HORIBA Scientific

Particle Characterization

Raman Spectroscopy

Size, Chemistry, and More: Raman and Laser Diffraction for Pharma Particle Analysis

Eunah Lee, Ph.D. and Jeff Bodycomb, Ph.D.

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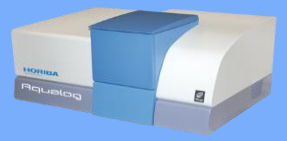
HORIBA Products for Biopharmaceuticals

SPRi

Binding Analysis

Fluorescence



Chemical Identification and Analysis

Raman

Molecular ID, Protein Secondary Structure

XRF

Elemental Analysis

Particle Analysis

Particle size distribution

Pharmaceutical Process

Drug Discovery | Pre-Clinical | Clinical | Submission | Launch

Submission – Technology Transfer

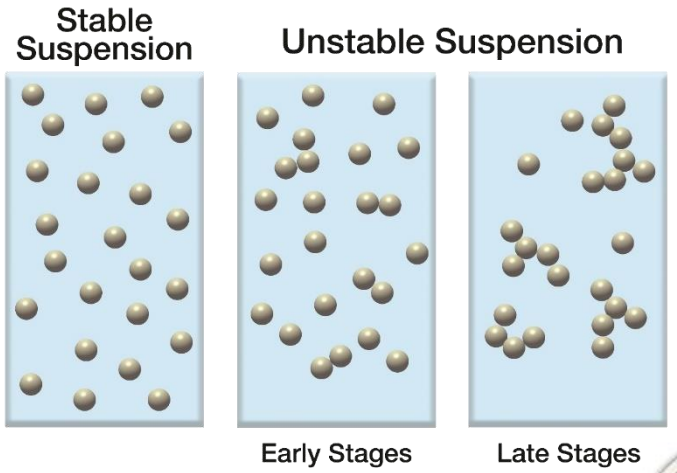
Drug Discovery | Pre-Clinical | Clinical | **Submission** | Launch

- Formulation per drug delivery system. For example
  - Peroral and topical: Verify API form, concentration and distribution.
    Particle size distribution depending on solubility and for dose uniformity
  - Inhalation and parenteral: Particle counts, size, shape and chemistry
- Formulation for scale up and manufacturing

Submission – Technology Transfer

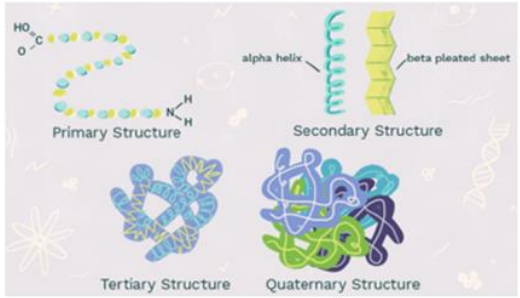
Drug Discovery | Pre-Clinical | Clinical | **Submission** | Launch

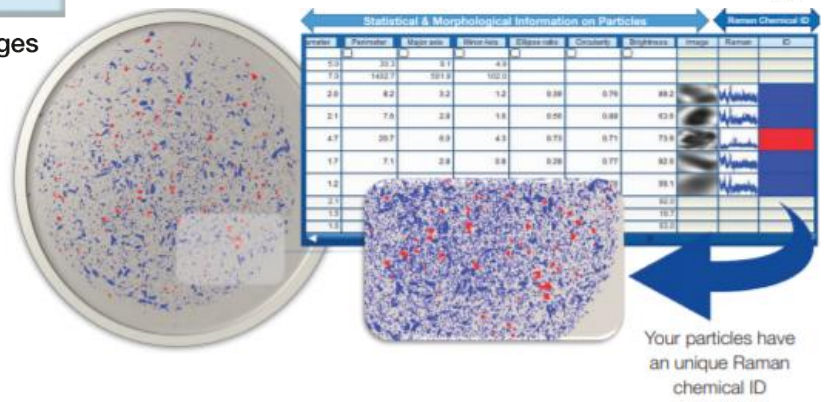
Particle analysis



Scale Up

- High concentration, aggregation
- Temperature
- pH
- Chemical reactions (solvent, reducing agent…)

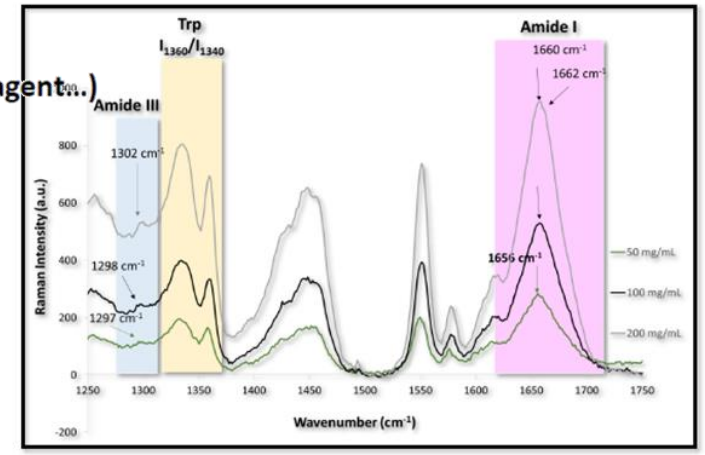




Nasal Spay: morphological classification and chemical identification of particles using ParticleFinder

Formulation development – OINDP

Raman microscopy



Sample	Tyr (I_{850}/I_{830}) Ratio	Amide III	Trp (I_{1360}/I_{1340}) Ratio	Amide I
50 mg/mL	1.04	1296 cm⁻¹	0.81	1656 cm⁻¹
100 mg/mL	1.28	1298 cm⁻¹	0.84	1660 cm⁻¹
200 mg/mL	1.44	1302 cm⁻¹	0.86	1660 cm⁻¹ 1662 cm⁻¹
Analysis	Increase in ratio signifies Tyr is more accessible to aqueous environment due to structure reorganization[1,3,4]	Shift in bands suggests a transition from α-helical to β-sheets[1,2,4]	An increase in ratio implies that Trp is well buried and closer to neighboring proteins[2]	Shift in bands and appearance of a shoulder peak at 1662 cm⁻¹ suggests a transition from α-helical to β-sheets.[1,2,4]

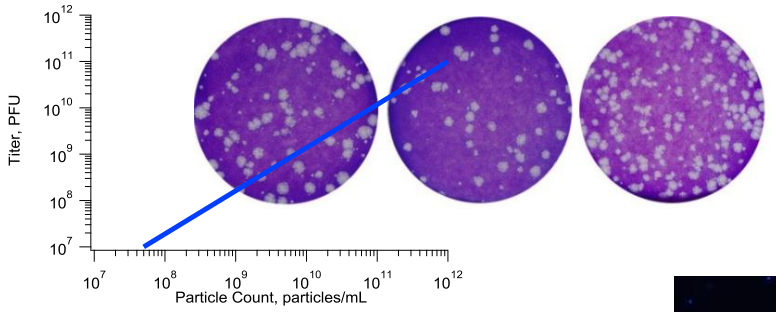
Pharmaceutical Process

- QC/QA tests including
  - Raw material screening
  - Failure tests
  - Content uniformity
  - Root cause analysis
- Surveillance is critical to monitor
  - Potential drug interactions and long term effects
  - Counterfeit drugs and patent infringement
- Research and investigation by regulatory entities

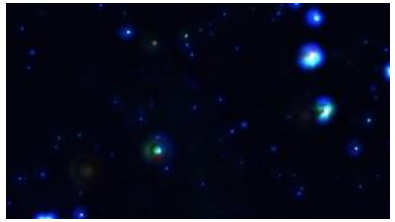
Launch – Continuous Verification

Drug Discovery | Pre-Clinical | Clinical | Submission | **Launch**

Particle analysis



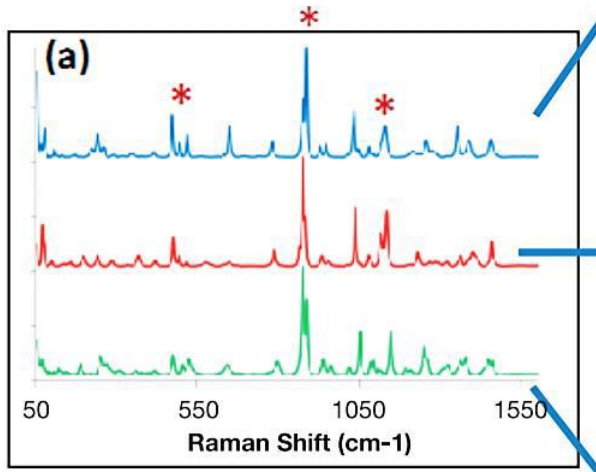
Vaccine Endpoint

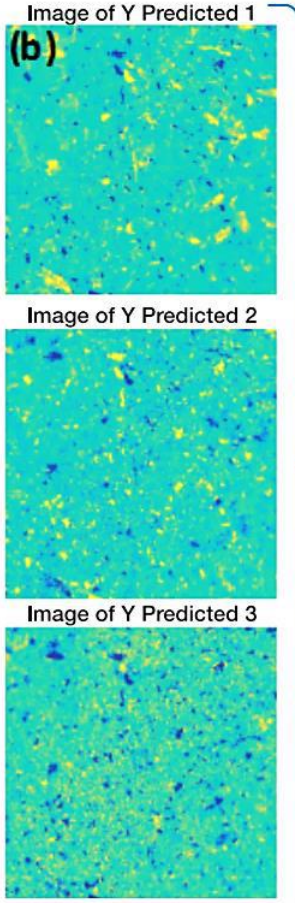


Rapid Vaccine Endpoint Determination
– 15 min vs. days

Raman microscopy

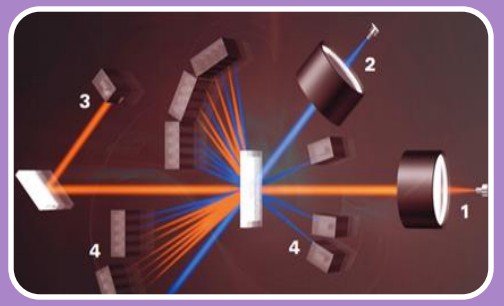
Polymorph Characterization





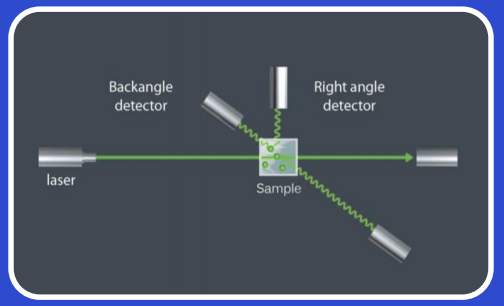
(a) Raman spectra of α, β, δ forms of mannitol;
(b) Images showing the spatial distribution of mannitol forms in powder blends

Particle Characterization Techniques



Laser Diffraction

- Scattering technique – large particles scatter intensely at narrow angles and small particles scatter weakly at wide angles
- Two color systems enable accurate measurement of small particles



Dynamic Light Scattering

- Scattering technique – Brownian motion induces changes in scattered light intensity
- Intensity is measured as a function of delay time and scattering angle
- Static light scattering

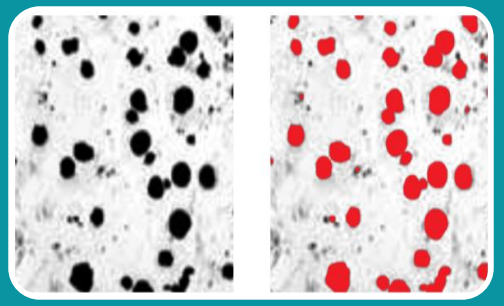


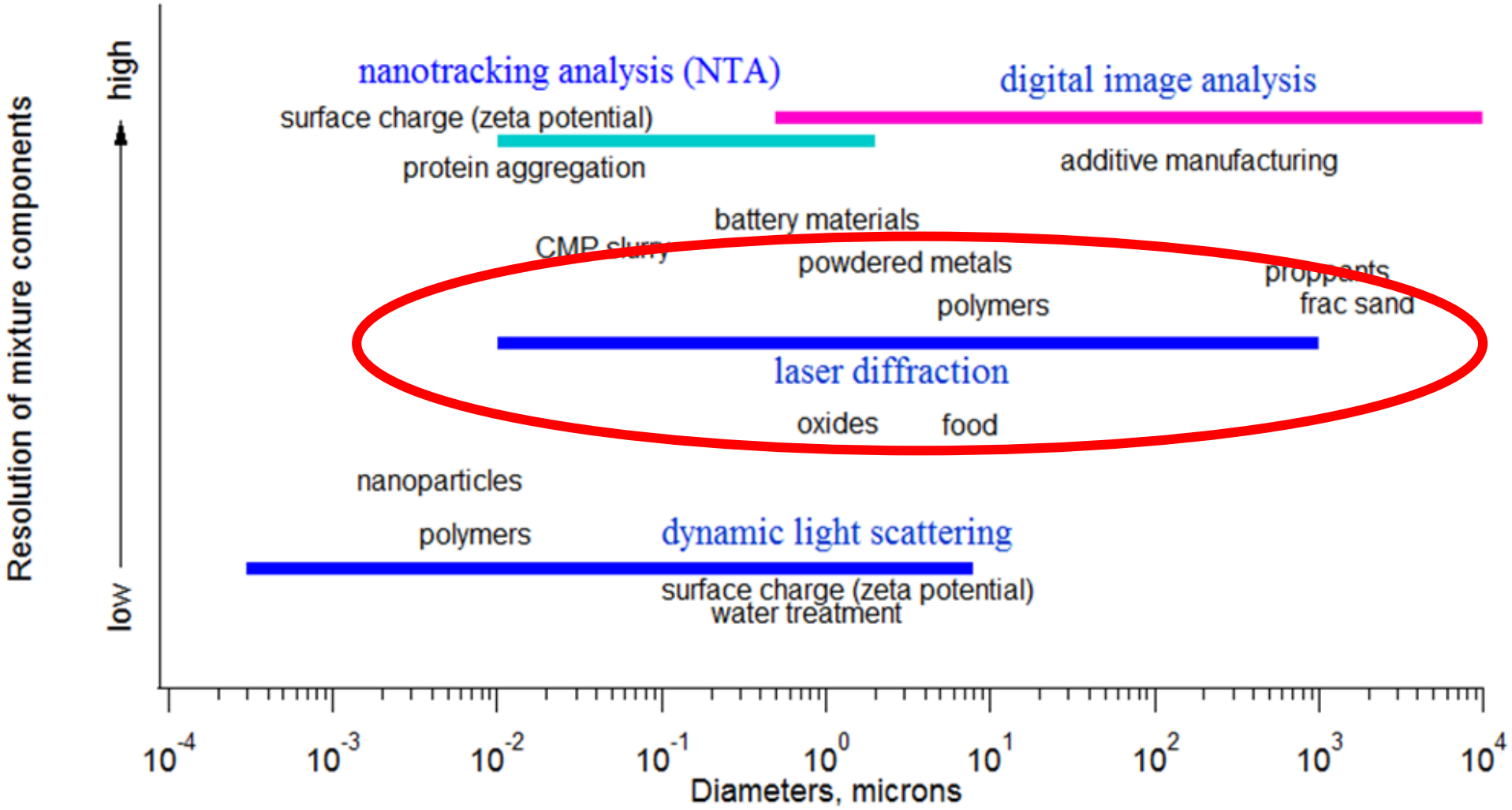
Image Analysis

- Static image analysis uses a microscope and digital camera to collect images of deposited particles
- Dynamic image analysis drops particles between light source and camera – projected shadows are recorded

