

# Application of Image Analysis extensions to processes of relevance to drug development

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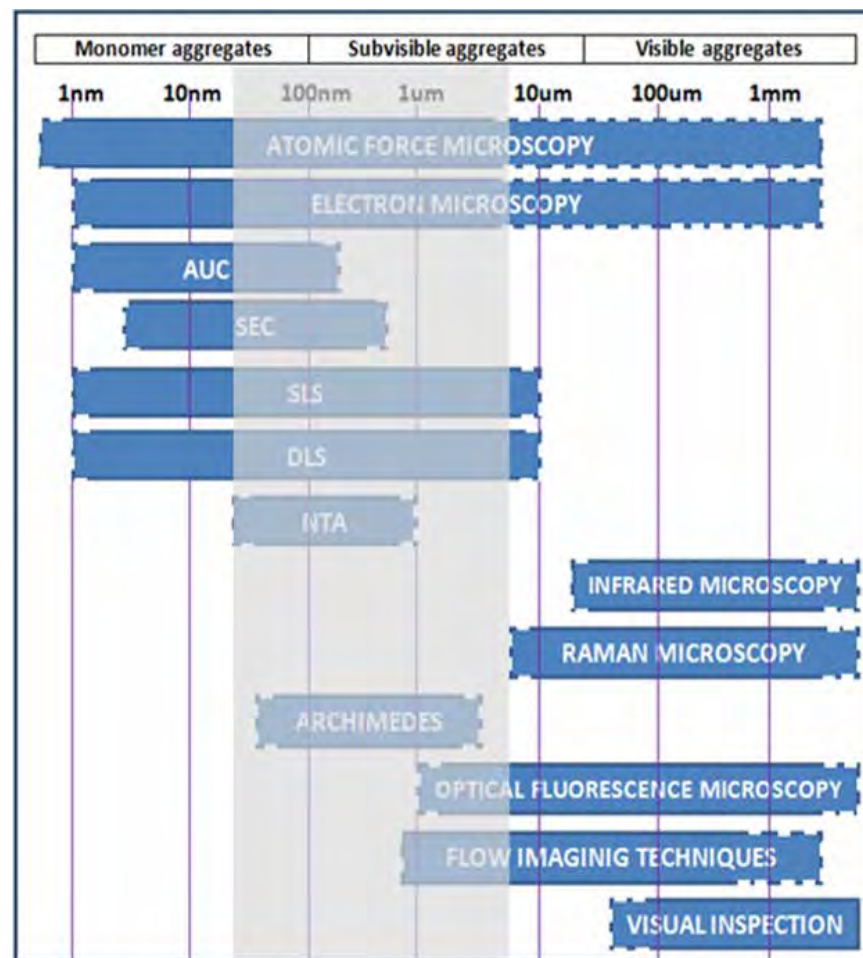
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# Proteinaceous aggregates characterisation

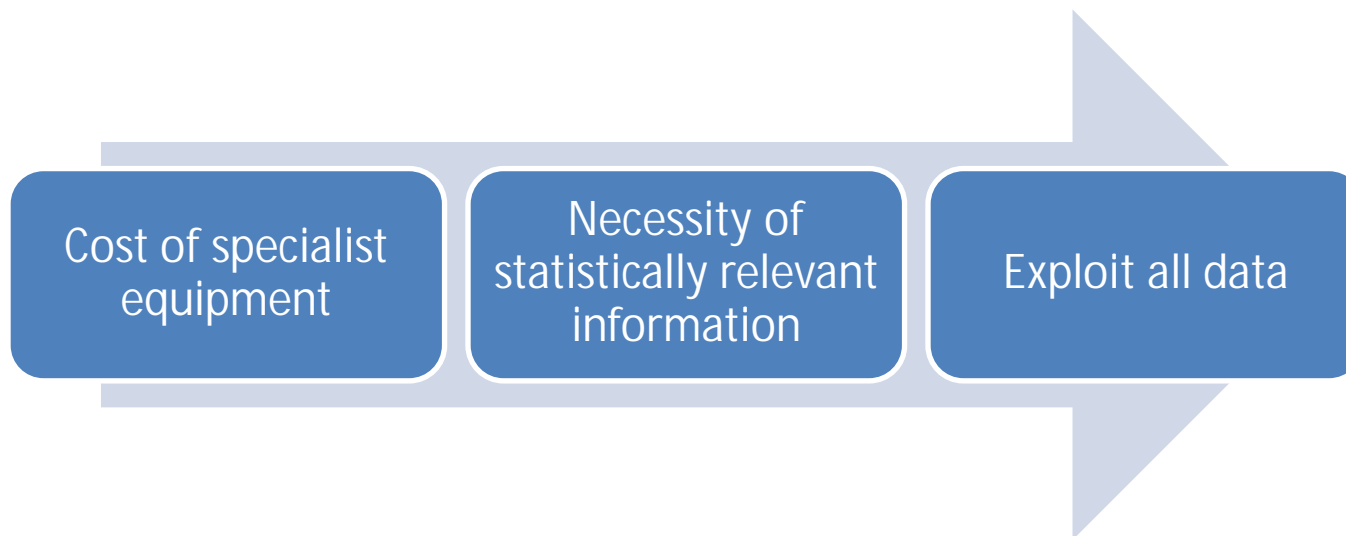
- Meet regulators requirements
  - USP chapter "Particulate matter in Injections" <788>: concentration limits for >10µm
- Provide additional information on presence of smaller aggregates
  - USP chapter <787> "Subvisible Particulate Matter in Therapeutic Protein Injections": monitor particles < 10µm
- Welcome information from orthogonal techniques (FDA)
- Adaptable to real formulations
- Contribute to prediction based on relevant stresses



