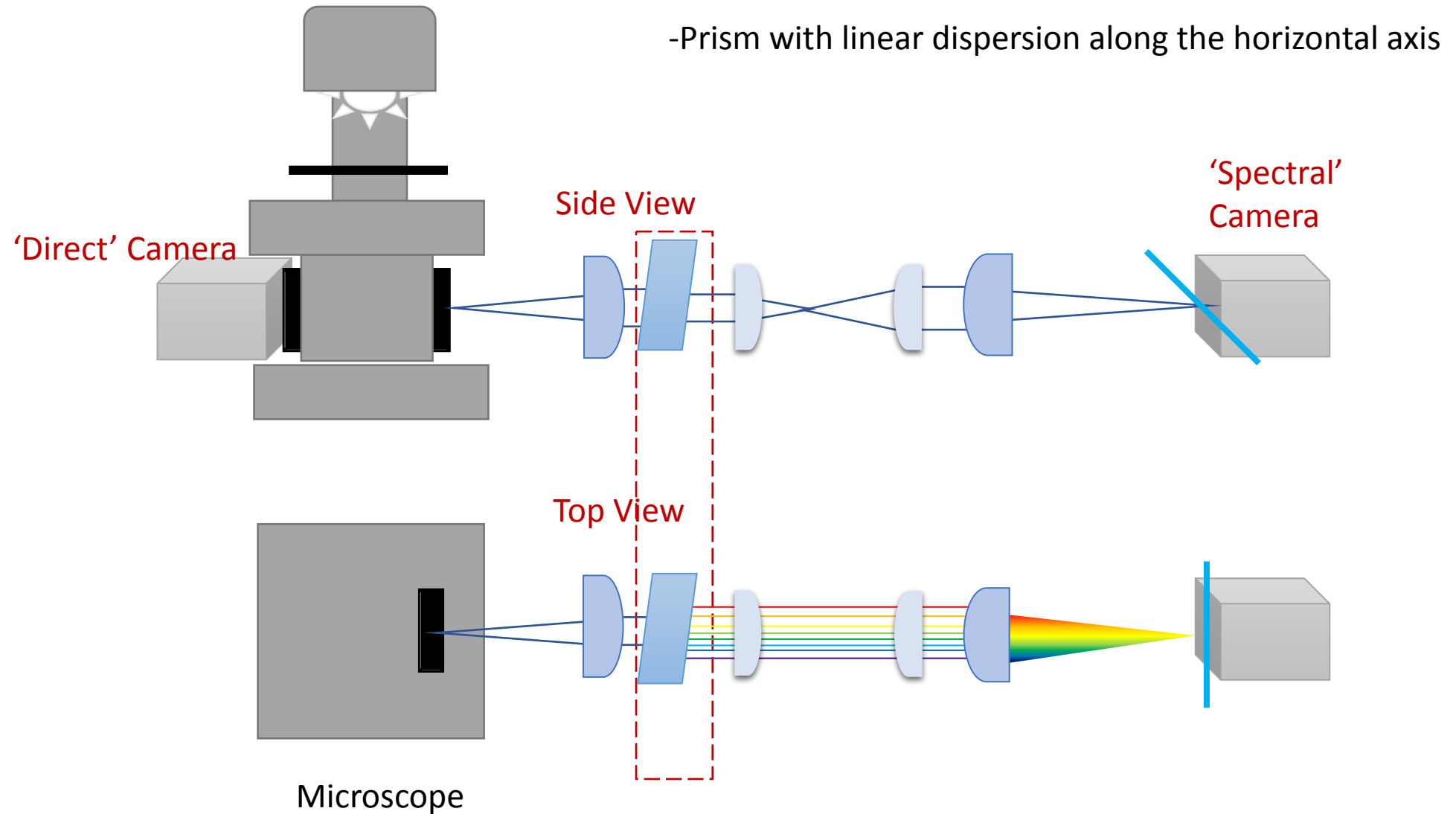
Requires a direct image and a dispersed (spectral) image to identify both position and color

☐ Acquires all spectral information simultaneously, no extra filters or switching lasers!
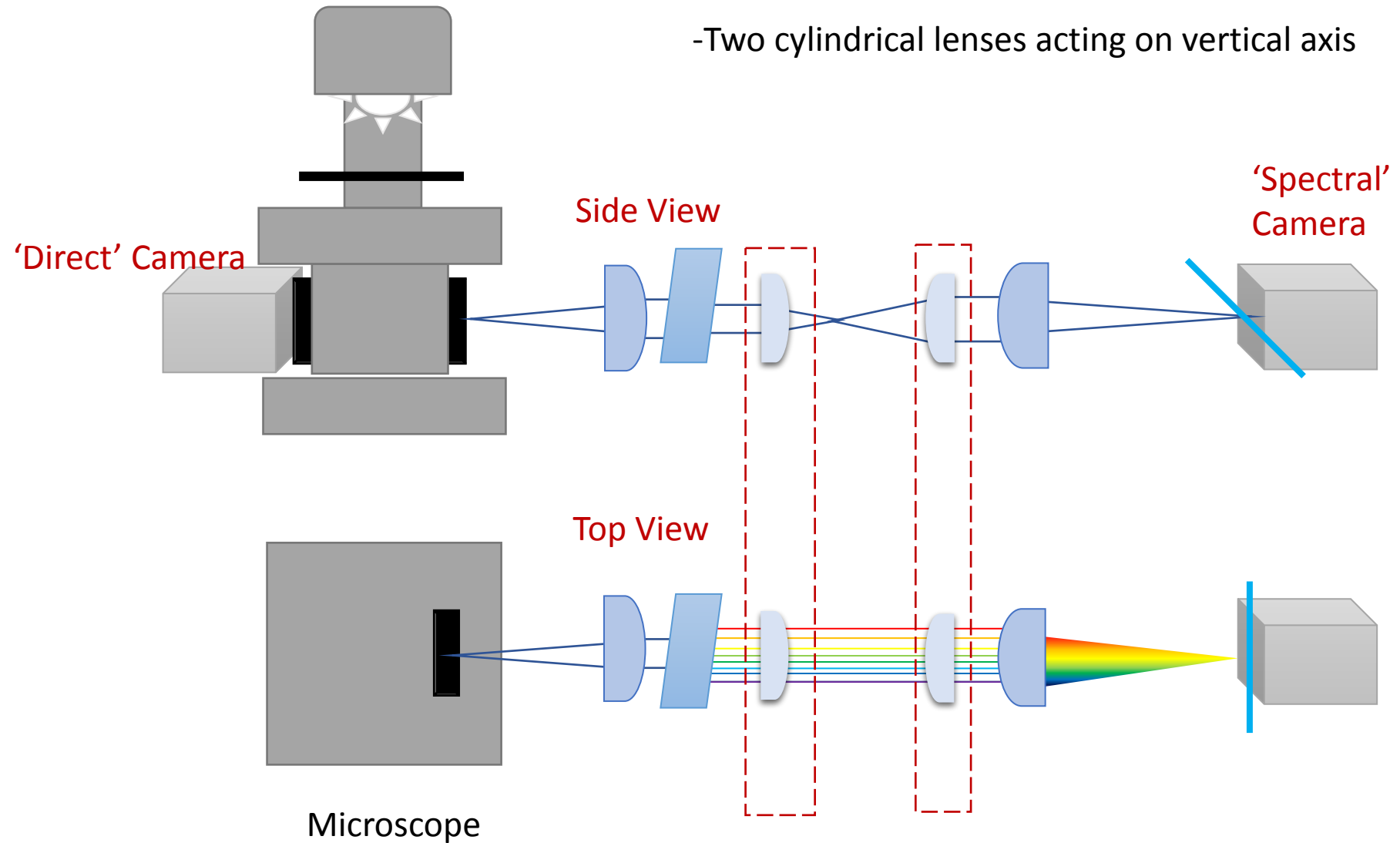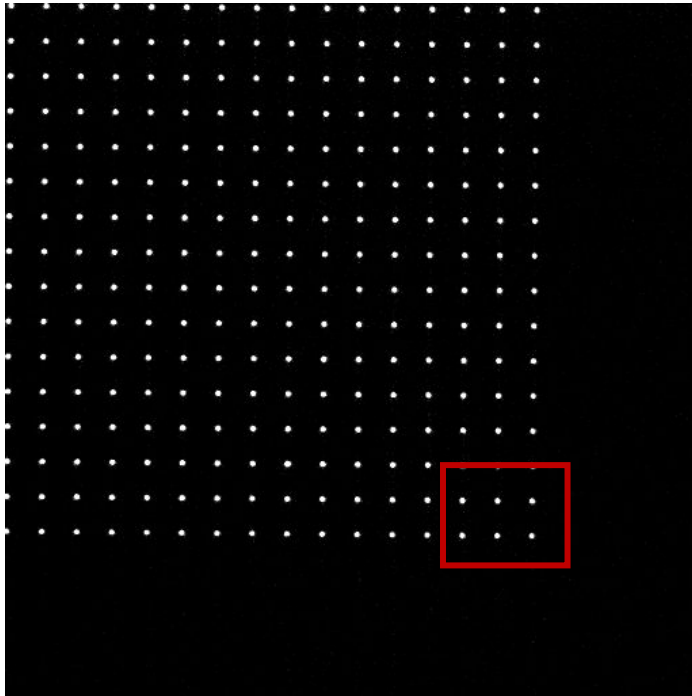
Optical System

'Direct' Camera

Side View

'Spectral' Camera

Top View

Microscope

Optical System

-Prism with linear dispersion along the horizontal axis

'Direct' Camera

Side View

'Spectral' Camera

Top View

Microscope

Optical System

-Two cylindrical lenses acting on vertical axis

'Direct' Camera

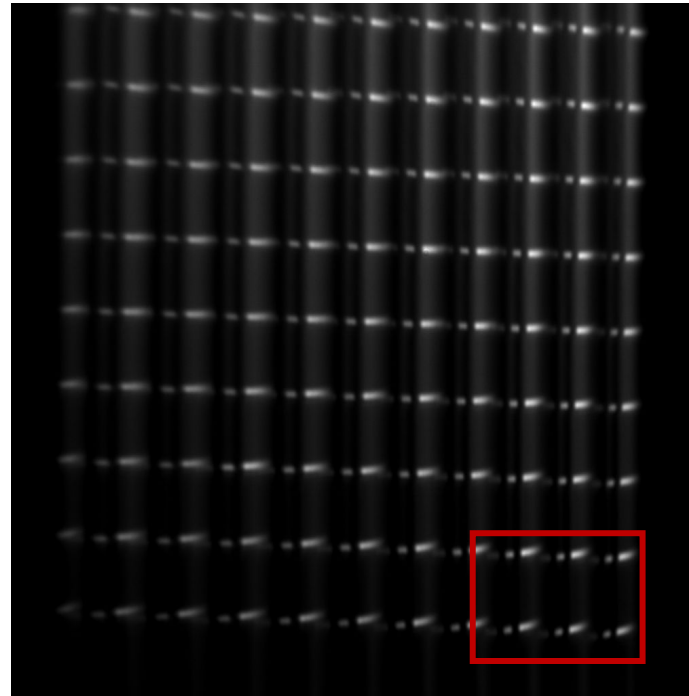Side View

'Spectral' Camera

Top View

Microscope

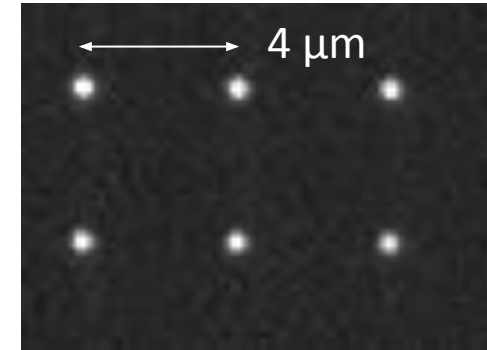# Nanogrid – 200 nm point sources
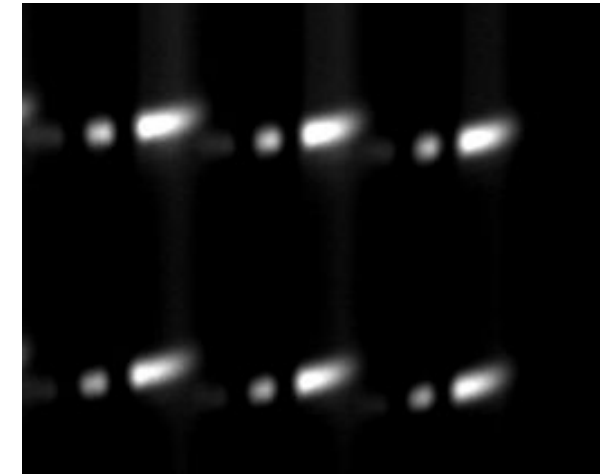
Direct