Laser Diffraction

- Laser diffraction overview
- LA960 overview
- A simple example – raw material analysis – one API, one excipient

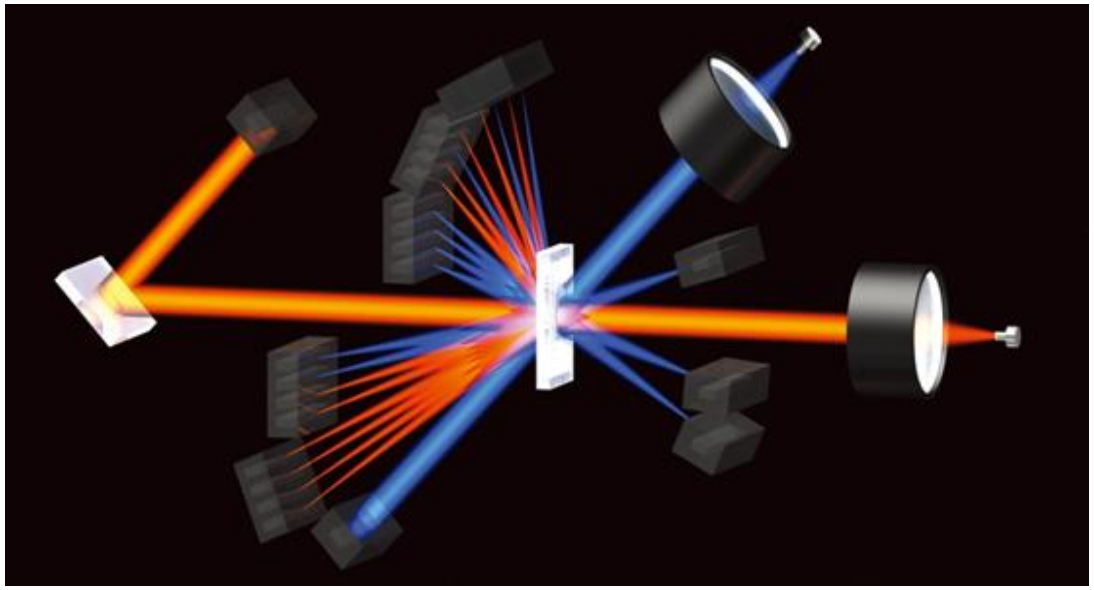
Perspective

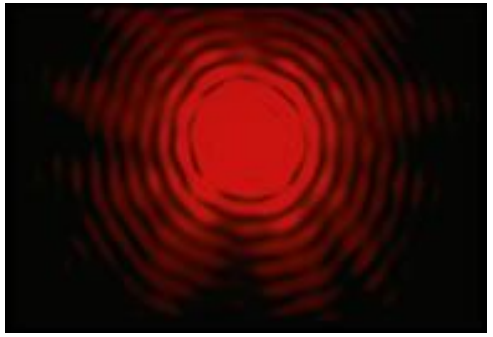


Laser Diffraction

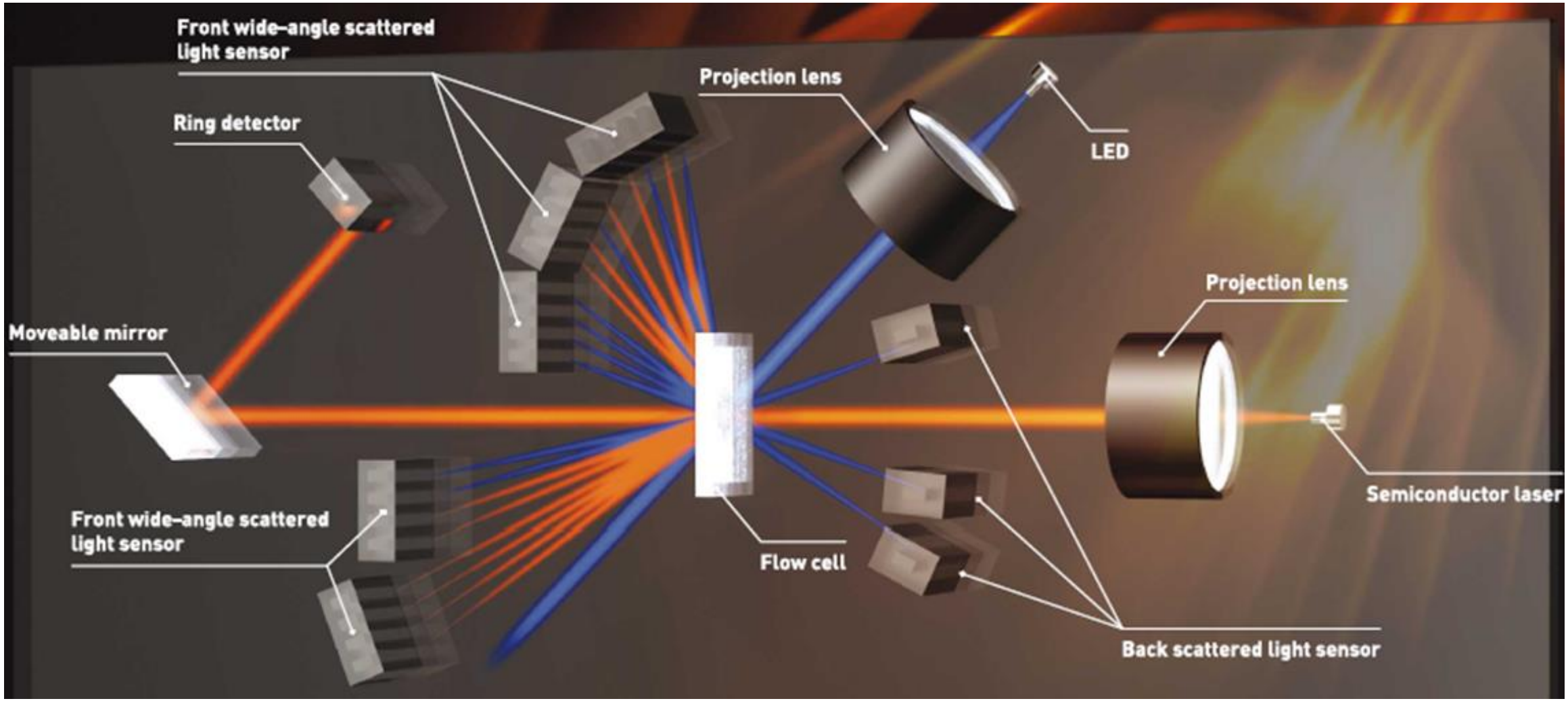
Convert scattered light as a function of angle to a particle size distribution

- **Quick, repeatable**
- **Powders, suspension**
- **Most common technique**



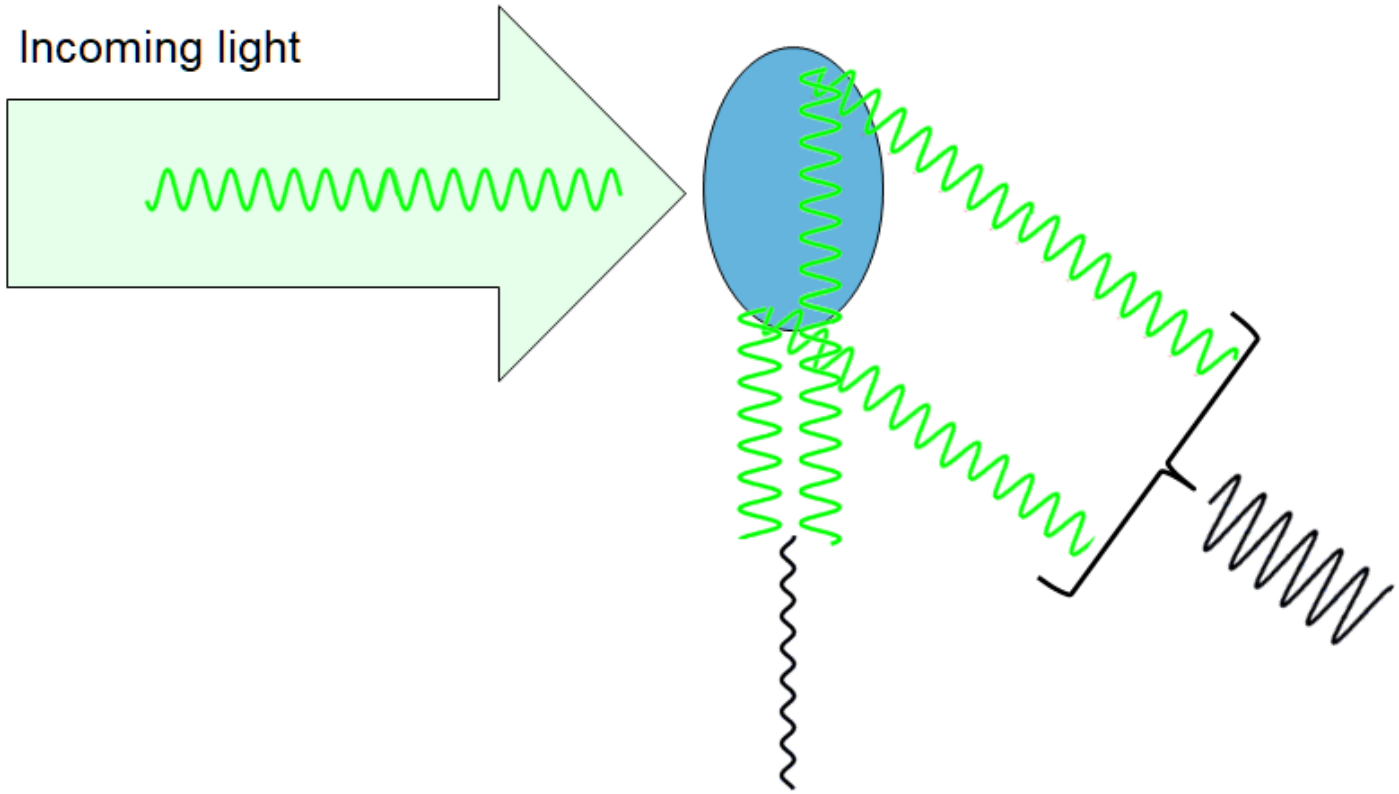


LA-960 Optics



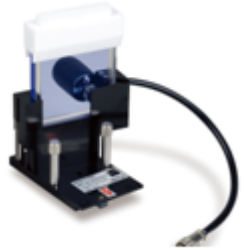
Path Length Difference

Diffraction effects arise due to scattering from various points in the particle (and, in the large particle limit only at the edges).

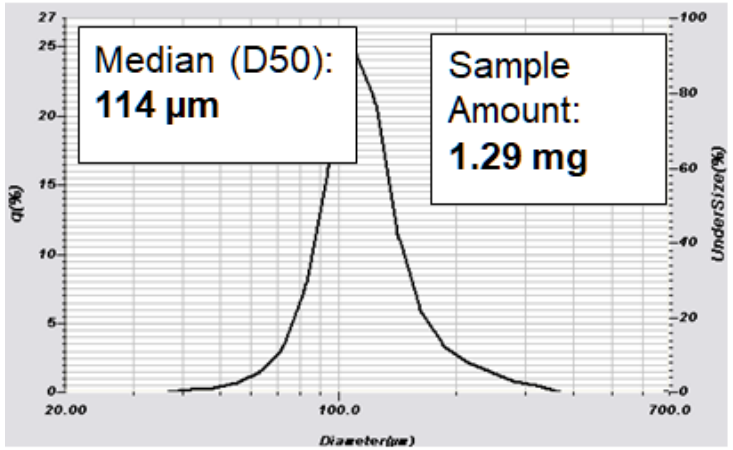


How much sample (wet)?

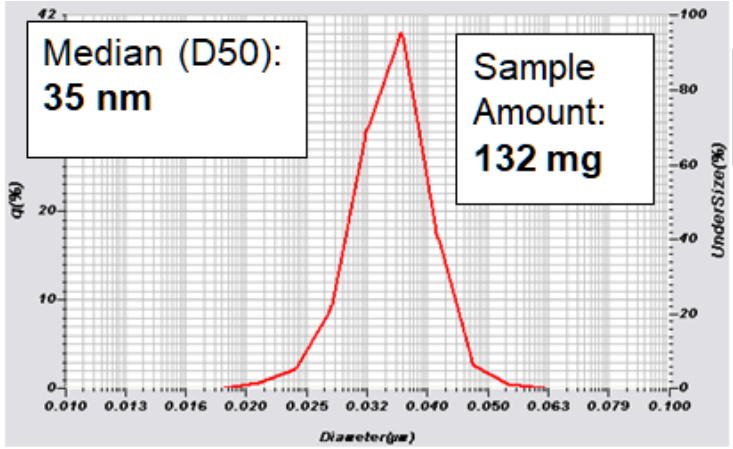
It depends on sample, but here are some examples:
Larger, broad distributions require larger sample volume
Lower volume samplers for precious materials or solvents.



