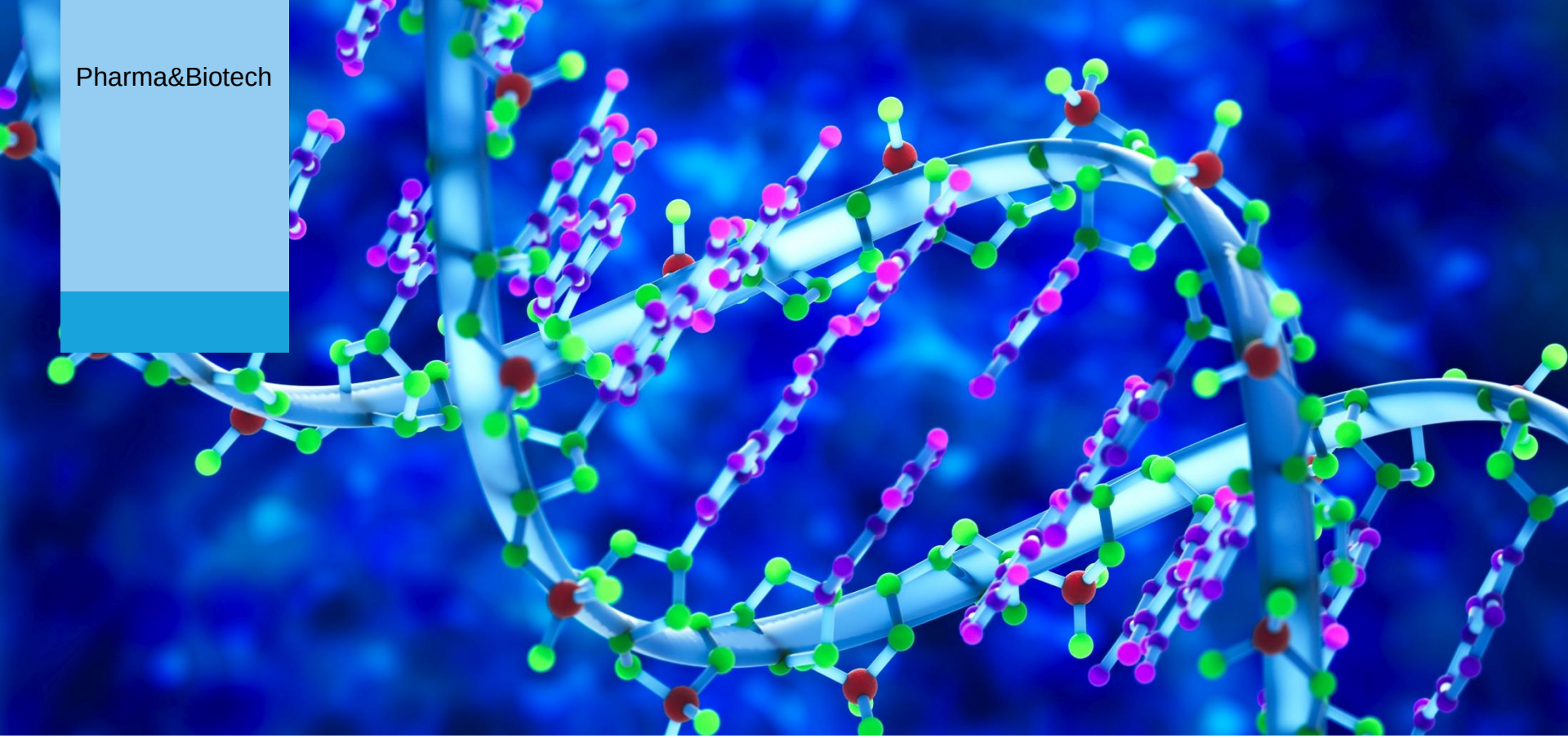
Pharma&Biotech



CRITICAL EVALUATION OF THE EMERGING ANALYTICAL METHODS FOR CHARACTERIZATION OF SUB-VISIBLE PARTICLES

The Known Unknowns in Subvisible Particle Characterization

Atanas Koulov

Lonza Drug Product Services

MCERSI Workshop | Baltimore | 05.12.2016

Lonza

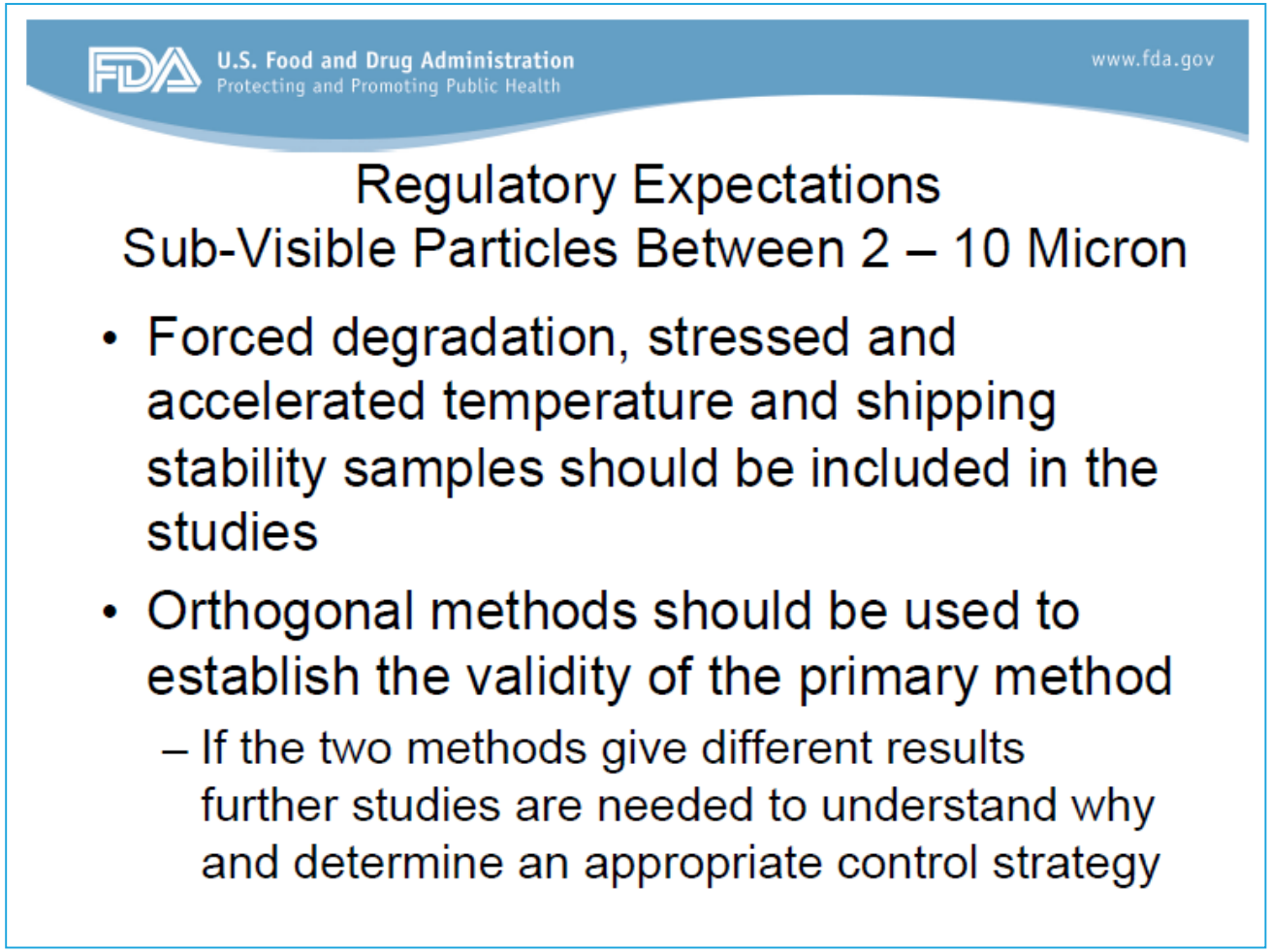
Forward-Looking Statements

Certain matters discussed in this presentation may constitute forward-looking statements. These statements are based on current expectations and estimates of Lonza Group Ltd, although Lonza Group Ltd can give no assurance that these expectations and estimates will be achieved. Investors are cautioned that all forward-looking statements involve risks and uncertainty and are qualified in their entirety. The actual results may differ materially in the future from the forward-looking statements included in this presentation due to various factors. Furthermore, except as otherwise required by law, Lonza Group Ltd disclaims any intention or obligation to update the statements contained in this presentation.

