Innopharma technology

HORIBA Scientific

Modern Particle Characterization Techniques Series

Image Analysis

Overview

Introduction

What is image analysis?

Common Questions

How Image Analysis is done

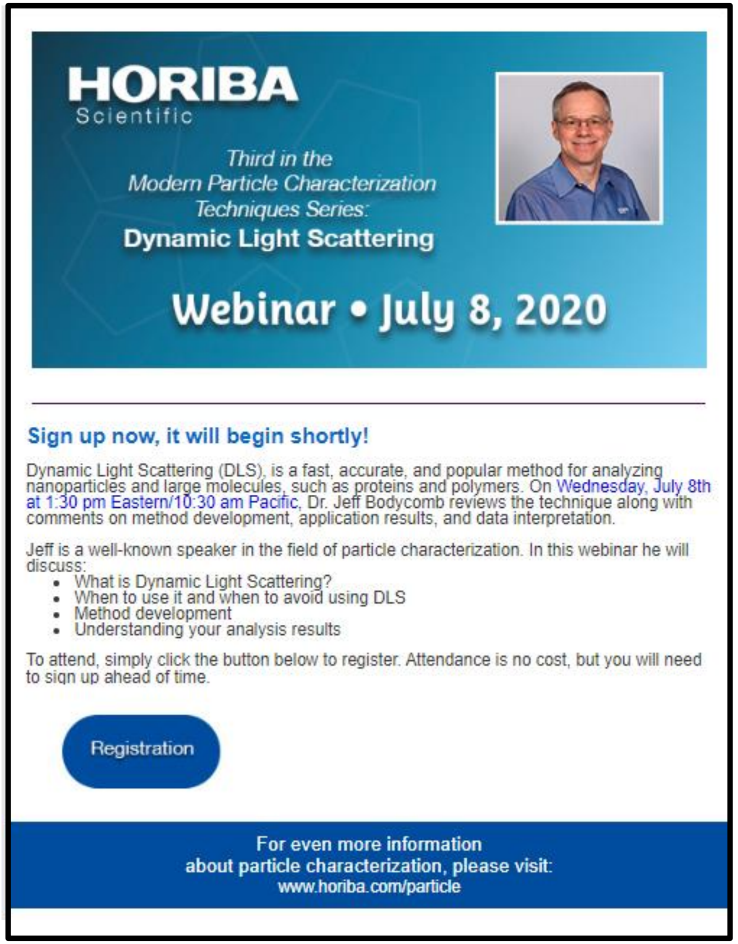
Future Perspectives

A word from our sponsor

Did you sign up for our newsletter?

Receive regular updates and news on the world of particle analysis.

Send us a chat or e-mail your desire to labinfo@horiba.com



HORIBA Scientific

Third in the Modern Particle Characterization Techniques Series:

Dynamic Light Scattering

Webinar • July 8, 2020

Sign up now, it will begin shortly!

Dynamic Light Scattering (DLS), is a fast, accurate, and popular method for analyzing nanoparticles and large molecules, such as proteins and polymers. On Wednesday, July 8th at 1:30 pm Eastern/10:30 am Pacific, Dr. Jeff Bodycomb reviews the technique along with comments on method development, application results, and data interpretation.

Jeff is a well-known speaker in the field of particle characterization. In this webinar he will discuss:

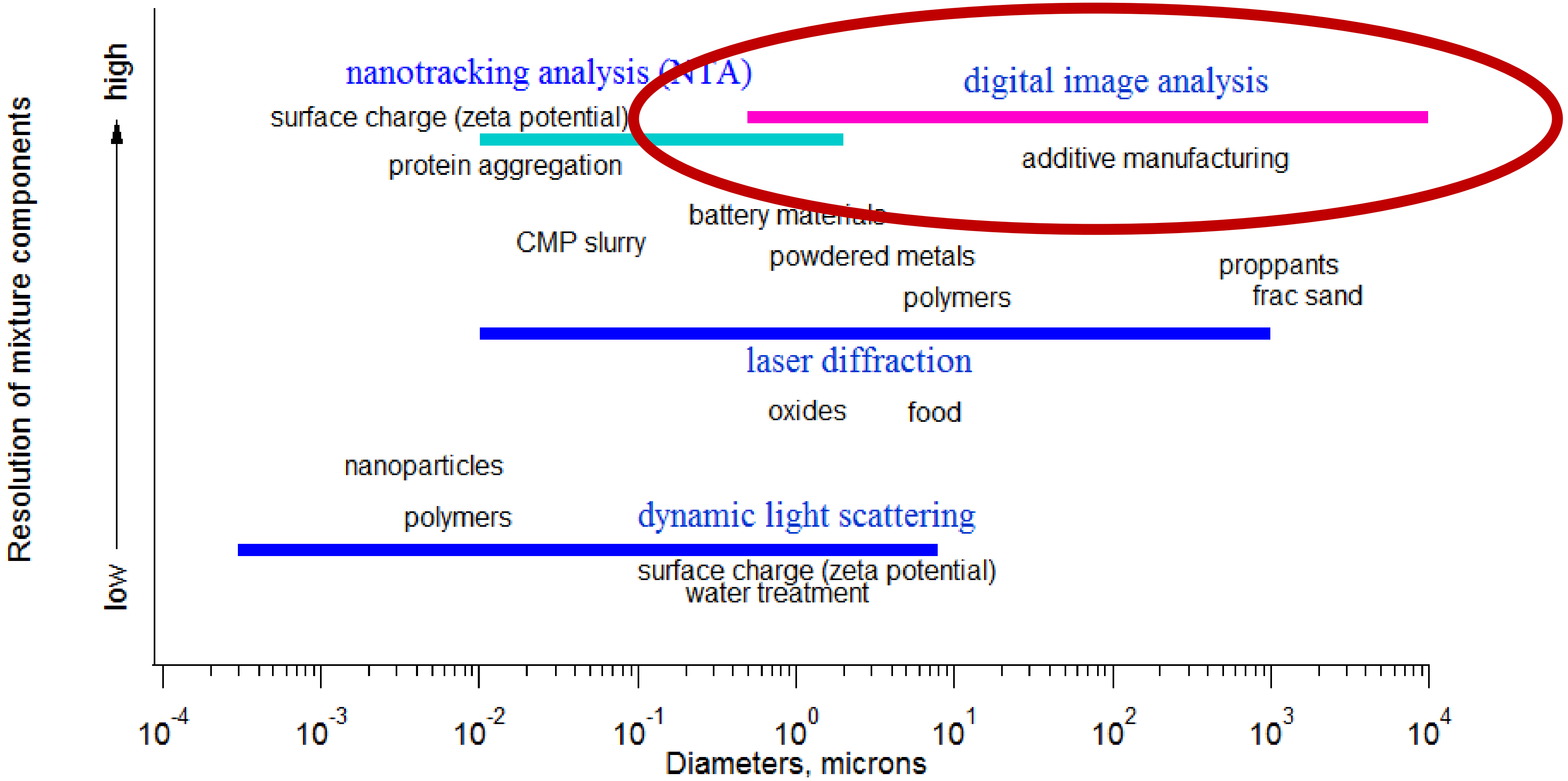
- What is Dynamic Light Scattering?
- When to use it and when to avoid using DLS
- Method development
- Understanding your analysis results

To attend, simply click the button below to register. Attendance is no cost, but you will need to sign up ahead of time.

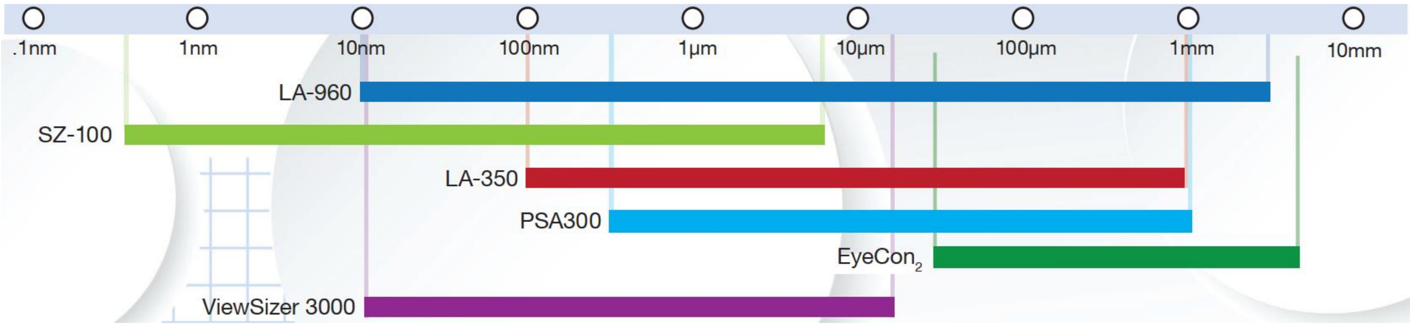
Registration

For even more information about particle characterization, please visit: www.horiba.com/particle

Sizing techniques

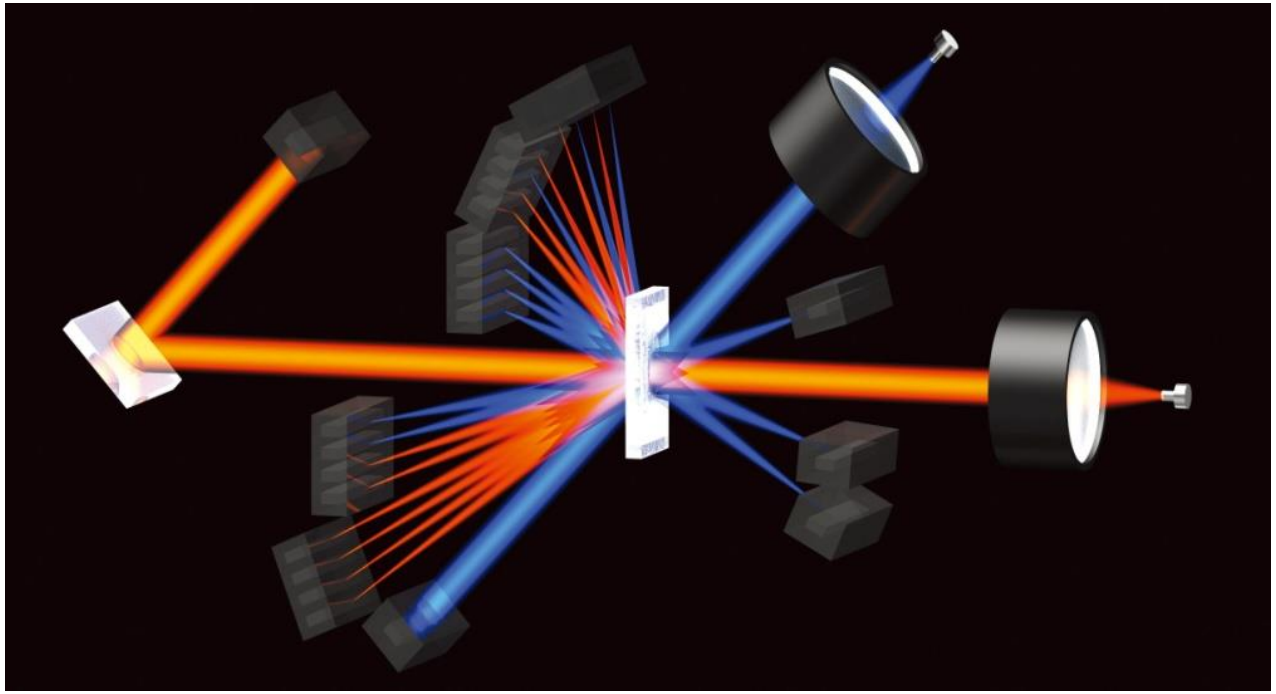


Particle Analysis – choices



	LA-960V2	LA-350	SZ-100V2	PSA300	ViewSizer 3000	EyeCon2	SA-9600
Technology	Laser Diffraction	Laser Diffraction	Dynamic Light Scattering, Electrophoretic Light Scattering, Debye Plot Method	Static Image Analysis	Nanoparticle Tracking Analysis**	Direct Imaging	BET Flowing Gas Adsorption and Desorption
Measurement Output	Particle Size	Particle Size	Particle Size, Zeta Potential, Molecular Weight	Particle Size and Shape	Particle Size	Particle Size and Shape	Surface Area
Measurement Range	10nm to 5000 µm	0.1 µm to 1000 µm	0.3 nm to 8 µm	0.5 µm to 1000 µm	10 nm to 15 µm	50 to 5500 µm	0.1m² to 50 m²
Typical Sample Amount*	~10 mg to 5 g	~10 mg to 5 g	100 µL to 3 mL	1 mg to 10 mg	350 µL to 1 mL	Continuous monitoring inline or benchtop	<1 g
External Dimensions	705 x 565 x 500 mm	297 x 429 x 376 mm	385 x 528 x 273 mm	686 x 508 x 305 mm	550 x 660 x 350 mm	250 x 128 x 132 mm	508 x 356 x 356 mm
Light Source/ Resolution	605 nm Laser Diode 405 nm LED	605 nm Laser Diode	532 nm Laser Diode 90°, 173° detectors	2.1 MP mono camera	445 nm blue laser, 520 nm green laser, 635 nm red laser with variable power output	12x3 High intensity, low energy RGB LEDs	NA

Laser diffraction



Laser Diffraction

- Particle size 0.01 – 3000 µm
- Converts scattered light to particle size distribution
- Quick, repeatable
- Most common technique
- Suspensions & powders

Laser diffraction

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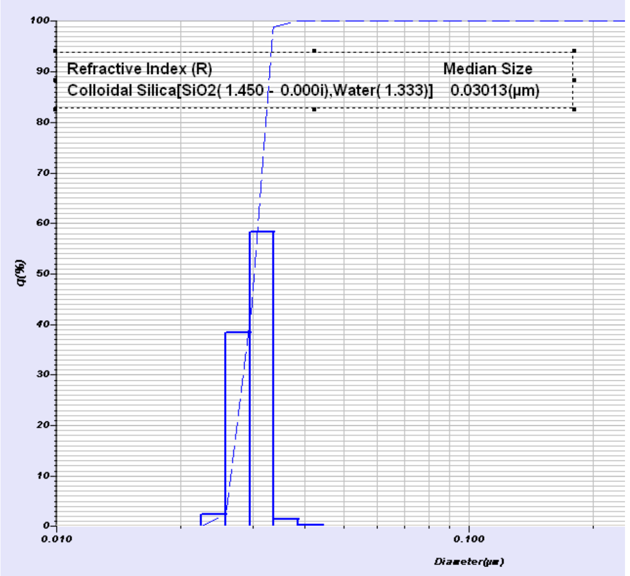
Suspension