512 x 512 pixels

Spectral

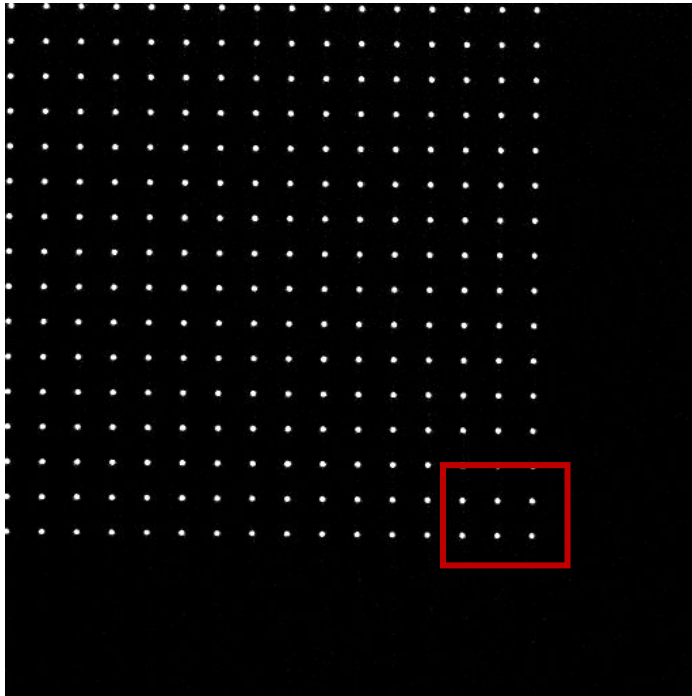1024 x 1024 pixels

4 µm

Direct

Spectral

Note:
All images taken with filter Cube
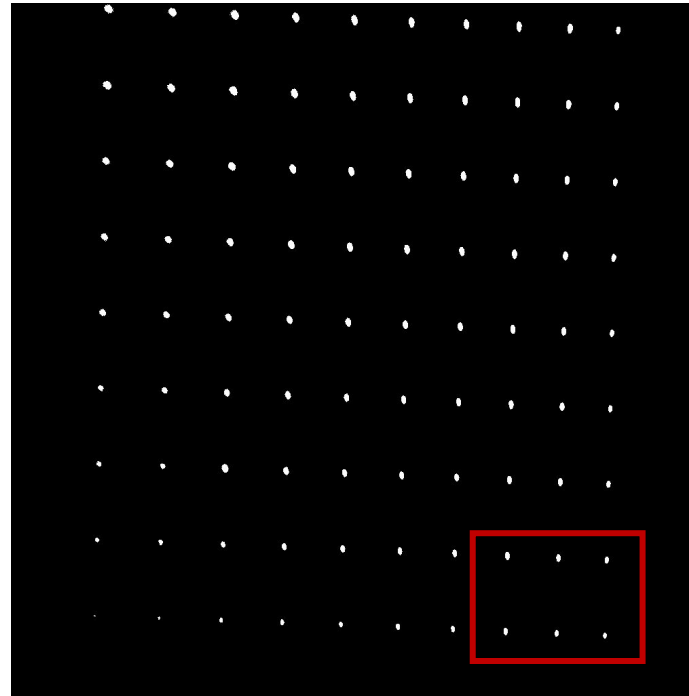with Semrock Dichroic Mirror
Reflects 405, 488, 561, 635 nm lasers
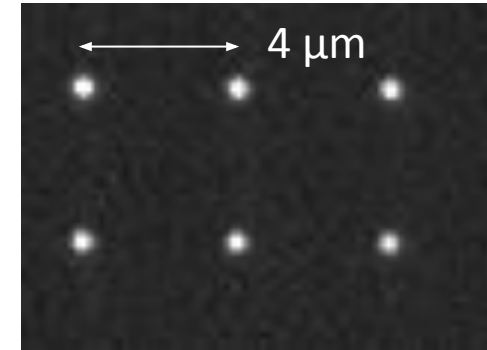
Nanogrid – 200 nm point sources

Direct

Spectral – 590 nm reference filter

4 µm

Direct

Spectral

Direct
160 nm/pixel
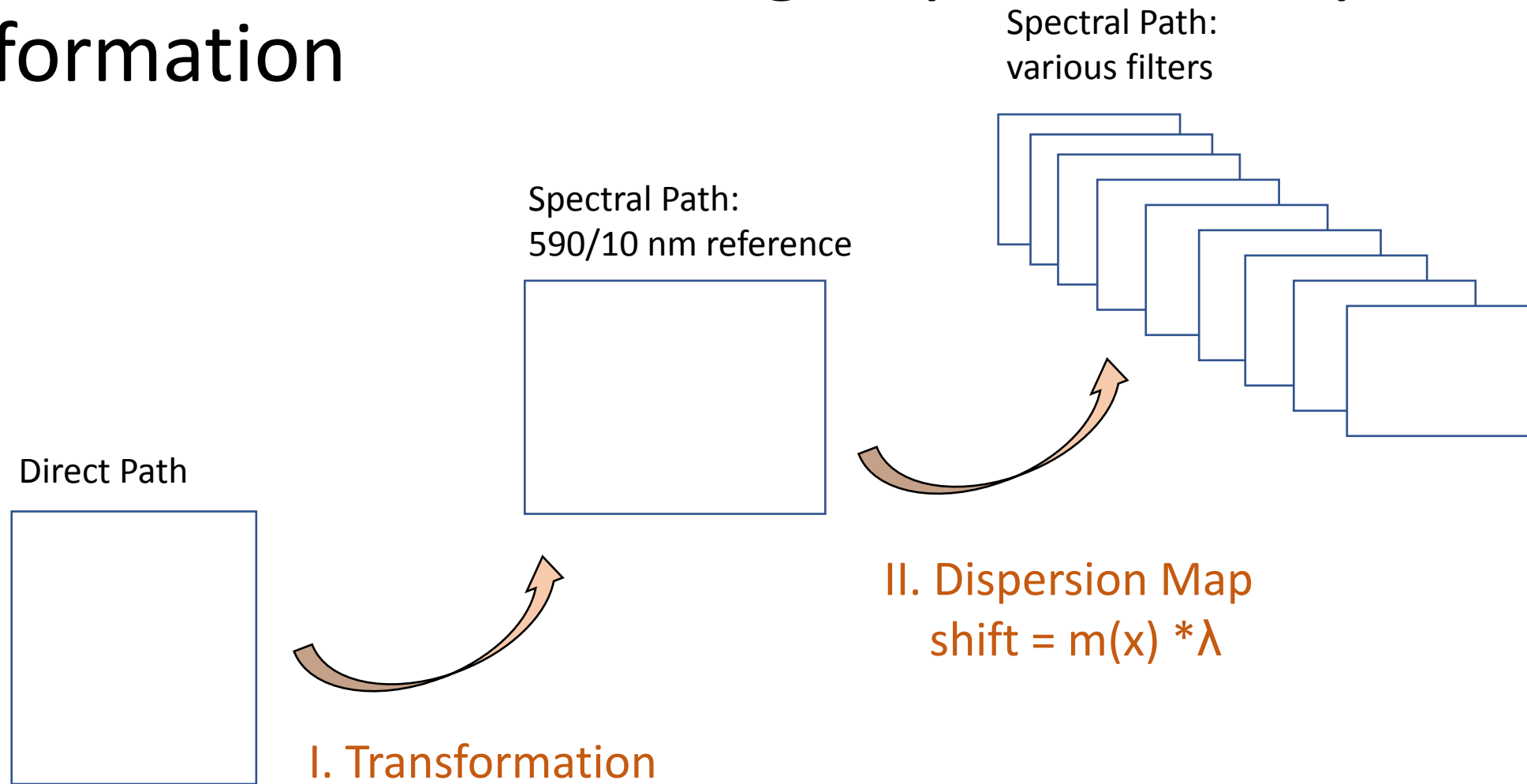
Spectral
x ~ 30-40 nm/px

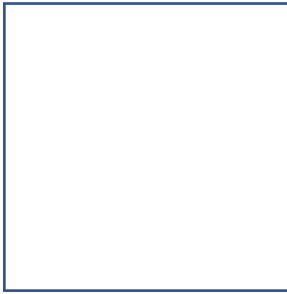512 x 512 pixels

1024 x 1024 pixels

Deconvolve to disentangle spatial and spectral information
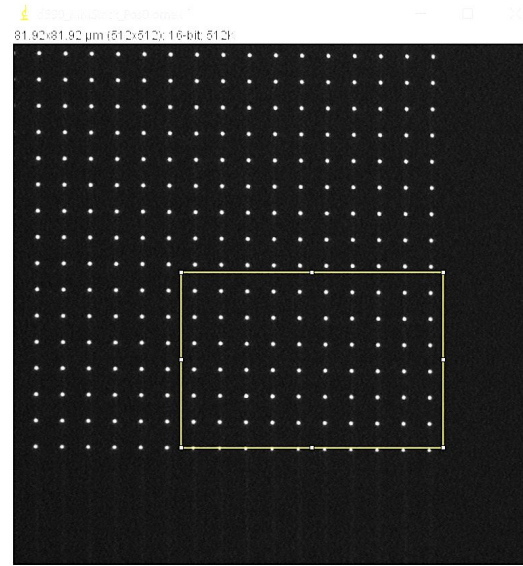
Spectral Path:
various filters

Spectral Path:
590/10 nm reference

Direct Path

I. Transformation

II. Dispersion Map
shift = m(x) *λ

I. Transformation

Direct Path

Spectral Path:
590/10 nm reference
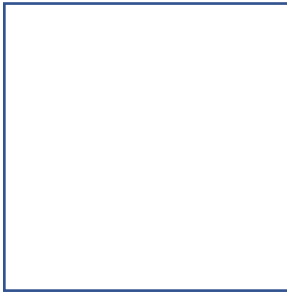

81.92x81.92 µm (512x512); 16-bit; 512k
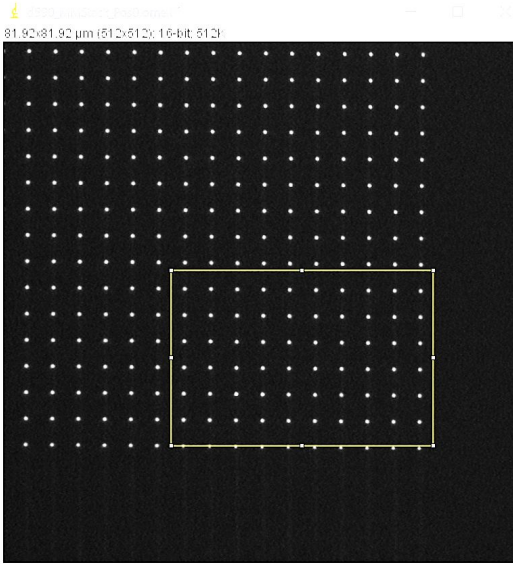
Spectral camera restriction

