

A New Form of Protein Aggregates: An Early Warning Sign of Polysorbate Degradation

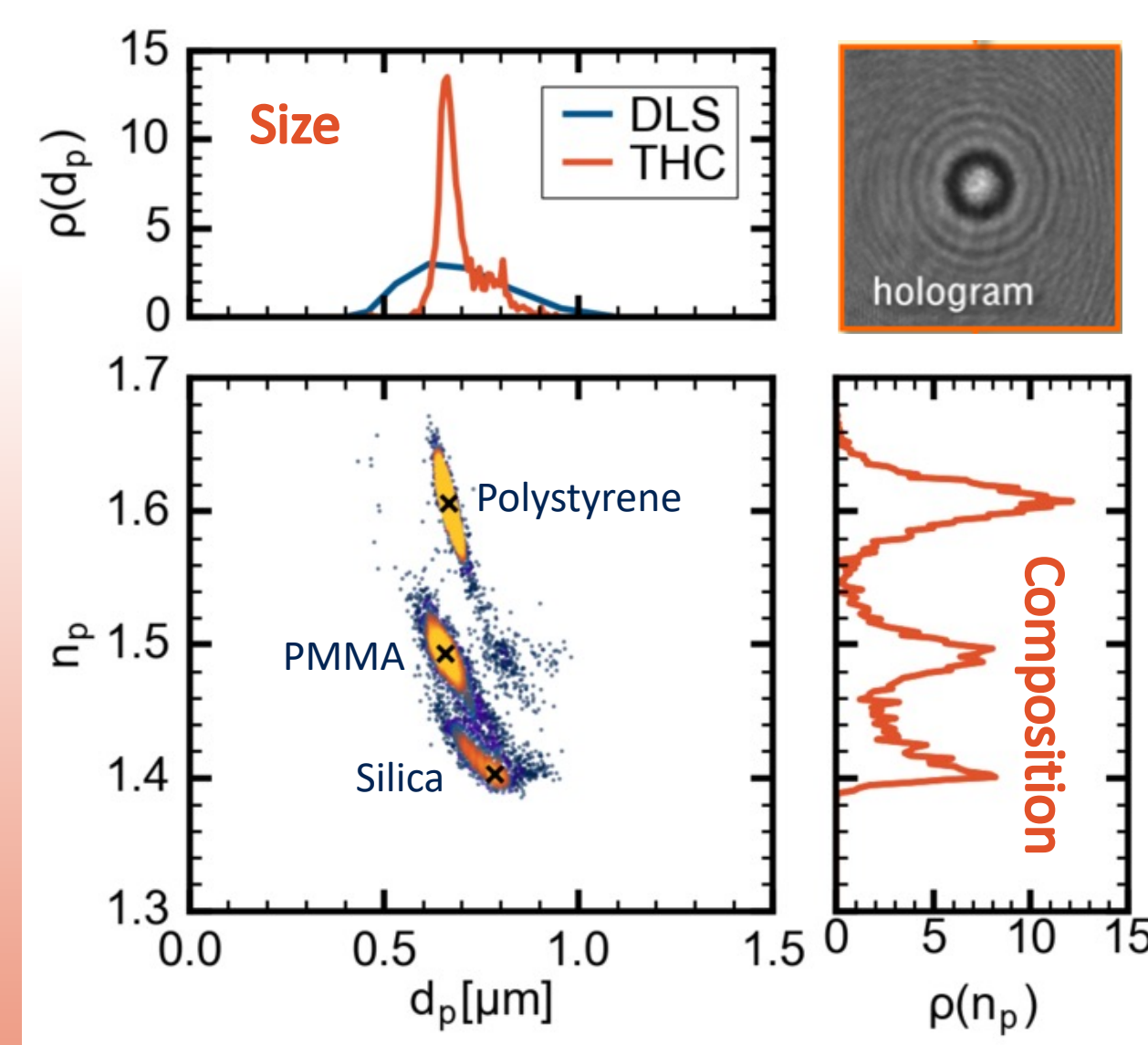
Valentina Montes-Flores, Juliana Lumer, and Laura A Philips
Spheryx, Inc., 330 East 38th Street, #48J, New York, NY 10016

Total Holographic Characterization (THC): A New Dimension of Information

- Each particle has a unique hologram signature
- Lorenz-Mie Theory is used to analyze the holograms and produces: Size and Refractive Index
- Accurate Concentrations
- Hologram symmetry reflects the shape of particles
- xCell disposable sample cell: no cross contamination & no cleaning

