Holographic Characterization of Protein Aggregates

Size, morphology and differentiation … one particle at a time (and fast)

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NYU CSMR

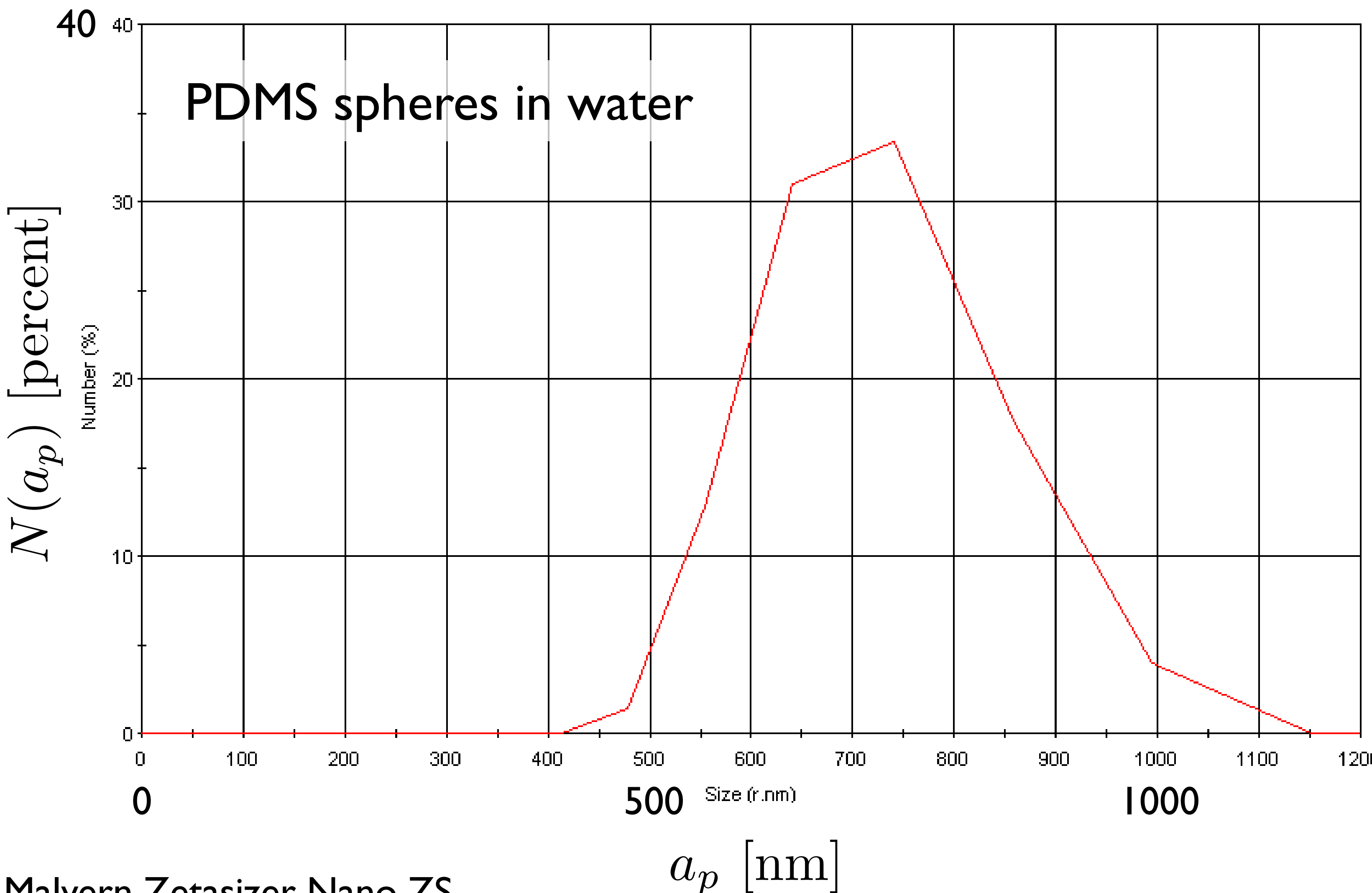
Holographic Characterization

Particle-resolved measurements of

- Radius: subvisible range
  200 nm to 10 ❍m
- Refractive index: proxy for composition
  Useful for differentiation
- Morphology
  Useful for differentiation
  Useful for analyzing aggregation mechanism
- Concentration
  Divide observed counts by measured sample volume
- Real-time processes
  30 measurements/second
  Complete analysis in 10 minutes

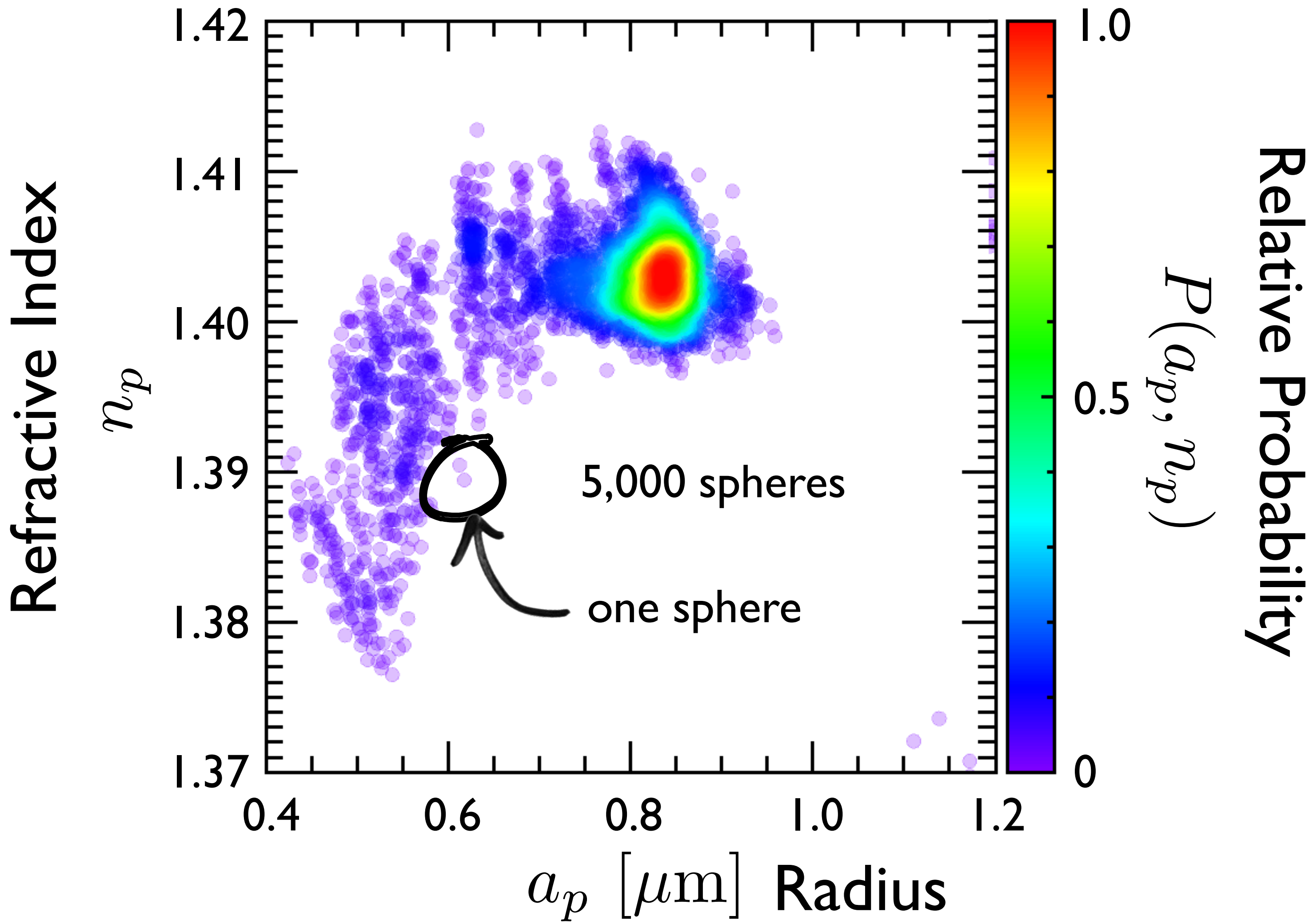
So, what's the problem?

Conventional Characterization (DLS)