Adapted from Zolls et al., J Pharm Sci, 2012

# Image analysis techniques

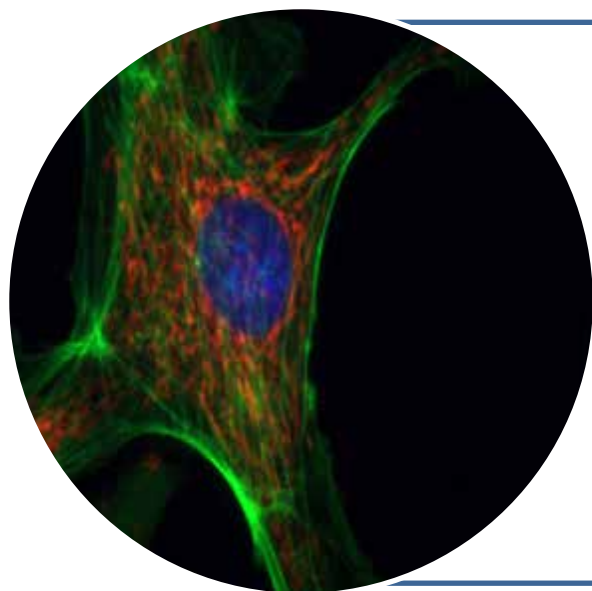
Recent considerations in life sciences



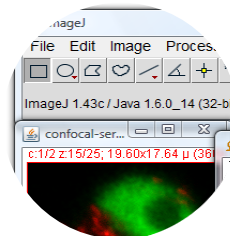
Typical example is fluorescence microscopy

# Fluorescence microscopy

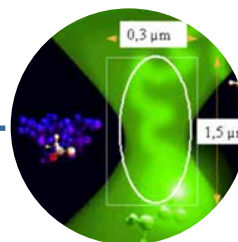
Moved from epifluorescence to STED, PALM over ~30 years



Initially high impact  
qualitative



Semi-quantification  
Image J



Quantification  
techniques: Fluorescence  
Correlation Spectroscopy

# Fluorescence microscopy related image analyses



Image correlation spectroscopy  
(Pedersen et al., early 90s)

Development of variants  
i.e. kICS, RICS etc...  
(Wiseman, Gratton)

Spatial Intensity  
Distribution Analysis  
(Wiseman group)