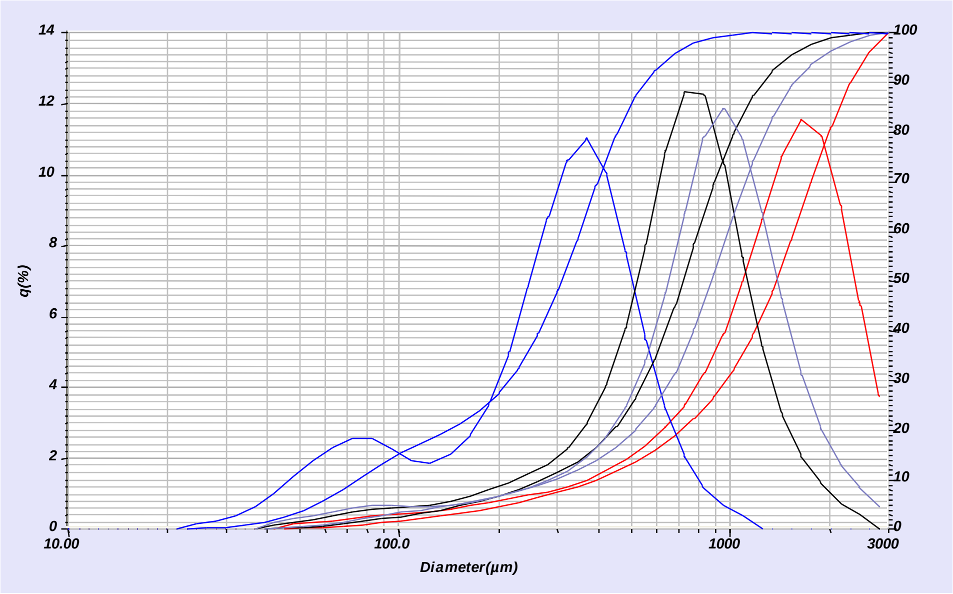
Silica
~ 30 nm

S.P.Area	:	2.0183E+6(cm²/cm³)
Mean Size	:	0.02990(μm)
Variance	:	5.0313E-6(μm²)
Median Size	:	0.03013(μm)
Mode Size	:	0.0302(μm)
Skewness	:	-0.2901

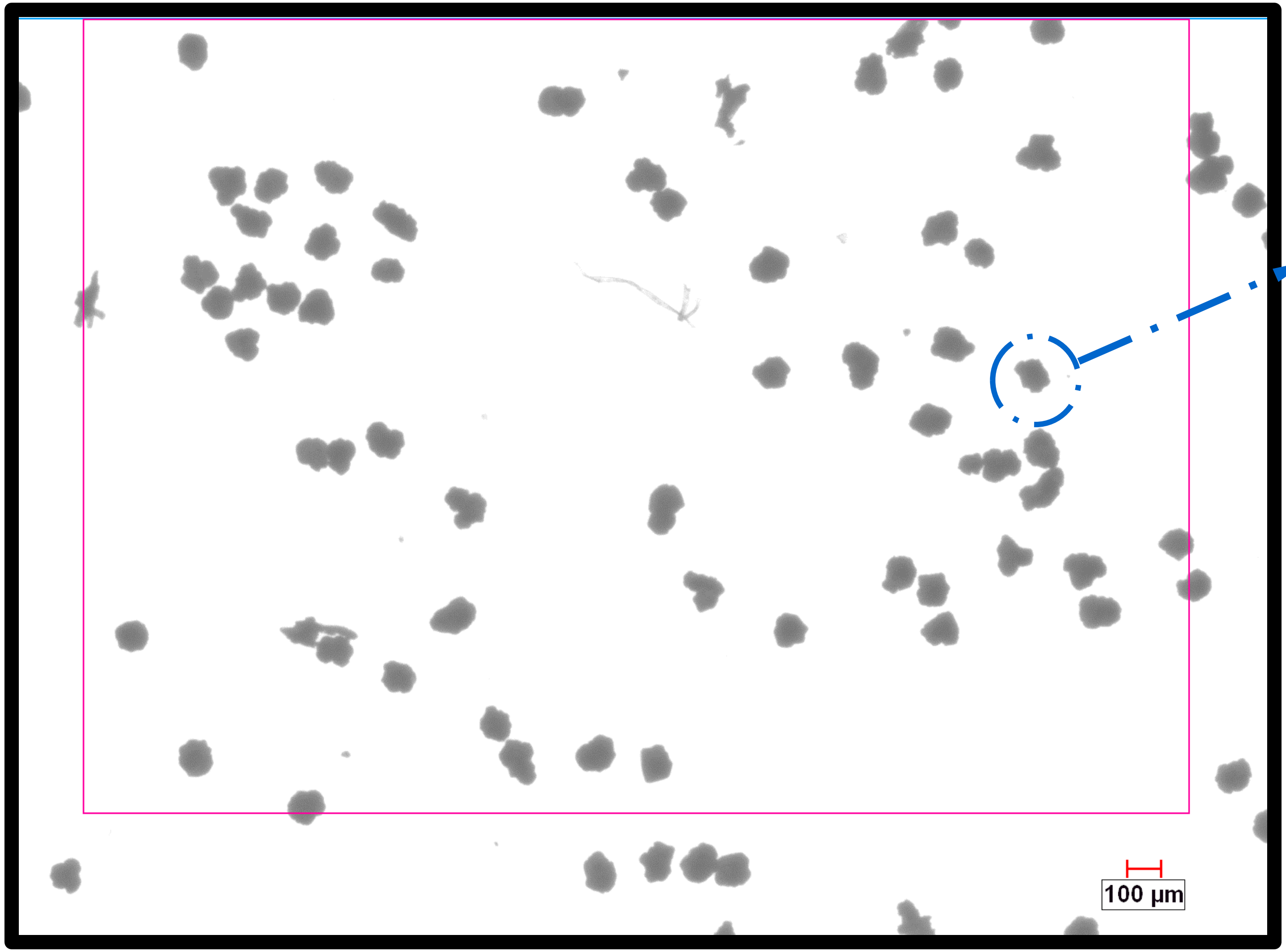


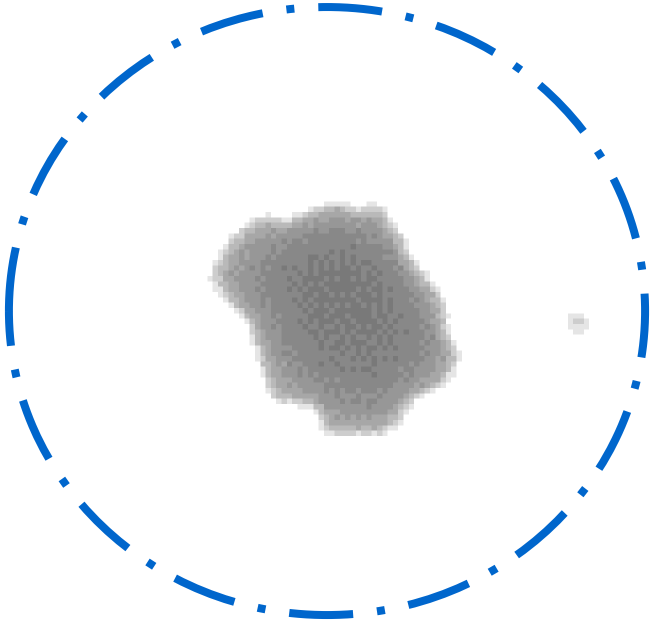
Powders

Coffee Results
0.3 – 1 mm



What is image analysis?

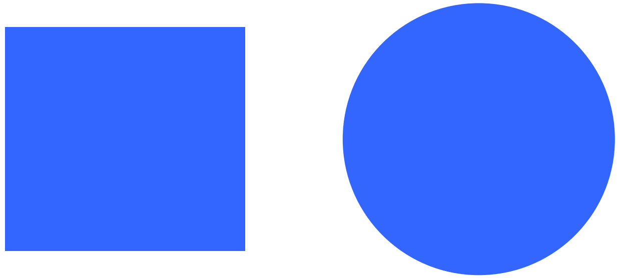




Take a picture, analyze for **size** and **shape**

Why image analysis?

- Replace sieves (really!)
- Verify/supplement laser diffraction results (orthogonal technique).
- Access larger sizes than laser diffraction
- Need shape information, for example due to importance of powder flow
- Particle size is a critical quality attribute (CQA) in the realm of Oral solid dosage (OSD) manufacture.
- Particle size directly relates to:
- Flowability
  - Blend uniformity
  - Compressibility
  - Dissolution rate
  - Bioavailability



Same size (cross section),
but behave very differently.

Why image analysis?

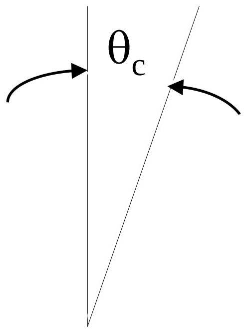
Replace Sieves

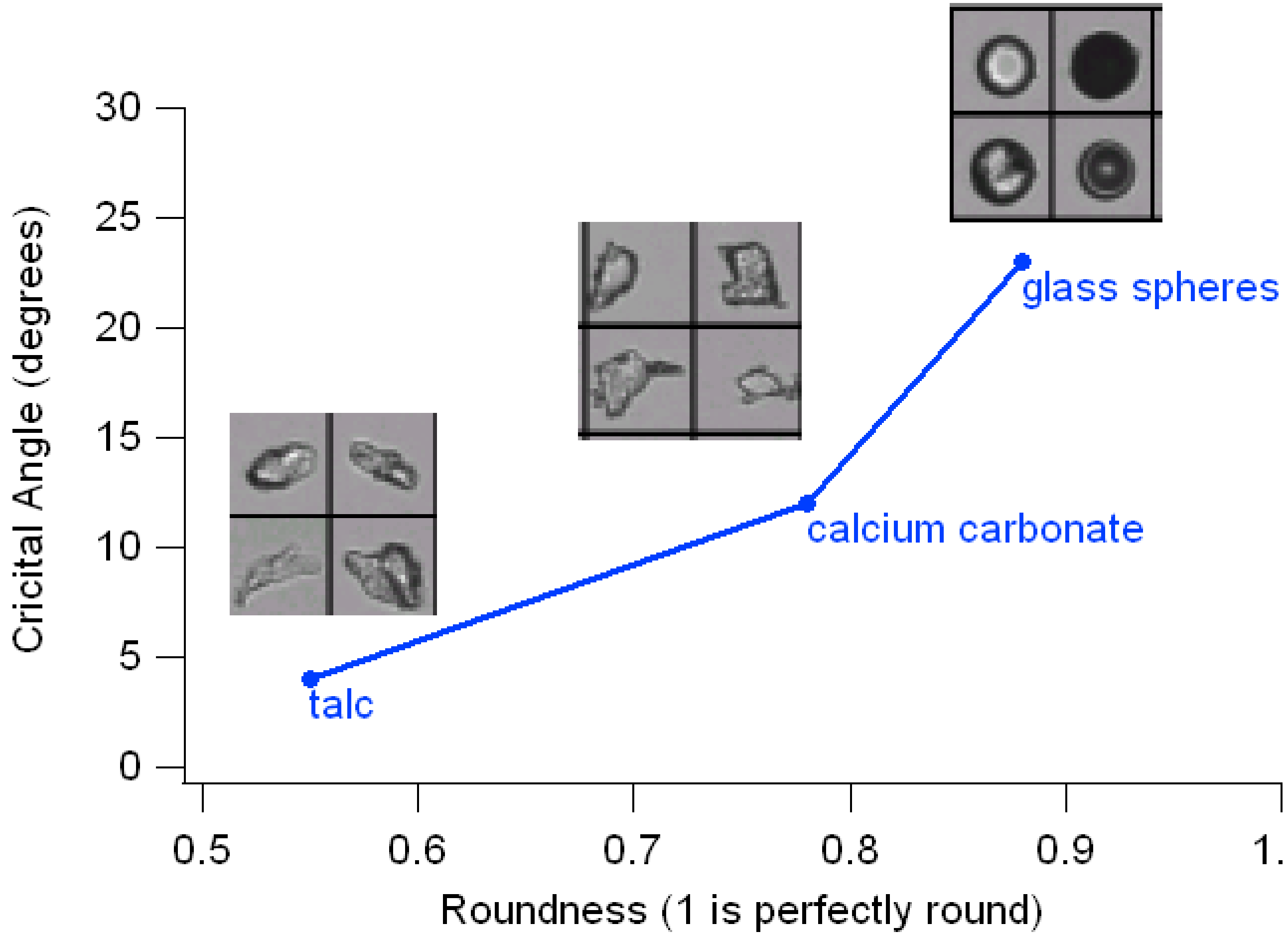
- Tend to wear over time. It is difficult to tell when sieve results are “drifting” due to wear
- Results depend on nature of shaking and loading leading to operator-to-operator variations in results
- Small number of size classes

Courtesy Wikipedia user © BMK (de.wikipedia.org)“
//commons.wikimedia.org/wiki/File:Laboratory_sieves_BMK.jpg

Effect of shape on flow

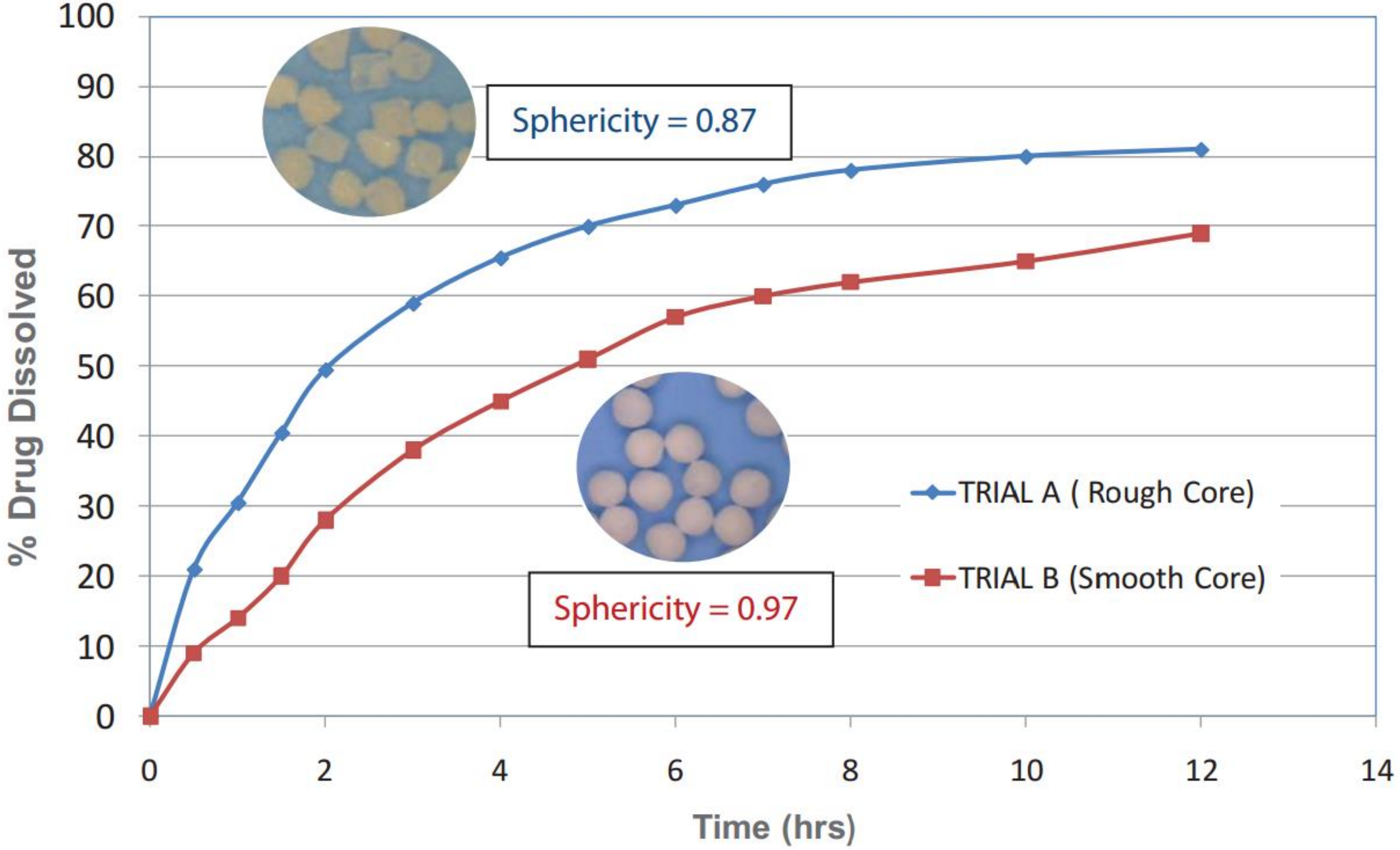
- Yes, I assumed density doesn't matter.
- Roundness is a measure based on particle perimeter.





