

Liposomal Doxorubicin Under Microconfinement and Microscopy

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Session Description and Objectives

- We introduce a method to measure multiple characteristics of particle-based therapeutics, including:
 - Suspension concentration
 - Particle size distribution
 - Encapsulation and dose
- Technical aspects of our work:
 - Device-enhanced optical microscopy
 - Single-particle tracking and sizing
 - Light-scattering and fluorescence
 - Precision, accuracy, and efficiency

Biography and Contact Information

FDA and NIST are working together to develop measurement methods for fundamental study and quality control of particle-based therapeutics

Kuo-Tang Liao

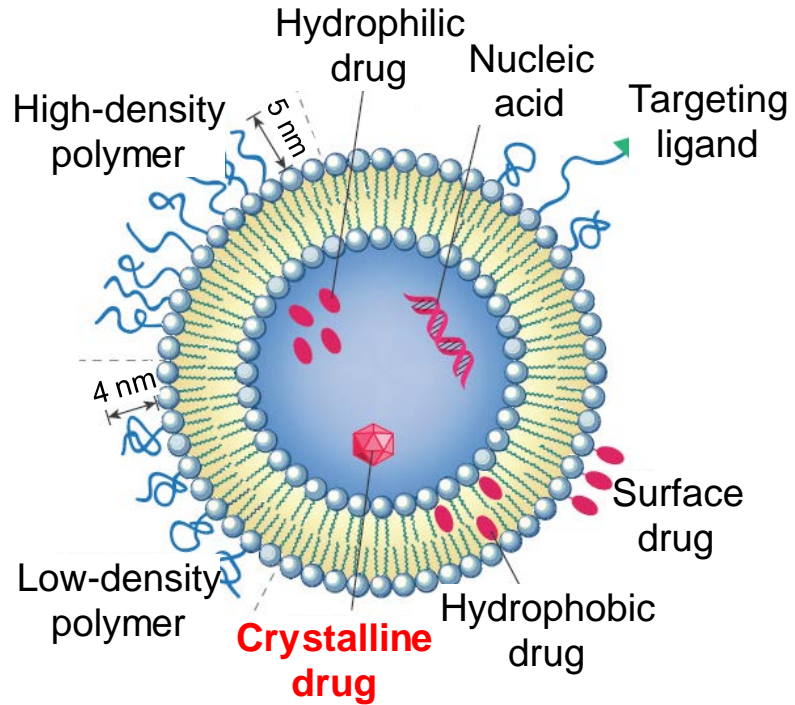
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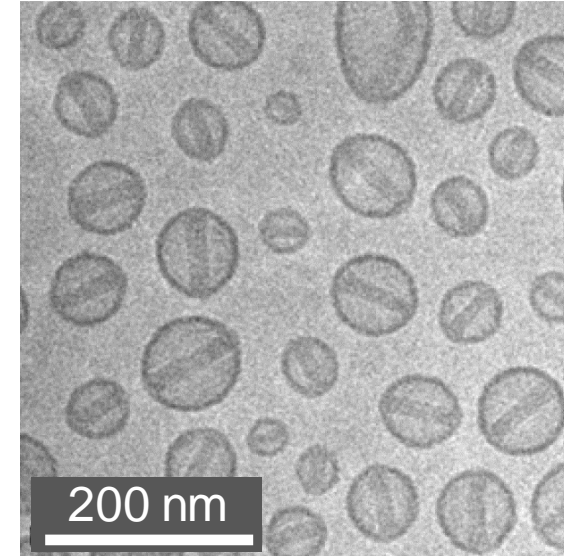
Liposomal formulations



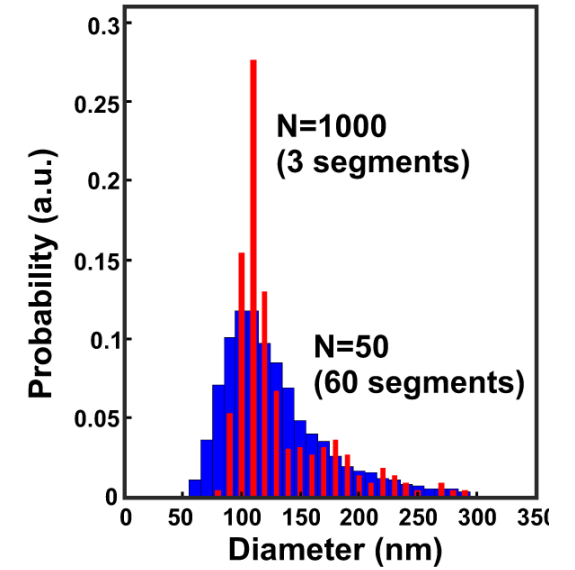
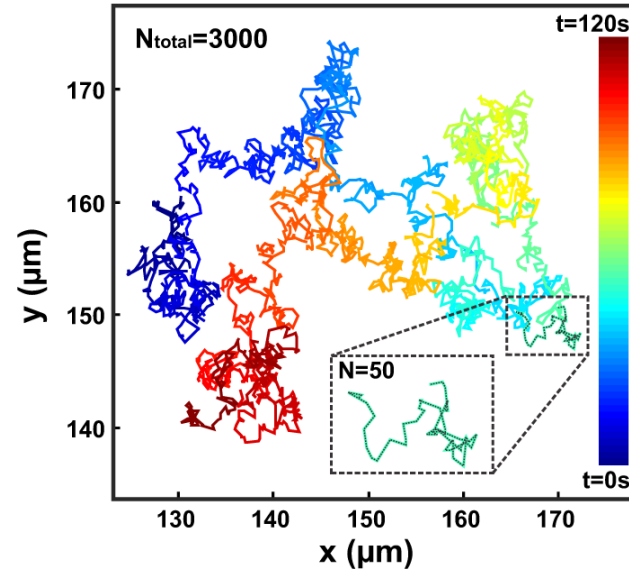
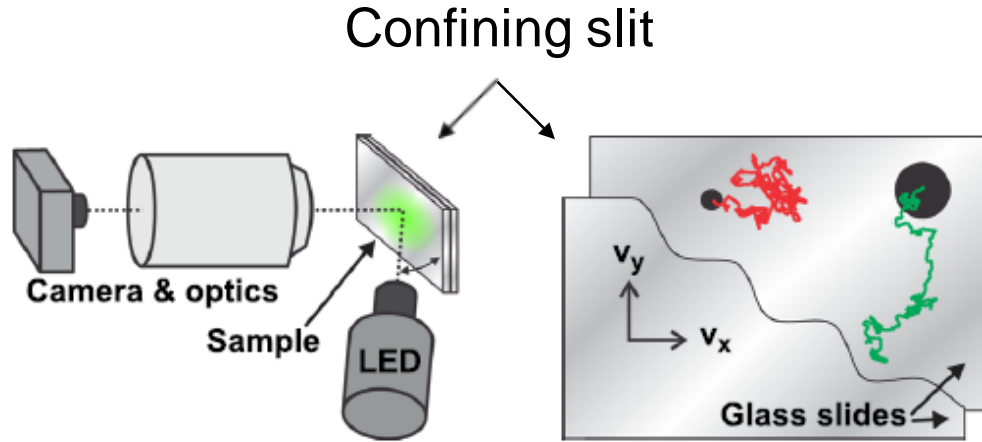
Generic vs. reference



Cryogenic electron microscopy



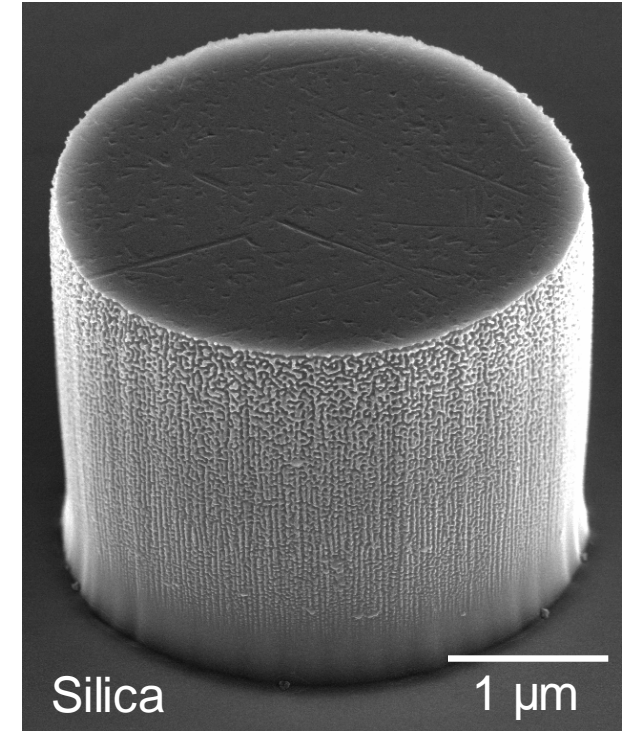
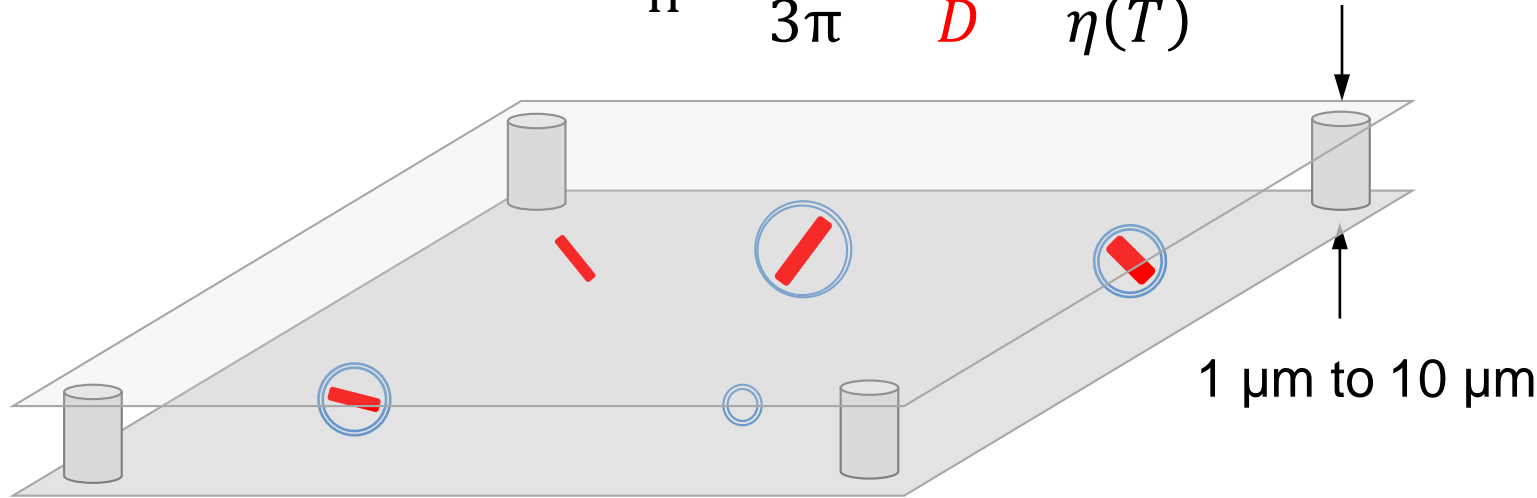
→ Electron microscopy is powerful but not practical for every measurement