Malvern Zetasizer Nano ZS

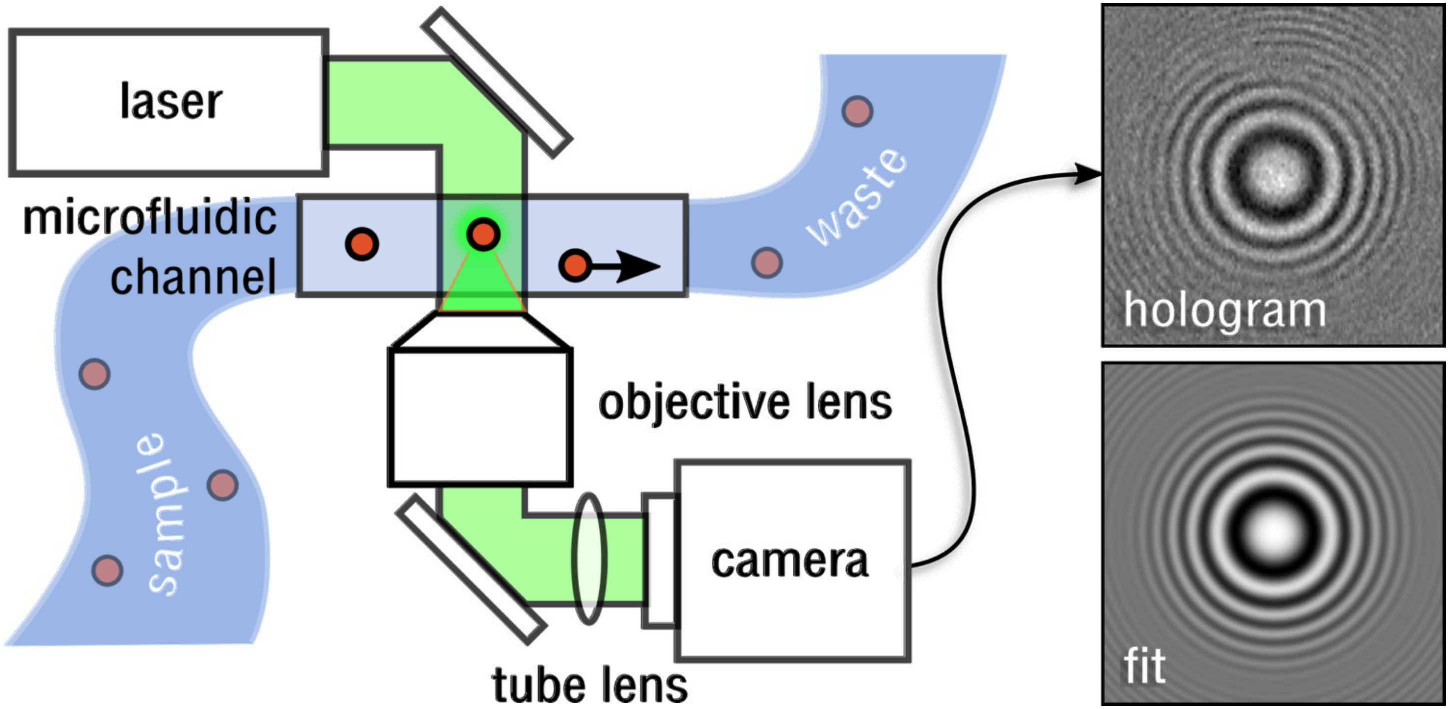
Holographic Characterization



Wang, *et al.*, *Soft Matter* **11**, 1062 (2015)

Holographic Tracking & Characterization

Lorenz-Mie Microscopy

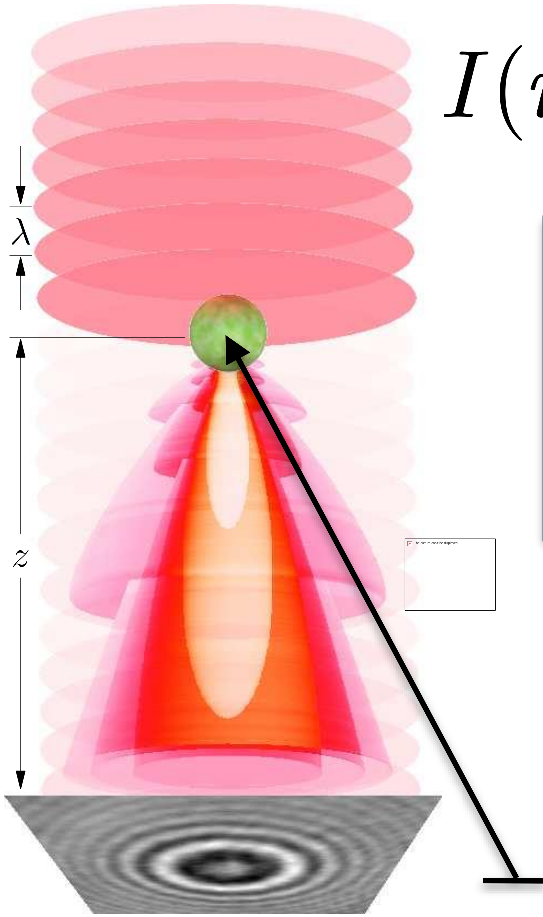


Parameters		
Tracking	3D Position	$\boldsymbol{r}_p(t)$
Characterization	Radius	a_p
	Refractive Index	n_p

Lee *et al.*, *Optics Express* **15**, 18275 (2007)

Holographic Tracking & Characterization

How Lorenz-Mie Microscopy works



Focal plane

$$I(\boldsymbol{r}) = |\boldsymbol{E}_0 + \boldsymbol{E}_s(k(\boldsymbol{r} - \boldsymbol{r_p}))|^2$$

Incident plane wave:

$$k = \frac{2\pi n_m}{\lambda}$$

Scattered field:

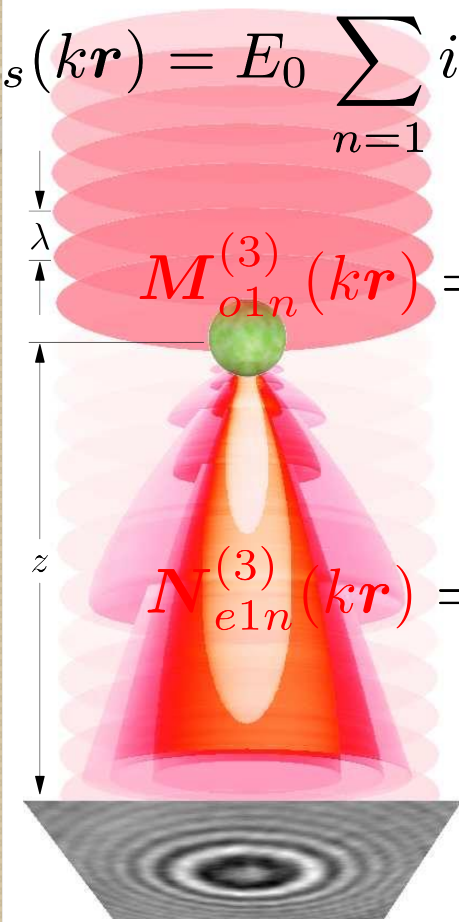
- $\boldsymbol{r}_p$: position
- a_p : radius
- n_p : refractive index

Lee *et al.*, *Optics Express* **15**, 18275 (2007)

Holographic Tracking & Characterization

How Lorenz-Mie Microscopy works

$$\boldsymbol{E}_s(k\boldsymbol{r}) = E_0 \sum_{n=1}^{\infty} i^n \frac{2n+1}{n(n+1)} \left(i\, a_n\, \boldsymbol{N}^{(3)}_{e1n}(k\boldsymbol{r}) - b_n\, \boldsymbol{M}^{(3)}_{o1n}(k\boldsymbol{r}) \right)$$



$$\boldsymbol{M}^{(3)}_{o1n}(k\boldsymbol{r}) = \frac{\cos\phi}{\sin\theta} P_n^1(\cos\theta)\, j_n(kr)\, \hat{\theta} - \sin\phi \frac{dP_n^1(\cos\theta)}{d\theta}\, j_n(kr)\, \hat{\phi}$$

$$\boldsymbol{N}^{(3)}_{e1n}(k\boldsymbol{r}) = n(n+1)\, \cos\phi\, P_n^1(\cos\theta) \frac{j_n(kr)}{kr}\, \hat{r} + \cos\phi \frac{dP_n^1(\cos\theta)}{d\theta} \frac{1}{kr} \frac{d}{dr}[r\, j_n(kr)]\, \hat{\theta} - \frac{\sin\phi}{\sin\theta} P_n^1(\cos\theta) \frac{1}{kr} \frac{d}{dr}[r\, j_n(kr)]\, \hat{\phi}$$

Lee *et al.*, *Optics Express* **15**, 18275 (2007)

Holographic Tracking & Characterization

How Lorenz-Mie Microscopy works

$$\boldsymbol{E}_s(k\boldsymbol{r}) = E_0 \sum_{n=1}^{\infty} i^n \, \frac{2n+1}{n(n+1)} \left(i \, a_n \, \boldsymbol{N}^{(3)}_{e1n}(k\boldsymbol{r}) - b_n \, \boldsymbol{M}^{(3)}_{o1n}(k\boldsymbol{r}) \right)$$

Lorenz-Mie scattering coefficients for a sphere:

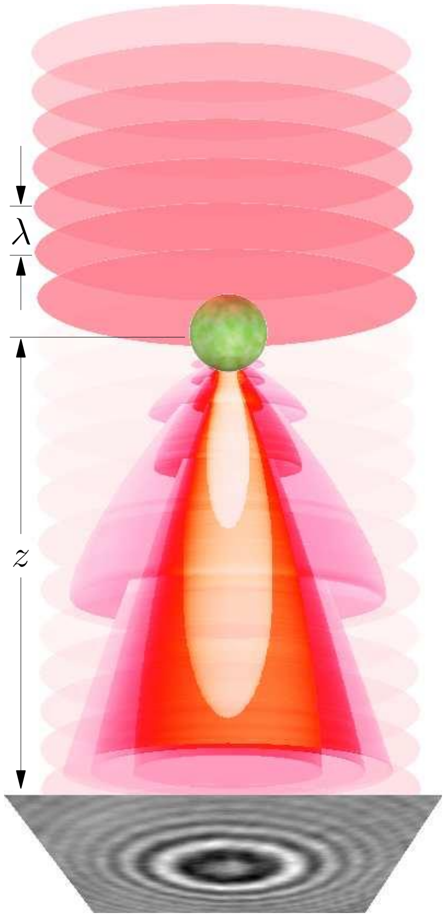
$$a_n = \frac{m^2 j_n(mka_p)\,[ka\, j_n(ka_p)]' - j_n(ka_p)\,[mka_p\, j_n(mka_p)]'}{m^2 j_n(mka_p)\,[ka_p\, h_n^{(1)}(ka_p)]' - h_n^{(1)}(ka_p)\,[mka_p\, j_n(mka_p)]'}$$

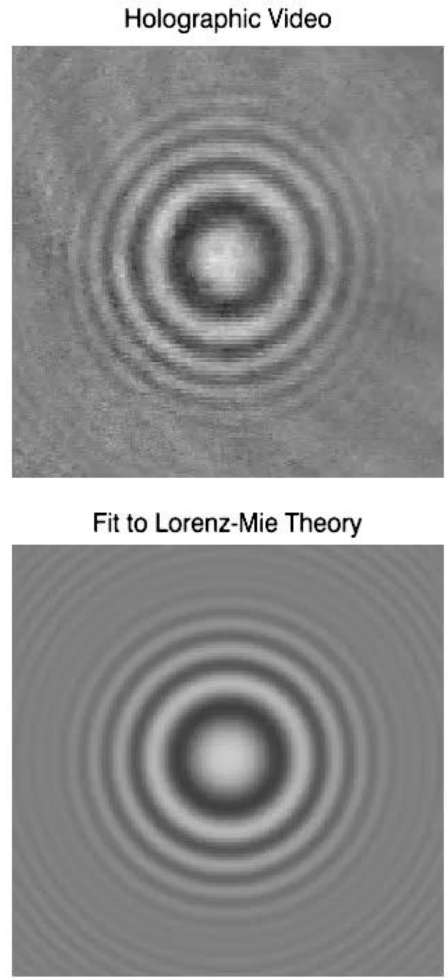
$$b_n = \frac{j_n(mka_p)\,[ka_p\, j_n(ka_p)]' - j_n(ka_p)\,[mka_p\, j_n(mka_p)]'}{j_n(mka_p)\,[ka_p\, h_n^{(1)}(ka_p)]' - h_n^{(1)}(ka_p)\,[mka_p\, j_n(mka_p)]'}$$