1. localize corresponding points on direct camera and spectral camera (590 nm)
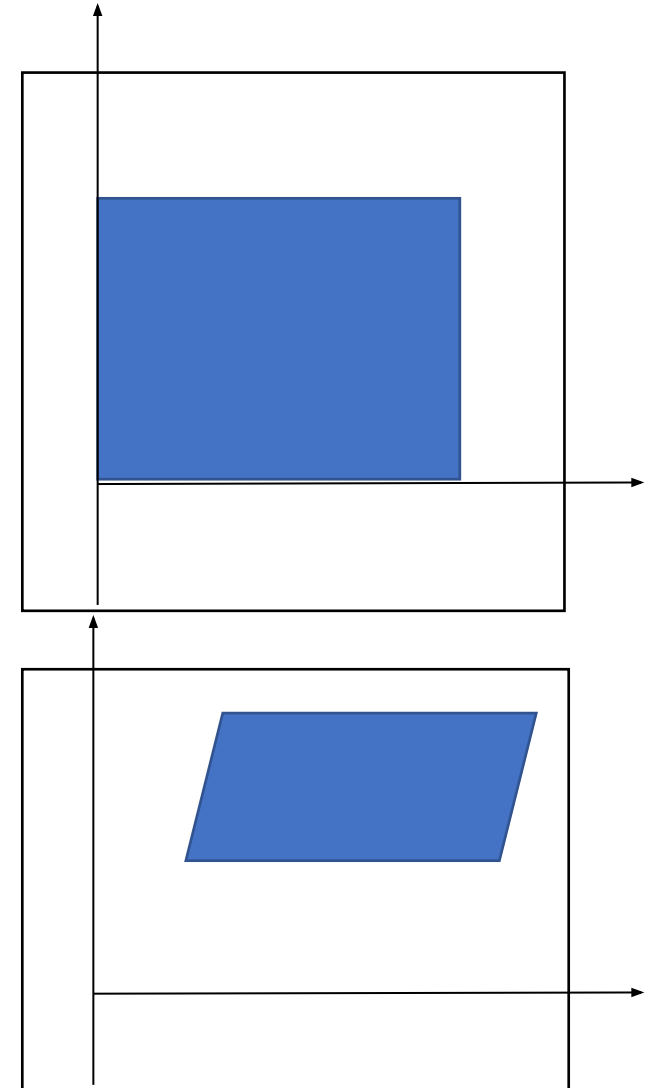
I. Transformation

Direct Path
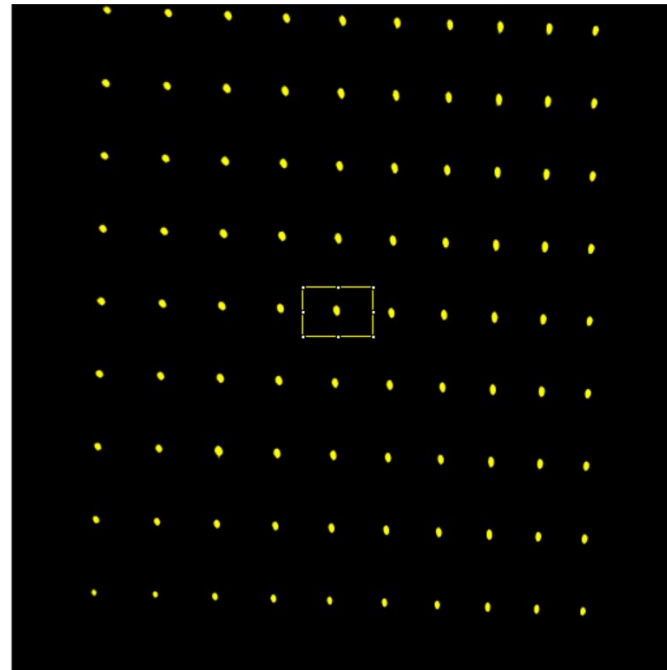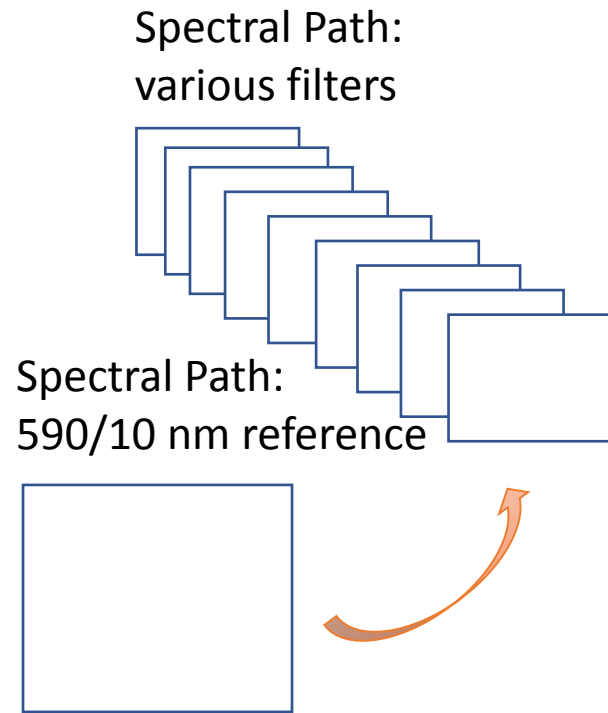
Spectral Path:
590/10 nm reference

81.92x81.92 μm (512x512); 16-bit; 512k

Spectral camera restriction

1. localize corresponding points on direct camera and spectral camera (590 nm)

II. Dispersion Map

Spectral Path:
various filters

Spectral Path:
590/10 nm reference

Spectral image using 590 nm filter,
false colored orange

590
reference

460   510 546   650 710

-41   -21 -14   0   18 26   px

Composite image of 6 filters (465-710 nm),
false colored to match approximately
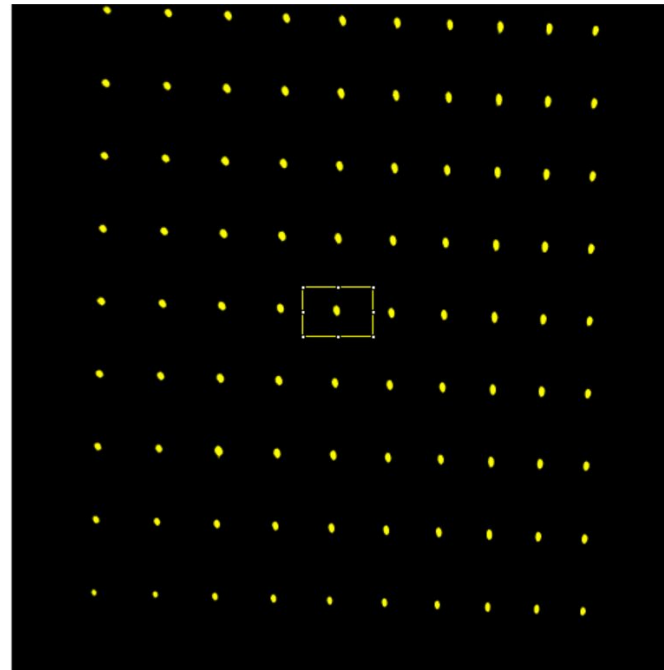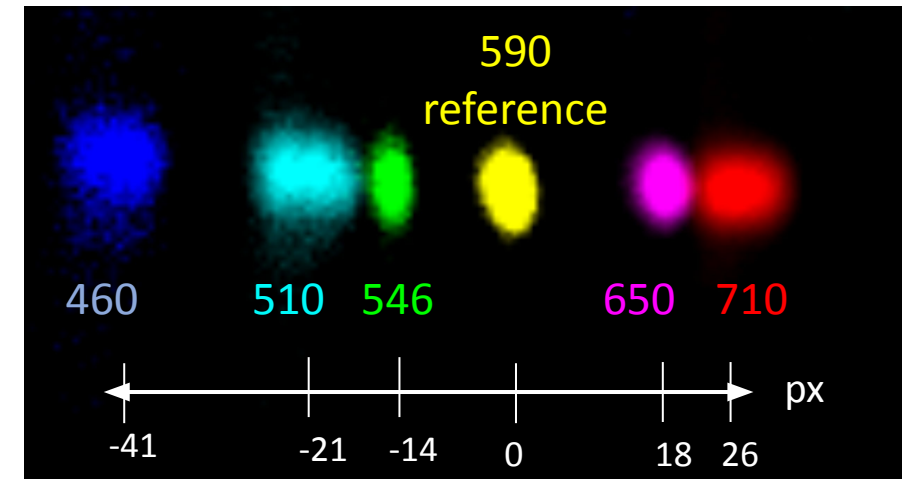
II. Dispersion Map

Spectral Path:
various filters
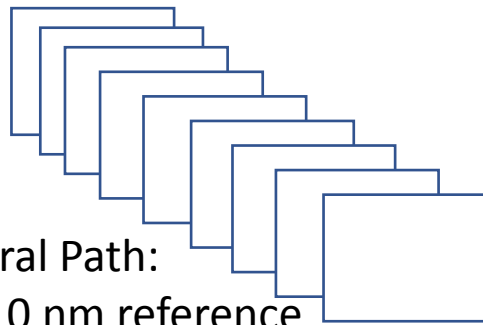
Spectral Path:
590/10 nm reference

As desired
linear dispersion ~ 3.6 nm/px!

$\lambda = 3.64 * \Delta x + 590$ nm

Spectral image using 590 nm filter,
false colored orange

590
reference

460   510 546   650 710

px

-41   -21 -14   0   18 26
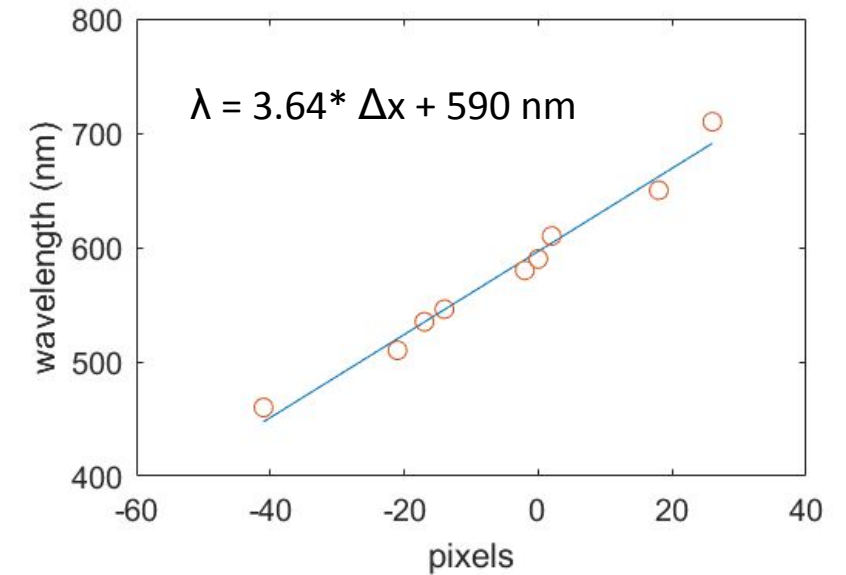
Composite image of 6 filters (465-710 nm),
false colored to match approximately