→ Confinement improves precision of NTA but errors approach 15%

Our idea: NTA in a slit with a pillar array

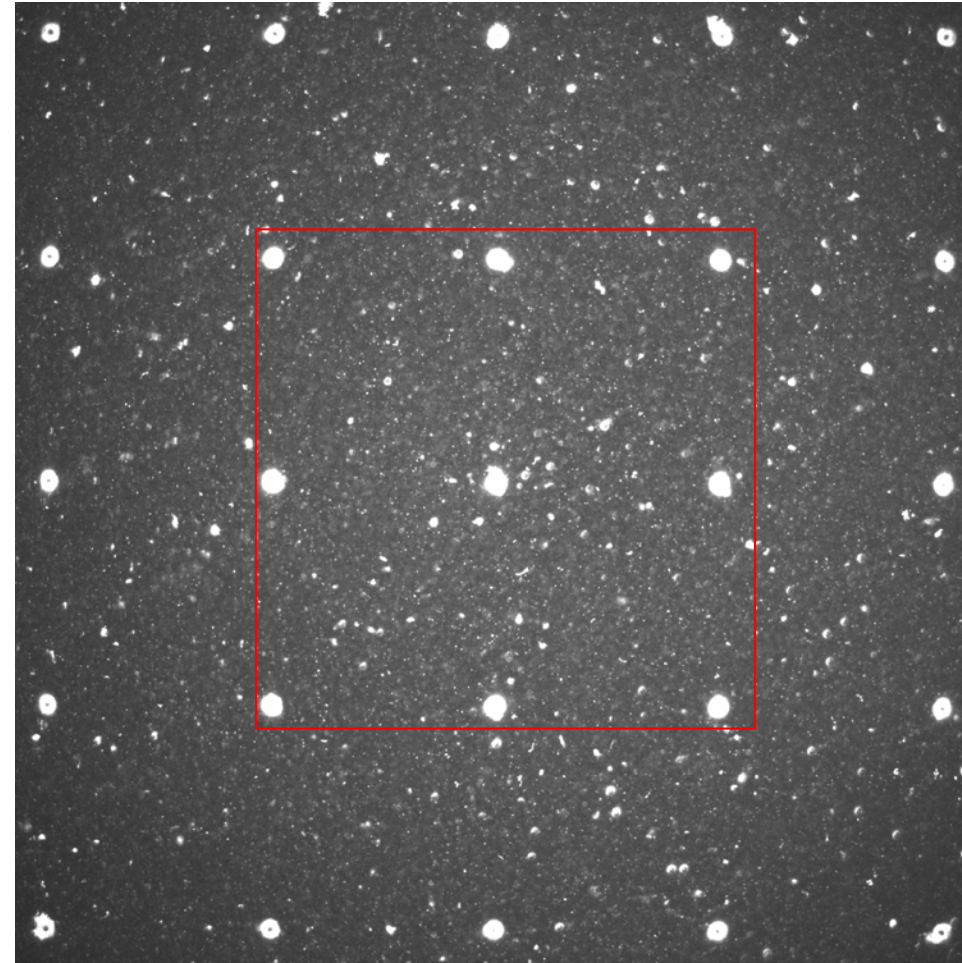
$$d_H = \frac{k_B}{3\pi} \cdot \frac{c(z)}{D} \cdot \frac{T}{\eta(T)}$$