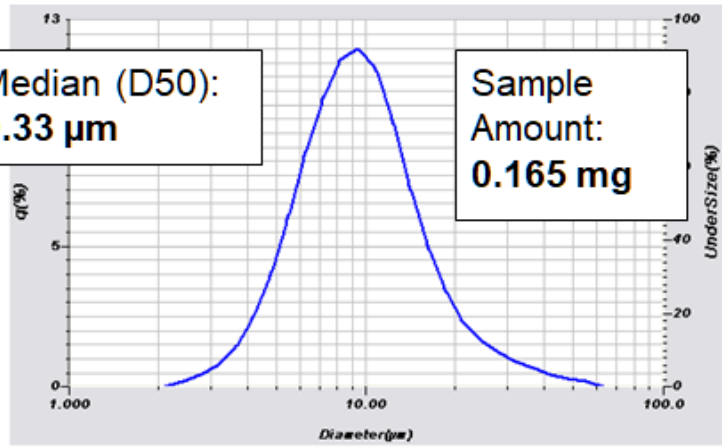
Sample Handlers	Volume (mL)
Aqua/SolvoFlow	180 - 330
MiniFlow	35 - 50
Fraction Cell	15
Small Volume Fraction Cell	10



Bio polymer



Colloidal silica



Magnesium stearate

Instrument to instrument variation

4 instruments

(real sample)

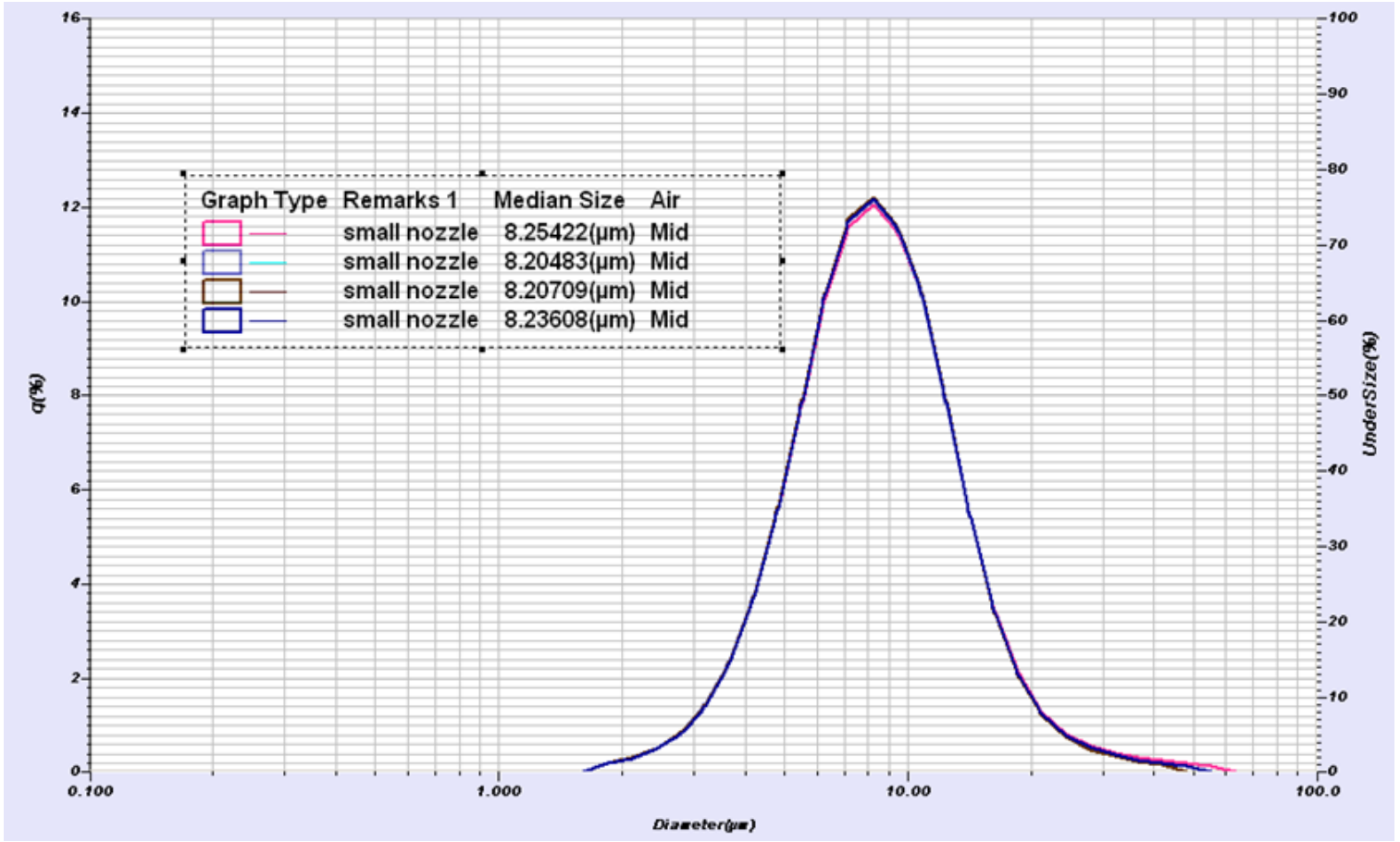
Formula-tion 1	Dmean	D5	D10	D50	D90	D95
Average (nm)	155	112	119	152	193	208
Std. Dev. (nm)	0.8	0.8	0.7	1.0	1.1	0.7
CV (%)	0.5	0.7	0.6	0.6	0.6	0.3

Formula-tion 1	Dmean	D5	D10	D50	D90	D95
Average (nm)	193	136	147	187	247	264
Std. Dev (nm)	1.5	0.5	0.4	0.6	0.4	1.1
CV (%)	0.8	0.4	0.3	0.3	0.2	0.4

Excipient: Mg Stearate

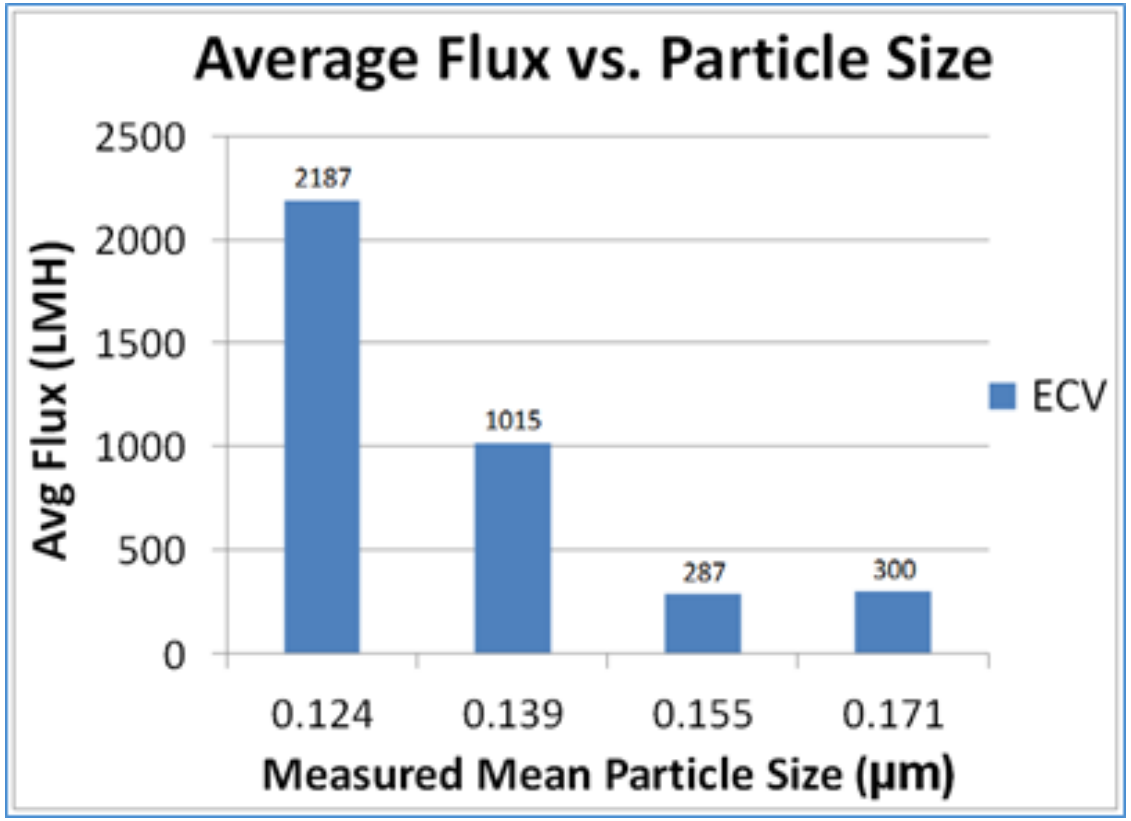
Measured as a dry powder, note tight repeatability.

Run	Median Size, microns
1	8.25
2	8.20
3	8.21
4	8.24
Mean	8.22
Std. Dev.	0.024
CoV	0.3%



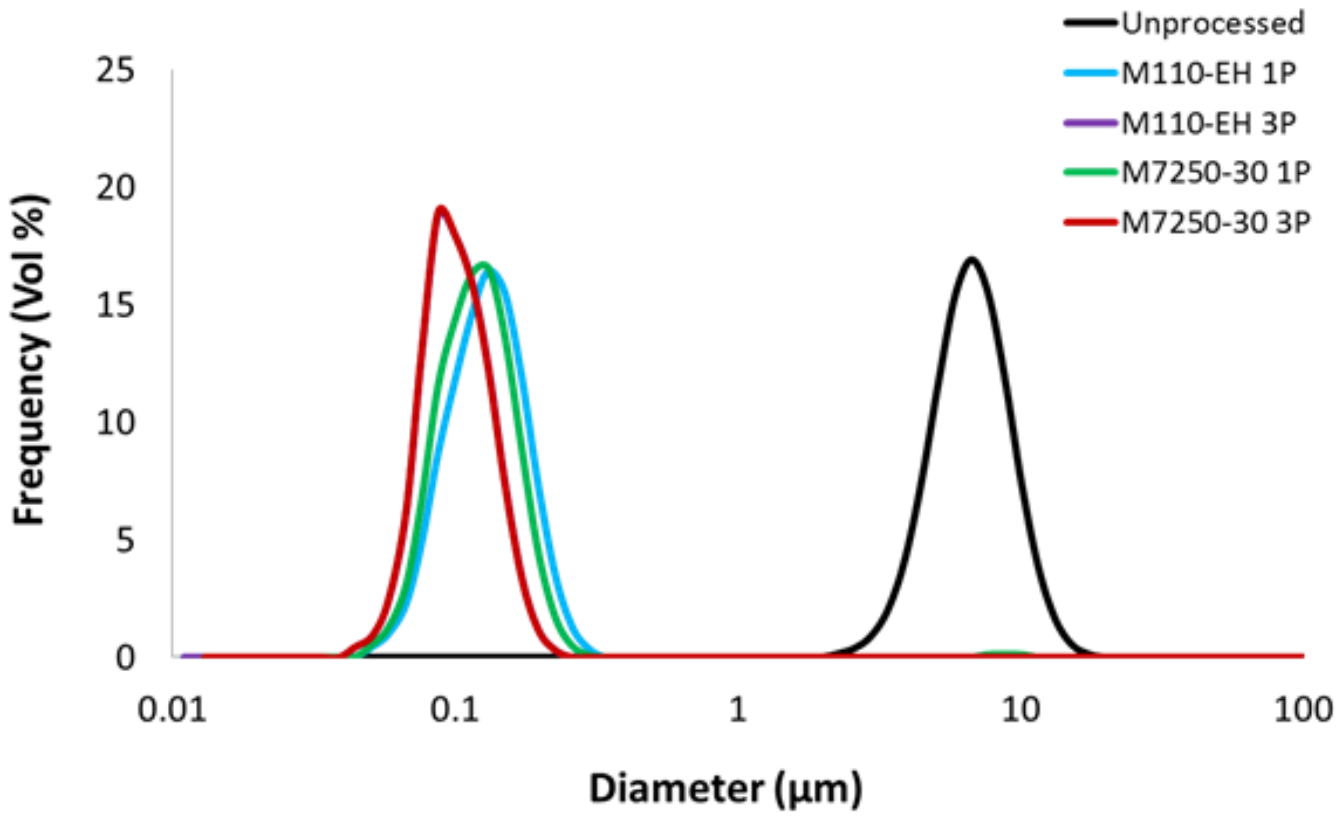
Nanoemulsion Vaccine Adjuvant

Smaller particle lead to better flow through a filter (e.g., filtration sterilization). Monitoring size helps downstream processing steps.



Nanoemulsion Vaccine Adjuvant

Squalane processed with Microfluidizer

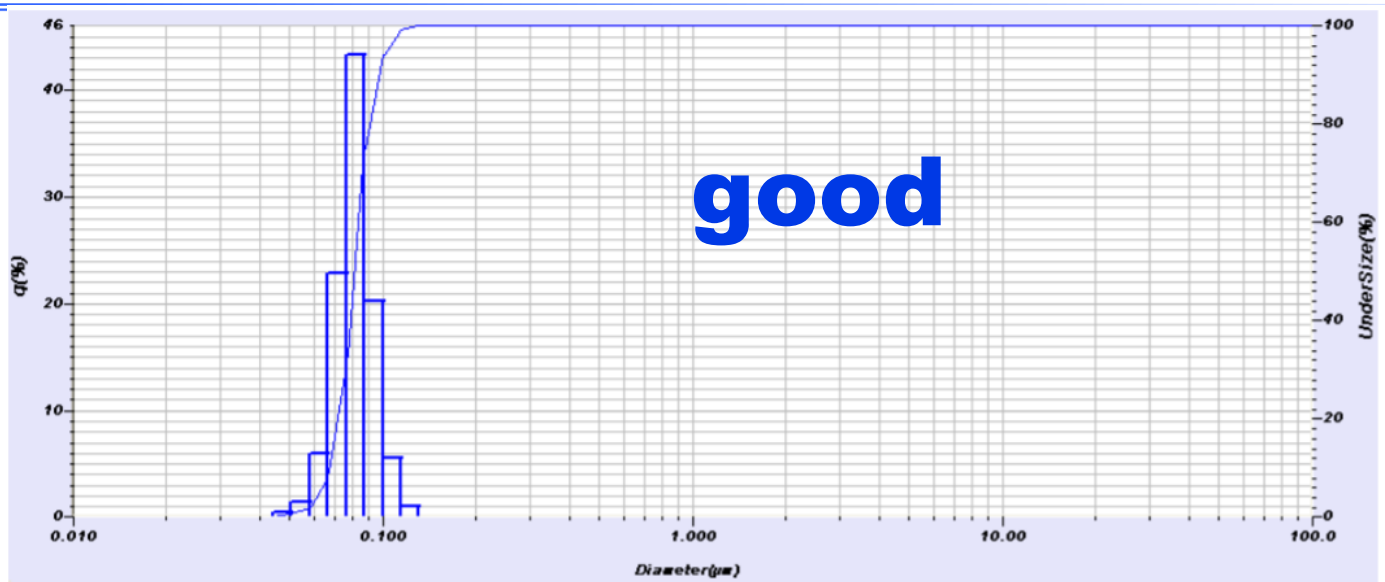


Effective measurements of particles over 1 micron to give consistent results from start to finish.