Mapping linear dispersion by imaging 7 filters and nanogrid

Linear Spectral Shift
Row 4, Col 3

Slope = -6.5

Wavelength [nm]

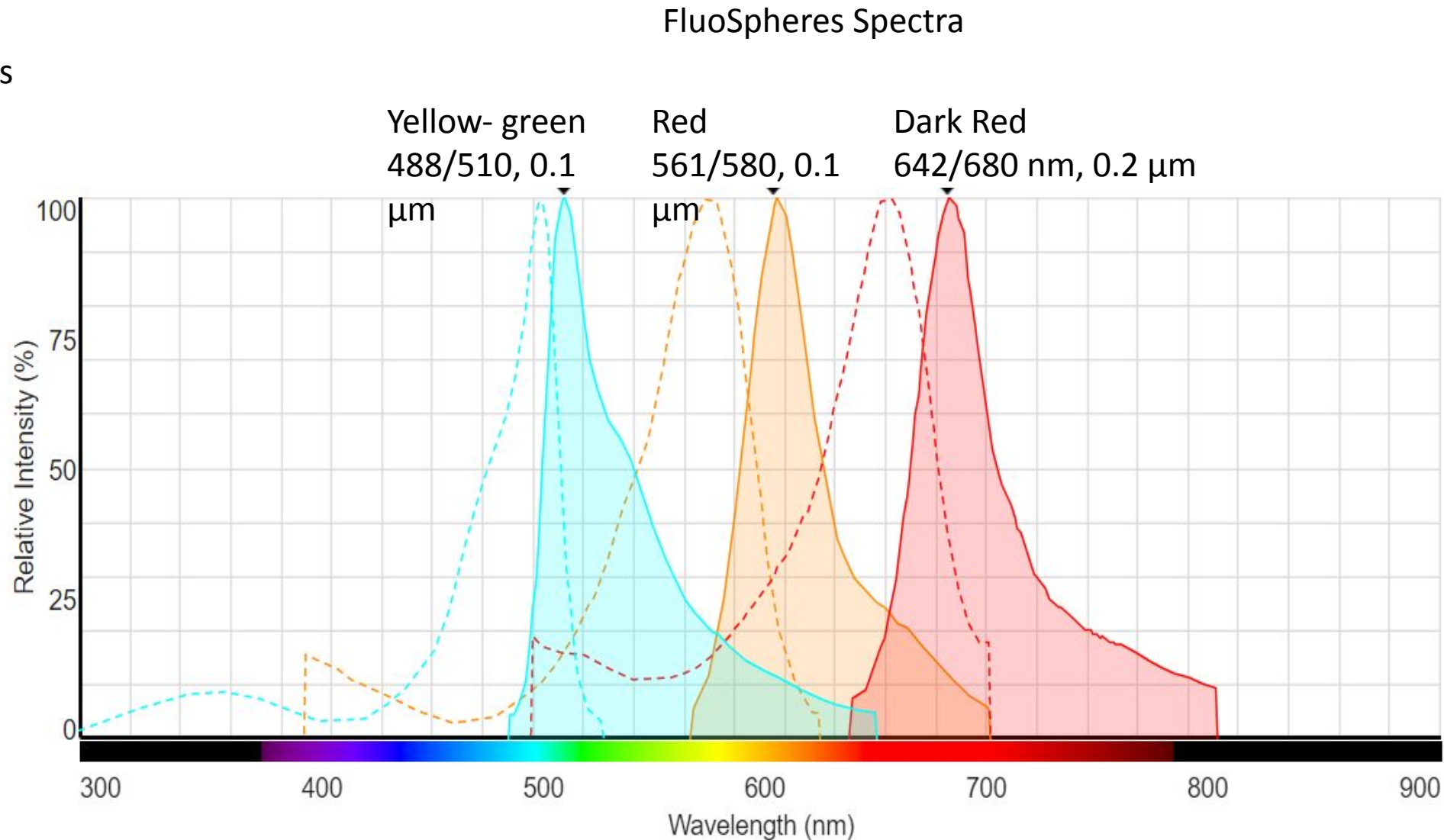X-Distance from Reference Coordinate [px]

Spectral *590 nm, Reference*

1024 x 1024 pixels
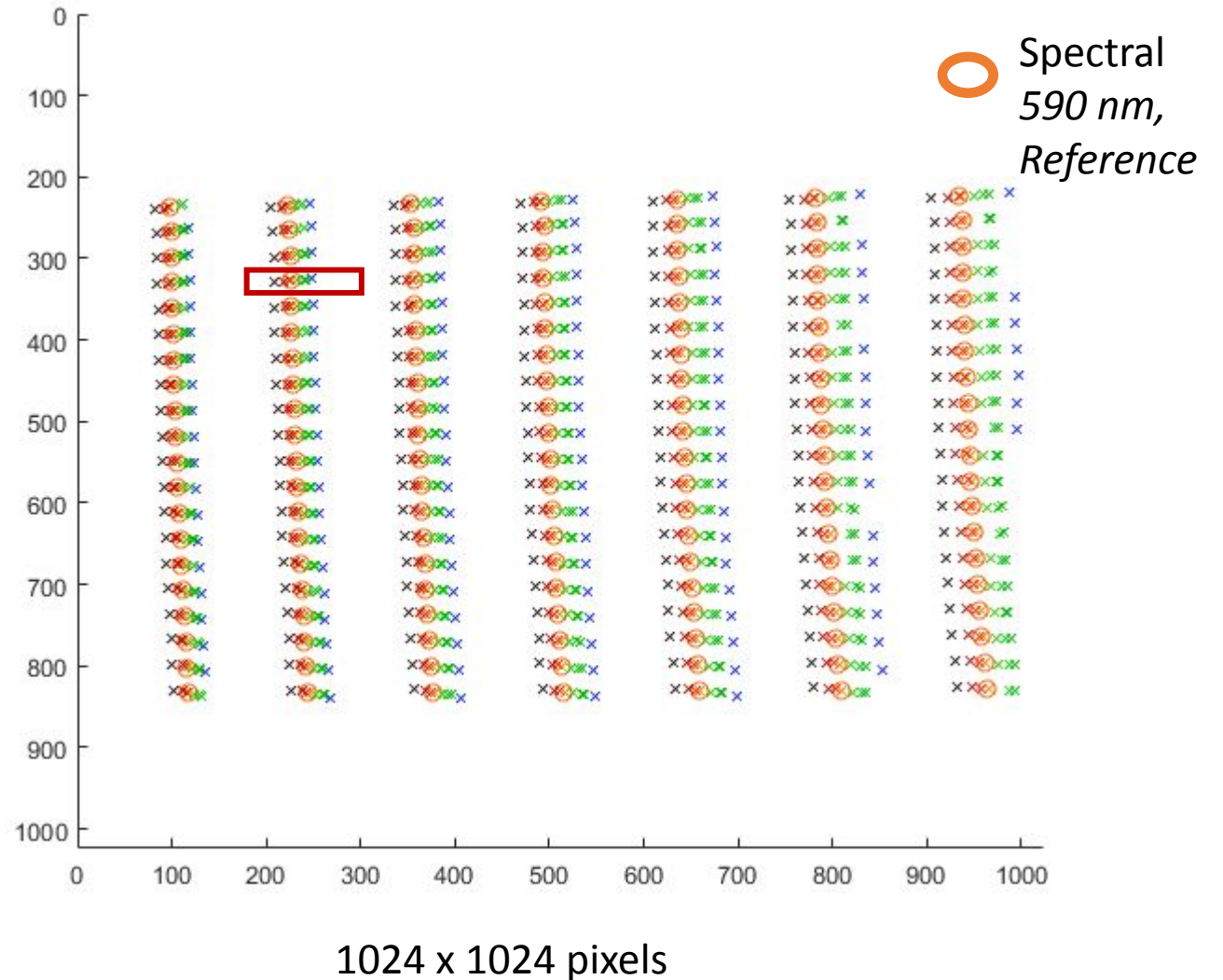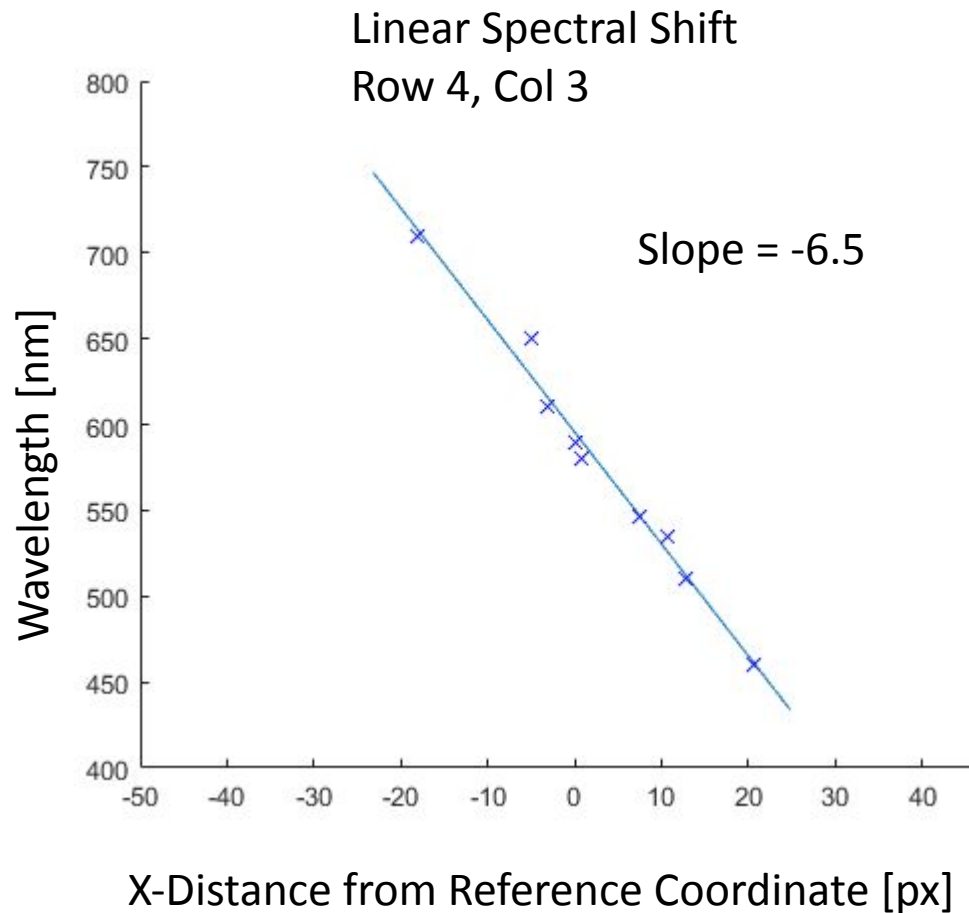
Three microbeads with single dyes

Goal:

Characterize three beads on mixed slides based on spectra

Direct Camera

FluoSpheres Spectra

Yellow- green
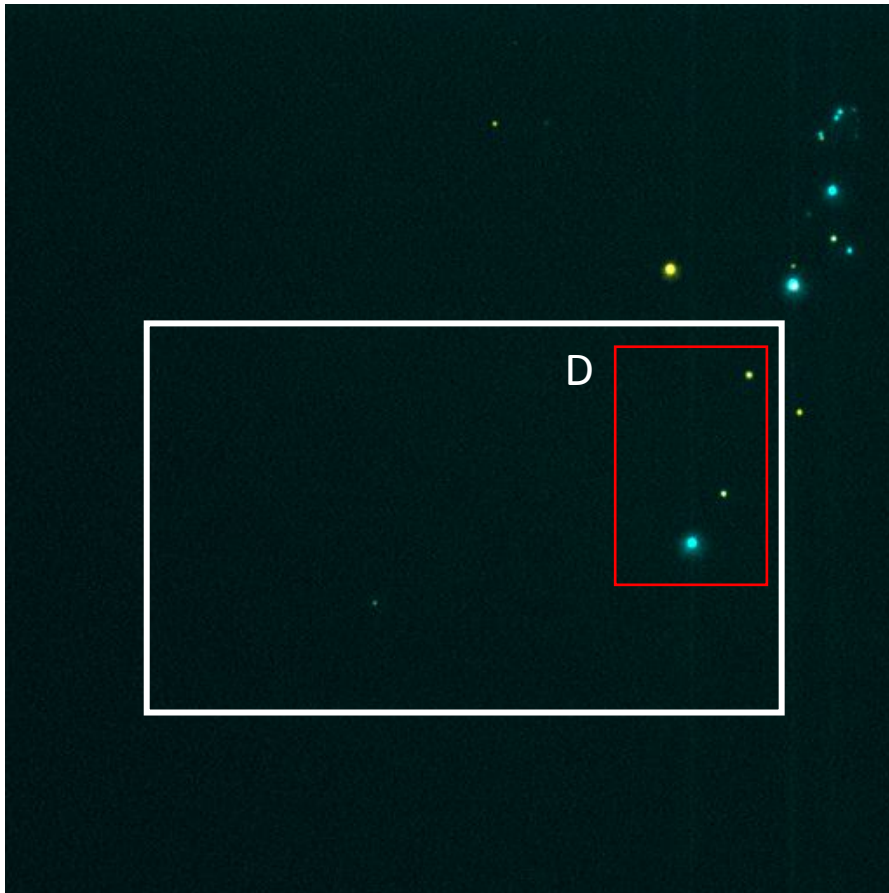488/510, 0.1 μm

Red
561/580, 0.1 μm
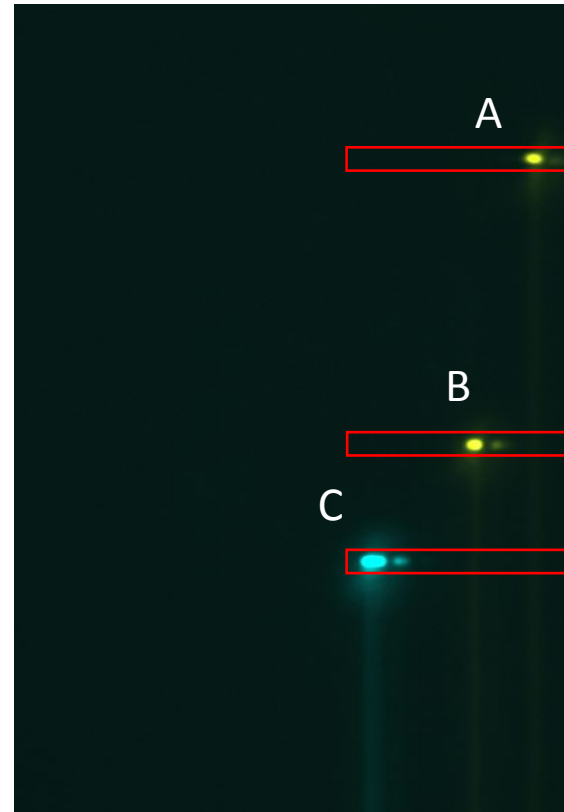
Dark Red
642/680 nm, 0.2 μm
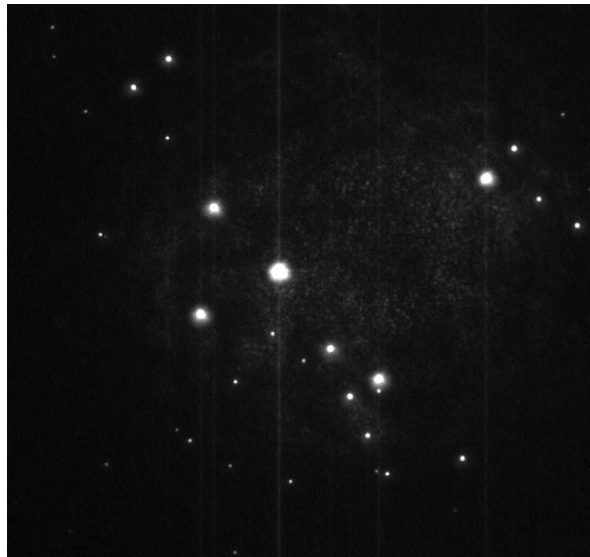
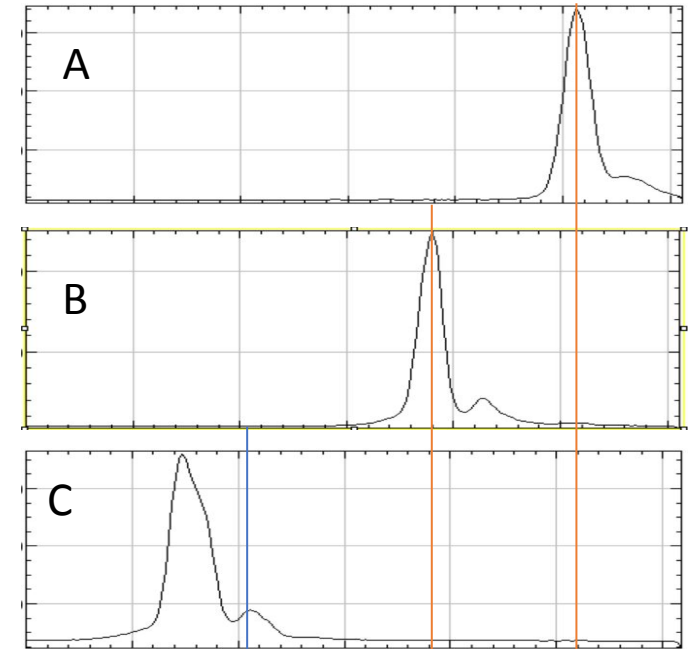# Yellow-green bead (cyan) appears more shifted left than the red bead (yellow)

Direct camera – false colored composite
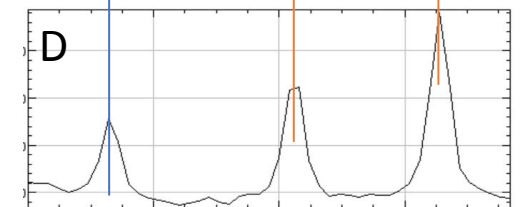
Spectral camera

Spectral camera intensity profiles

Direct camera intensity profiles

488 nm yellow-green bead spectrum
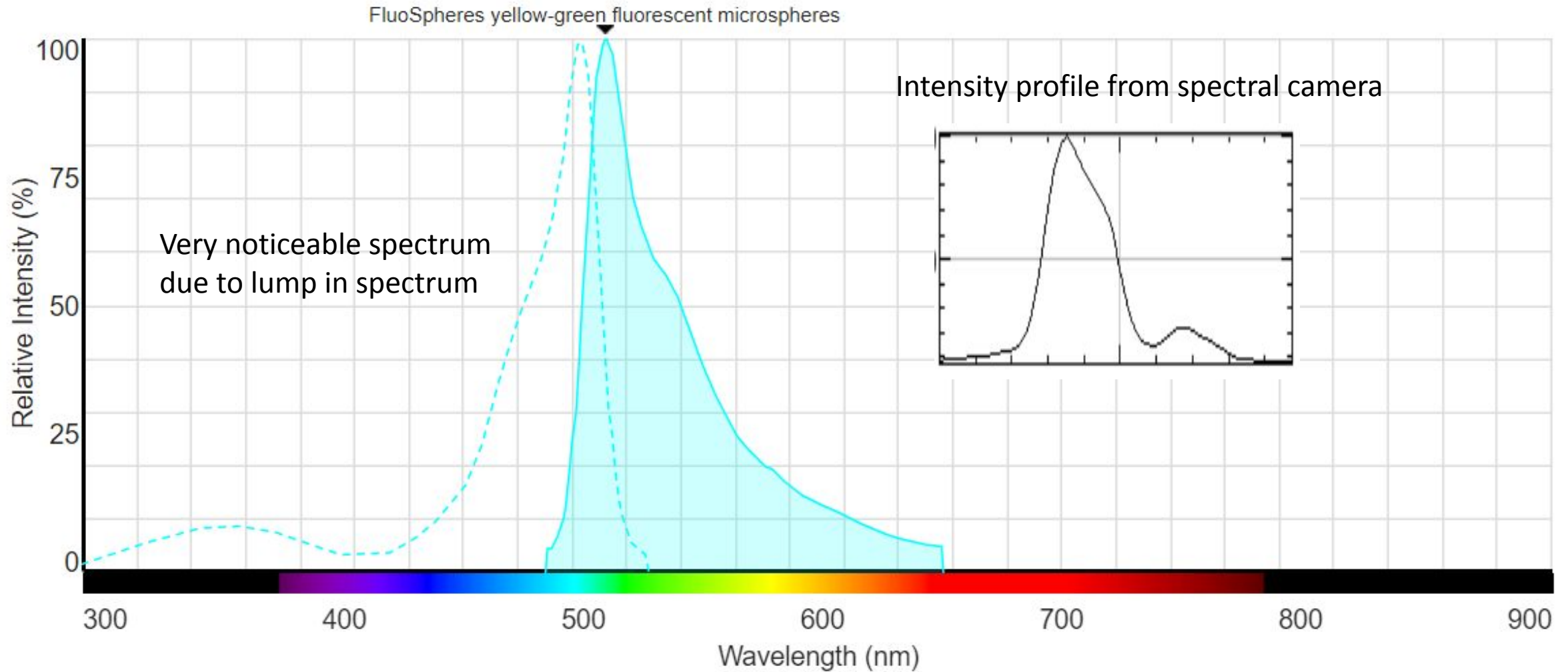
FluoSpheres yellow-green fluorescent microspheres

Intensity profile from spectral camera

Very noticeable spectrum
due to lump in spectrum

Relative Intensity (%)

Wavelength (nm)

Imaging fixed tri-color beads

Direct Camera

Spectral Camera

642, 561, **488**

642, **561**, 488

642, 561, 488

642, 561, 488

Laser Lines (nm)

Dispersion:
Red ---> Blue spread

Imaging fixed tri-color beads

Direct Camera

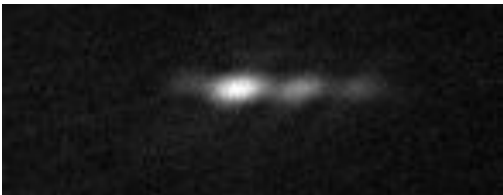Spectral Camera

642, 561, **488**

642, **561**, 488

642, 561, 488

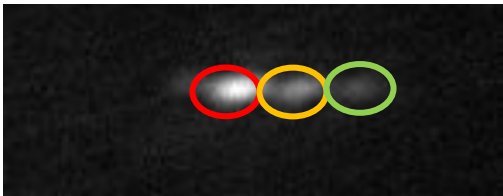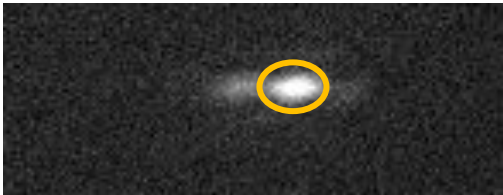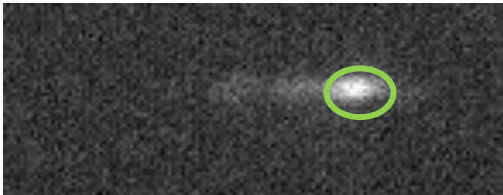**642, 561, 488**

Laser Lines (nm)

Dispersion:
Red ---> Blue spread

Future directions - Quantum Dots?