Subvisible Particles – Why Measure?

- Subvisible particles are likely to be present in parenteral drug products
- Biological consequences?
- Historically, SvP >10µm and >25µm have been monitored in parenterals (USP<788>)
- Most recently, regulatory expectations for particle characterization are being extended to particles <10µm and even <1µm
- A number of new technologies have emerged over the last decade, but their performance is not well understood

Subvisible Particles – Why Measure?

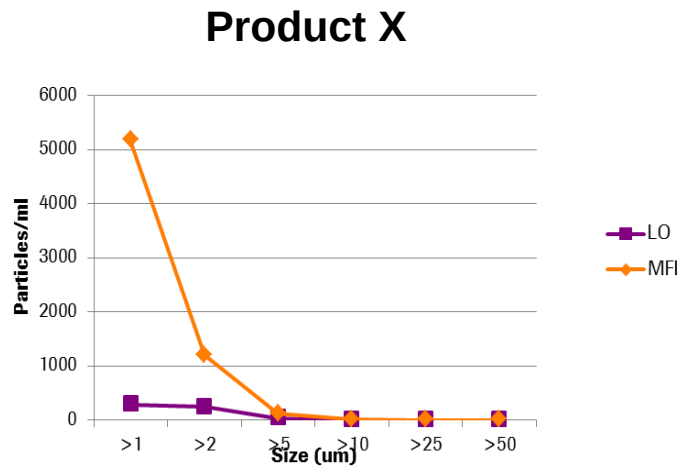


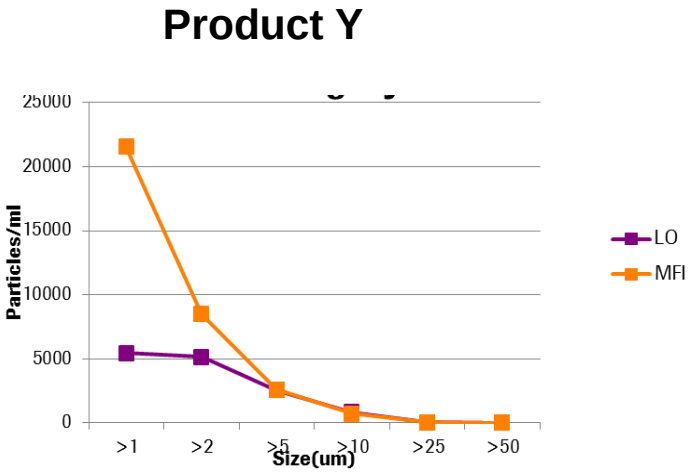
S. Kirshner, USFDA
Breckenridge CO, 2014
Workshop on Aggregation and Immunogenicity

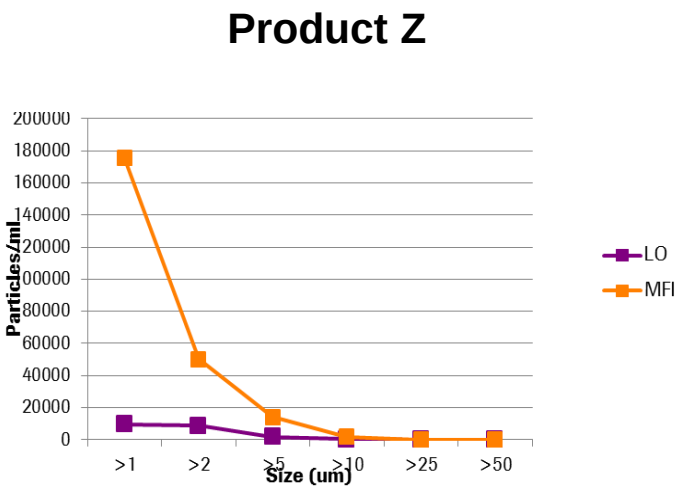
Subvisible Particle Methods – How to Measure?

- Which methods are “orthogonal”?
- Are we confident in method performance?
- How do we setup (product-specific) limits for SvP?

Subvisible Particles – How to Measure?





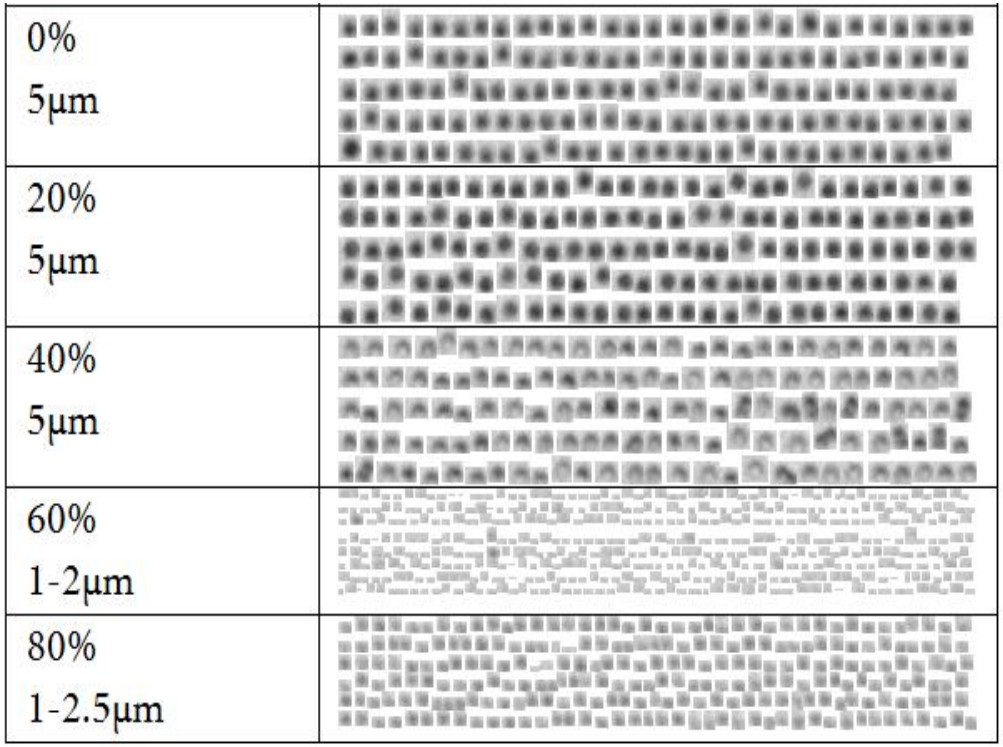


Different methods – different results. Why?