Multiparticulate drugs

Shape affects drug release profile



Common Questions

How many particles?

Minimum Particle Size

Maximum Particle Size

Accuracy

<USP> 776: Mean

$$u = z\left(\frac{s}{\sqrt{n}}\right)$$

$$n = \left(\frac{sz}{u}\right)^2$$

u=uncertainty
z=confidence coefficient (often ~2, see a statistics book)
s=standard deviation of distribution (width)
n=number of particles measured

Example: for uncertainty of ± 5 µm with st dev= 20 µm
Must measure 61 particles

$$n = \left(\frac{sz}{u}\right)^2 = \left(\frac{(20)(1.96)}{5}\right)^2 \approx 62$$

Implies normal particle size distribution, greater than 30 particles, and known standard deviation.

<USP> 776: Standard Deviation

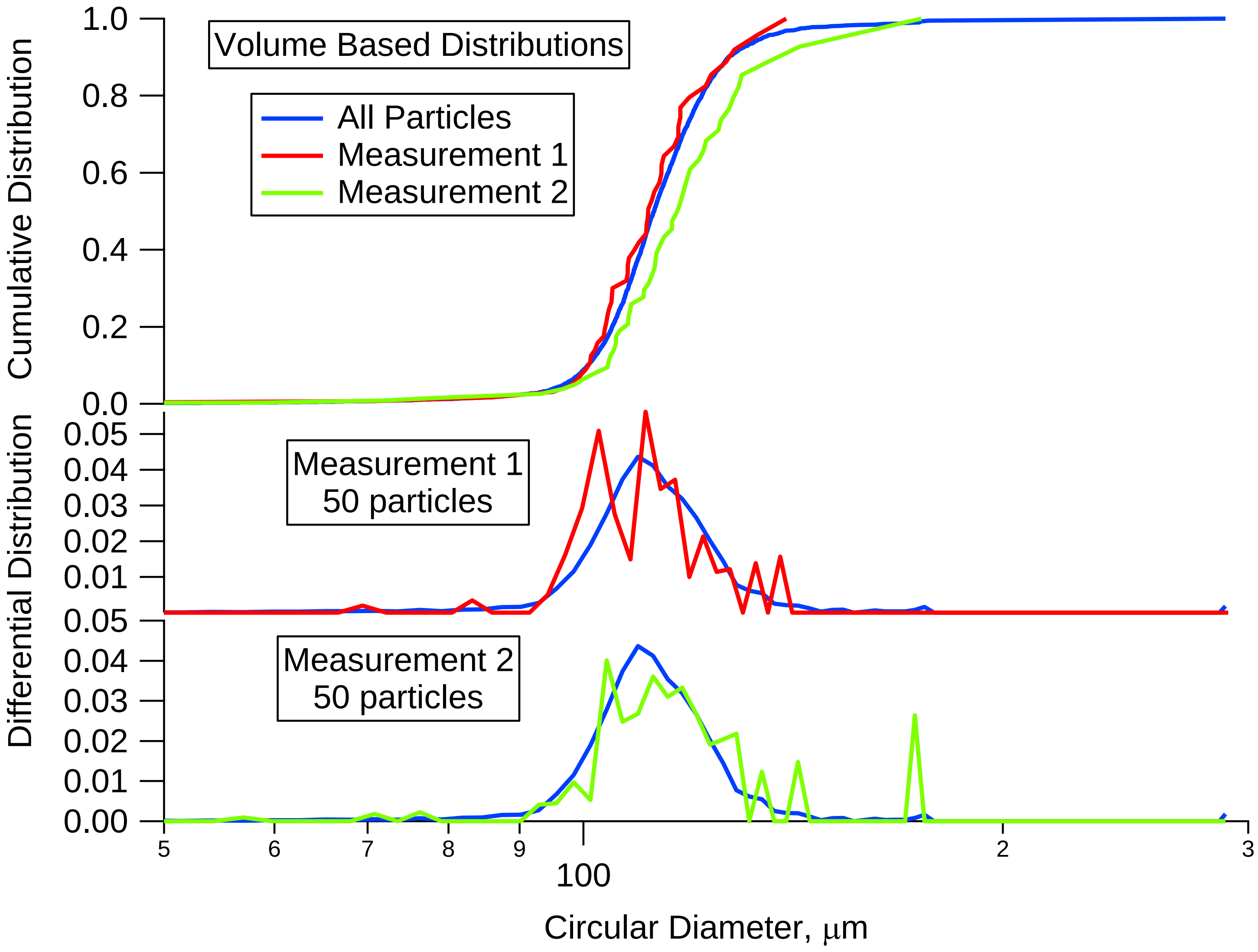
$$\sqrt{s^2\left(\frac{n}{\chi_a^2}\right)} < \sigma < \sqrt{s^2\left(\frac{n}{\chi_b^2}\right)}$$

χ=Moment of chi squared distribution (see a statistics book)
s=estimated standard deviation of distribution (width)
n=number of particles measured

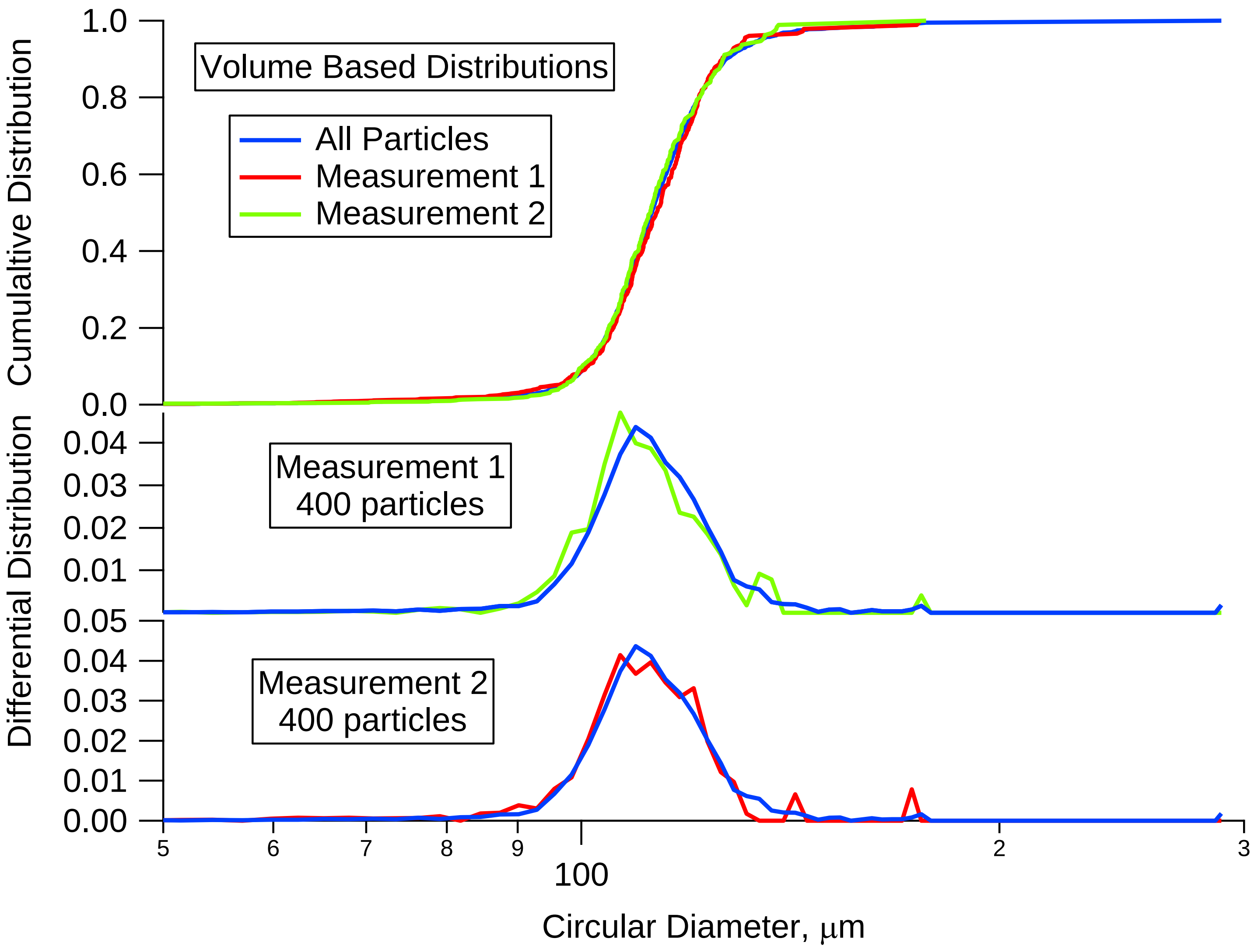
These limits are asymmetric around the standard deviation.

Assumes normal particle size distribution, greater than 30 particles, and known standard deviation.

