HORIBA Scientific
Particle Characterization

Laser Diffraction Method Development

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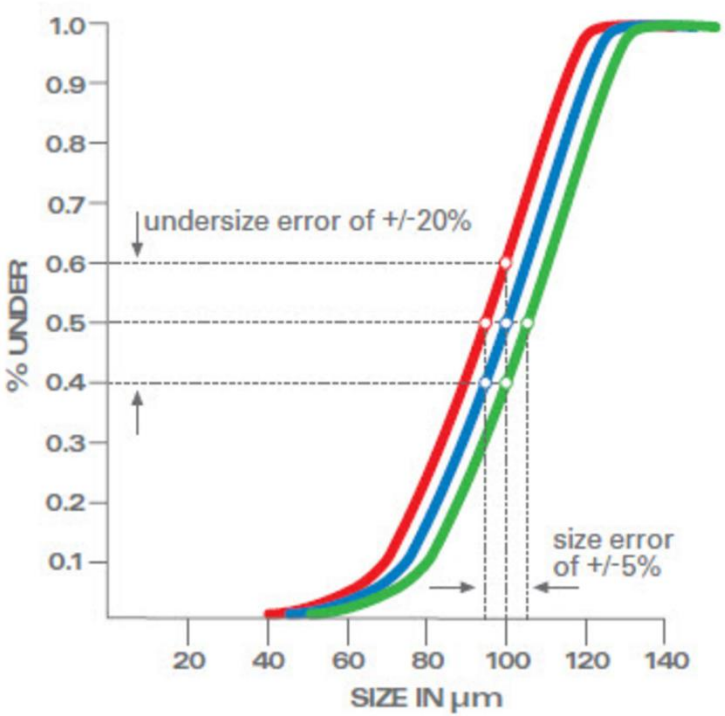
Method development overview

- Consider Goal(s): Reproducible method that tracks product performance
- Choose measurement approach (dry vs. suspension)
- Choose Refractive Index (real and imaginary)
- Vary measurement settings that can influence result
  - Dry: measurement duration, concentration, air pressure
  - Wet: sampler selection, dispersion, duration, concentration, energy (mixing + ultrasound)
  - Rough out working values
  - Systematically test near working values.
- Test method (reproducibility)
  - Meet ISO, USP or internal guidelines
  - Check COV at d10, d50, d90

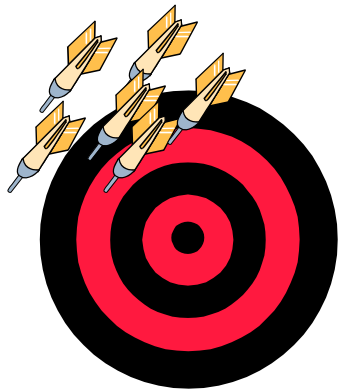
Goals

- Reproducible method that tracks product performance
- Accuracy: tricky subject, is it the “real” particle size?
- Repeatability: liquid suspension re-circulating in sampler
- Reproducibility: prepare, measure, empty, repeat
- Resolution: optimize to find second populations
- Match historic data (sieves), but quicker, easier technique

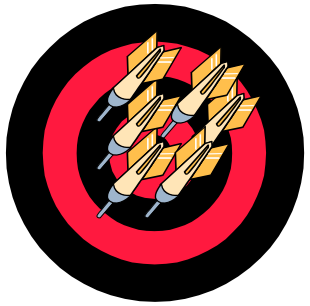
- Use structured approach for any decision/choice that may influence result
- Have data to support selections made
- Document process so colleagues understand your choices



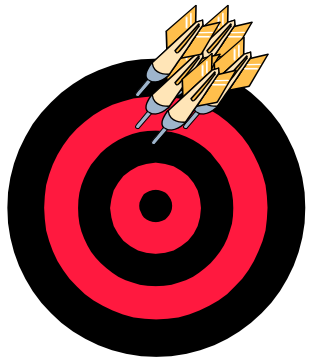
Accuracy vs. Precision



LOW ACCURACY
LOW PRECISION



HIGH ACCURACY
LOW PRECISION



LOW ACCURACY
HIGH PRECISION

HIGH ACCURACY
HIGH PRECISION

(A) Low accuracy, low precision measurements form a diffuse, off-center cluster
(B) Low accuracy, high precision measurements form a tight off-center cluster
(C) High accuracy, low precision measurements form a cluster that is evenly distributed but distant from the center of the target
(D) High Accuracy, high precision measurements are clustered in the center of the target.

Accuracy

Is it the "real particle size"?

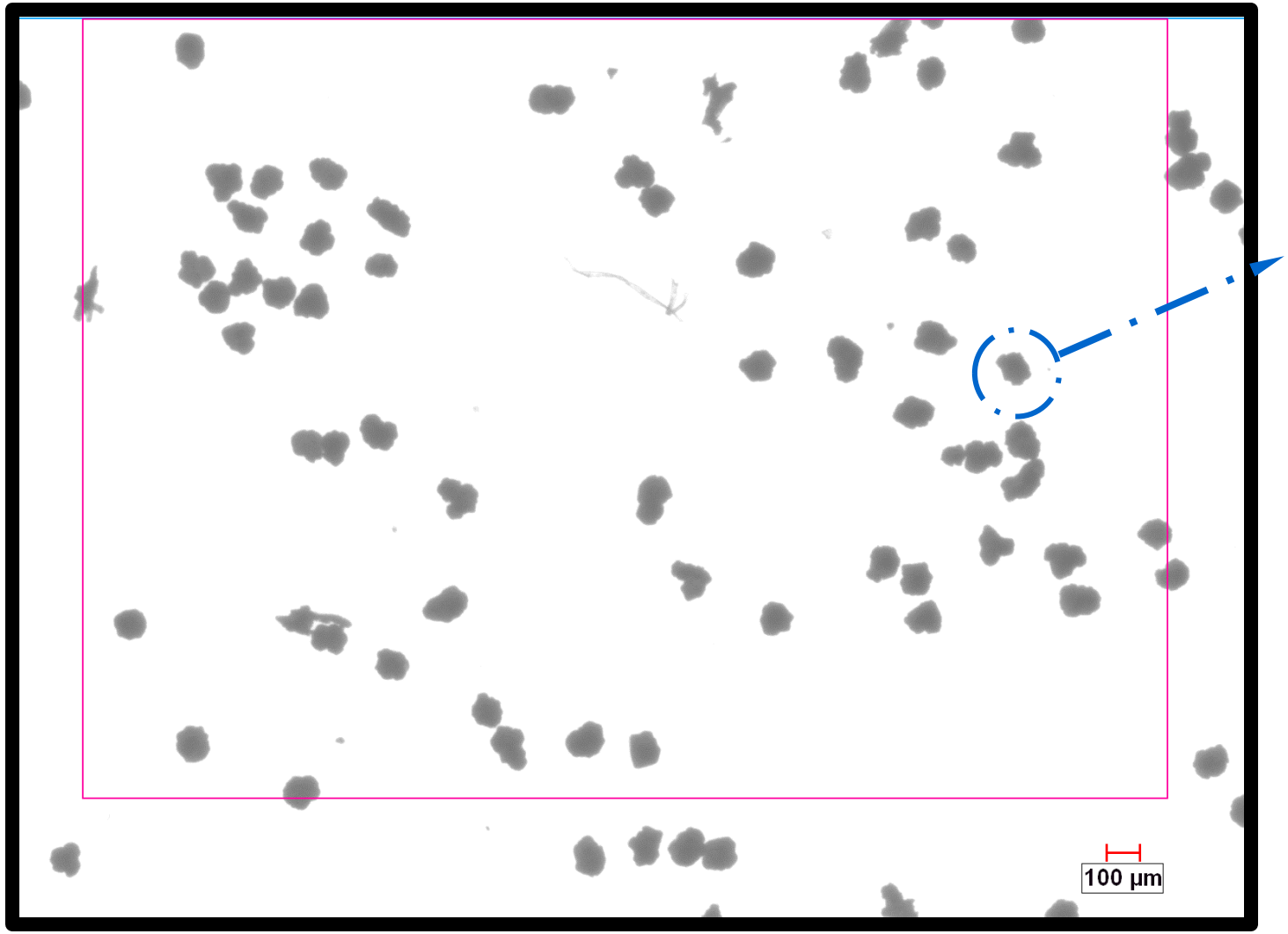
- Comparison to referee technique
- Microscope (image analysis) is referee technique for particle characterization
- For submicron particles, also consider DLS and Nanoparticle Tracking analysis, though TEM images are more convincing.

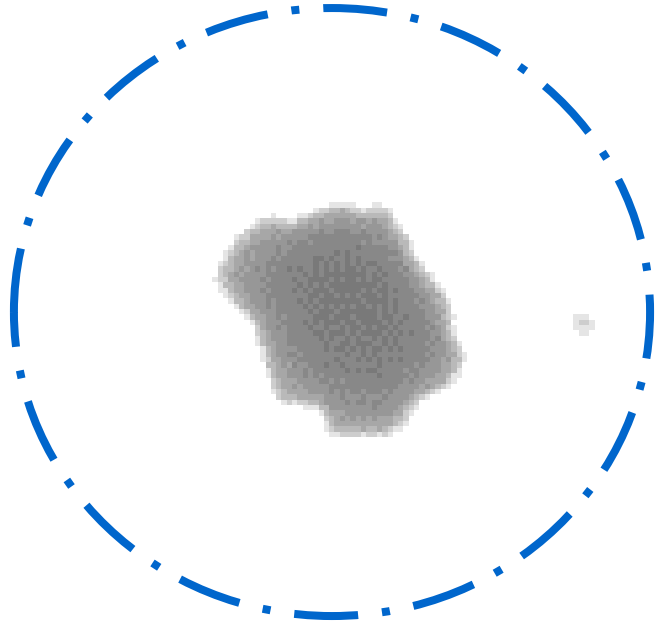
- Compare to sieving or historical data

Focus on precision, in the worst case, you can build a correlation.

Adjustments to analysis (e.g., refractive index) are easier with good precision.
Very hard to build a correlation without precision.

Image Analysis





Take a picture, analyze for **size** and **shape**

Types of Precision

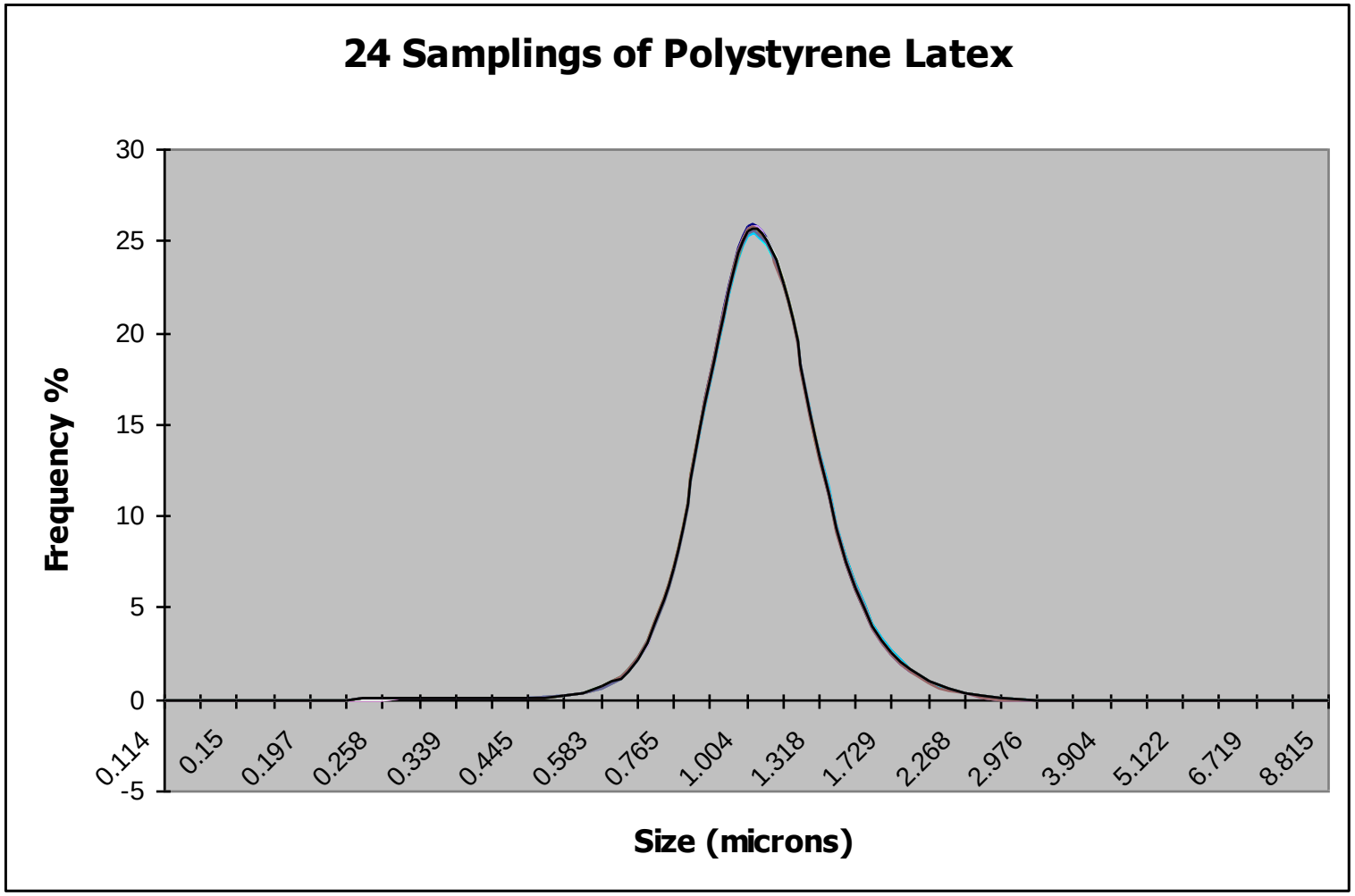
Repeatability

- Prepare sample, add to wet sampler, re-circulate, measure same multiple times (suspensions only)
- Provides limited information (mostly a test of analyzer performance)
- First test of a method

Reproducibility

- Prepare sample, measure, drain, repeat (suspensions + dry)
- Distinguishes great methods

Reproducibility



Yes, we emptied instrument between each analysis.

