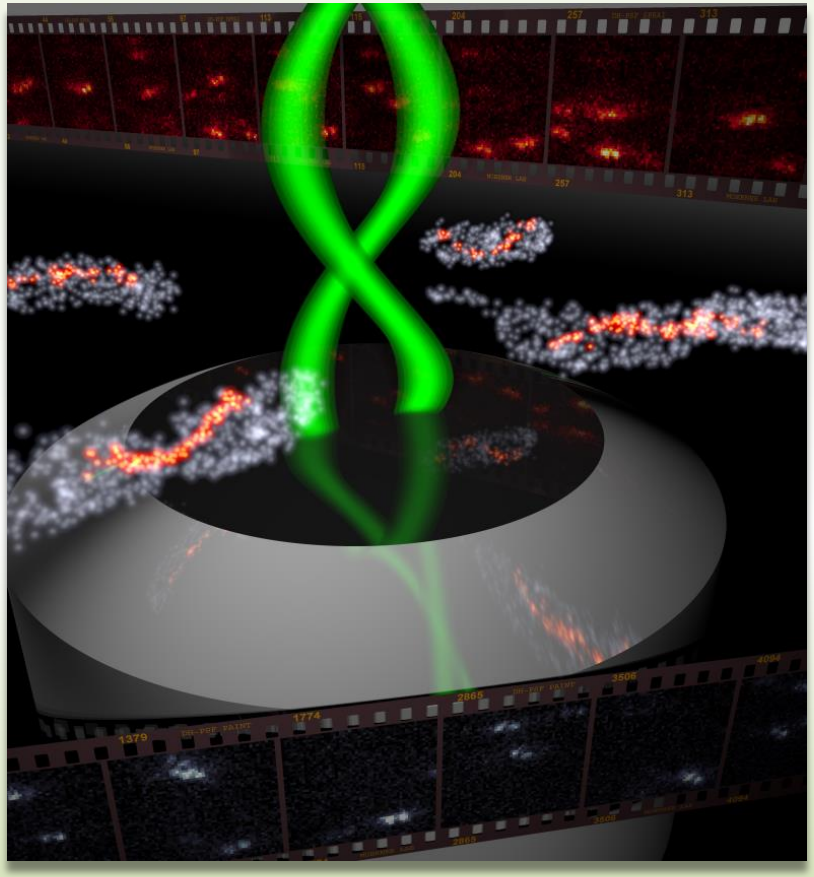
Experiment 2:
Single-Molecule Signal Identification