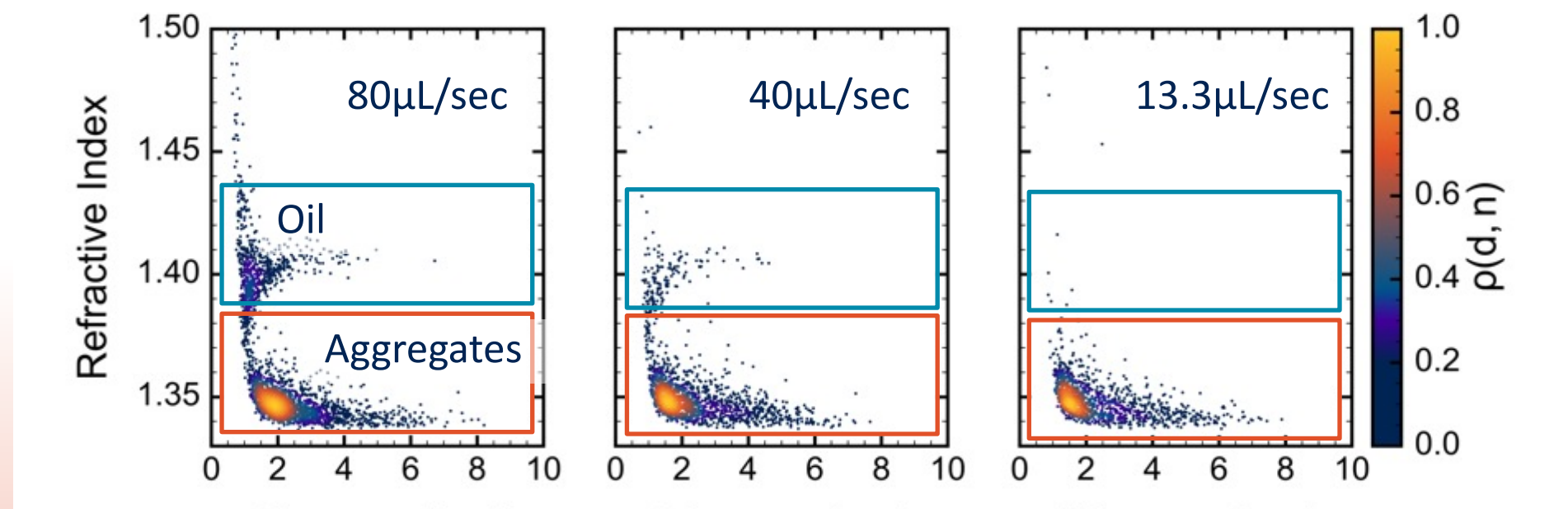


- Size: 500nm – 10µm
- Concentration: 500 – 10⁷ particles/mL
- Sample size: 30 µL/60µL
- Viscosity: 1-40 cP
- Measurements times: 10-15 min



-THC distinguishes composition by refractive index
-Simultaneous measurement of multiple components of heterogeneous mixtures

Quantitating Each Contaminant Type Independently



- IgG sample ejected from a syringe at 3 speeds

Concentration (Particle/mL x10 ⁴)			
Sample	Protein	Oil	Total
Slow	35	2	38
Medium	40	6	47
Fast	60	63	130

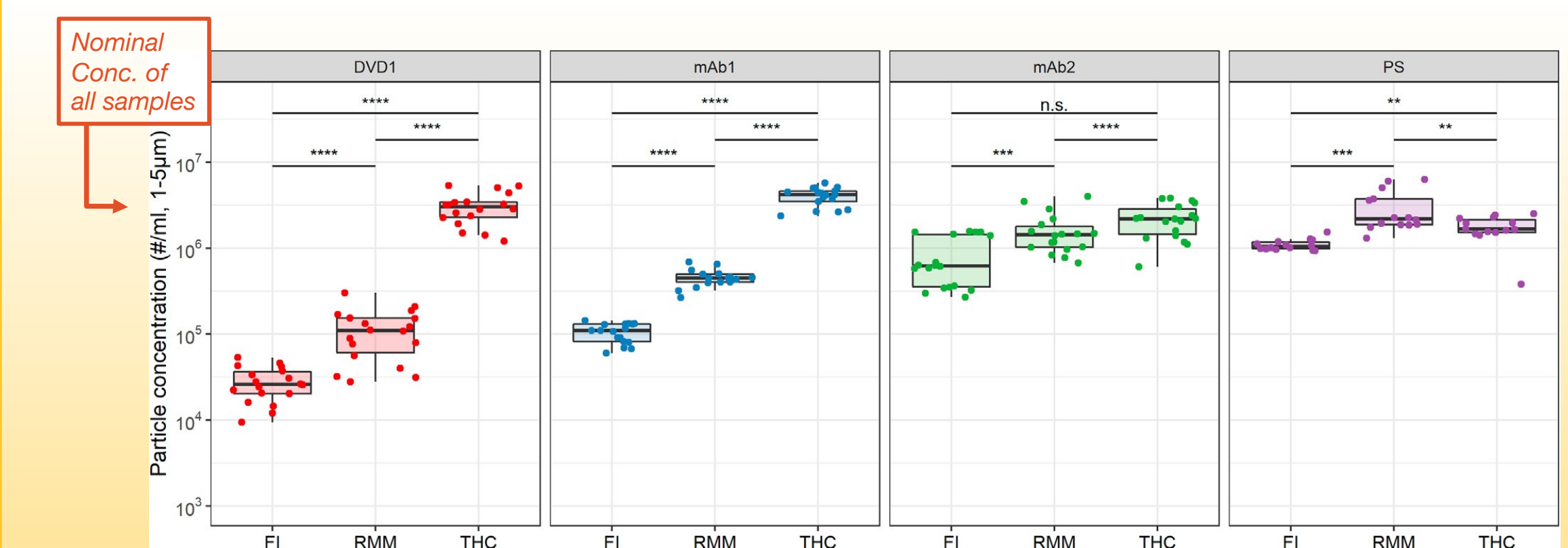
accurate #particles
accurate volume = accurate concentration

Manufacturing Applications



User interface reports concentrations of multiple contaminants simultaneously for GMP environments

10x More Accurate Results for Small Particles

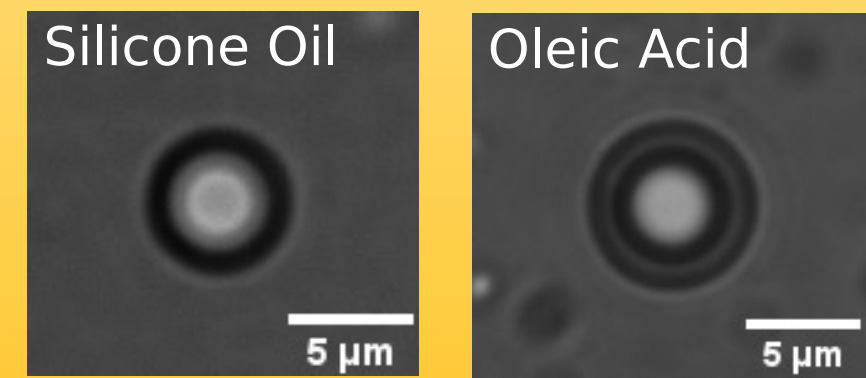


FROM: J. of Pharm. Sci., 112, 2023, p.985-990. The Strengths of Total Holographic Video Microscopy in Detecting Sub-Visible Protein Particles in Biopharmaceuticals: A Comparison to Flow Imaging and Resonant Mass Measurement. Rahn et al., AbbVie Deutschland GmbH & Co.