→ Precision, accuracy, efficiency

Liposomal doxorubicin under test

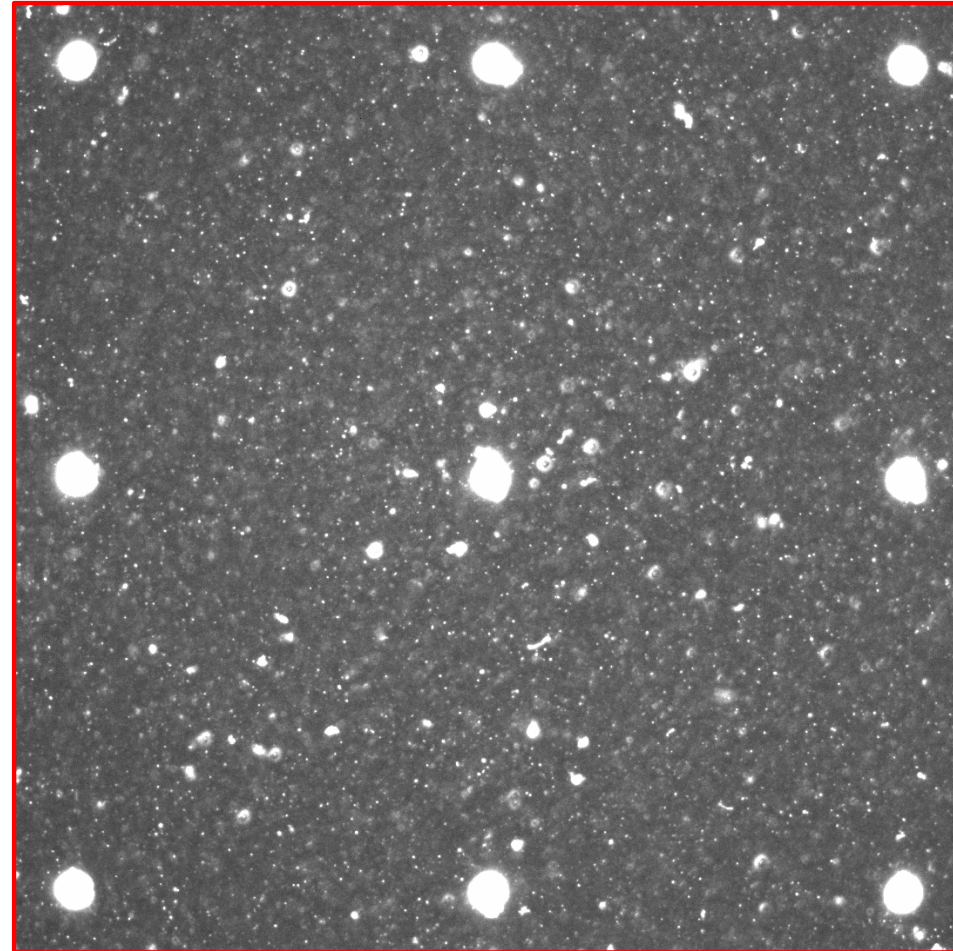
Darkfield



150 μm

Liposomal doxorubicin under test

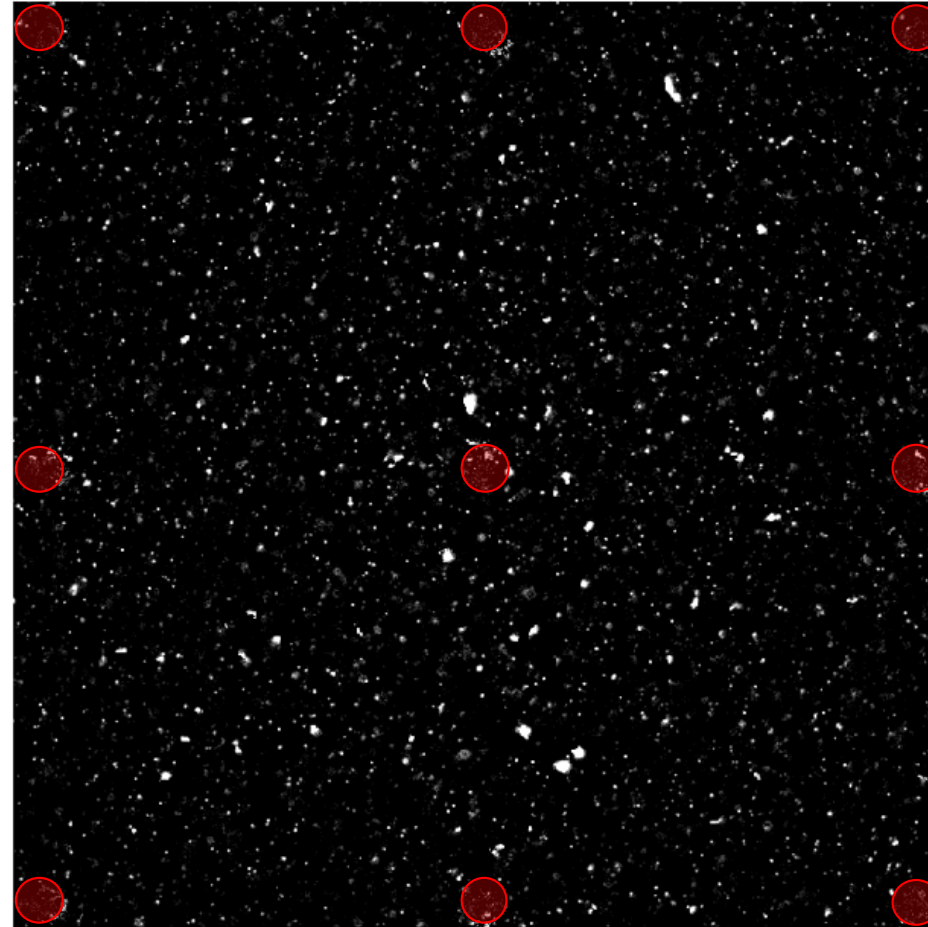
Darkfield



50 μ m

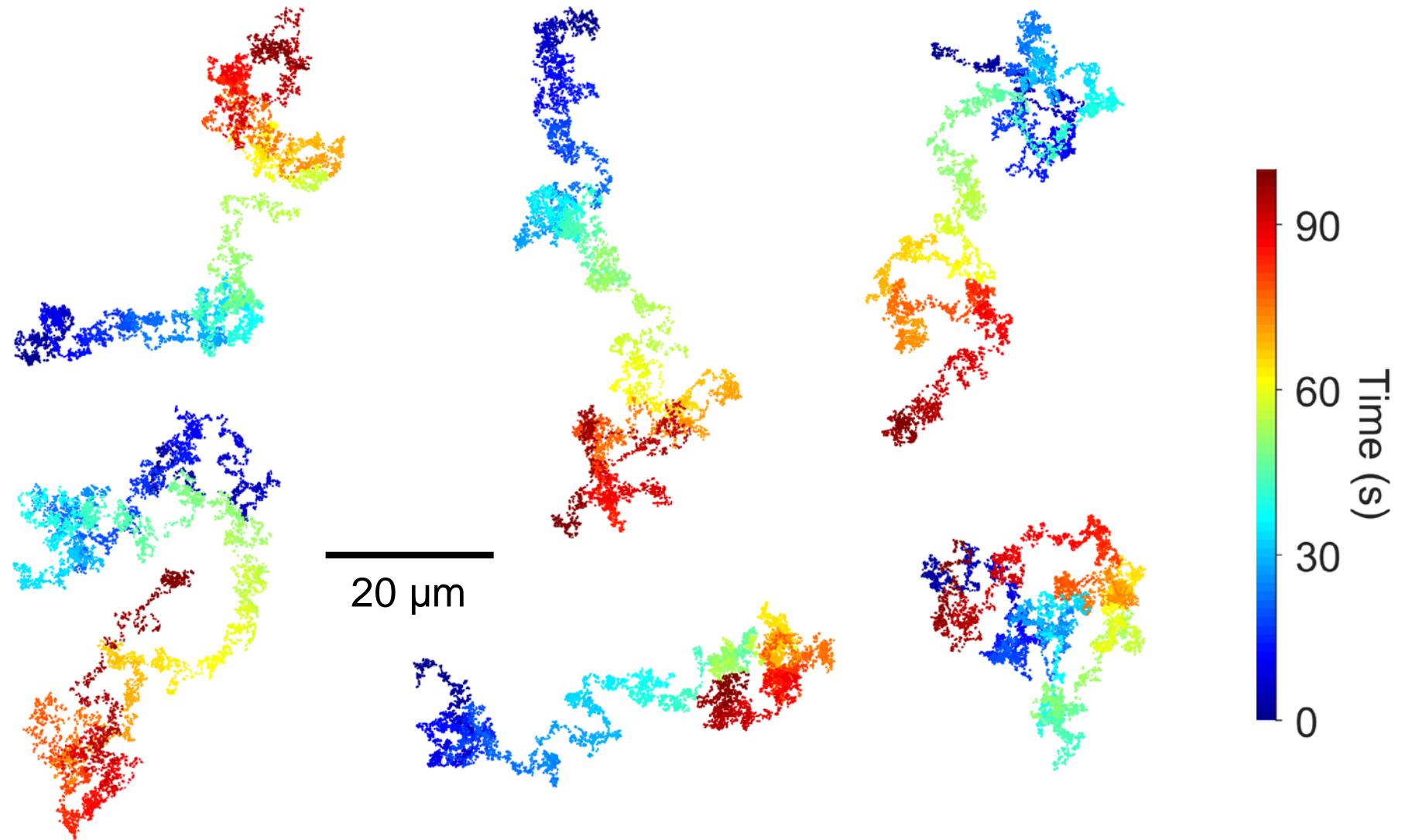
Liposomal doxorubicin under test

Darkfield after background subtraction

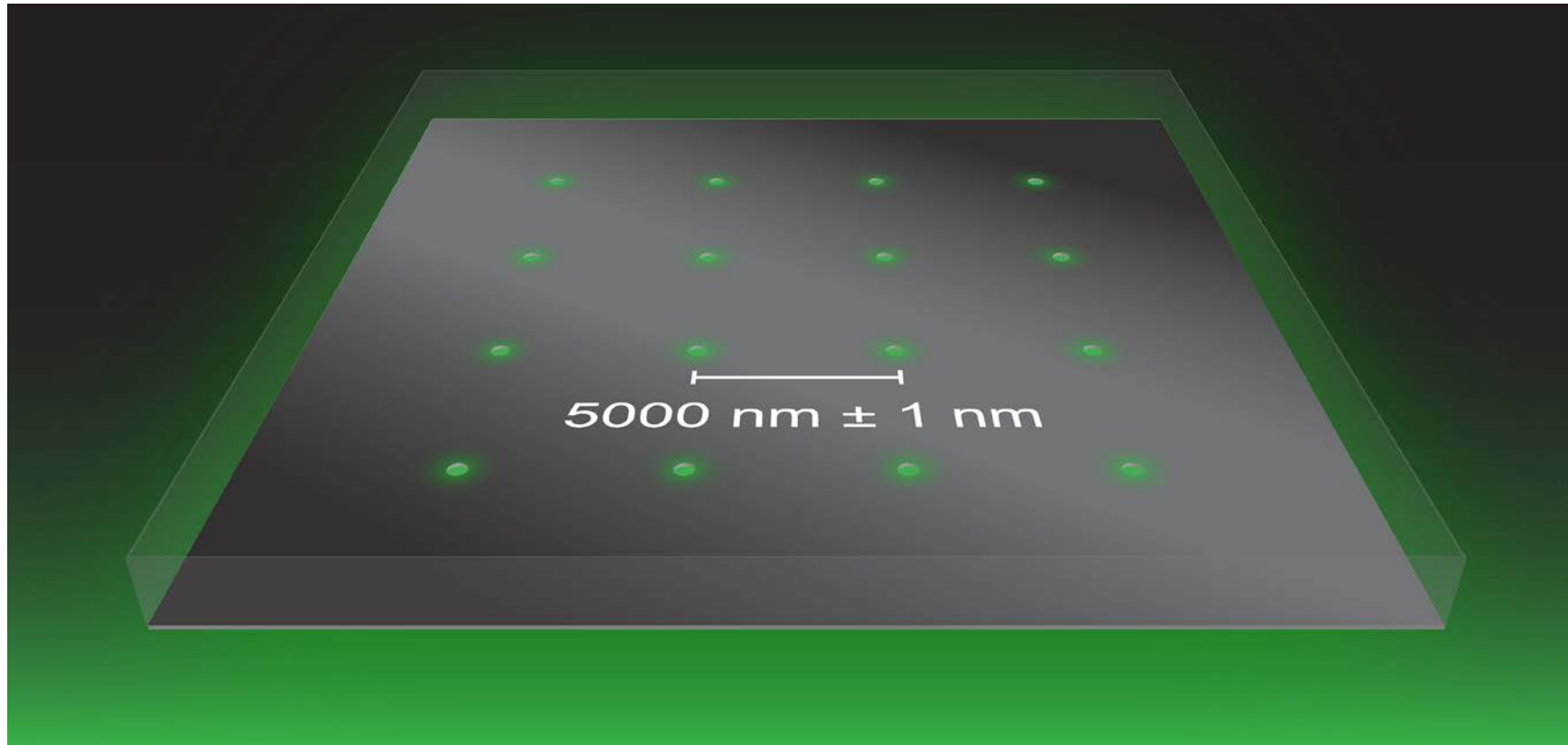


50 μ m

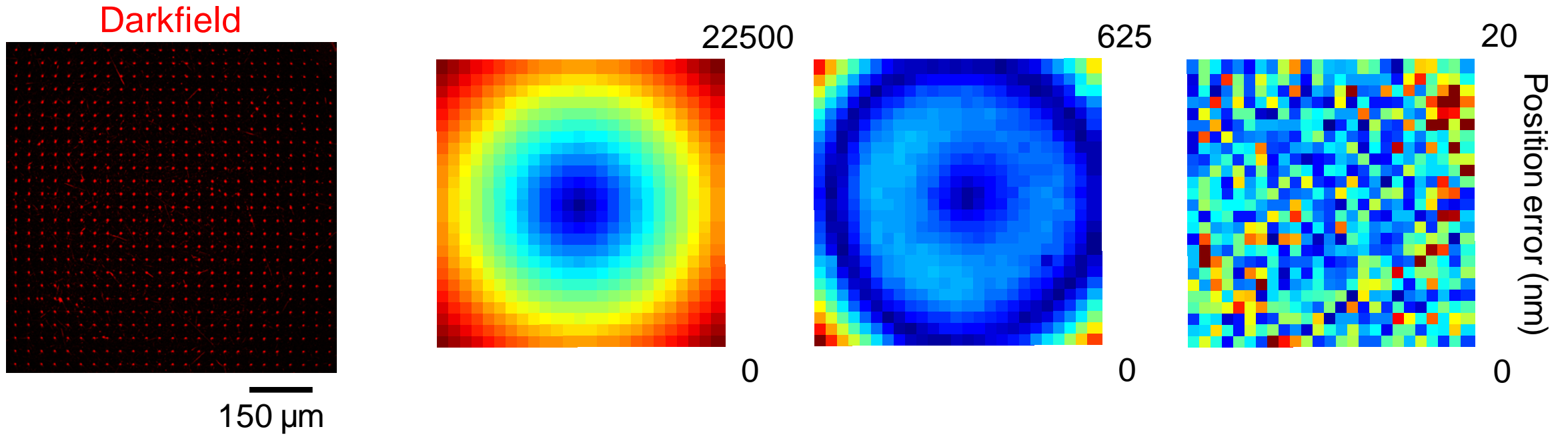
Random walks in two dimensions



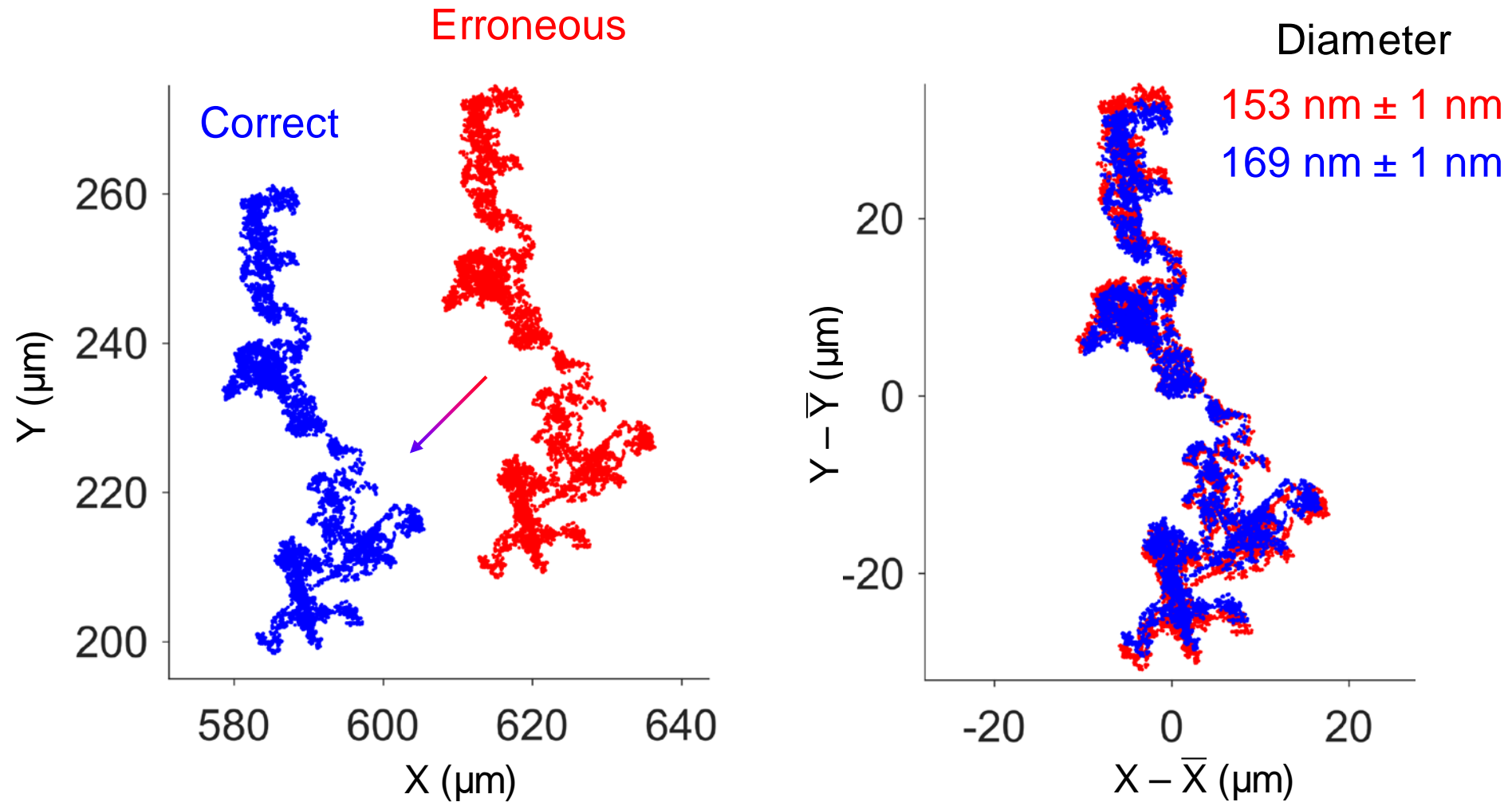