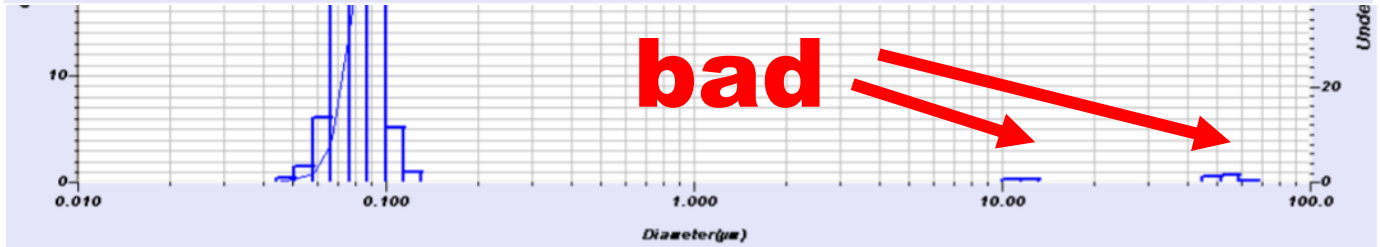
PLA for drug delivery

Polylactide nanoparticles, good vs bad batch.

D10, d50, d90 the same. Used volume mean as criteria.



D(v,0.1)	:	0.06752(μm)	Mean Size	:	0.08172(μm)
D(v,0.5)	:	0.08108(μm)	Median Size	:	0.08108(μm)
D(v,0.9)	:	0.09753(μm)	Mode Size	:	0.0812(μm)

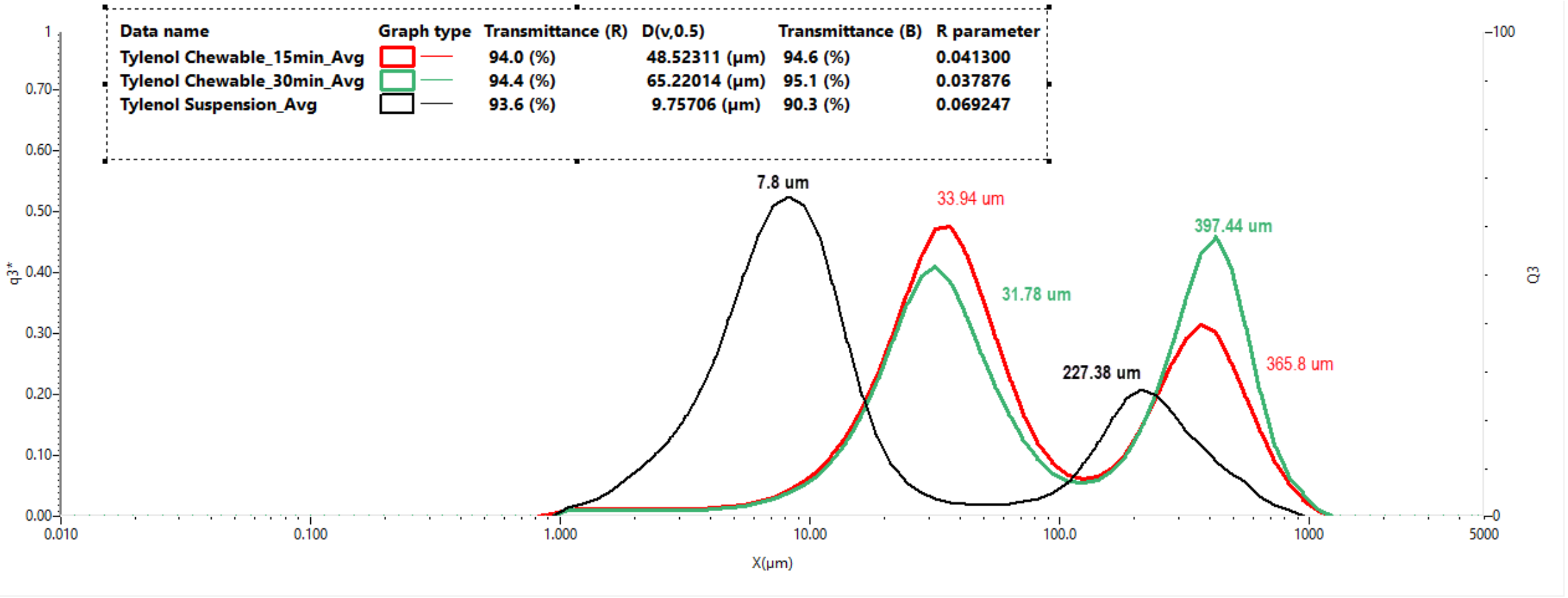


D(v,0.1)	:	0.06746(μm)	Mean Size	:	0.73105(μm)
D(v,0.5)	:	0.08112(μm)	Median Size	:	0.08112(μm)
D(v,0.9)	:	0.09825(μm)	Mode Size	:	0.0811(μm)

An example – Children's tylenol

- Chewable in Bubble gum flavor:
  - Active Ingredient: Acetaminophen 160 mg in each tablet
  - Inactive ingredients: anhydrous citric acid, cellulose acetate, crospovidone, D&C red no. 7 calcium lake, dextrose, flavor, magnesium stearate, povidone, sucralose
- Suspension in Grape flavor:
  - Active ingredient: Acetaminophen 160 mg in each 5 mL
  - Inactive ingredients: anhydrous citric acid, D&C red no. 33, FD&C blue no. 1, flavors, glycerin, high fructose corn syrup, microcrystalline cellulose and carboxymethylcellulose sodium, purified water, sodium benzoate, sorbitol solution, sucralose, xanthan gum

Laser diffraction analysis results



Red and **Green**: Chewable in Bubble gum flavor

Black: Suspension in Grape flavor

Sample Preparation for Image Analysis

Powders

Vacuum-induced dry powder dispersion

Particulate in suspension

Vacuum filtration with filter insert/holder

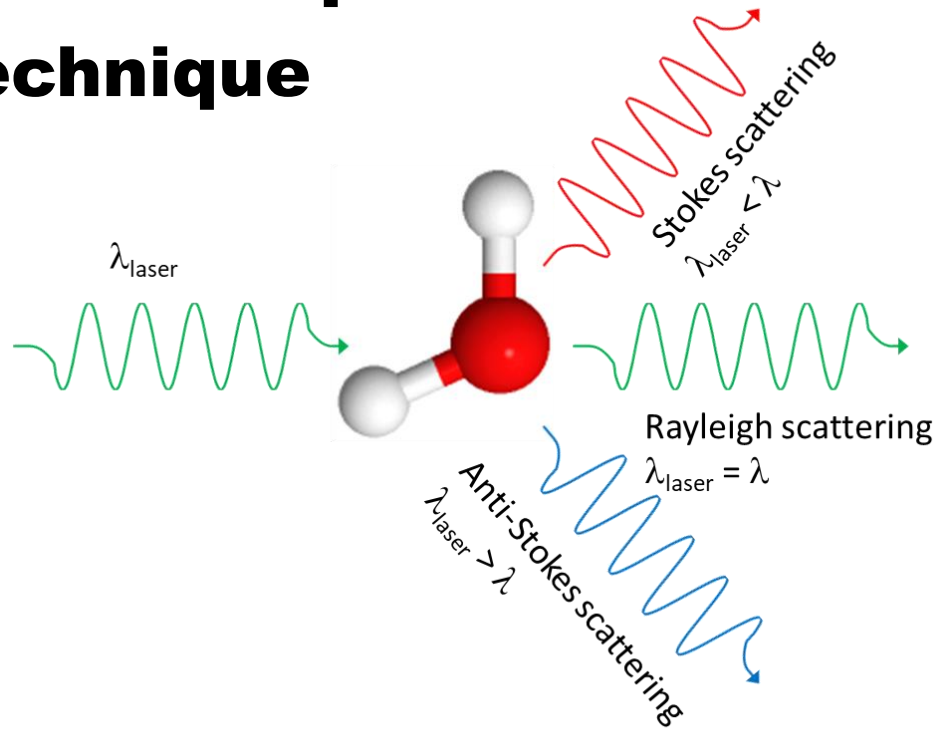
Aerosols/Sprays/Inhalers

Dispense particulate directly on substrate (glass/metal)

Adding Chemical ID to Particle Characterization

Raman spectroscopy

- **Inelastic light scattering**
- **Probe molecular vibrations within a sample**
- **Non-invasive, non-destructive technique**
- **No sample preparation**



XploRA confocal Raman microscope

- Excellent Performance
- High sensitivity
- Ultimate spatial resolution
- Multimodal optical microscopy
- Compact design

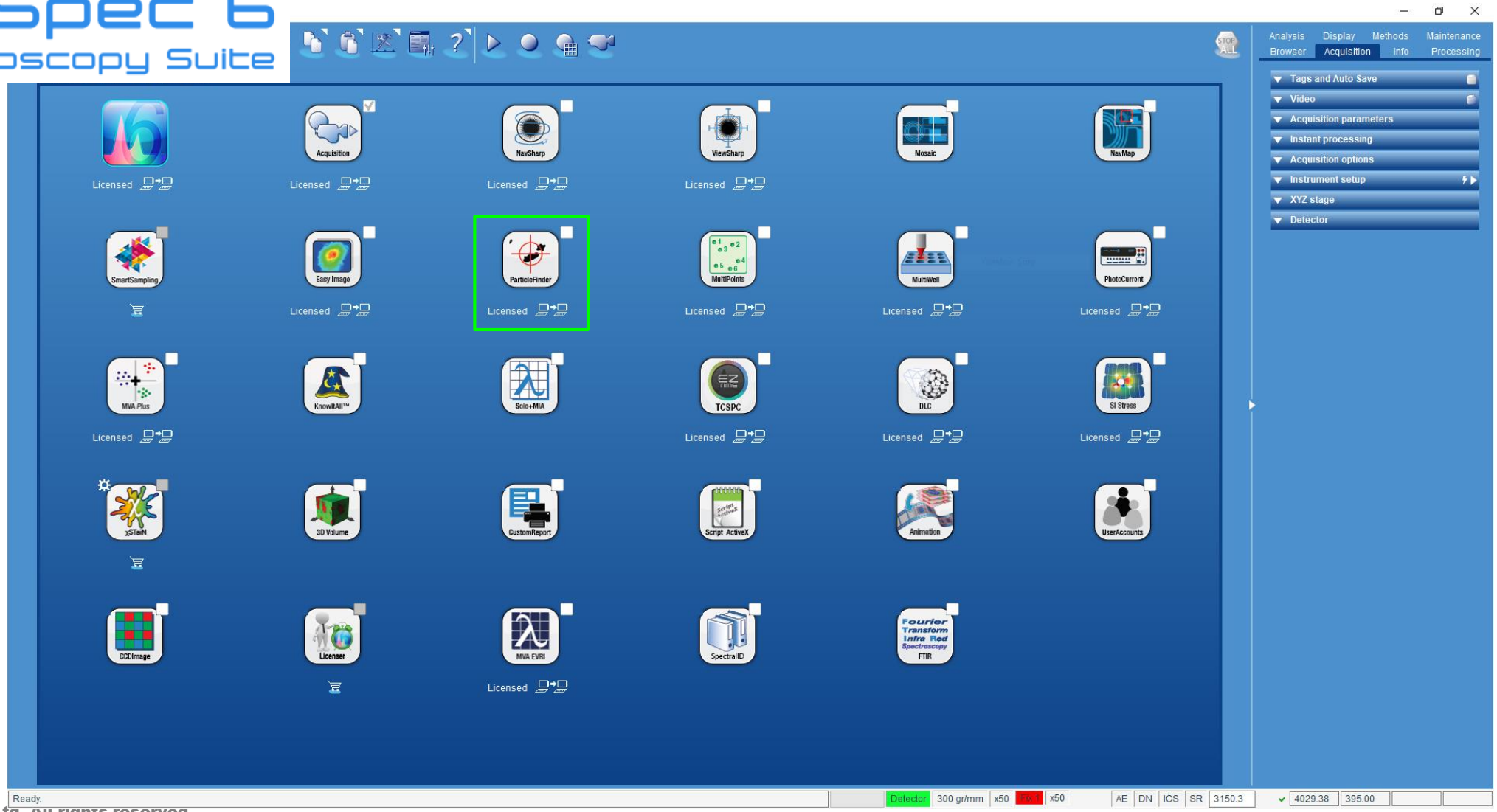
- Technical Evolution
- Multivariate analysis
- SWIFT/SWIFT XS
- Particle Finder
- User Account Control
- Multiwell module
- KnowItAll database searching

- Extreme Extension
- AFM-Raman
- Photoluminescence
- Macro accessory
- Remote sampling
- EMCCD options

- Advanced Automation
- OneClick Raman operation
- Laser switching
- Autocalibration
- Extended video montage/mosaic
- EasyNav

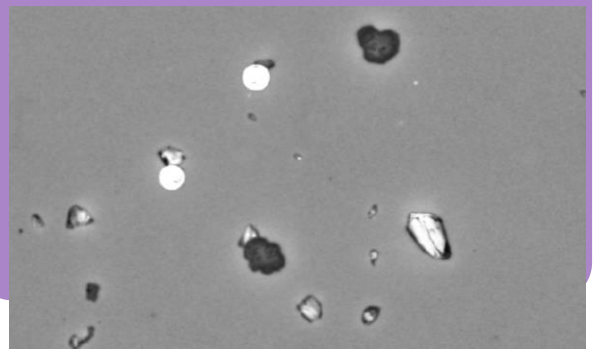
Soleil confocal Raman microscope

LabSpec 6 Spectroscopy Suite

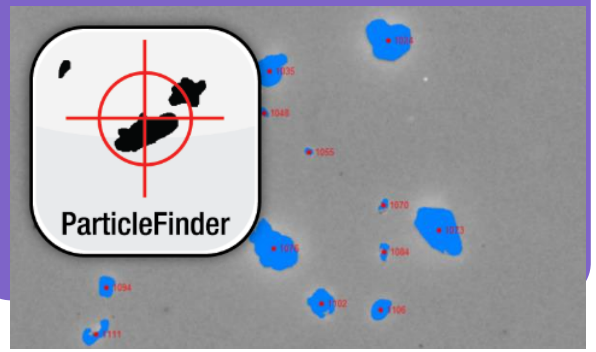


Particle Correlated Raman Spectroscopy (PCRS)