Reproducibility

Reproducibility: prepare, measure, empty, repeat

What would be good reproducibility?

- Look at accepted standards
- Measure 3 or more times, calculated COV at d_{10}, d_{50}, d_{90}
  - COV (RSD) = st dev/mean * 100
- ISO13320
  - COV < 3% at median d_{50}
  - COV < 5% at d_{10} & d_{90}
- USP<429>
  - COV < 10% at median d_{50}
  - COV < 15% at d_{10} & d_{90}

Note: double all limits when d_{10}, d_{50}, or d_{90} < 10 microns

Repeatability

Test repeatability first!

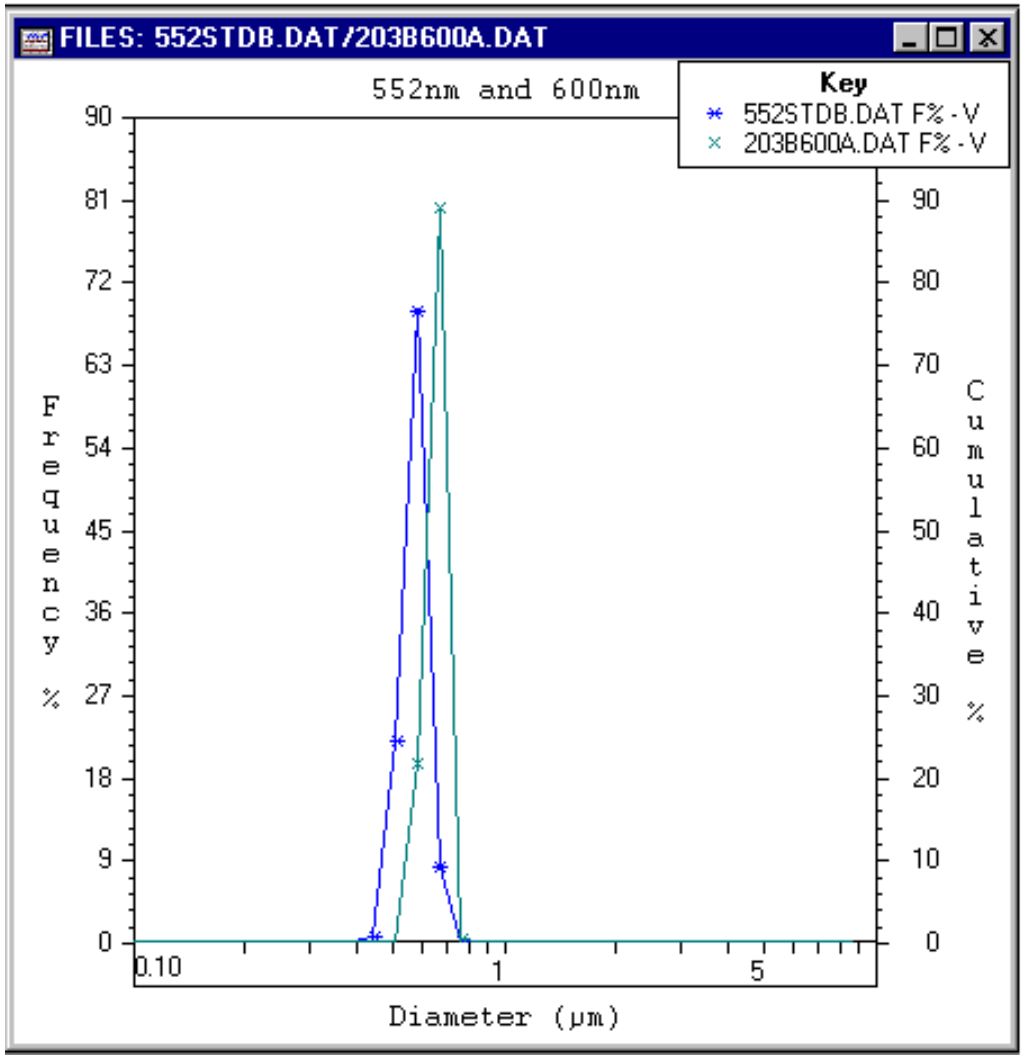
If possible, check repeatability by leaving sample in the analyzer and running a multiple times.

This lets you separate issues with reproducibility from repeatability. Your reproducibility is unlikely to be better than repeatability.

Resolution

- Ability to measure small differences in particle size.
- Small differences between successive samples (different production lots) are most important. This is tied to reproducibility.
- Detection limit of small amount of material outside of main size distribution.
- Best defined by user’s real-world requirements.

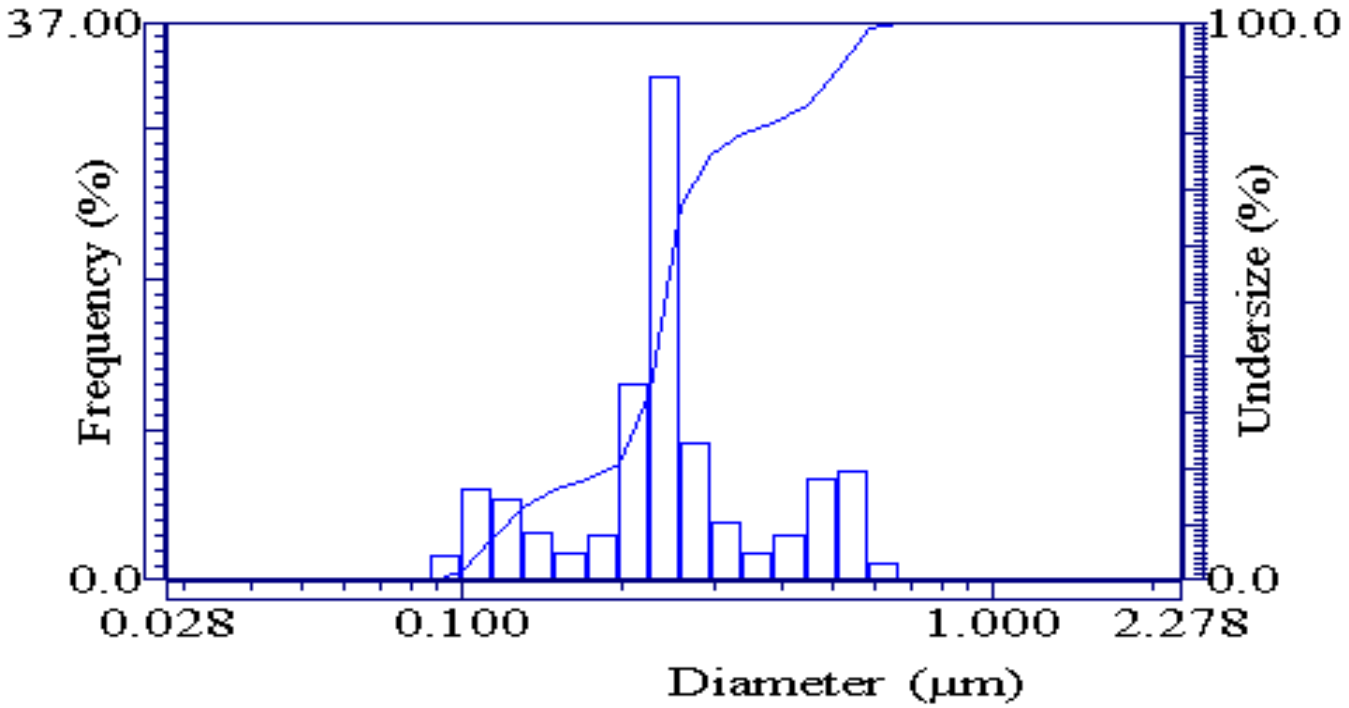
High Resolution?



- Resolve size difference between two materials of similar size
- 552nm and 600nm PSL
- Measured separately: high resolution
- Measure mixed together: peaks would blend and you cannot discern two populations.

- Directly connected to reproducibility. If your result is +/-5%, then you can't resolve a 2% change.

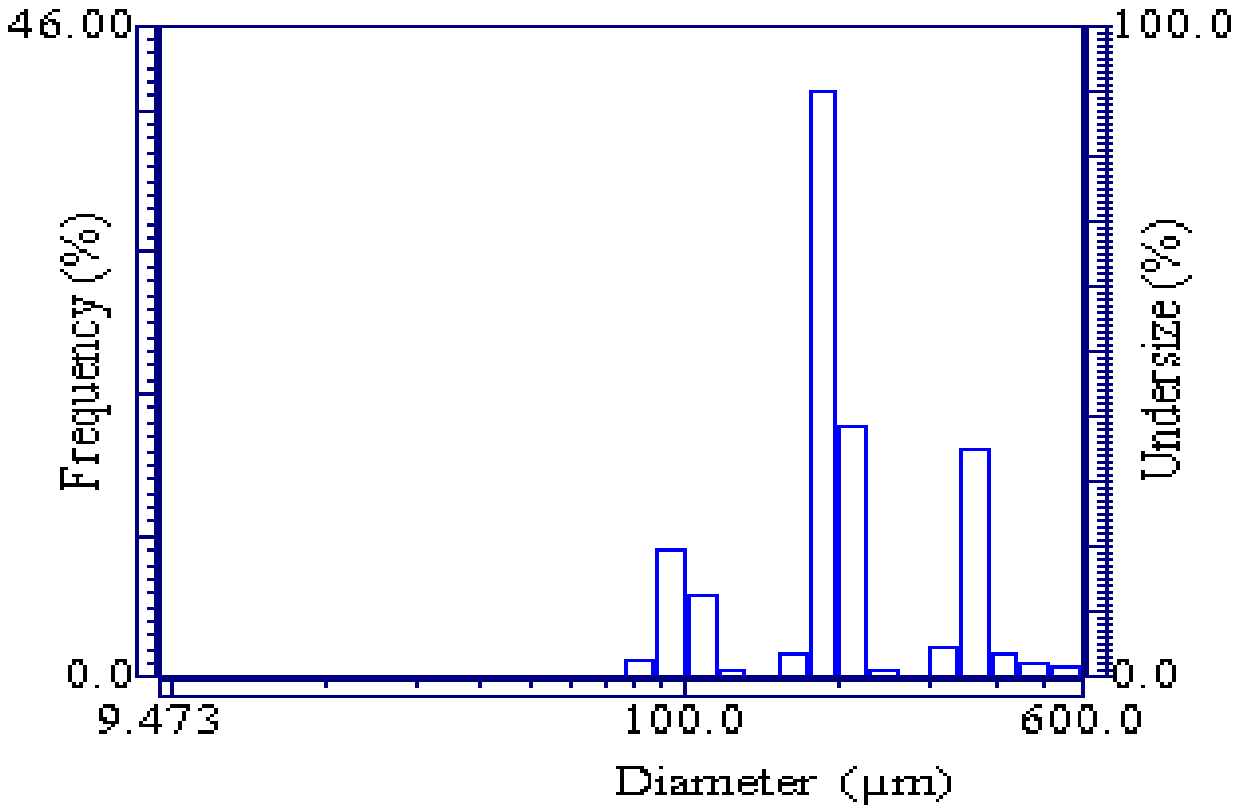
Separate peaks



83nm,
204 nm,
503nm
PSL

Resolution of multiple modes in a single sample
Next peak at least 2x of previous size

Separate Peaks

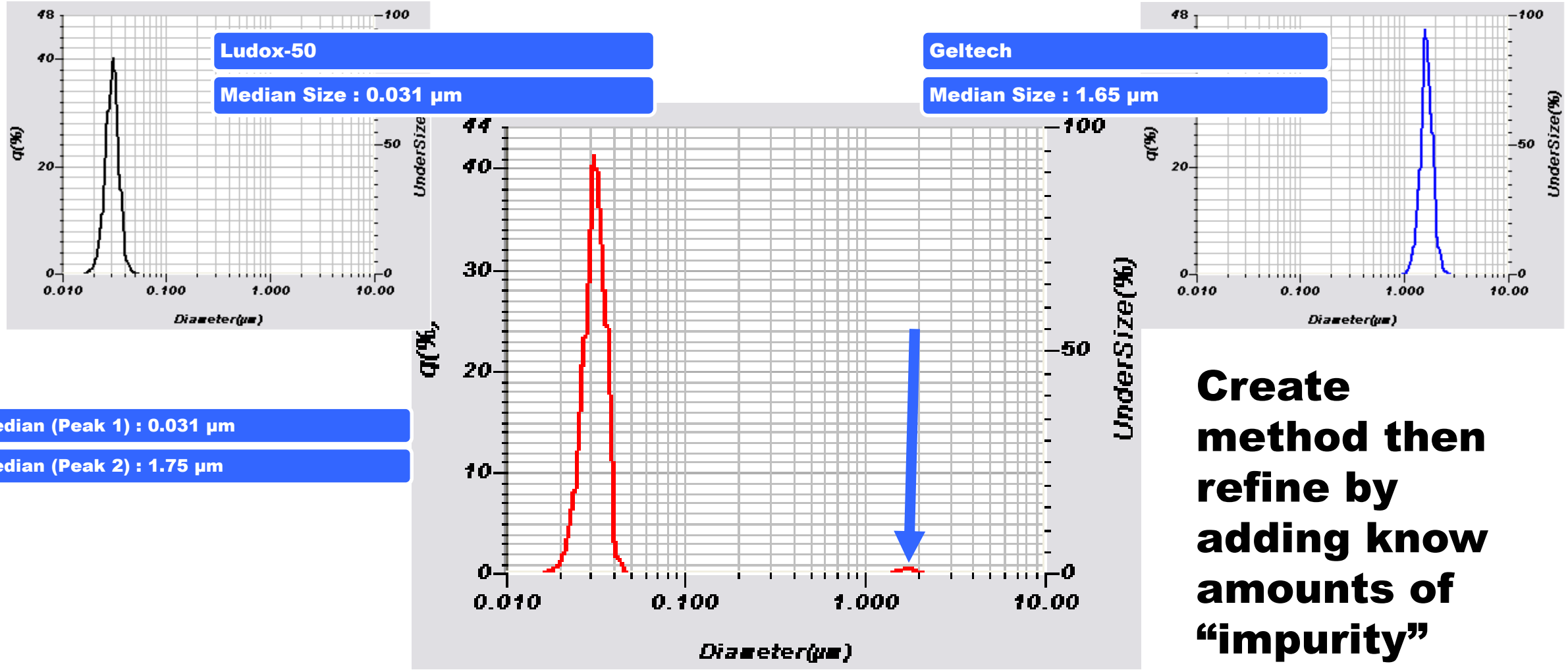


100µm,
200µm,
400µm
glass beads

Next peak 2x of previous size

Resolution is independent of where you are on size scale

Identifying Trace Impurities



0.05wt% Geltech 1.5 in LUDOX

Create method then refine by adding know amounts of "impurity" and testing.

