Multi-modal Single-molecule Imaging with Continuously Controlled Spectral-resolution (CoCoS🌴) Microscopy

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NanoBioPhotonixLab
Fluorescence microscopy: Color as a contrast mechanism

Single molecules interactions

Single molecule Förster Resonance Energy Transfer (smFRET)

Optical schemes for wide-field color detection

Filter wheel

Color channel split-view

Spectral split-view

✓ Space
✓ Time
✗ Time
✗ Space

✓ Time
✓ More colors
✗ Space (<20×20μm²)
✗ SNR

CoCoS
Optical Setup

✓ Large FOV (130×130 μm²)

✓ Almost no photon loss

✓ Simultaneous multi-color acquisition

✓ Simple add-on (maintains optical axis)
Operation principle

Double Amici prism (direct vision prism)

Atmospheric chromatic aberration correction in astronomy

RPA = relative prism angle

\[ D(\lambda) = 2D_{SP}(\lambda) \cdot \sin\left(\frac{180^\circ - RPA}{2}\right) \]

Continuously Controlling Spectral-resolution (CoCoS)

Three CoCoS modalities:

- **Localization** (no dispersion)
- **Color detection** (minimal dispersion)
- **Spectroscopy** (fully detailed spectra)

SNR & Throughput

Spectral-resolution

0.5 s
Continuously Controlling Spectral-resolution (CoCoS)

Choosing the ideal spectral-resolution for color detection:

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<th>AF 405</th>
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<th>CY 3</th>
<th>ATTO 550</th>
<th>AF 555</th>
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<th>CF 640R</th>
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1. Alexa Fluor 350
2. Alexa Fluor 405
3. Alexa Fluor 430
4. Alexa Fluor 488
5. Alexa Fluor 500
6. Alexa Fluor 514
7. Alexa Fluor 532
8. Alexa Fluor 546
9. Alexa Fluor 561
10. Alexa Fluor 594
11. Alexa Fluor 647
12. IRDye 800CW
13. FITC
14. FITC
15. FITC
16. FITC
17. ALEXA FLUOR 647
18. ALEXA FLUOR 647
19. ALEXA FLUOR 647
20. ALEXA FLUOR 647

180°: Full cancelation
0°: Max dispersion
178° 176° 174° 166° 162° 172° 170° 168° 164° 160° 140°
Application 1: **Color detection**

Single microRNA identification using 4-color NanoString barcodes

In collaboration with the Shomron lab

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**a.**

- hsa-miR-150-5p
- hsa-miR-142-3p
- hsa-miR-223-3p

**b.**

- **180°**
- **176°**
- **176°**

NanoString sequential acquisition  
CoCoS single snapshot matrix decoding
Barcode decoding in a single snapshot

- Single-snapshot decoding: 4-fold increased throughput
- More colors - No time cost: >410-fold increase in available barcode combinations

In collaboration with the Shomron lab
Application 2: Spectroscopy

Resolving the shades of far-red dyes (spectral detection with a single laser excitation)

Can we resolve multiple far-red dye within the same color channel?
### a. Intensity

- **180° (no dispersion)**
  - Localization image

- **120° (optimal dispersion)**
  - Spectral image

- **±30° prism rotation**

### b. Overlaid localization and spectral FOV

#### Classified localization FOV

- CF640R
- AF647
- Cy5.5

#### Overlaid localization and spectral FOV

- CF640R
- AF647
- Cy5.5

### c. Normalized intensity

- **CF640R**
  - Mean correlation factor: 0.99

- **AF647**
  - Mean correlation factor: 0.98

- **Cy5.5**
  - Mean correlation factor: 0.96

### d. Wavelength [nm]

- **Normalized intensity**

Wavelength range: 640 to 780 nm
Future application: 6 targets with a single excitation

- <6nm spectral-resolution – full spectral characterization
- Reduced phototoxicity for live samples
- Reduced optical complexity and costs (single excitation, no chromatic aberrations)
Application 3: Measuring nm distances with smFRET

- Single molecule sensitivity in Epi-illumination (no TIRF)
- >6-fold increase in throughput over standard dual-color smFRET
- >4-color smFRET with ultra-high throughput

In collaboration with the Craggs lab
Thanks!

**New Results**

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Jonathan Jeffet, Yael Michaeli, Dmitry Torchinsky, Ifat Israel-Elgali, Noam Shomron, Timothy D. Craggs, Yuval Ebenstein

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Thanks!
**Veronica Labrador**  5 minutes ago
How would this technique work in a high density fluorophore situation?

**Denitza Denkova**  5 minutes ago
Is there any limitation on how sparse/dense your sample labelling can be?

**Julien Colombelli**  5 minutes ago
In SMLM to Cocos combination, how is the maximum density of single molecules affected compared to a normal SMLM experiment: should it be n (?) times lower in order to not suffer overlap?

**Sebastien Tosi**  4 minutes ago
Does the order of detection of the labels matters for barcode classification? If so, how do you deal with barcode orientation?

**Julien Colombelli**  4 minutes ago
With aligned prisms for no dispersion, does the PSF suffer and downgrades the SMLM localization precision?