Acquire Optical Image



Auto Detect Particles

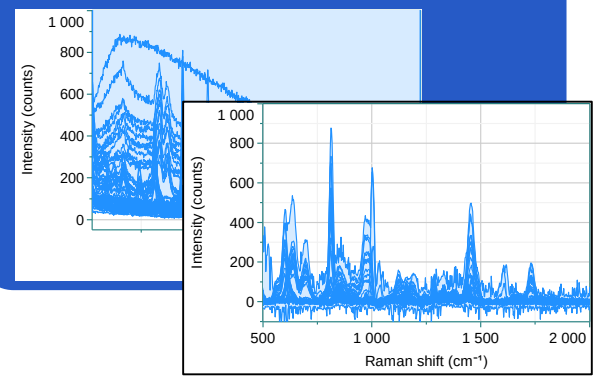


Particle morphology

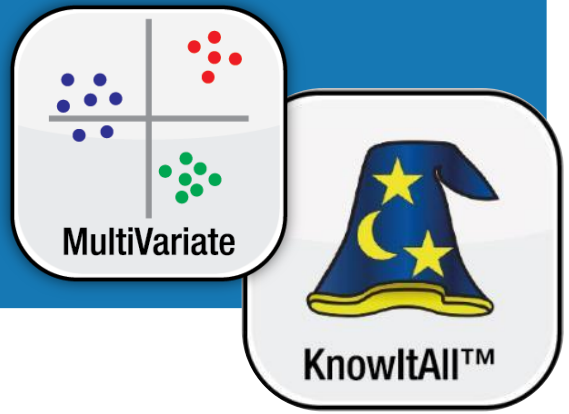
Results

Index	X pos	Y pos	Area	Diameter	Perimeter
	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	300.0	0.0
1(767)	7359.3	556.8	10741.7	116.9	460.9
2(805)	-1875.6	1434.4	27284.7	186.4	698.7
3(193)	12194.0	-9826.5	16293.5	144.0	855.8
4(428)	11847.3	-4930.7	209507.3	516.5	2317.7

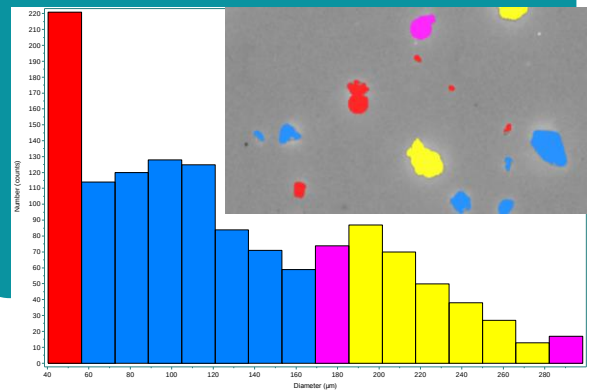
Spectral acquisition



Chemical ID

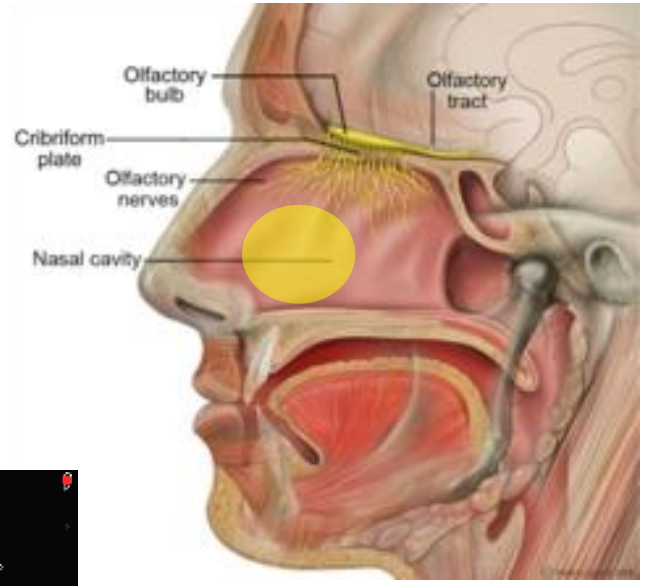


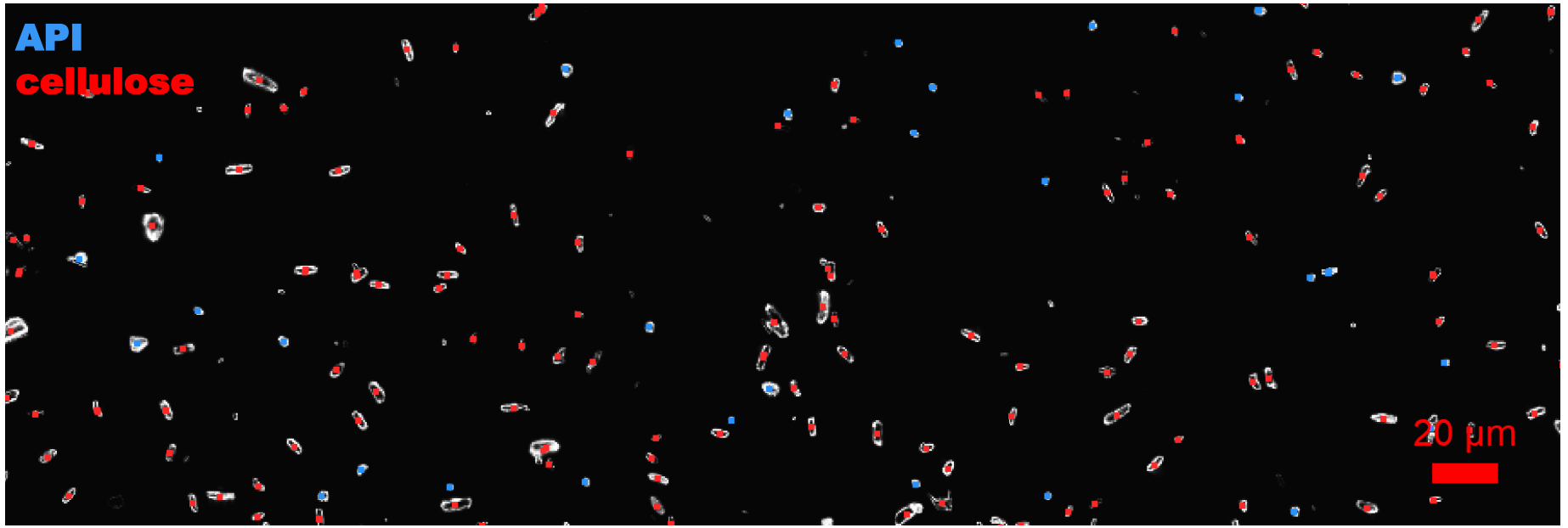
Statistical analysis



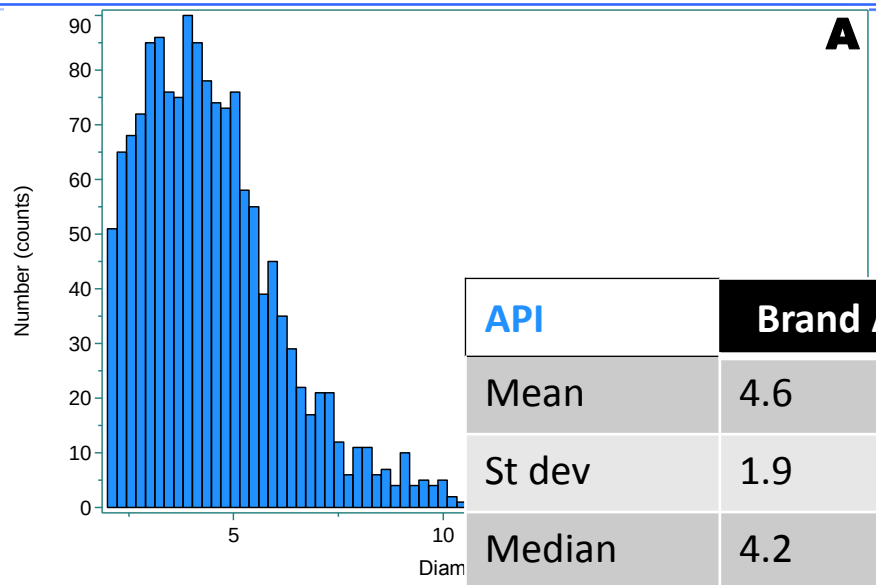
An example – Nasal Spray

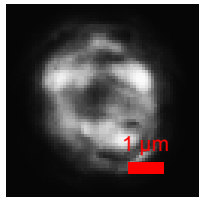
- Spray action defines aerosol droplet size and dictates deposition area
- Particle size (single particle/agglomerate) effects API uptake and bioavailability



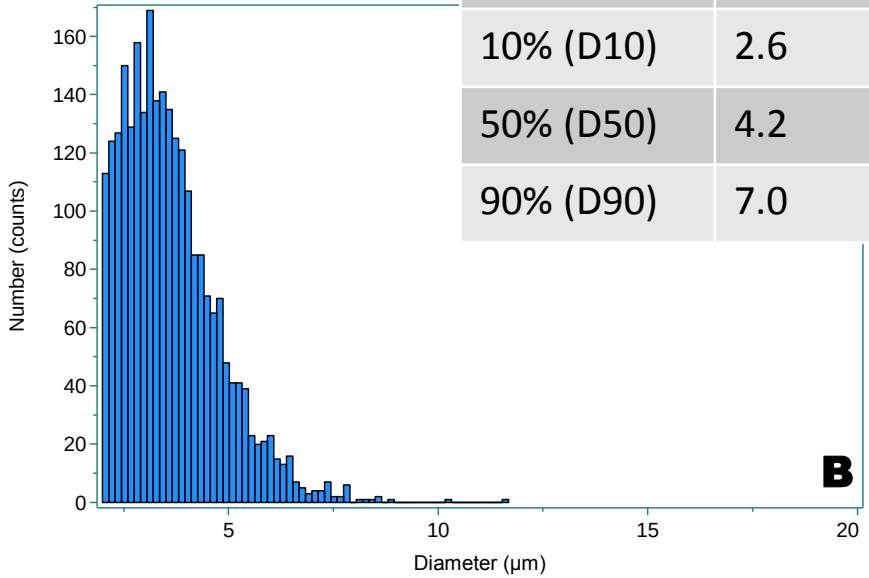


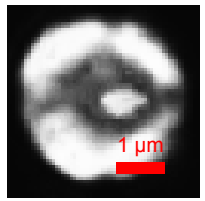
Particle Diameter

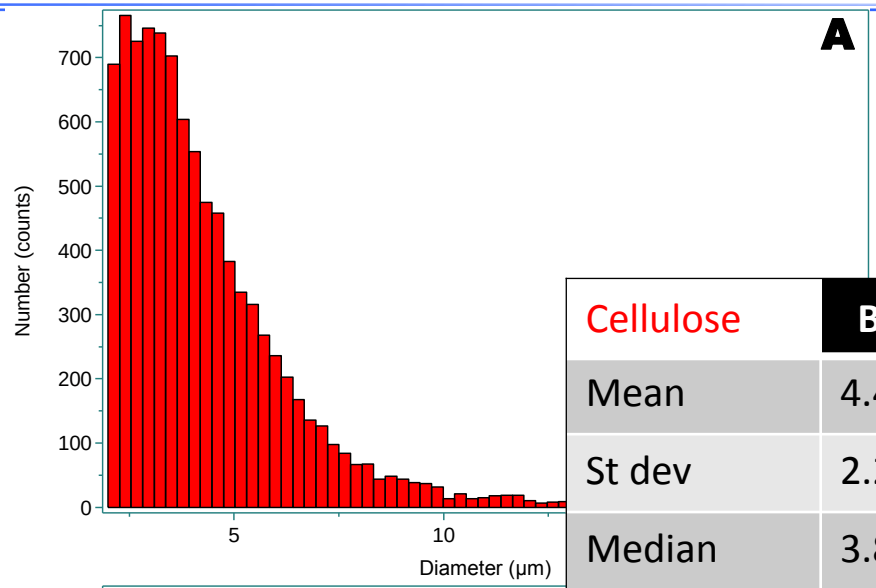


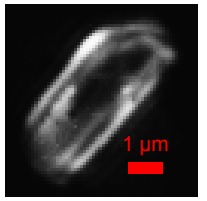


API	Brand A	Brand B
Mean	4.6	3.6
St dev	1.9	1.2
Median	4.2	3.4
10% (D10)	2.6	2.3
50% (D50)	4.2	3.4
90% (D90)	7.0	5.2

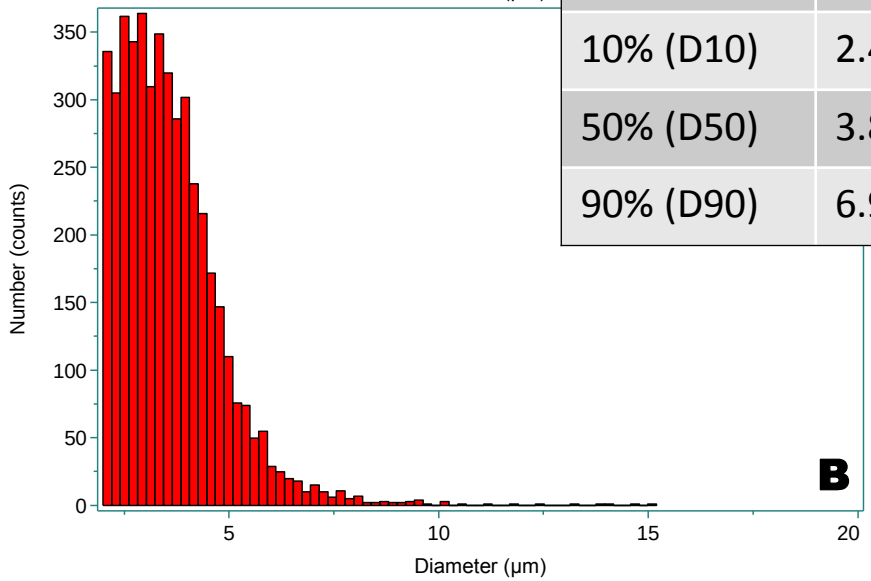


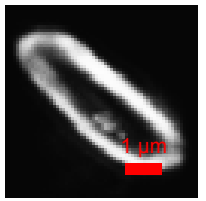






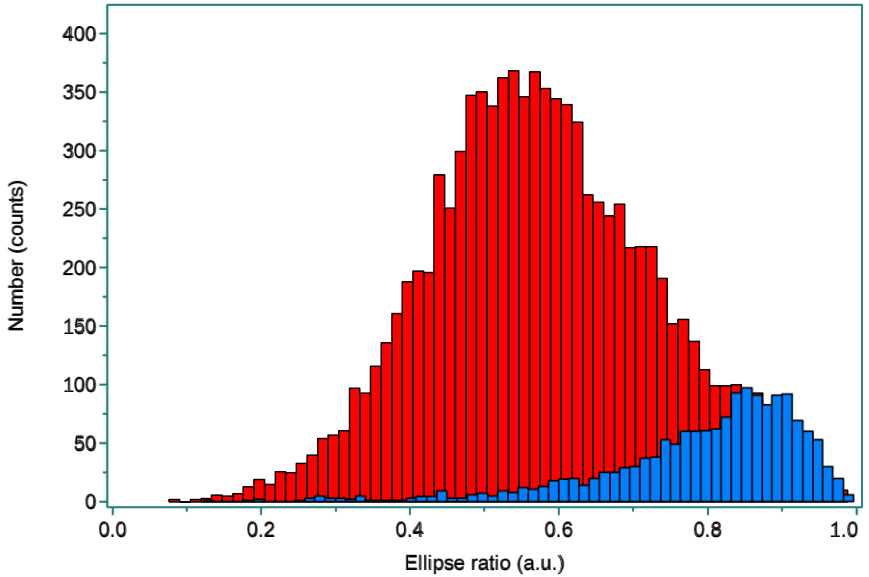
Cellulose	Brand A	Brand B
Mean	4.4	3.6
St dev	2.2	1.2
Median	3.8	3.4
10% (D10)	2.4	2.3
50% (D50)	3.8	3.4
90% (D90)	6.9	5.1

