Total Holographic Characterization: PS80 Degradation and Gene Therapy Filling Efficiency

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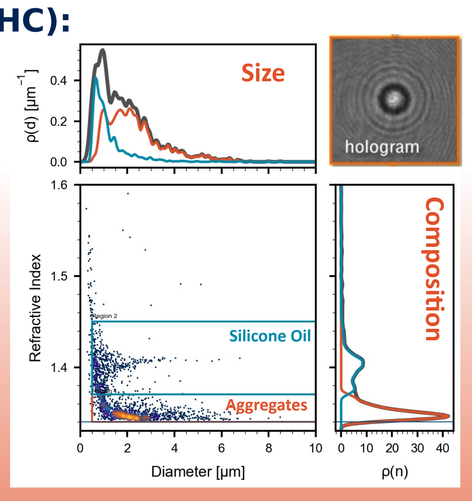
Total Holographic Characterization (THC):
A New Dimension of Information

- 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 the particle
- xCell disposable sample cell: no cross contamination & no cleaning

xSight

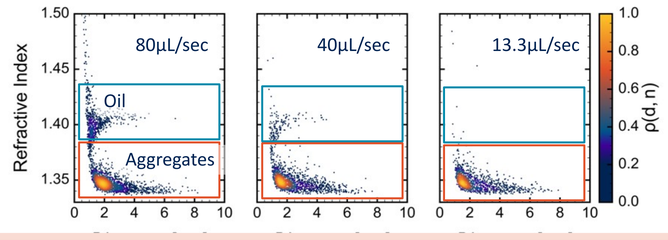
- Size: 500nm – 10µm
- Concentration: 10^4 – 10^7 particles/mL
- Sample size: 30 µL
- Viscosity: 1-25 cP
- Measurements times: 10-15 min (typical)



-THC distinguishes composition by refractive index

-Simultaneous measurement of multiple components of heterogeneous mixtures

Quantitating Each Contaminant Type Independentlv



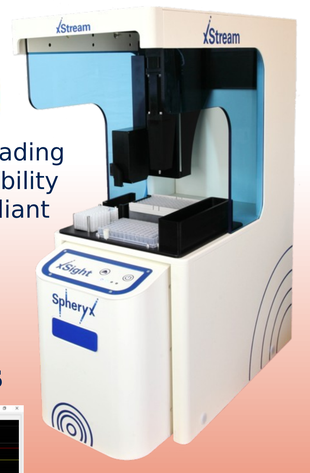
- IgG sample ejected from a syringe at 3 speeds

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

xStream

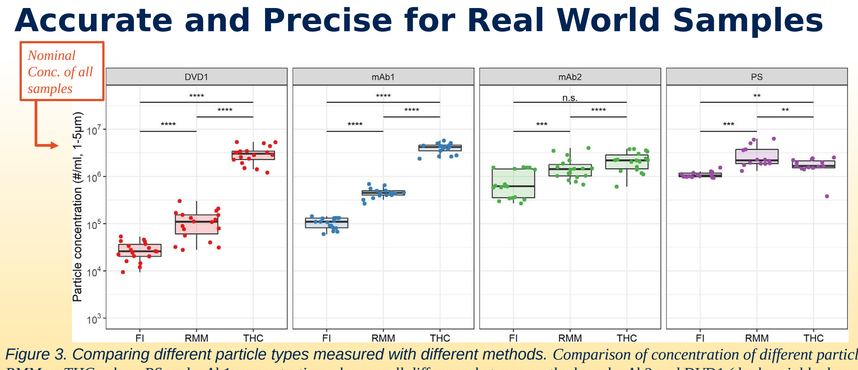
- Automated sample loading
- 96 well plate compatibility
- CFR 21 Part 11 Compliant Software Suite



Manufacturing Applications

User interface reports concentrations of multiple contaminants simultaneously for GMP environments

Accurate and Precise for Real World Samples

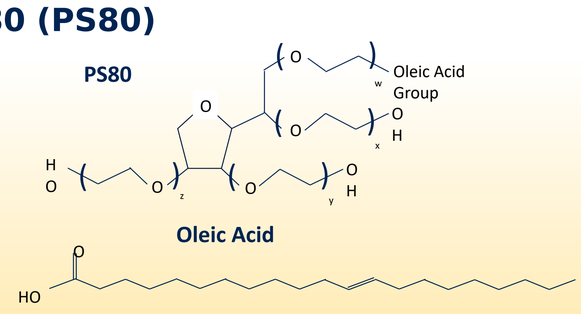


FROM: J. of Pharm. Sci., 112, 2023, p 985-990. The Strengths of Total Holographic Video Microscopy in Detecting Sub-Visible Protein Particles in Biopharmaceuticals: A Comparison to Flow Imaging and Resonant Mass Measurement, Rahn et al., AbbVie Deutschland GmbH & Co.