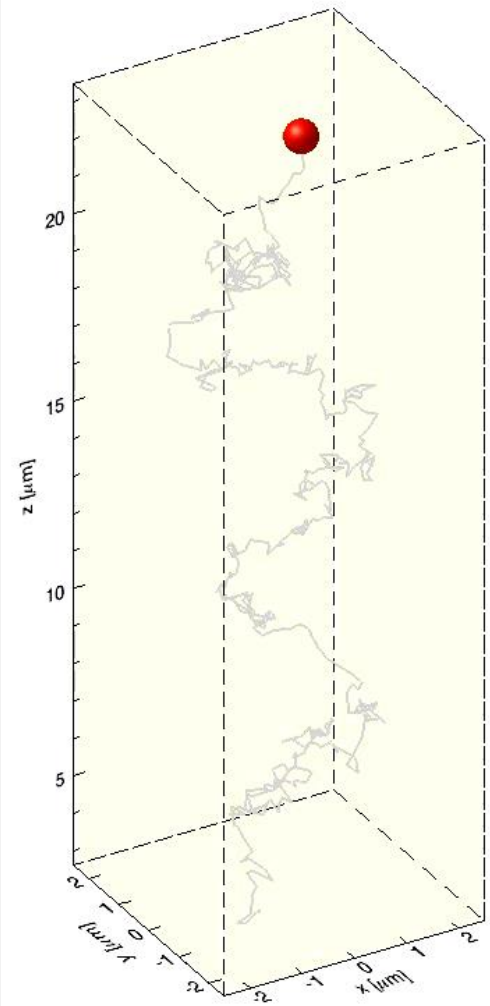
- relative radius: ka_p
- refractive index: $m = \dfrac{n_p}{n_m}$

Lee *et al.*, *Optics Express* **15**, 18275 (2007)

Lorenz-Mie Microscopy





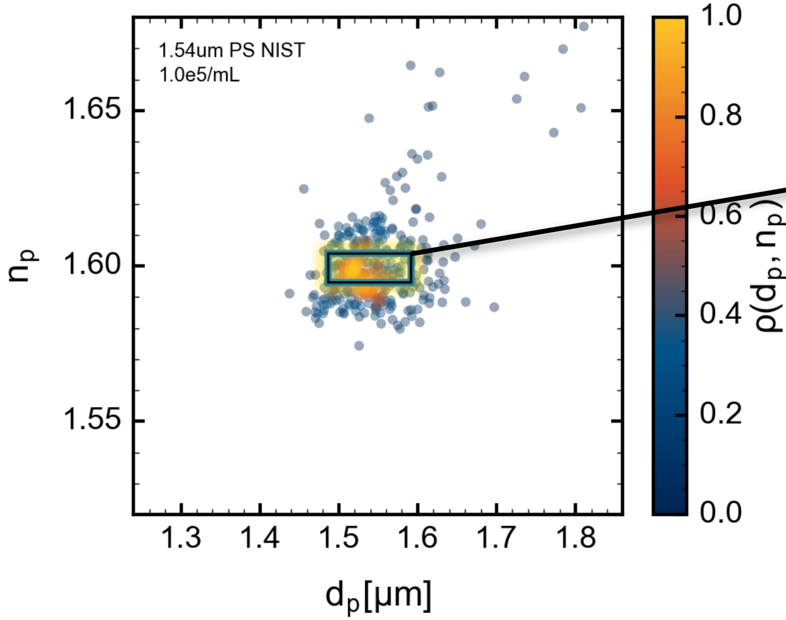


Lee *et al.*, *Optics Express* **15**, 18275 (2007)

Holographic Tracking & Characterization

Independently verified performance

Property	Precision	Range
3D Position	$\Delta \boldsymbol{r}_p \leq 3$ nm	$100 \times 100 \times 100\ \mu\text{m}^3$
Radius	$\Delta a_p \leq 2$ nm	200 nm $- 20\ \mu$m
Refractive Index	$\Delta n_p \leq 10^{-3}$	$1 - 5$
Concentration	$\Delta c_p \approx \sqrt{c_p}$	$10^3\text{mL}^{-1} - 10^8\text{mL}^{-1}$



NIST Traceable Particles
$d_p = 2\,a_p = 1.54 \pm 0.05\ \mu\text{m}$
$n_p = 1.598 \pm 0.005$

Bangs Labs #12035

Spheryx

Krishnatreya *et al.*, *Am. J. Phys.* **82**, 23 (2014)

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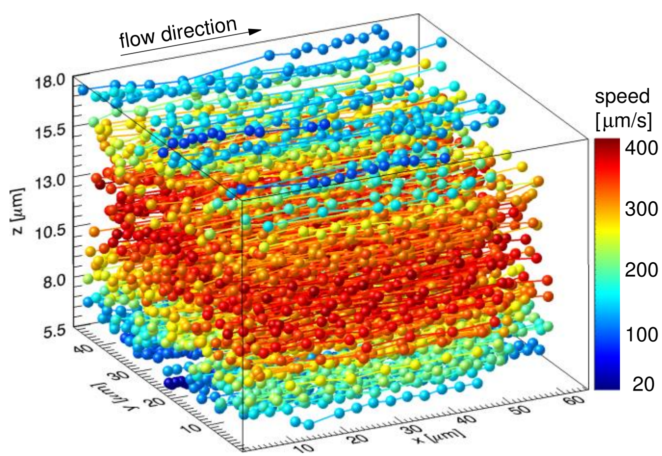
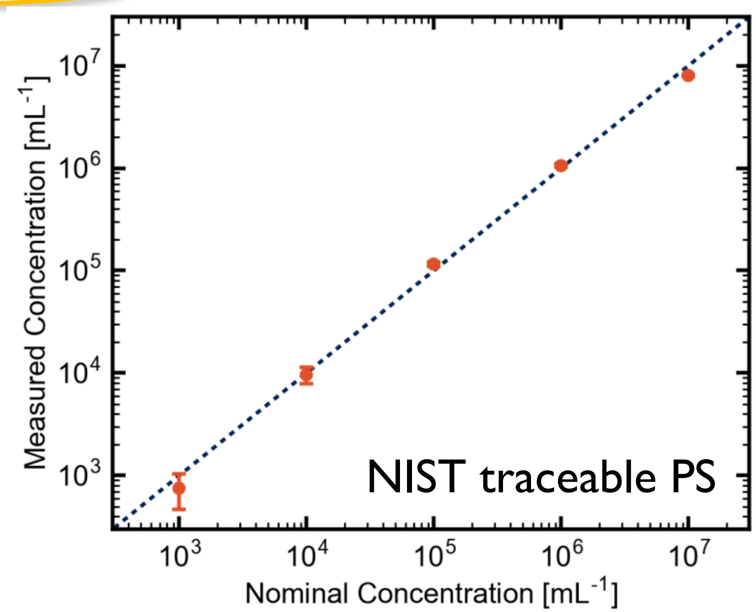
Holographic Tracking & Characterization

Independently verified performance

Property	Precision	Range
3D Position	$\Delta \boldsymbol{r}_p \leq 3$ nm	$100 \times 100 \times 100\ \mu\text{m}^3$
Radius	$\Delta a_p \leq 2$ nm	200 nm $- 20\ \mu$m
Refractive Index	$\Delta n_p \leq 10^{-3}$	$1 - 5$
Concentration	$\Delta c_p \approx \sqrt{c_p}$	$10^3\,\text{mL}^{-1} - 10^7\,\text{mL}^{-1}$