Aperture array for reference positions



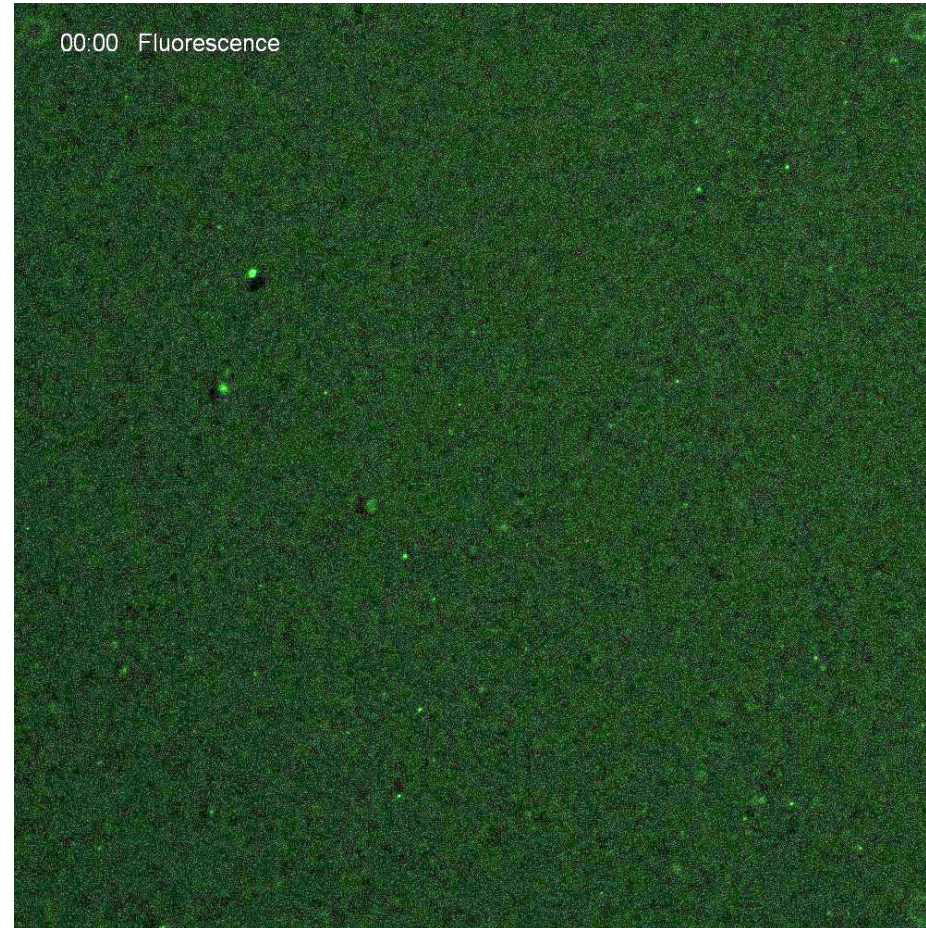
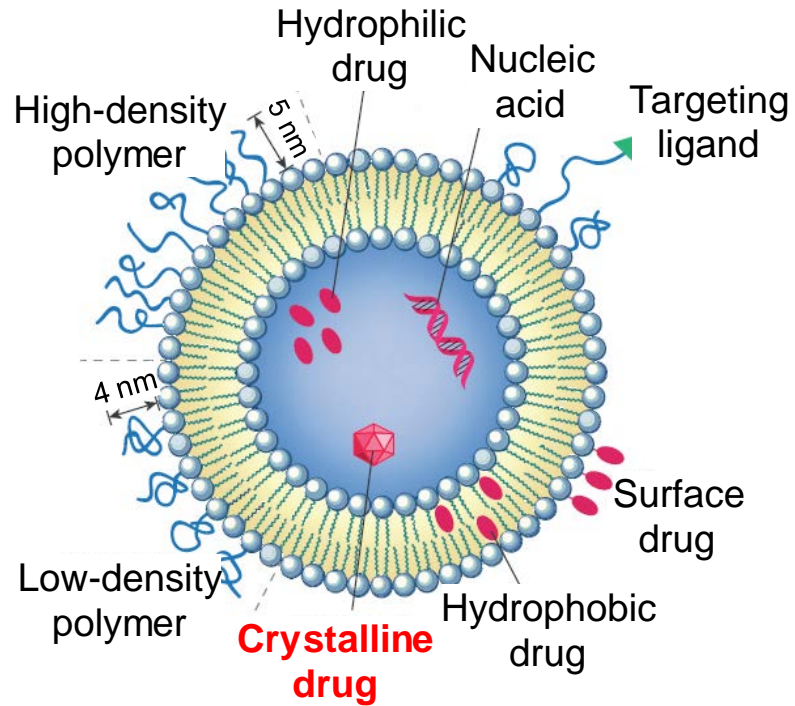
Pillar array for reference positions



Accurate tracking and sizing of particles



Encapsulation and dose measurements



50 μm

Acknowledgments



Craig R. Copeland



Samuel M. Stavis



Ja Hye Myung



Darby Kozak

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Toward precise, accurate, and efficient measurements of multiple characteristics of particle-based therapeutics

Questions?