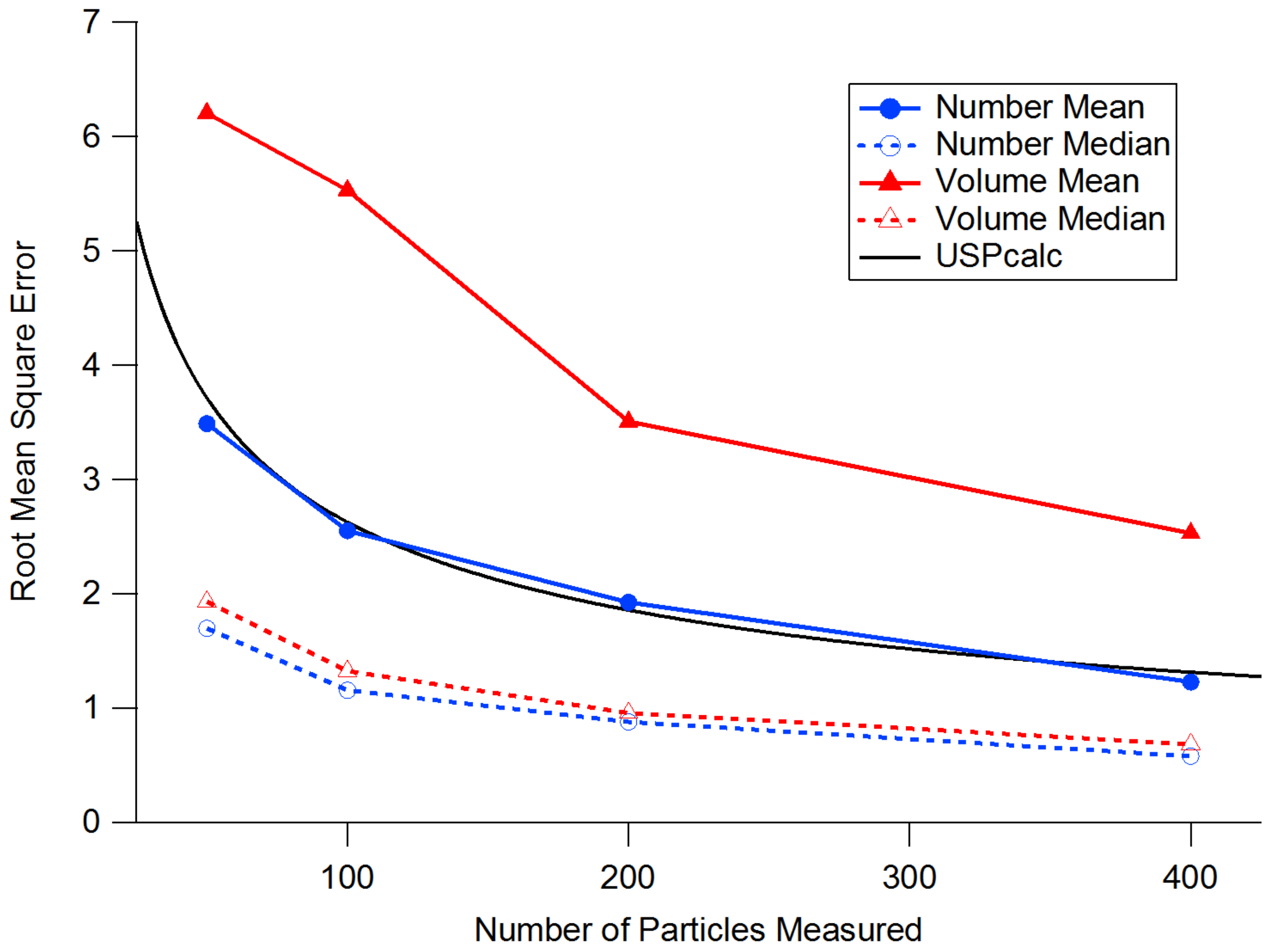
50 particles



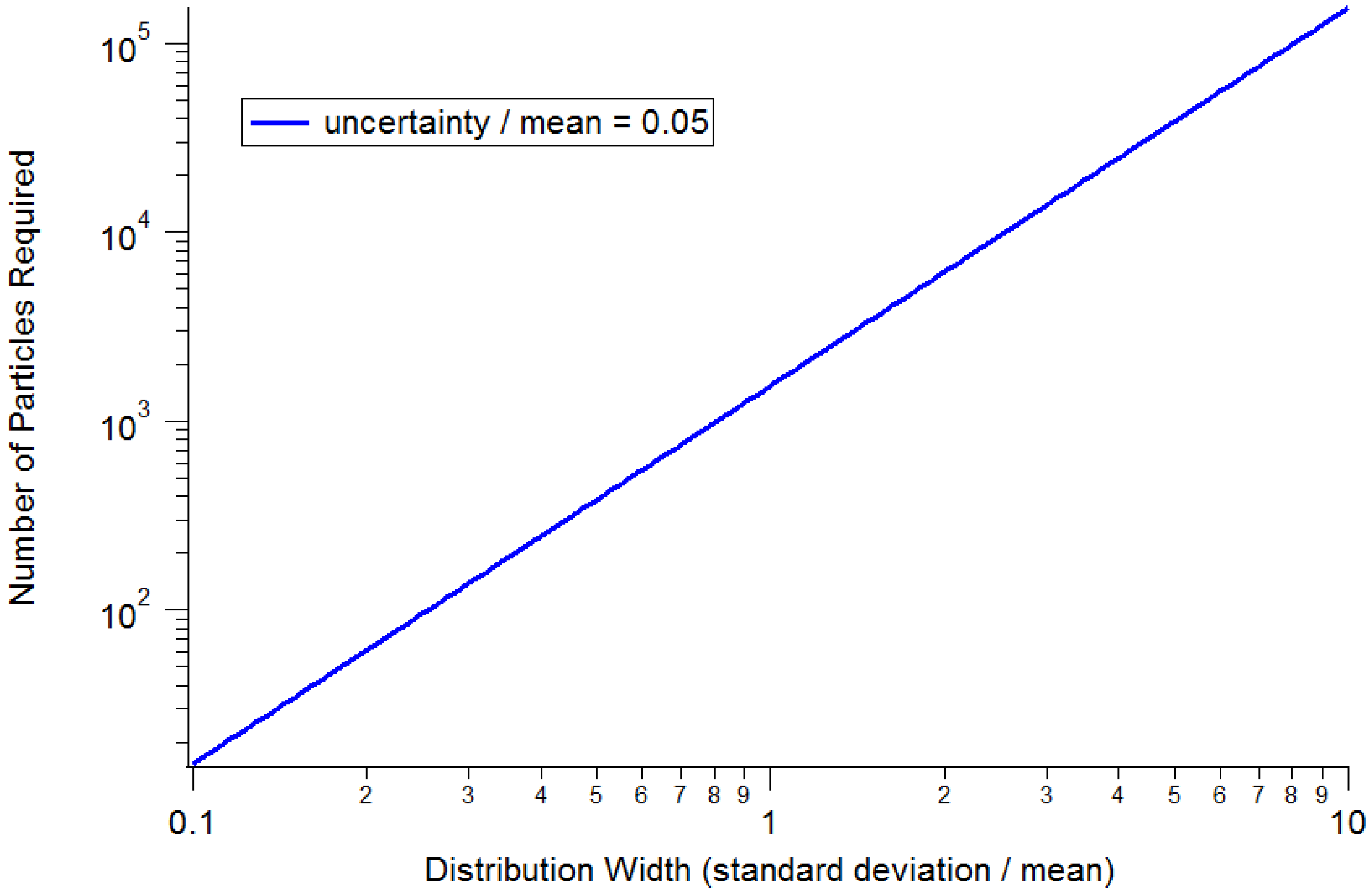
400 particles



Compare With USP



How Many Particles?



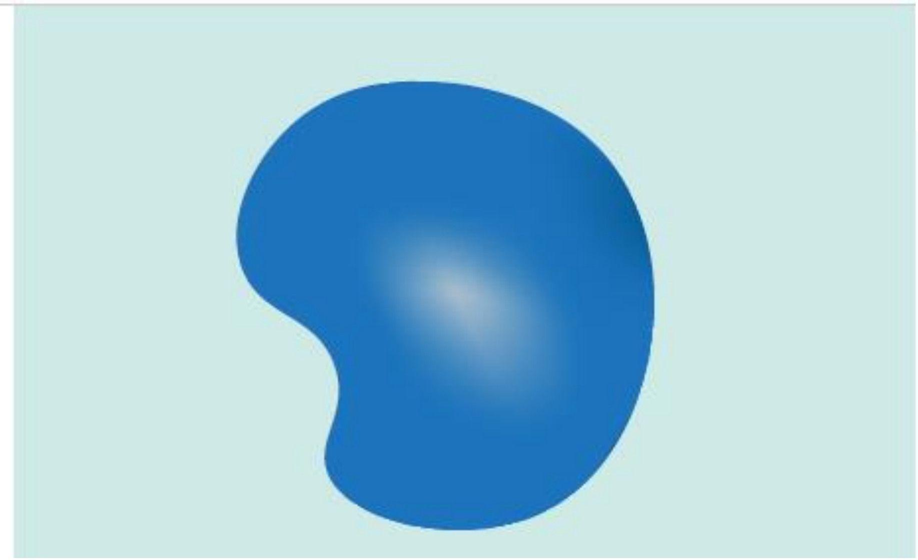
- Some materials have a distribution such that SD/Mean ~ 1.
- To obtain reliable mean values, measure ~1500 particles.
- To obtain more details about the distribution, (10x?) more particles need to be measured.

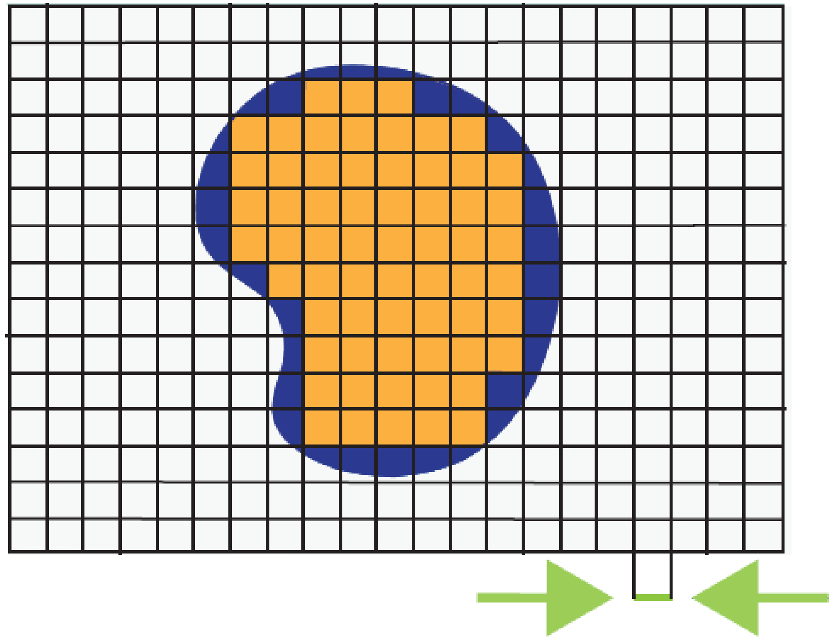
Over to Darren…

Minimum Size: Pixels

Detection of particles

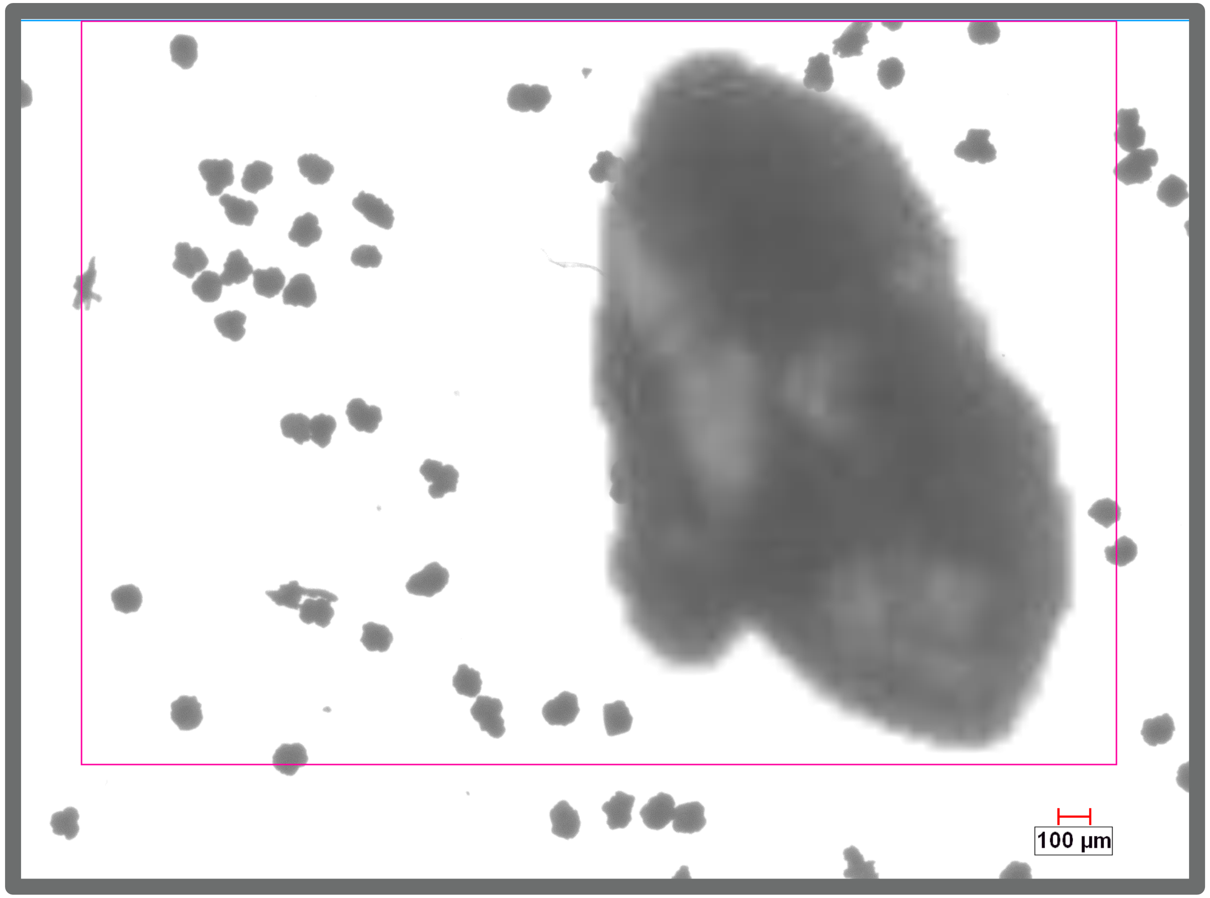
One pixel is element of a projection when at least half of the pixel is covered.





Maximum size

Large particles cannot have been measured properly even they fit in the frame.

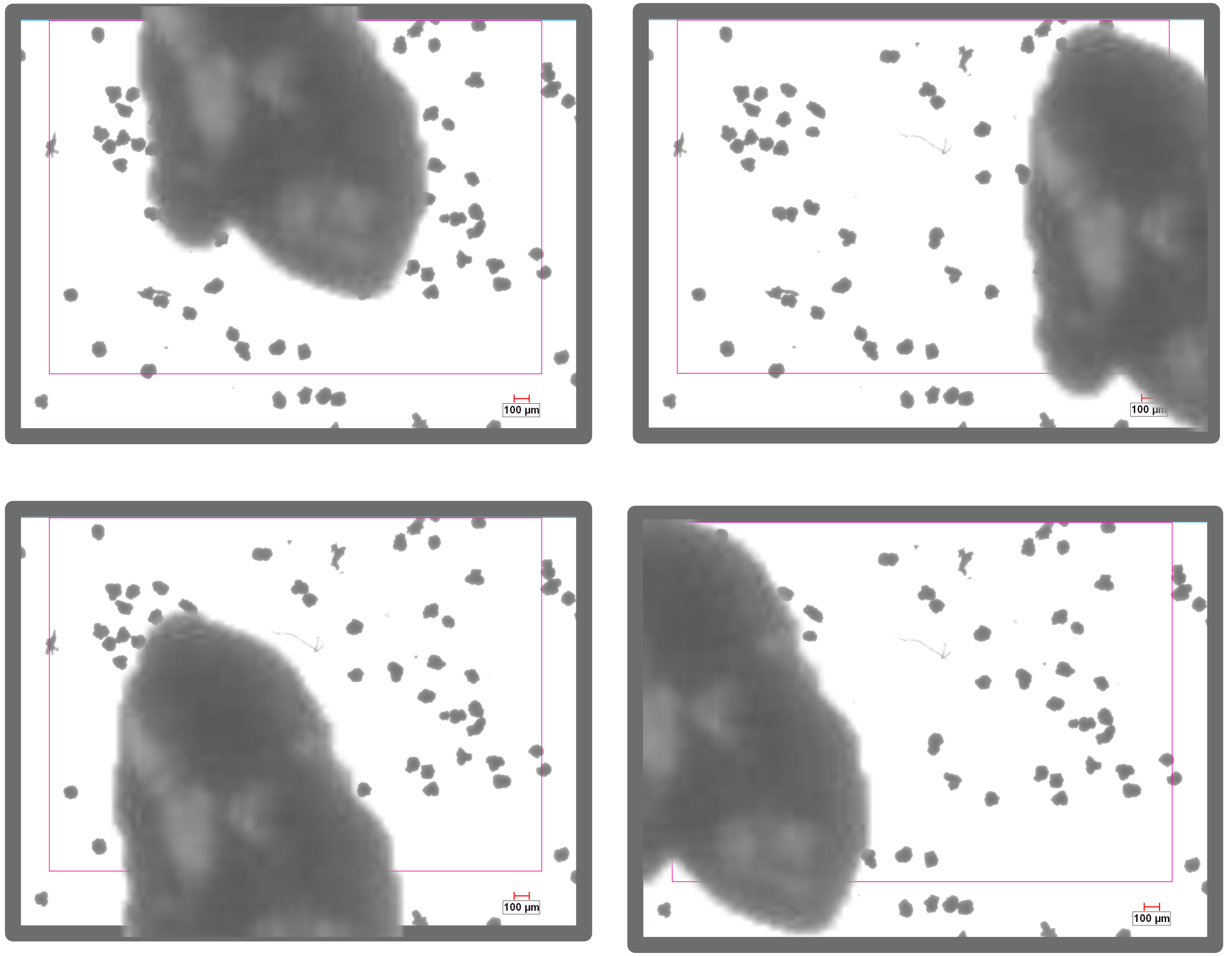


Maximum size

The probability of large particles touching the edge of the frame is higher than for smaller particles.