GahlmannLab
University of Virginia
Department of Chemistry

October 15, 2015

www.gahlmannlab.com

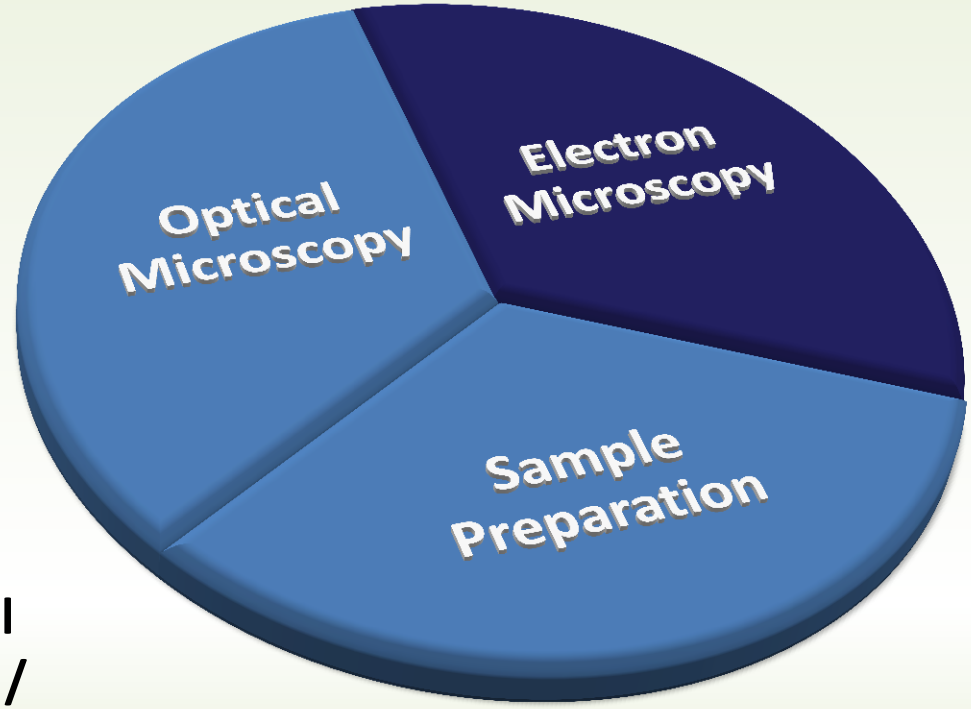
GahlmannLab Structure

Lasers, detectors, optical signal processing

Cryo-electron tomography

FEI Titan Krios

Fluorescent probes, photo-physics



Instrument automation

Substrate manufacture

Computational image analysis / data processing / model building

Cell culture,

Mutant strains

Labeling strategies

Single-Molecule Imaging Overcomes Diffraction Limit

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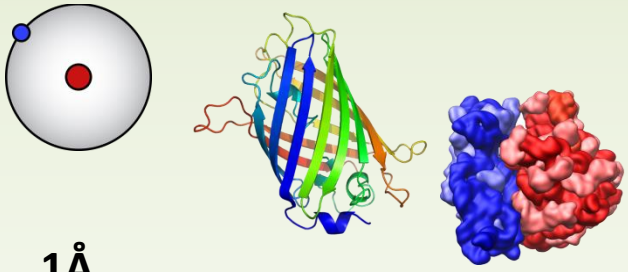
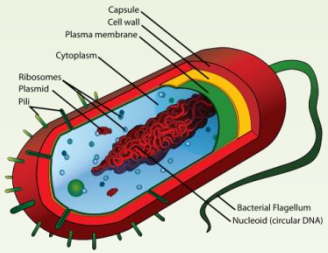
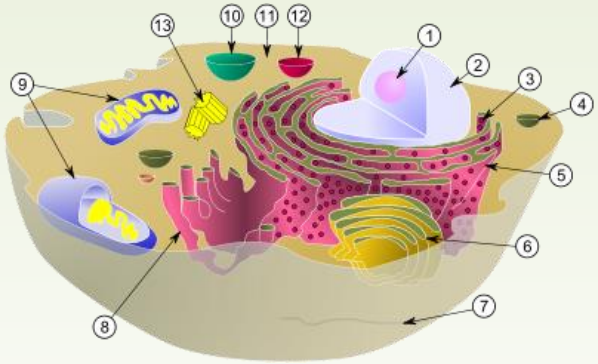


Abbe's Diffraction Limit (~ 300 nm)





