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Wilson College of Textiles

Integrating Spectroscopic Single-Molecule Localization Microscopy with DNA-PAINT for Simultaneous Multiplexing Super-Resolution Microscopy

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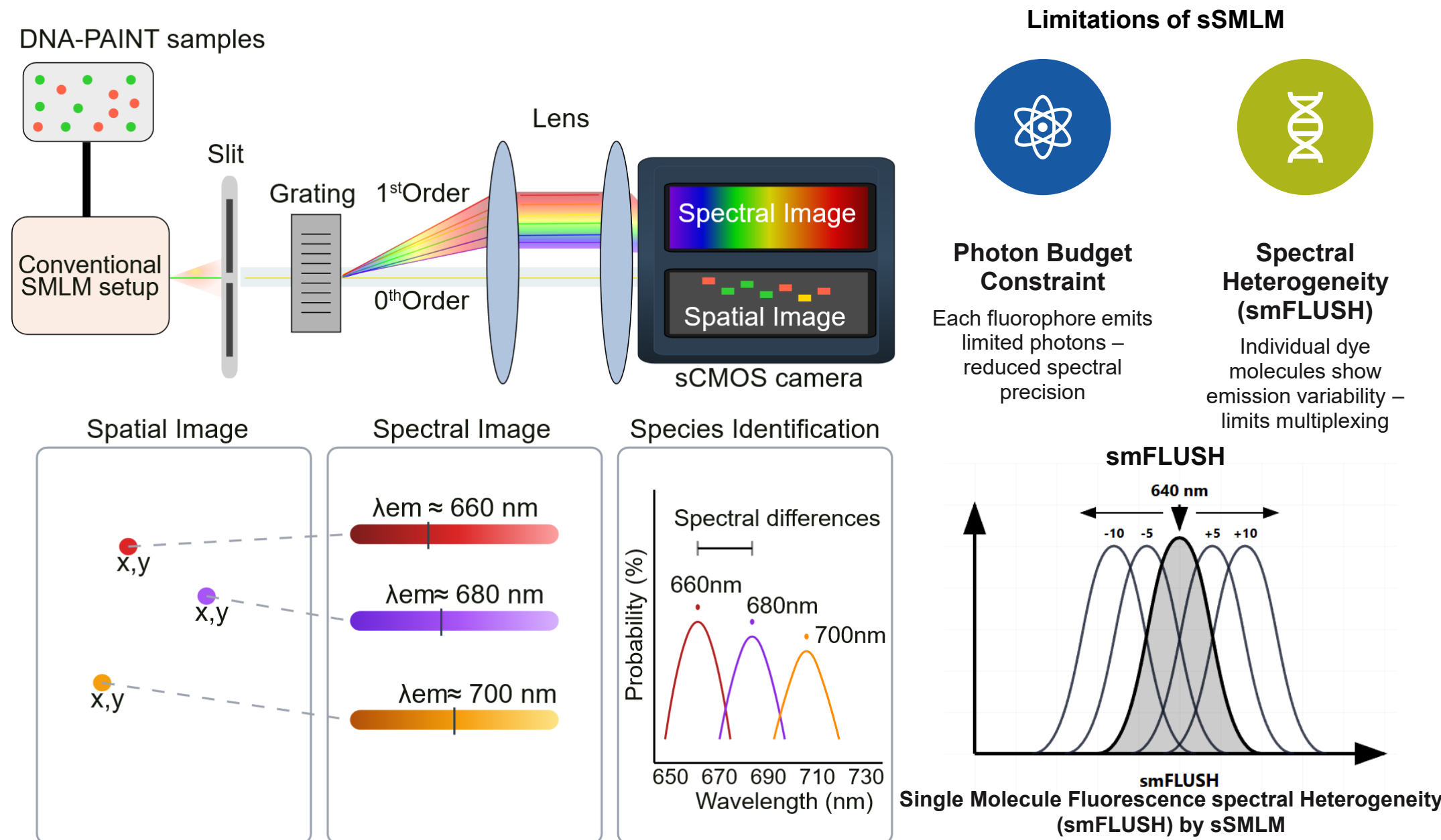
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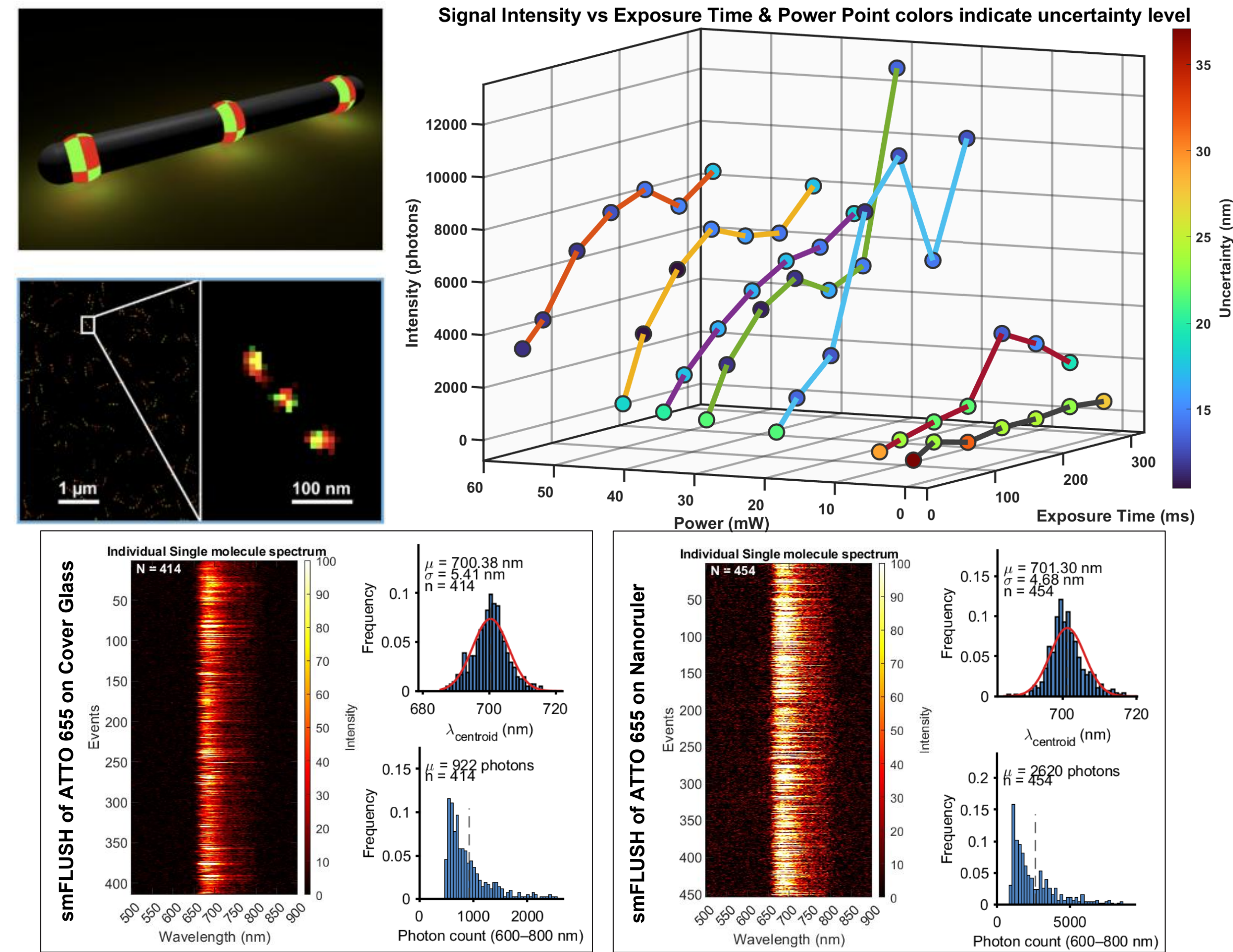
Joint Department of **BIOMEDICAL ENGINEERING**
UNC CHAPEL HILL NC STATE UNIVERSITY