Dry vs wet?

Wet:

- **if your sample is already in liquid dispersion**
- **smaller particles (<10 microns>) that need liquid to keep agglomerates apart**
- **need to use surfactants**

Dry:

- **sample is already a dry powder**
- **larger particles**

Wet Method Development

Workflow

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First determine RI

Choose dispersant (water, surfactants, hexane, etc.)

Sampler selection: sample volume

Pump & stirrer settings

Concentration

Measurement duration

Does the sample need ultrasound?

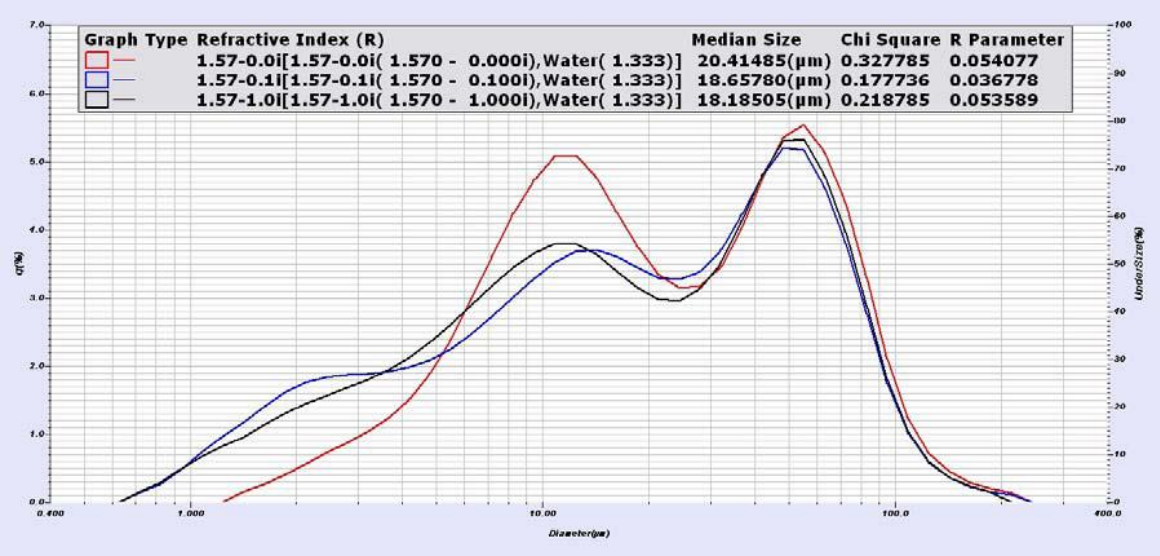
- Document size-time plot
- Disperse sample, but don’t break particles
- Check for reproducibility

Refractive Index

Refractive index affects accuracy, but not repeatability

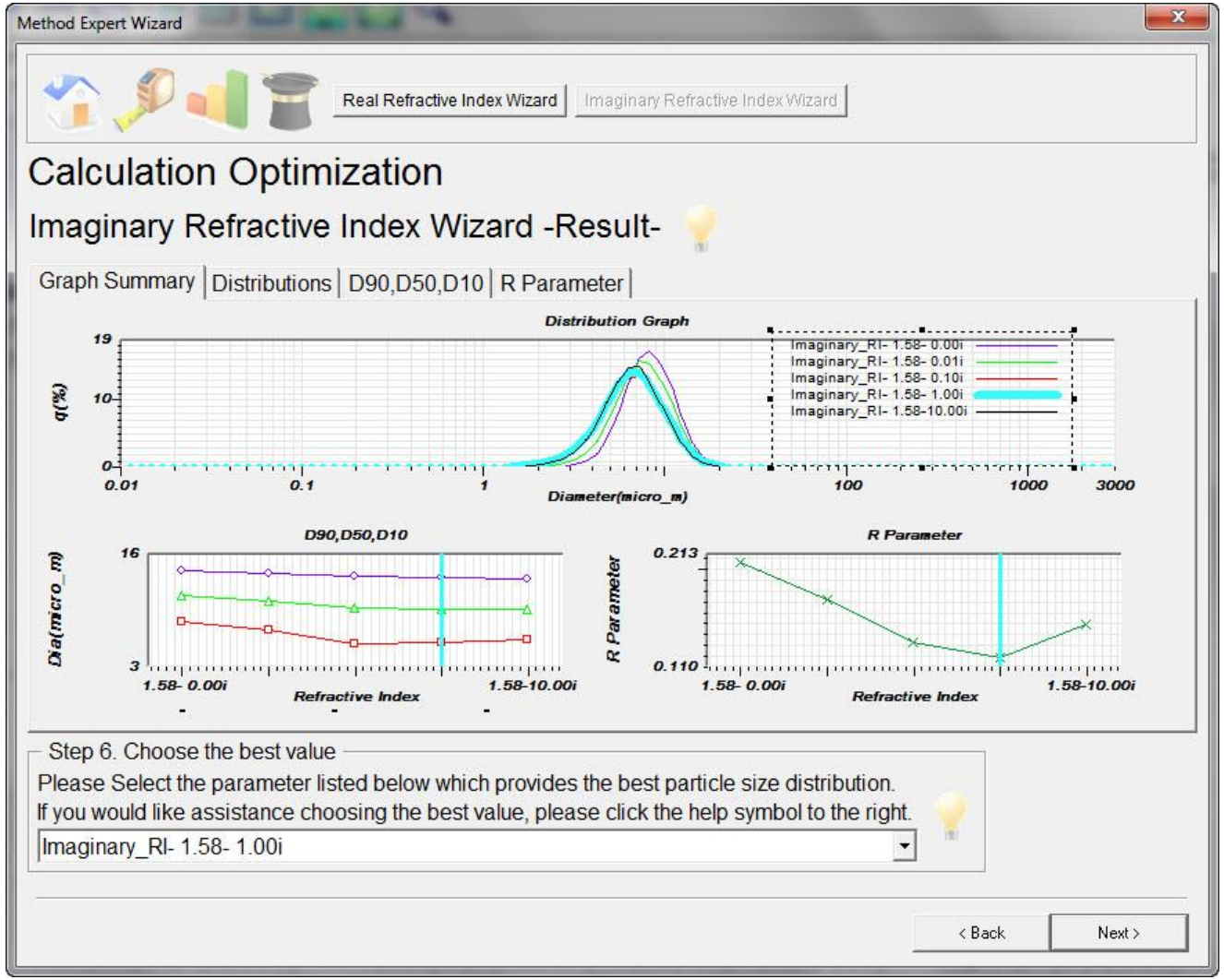
Real component via literature or web search, Becke line, etc.

Measure sample, vary imaginary component to see if/how results change, choose value that minimizes R parameter error calculation



More in the refractive index webinar at :
https://www.horiba.com/en_en/products/by-segment/scientific/particle-characterization/particle-analysis-webinar-series/

LA-960 Method Expert



Dispersant Choice

Which liquid?

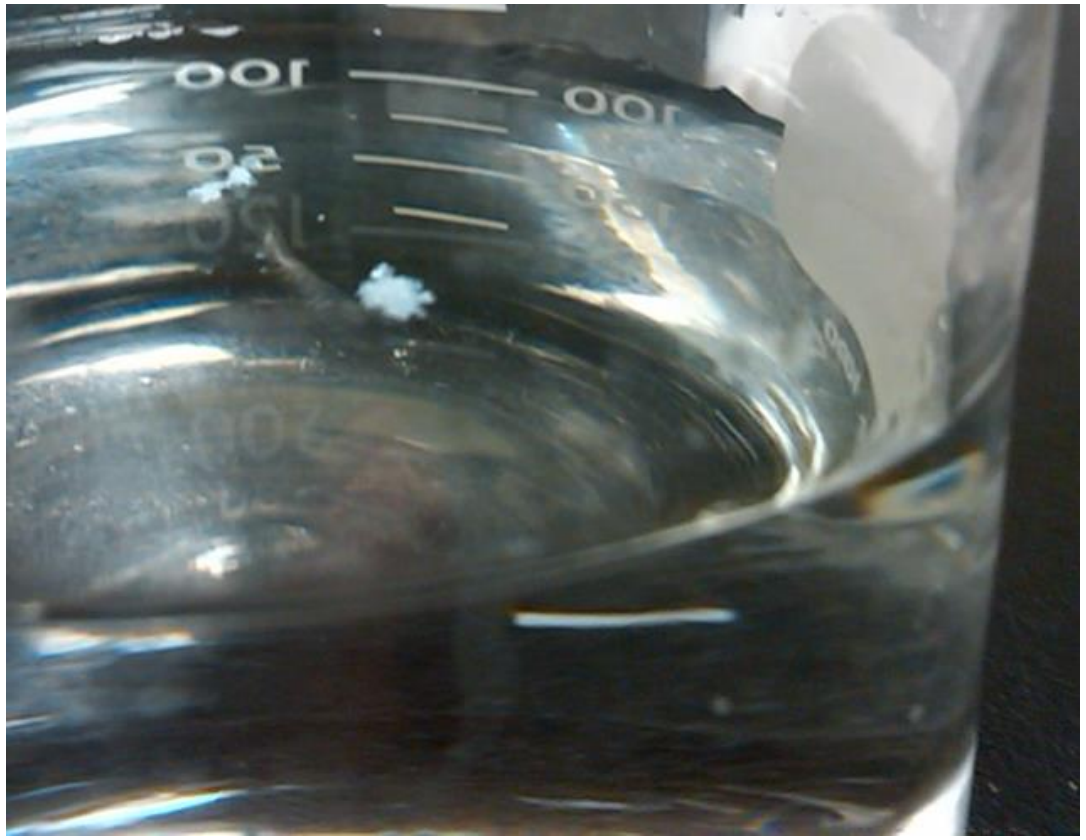
Water is easy to handle and inexpensive so it is quite popular.

Choose organic solvents when dispersion in water is difficult or if particles are water soluble.

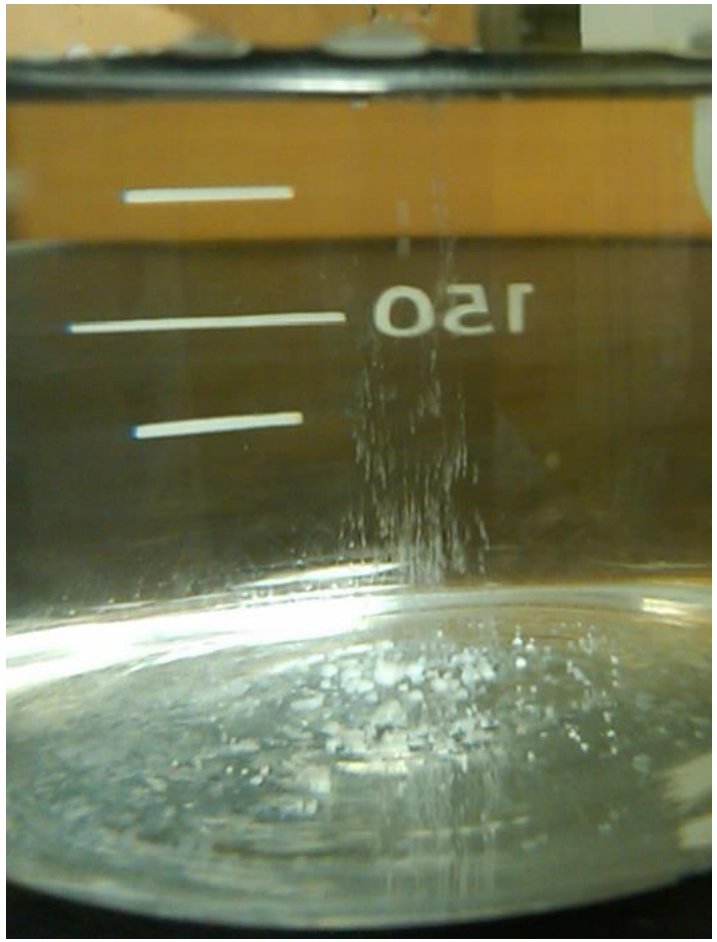
Poor wetting can lead to poor accuracy (evaluating loose agglomerates rather than primary particles) and repeatability (variatins in agglomeration.

Set up vials or beakers with each candidate dispersant. Gently put a little bit of fine particle on the top of each.