Koulov et al., IABS 2nd particle workshop Nov 2015

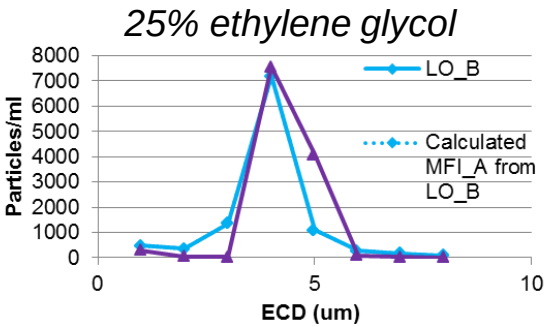
Subvisible Particles – How to Measure?

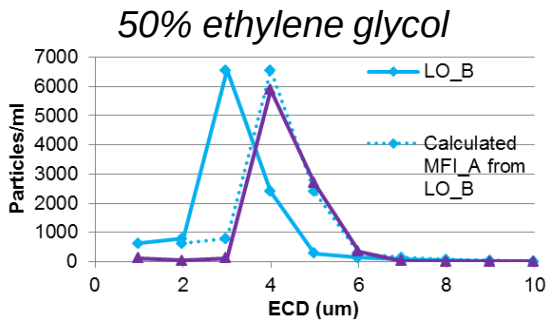
5µm silica particles in sucrose solutions

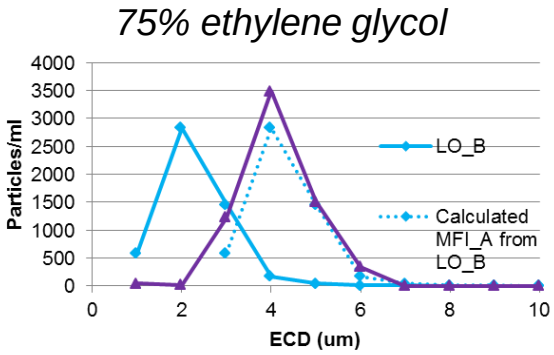


These methods are not truly orthogonal!

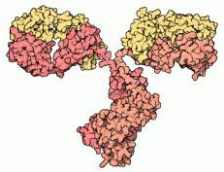
Koulov et al., IABS 2nd particle workshop Nov 2015



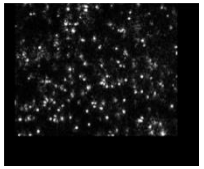




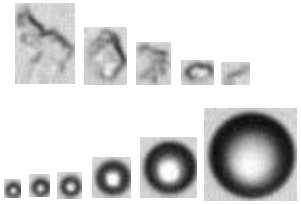
Subvisible Particles – Size Distribution?



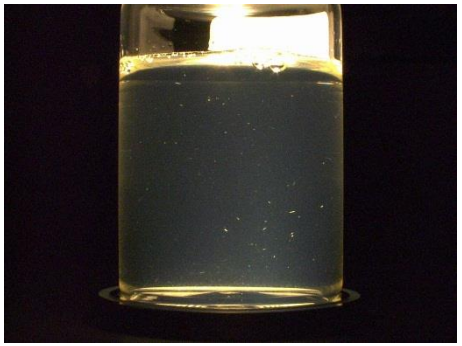
Ab monomer
(~5nm)



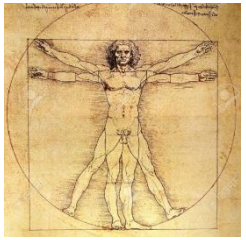
Nanoparticles
(~50nm)



Sub-visible (microscopic)
particles (~1µm)



Visible particles
(~300µm)



A human

A blue whale

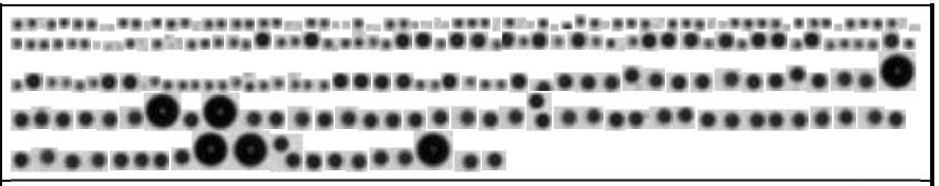
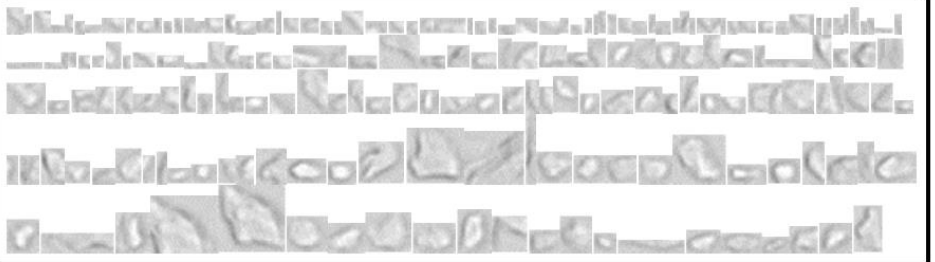
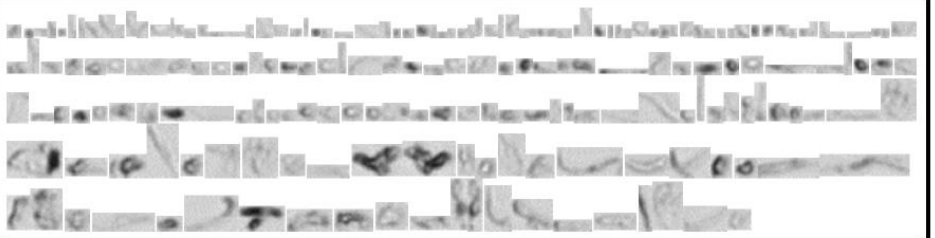
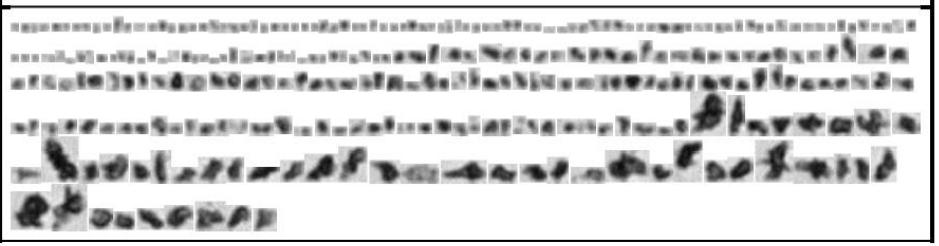
Mount Pilatus
(Tomlishorn)

Oberon
(moon of Uranus)

Wait, this doesn't sound so simple!

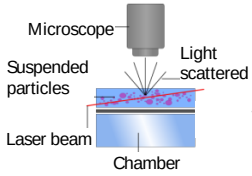
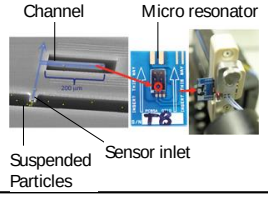
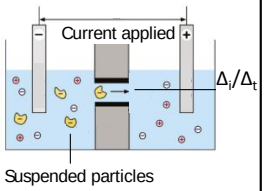
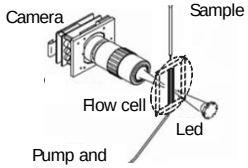
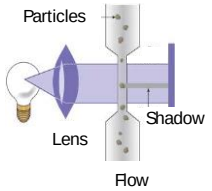
Subvisible Particles: Same, but Different

Table 1 Randomly selected and representative MFI images of the particle models used

Latex mixture	
BSA	
mAb model A	
mAb model B	

Rios et al., 2006, J Pharm Sci (in press)