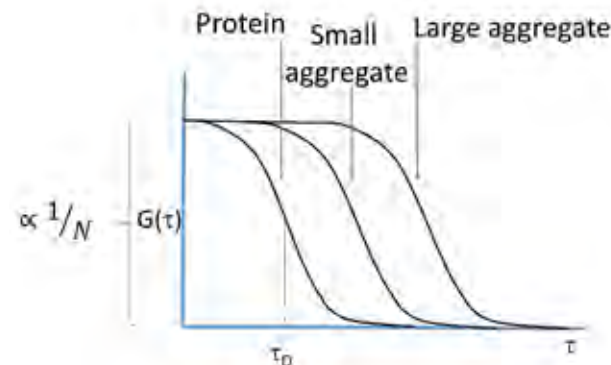
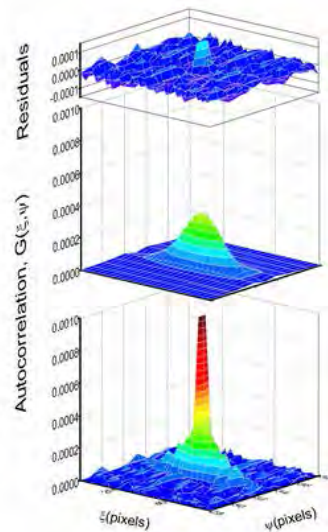
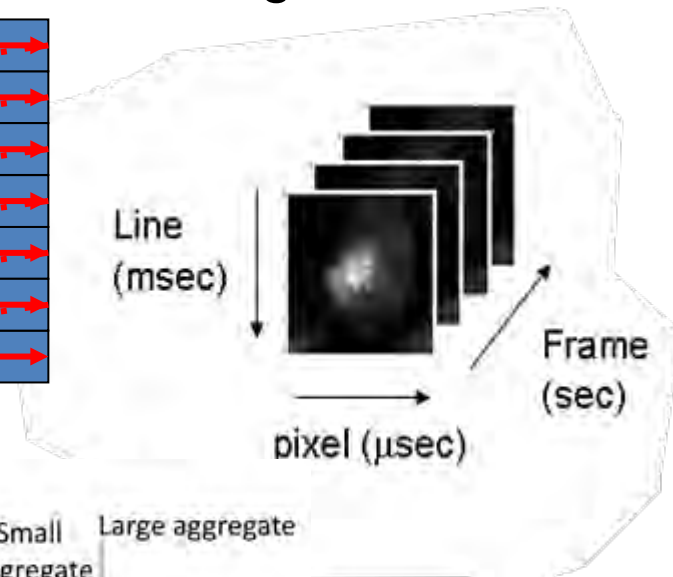
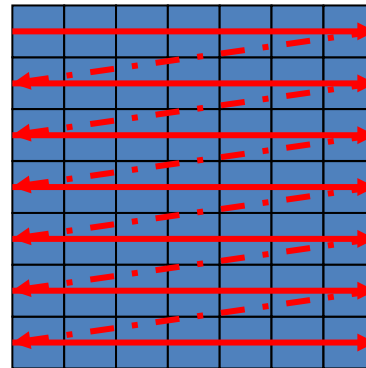
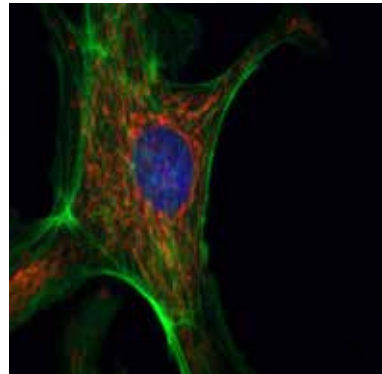
Number and Brightness  
(Gratton)

and many others...