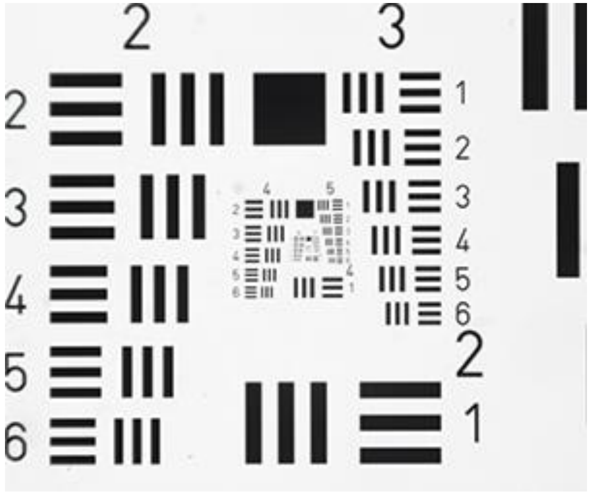
→ Large particles cannot have been measured sufficiently.

→ Upper limit of measurement range

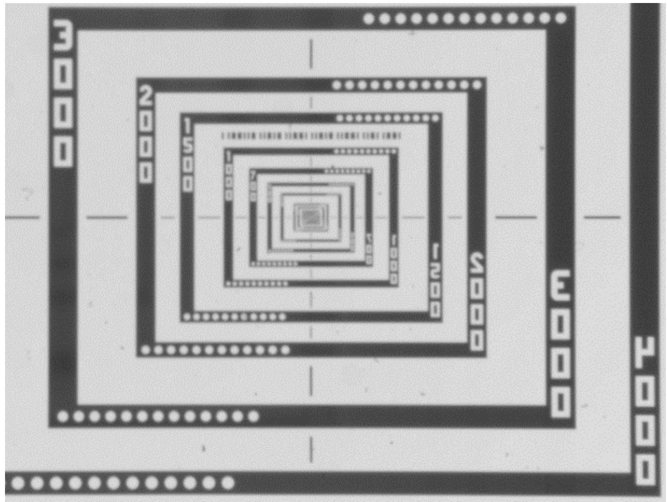


Accuracy

- Is that the “real size”?
- Image analysis uses actual pictures to extract size.
- Calibration with a reticle.



Air force target

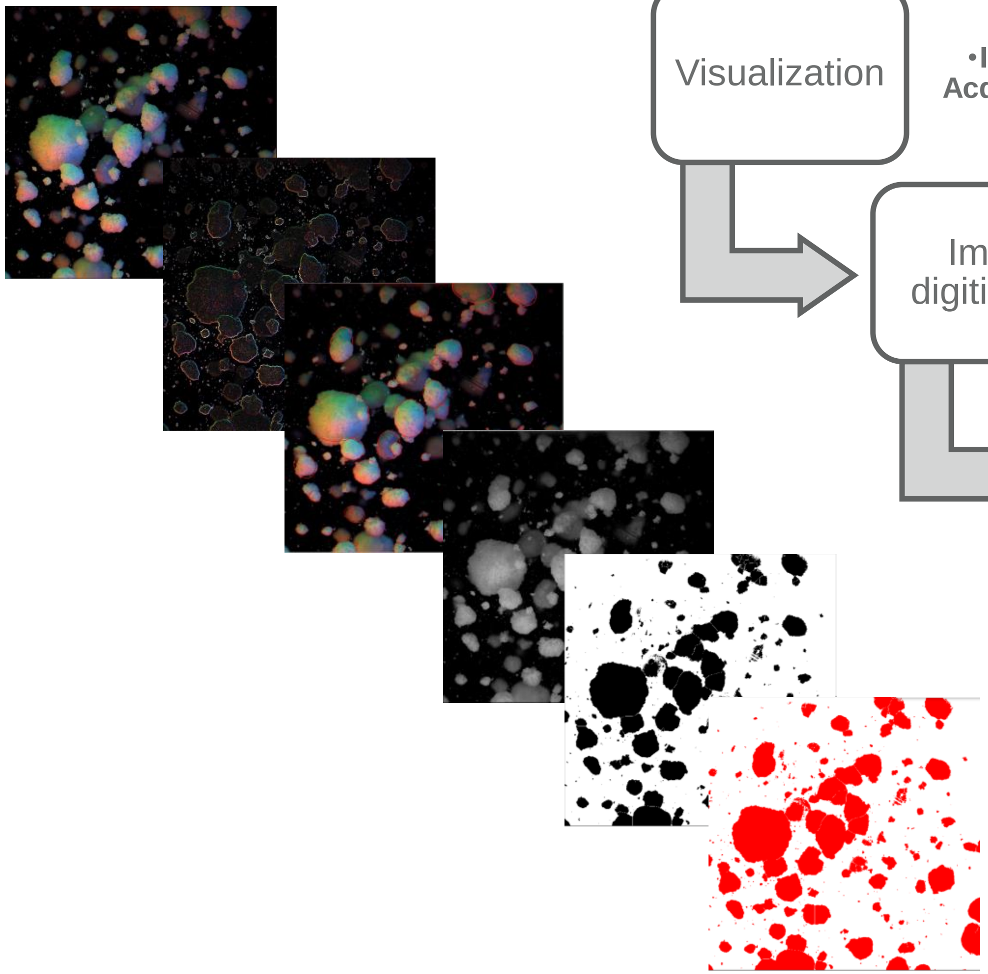


PSA300 calibration target

How Image Analysis is done

- IA is the techniques of processing images through algorithms that manipulate the numerical representation of the images to obtain useful information.
- Output information may be an image or information associated with that image, such as data on features, characteristics, bounding boxes, or mask
- With the ability to measure particles in a number of ways comes the decision of what way to report those measurements.

Main Steps in Image Analysis



- Visualization — •Image Acquisition
- Image digitization — •Thresholding
- Image Processing — •Segmentation
- Feature of Interest — •Measurement
- Data — •Statistical Analysis

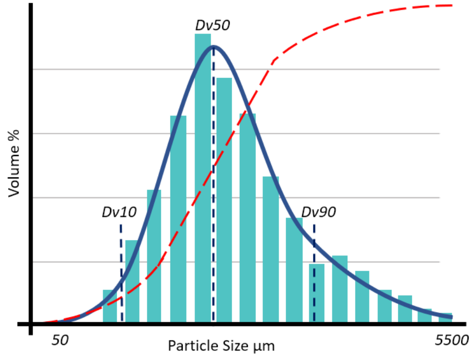


Image Capture

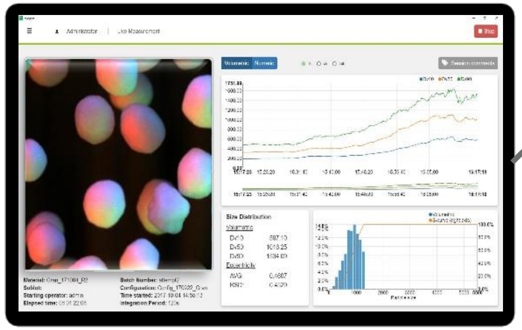


Image Processing

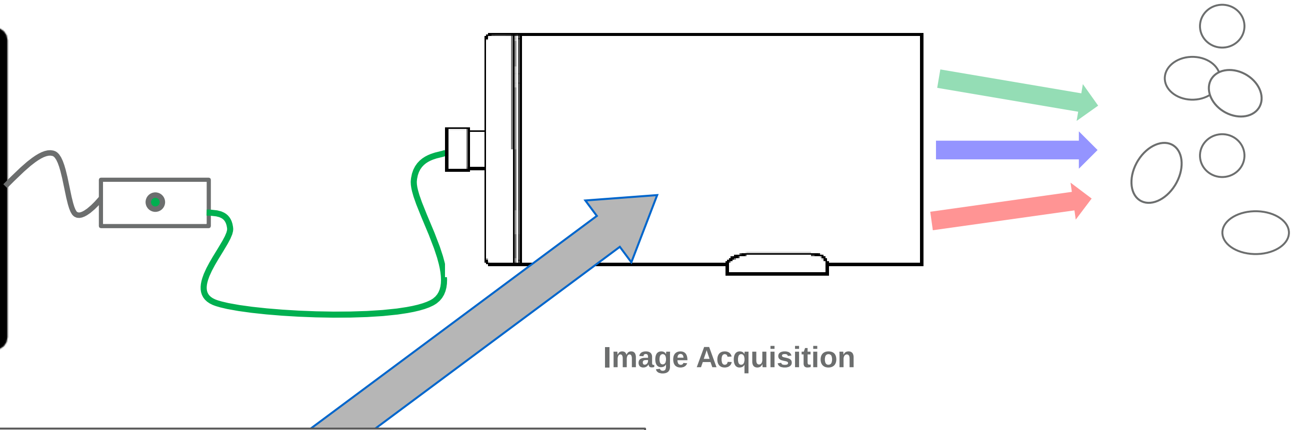


Image Acquisition

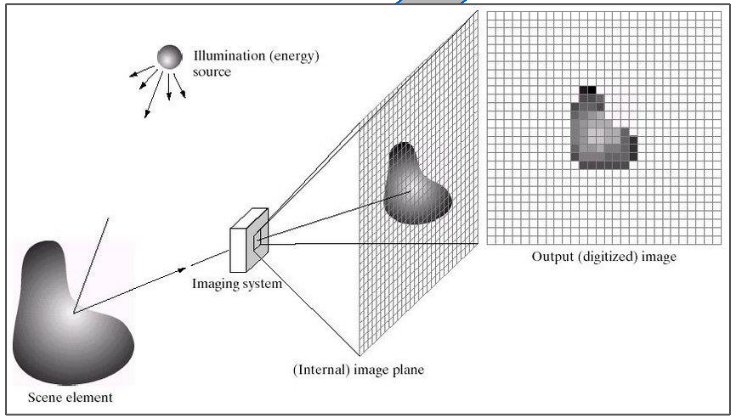
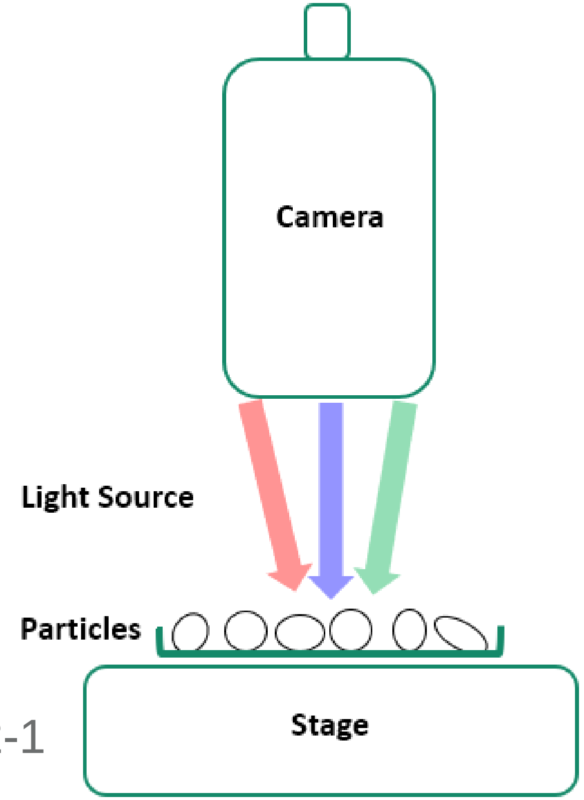
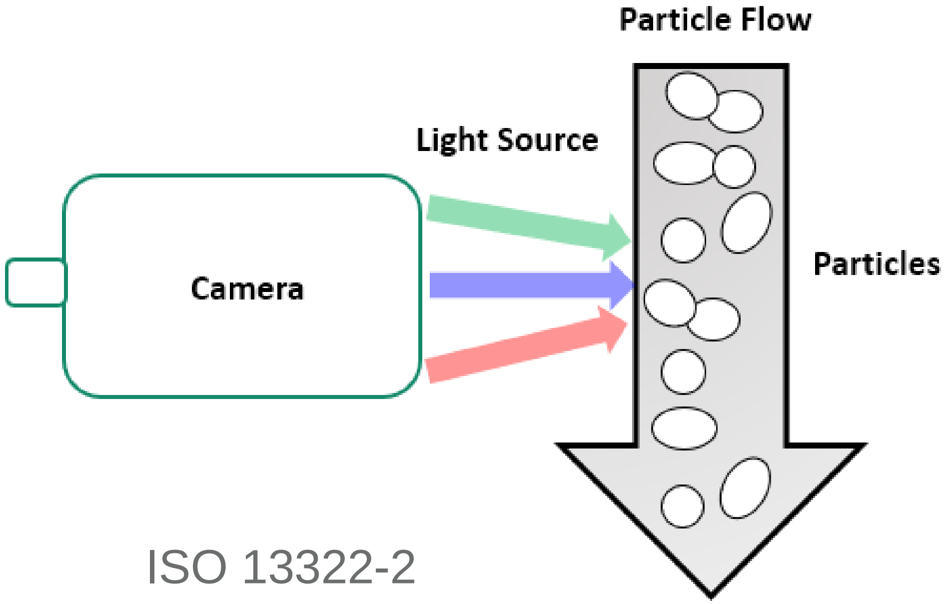


Image digitization

Image Capture



ISO 13322-1



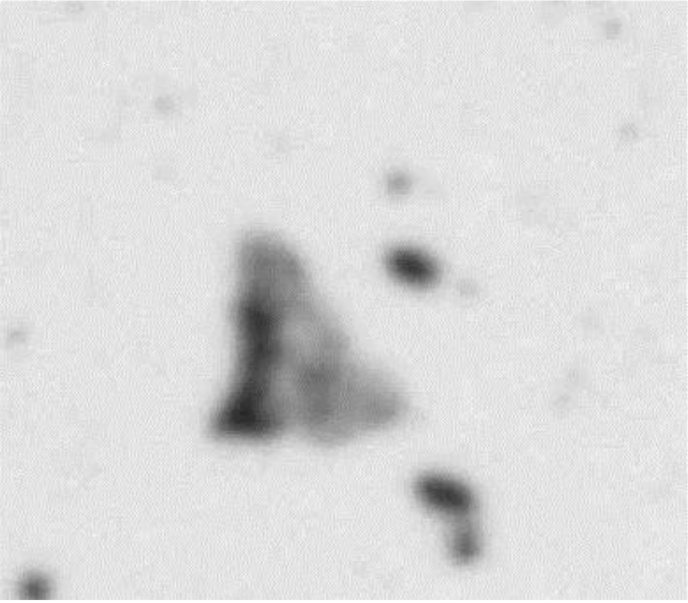
ISO 13322-2

Static and Dynamic IA: one is where the particles measured are stationary, the other measures particles in motion

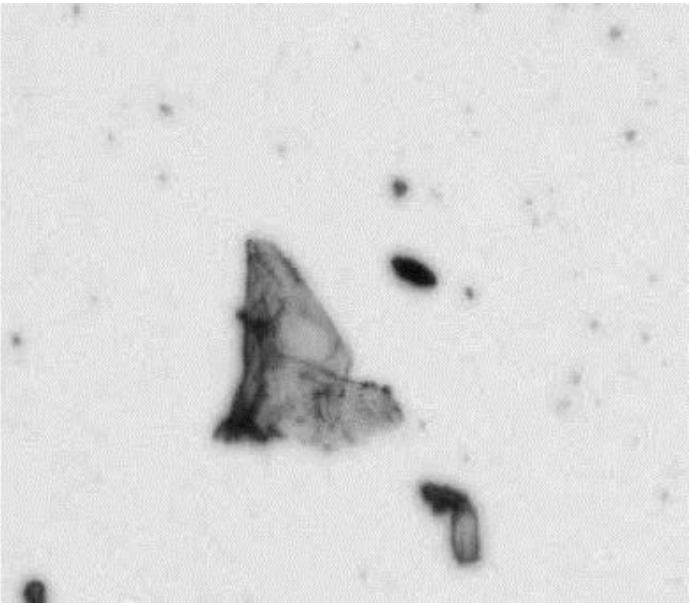
Acquiring images

We want a good microscope and nice sharp images.
Pay attention to lighting and focus.