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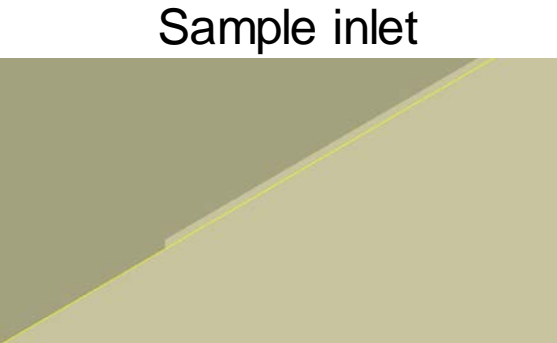
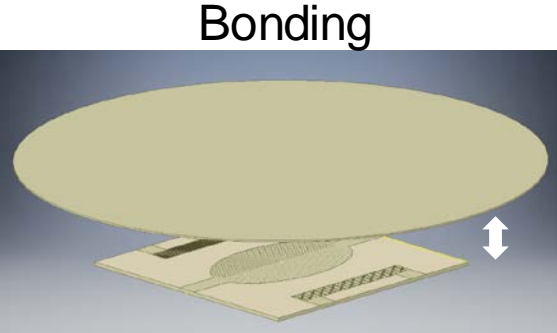
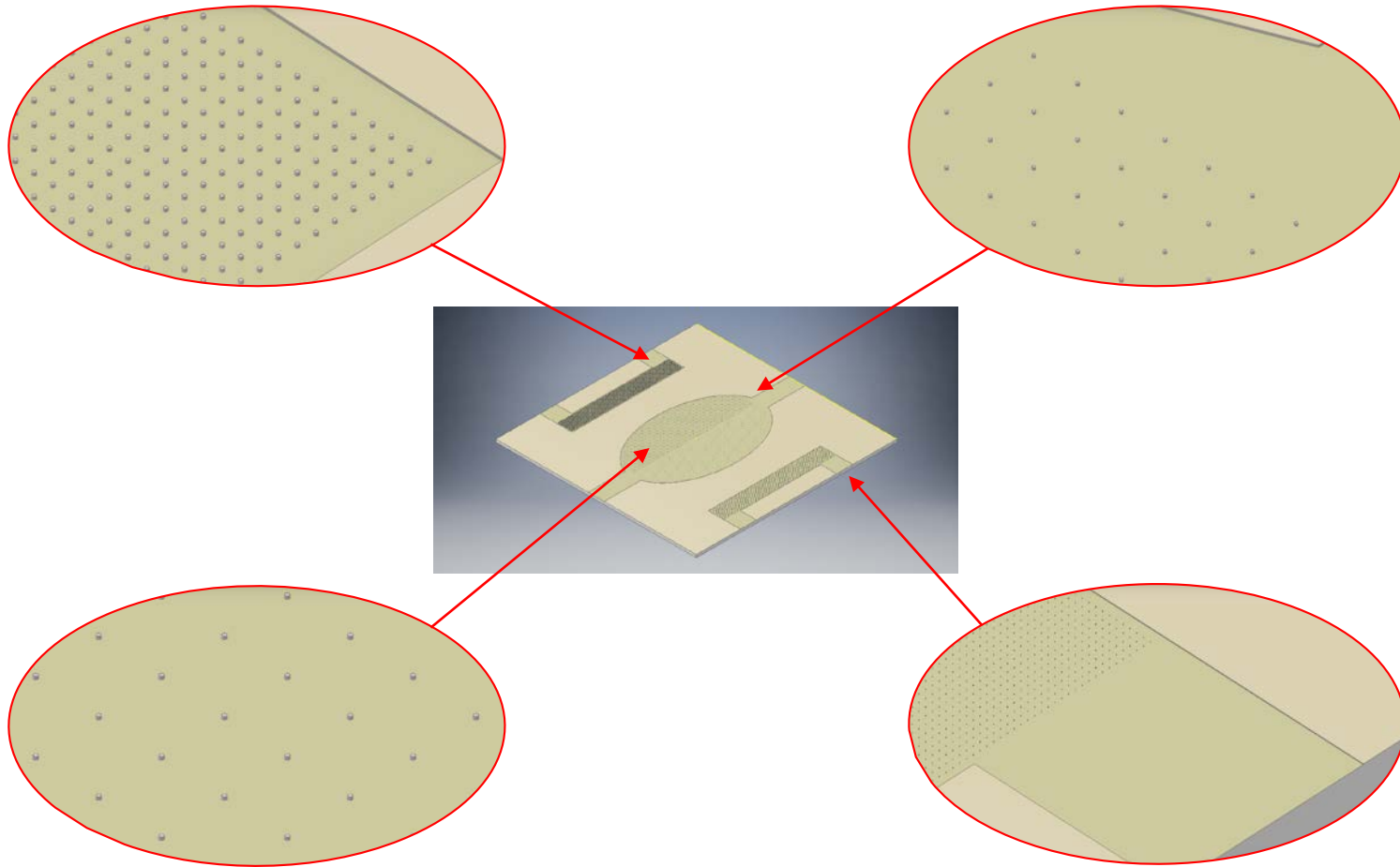
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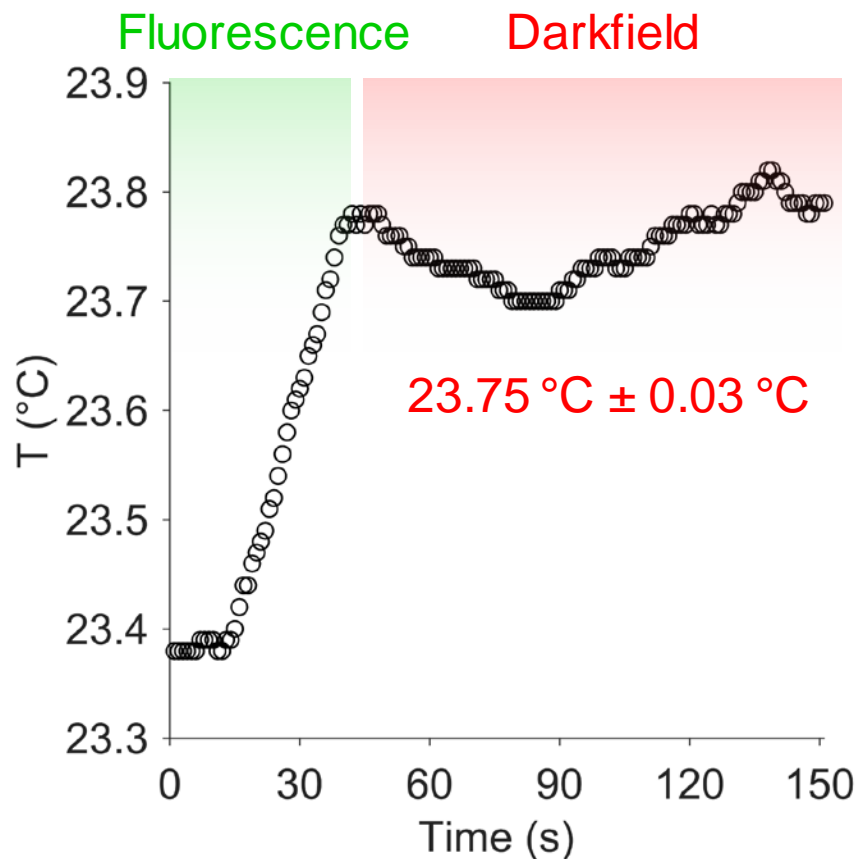
- X- ray Scattering
- Atomic Force Microscopy
- Dynamic Light Scattering
- Nanoparticle Tracking Analysis