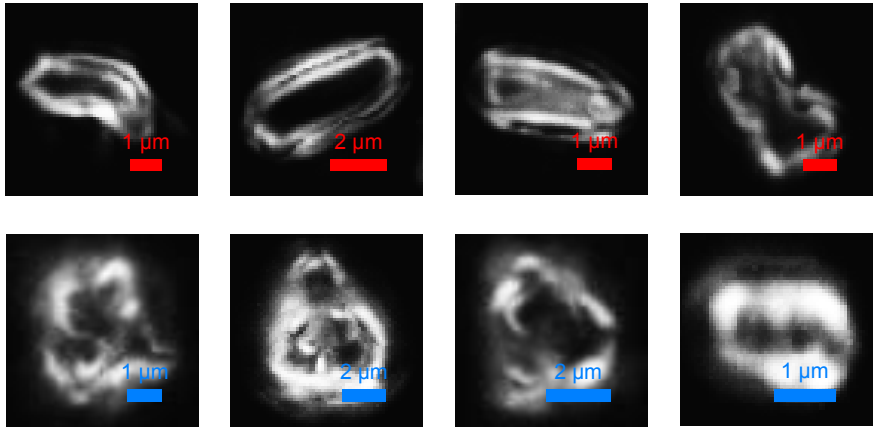




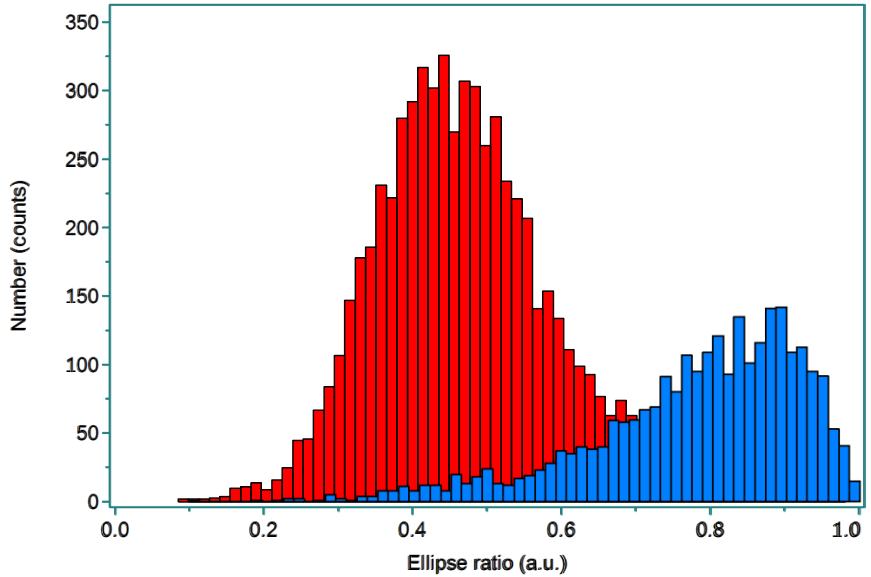
Ellipse Ratio

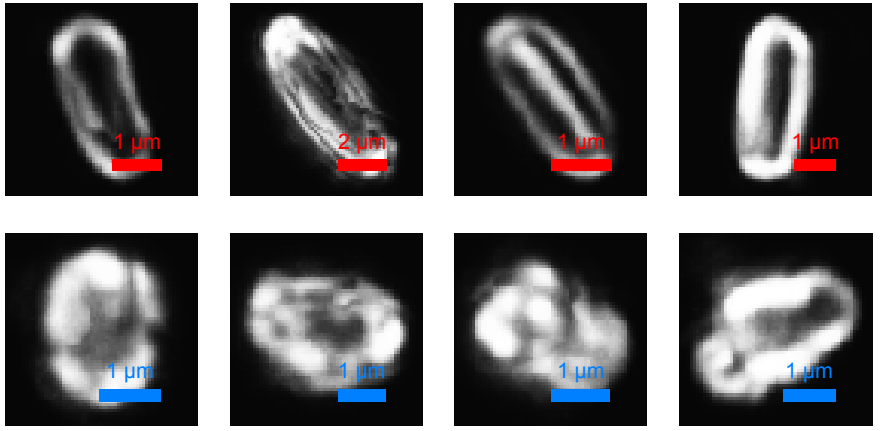
Brand A



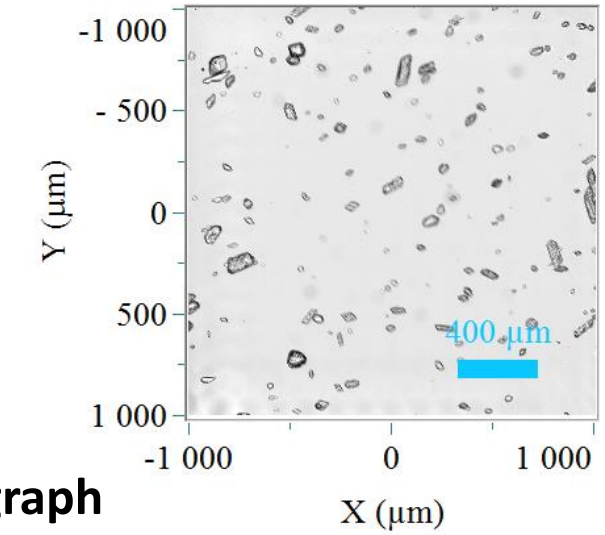


Brand B

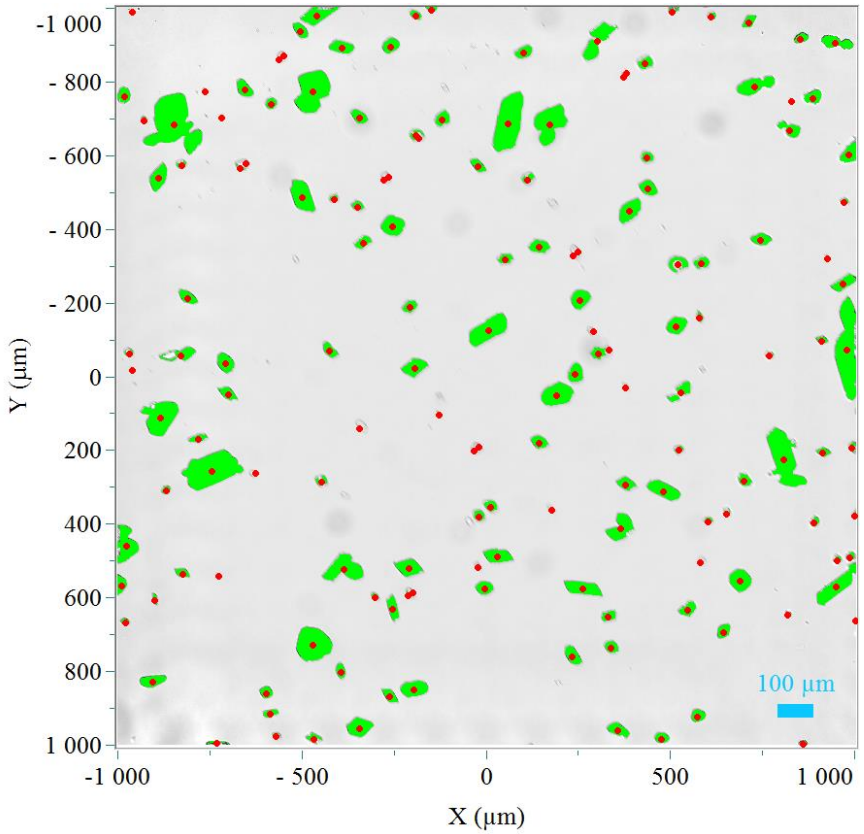




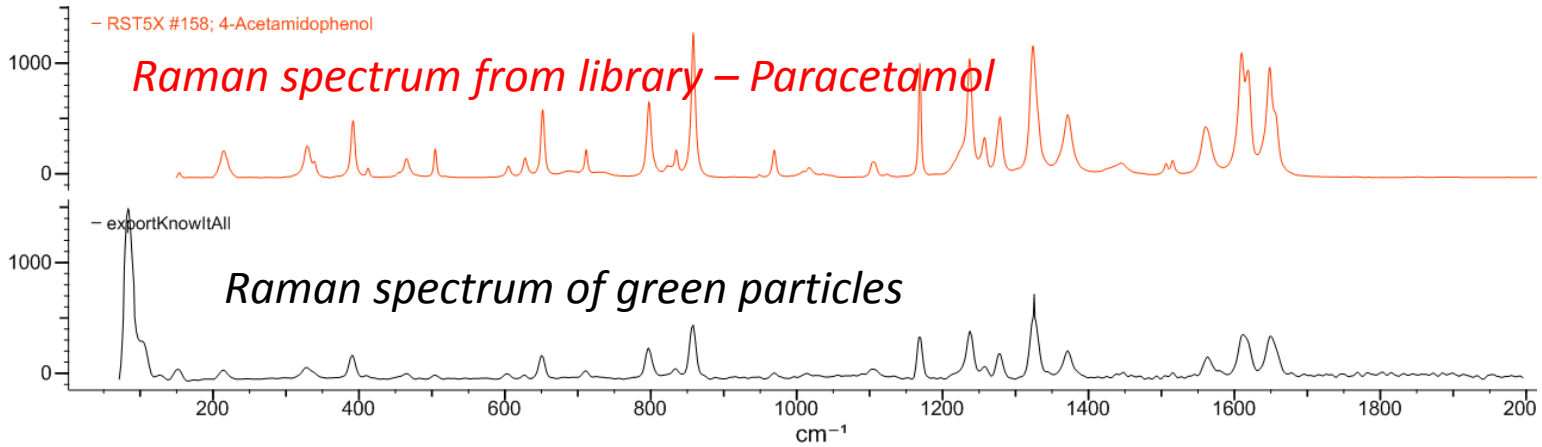
An example – Paracetamol in suspension



Micrograph transmitted, brightfield



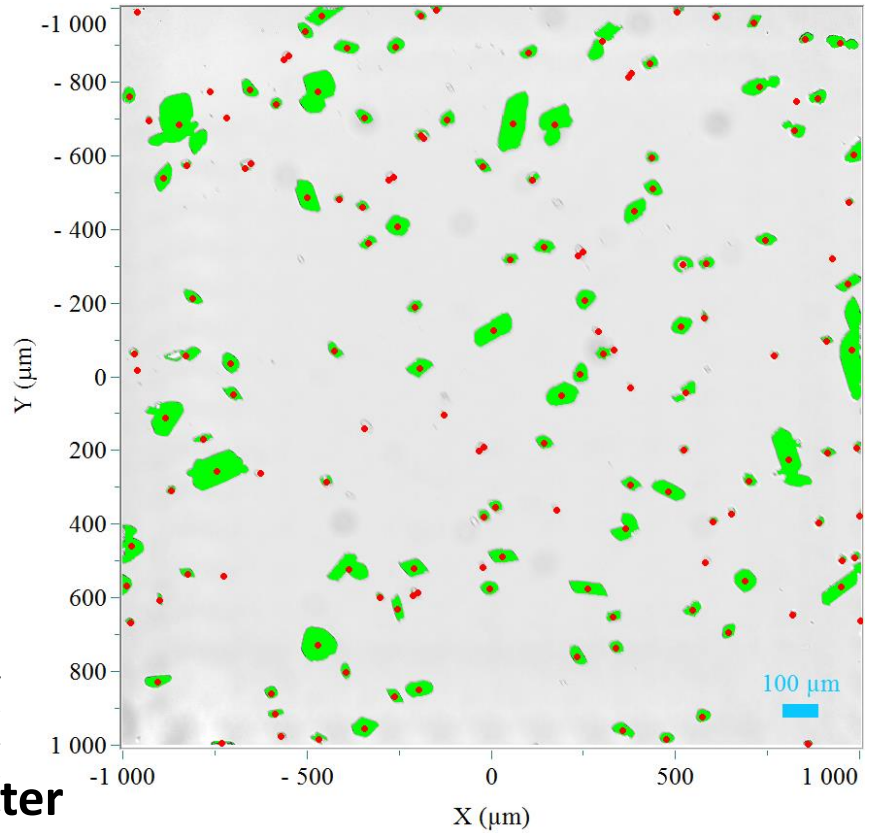
Particles highlighted in green



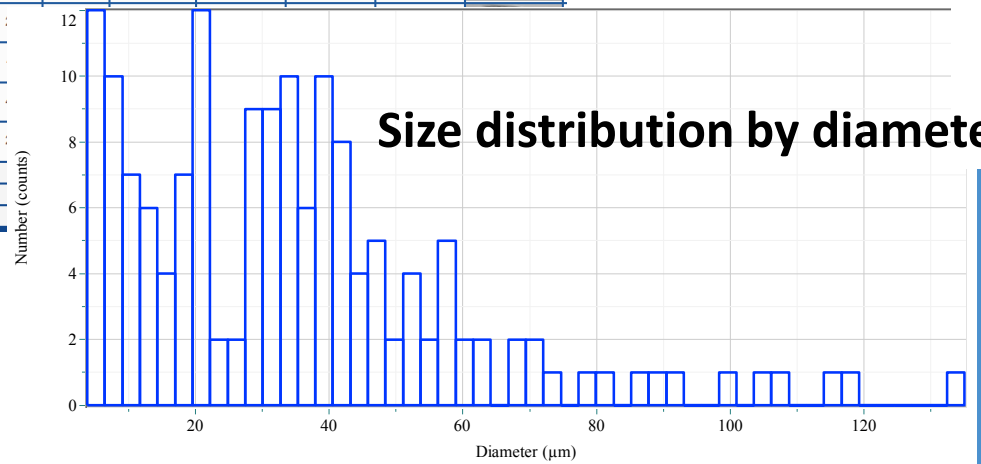
Particle characteristics and distribution