Analytical Toolbox – Different Tools for Different Jobs

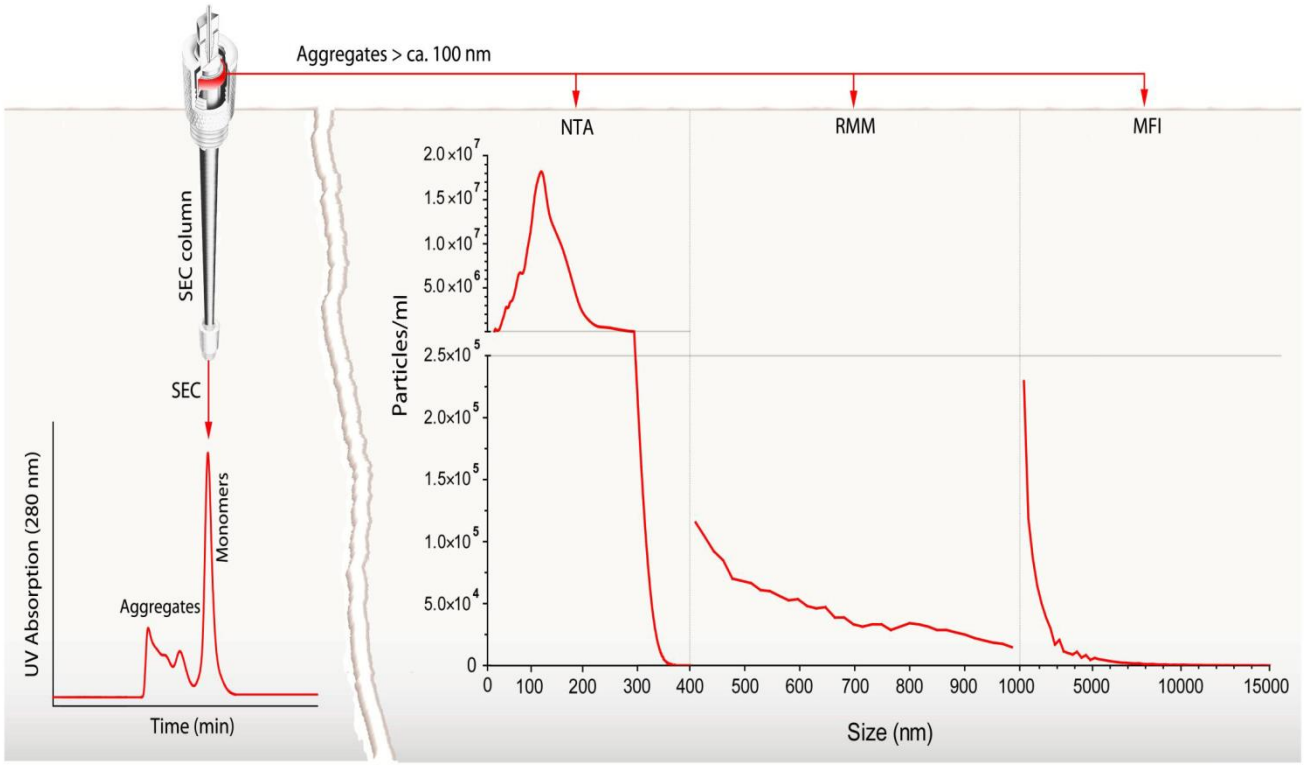
		Nano track analysis	Resonant mass	Coulter counter	Flow imaging microscopy		Light obscuration
							
		NTA	Archimedes	CC	MFI	FC	HIAC
Principle		Tracking of Brownian motion of individual particles 	Changes in frequency due to added mass 	Changes in resistance due to volume displacement 	Weighing of single particles passing through a flow cell 		Drop in current due to the amount of light blocked 
Raw data		Video**, #/mL/size	#/mL/size, particle buoyancy	#/mL/size	#/mL/size, images**, particle morphology		#/mL/size
Optimal size rage [um]*	0.03						
	0.05						
	0.20						
	0.30						
	0.60						
	0.50						
	0.80						
	1.00						
	2.00						
	5.00						
	18.0						
	25.0						
Optimal sample concentration *[particles/mL]**		$3x10^8$ - $1x10^9$, ~20-70 centers per frame	< $8x10^6$	~ $2x10^5$, coincidence < 5%	MFI: < $9x10^4$ FC: < $1.5x10^6$		< $1x10^4$

* As for the supplier. In all the cases, the optimal sample concentration is much more higher than the typically found in non stressed high concentrated protein samples or in stressed samples at relevant conditions ** Further analysis needed to get #/mL/size ▨ Informative data

Rios et al., 2016, Pharm Res, 33: 450-

Some of these methods are truly orthogonal!

Subvisible and Submicron Particle Measurement Methods: Same, but Different!



Filipe et al., 2013, TrAC, 49: 118-

Subvisible Particle Measurement Methods – Do We Understand their Analytical Performance?

“The ability of discerning high quality unavoidably implies the ability of identifying shortcomings.”

Edsger Dijkstra

Precision of SvP Characterization Methods

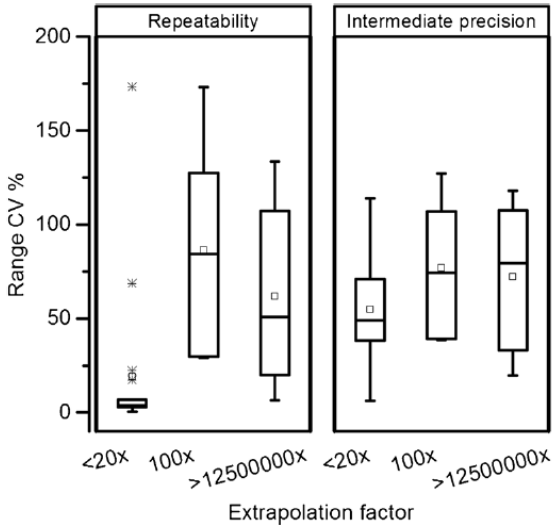


Fig. 1 Precision of subvisible particle methods in relation to the applied extrapolation factors. Syringes containing protein formulation stored for 2 months at 2–8°C were used for precision assessment. Results, reported as CV% were plotted against the corresponding extrapolation factors. Factors used were <20x for HIAC, MFI and CC. 100x for RMM. 12500000x for NTA.

Table V Sample volume and applied extrapolation factors to report final particle concentration normalized to 1 mL of the different instruments are summarized

Instrument	Measurement volume, V (mL)	Extrapolation factor, $1/V$ (mL^{-1})
HIAC	>1	1.0x
MFI	0.6	1.6x
CC	0.05	20x
RMM	0.01	100x
NTA	0.00000008	12500000x