Poor wetting: particles don’t break surface tension



Good wetting: particles separate and stream through surface

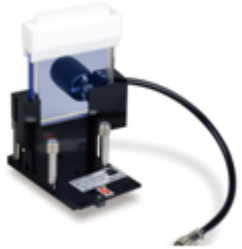


Sample Handling

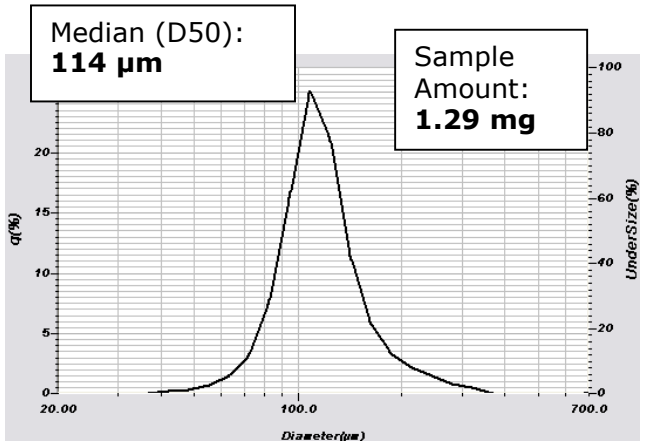
Agitation and sampling (sample volume) affect repeatability.

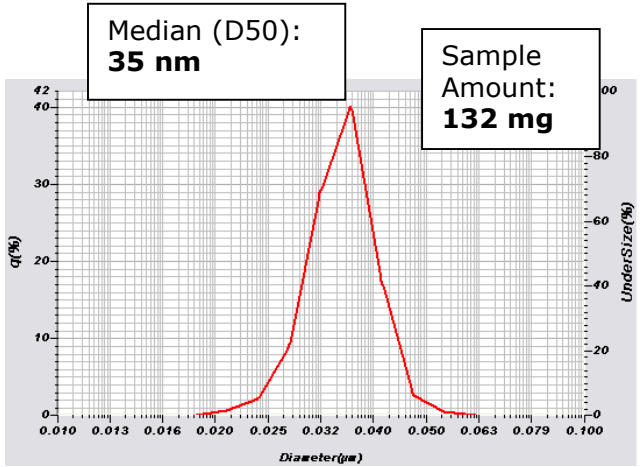
Larger, broad distributions require larger sample volume

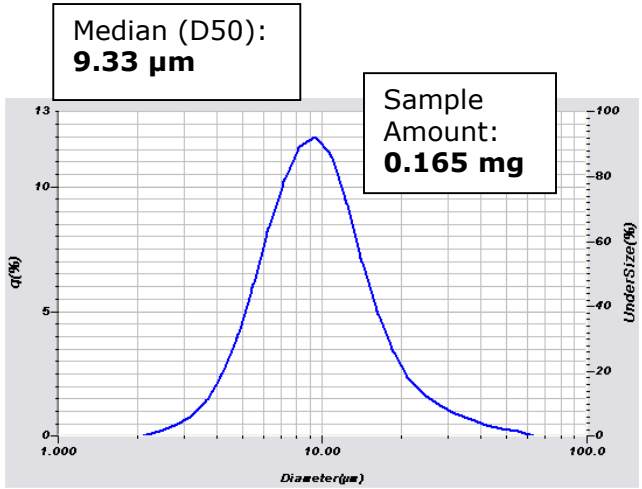
Lower volume samplers for precious materials or solvents



LA-960 Sample Handlers	Dispersing Volume (mL)
Aqua/SolvoFlow	**180 - 330**
MiniFlow	**35 - 50**
Fraction Cell	**15**
Small Volume Fraction Cell	**10**

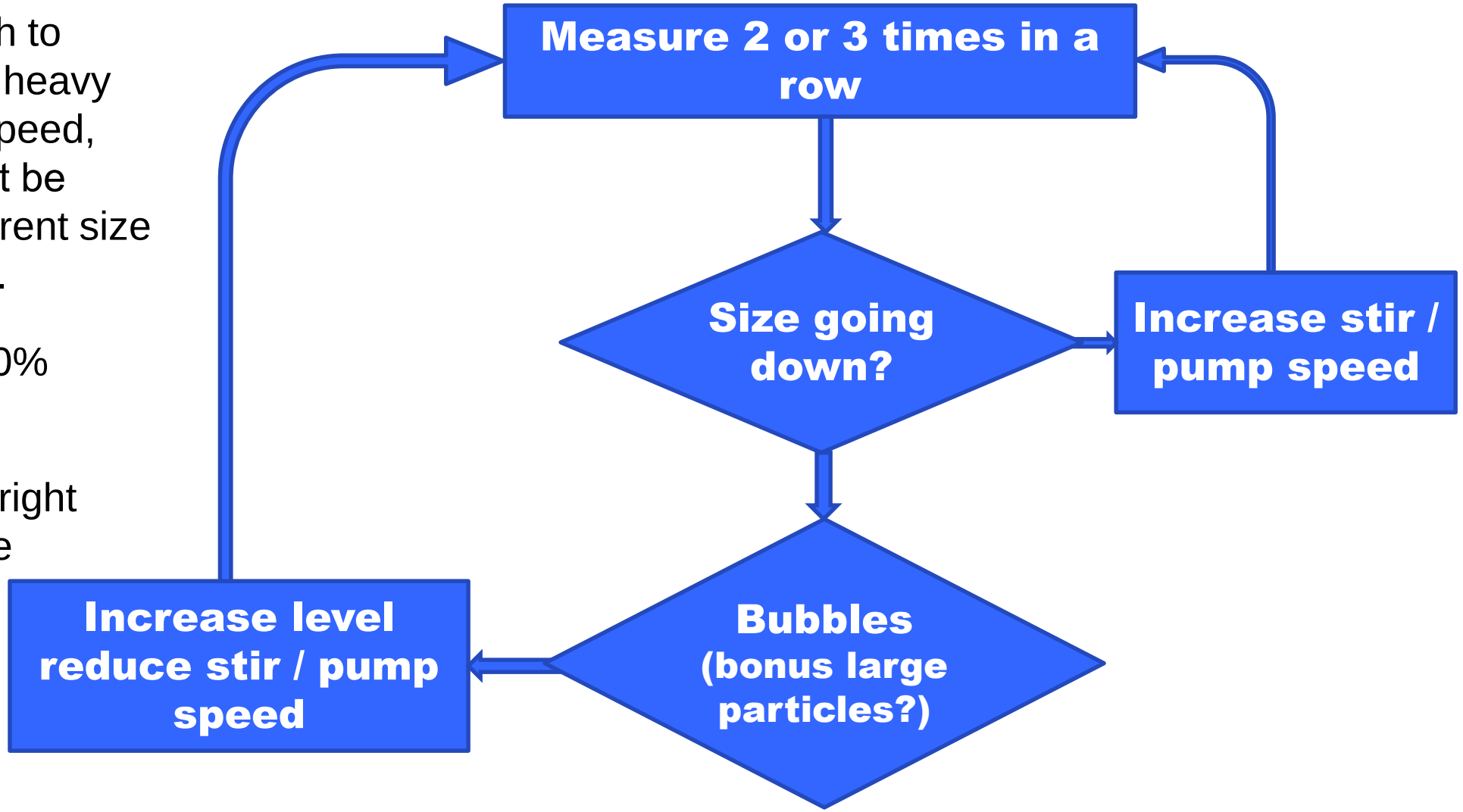






Pump and Stir Start

- Must be high enough to suspend & circulate heavy particles-if too low speed, larger particles won't be measured and apparent size decreases with time.
- Add sample to 85-90% transmission.
- Do this to get in the right neighborhood before systematic study.



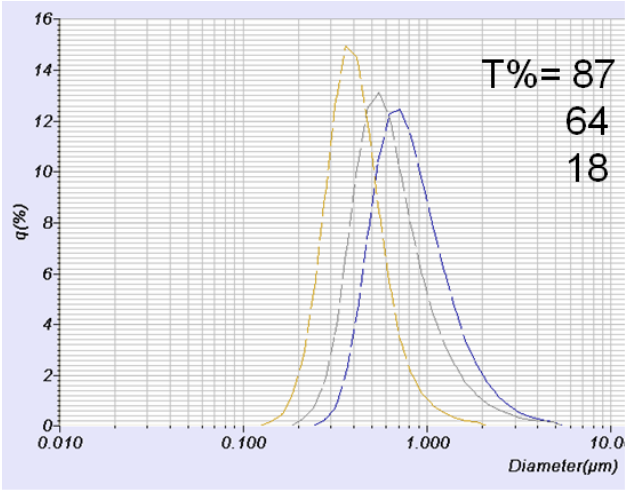
Concentration

Laser diffraction works best in limit of zero concentration

High enough for good S/N ratio

Low enough to avoid multiple scattering

Typically 95 – 80 %T



Rough out pump/stir

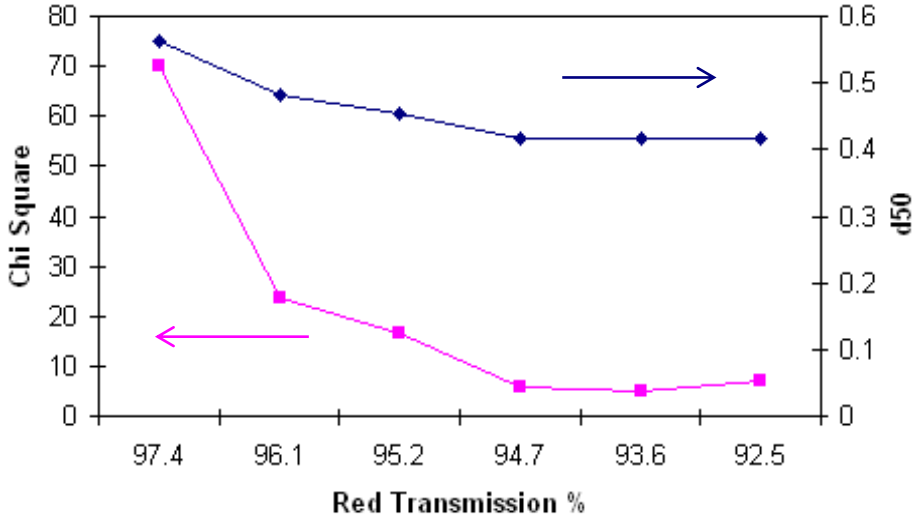
Add sample to ~95% T (low concentration)

Measure size

Add another drop to go to ~90% T→ Measure

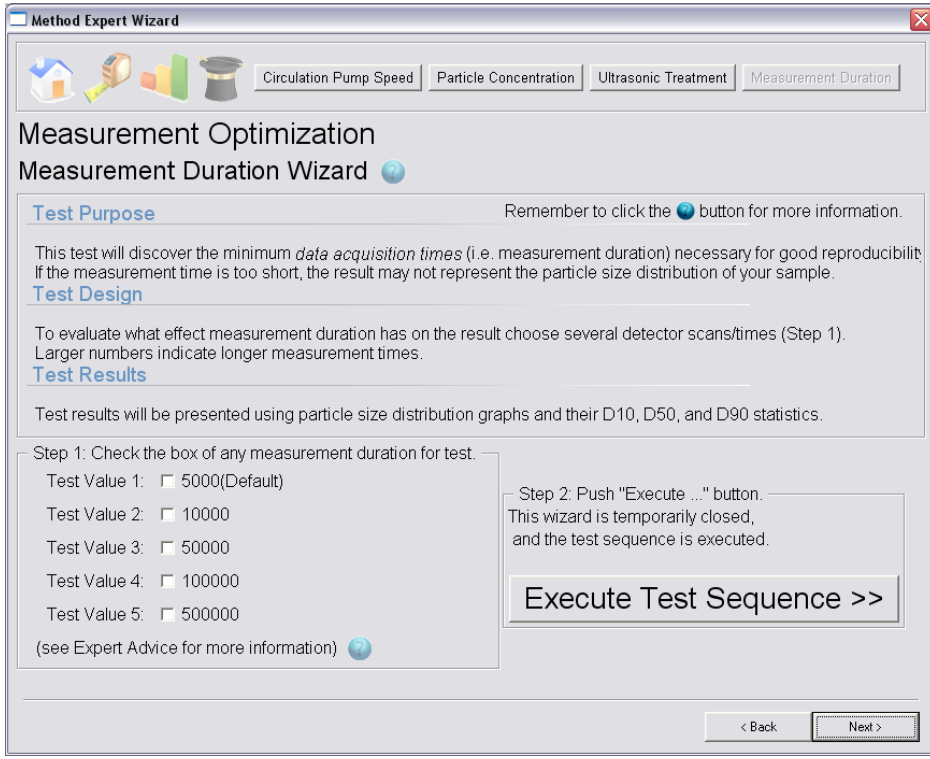
Add another drop to go to 85% T → Measure

Review results (see graph). Size changes with T% or extra peaks? Concentration too high, T% too low.



Duration

- Long enough for reproducibility
- Typically 5 sec (almost always…)
- Longer time for large, broad distributions
- Can be automated in software
- Could be used for robustness testing during method validation



Check repeatability. If size is varying at random, try increasing duration or concentration.