Pros

- Very narrow spectra
- Excitable by 405 nm laser
- Blink when isolated or can stably fluoresce
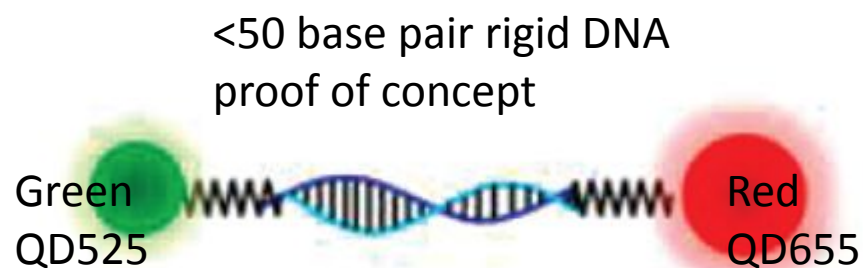- Stable for a long time
- Bright!
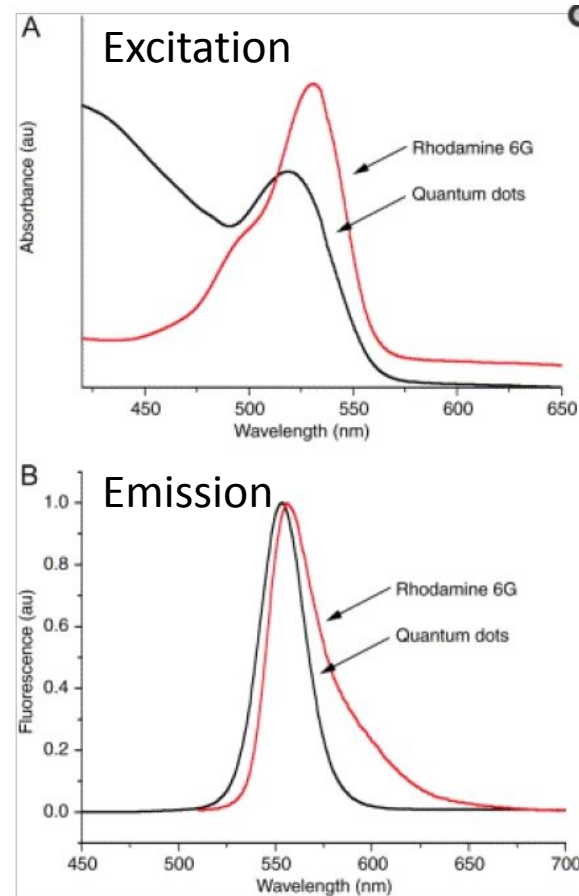
Cons

- Much larger (tens of nm)
- Spectral blueing in oxygen environment and toxic to cells (mostly fixed)

Chan et al. Luminescent quantum dots for multiplexed biological detection and imaging. *Current opinion in biotechnology*. 2002; 13:40-46

Quantum dot pairs used as DNA ruler with superlocalization

<50 base pair rigid DNA proof of concept

Green QD525

Red QD655

0th order (straight)

1st order (deflected)
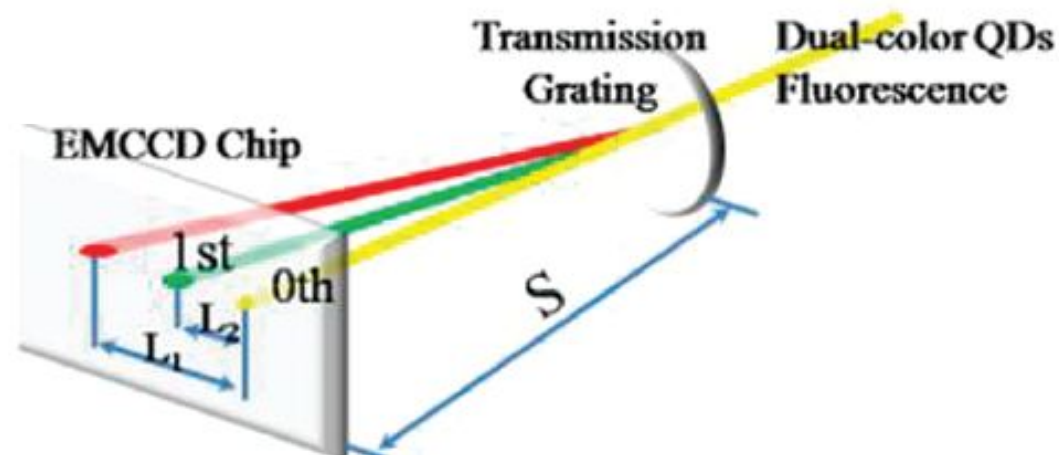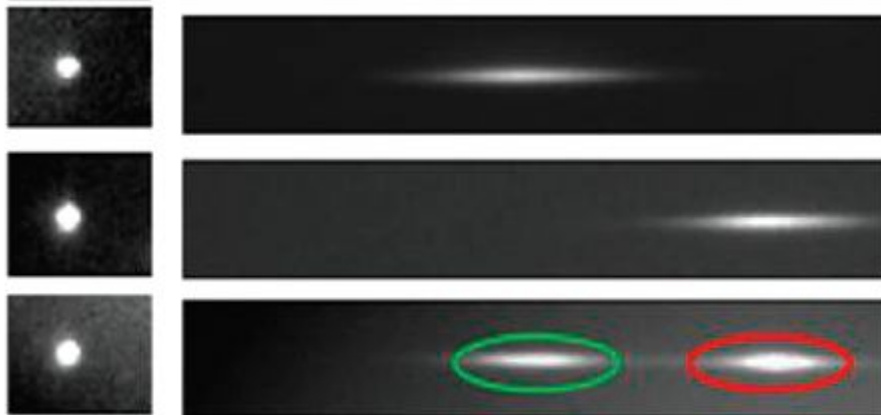
Superlocalization Spectral Imaging Microscopy of a Multicolor Quantum Dot Complex

Xingbo Shi,[†] Zhongqiu Xie,[†] Yuehong Song,[†] Yongjun Tan,[†] Edward S. Yeung,[†] and Hongwei Gai*[,†,‡]

Quantum dot pairs used as DNA ruler with superlocalization

0th order
(straight)

1st order
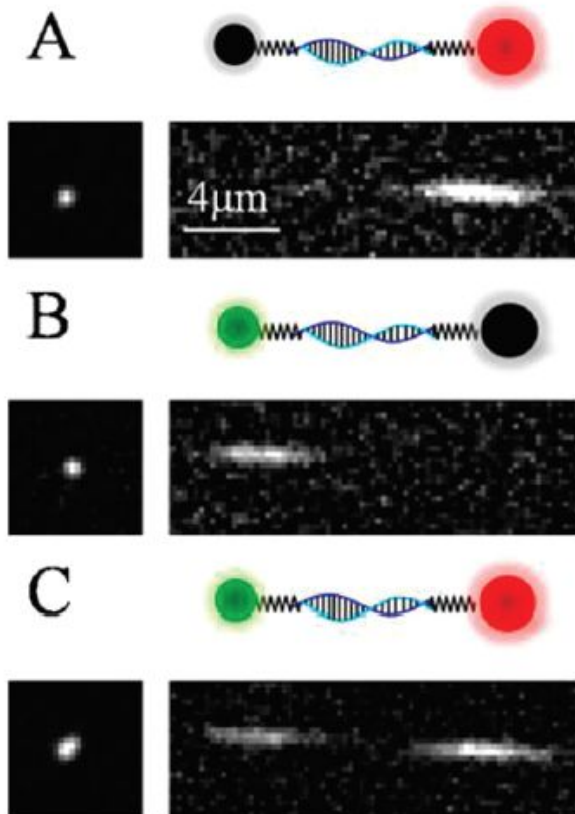(deflected)

1st order intensities

Localization model

Immobile QD
as reference

Quantum dot pairs used as DNA ruler with superlocalization

0th order (straight)   1st order (deflected)

1st order intensities

Localization model

4μm

A

Intensity

0   200   Time (s) 400   600

Localize!

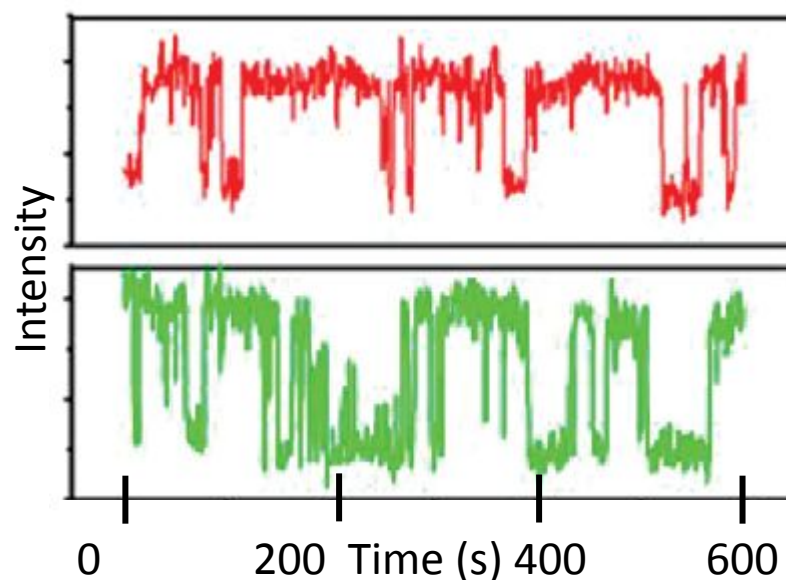Immobile QD as reference

Localize red when green is off

Quantum dot pairs used as DNA ruler with superlocalization

0th order
(straight)

1st order
(deflected)

1st order intensities

Localization model

B

Intensity

0   200 Time (s) 400   600

Localize!

Immobile QD
as reference

Localize green when red is off

Xingbo Shi,[†] Zhongqiu Xie,[†] Yuehong Song,[†] Yongjun Tan,[†] Edward S. Yeung,[†] and Hongwei Gai*[,‡]

Quantum dot pairs used as DNA ruler with superlocalization
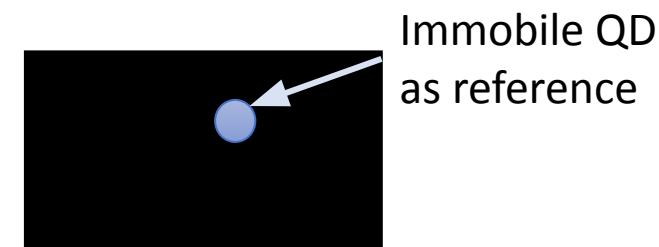
0th order (straight) 1st order (deflected) 1st order intensities Localization model

Conclusion:
estimated distance of 3 DNA lengths
within 2 nm of accepted value,
each with less than 7 nm uncertainty

Current hyperspectral setup may use similar QD pairs/triplets for **spectral barcoding** (…no superlocalization)

Current hyperspectral setup may use similar QD pairs/triplets for **spectral barcoding** (…no superlocalization)

Marker Type	QD Spectra	Spectral Camera Intensity Profiles
460 + 650 QDs 1:1		
525 + 650 QDs 1:1		
590 + 650 QDs 1:1		
525 + 650 QDs 1:2		

400 nm — Visible Light — 800 nm

Current hyperspectral setup may use similar QD pairs/triplets for **spectral barcoding** (…no superlocalization)

Visible Light

400 nm — 800 nm

Marker Type   QD Spectra   Spectral Camera Intensity Profiles

Marker Type	QD Spectra	Spectral Camera Intensity Profiles
460 + 650 QDs 1:1		60 px
525 + 650 QDs 1:1		40 px
590 + 650 QDs 1:1		20 px
525 + 650 QDs 1:2		40 px

How to distinguish these two?