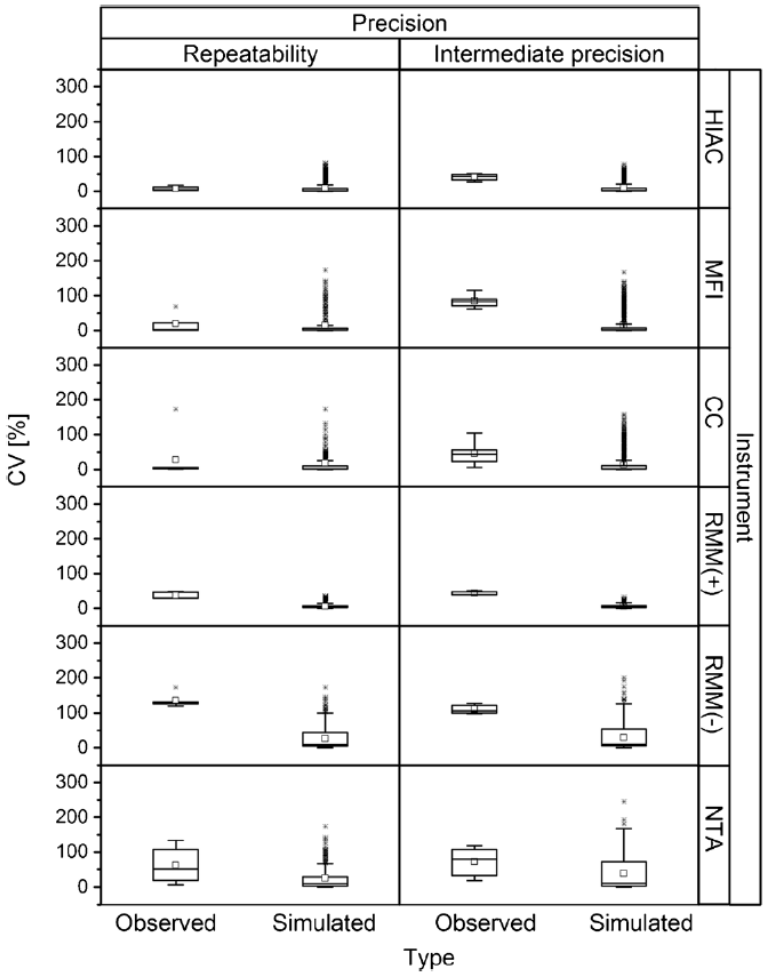


Fig. 2 Comparison of the experimentally measured and simulated (using Poisson distribution) CV% values per instrument and type of precision analysis. For additional details, please refer to Materials and Methods.

Rios et al., 2016, Pharm Res, 33: 450-

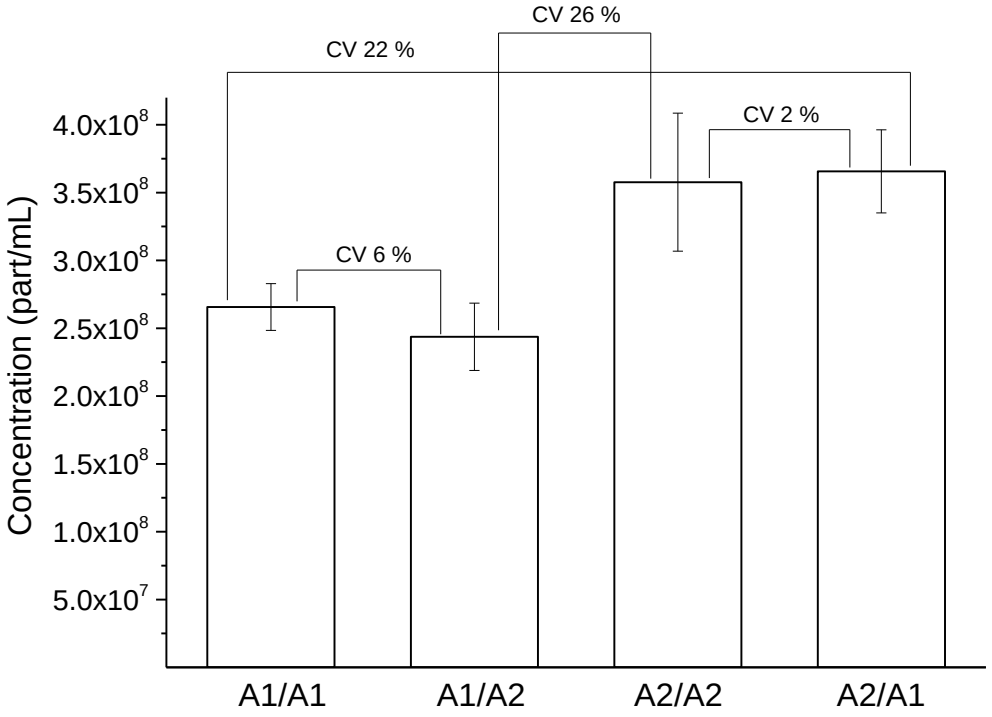
Example: Nanoparticle Tracking Analysis

Video recording and video analysis parameters of the measurement of a protein sample identically prepared and independently measured by two different analysts in different days.

Video recording	Analyst 1 (A1)						Analyst 2 (A2)					
	Video 1		Video 2		Video 3		Video 1		Video 2		Video 3	
Shutter	1265		1265		1265		299		299		299	
Gain	253		283		268		299		377		377	
Video analysis	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Blur	7	7	7	7	7	7	7	9	7	9	7	9
Detection Threshold	7	9	8	11	8	10	14	12	14	13	14	11
Min Track Length	10	10	10	10	10	10	10	10	10	10	10	10
Min Expected Size	50	100	50	100	50	100	100	50	100	50	100	100
Results	Mean		Stdesv				Mean		Stdesv			
Concentration	2.66E+08		1.72E+07				3.58E+08		5.09E+07			
Size	139		30				141		28			

Koulov et al., Biotherapeutic Analytical Summit 2015

Example: Nanoparticle Tracking Analysis



Intermediate precision – video recording setup has much higher impact then post-processing

Koulov et al., Biotherapeutic Analytical Summit 2015

What do We Need to Pay Close Attention To?

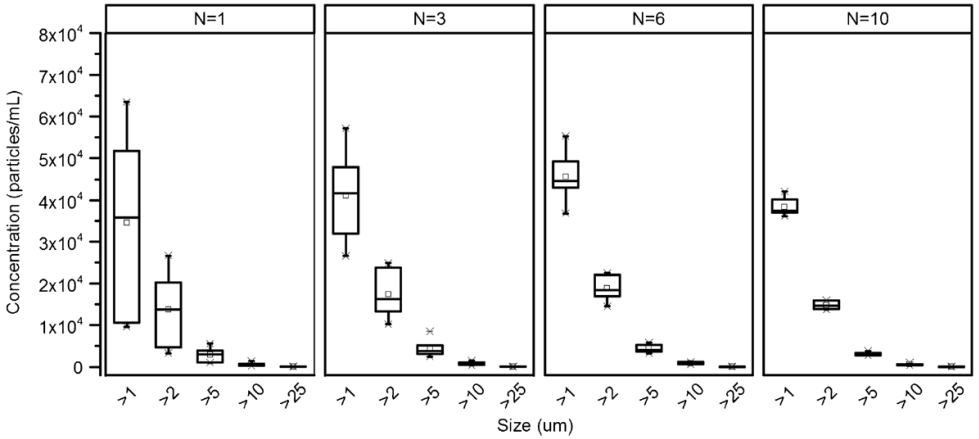
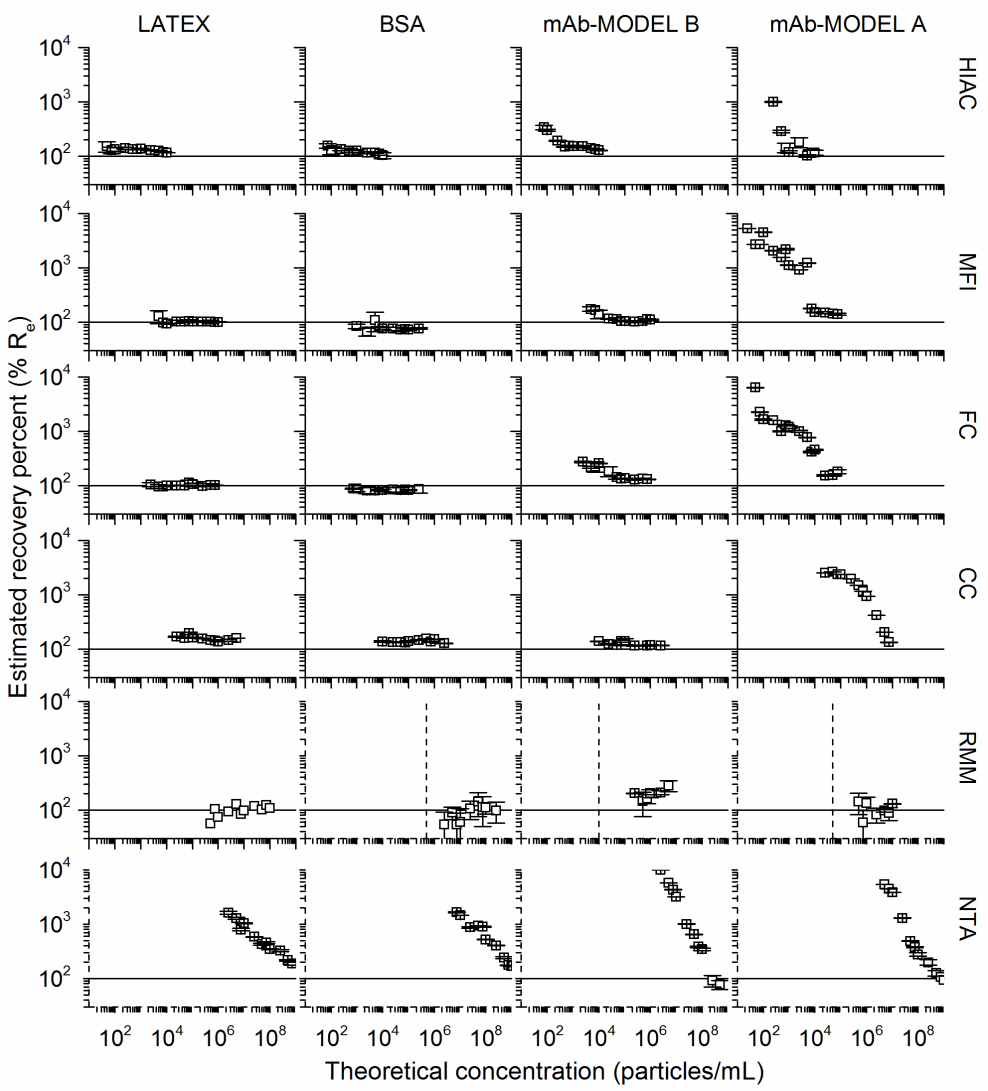