The design of microfluidic device

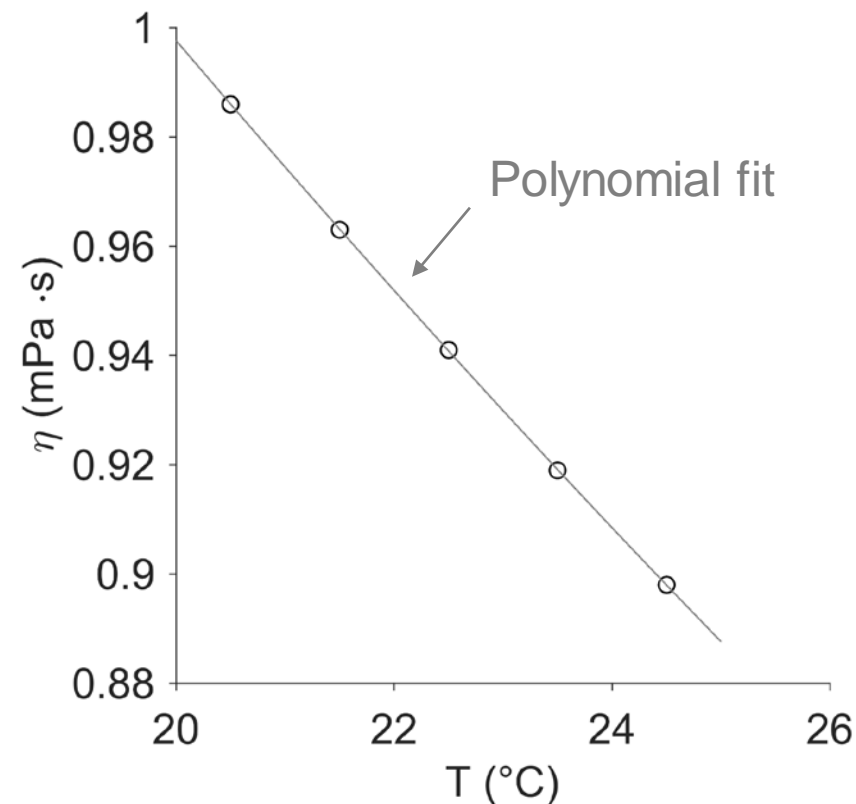


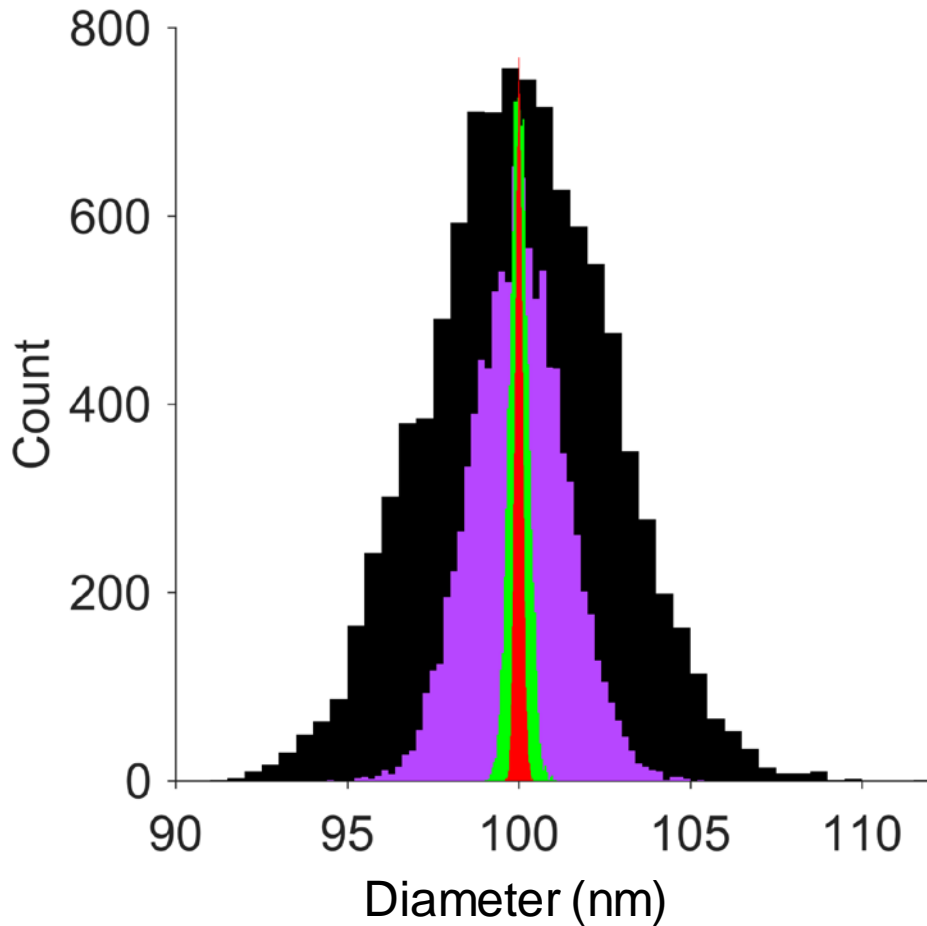
Inference of liquid media properties

Thermometry



Viscometry





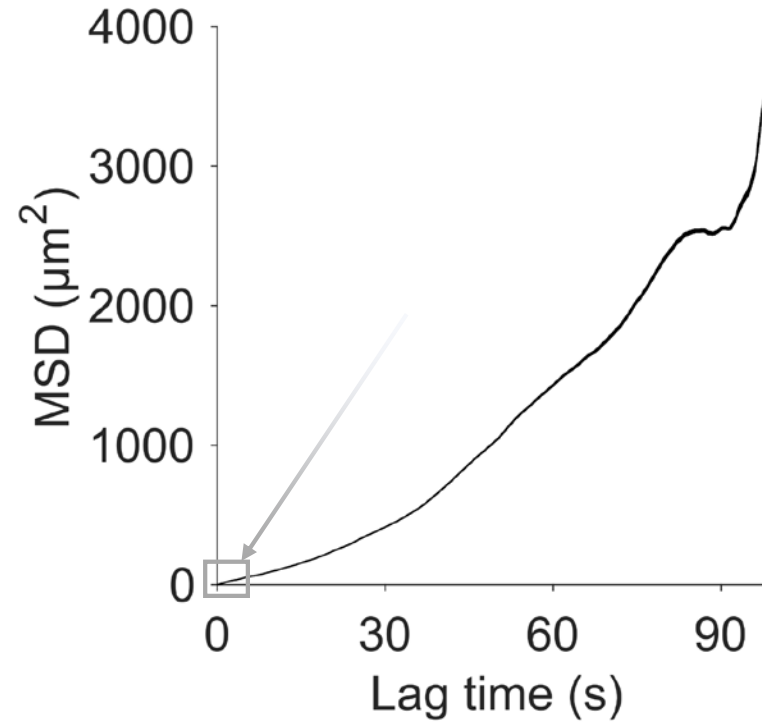
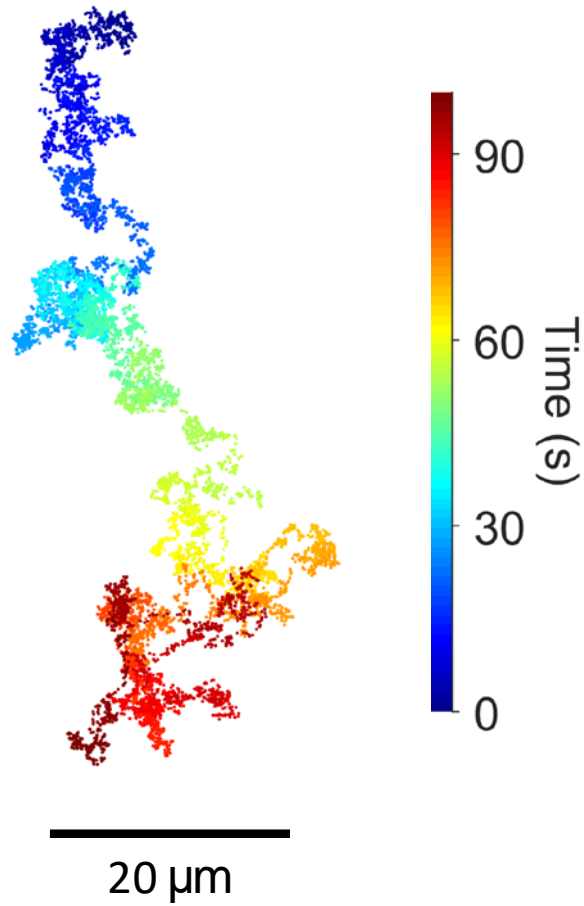
$$d_H = \frac{k_B}{3\pi} \cdot \frac{c(z)}{D} \cdot \frac{T}{\eta(T)}$$

Temperature standard deviation (°C)	Diameter mean (nm)	Diameter standard deviation (nm)
0.04	100.00	0.11
0.10	100.00	0.27
0.50	99.99	1.35
1.00	100.02	2.66

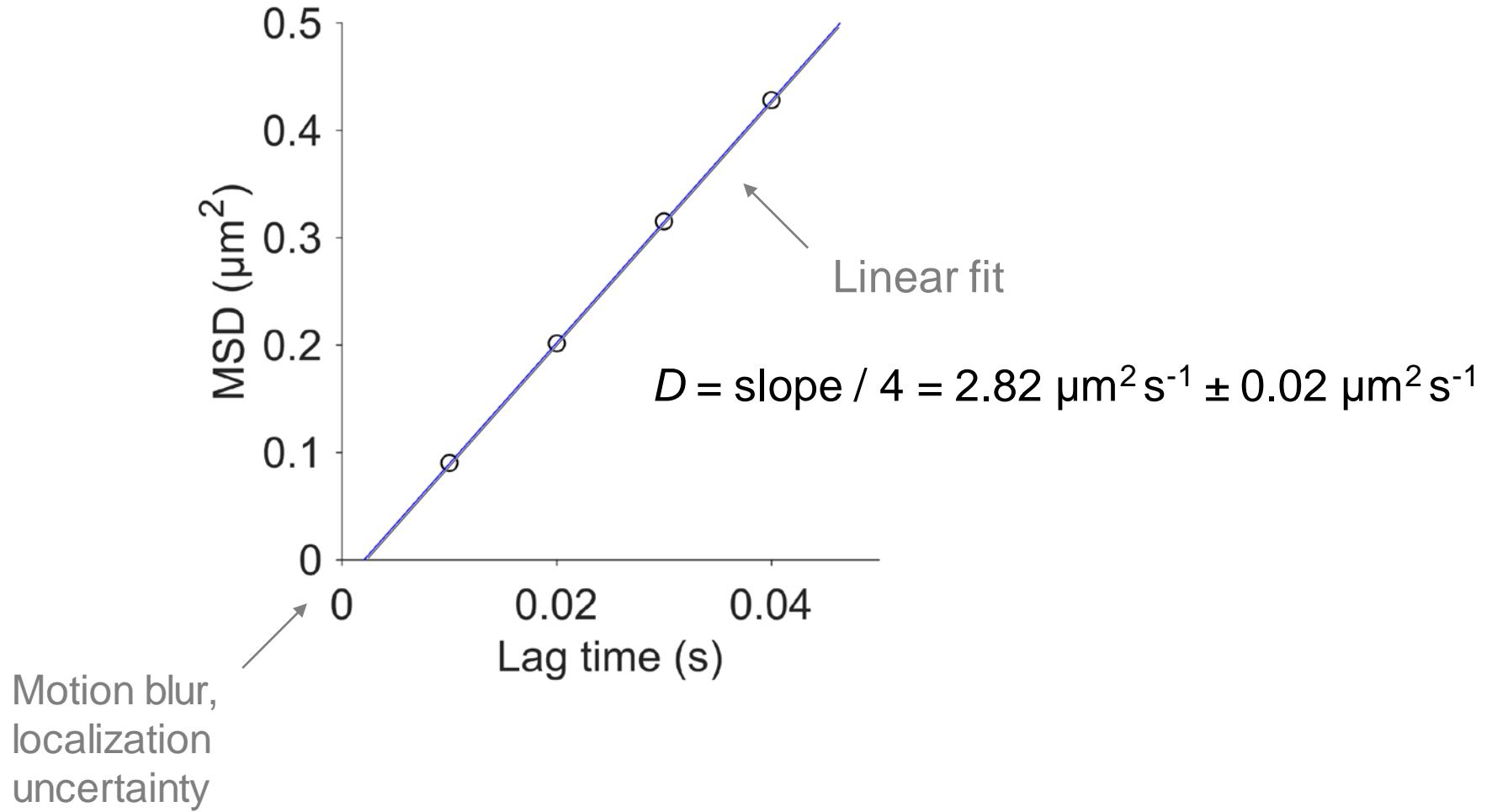
Mean square displacement (MSD) analysis

$$MSD = \langle \text{Displacement}^2 \rangle_{n\Delta t_E} = 4D \underbrace{(n\Delta t_E)}_{\text{Lag time}}$$