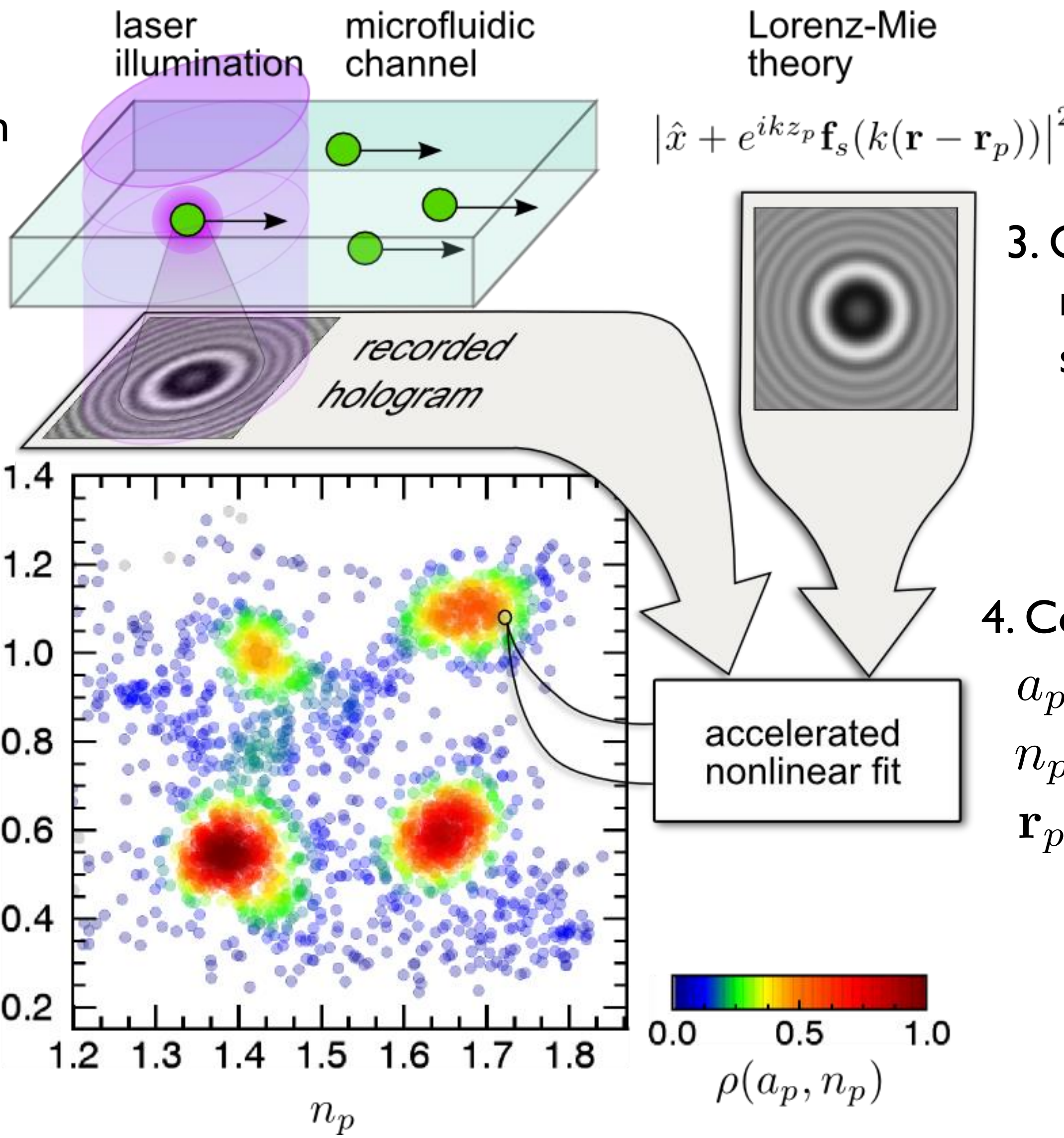
Measured flow volume
(no calibration)



Krishnatreya *et al.*, *Am. J. Phys.* **82**, 23 (2014)

Automated Holographic Characterization

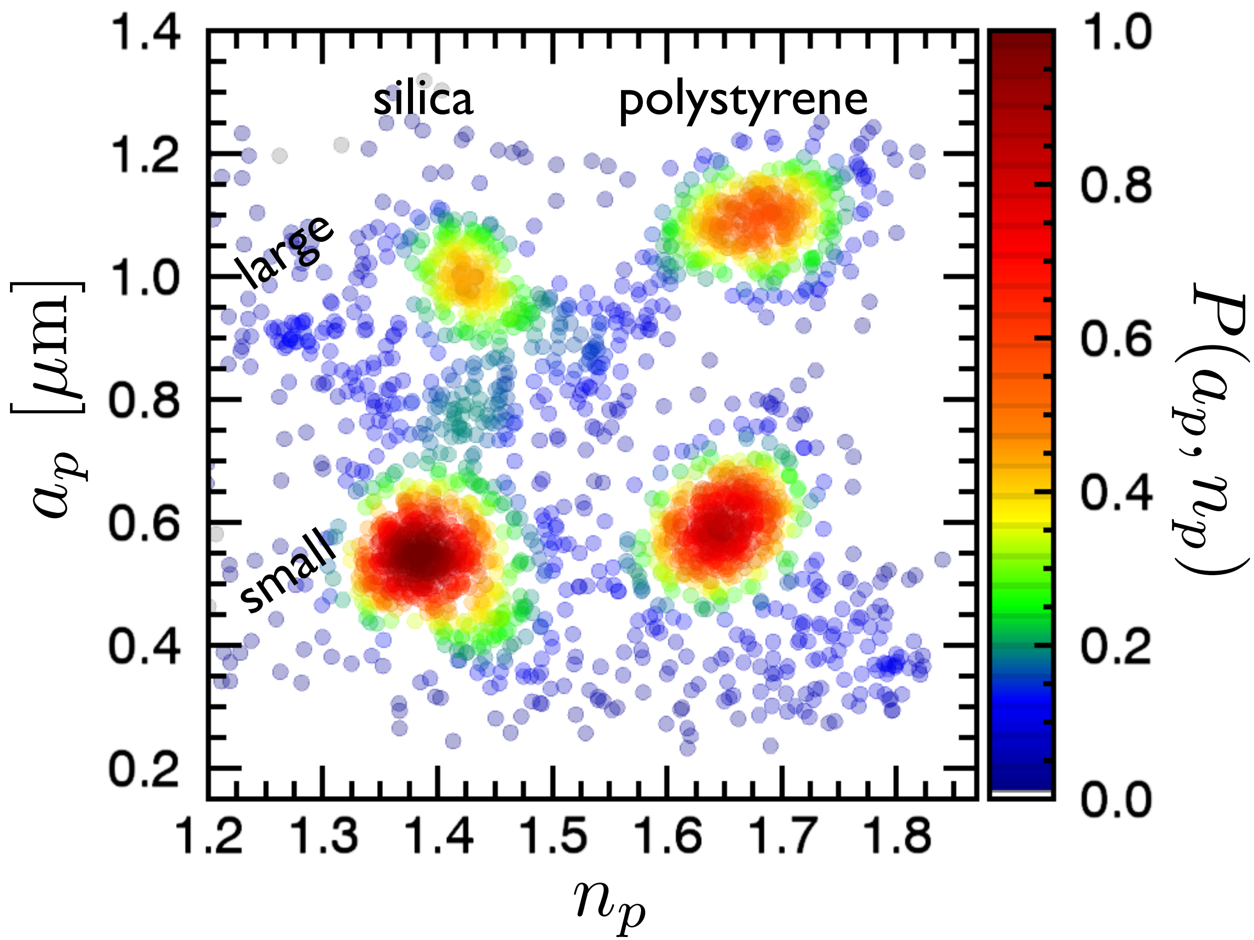
1. Particles pass through laser beam in microfluidic channel
2. Microscope records interference pattern



3. Compare measurement to scattering theory
4. Comparison yields
   - a_p : radius
   - n_p : refractive index
   - $\mathbf{r}_p$: 3D position
5. Build statistics: Each point characterizes one particle

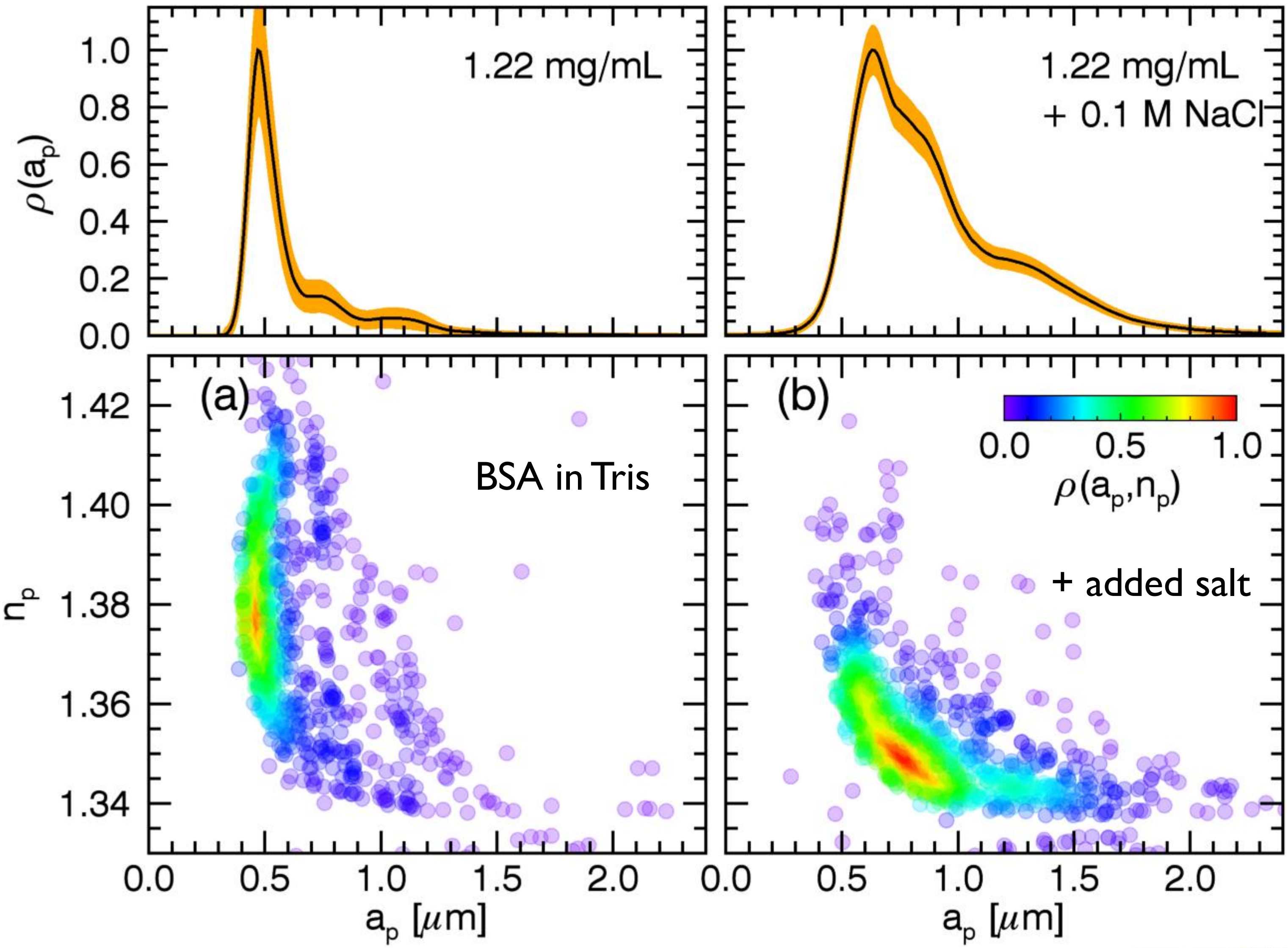
Size/refractive index distribution: for 2,500 spheres acquired in 10 minutes