Figure 3. Comparing different particle types measured with different methods. Comparison of concentration of different particles measured using FI, RMM or THC, where PS and mAb1 concentrations show small difference between methods and mAb2 and DVI/D1 (dual variable domain) particle concentration seem to be highly dependent on the method of choice. Stated p-values of the pairwise comparisons were done using Wilcoxon rank sum test, where "n.s.", "**", "****", and "*****" represents p>0.05, p<0.01, p<0.001, and p<0.0001 respectively. Data shown for particles between 1 and 5 µm.

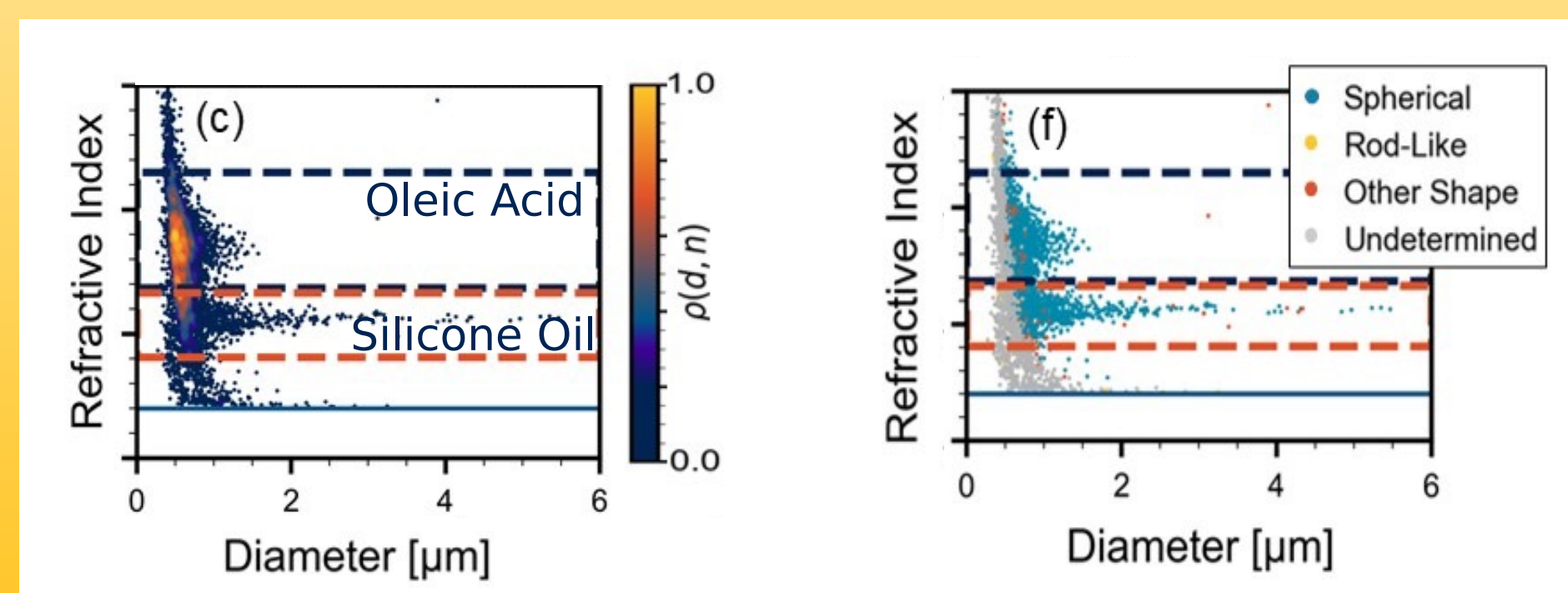
Silicone Oil vs. Other Emulsions

Light Microscope Images

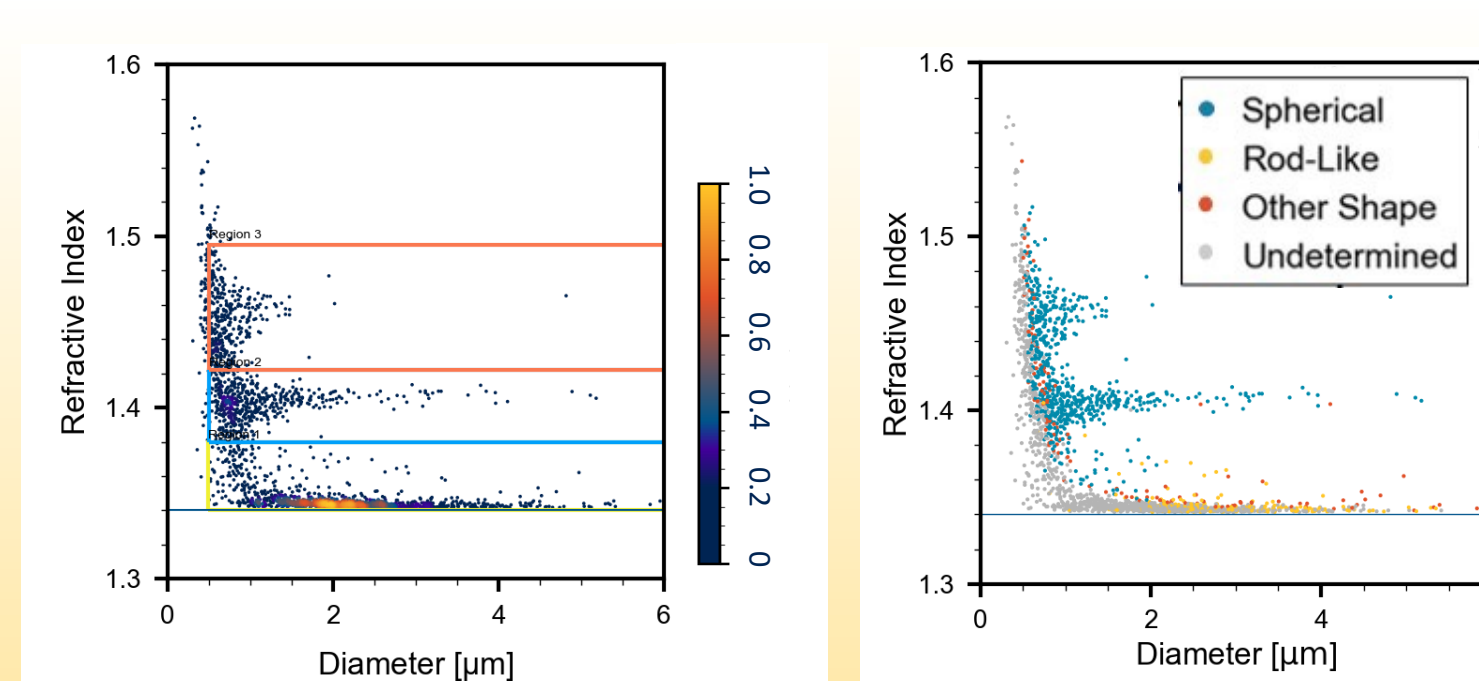


Different emulsion droplets are not distinguishable

THC distinguishes silicone oil and oleic acid by index, when they are the same shape and size



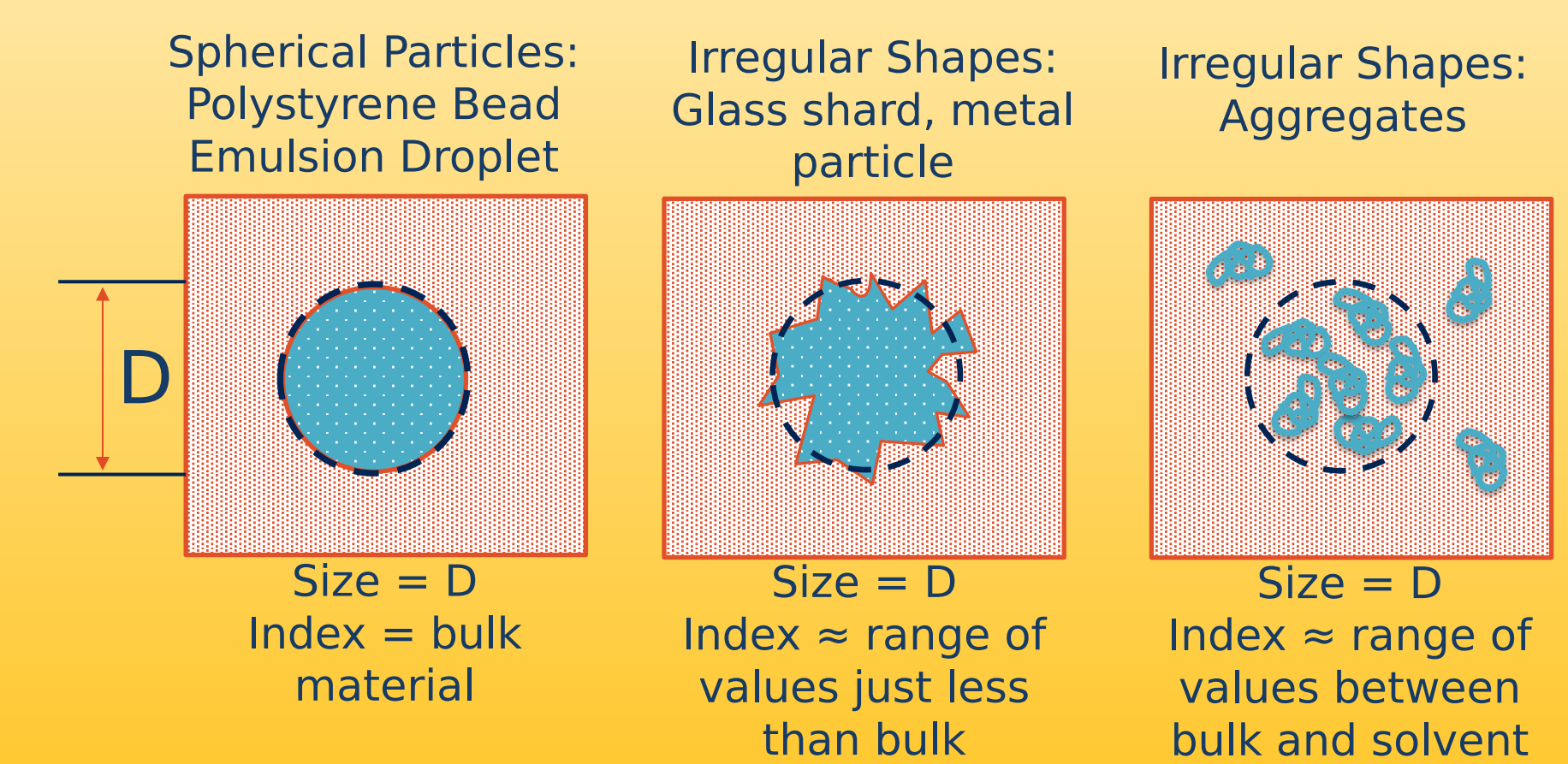
Particle Morphology: Another Dimension of Information