Ultrasonic Dispersion

Adding energy to break up agglomerates – disperse to primary particles, without breaking particles

Similar to changing air pressure on dry powder feeder

Typically set to 100% energy, vary time (sec) on

Investigate tails of distribution

- High end to see if agglomerates removed
- Small end to see if new, smaller particles appear (breakage)

Test reproducibility, consider robustness

Note:

- Do not use on emulsions
- Can cause thermal mixing trouble w/solvents - wait
- Use external probe if t> 2-5 minutes

More in the sampling and dispersion webinar at :
https://www.horiba.com/en_en/products/by-segment/scientific/particle-characterization/particle-analysis-webinar-series/

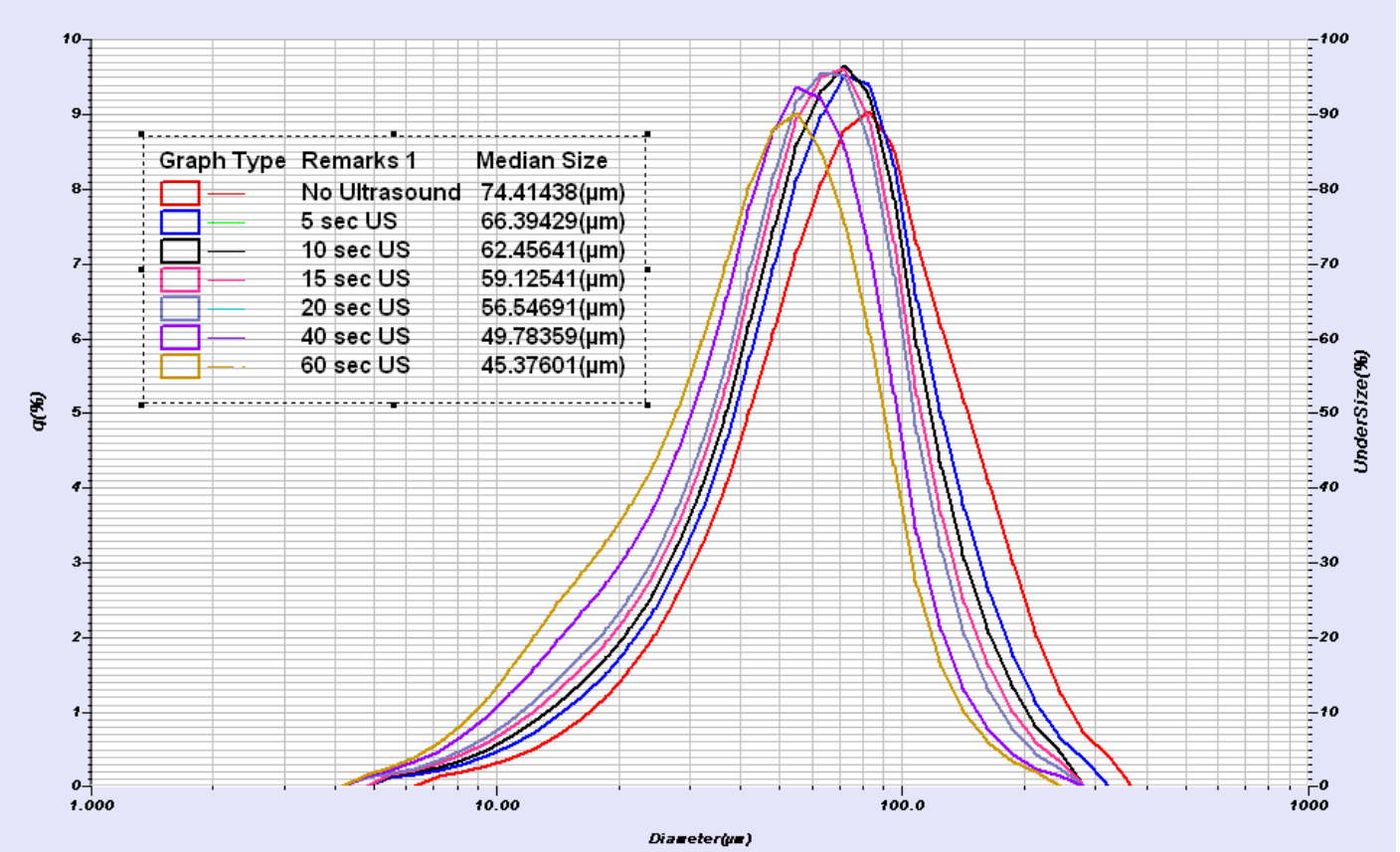
Ultrasound

On board ultrasound can break up loose agglomerates.

Get sample at middle of T% range and rough pump and stir.

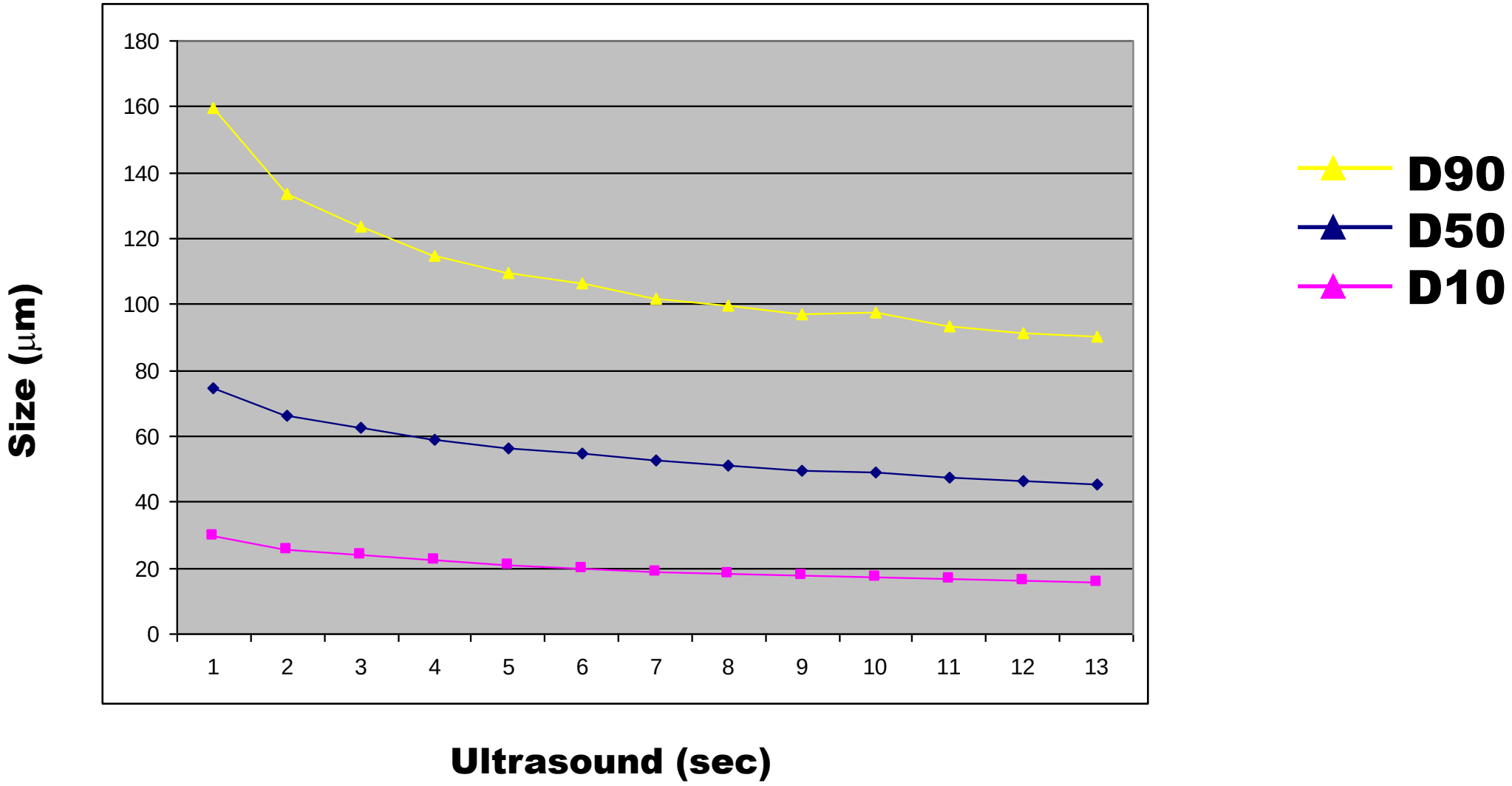
Measure
Ultrasound 10 sec→ measure
10 more seconds → measure
Etc.

Review results. No significant change means way too little or you don't need it. If changing, go to where size stops changing.



If you need a lot of ultrasound, use an external high power unit.

Plot summary data

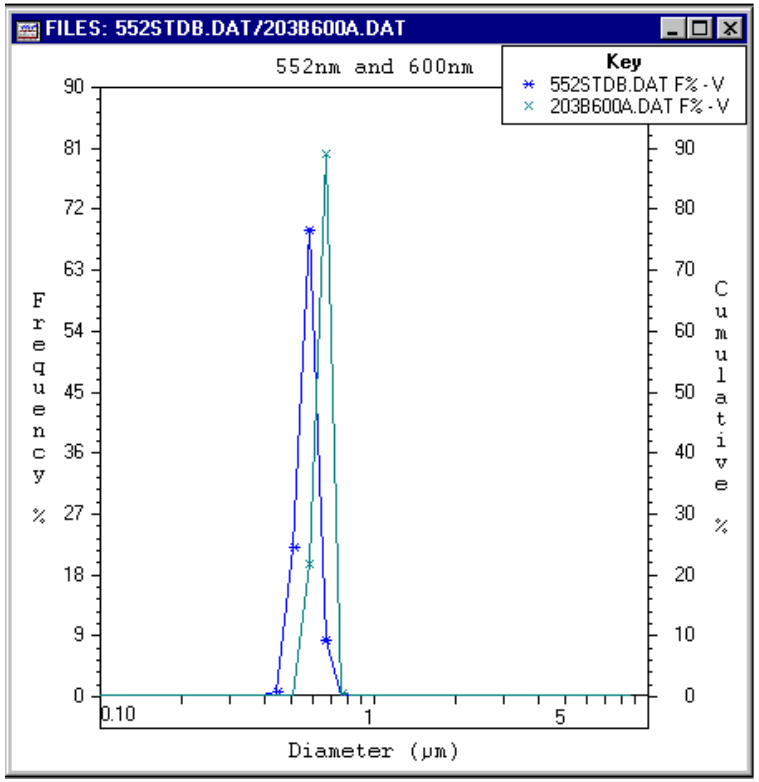


Reproducibility

Need the same answer after drain and refill.

Driven by

- Concentration repeatability/sensitivity→ T%
  - Hard to hit the same T%, predilute before adding
- Sampling
  - Ensure the suspension is well mixed to entrain all particles, even large ones that settle.
- Suspension stability (e.g., agglomeration or dissolution with time)
- Check rinsing here. Do you get your old baseline back?

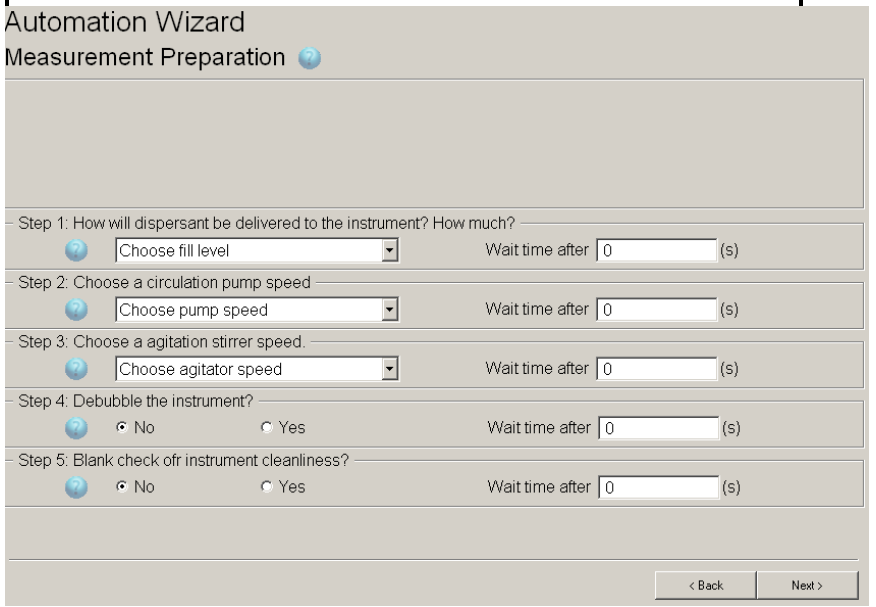


Systematic reporting

- Systematically measure at several settings & select optimum (repeatability)
- Pump +/-1
- Stir +/-1
- Ultrasound +/- 10 seconds
- T% +/- 5%

- Write a report to yourself so you understand your choices.

Exp #	Agitation	Circulation	$D_{mean\ (nm)}$	D_{10} (nm)	D_{90} (nm)
1	1	1	187.03	137.5	245.7
2	1	3	184.23	135.9	242.1
3	3	1	187.28	137.8	245.8
4	3	3	184.61	136.1	242.5
5	1	1	185.32	136.3	243.7
6	1	3	184.04	135.8	241.8
7	3	1	184.13	135.8	241.9
8	3	3	184.98	136.4	242.9