No



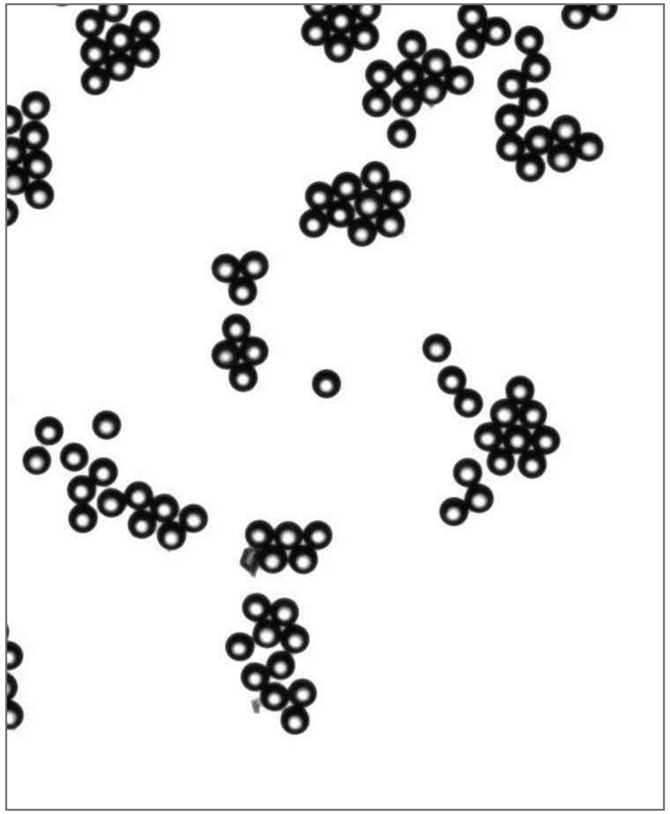
Yes



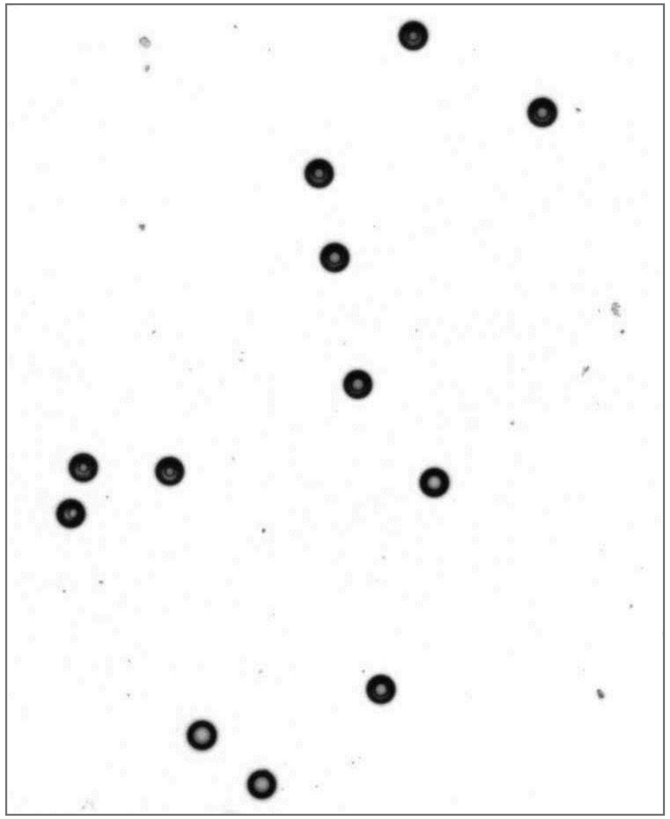
Dispersing a sample (for acquisition)

Often want to spread particles out so that they don’t touch.

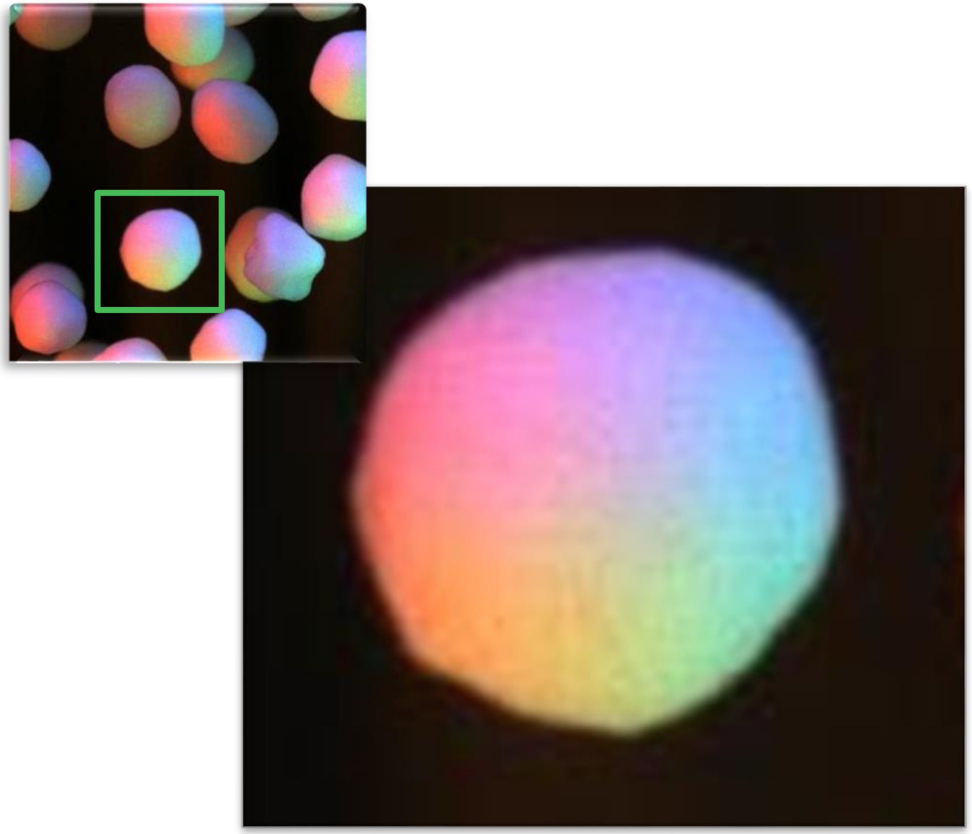
No



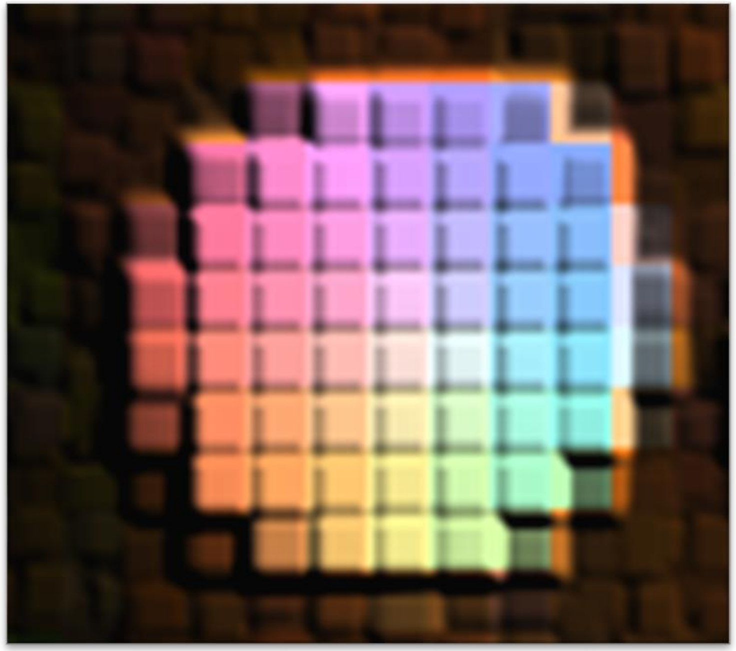
Yes



Analog to Digital



Analog Image



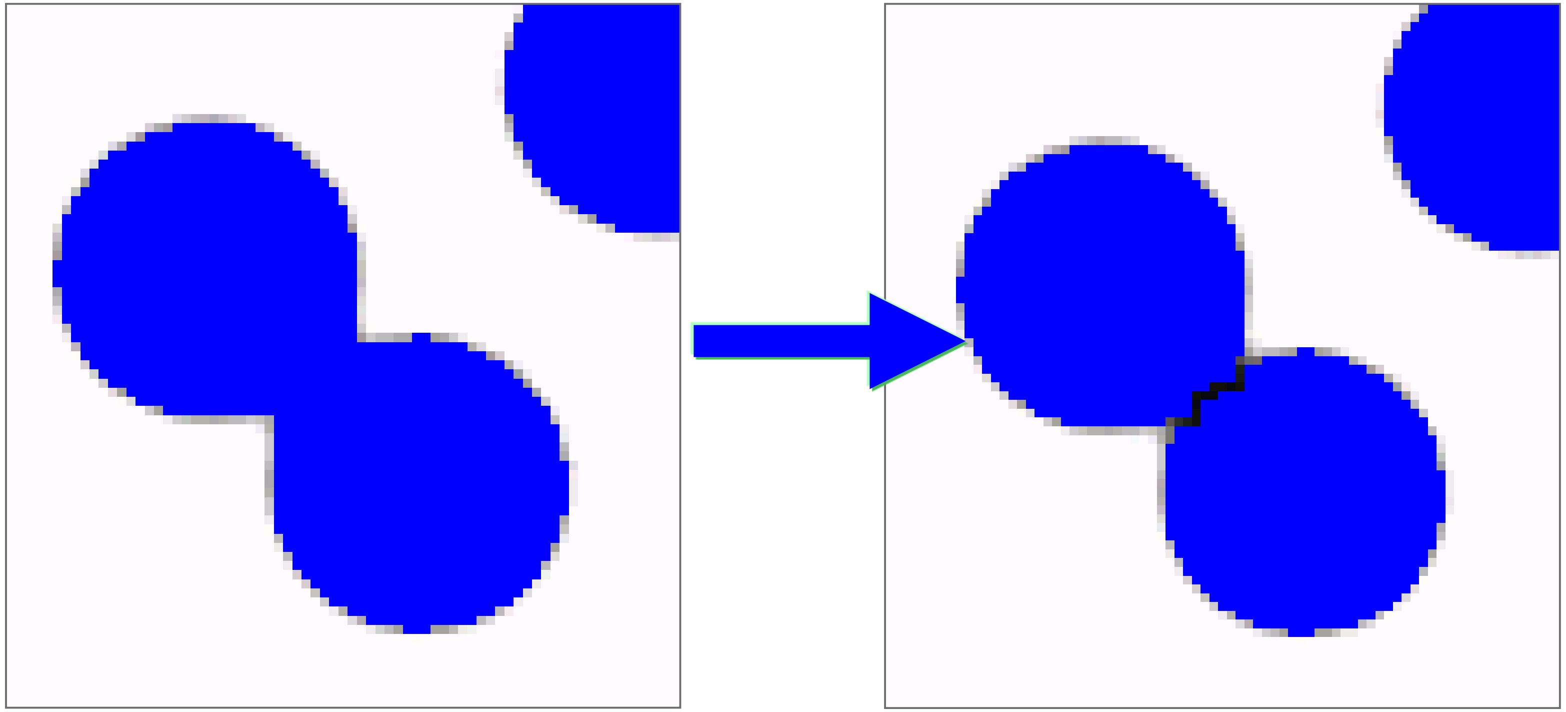
Digital Image

0	0	0	0	0	0	0	0	0	0
0	0	0	80	100	97	180	190	0	0
0	0	222	90	150	110	240	255	180	0
0	80	245	80	100	120	205	210	230	0
0	15	210	111	96	63	201	222	240	0
0	180	190	135	97	91	110	2	230	0
0	240	255	205	180	190	55	255	219	0
0	205	210	175	240	255	110	150	55	0
0	0	45	185	205	210	88	255	0	0
0	0	0	0	0	0	0	0	0	0

Pixel Quantization

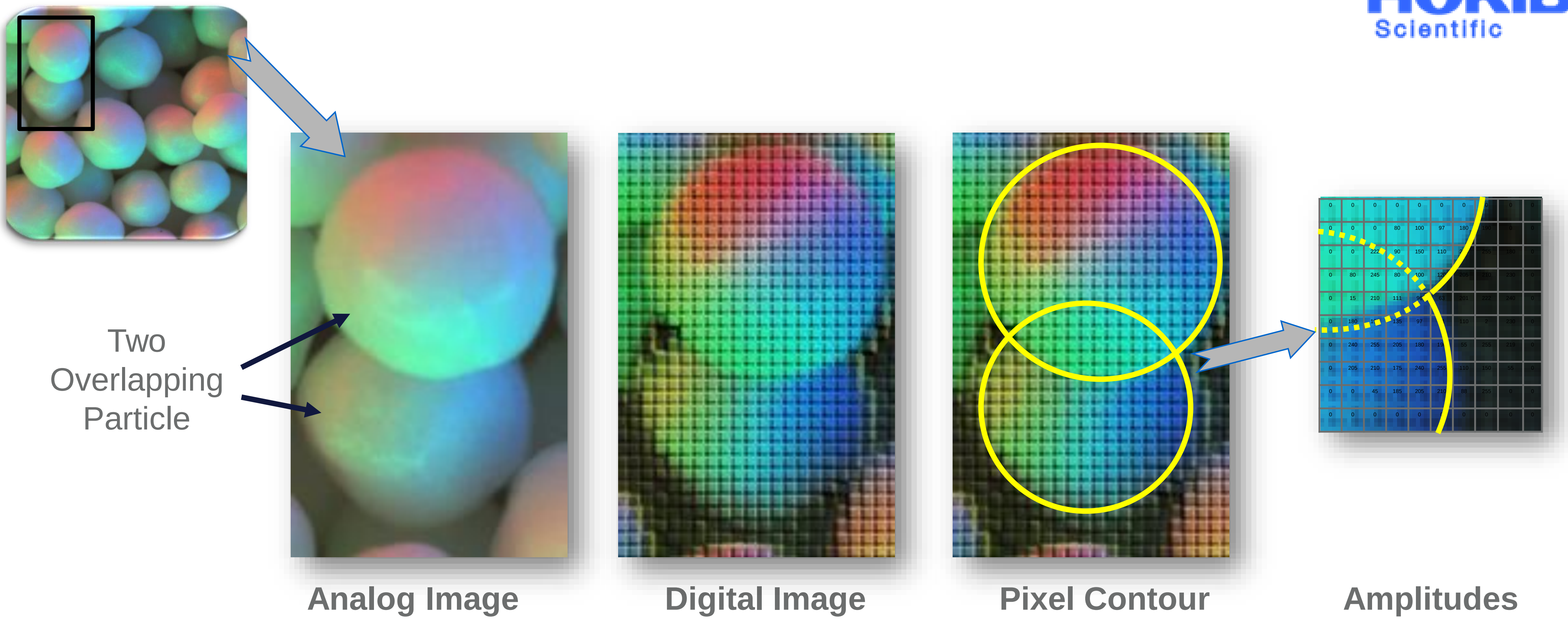
Segmentation

This can be done with software rules (such as look at curvature), but generally, it is better to have a good dispersion.