In house RICS software ManICS

# RICS: HOW IT WORKS

# Image Correlation Spectroscopy: Using the information contained in images

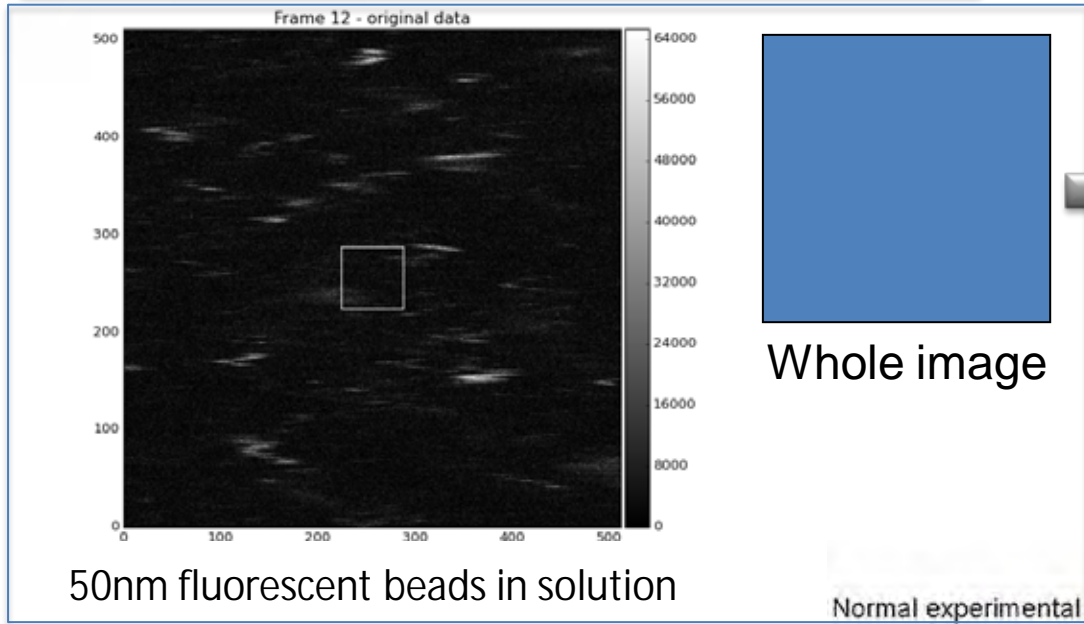


Adapted from Digman et al, Biophys J, 2005

Hamrang et al., J Pharm Sci, 2012



ICS does not track particles: it considers/correlates fluorescence fluctuations. Fundamentally different from NTA

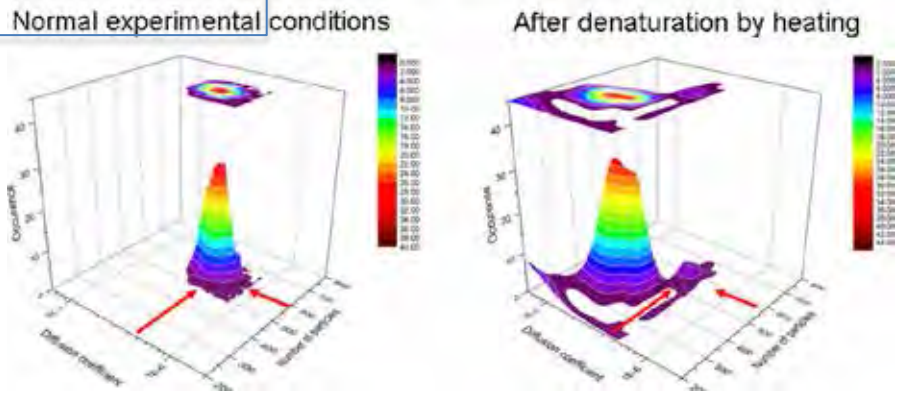


50nm fluorescent beads in solution

1µM Alexa Fluor BSA in PBS

Proof of concept:  
heat stressed AF488-BSA solution

Hamrang et al., J. Pharm. Sci. (2012)





# STUDY OF AGGREGATION PROPENSITY

# RICS: move towards real formulations

- High concentration of proteins
- Excipients and contaminants
- High vs low concentration

RICS:  
Short optical path  
Backwards  
Only label aggregate  
Hydrophobicity

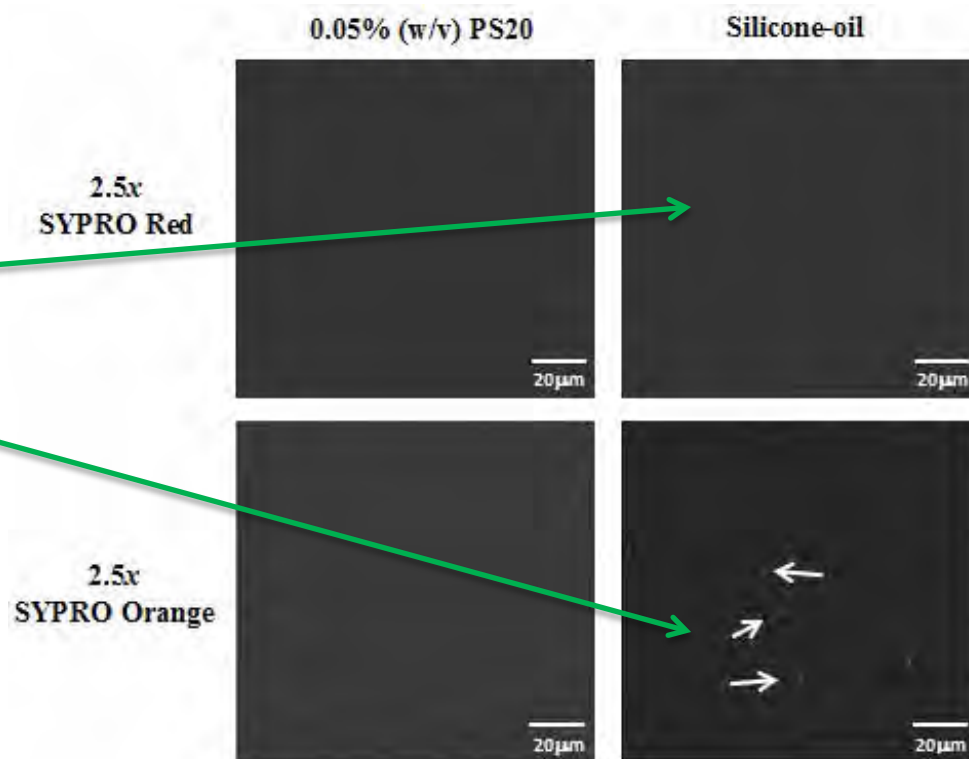
Important choice  
Extrinsic fluorescent dyes to label:  
(1) Aggregates  
(2) The medium