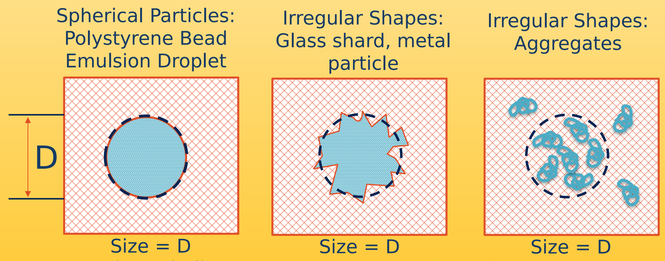
*Figure 3. Comparing different particle types measured with different methods. Comparison of concentration of different particles measured using FI, RMM or THC, where PS and mAb1 concentrations show small difference between methods and mAb2 and DVD1 (dual variable domain) particle concentration seem 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.*

Oleic Acid: Degradation Product of Polysorbate 80 (PS80)

- PS80: most frequently used surfactant in biologics
- PS80 produces Oleic Acid as it degrades
- PS80 aging limits shelf life of biologics
- Refractive index (n) measures composition.
- Oleic acid emulsion has a unique index of refraction (n = 1.46)
- Other forms of oleic acid create aggregates that appear near the index of refraction of the medium

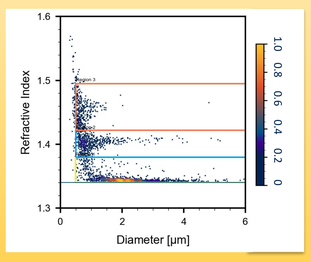


Effective Sphere Model



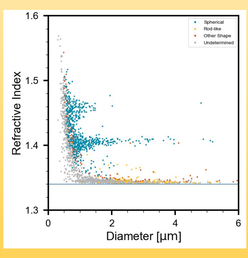
xCell disposable sample chip ensures
-easy loading
-no cleaning
-no cross contamination

Particle Morphology: Another Dimension of Information

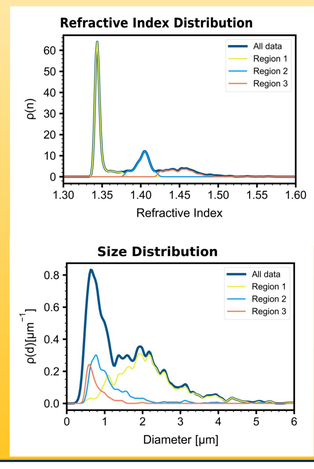


Color Reflects Particle Density

Yellow is the highest density of particle
Navy blue is the lowest density of particles



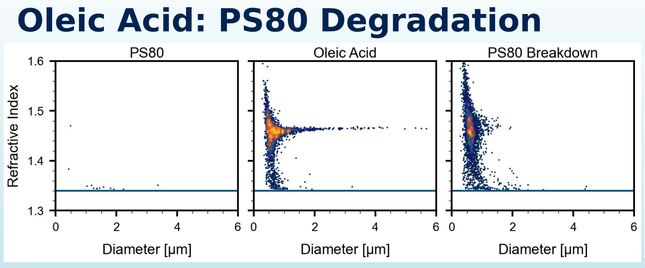
Color Reflects Particle Morphology

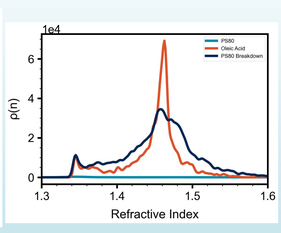


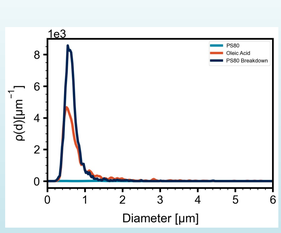
- Distinguish protein aggregates from emulsion droplets by index and shape
- Distinguish different emulsions by index
- Determine size distributions of each contaminant in a single measurement