1Å

1nm

10nm

100nm

1µm

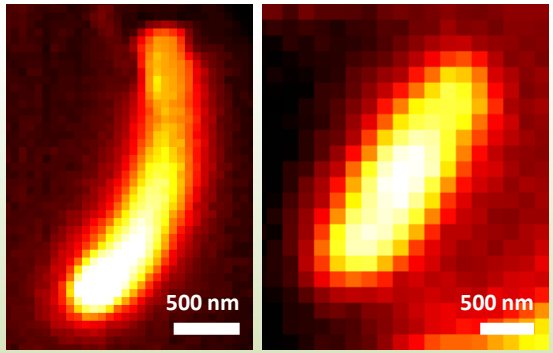
10 µm

100µm

Cryo-electron tomography

Single-molecule fluorescence microscopy

Fluorescence microscopy



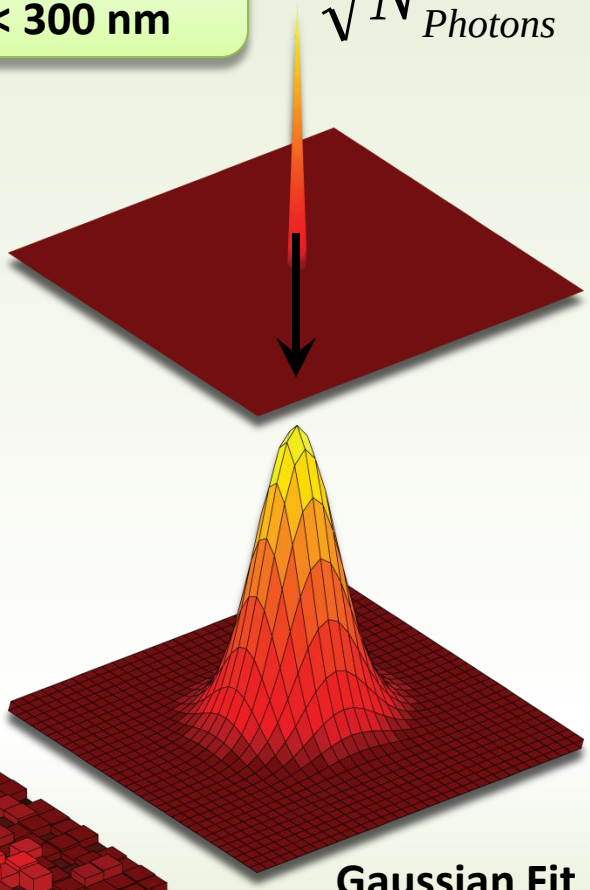
2 Key Concepts

Concept # 1 – Localize Single-Molecules

Uncertainty in Position

10 – 40 nm << 300 nm

$$\sim \frac{1}{\sqrt{N_{Photons}}}$$



Gaussian Fit

Diffraction Limited Image

Concept # 2 – Control Emitter Concentration

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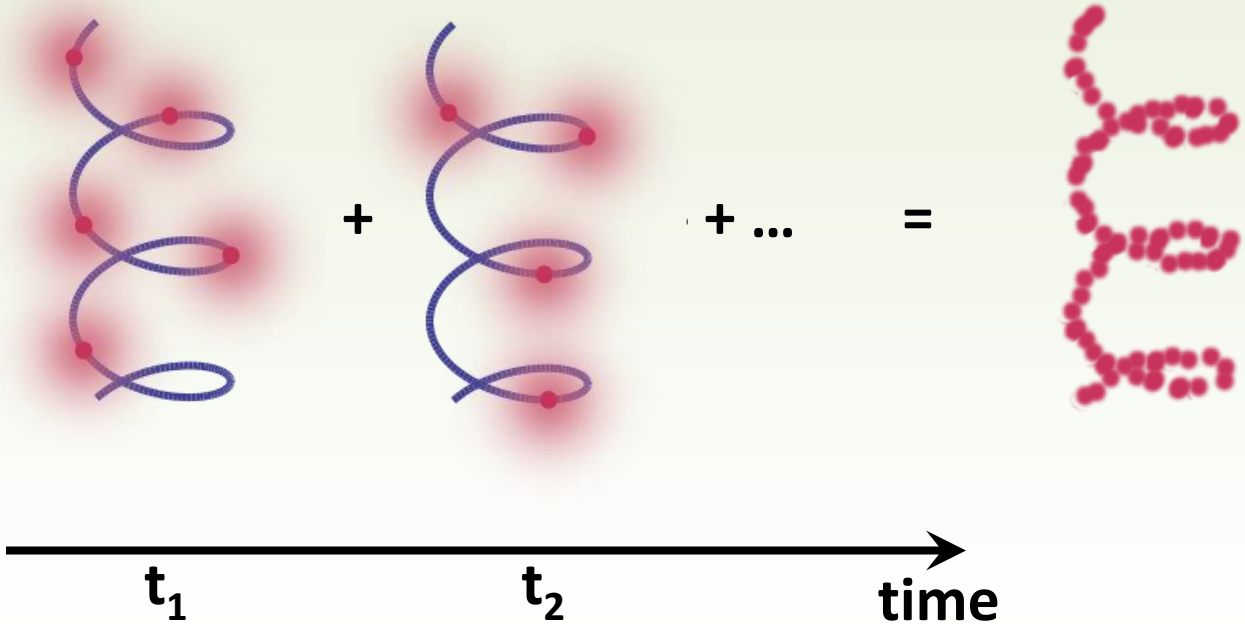
Epifluorescence