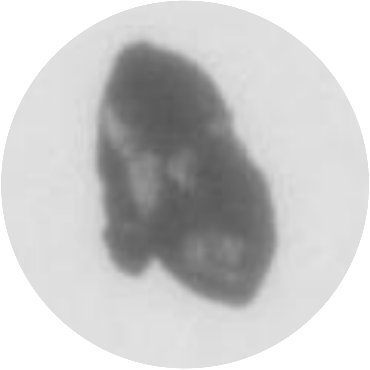


There are clever illumination schemes to help with this…

Overlapping (Segmentation)

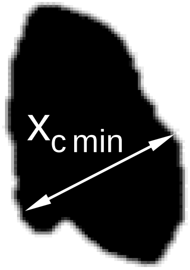


Measurement: Particle Size



$x_{c\,min}$

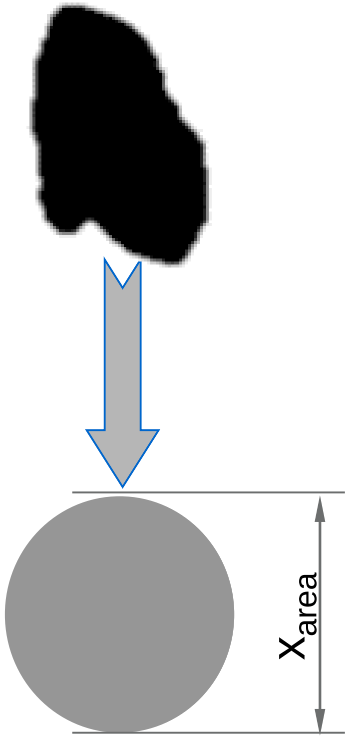
"width"



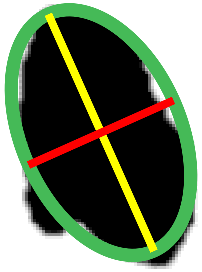
Width is best suited for comparison with sieves !

x_{area}

"diameter over projection surface"



"length"



Major, minor diameters

Shape parameters can be calculated!

Measurement: Particle Shape

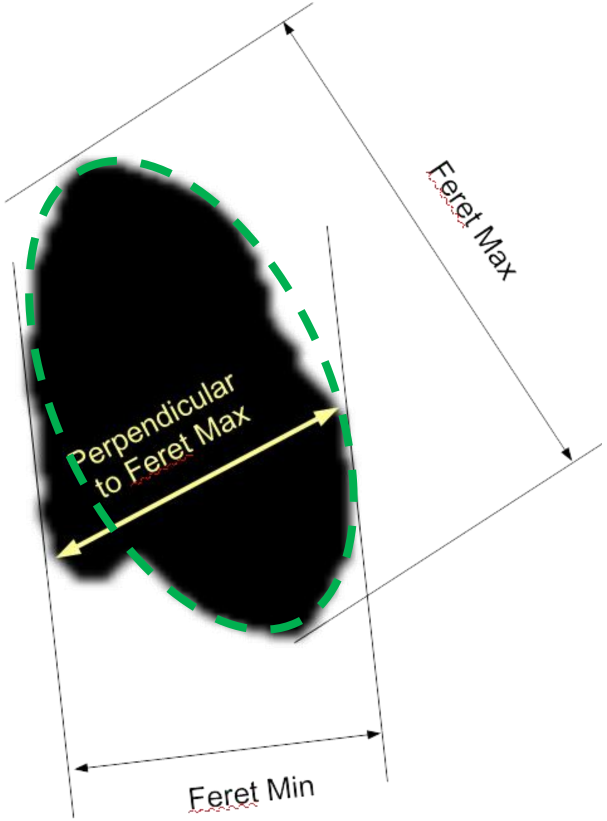
Aspect ratio

= Feret Min / Feret Max

= Feret Max / Feret Min

= Feret Max / Feret Perp. to Feret Max

Three different numbers



Eccentricity

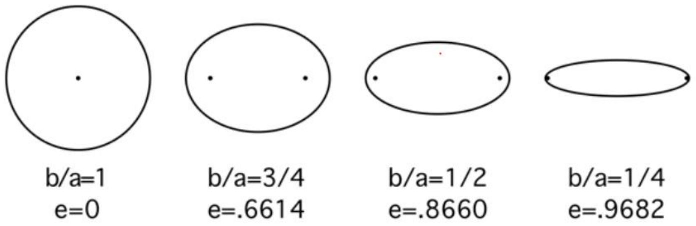


Figure 3: Representation of how eccentricity changes with respect to major (a) & minor (b) diameters

Eccentricity is measured between 0 – 1
0 being completely spherical, major diameter = minor diameter

$$eccentricity\ (e) = \sqrt{1 - \left(\frac{D_{min}}{D_{max}}\right)^2}$$

Measurement: More particle shapes

Roundness

A shape measure that quantifies the "roundness" of an object's edges:

$$\frac{4 \times \text{Area}}{(\pi \times L \times L)}$$

Compactness

Ratio of area over convex perimeter:

$$\frac{4\pi\ A}{\text{Convex perimeter}^2}$$

Roughness

A shape measure that quantifies the jaggedness of an object's edges:

$$\frac{\text{Convex perimeter}}{\text{Perimeter}}$$

Fractal Dimension

Numerical characterization of irregular contours through fractal geometry.

$$P = P_c \delta^{1-D}$$

D is the Fractal Dimension, d is the unit length of the scale used for the measurement and P is the perimeter of the object (1<D<2).

Aspect Ratio

Ratio of length over width.

$$\frac{\text{Length of longest feret}}{\text{Length of shortest feret}} = \frac{\text{Length}}{\text{Width}}$$

Sphericity

Estimate of the sphericity of an object:

$$\frac{4\,\pi\,A}{P^2}$$

The PSA300

- Turnkey System
  - More time getting results and less time engineering
- Automated
  - Faster
  - Less operator labor
  - Less operator bias
- Powerful Software Features
  - Image Enhancement
  - Particle separation
- Separate Disperser Option
  - More flexible sample preparation

The Eyecon$_2$

- Real-Time particle Size Distribution and shape
- Use in:
  - Research & development (QbD/DoE/CPP/CQA)
  - Scale up
  - Tech transfer

  Use on:
  - Fluidised Bed Coating, Granulation, Drying
  - Twin Screw Granulation
  - Roller Compaction/Milling
  - Extrusion, Spheronisation

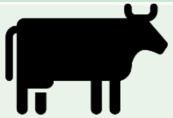
Conclusions

- Image Analysis is good for
  - Replacing Sieves
  - Size
  - Shape
  - Supplementing other techniques

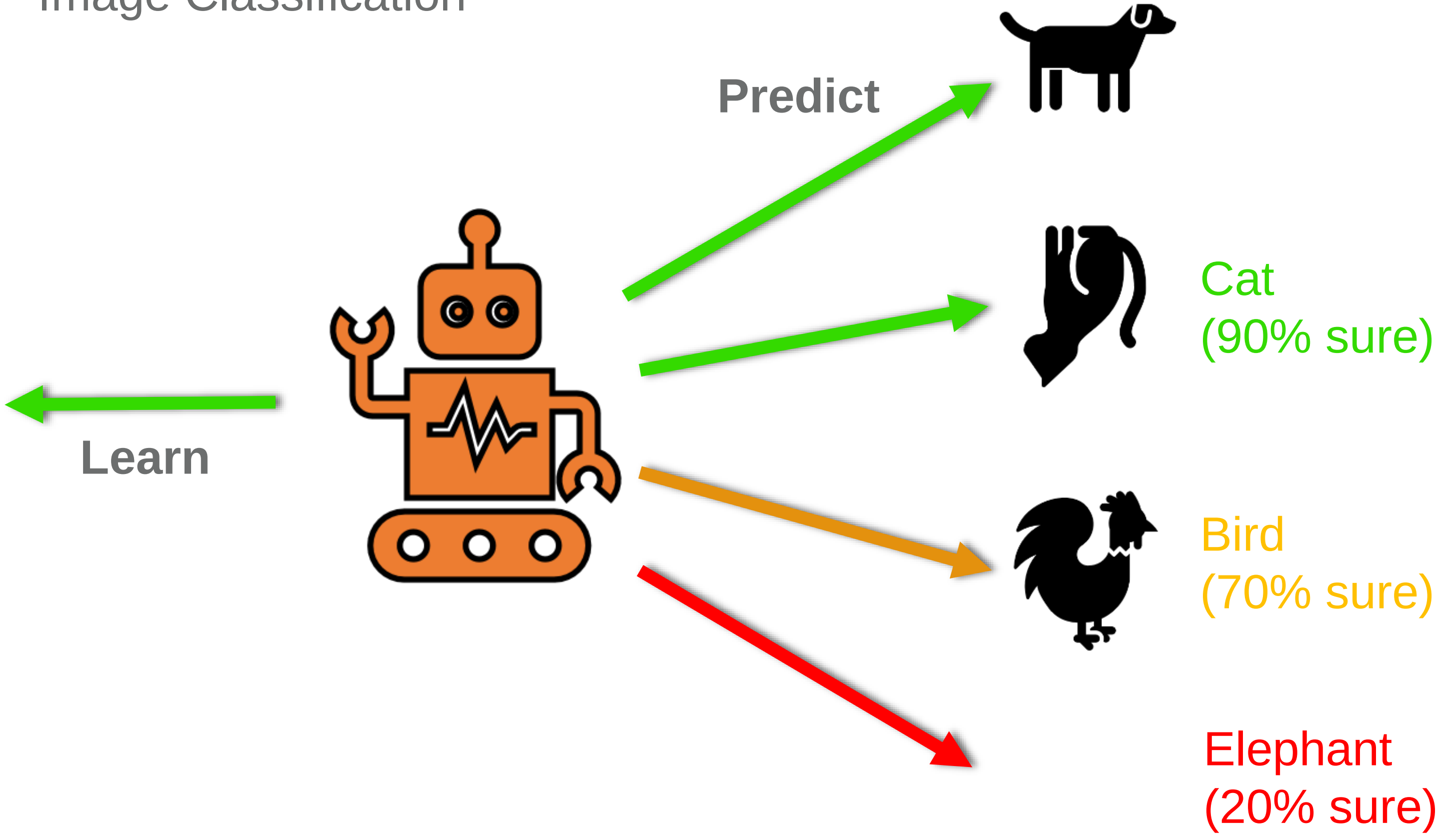
- Watch out for
  - Sample preparation
  - Image quality
  - Measure enough particles

Where is PSA and image analysis going?

Machine Learning – Supervised Example

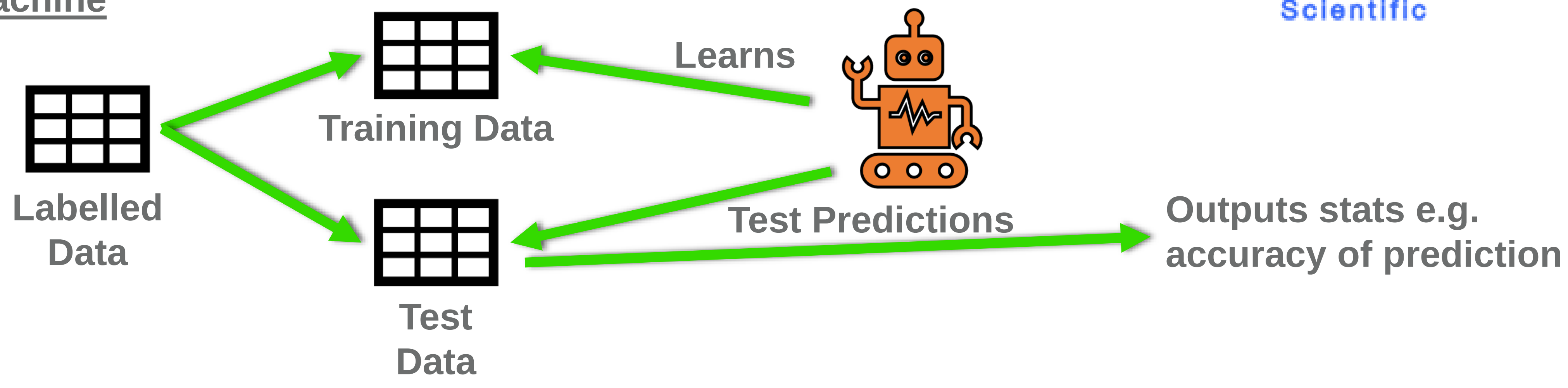
Image	Label
	Dog
	Cat
	Bird
	Cow
	Elephant
	Rat
	Tortoise