Characterizing Colloidal Mixtures



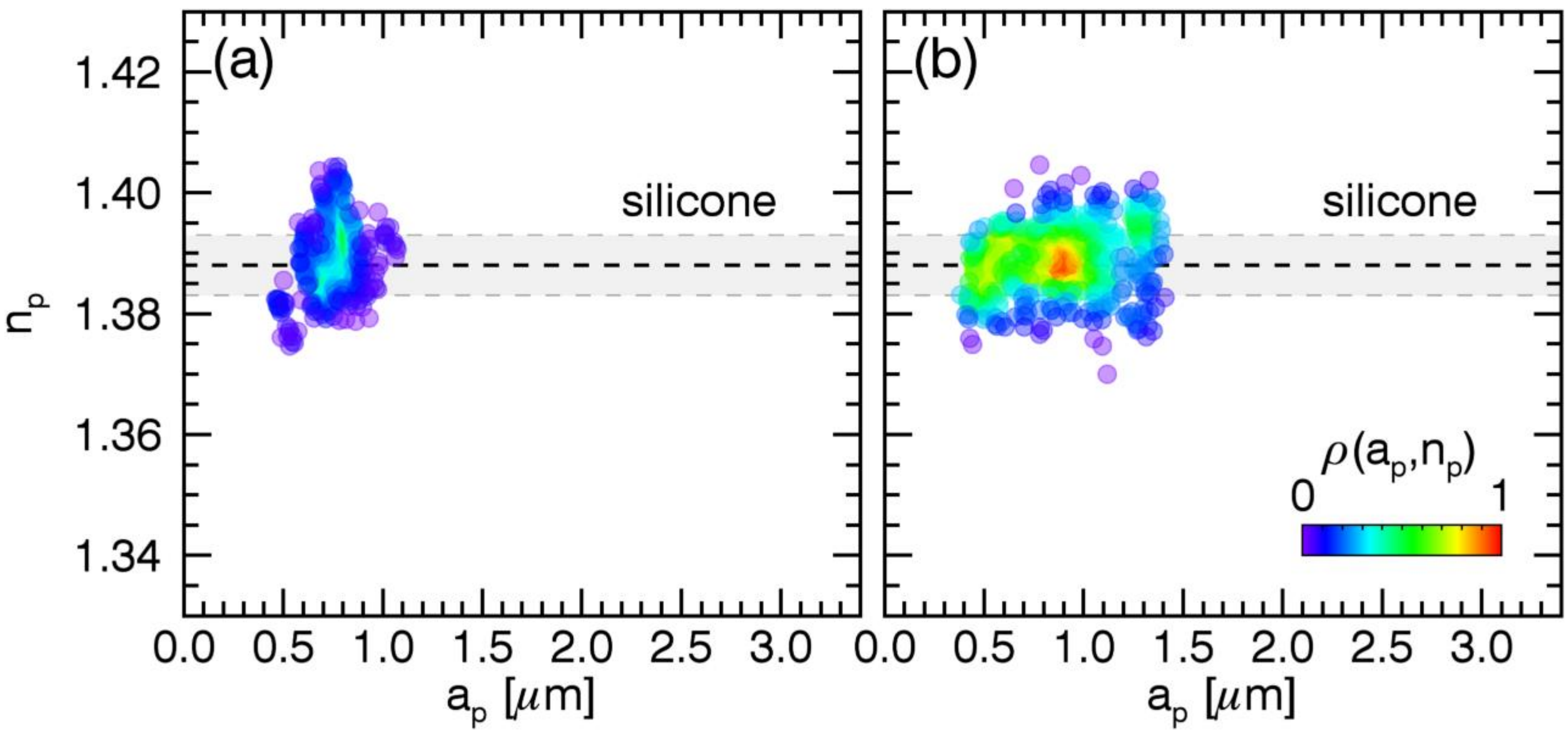
Yevick, Hannel & Grier, *Optics Express* **22**, 22864 (2014)

Characterizing Protein Aggregates



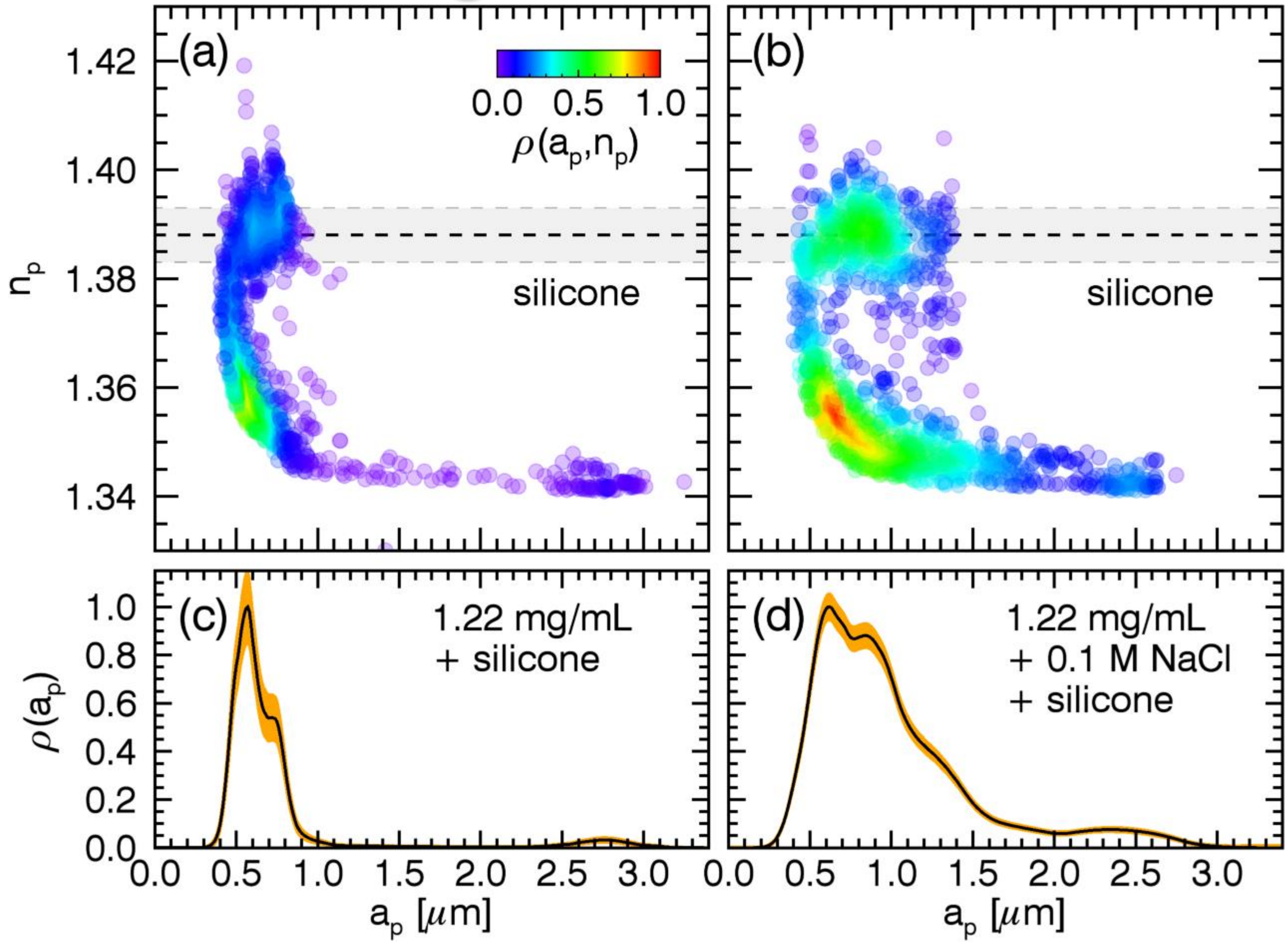
Wang *et al.*, *J. Pharm. Sci.* **105**, 1074 (2016)

Differentiating Silicone Oil Droplets



Wang *et al.*, *J. Pharm. Sci.* **105**, 1074 (2016)

Characterizing Protein & Contaminants



Wang *et al.*, *J. Pharm. Sci.* **105**, 1074 (2016)

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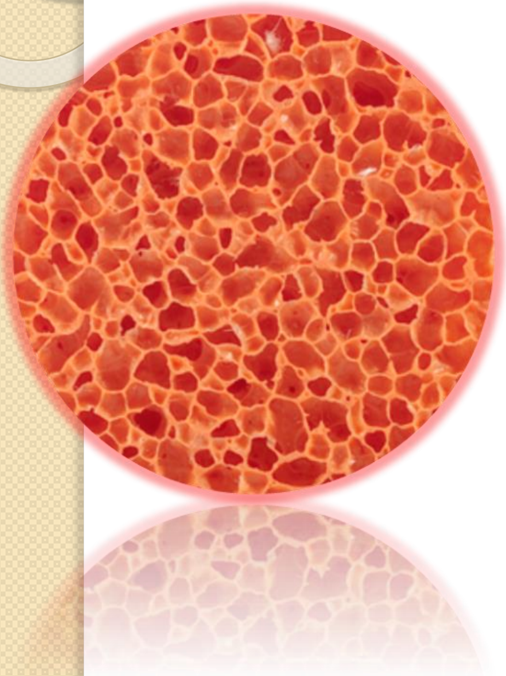
Effective Medium Theory