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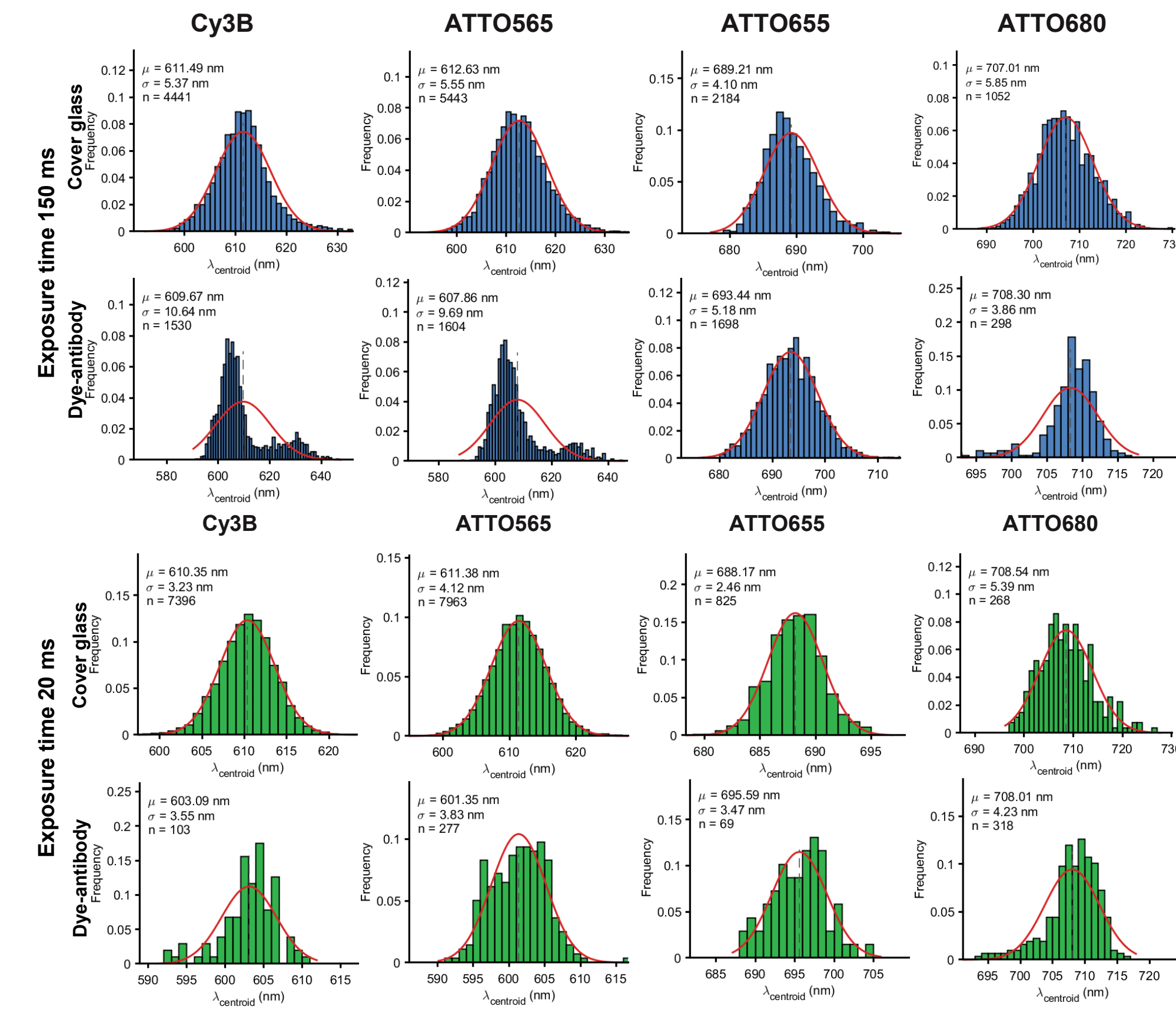
Multiplexing by Spectroscopic SMLM (sSMLM)