Color = Particle Density Color = Particle Morphology

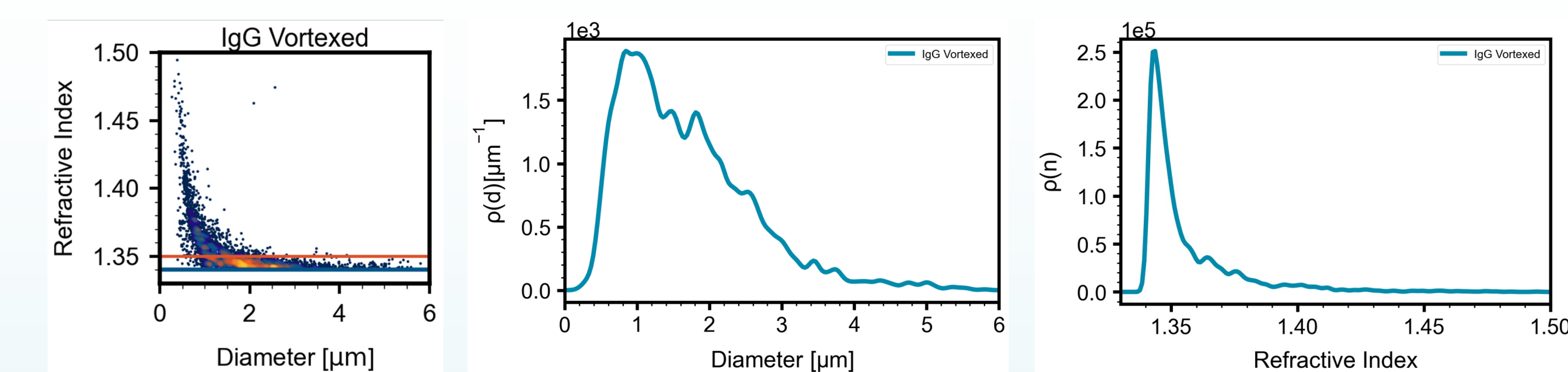
- Distinguish aggregates and emulsion droplets by index and shape
- Distinguish emulsions by index
- Size distributions of each contaminant in a single measurement

Effective Sphere Model



Protein Aggregation Detection and Analysis

Typical Protein Aggregation Behavior under Stress



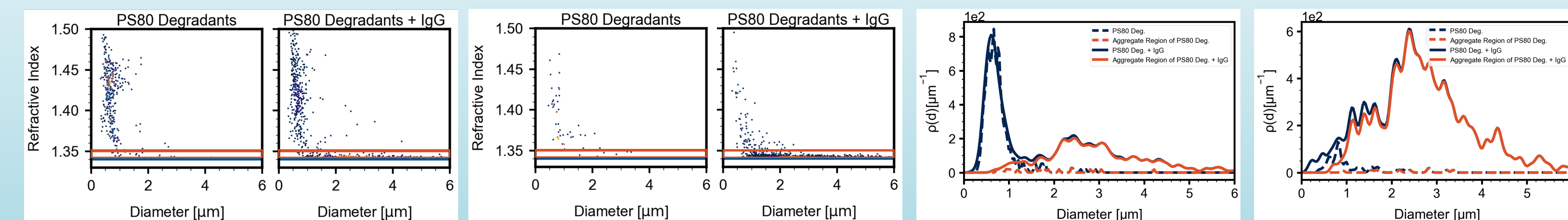
- Protein aggregates form when biologics are exposed to thermal or mechanical stresses
- Shown is an example of IgG in sodium acetate (1mg/1mL) stressed by vortexing
- The concentration of aggregates increases monotonically as aggregates get smaller
- This THC signature is typical of protein aggregation under thermal or mechanical stresses
- Protein aggregates appear close to the refractive index of the buffer

Protein Aggregation Behavior in the Presence of Polysorbate Degradants

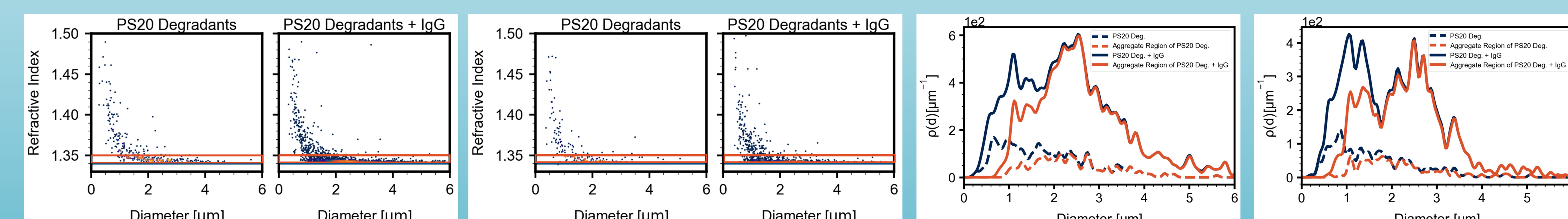
- When a biologic formulation is exposed to degradants of polysorbate (PS80 or PS20) a new THC feature is observed
- The new feature appears as a peak in the size distribution located between 1-3 µm with a refractive index just above the medium refractive index