## Aggregate labelling optimisation

- Sufficient signal to noise ratio
- Specific to aggregates (does not label silicone oil droplets or surfactant)
- Photostable
- Rule out autofluorescence of components e.g. silicone oil droplets

- SYPRO<sup>®</sup> red (2.5x) does not label silicone oil droplets

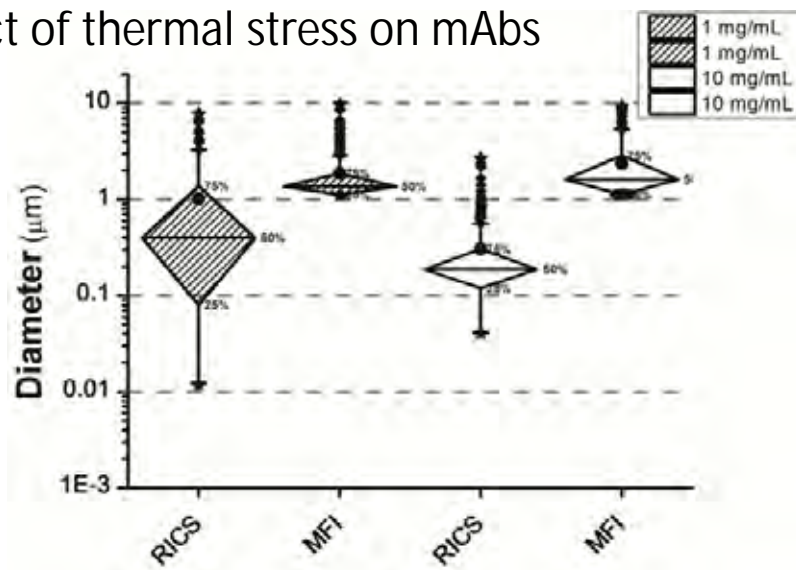
- SYPRO<sup>®</sup> orange (2.5x) labelled silicone oil droplets



# Evaluation in simple solutions

- Validation and benchmarking of RICS against standard techniques (DLS, MFI) in buffered solutions
- Two types of stress were imposed to mAbs solutions (heat and freeze thaw cycles)
- Sypro red is added after stress

Effect of thermal stress on mAbs



Concentration (mg/mL)	1	10
Treatment	Z-average Diameter (nm)	
NT	14.60±0.74	10.71±0.10
58 °C	33.40±0.20	30.23±0.00