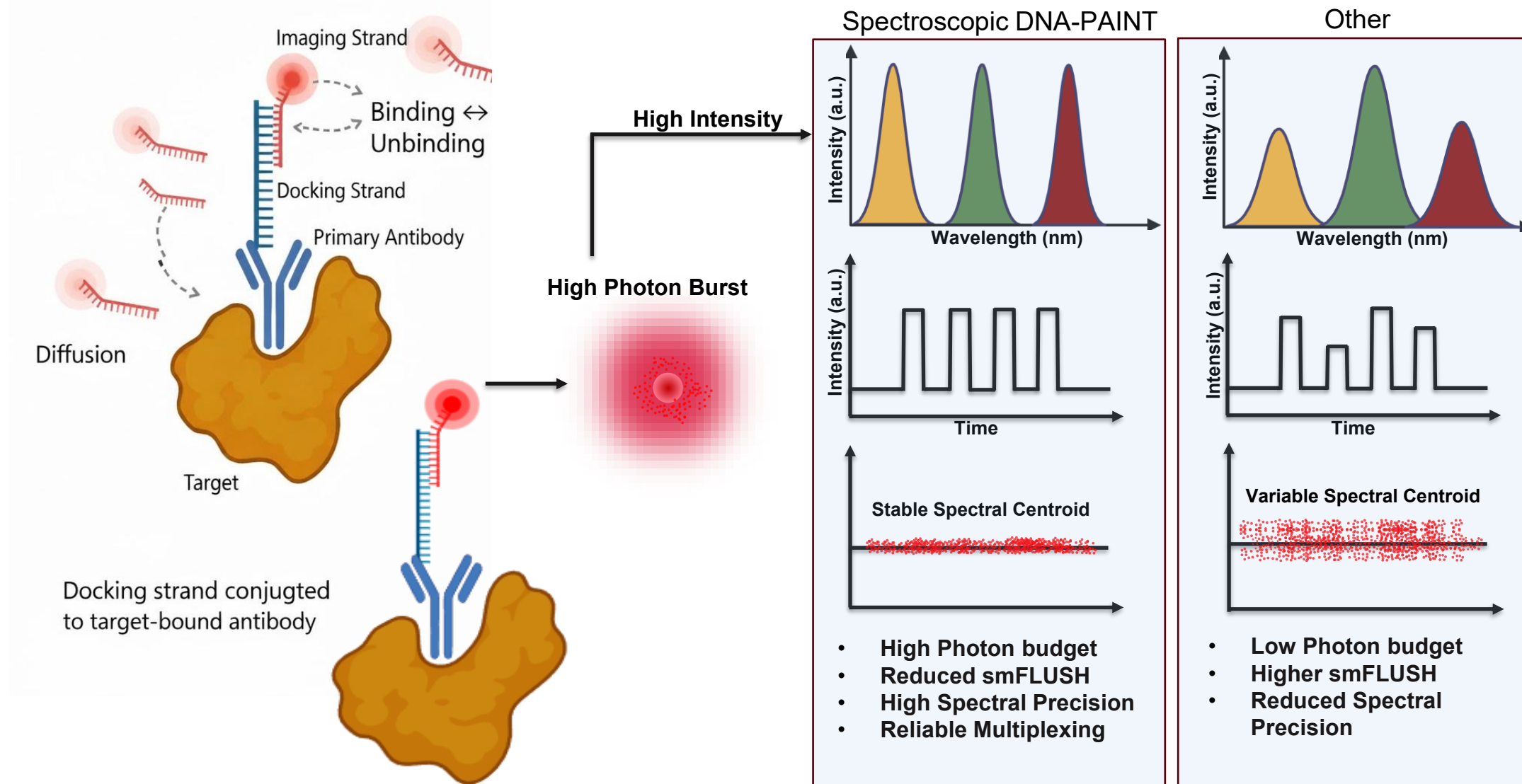
Nanoruler Calibration and Optimization