Fig. 3 Protein particle concentration variability as a function of pool size. Comparison of the variability of 6 independently prepared samples of commercial proteins. The content of a number N of prefilled syringes was pooled and analyzed by MFI.

Rios et al., 2016, 33: 450-

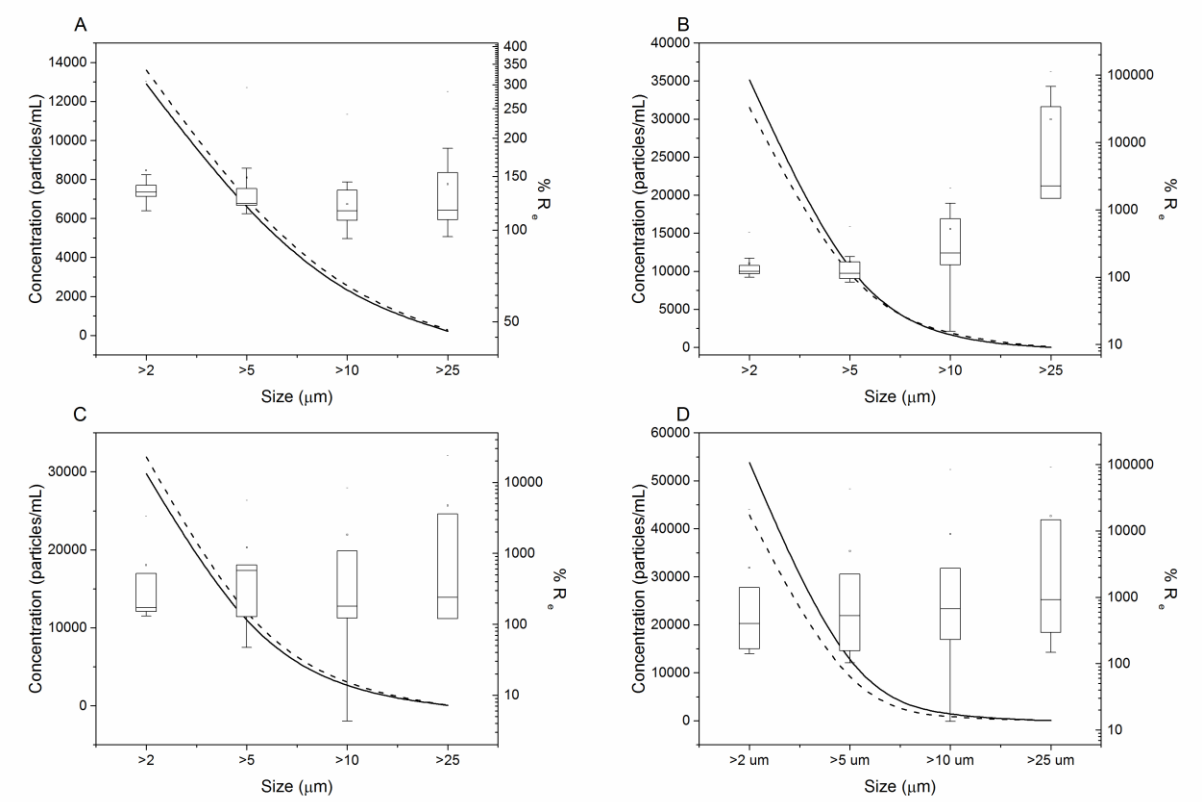
- Inherent method variability of SvP methods:
  - Large extrapolation factors in sub-µm methods
  - Sample prep (e.g. pooling)
  - Method-specific factors
- Evaluation of method performance is essential and may require major efforts, significant resources and expert knowledge

Accuracy of SvP Characterization Methods



Rios et al., 2016, J Pharm Sci , 105(7):2042-52

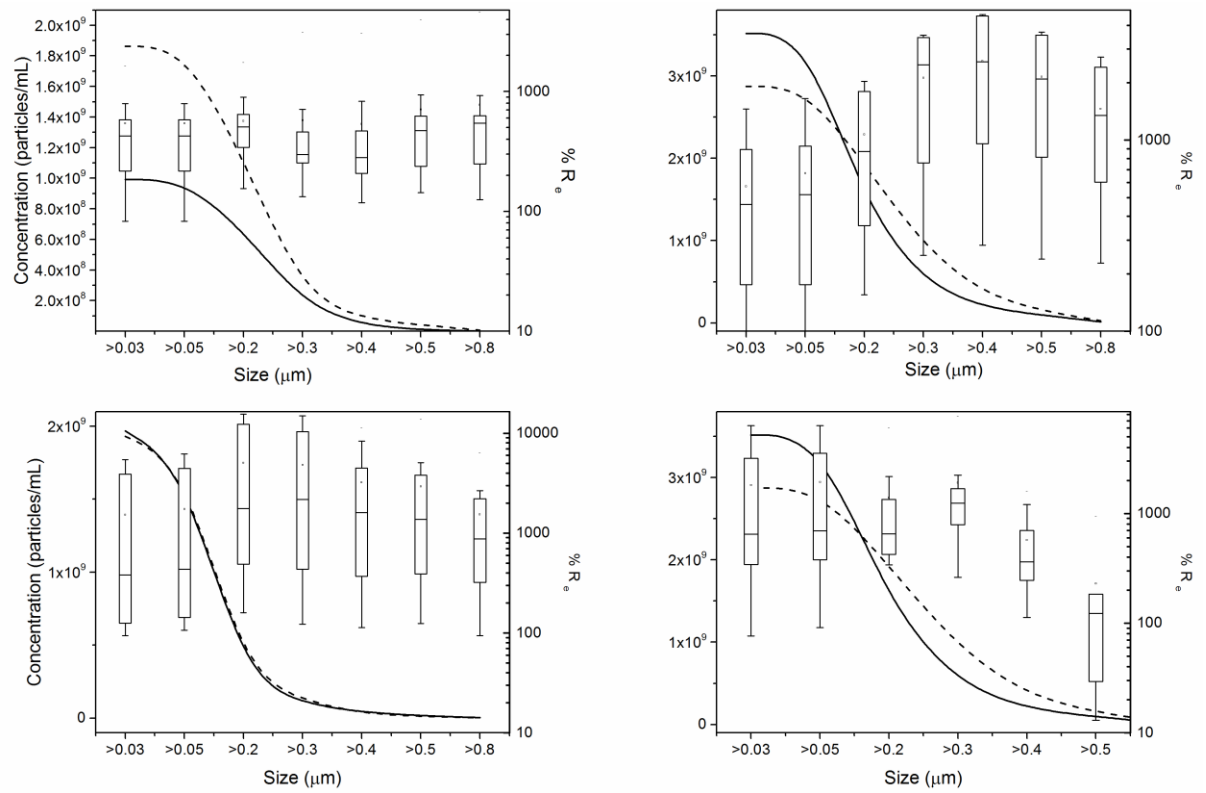
Accuracy of SvP Characterization Methods