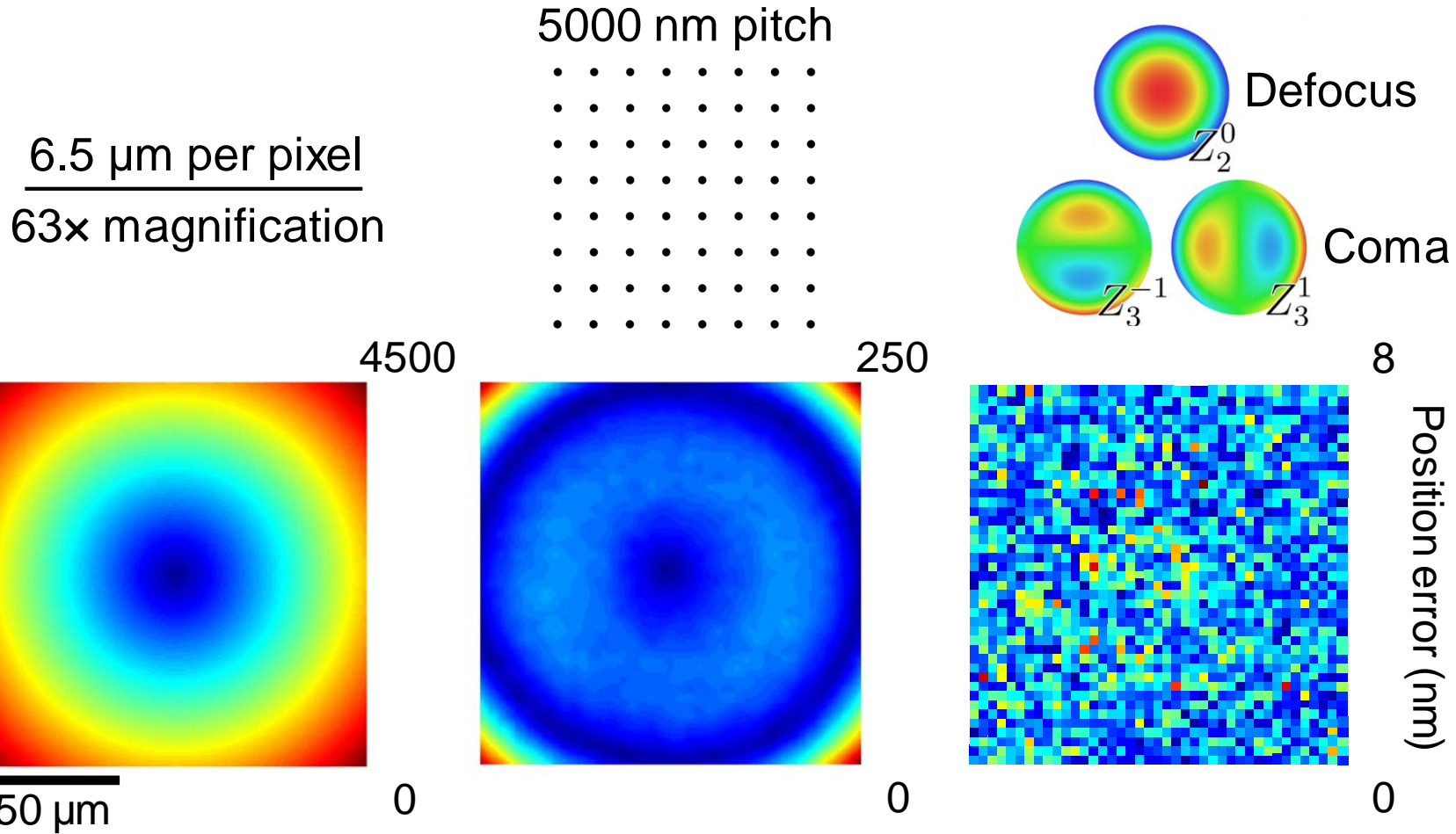
Lag time



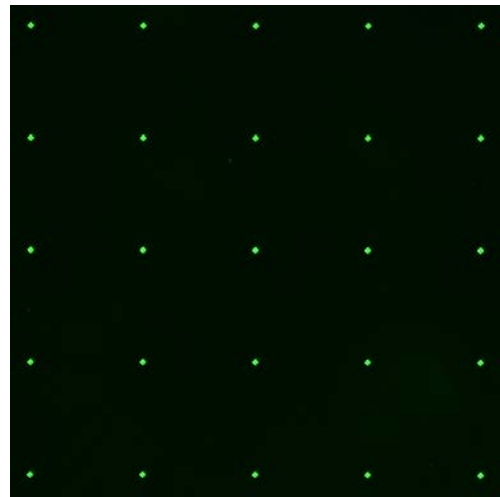
Mean square displacement (MSD) analysis



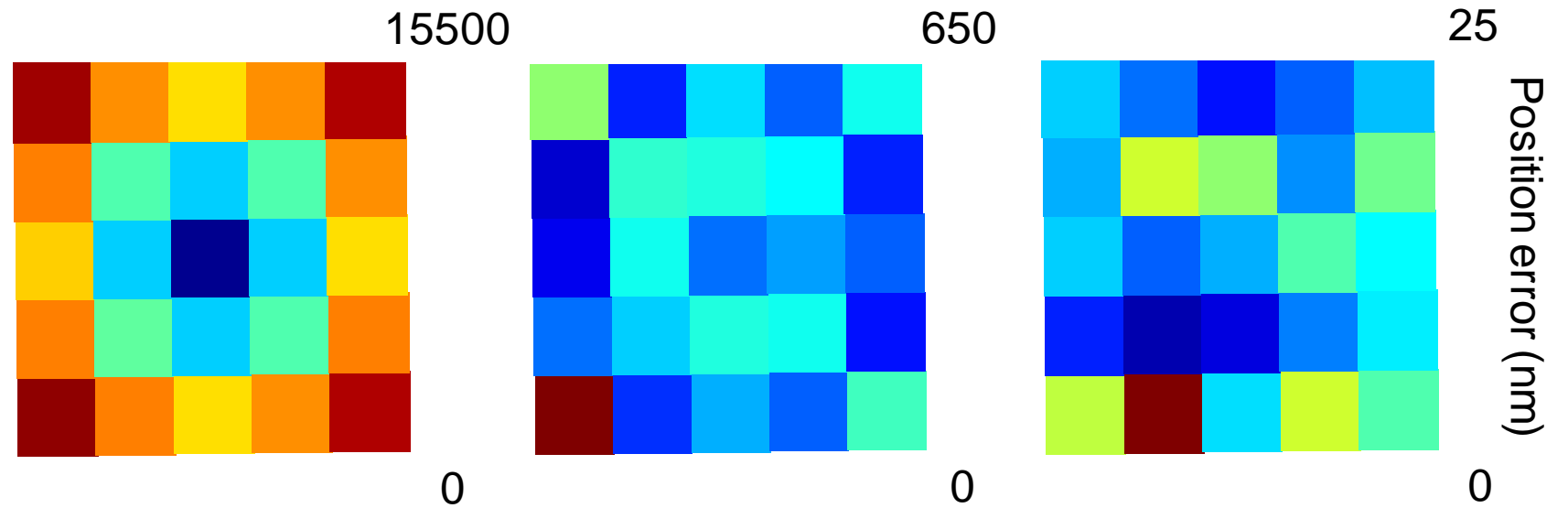
The necessity of widefield calibration



Inverse fluorescence

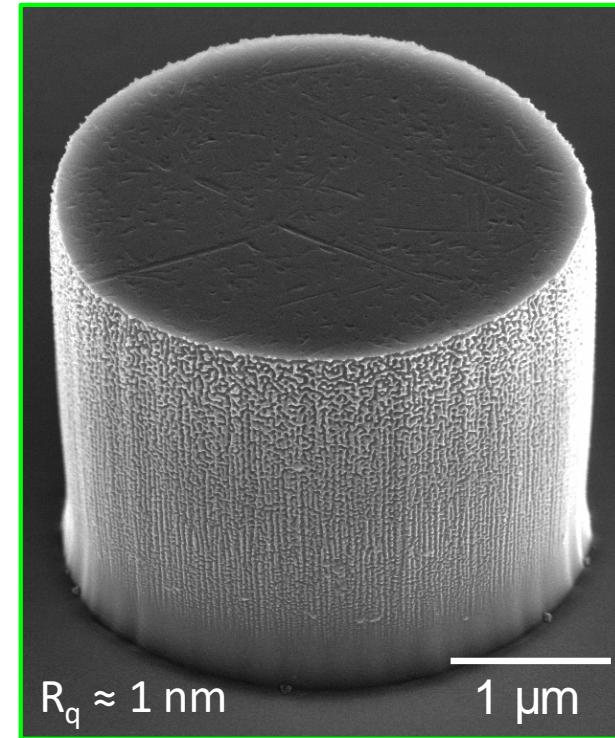
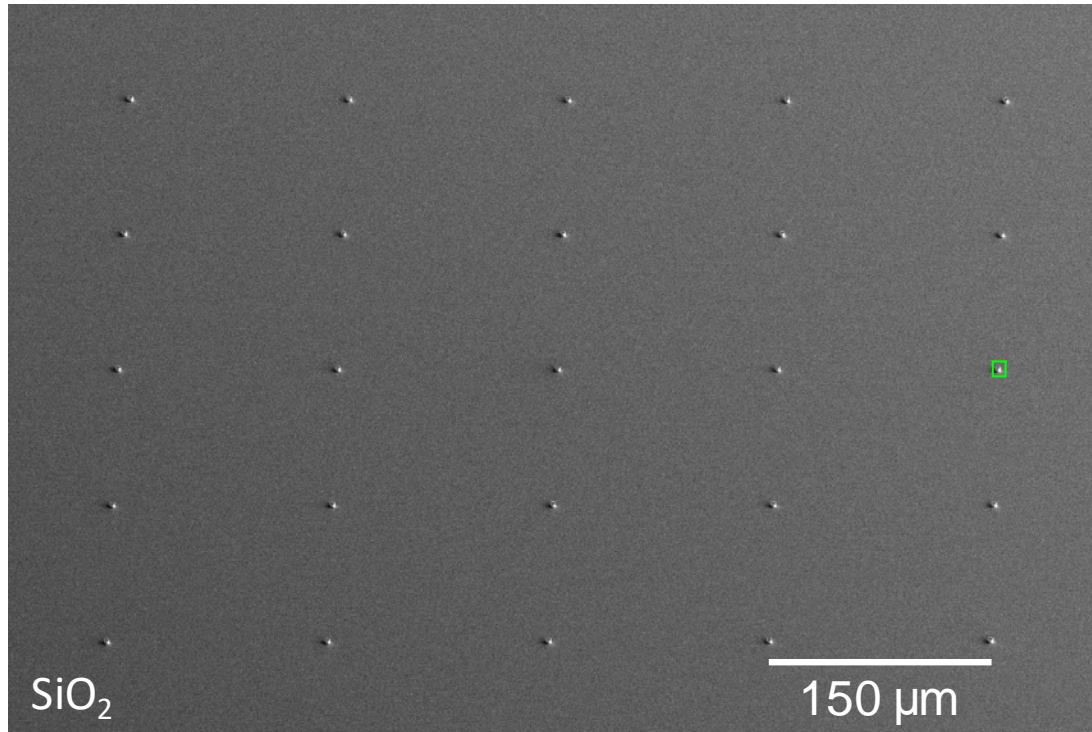