t = 0 t = 1 day t = 0 t = 1 day

PS80



PS20

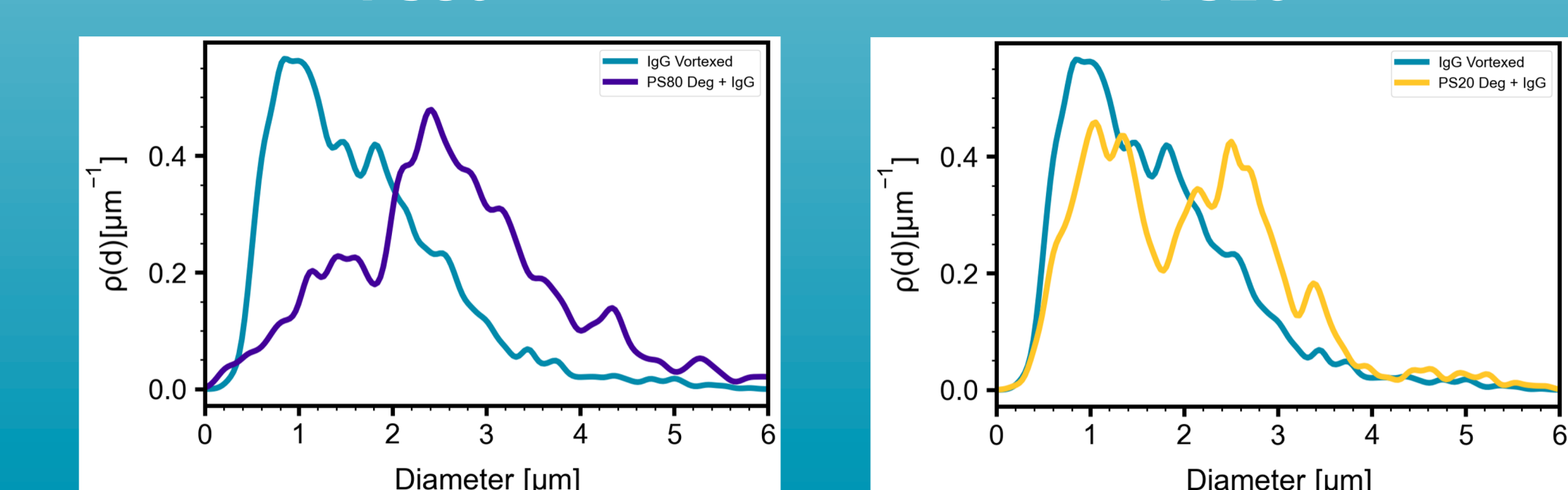


Polysorbate Induces Different Aggregation Behavior than Other Stresses

Degradation vs. Vortexing

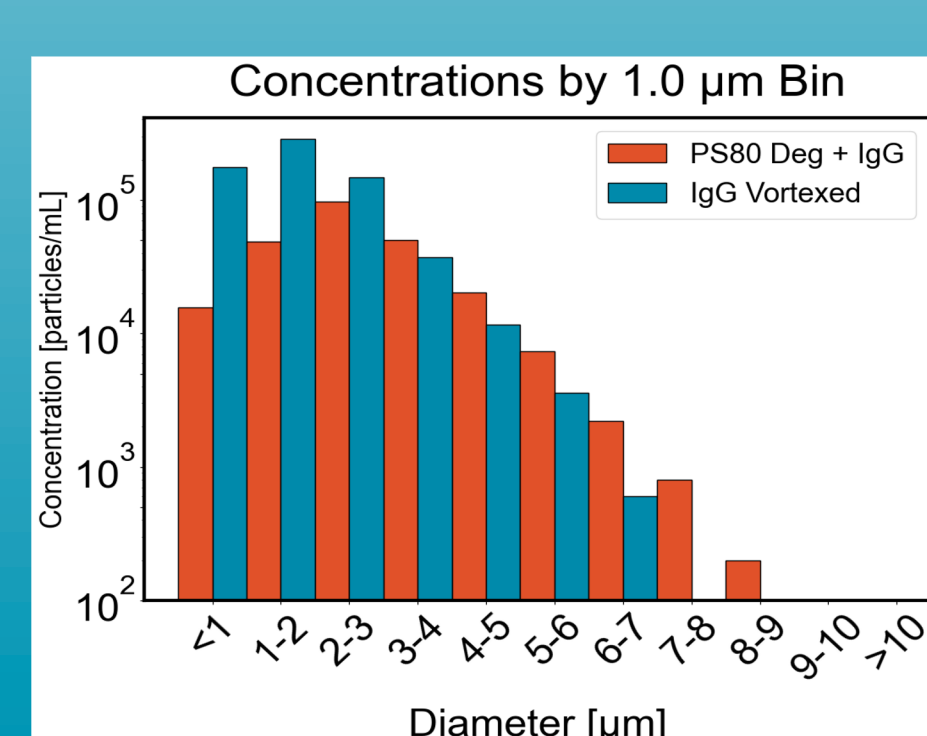
PS80

PS20



- A peak in the size distribution consisting of particles near the index of the buffer, with a maximum of 1-3 µm is an indicator of polysorbate degradation.

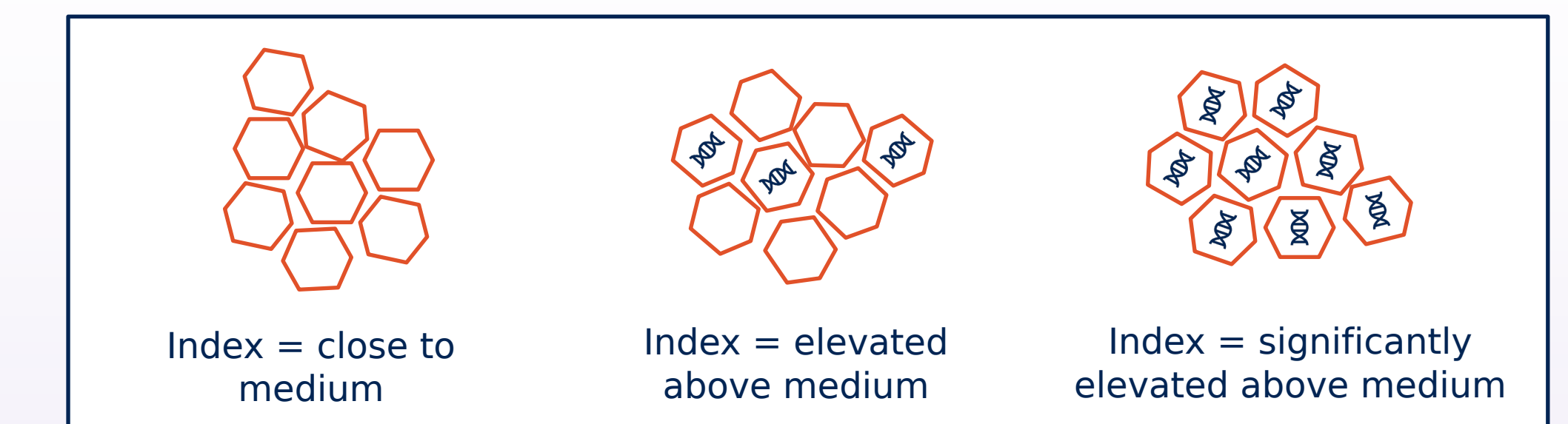
- High resolution at small sizes is needed to identify this early warning sign of polysorbate degradation to distinguish it from aggregates formed from other stresses.