Porous particle



Material: Refractive index: n

Volume fraction: ϕ

Medium: Refractive index: n_m

Effective refractive index: n_p

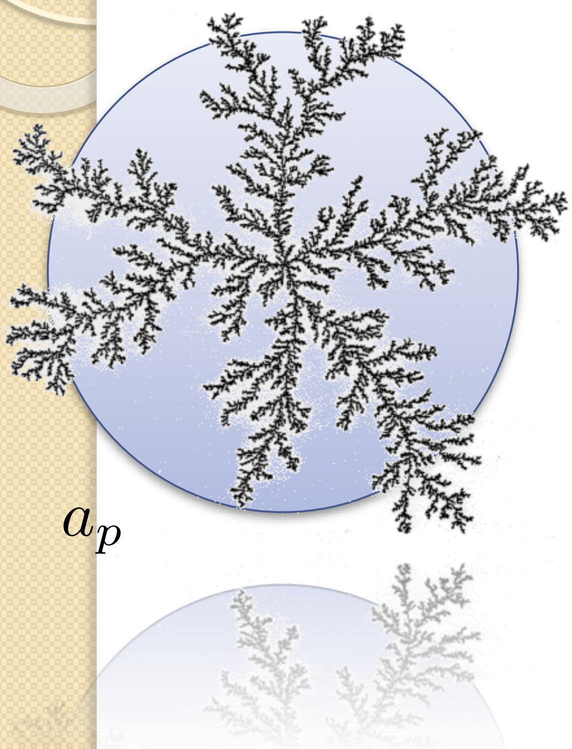
$$f(n_p) = \phi f(n) + (1 - \phi) f(n_m)$$

Lorentz-Lorenz factor:

$$f(n) = \frac{n^2 - n_m^2}{n^2 + 2n_m^2}$$

Cheong *et al.*, *Soft Matter* **7**, 6816 (2011)

Fractal Aggregates



Volume fraction:

$$\phi = \left(\frac{a_0}{a_p}\right)^{3-D}$$

Effective Medium Theory:

$$f(n_p) = \phi f(n) + (1-\phi) f(n_m)$$

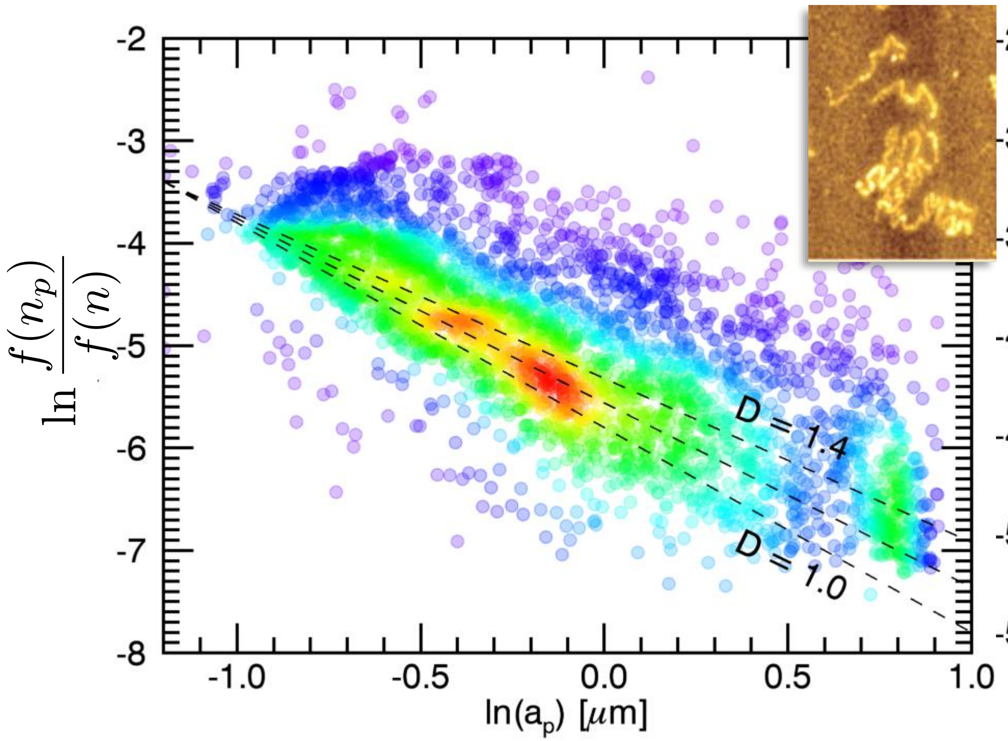
Size-Index Scaling:

$$\frac{f(n_p)}{f(n)} = \left(\frac{a_0}{a_p}\right)^{3-D}$$

Wang *et al.*, *Soft Matter* **12**, 8774 (2016)

Fractal Scaling of Protein Aggregates

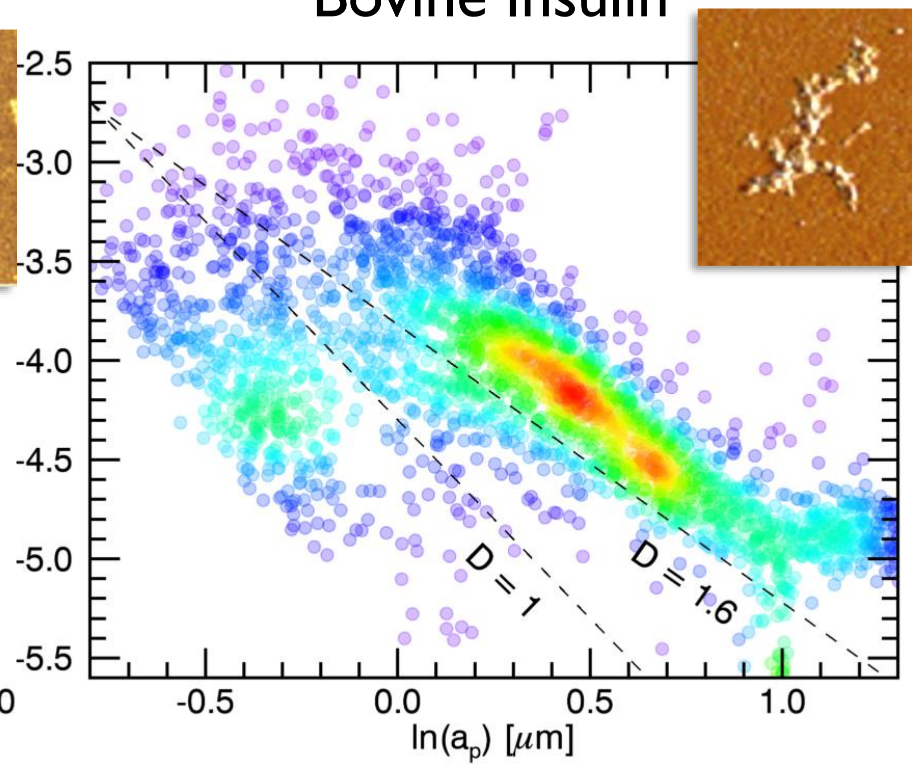
Bovine Serum Albumin



$D = 1.1 \pm 0.1$

Filamentary clusters

Bovine Insulin

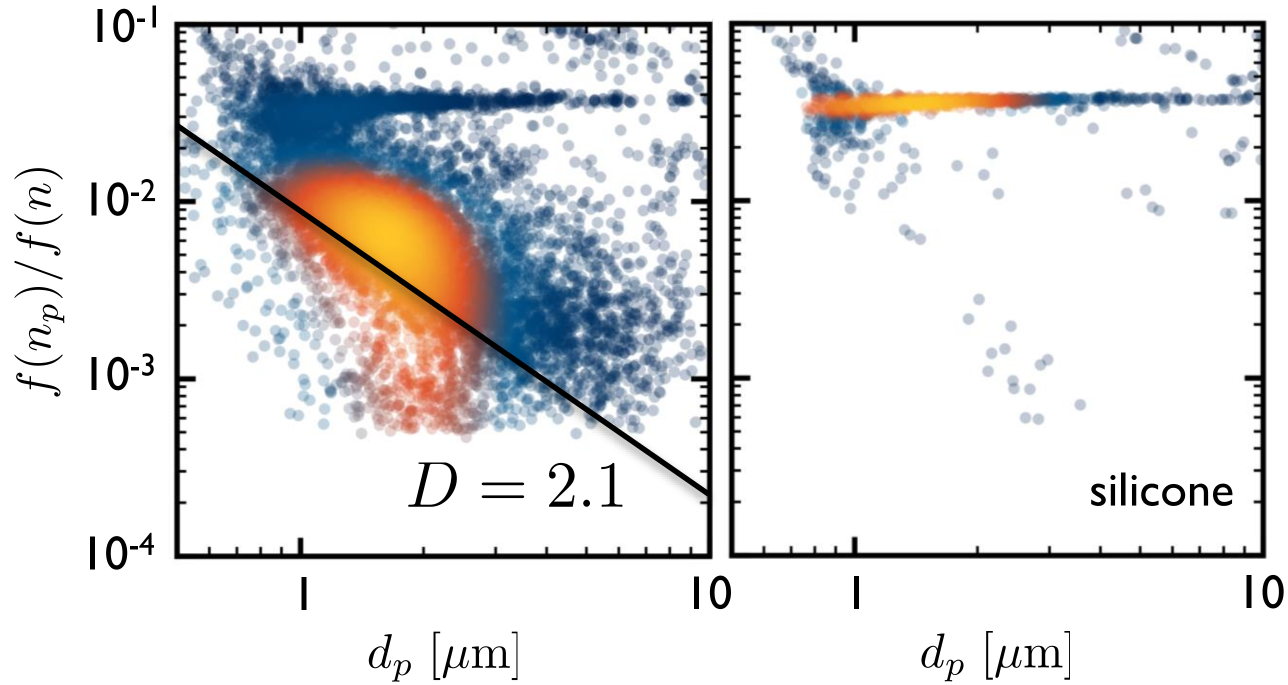


$D = 1.6 \pm 0.1$

Cluster-cluster aggregates

Wang *et al.*, *Soft Matter* **12**, 8774 (2016)

Holographic Characterization of Human IgG



Large fractal dimension: compact clusters
Differentiation between protein aggregates and silicone droplets

Spheryx

Ruffner *et al.*, *unpublished* (2016)

NYU CSMR

Holographic Characterization

- Detects, counts and characterizes subvisible protein aggregates *in situ*
- Differentiates by composition & morphology
- Fast, time-resolved measurements
- Independently verified precision & accuracy
- Minimal calibration

Comparison with Industry Standard

DLS (Malvern Zetasizer Nano)