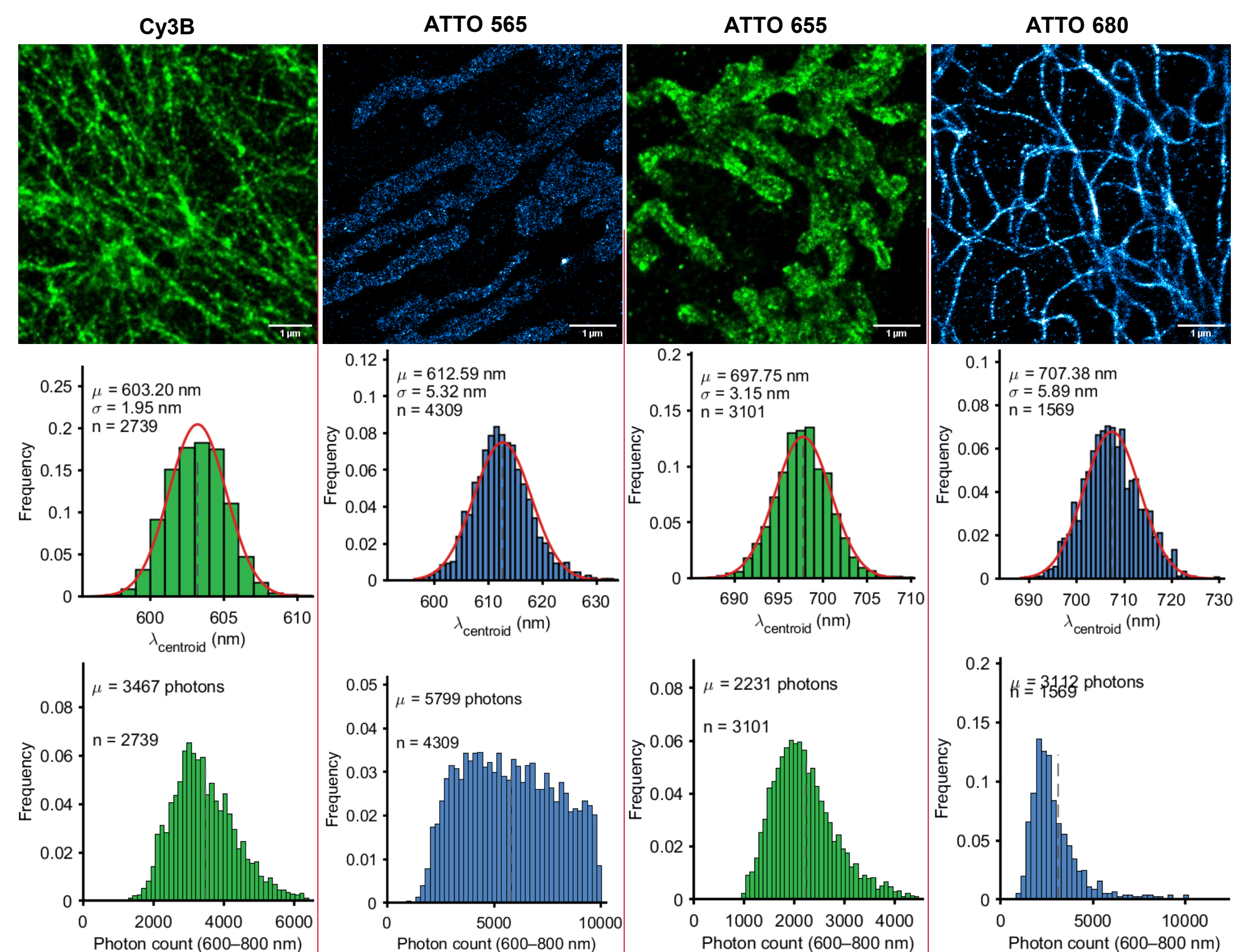
smFLUSH results on cover glass and dye-antibody conjugation