THC for Gene Therapy: Preliminary Results

xSight Application

Challenge: 20-30 nm capsids Solution: Aggregates of capsids



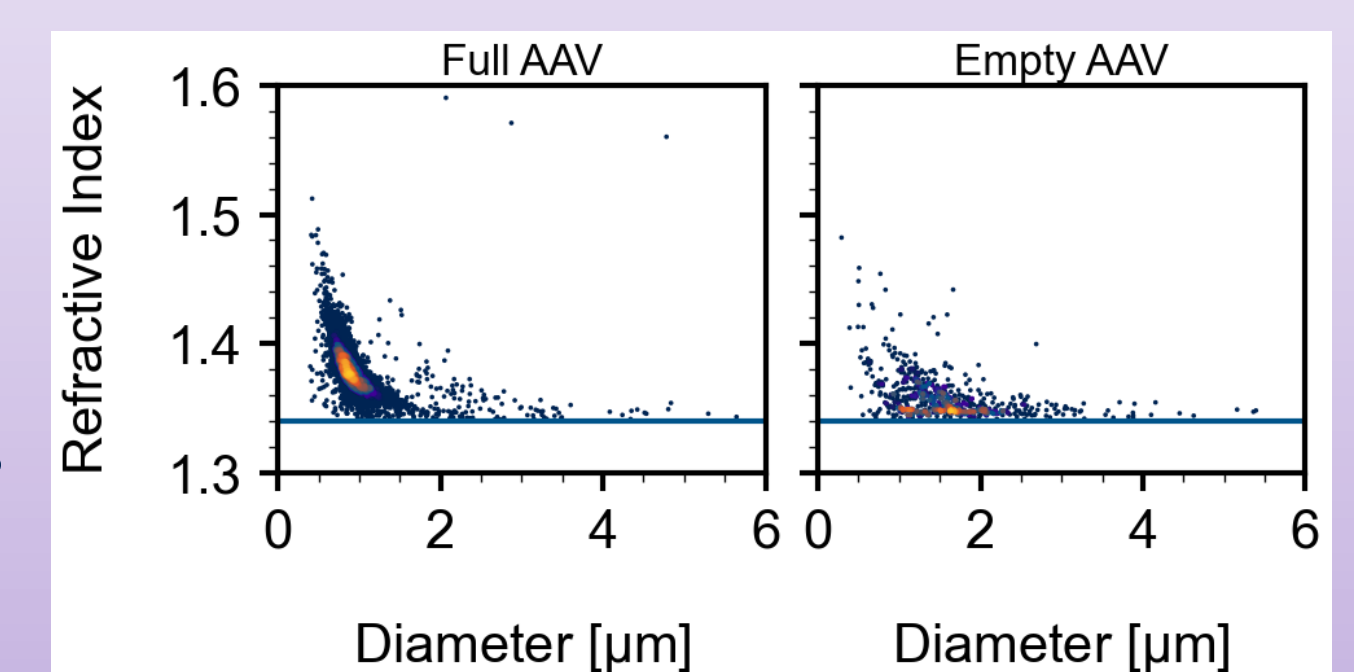
Filling ratio of capsids carrying viral vector: a CQA

Empty or partially filled capsids:

- Impurities with unclear clinical impact
- Decrease efficacy
- Possible immune response

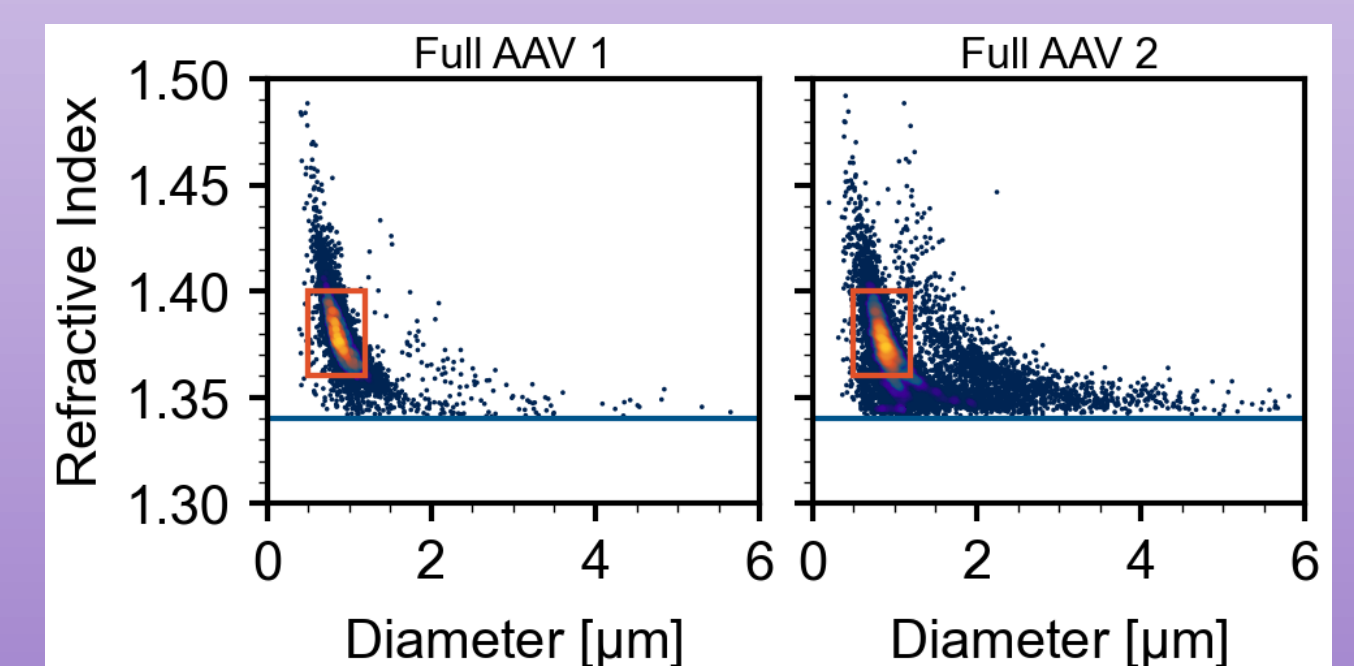
Needed: Technology to measure capsids quickly & reliably for product & process development, process control, release testing.