Current hyperspectral setup may use similar QD pairs/triplets for **spectral barcoding** (…no superlocalization)

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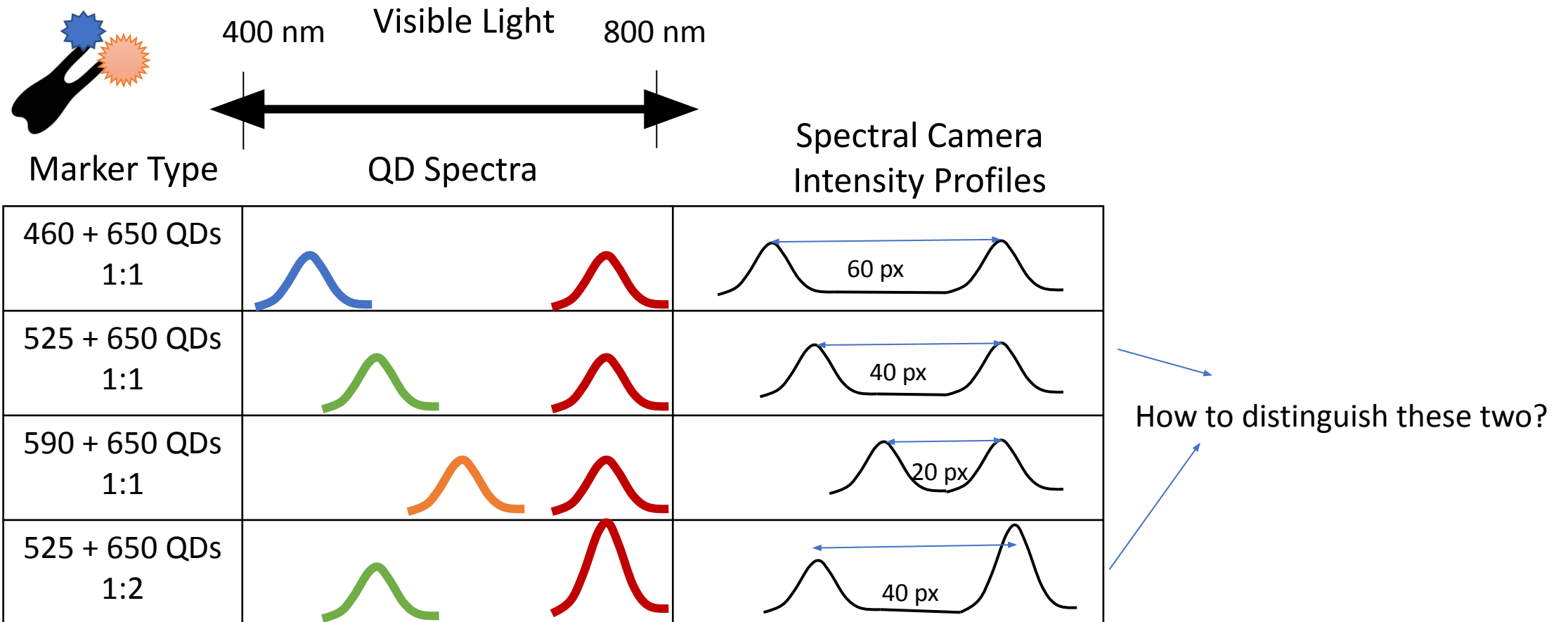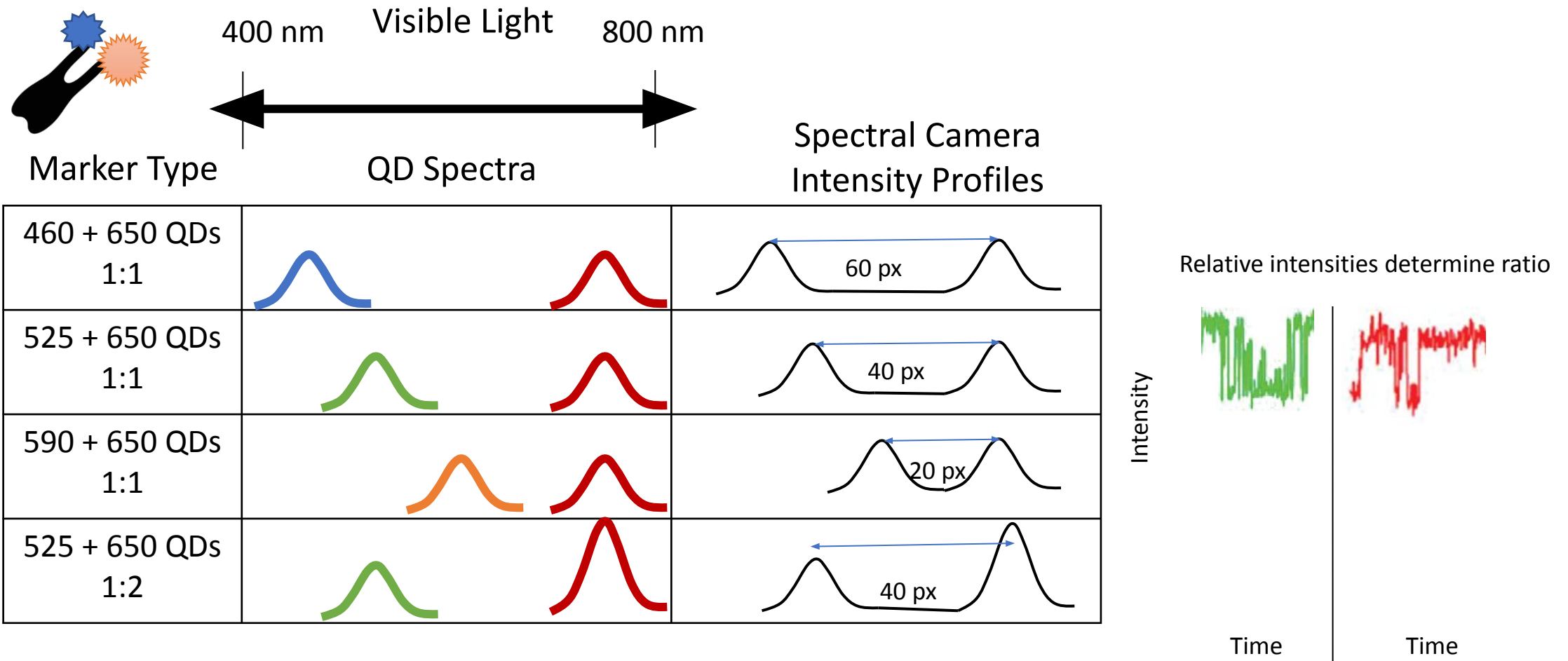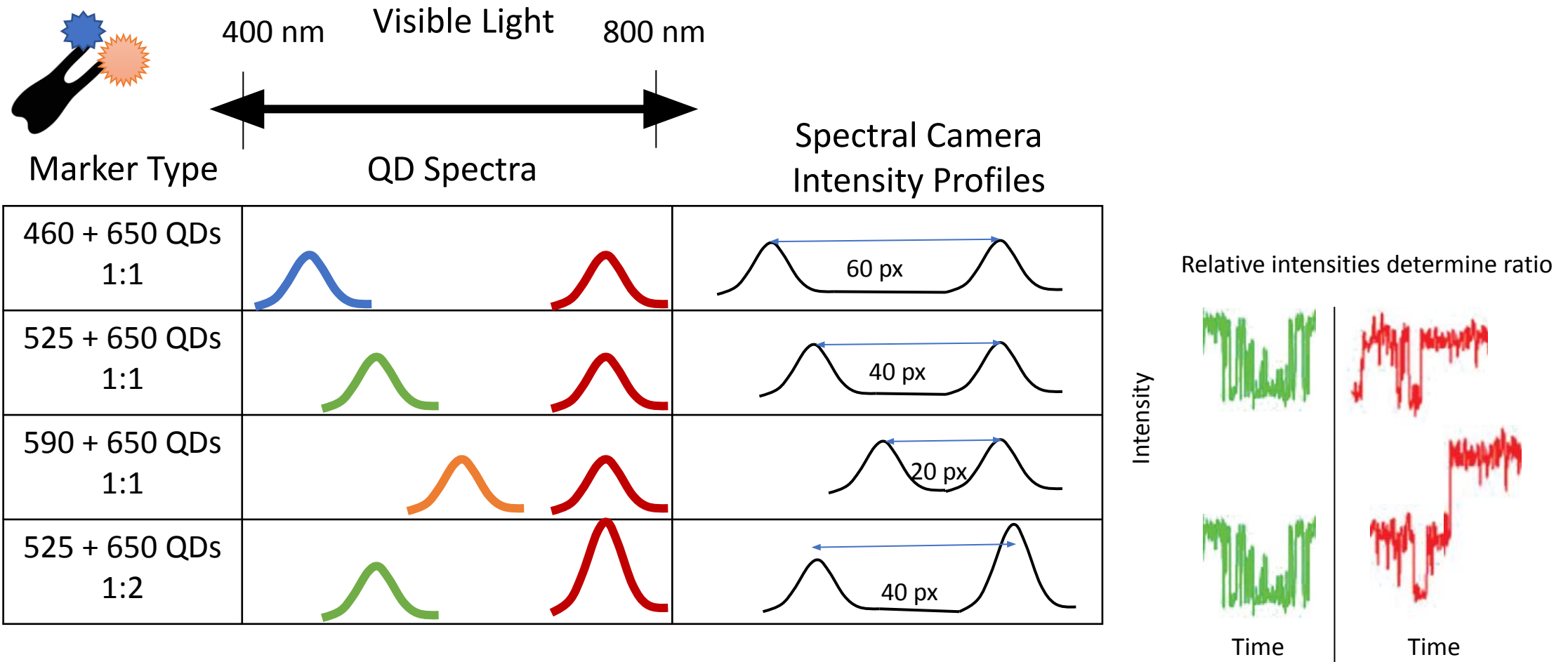| Marker Type	QD Spectra		Spectral Camera Intensity Profiles
460 + 650 QDs 1:1			60 px
525 + 650 QDs 1:1			40 px
590 + 650 QDs 1:1			20 px
525 + 650 QDs 1:2			40 px