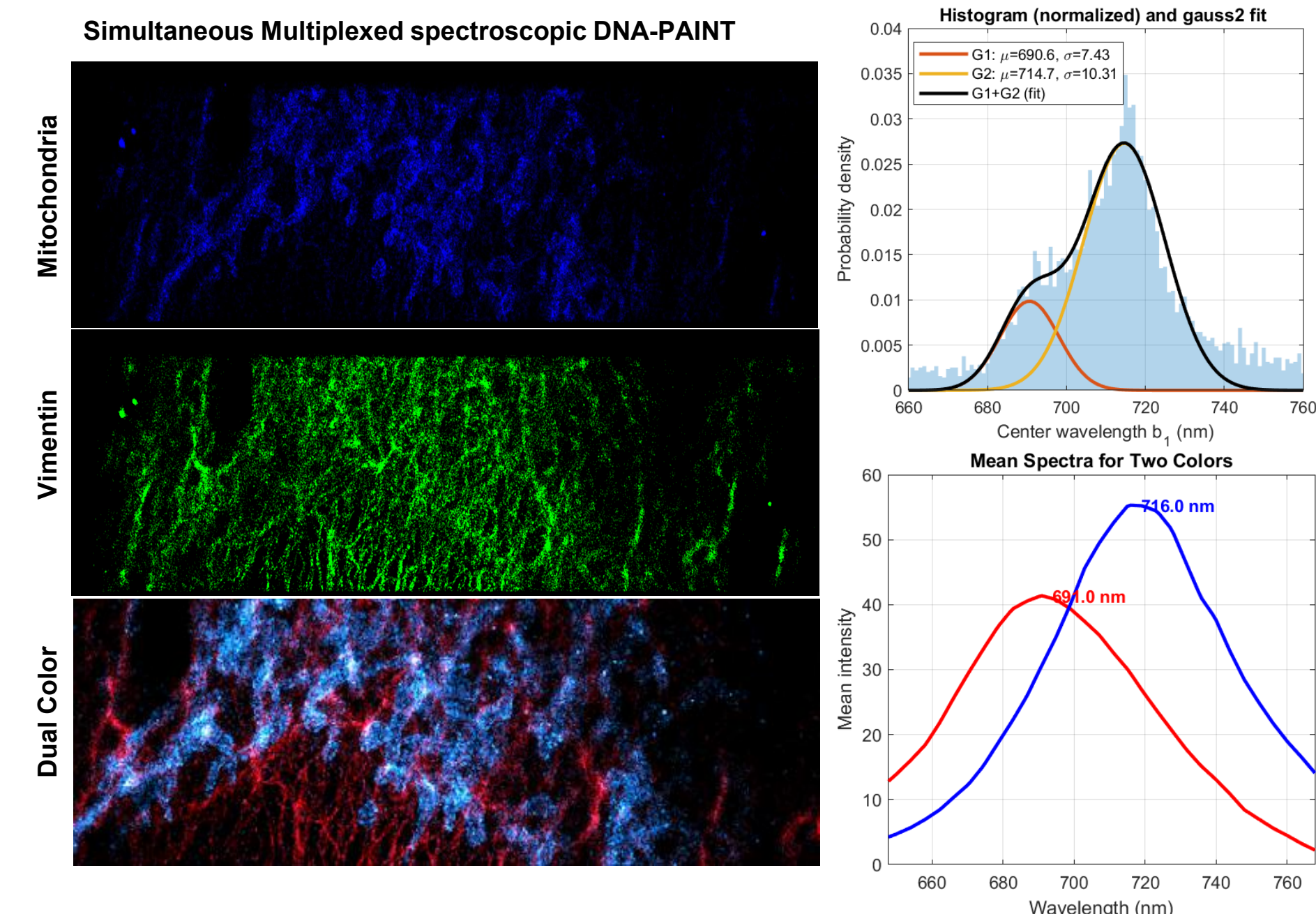
DNA PAINT



Single Color Spectroscopic DNA PAINT



Dual Color Spectroscopic DNA PAINT



Experimental Design

- Nanoruler calibration & optimization:** DNA-origami nanorulers from Massive Photonics were used to optimize imaging parameters for our system and benchmark single-molecule spectral stability.
- smFLUSH assessment under DNA-PAINT:** Single-molecule spectra of ATTO655 were quantitatively compared under DNA-mediated binding versus bare glass to evaluate spectral centroids, dispersion, and photon budget.
- Cellular validation of spectral stabilization:** DNA-PAINT imaging of fixed cells (mitochondria and Vimentin labeling) confirmed reduced smFLUSH and stable spectral centroids and higher photon budget in biological environments.
- Simultaneous multiplexed spectroscopic DNA-PAINT imaging:** Established spectral separability enabled single-acquisition, multi-target (mitochondria & vimentin) spectroscopic DNA-PAINT without sequential imaging.

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References

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