- Full and empty AAV capsid aggregates have different THC signatures



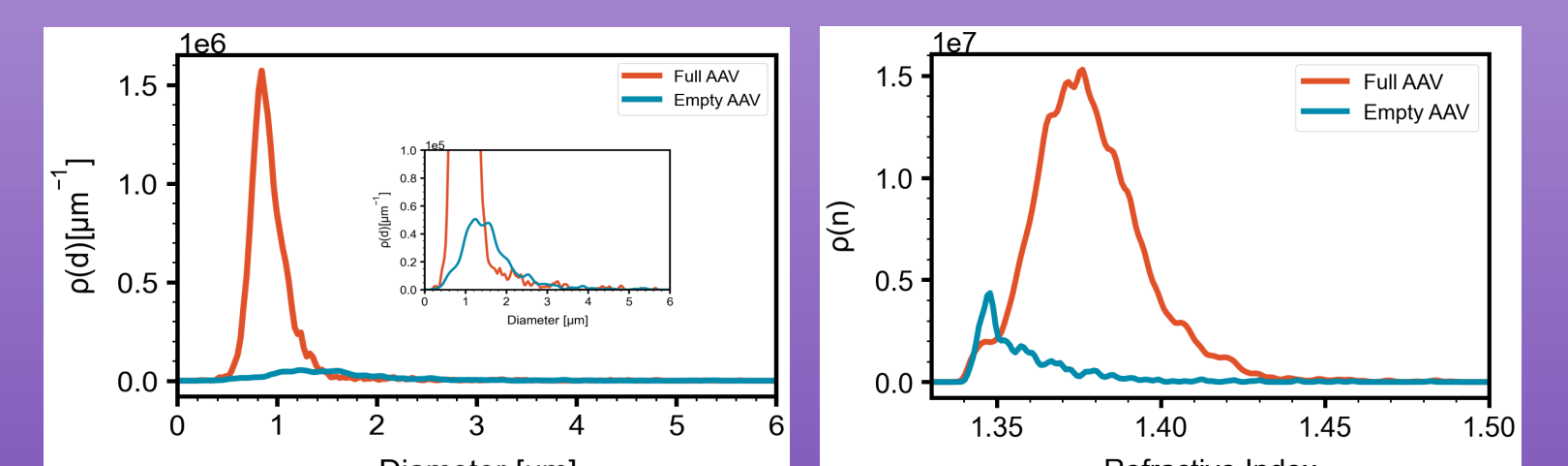
- Full capsid aggregates are smaller and higher index

- Empty capsid aggregates are larger and lower index

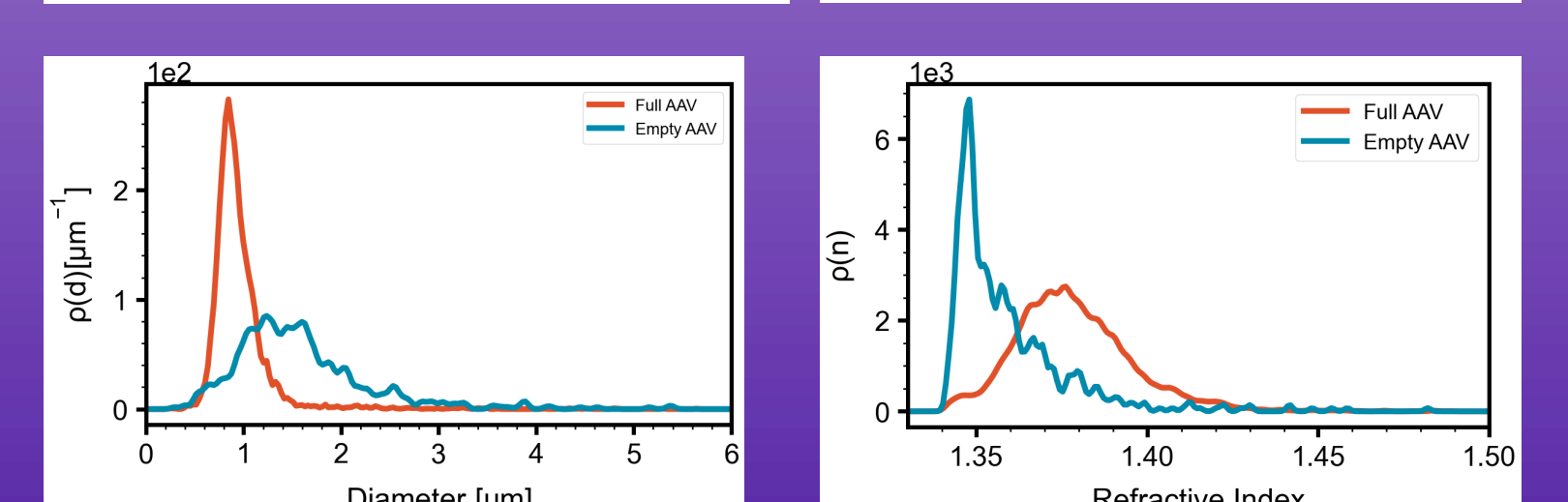


- Two different Full AAV standards show the same THC signature (orange box)

Comparison of Full vs. Empty Capsids (unnormalized)



Comparison of Full vs. Empty Capsids (normalized)



Collaboration with KBI BIOPHARMA

For more information contact us at: info@spheryx.solutions or visit our website at: www.spheryx.solutions