~500 nm

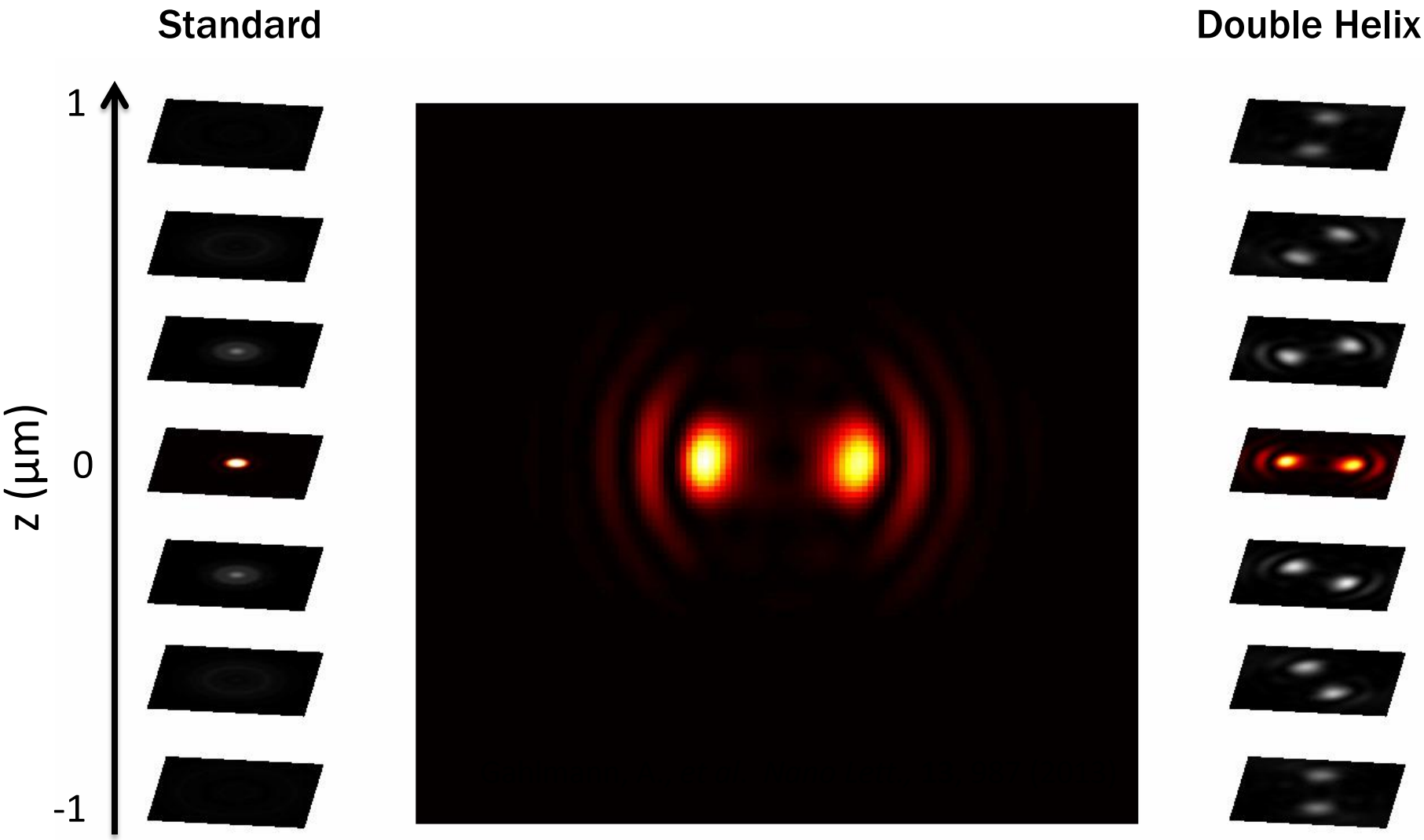
Active control of emitter concentration



PALM	→	Betzig, E., *et al.* *Science* **313**, 1642 (2006).
FPALM	→	Hess, S.T., *et al.* *Biophys. J.* **91**, 4258 (2006).
STORM	→	Rust, M.J., *et al.* *Nat. Methods* **3**, 793 (2006).