Size, shape and location of individual particles

Include	Class	Index	X pos	Y pos	Area	Diameter▲	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Brightness	Image
Use filtering			☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Filter limits(min)													
Filter limits(max)													
☐		1(27)	-847.7	-684.6	14046.8	133.7	695.0	165.8	136.5	0.82	0.60	125.0	
☐		2(67)	976.2	-72.6	11122.7	119.0	793.5	286.8	55.3	0.19	0.47	114.0	
☐		3(99)	-745.6	254.6	10931.0	118.0	469.0	163.1	88.1	0.54	0.79	121.2	
☐		4(26)	58.6	-687.9	9291.8	108.8	448.1	176.2	68.7	0.39	0.76	123.6	
☐		5(91)	805.3	223.8	9014.2	107.1	608.4	183.6	68.4	0.37	0.55	125.8	
☐		6(18)	-472.2	-772.6	7854.7	100.0	388.7	125.2	84.1	0.67	0.81	100.7	
☐		7(140)	-470.0	727.3	6946.0	94.0	334.5	97.8	90.9	0.93	0.88	90.8	
☐		8(88)	-883.6	111.5	6253.9	89.2	391.1	103.5	81.2	0.78	0.72	131.1	
☐		9(30)	170.9	-685.0	6190.7	88.8	374.2	113.4	76.4	0.67	0.75	116.3	
☐		10(70)	4.2	-125.4	5317.9	82.3	331.9	127.0	54.9	0.43	0.78	133.7	
☐		11(117)	-386.8	522.4	4890.0	78.9	369.8	109.8	70.7	0.64	0.67	135.7	
☐		12(121)	947.3	569.5	4488.3	75.6	410.4	154.4	39.4	0.26	0.58	124.0	
☐		13(85)	190.3	50.1	4106.3	72.3	264.5	89.0	59.2	0.66	0.86	133.3	
☐		14(48)	-499.9	-487.7	3998.6	71.4							
☐		15(114)	-976.9	456.9	3919.3	70.6							
☐		16(8)	300.2	-910.6	3872.2	70.2							
☐		17(21)	726.2	-787.5	3323.7	65.1							
Mean			15.0	-46.8	1539.7	36.5							
Stdev			600.1	595.3	2226.7	25.1							
Median			7.3	-46.6	904.2	33.9							



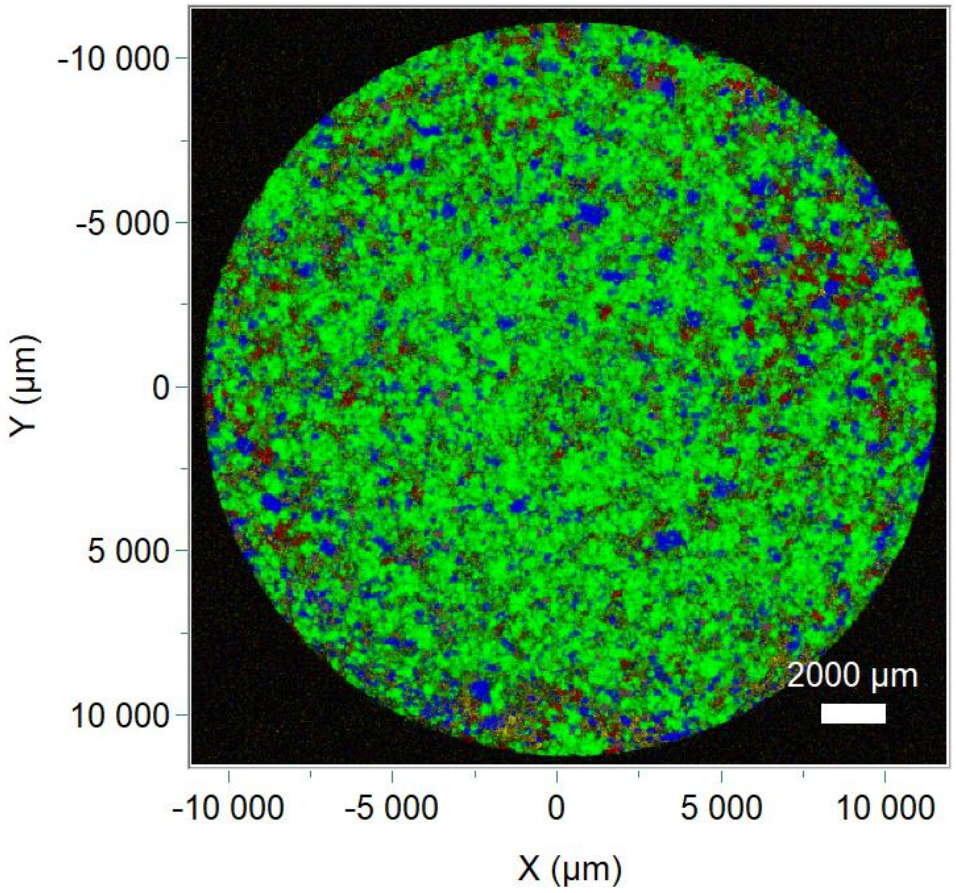
Size distribution by diameter



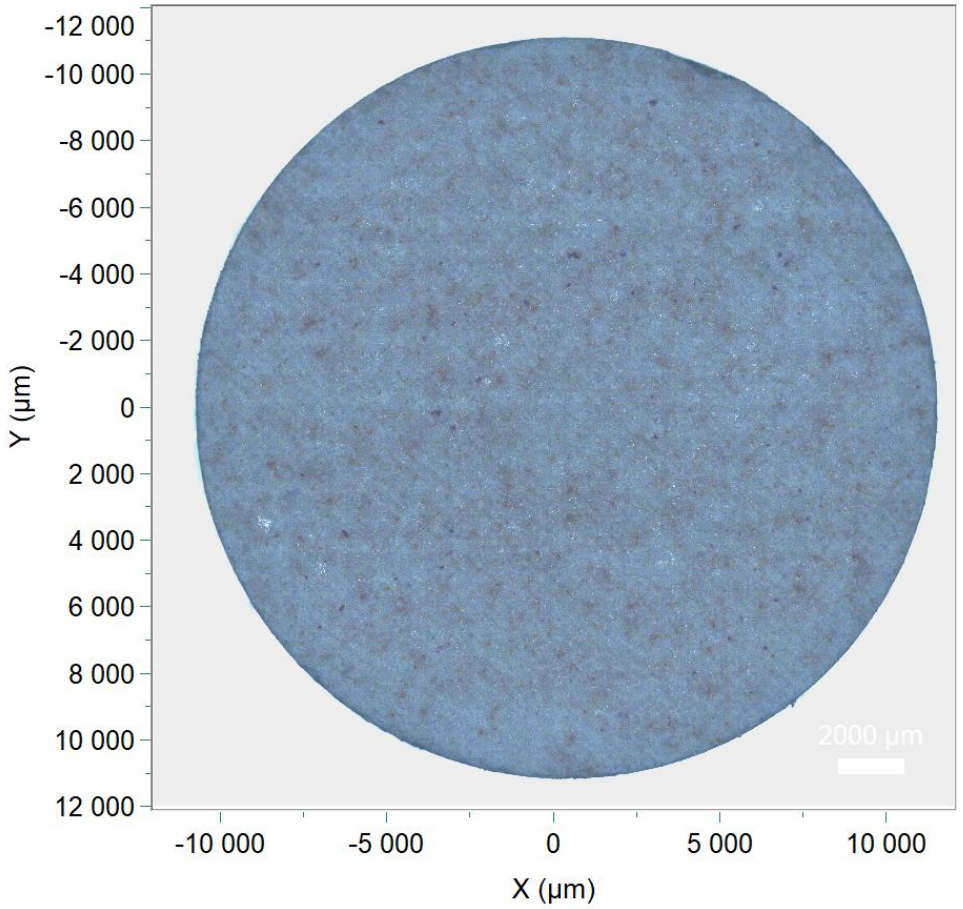
Count	156	Corrected count	0
Bin	50	10 percentile	8.94384
Mean	36.4507	50 percentile	33.9193
StDev	25.1342	90 percentile	70.641
Median	33.9294		