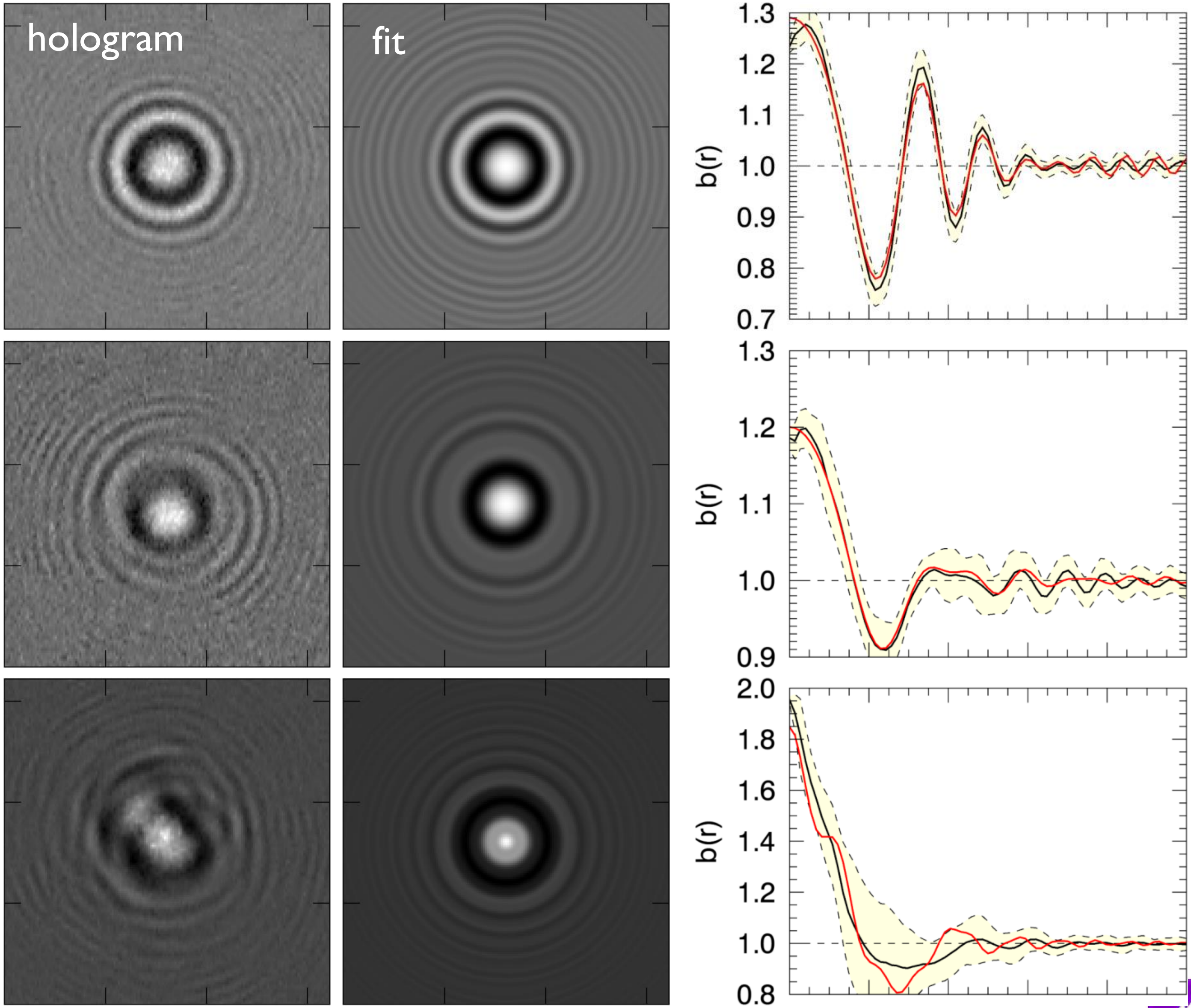
diameter [μm]	manufacturer	Zetasizer [μm]	stdev(ap)	HVM(ap) [μm]	stdev(ap)	HVM(np) [μm]	Porosity
15.17±0.14	PS-research Particle	-	-	15.282	0.6	1.599±0.02	1%
12	PS-research Particle	-	-	12.07	0.13	1.597±0.004	1%
10.02±0.08	PS-research Particle	-	-	9.928	1.1	1.605±0.008	1%
1.51±0.045	Polyscience	-	-	1.634	0.04	1.576±0.008	12.10%
0.995±0.021	Duke scientific	0.972	0.13	1.1	0.01	1.58±0.005	6%
0.701±0.006	Duke scientific	0.8166	0.113	0.824	0.04	1.565±0.016	25%
0.304±0.006	Duke scientific	0.37	0.088	0.482	0.03	x	x
0.06±0.0025	Duke scientific	0.082	0.045	0.078	0.02	x	x

1. Consistent mean radius results in common range
2. HVM also works for larger particles
3. HVM results have smaller (better) instrumental spread
4. HVM also yields refractive index (porosity)

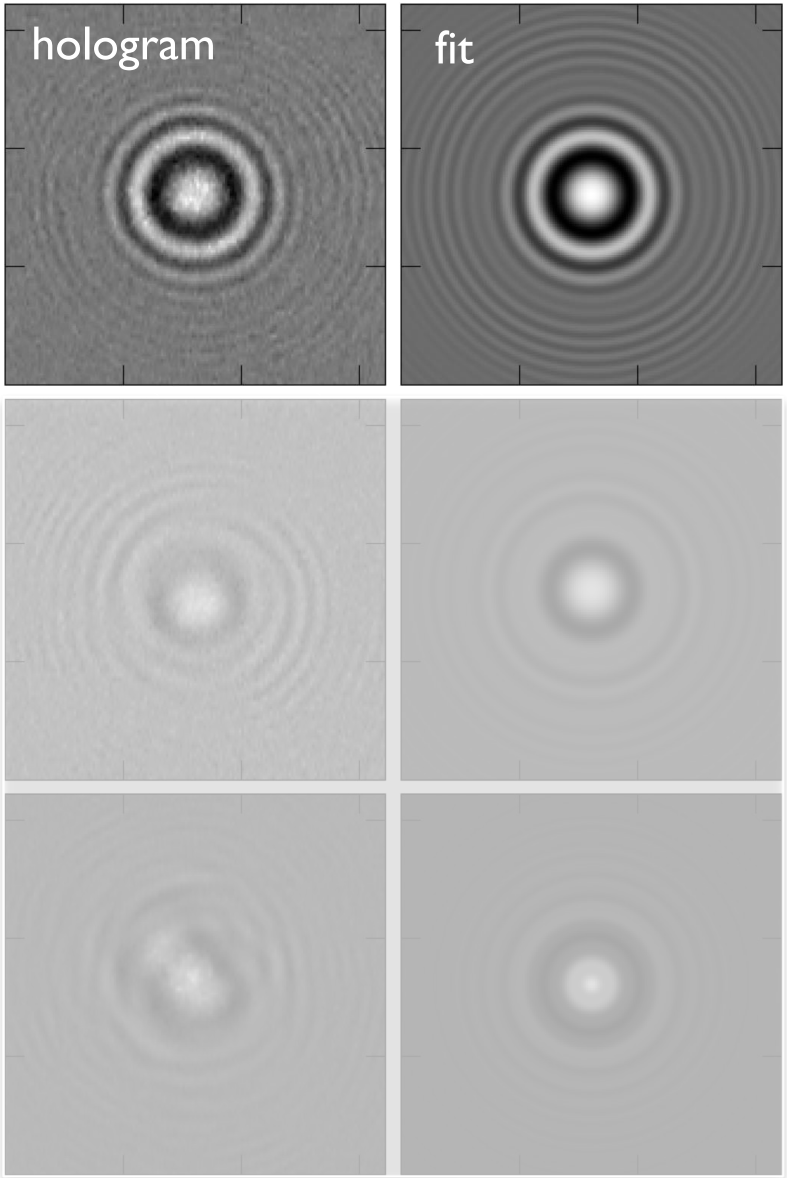
Cheong *et al.*, *Opt. Express* **17**, 13071 (2009)

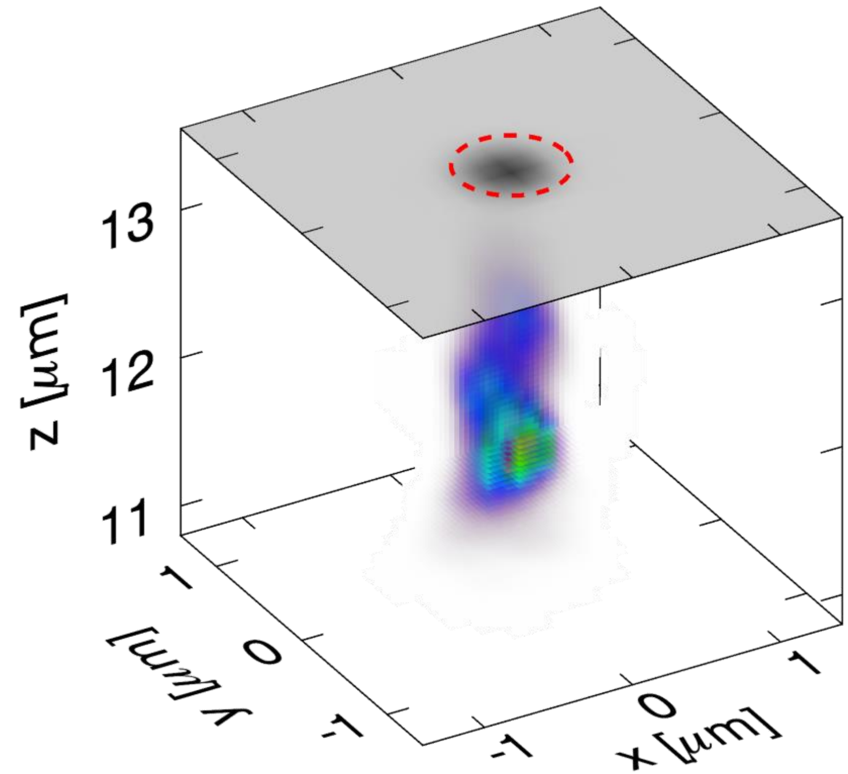
Cheong, Xiao, Pine & Grier, *Soft Matter* **7**, 6816 (2011)

So, what do our clusters look like?