150 μm



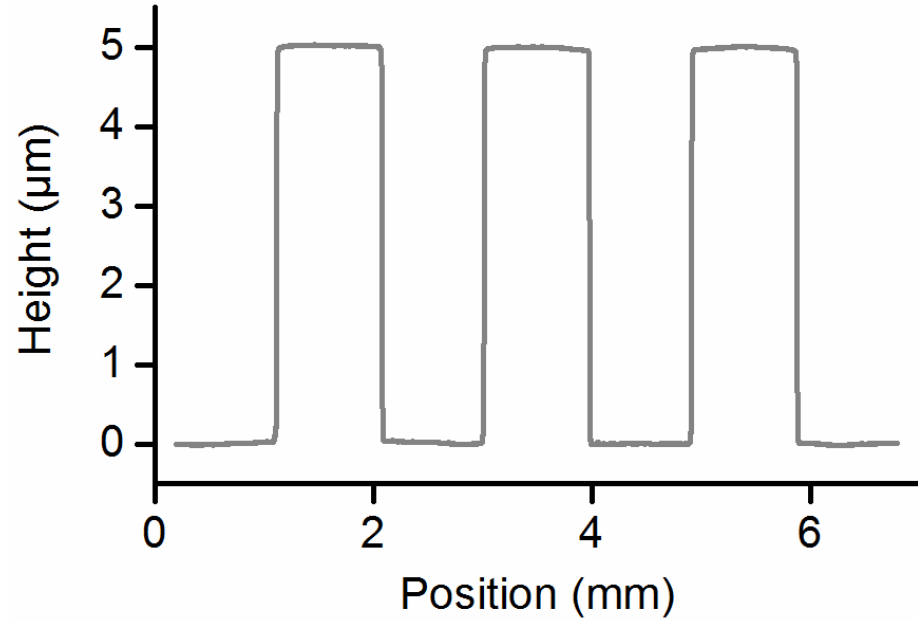
First function – depth control

SiO₂ (20 nm) – Cr (200 nm) hard mask

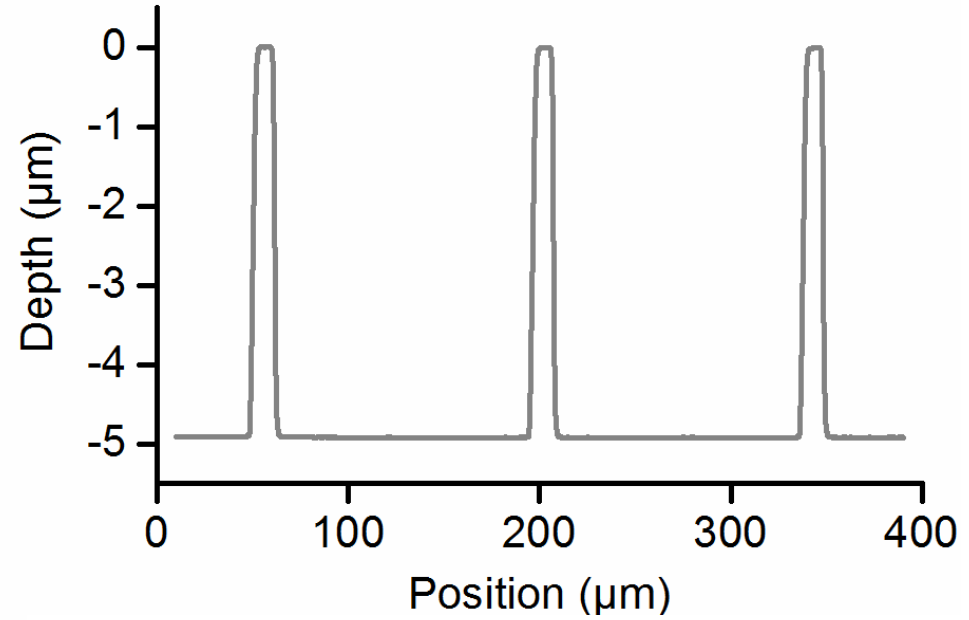


SF₆ – C₄F₈ ICP – RIE 2750 W

Reference material

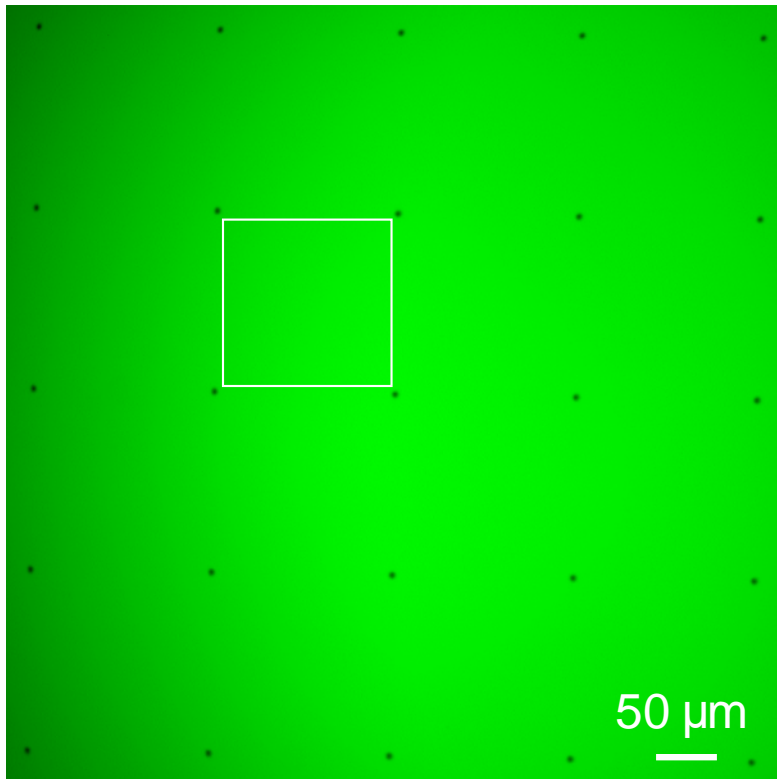


Pillar array



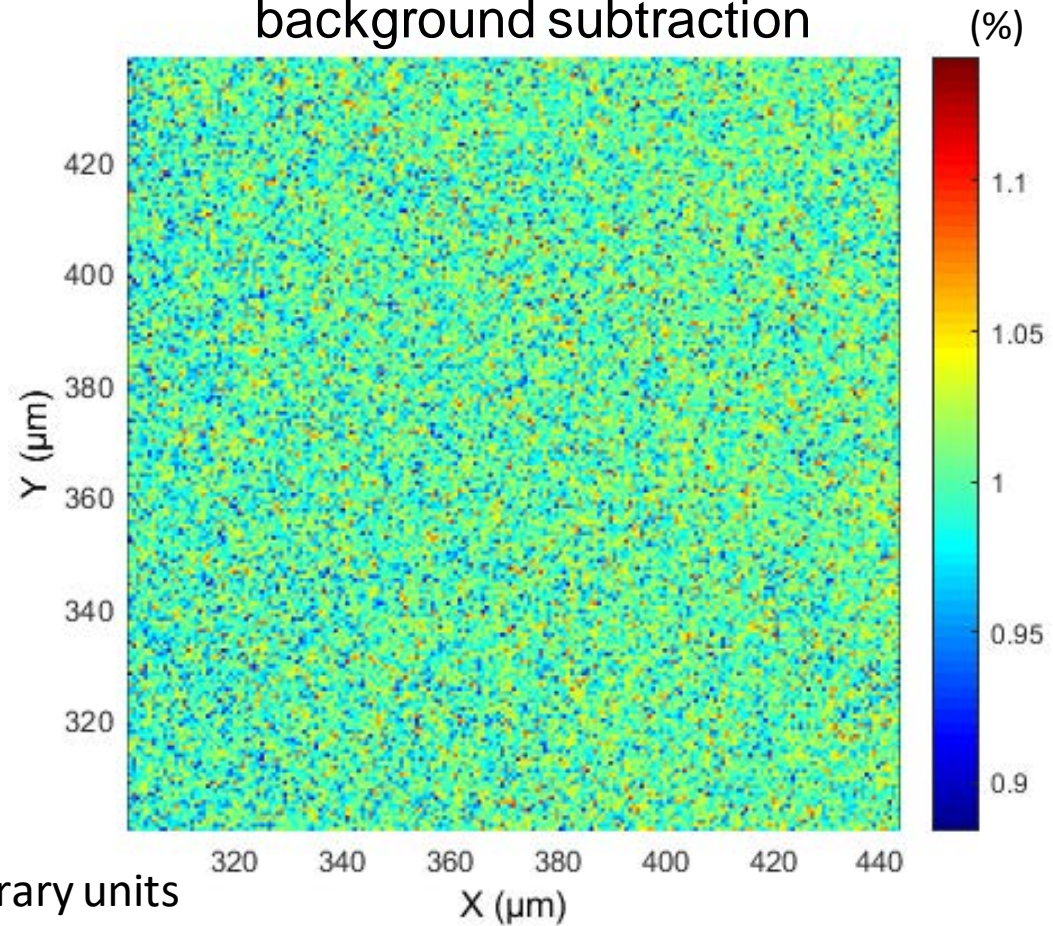
Etch depth = $4.871 \mu\text{m} \pm 0.009 \mu\text{m}$

Fluorescence

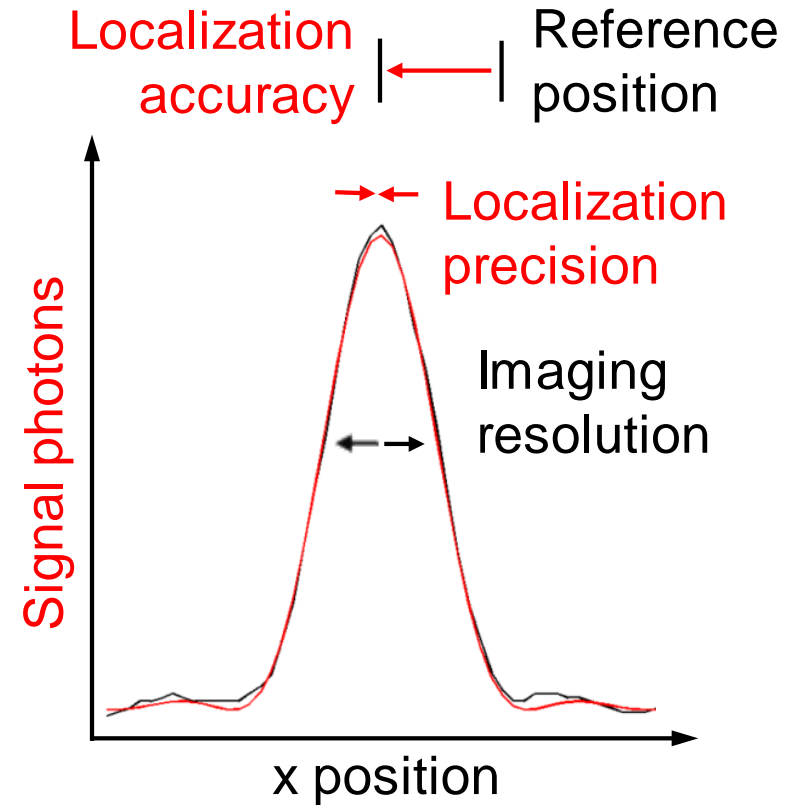
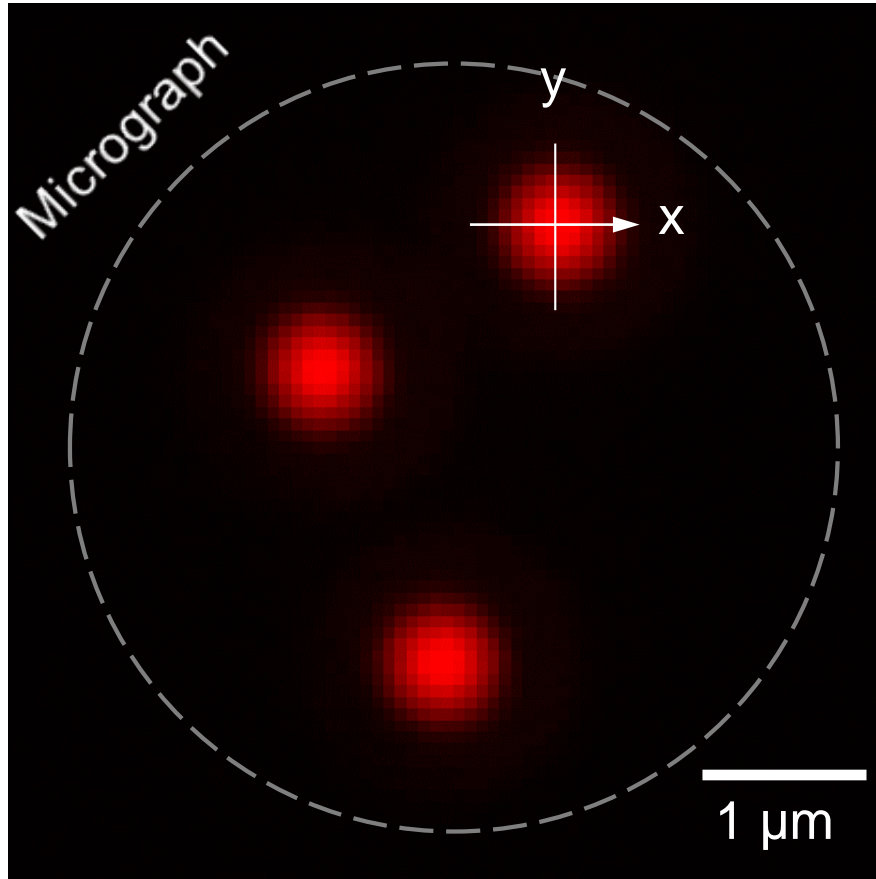
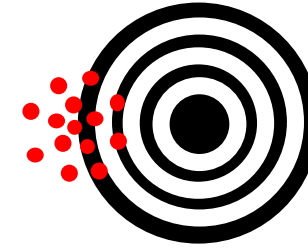


(58412 ± 2638) arbitrary units
(mean \pm standard deviation)

After flatfield correction,
background subtraction



Second function – localization accuracy



Representative diameter distribution