Example: Supervised Image Classification

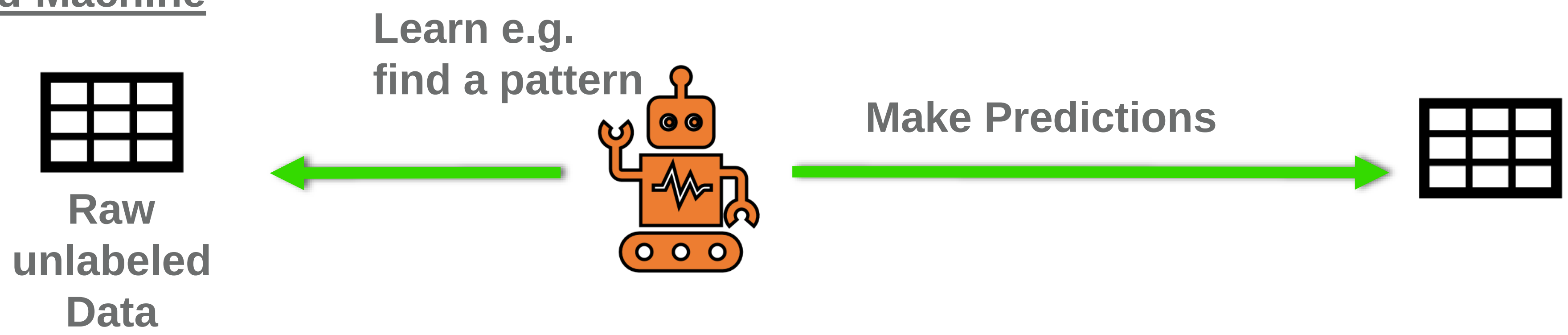


Machine Learning – Supervised vs Unsupervised

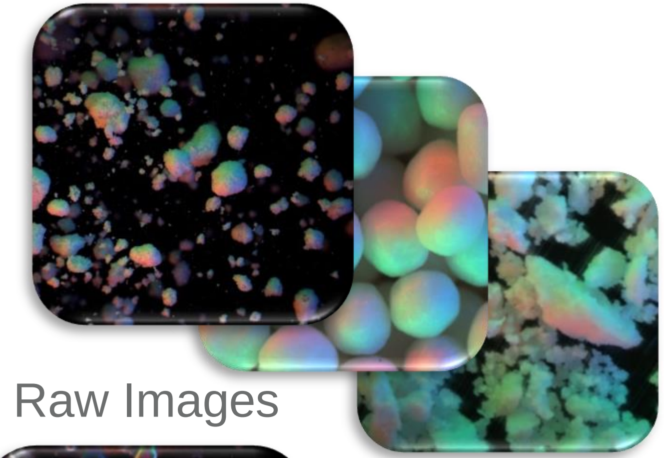
Supervised Machine Learning



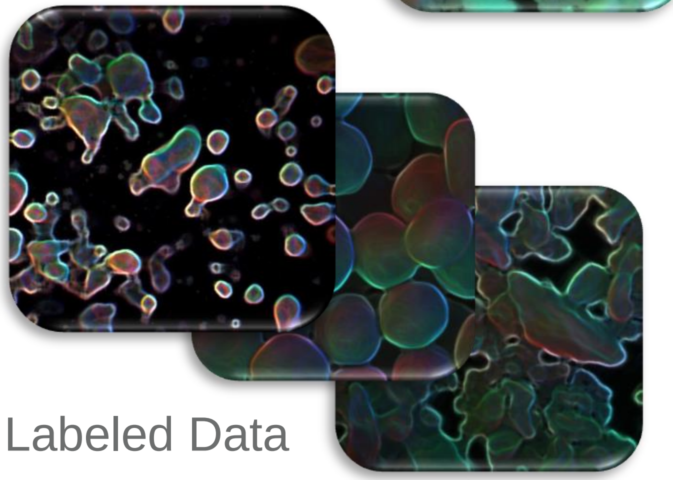
Unsupervised Machine Learning



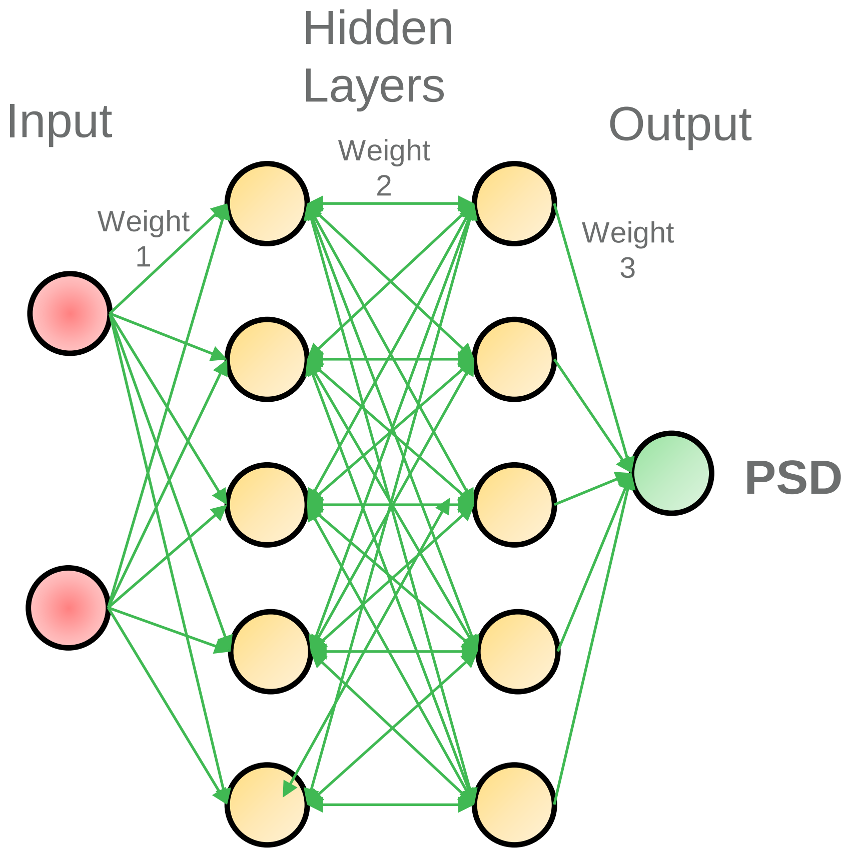
Neural Networks

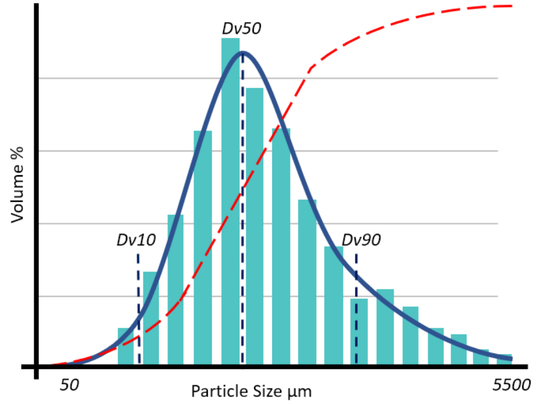


Raw Images

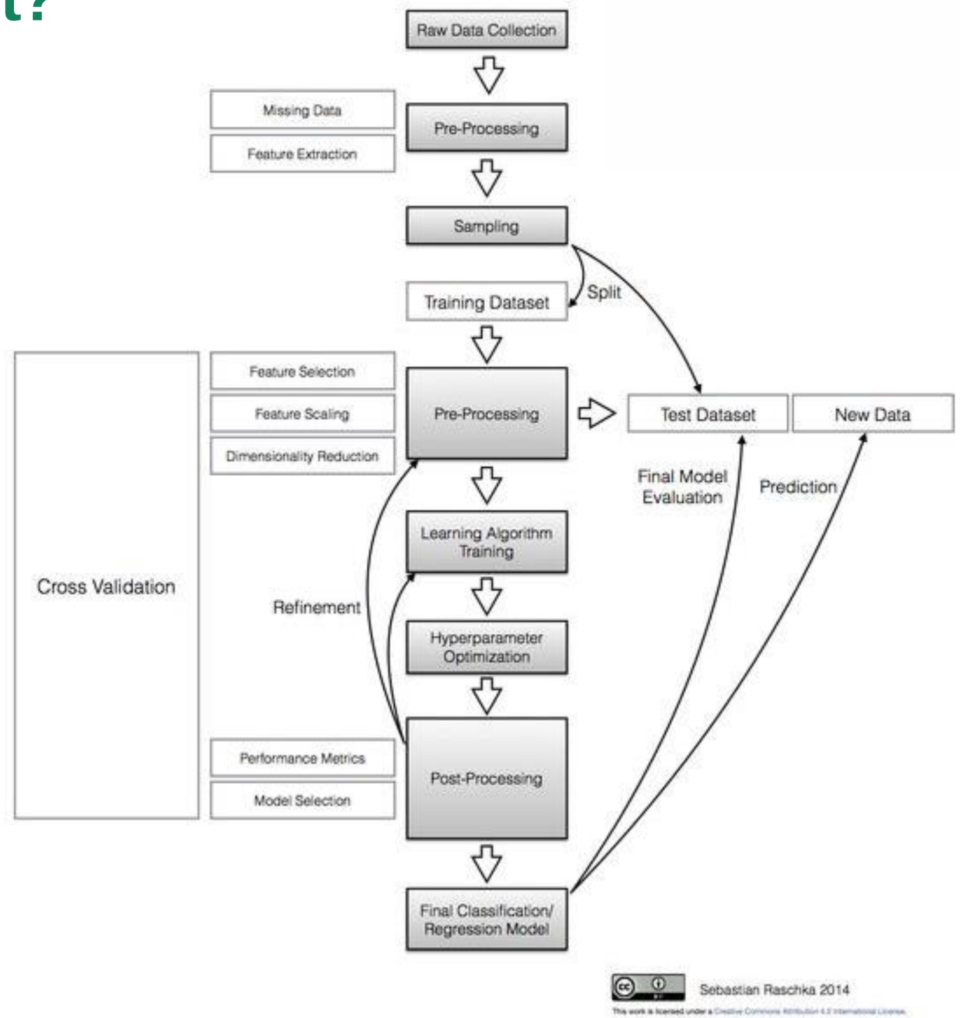


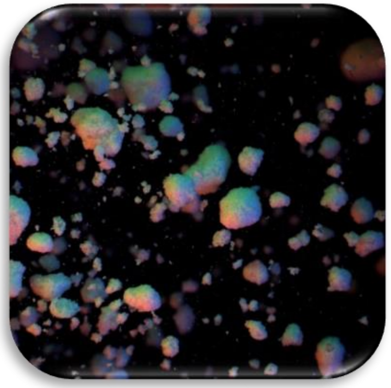
Labeled Data

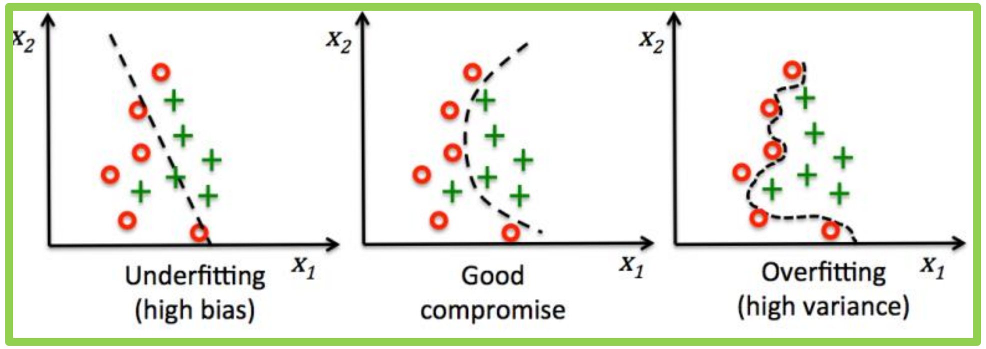


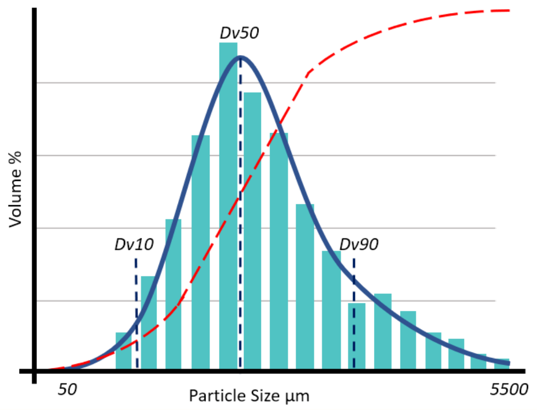


Machine Learning Easy Right?

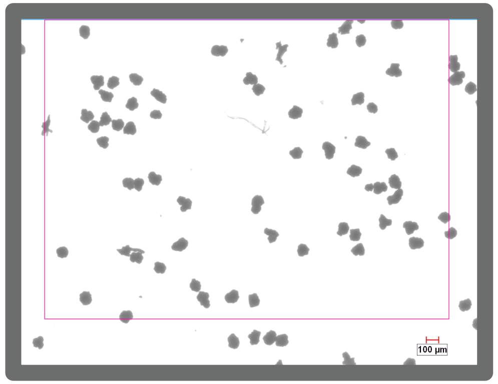


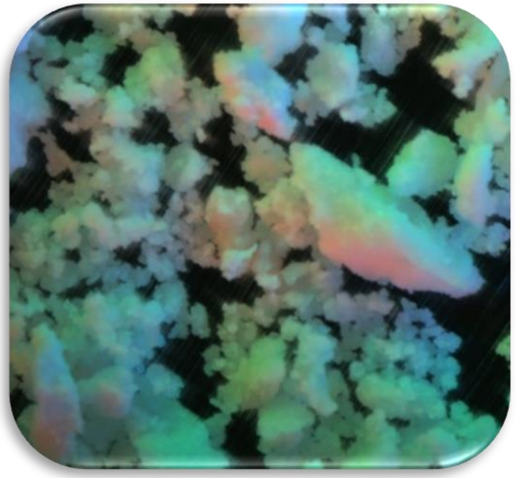






Questions?





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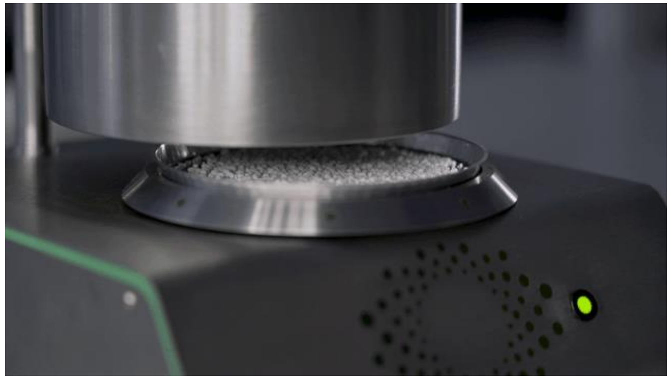

Thank you