An example – Vitamin C in tablet

Raman image, 10 ms/spectrum

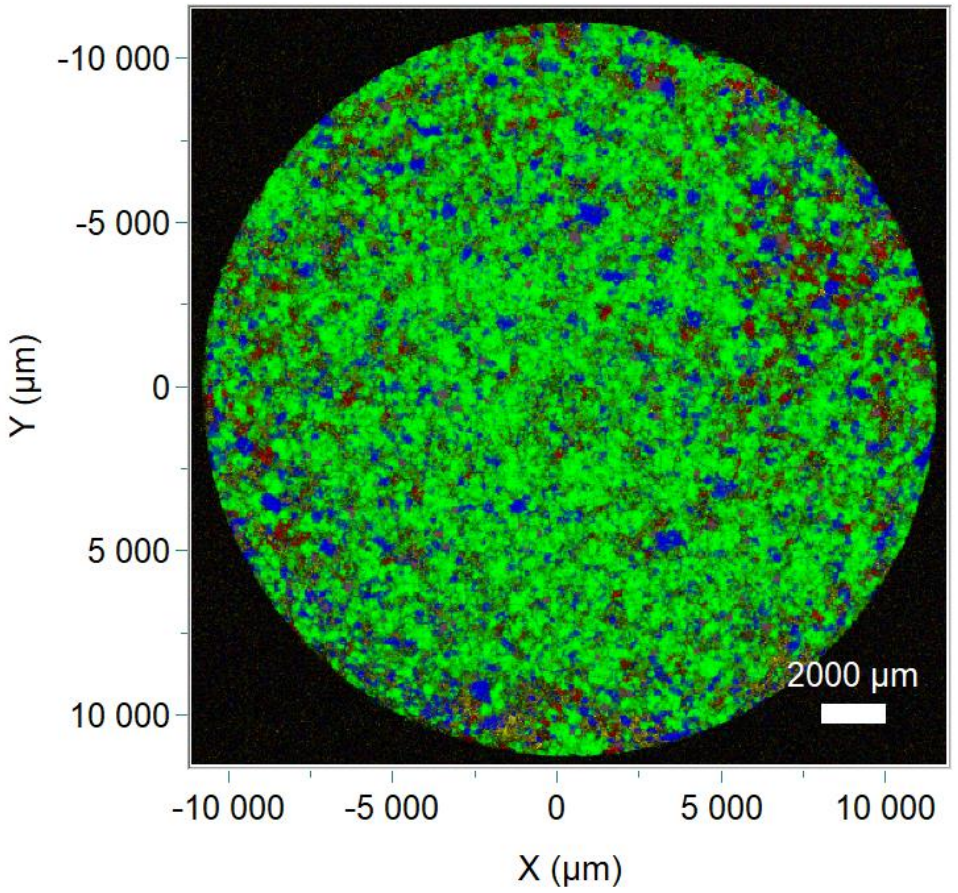


Micrograph, reflected, brightfield

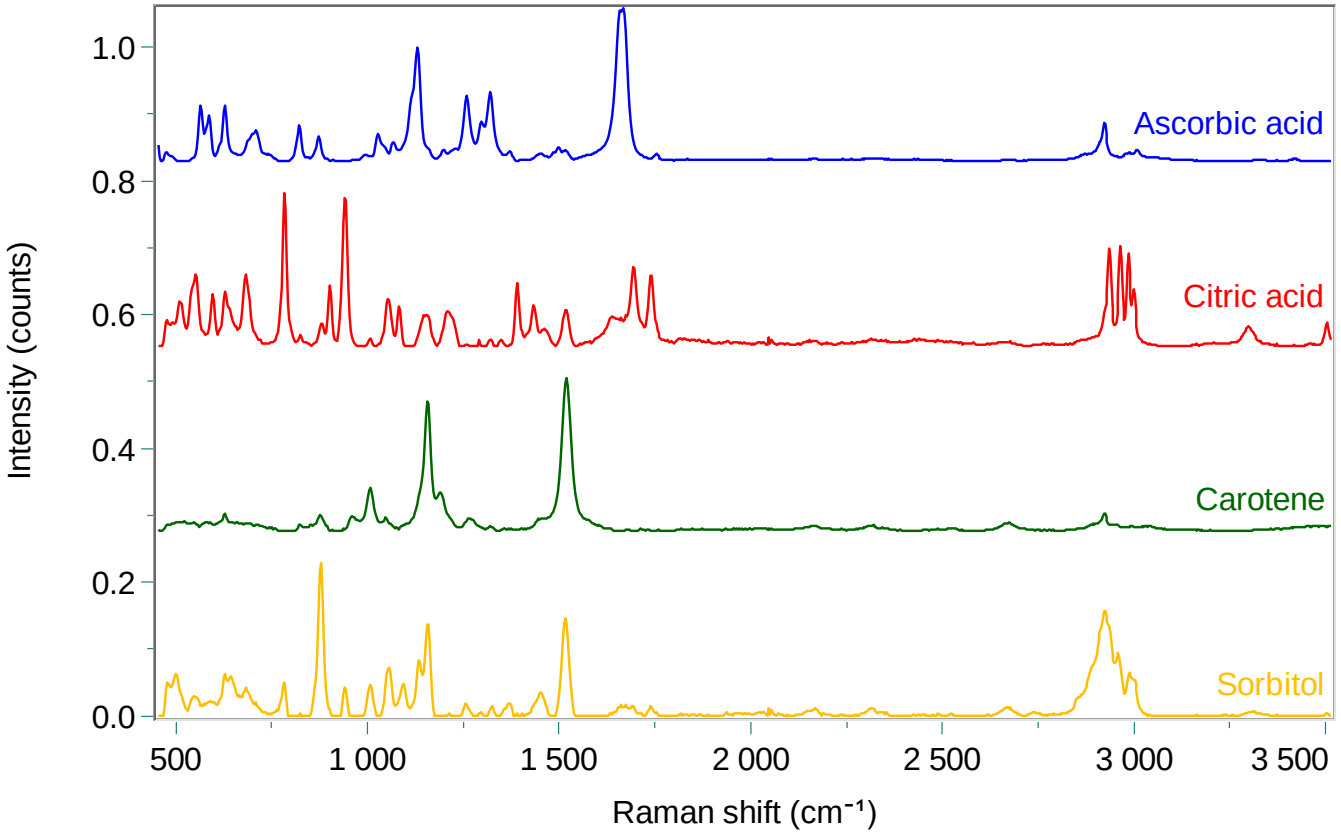


Particles embedded in matrix

Raman image, 10 ms/spectrum

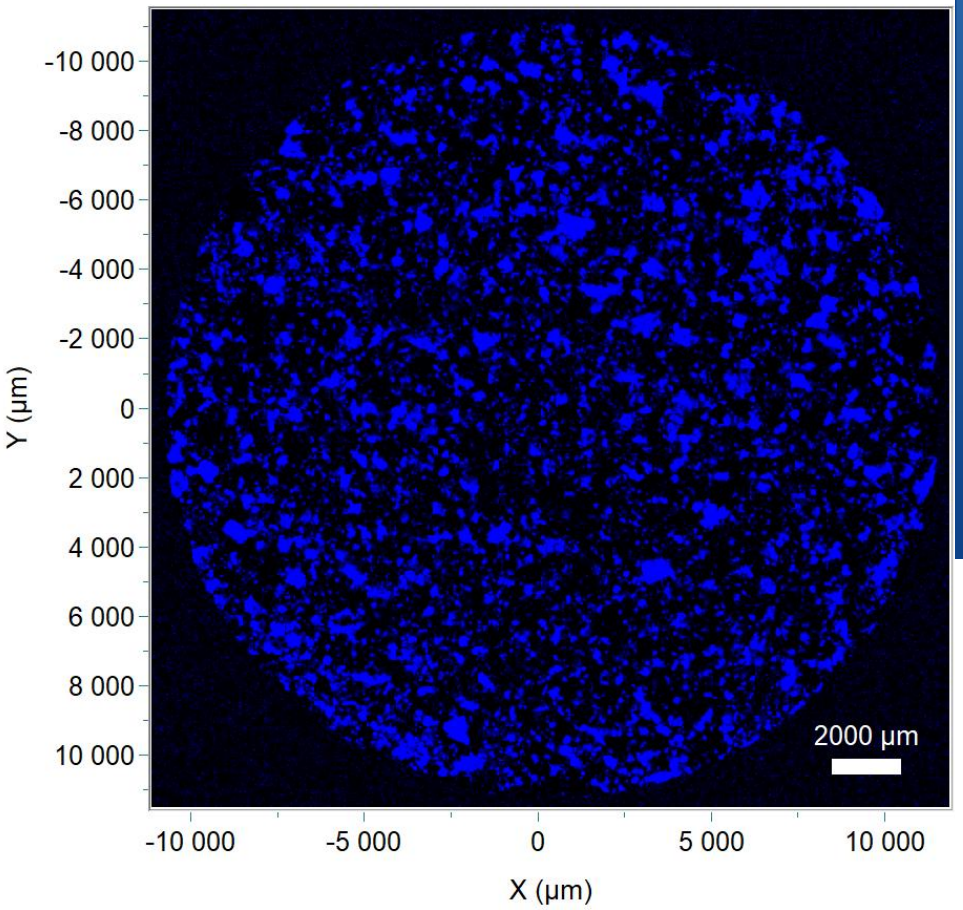


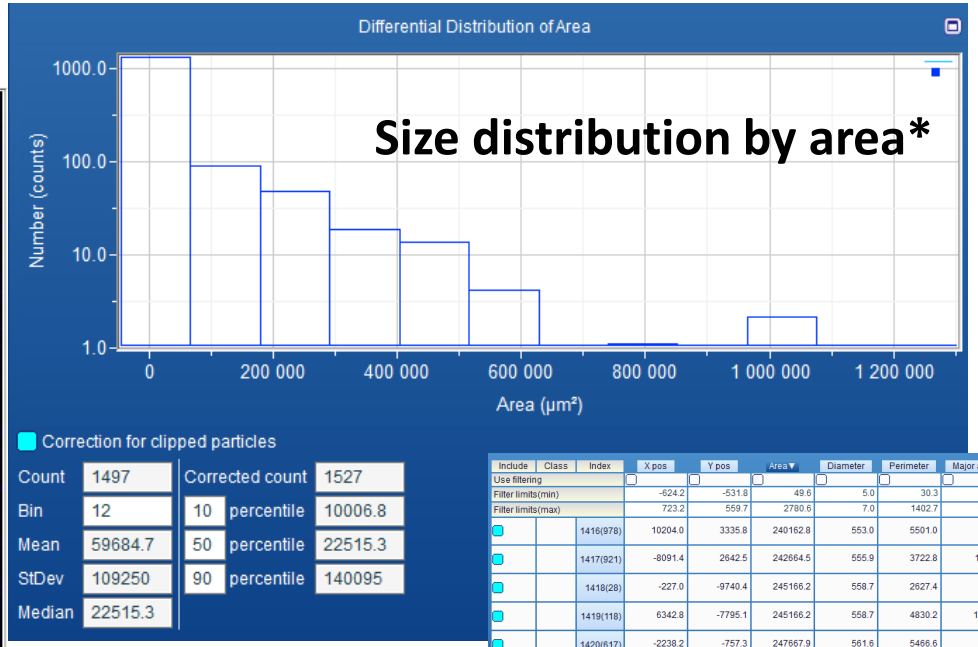
Representative spectra

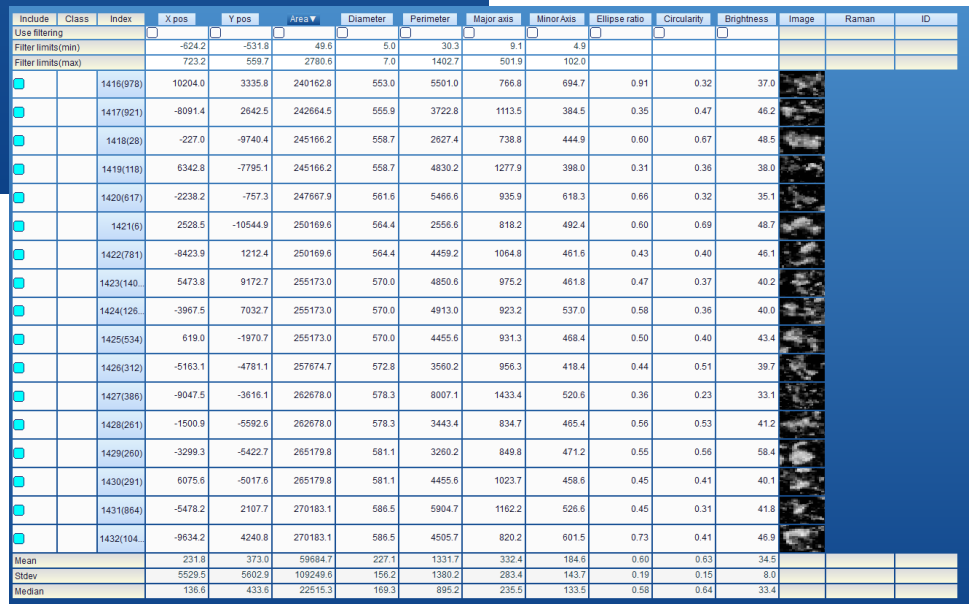


Particle characteristics and distribution

Raman image, ascrobic acid







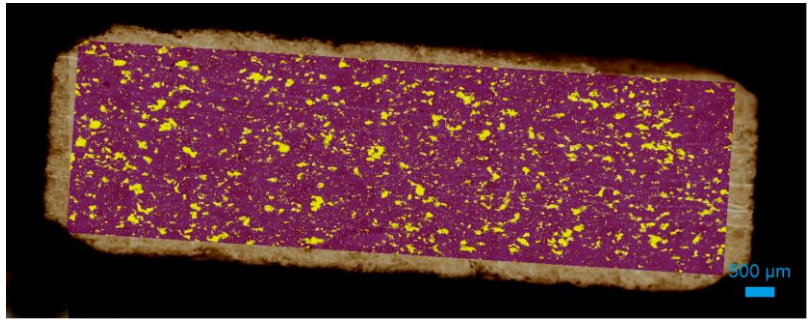
Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Brightness	Image	Raman	ID
Use filtering															
Filter limits(min)			-624.2	-531.8	49.6	5.0	30.3	9.1	4.9						
Filter limits(max)			723.2	559.7	2780.6	7.0	1402.7	501.9	102.0						
		1416(978)	10204.0	3335.8	240162.8	553.0	5501.0	766.8	694.7	0.91	0.32	37.0			
		1417(921)	-8091.4	2642.5	242664.5	555.9	3722.8	1113.5	384.5	0.35	0.47	46.2			
		1418(28)	-227.0	-9740.4	245166.2	558.7	2627.4	738.8	444.9	0.60	0.67	48.5			
		1419(118)	6342.8	-7795.1	245166.2	558.7	4830.2	1277.9	398.0	0.31	0.36	38.0			
		1420(617)	-2238.2	-757.3	247667.9	561.6	5466.6	935.9	618.3	0.66	0.32	35.1			
		1421(6)	2528.5	-10544.9	250169.6	564.4	2556.6	818.2	492.4	0.60	0.69	48.7			
		1422(781)	-8423.9	1212.4	250169.6	564.4	4459.2	1064.8	461.6	0.43	0.40	46.1			
		1423(140...	5473.8	9172.7	255173.0	570.0	4850.6	975.2	461.8	0.47	0.37	40.2			
		1424(126...	-3967.5	7032.7	255173.0	570.0	4913.0	923.2	537.0	0.58	0.36	40.0			
		1425(534)	619.0	-1970.7	255173.0	570.0	4455.6	931.3	468.4	0.50	0.40	43.4			
		1426(312)	-5163.1	-4781.1	257674.7	572.8	3560.2	956.3	418.4	0.44	0.51	39.7			
		1427(386)	-9047.5	-3616.1	262678.0	578.3	8007.1	1433.4	520.6	0.36	0.23	33.1			
		1428(261)	-1500.9	-5592.6	262678.0	578.3	3443.4	834.7	465.4	0.56	0.53	41.2			
		1429(260)	-3299.3	-5422.7	265179.8	581.1	3260.2	849.8	471.2	0.55	0.56	58.4			
		1430(291)	6075.6	-5017.6	265179.8	581.1	4455.6	1023.7	458.6	0.45	0.41	40.1			
		1431(864)	-5478.2	2107.7	270183.1	586.5	5904.7	1162.2	526.6	0.45	0.31	41.8			
		1432(104...	-9634.2	4240.8	270183.1	586.5	4505.7	820.2	601.5	0.73	0.41	46.9			
Mean			231.8	373.0	59684.7	227.1	1331.7	332.4	184.6	0.60	0.63	34.5			
Stdev			5529.5	5602.9	109249.6	156.2	1380.2	283.4	143.7	0.19	0.15	8.0			
Median			136.6	433.6	22515.3	169.3	895.2	235.5	133.5	0.58	0.64	33.4			

particles smaller than 100 µm × 100 µm are excluded

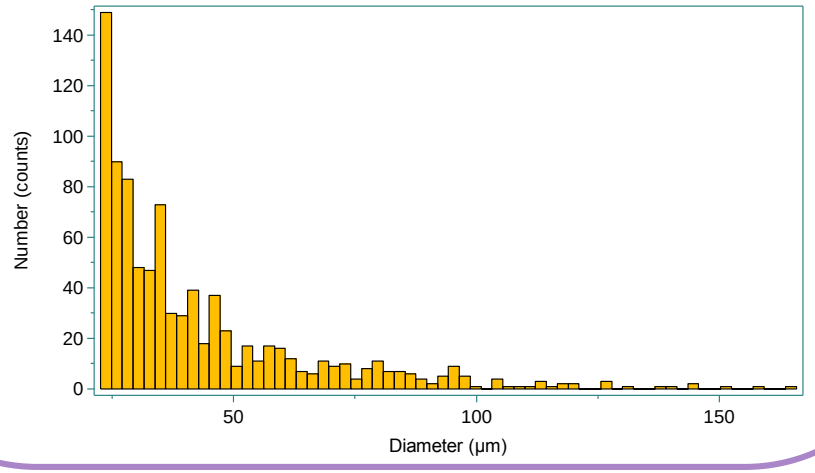
Size, shape and location of individual particle

Other Pharmaceutical and Cosmetic Examples

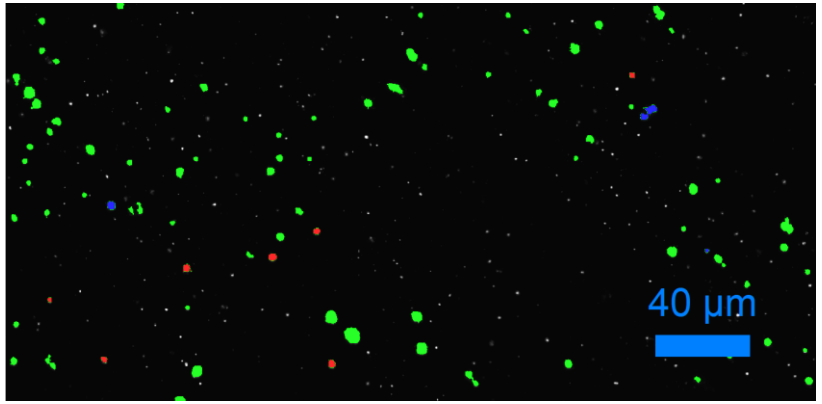
Raman Chemical Imaging – Tablet

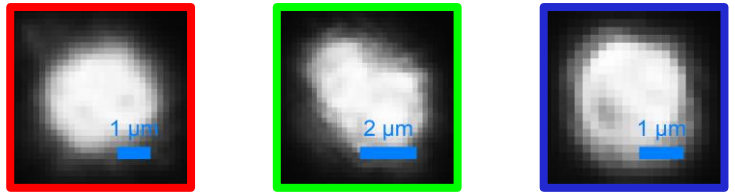


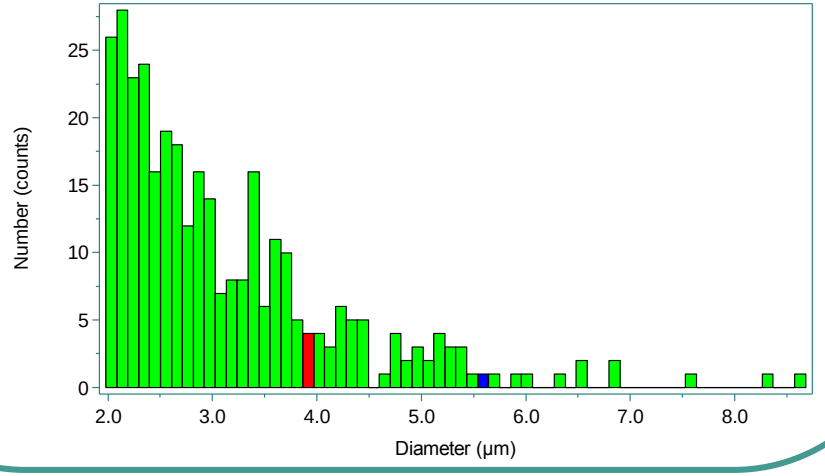
1000 µm



Topical Creams - Sunscreen







Particle Characterization: LA960 vs. XploRA PLUS

	LA960	XploRA PLUS
Technology	Laser diffraction	Static imaging + Raman imaging
Size range	10 nm to 5 mm	500 nm to 1 mm
Sample form	Good for dry or wet	Prefers dry, stationary
Shape	Possible	Possible
Chemical ID	No information	Yes
Speed	60 s per run, zillion particle per run Drum -> sampling from different parts, mix them well -> subsample -> run	Moderate
	Mixture -> sampling from different parts -> run them one -> compare statistics -> mixing quality	Run the same samples on Raman and see if chemical ID matches size distribution variation
Tandem analysis	Screening for outliers in batches	Root cause analysis