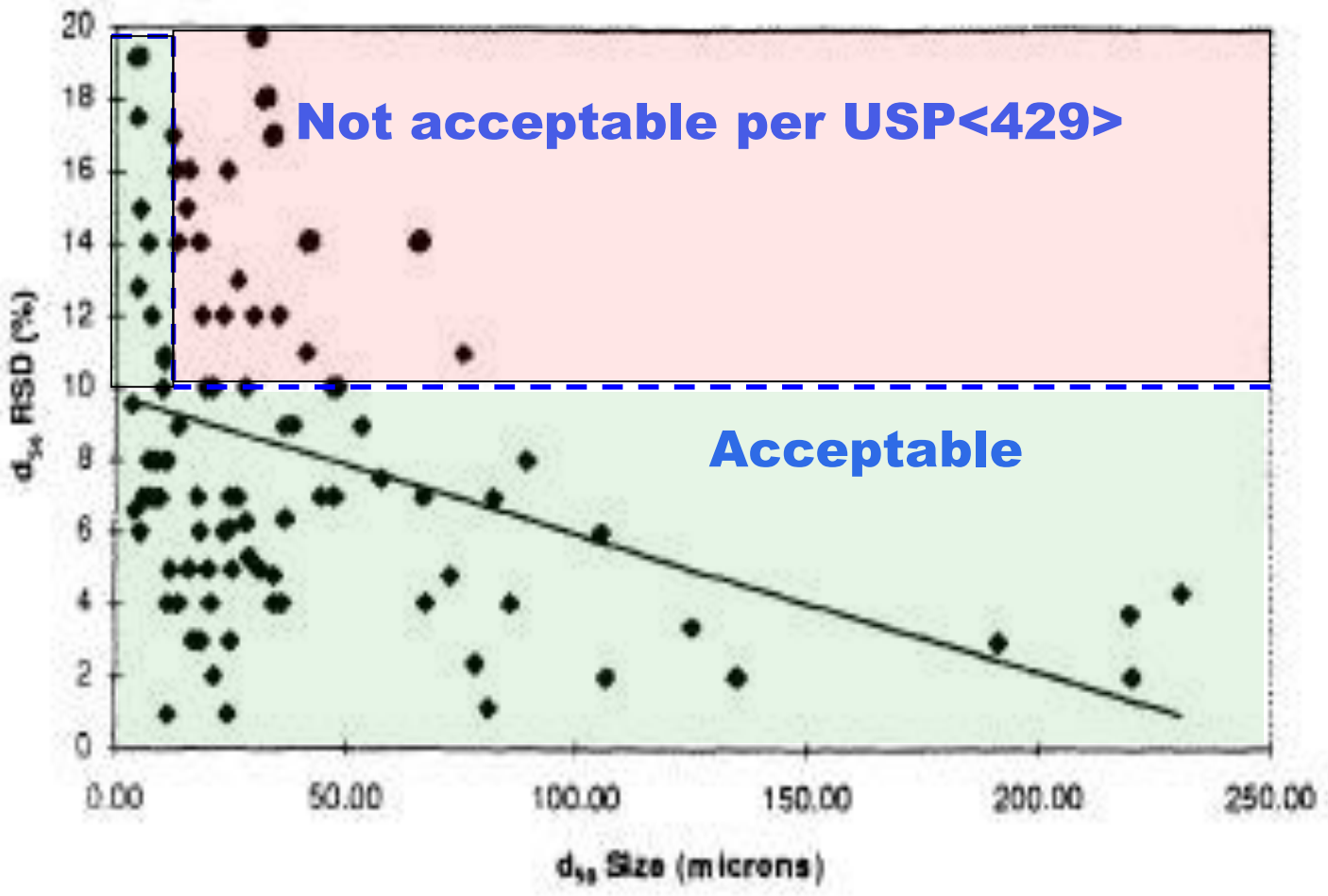
Reproducibility

58 methods

Laser diffraction for PSD

If RSD for d50 < 20%, then acceptable for QC environment

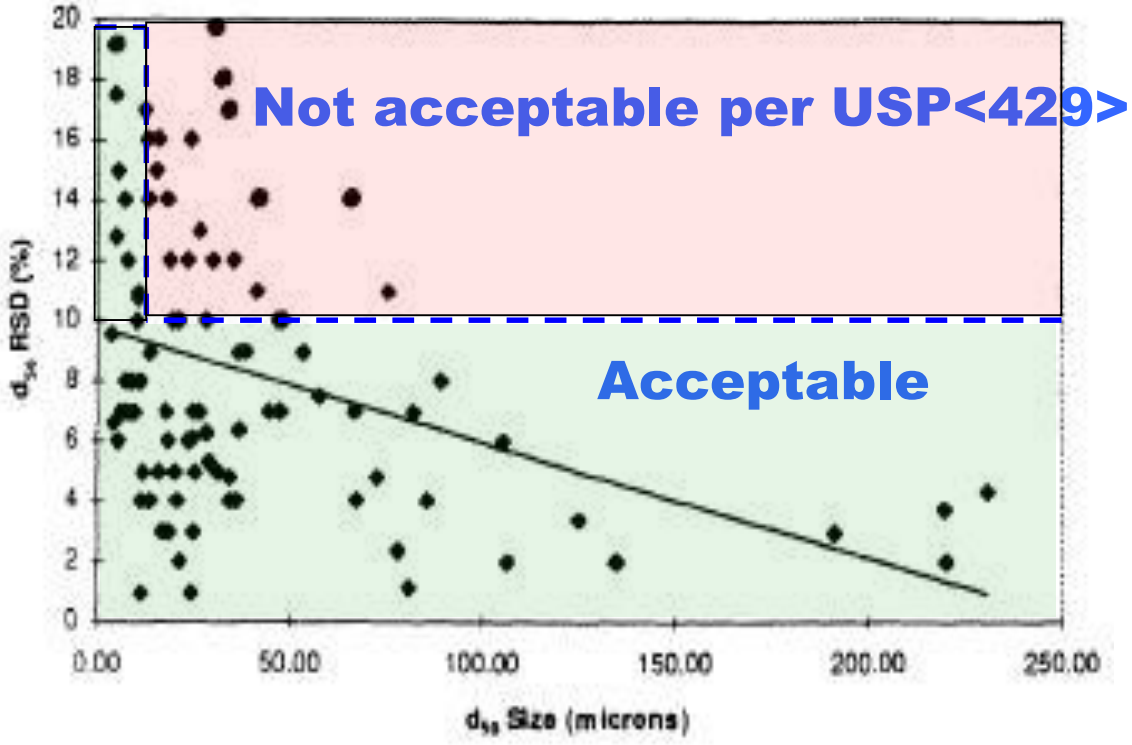
Note: RSD increases with decreasing size

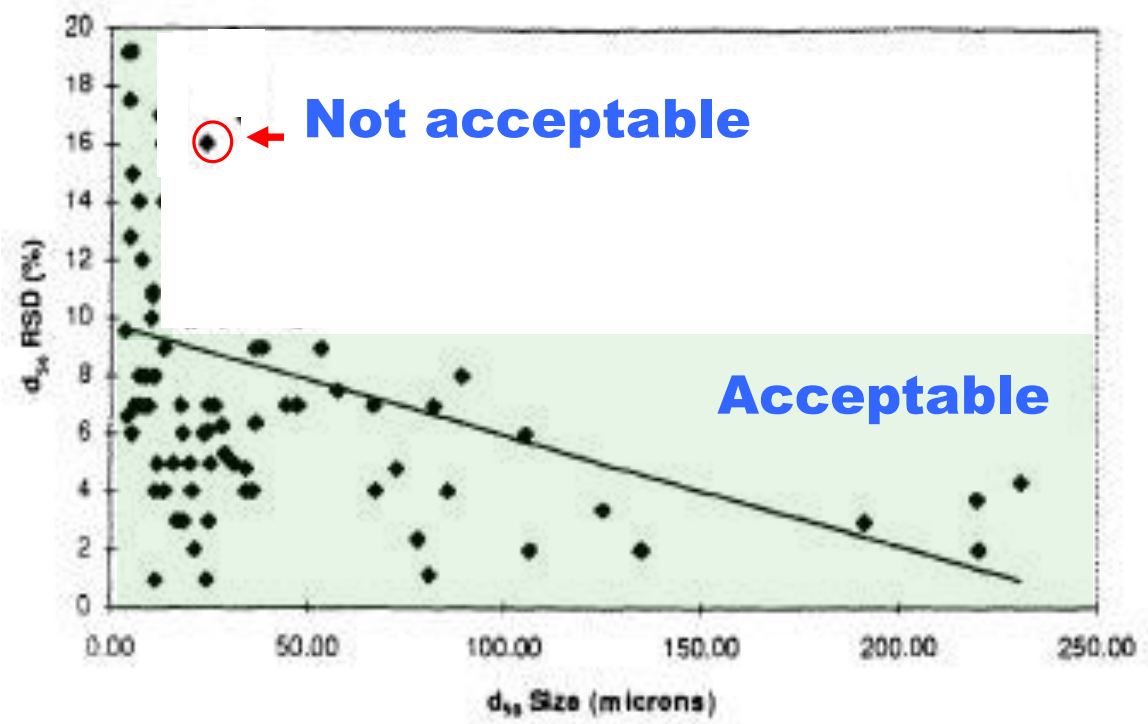


*Barber, Keuter, and Kravig, A Logical Stepwise Approach to Laser Diffraction Particle Size Distribution Analysis Methods Development and Validation Pharmaceutical Development and Technology, 3(2), 153-161 (1998)

Sampler Selection

Remove points from not acceptable region using Fraction Cell





Systematic analysis gives good hint about fraction cell use and importance of pumping for larger particles.

Automation

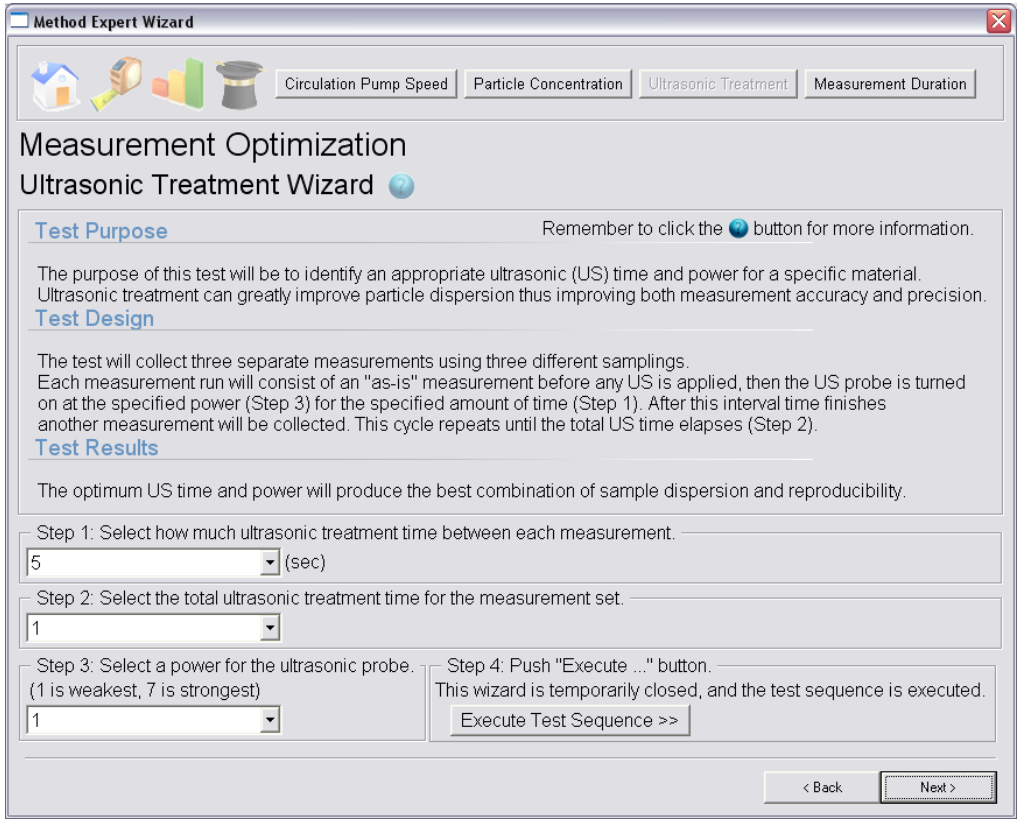
LA-960 Method Expert will systematically vary measurement parameters:

Refractive Index

Pump

Ultrasound

Generate result graphs



Dry Method Development

Workflow

First get sampling right & determine RI

Measure at 3 different pressures (low, medium, high)

Determine optimum pressure based on good dispersion while not breaking particles

Can also compare dry vs. wet measurements

Adjust other settings to optimize

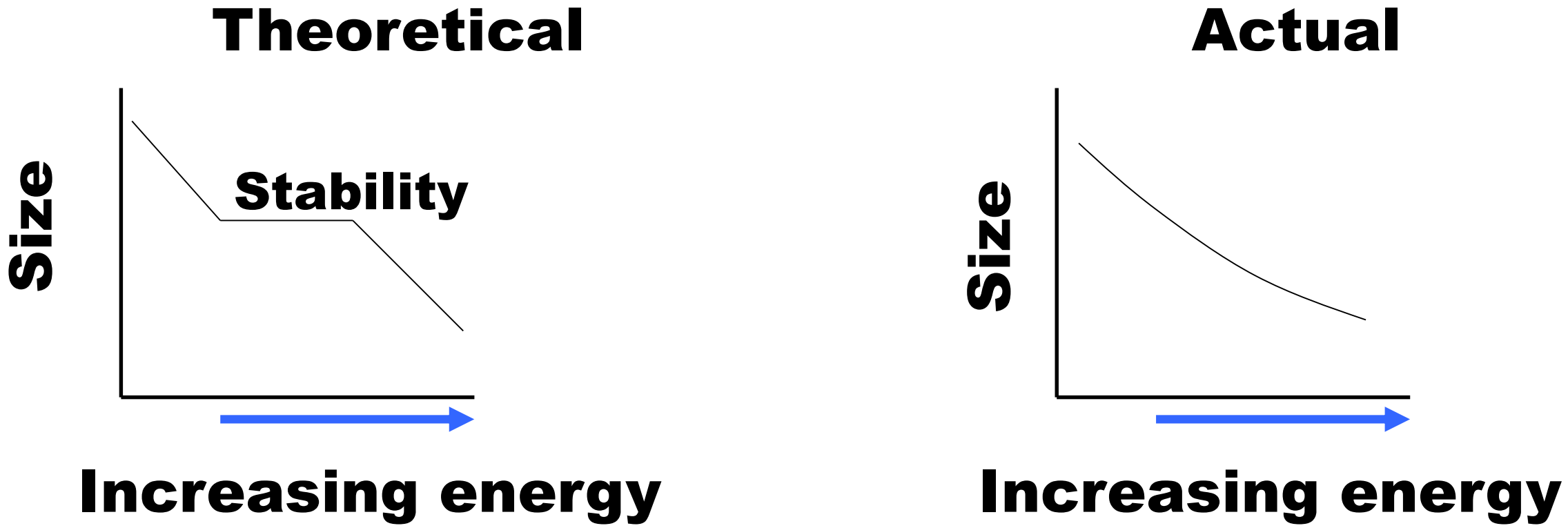
- sample concentration (T%)
- duration (amount of material)

Ideally measure all of powder placed into the sampler

- Segregation can occur on vibrating tray
- Constant mass flow rate important for stable concentration (T%) during measurement