2014 Nobel Prize in Chemistry

Optical Manipulation Enables 3D Imaging

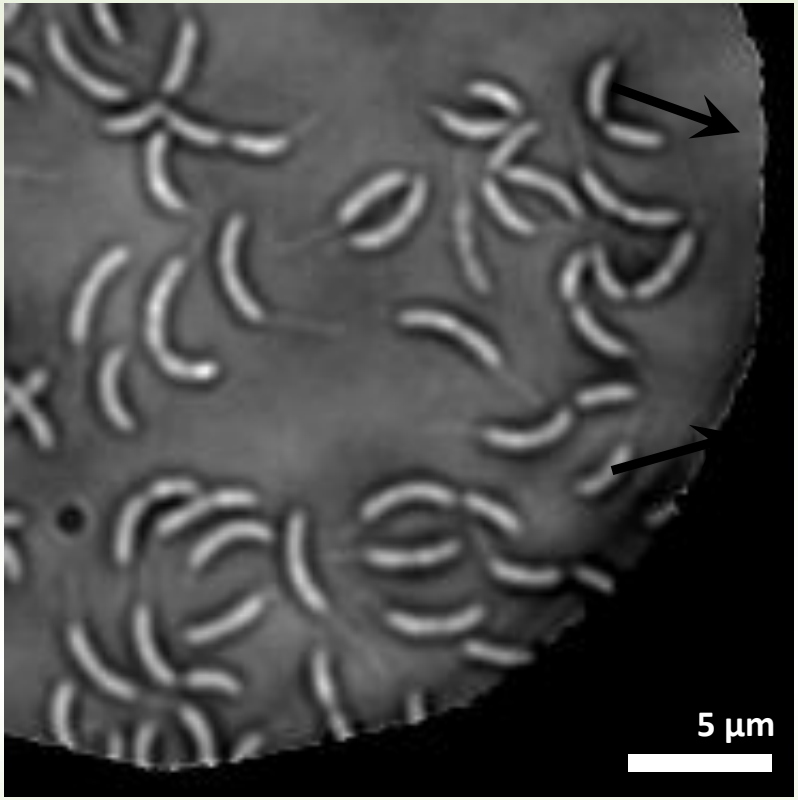


Piestun Lab, University of Colorado

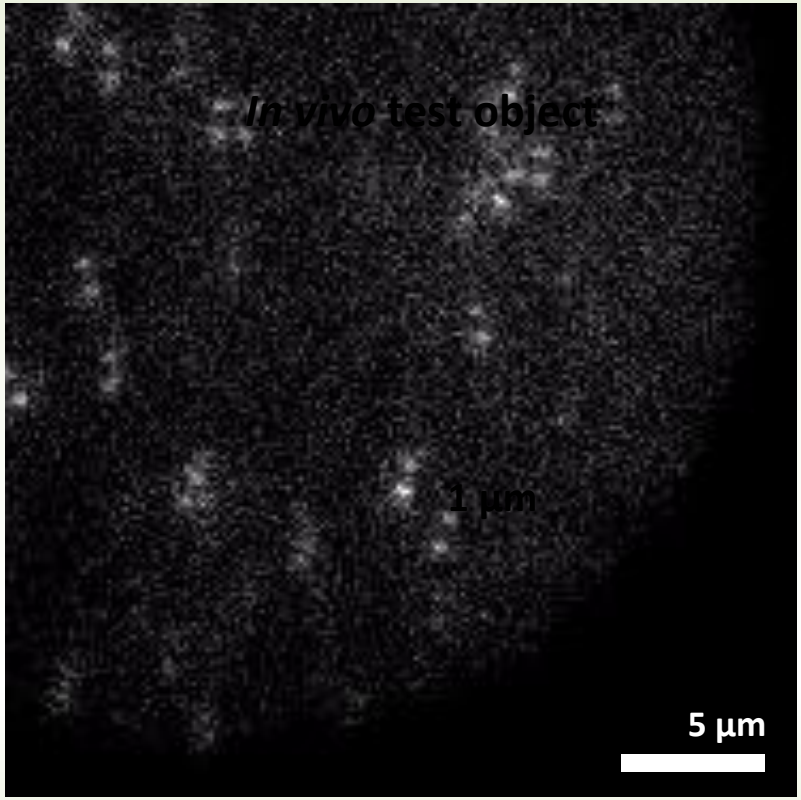
Pavani, S.R.P. & Piestun, R. *Opt. Exp.* **16**, 22048 (2008)