Example 1: Light Obscuration

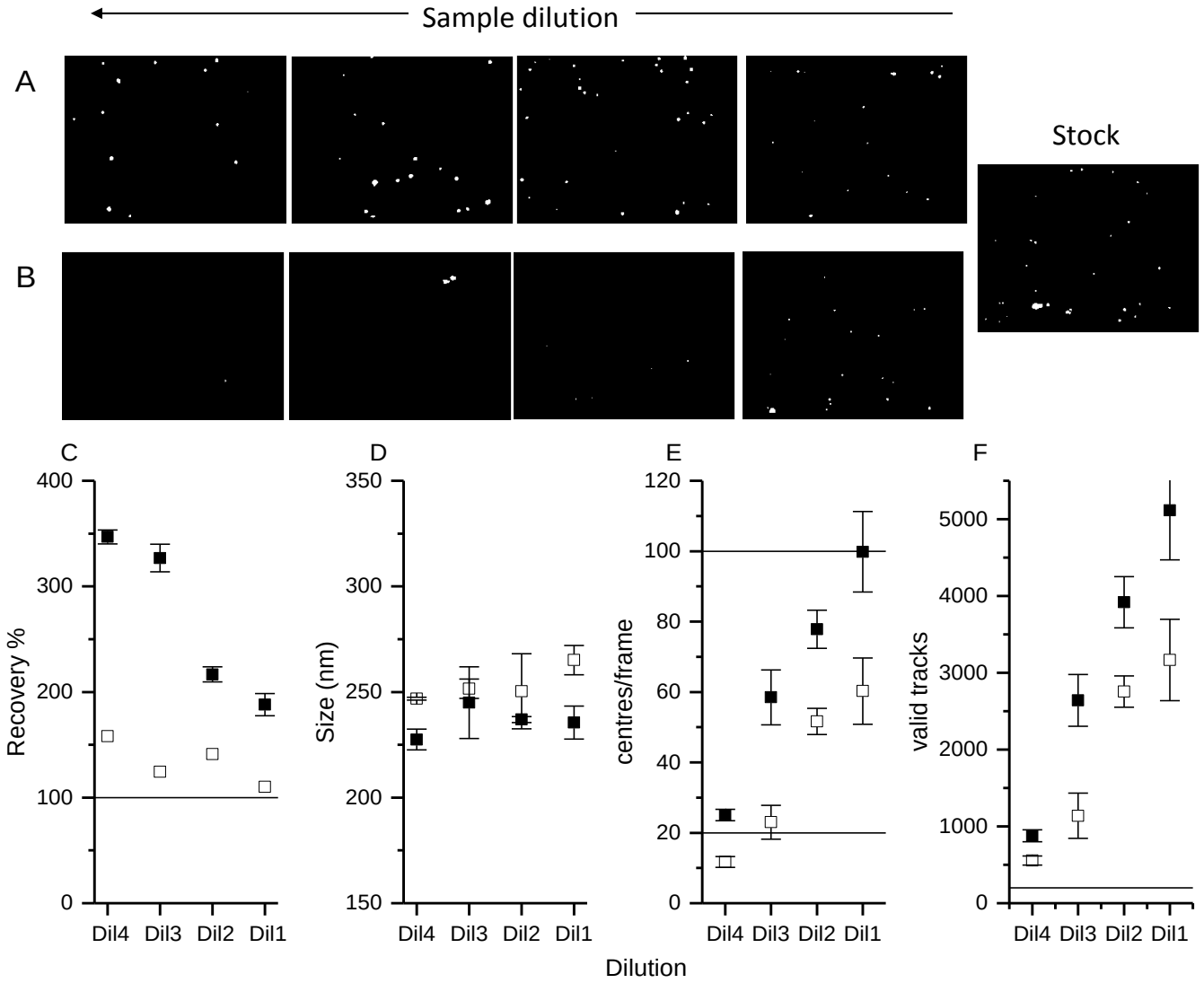
Rios et al., 2016, J Pharm Sci , 105(7):2042-52

Accuracy of SvP Characterization Methods



Example 2: Nanoparticle Tracking analysis

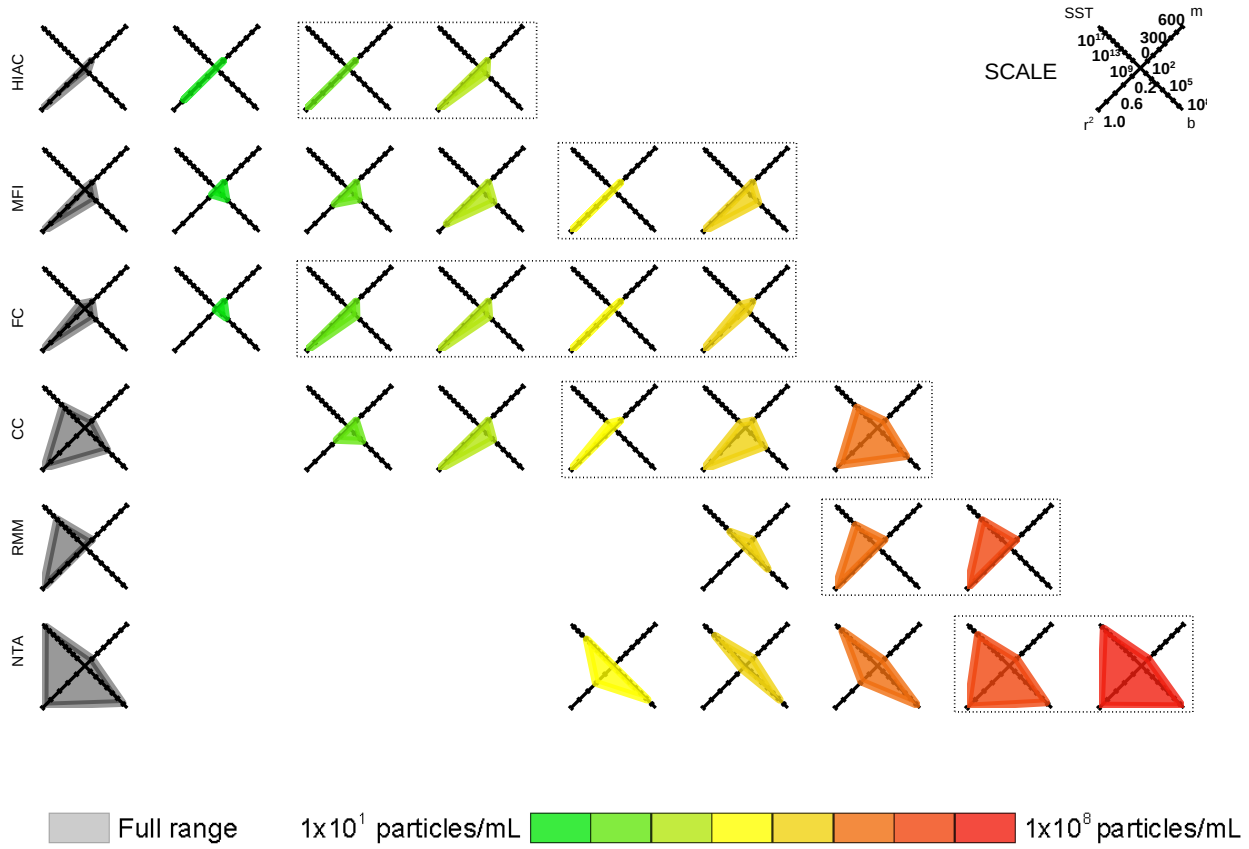
Rios et al., 2016, J Pharm Sci , 105(7):2042-52