Once settings chosen, test reproducibility

Dispersion vs Breakage

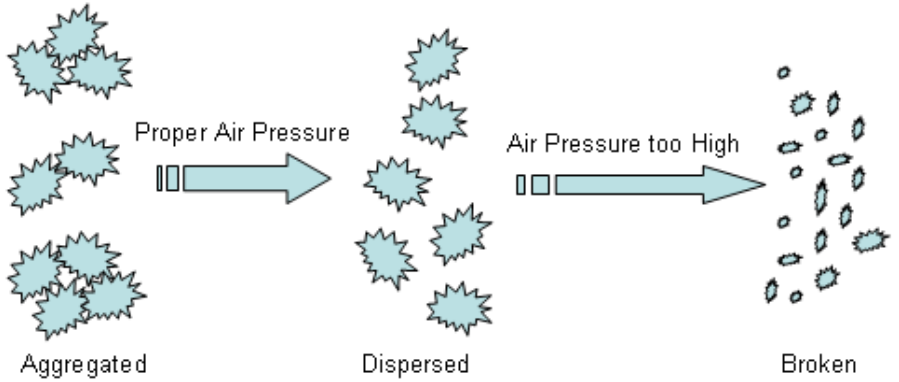


Higher air pressure or longer ultrasound duration

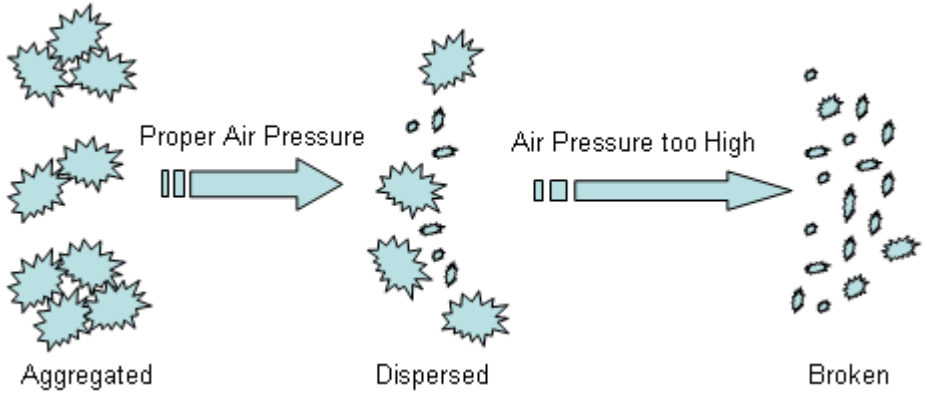
Dispersion vs Breakage

- **Dispersion and milling can be parallel rather than sequential processes**

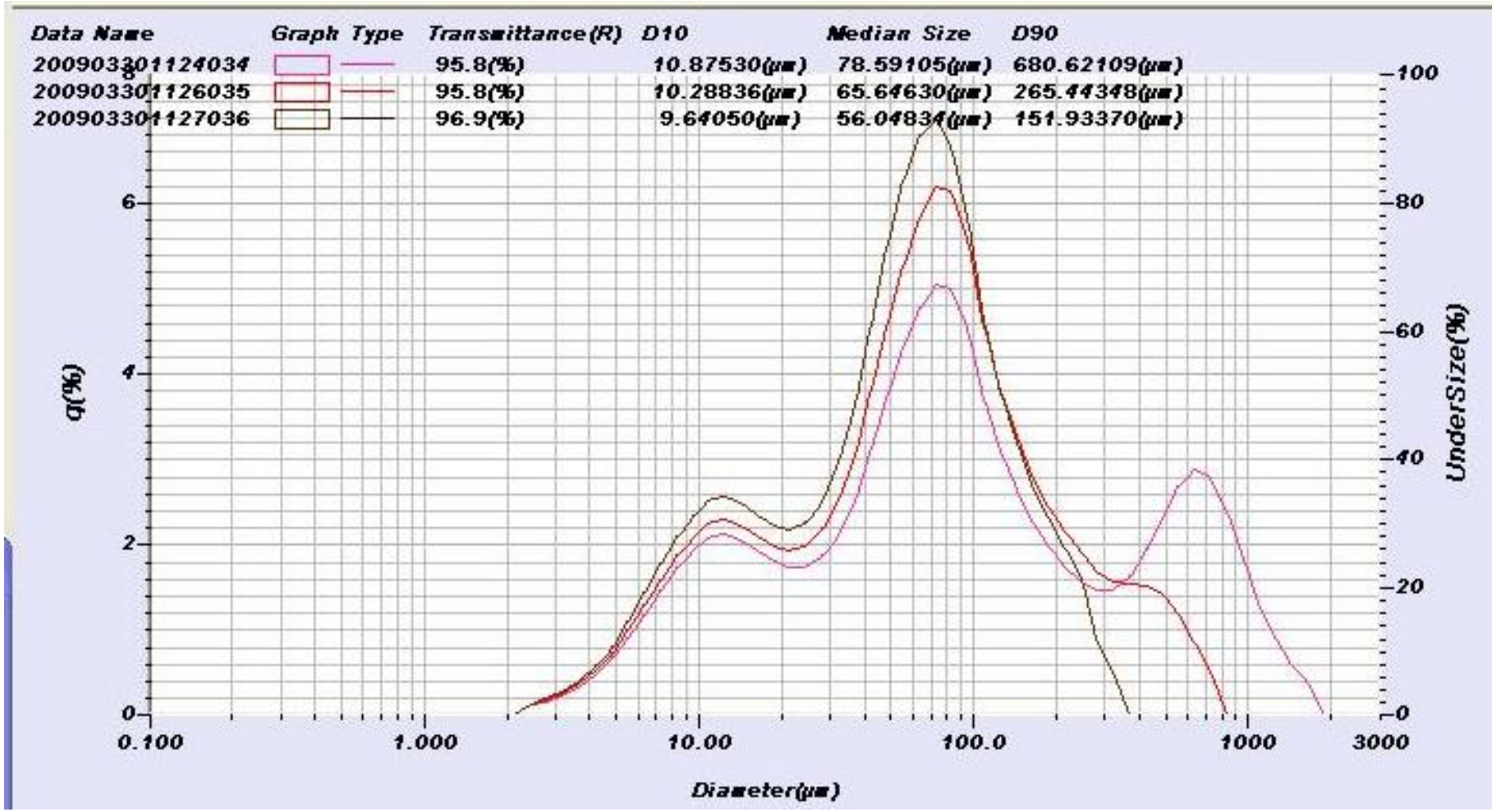
Theoretical



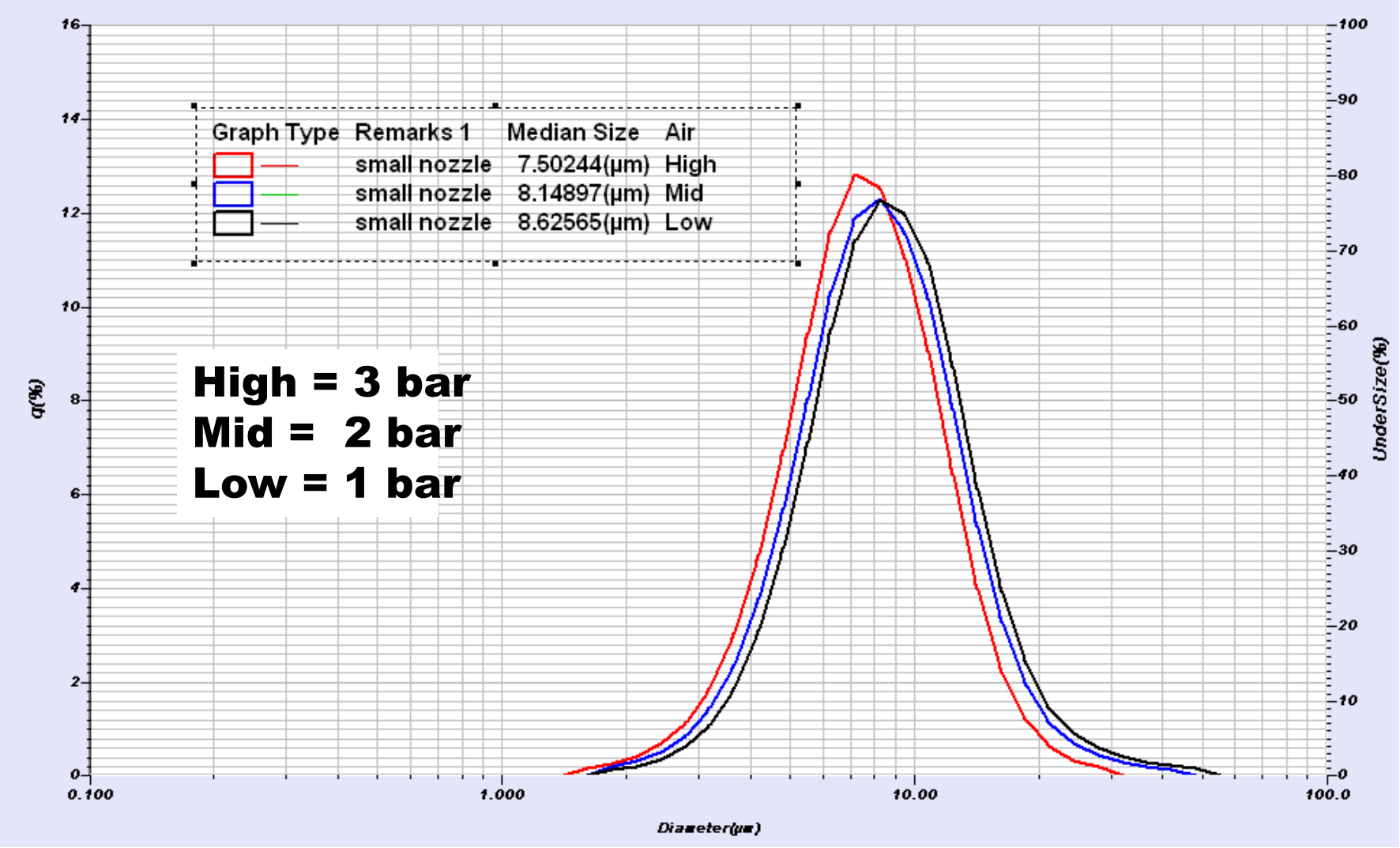
Actual



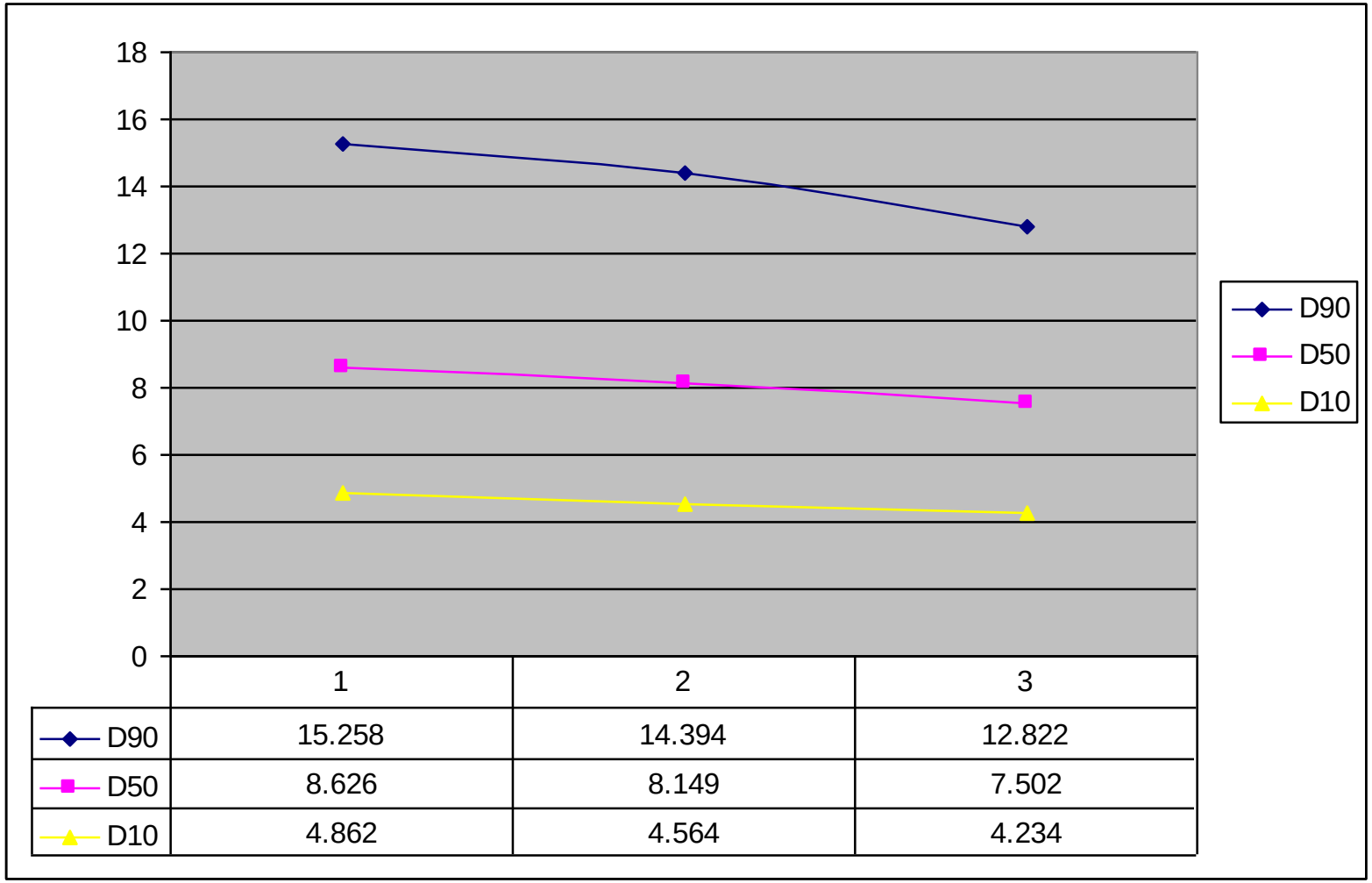
Pressure Titration



Mg Stearate

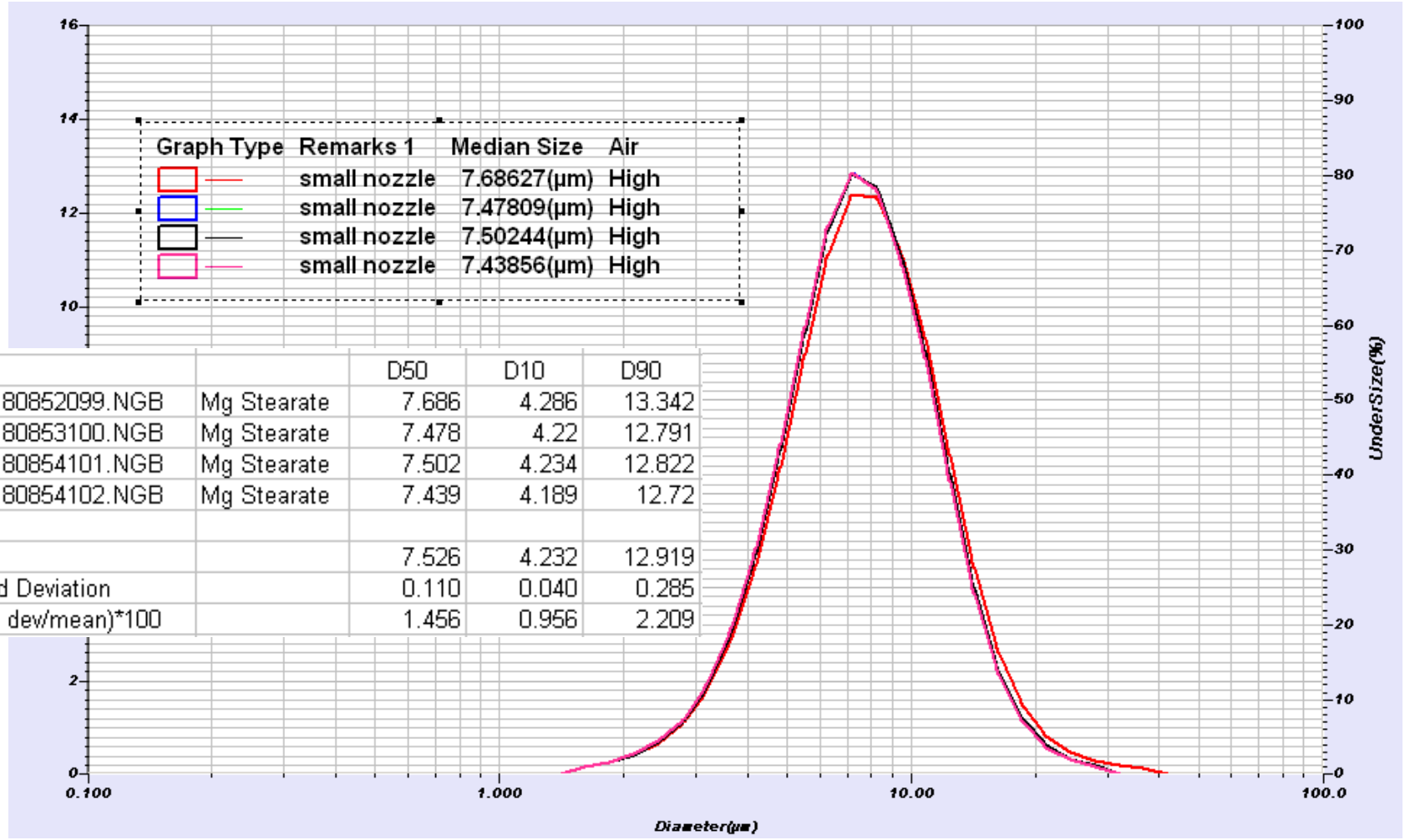


Mg Stearate



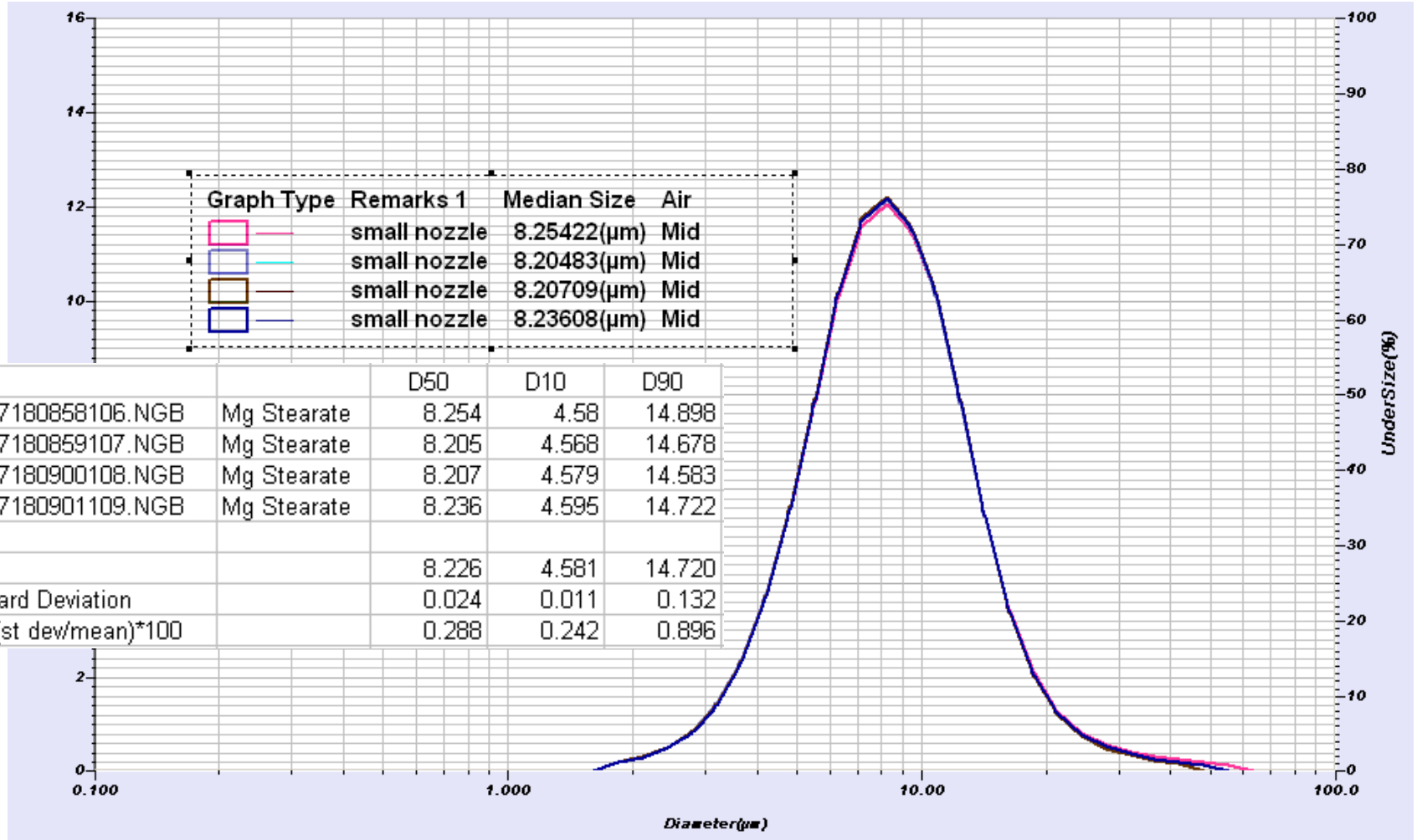
	1	2	3
D90	15.258	14.394	12.822
D50	8.626	8.149	7.502
D10	4.862	4.564	4.234

Reproducibility at 3 Bar



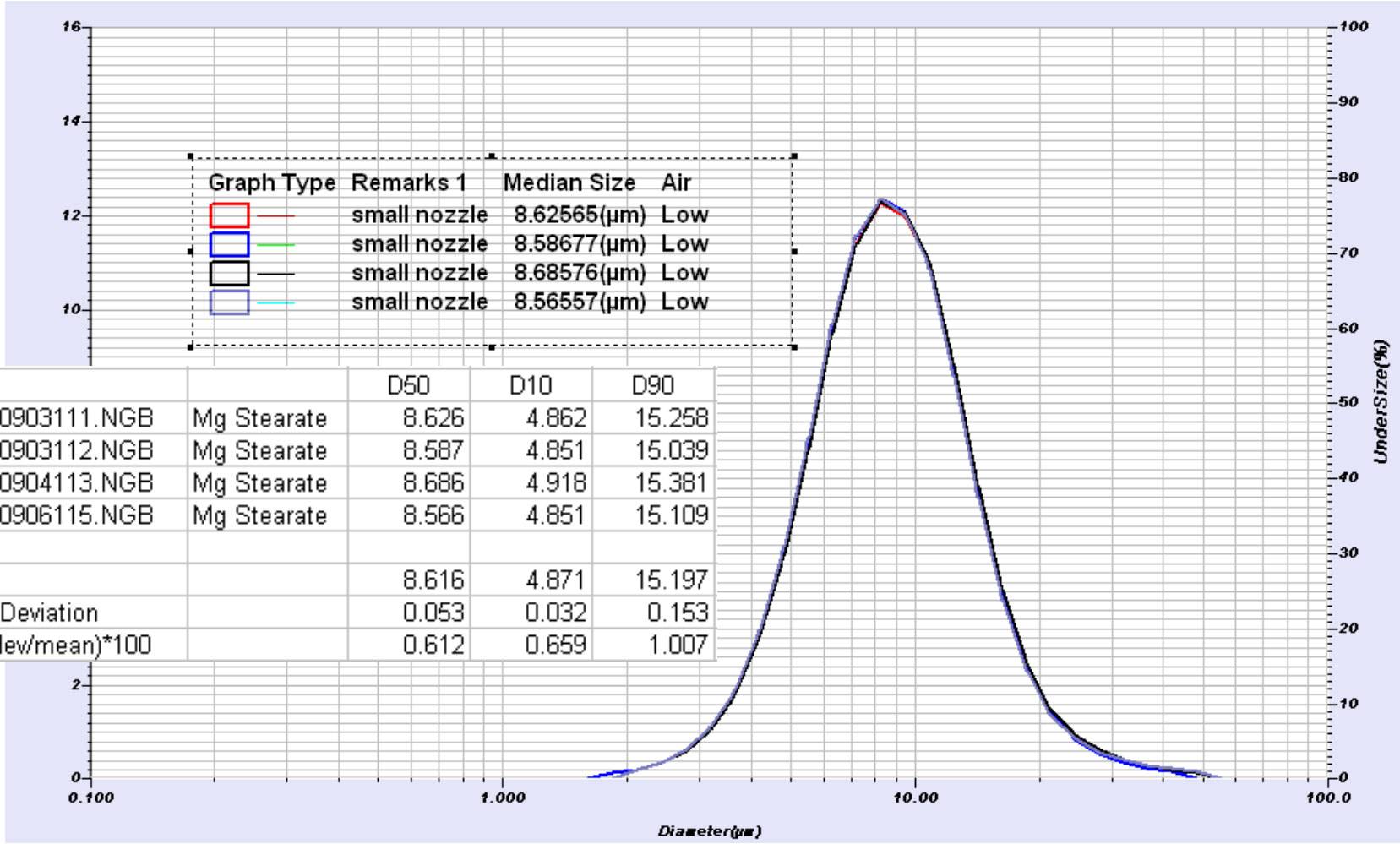
		D50	D10	D90
200707180852099.NGB	Mg Stearate	7.686	4.286	13.342
200707180853100.NGB	Mg Stearate	7.478	4.22	12.791
200707180854101.NGB	Mg Stearate	7.502	4.234	12.822
200707180854102.NGB	Mg Stearate	7.439	4.189	12.72
Mean		7.526	4.232	12.919
Standard Deviation		0.110	0.040	0.285
COV (st dev/mean)*100		1.456	0.956	2.209

Reproducibility at 2 Bar



		D50	D10	D90
200707180858106.NGB	Mg Stearate	8.254	4.58	14.898
200707180859107.NGB	Mg Stearate	8.205	4.568	14.678
200707180900108.NGB	Mg Stearate	8.207	4.579	14.583
200707180901109.NGB	Mg Stearate	8.236	4.595	14.722
Mean		8.226	4.581	14.720