Visible Light
400 nm — 800 nm
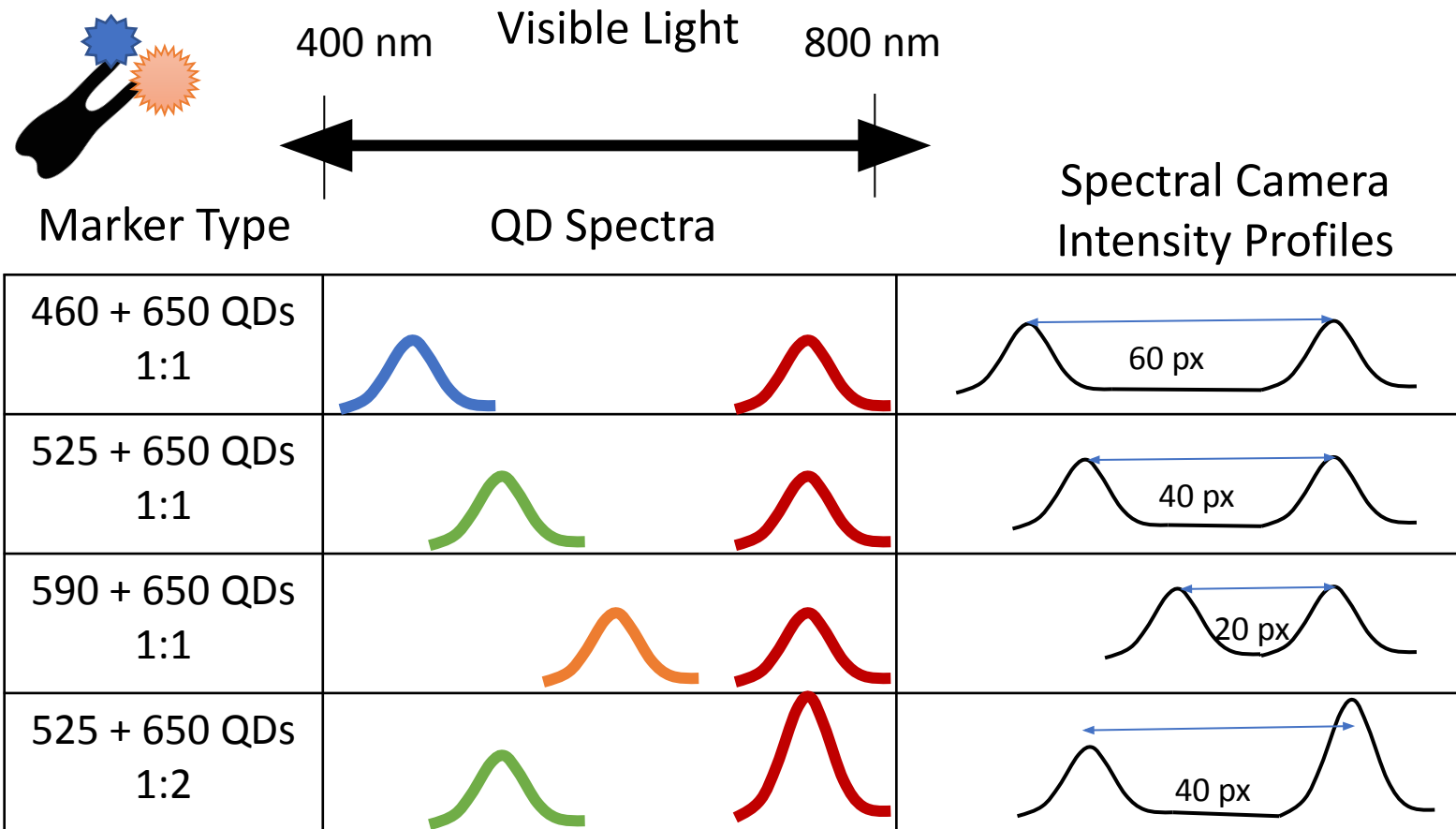
Spectral Barcodes!

Relative intensities determine ratio
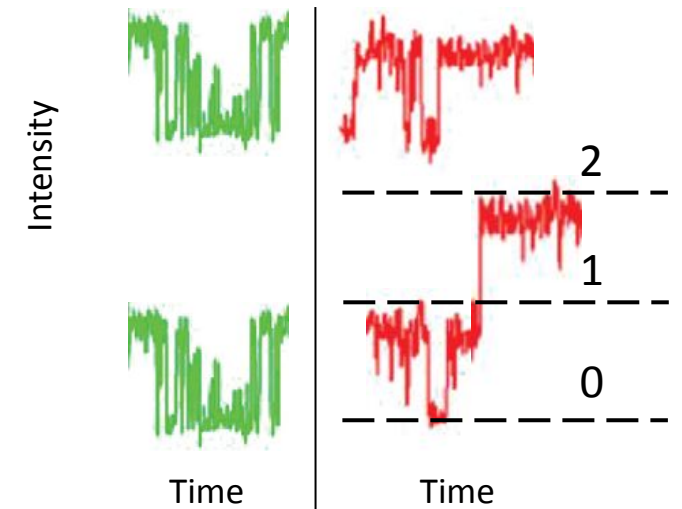
Intensity / Time

2
1
0
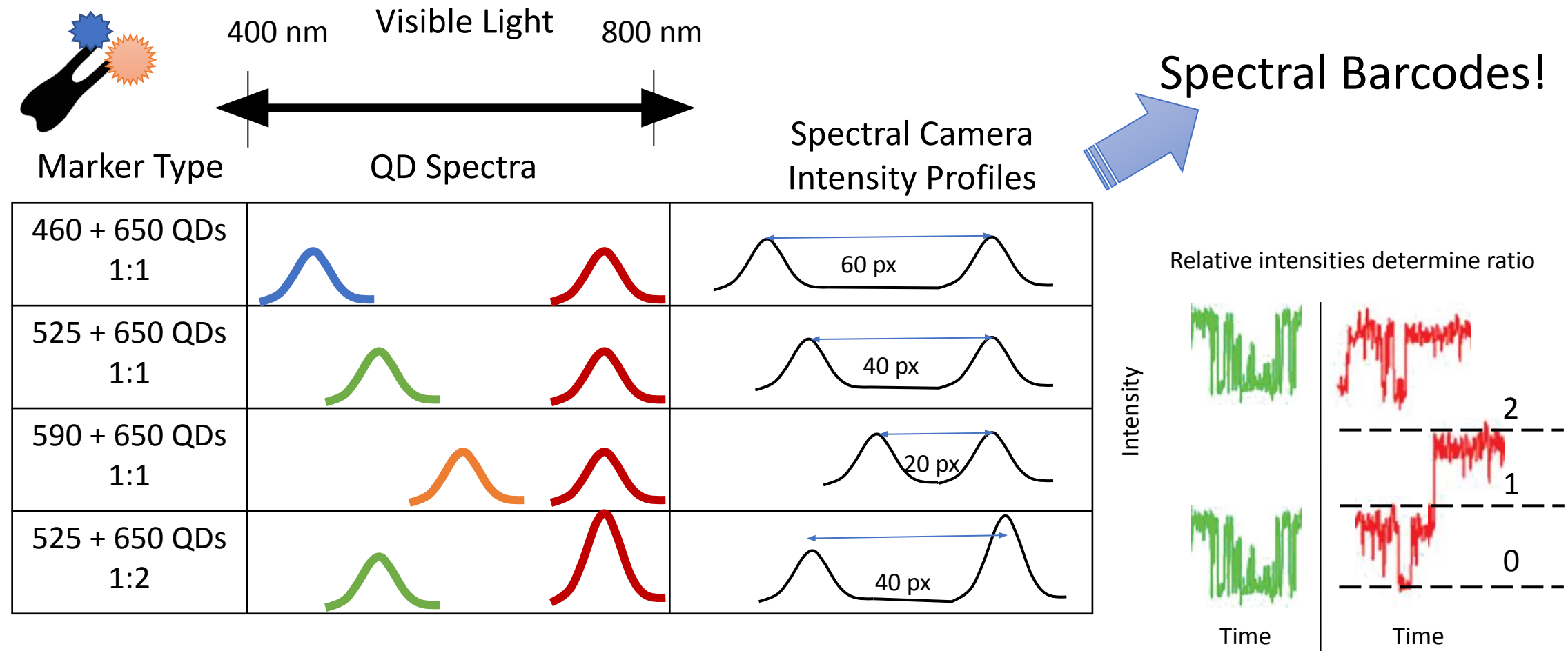
Hyperspectral imaging –
what it (maybe) can and can't do

Requirements:

-fixed reference positions
for direct camera

-sparse but bright

- >50 ms exposure

-no blinking,
except for spectral barcodes

-no sub-pixel localization
experiments ☹

Hyperspectral imaging –
what it (maybe) can and can't do

Requirements:

-fixed reference positions
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experiments ☹

Dynamics

Fixed objects with reference color (tagged in blue)
and binding fluorescent probes (green/red)

☐ relative intensities reveal binding dynamics

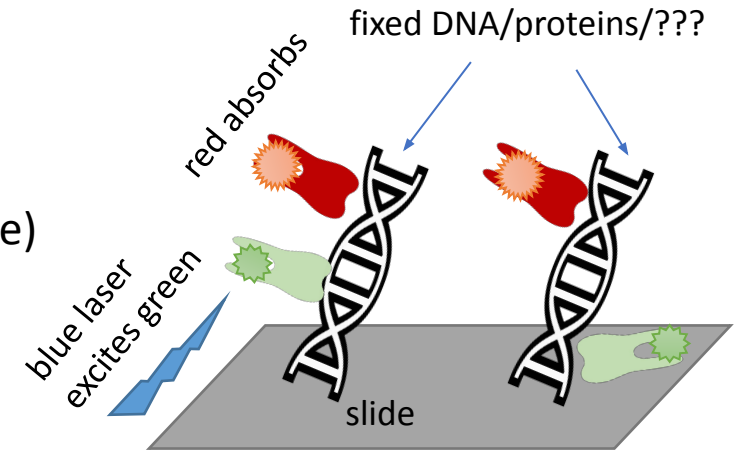# Hyperspectral imaging –
what it (maybe) can and can't do

Requirements:

-fixed reference positions for direct camera

-sparse but bright

- >50 ms exposure

-no blinking, except for spectral barcodes

-no sub-pixel localization experiments ☹

Dynamics

Fixed objects with reference color (tagged in blue) and binding fluorescent probes (green/red)
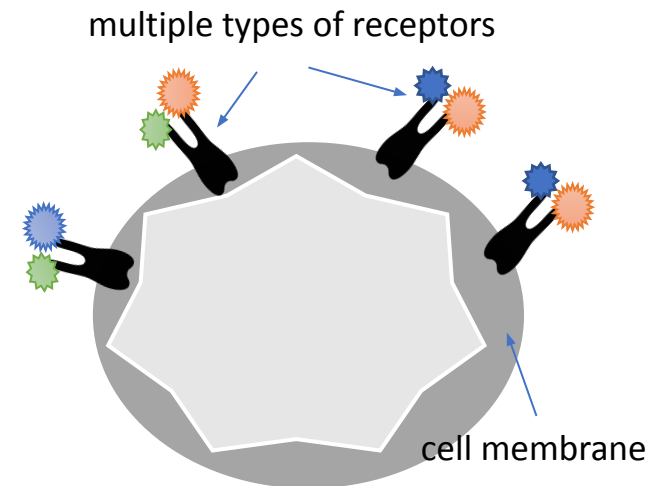
☐ relative intensities reveal binding dynamics

red absorbs

fixed DNA/proteins/???

blue laser excites green

slide
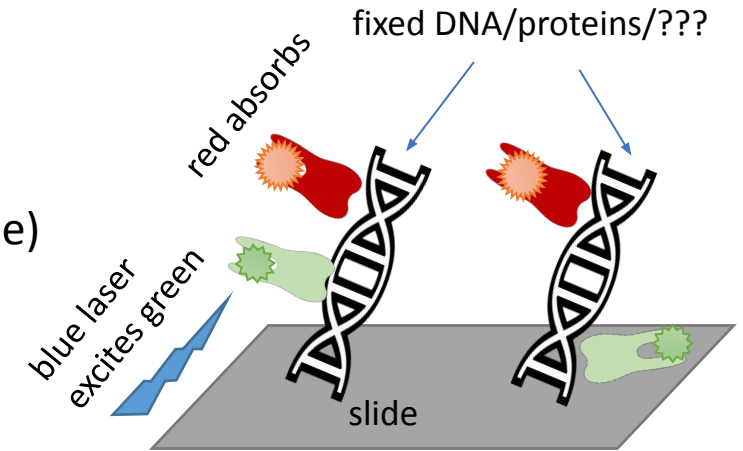
Multiplex Imaging

Blinking QD complexes bind to receptors on membrane

☐ frequency/type of cell receptors reveal information about cell

multiple types of receptors

cell membrane

Thank you!

<3