So, what do our clusters look like?





Holographic Deconvolution Microscopy

Dixon *et al.*, *Opt. Express* **19**, 16410 (2011)
Chen *et al.*, *J. Pharm. Sci.* **105**, 1074 (2016)

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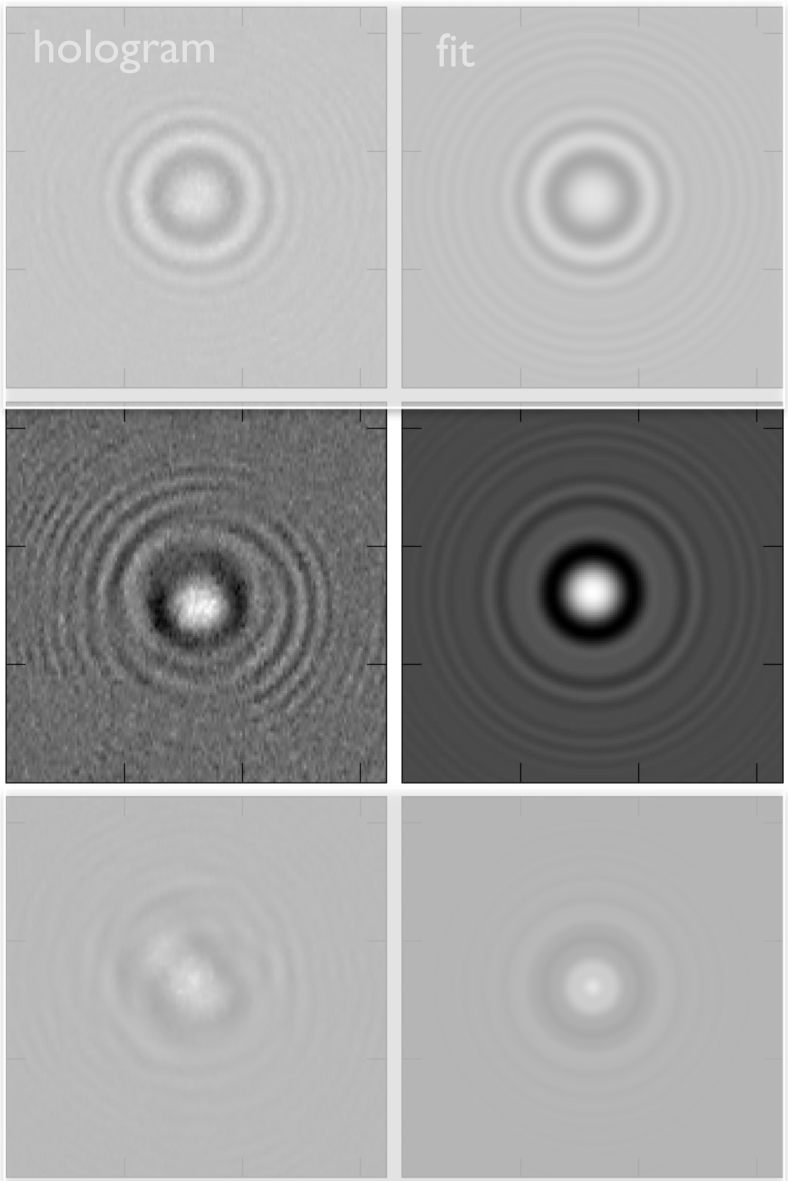
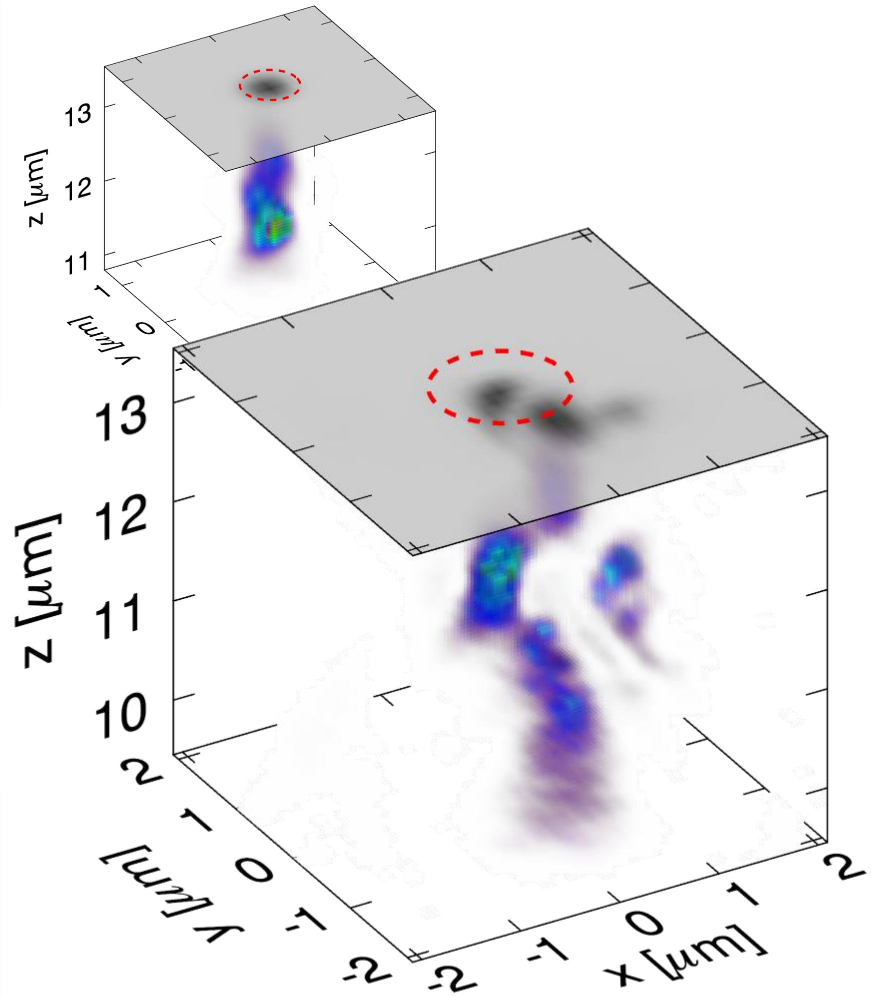
So, what do our clusters look like?





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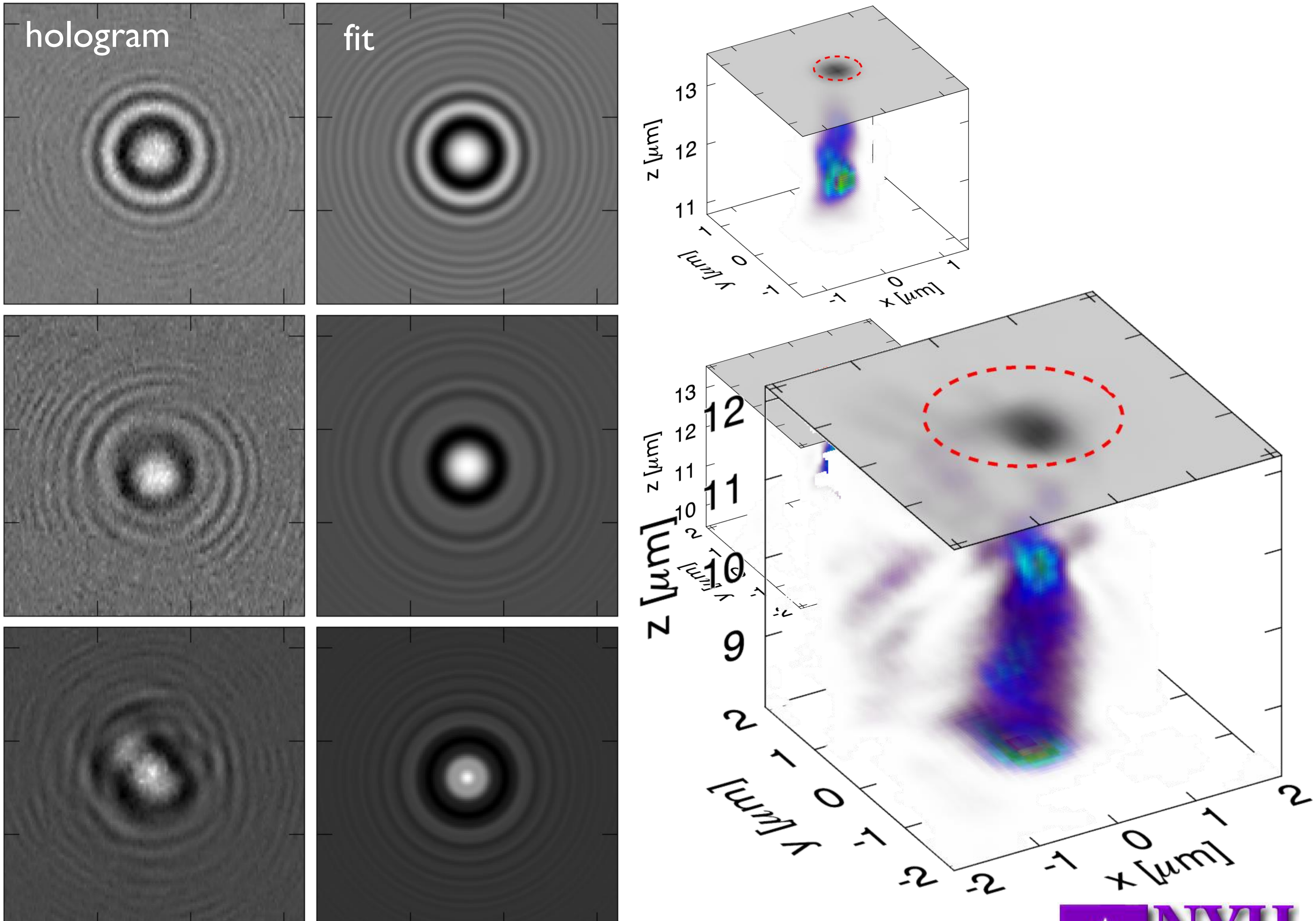

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