Standard Deviation		0.024	0.011	0.132
COV (st dev/mean)*100		0.288	0.242	0.896

Reproducibility at 1 Bar



		D50	D10	D90
200707180903111.NGB	Mg Stearate	8.626	4.862	15.258
200707180903112.NGB	Mg Stearate	8.587	4.851	15.039
200707180904113.NGB	Mg Stearate	8.686	4.918	15.381
200707180906115.NGB	Mg Stearate	8.566	4.851	15.109
Mean		8.616	4.871	15.197
Standard Deviation		0.053	0.032	0.153
COV (st dev/mean)*100		0.612	0.659	1.007

Summarize Reproducibility

Pressure	D10 COV	D50 COV	D90 COV
1 Bar	0.659	0.612	1.007
2 Bar	0.242	0.288	0.896
3 Bar	0.956	1.456	2.209

This is reproducibility; Sampling is important!

Must have representative sample

T%, quantity

T% is done much like you did with wet. Vary T% setting and monitor results

Sample Quantity. Vary quantity and monitor results.

Summary

Design for maximum precision.

Follow guidelines in standards.

Liquid Suspensions: wet, disperse

Investigate system settings: concentration, agitation, ultrasound.

Dry Powders: select air pressure

Must have representative sample.

Systematically evaluate the method.

Prepare a report in case there are questions later.

Thank you

Thank you

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

धन्यवाद

Grazie

Merci

谢谢

நன்ற

ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

شُكْرا

Tack ska ni ha

Большое спасибо

Danke

Gracias