Nanotracking analysis: Influence of the operator – video recording settings

Koulov et al., Biotherapeutic Analytical Summit 2015

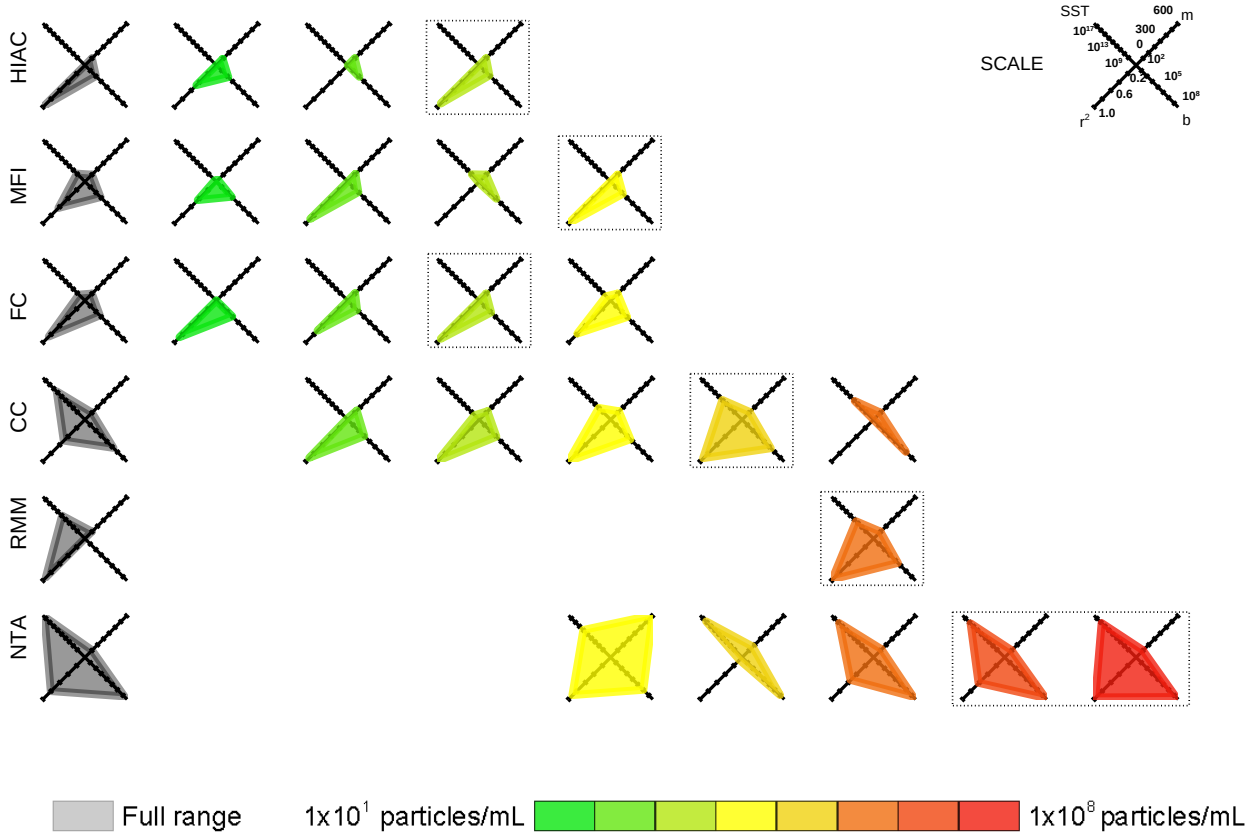
Linearity of SvP Characterization Methods



Example 1: Latex beads

Rios et al., 2016, J Pharm Sci , 105(7):2042-52

Linearity of SvP Characterization Methods

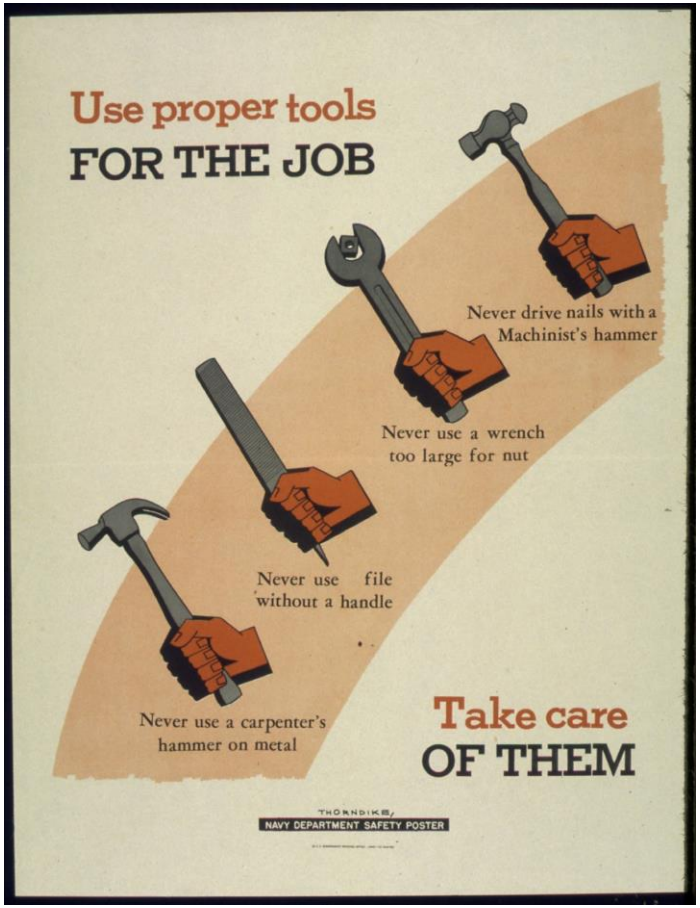


Example 1: mAb model A

Rios et al., 2016, J Pharm Sci , 105(7):2042-52

What Do We Do?

- How do we “cover” the entire SvP range?
  - ~~Easy, just measure everything~~
- Evaluation of method performance is essential and requires major efforts, significant resources and expert knowledge
- Different tools for different jobs:
  - Product Quality (SvP measurements for submission dossiers)
  - Product characterization (e.g formulation or device development)



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  Nadine Ris
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  Christof Finkler

Thank you

Drug Product Services

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