3D Image Registration - Validation

White Light Image

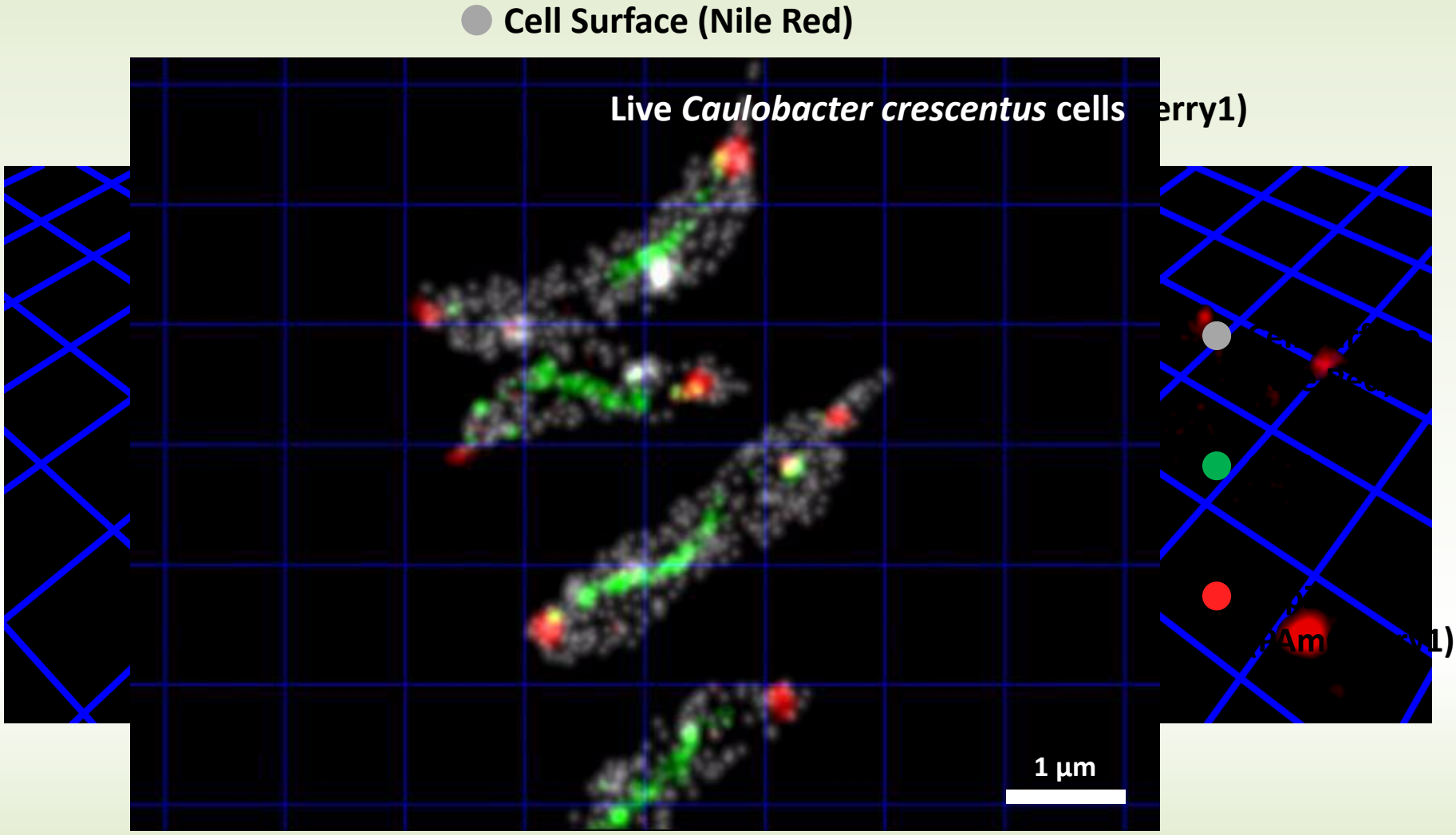


Single CreS (eYFP) molecules



Gahlmann, A., *et al*. *Nano Lett.* **13**, 987 (2013)

Accurate 3D Multicolor Imaging Now Possible



Gahlmann, A., *et al*. *Nano Lett.* **13**, 987 (2013)