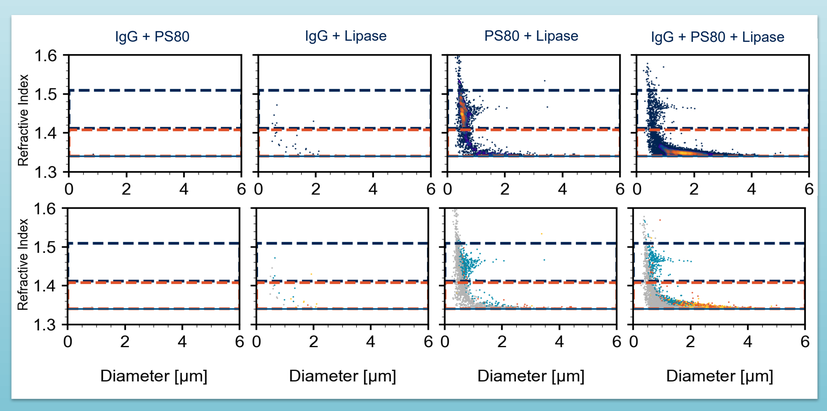
THC Measurements of Polysorbate 80 Degradation

Oleic Acid: PS80 Degradation









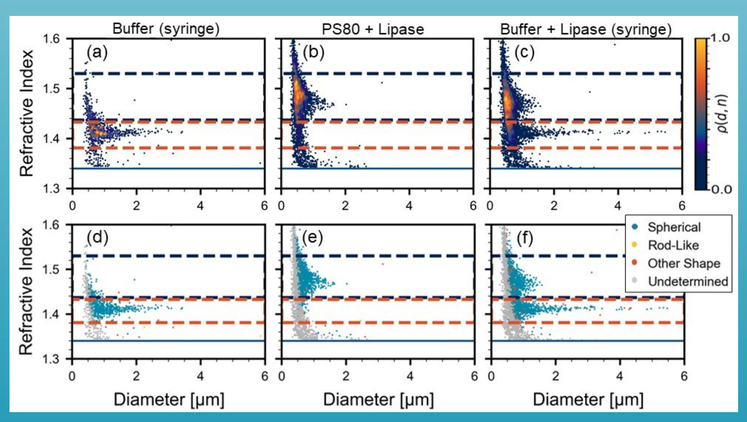
No particles in model biologic: IgG + PS80 in buffer

Lipase does not degrade IgG

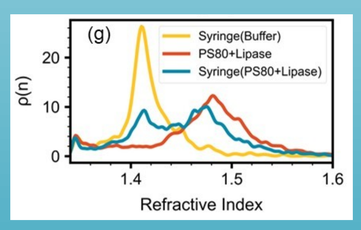
Lipase degrades PS80 producing oleic acid

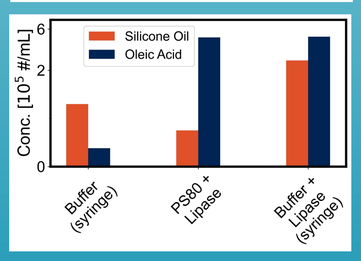
Lipase in model biologic degrades PS80 producing oleic acid and protein aggregates

Identifying Silicone Oil vs. Other Emulsions

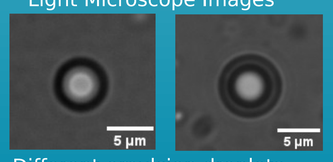


THC distinguishes the 2 emulsions, silicone oil and oleic acid by their refractive indexes, even when they are the same shape and size





Light Microscope Images

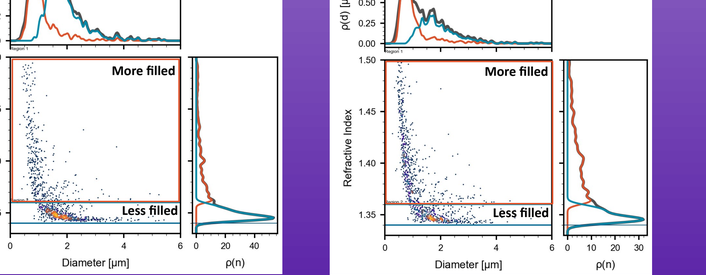


Different emulsion droplets are not distinguishable

Gene Therapy Application: Capsid Filling Efficiency Determination

Measurement Principle

Measurement: Quantitative evaluation of capsid content
Current Methods: UV Absorbance; SEC-MALS; AUC; cryo-EM; TEM; CDMA



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

- Filled capsids (orange) have a higher index than other material (blue)
- In a single 10 min measurement the filling efficiency can be measured

- Filled capsids (orange) have a higher index than other material (blue)
- Sensitizer increases the ratio of filled to empty capsids

Collaboration with OHRI, Ottawa, Canada: Jean-Simon Diallo, Elena Godbout, Rozanne Arulanandam

Standards of known filling fractions can be used to create quantitative measurements of filling efficiency in a measurements that takes 10 min without any special sample preparation

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