Hamrang et al., J Pharm Sci (2015), 104, 2473-2481

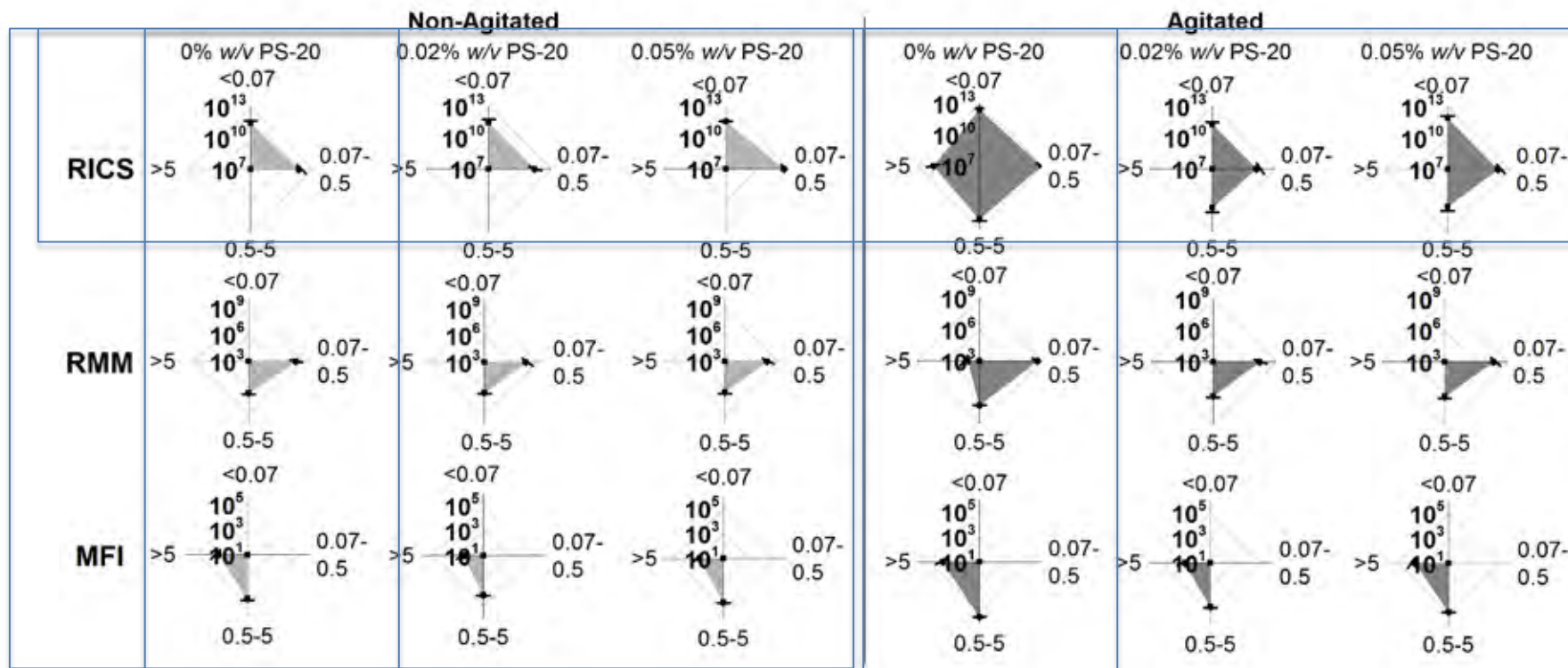
Similar trends were observed following freeze thaw cycles on mAb solutions

# Move towards real formulations (PFS)

Differentiation between proteinaceous and non-proteinaceous sub visible particles



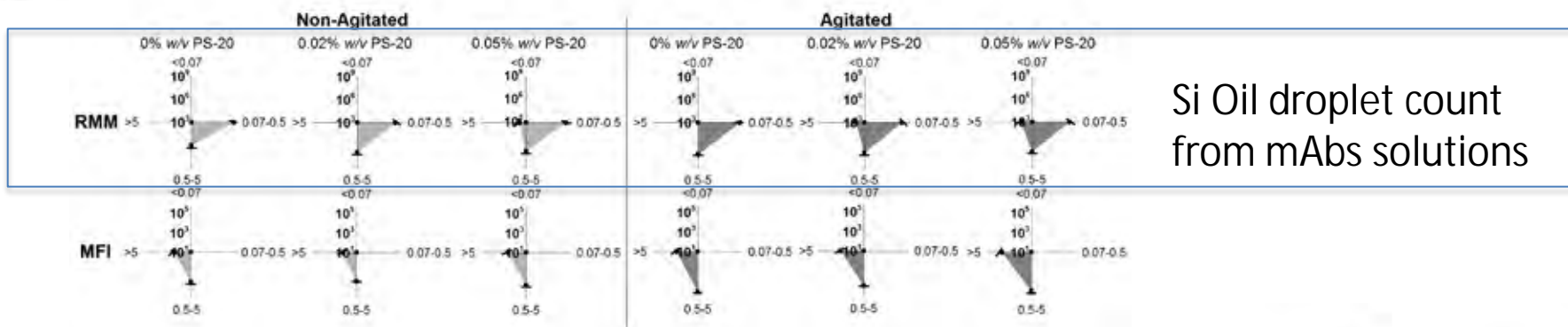
mAbs aggregates



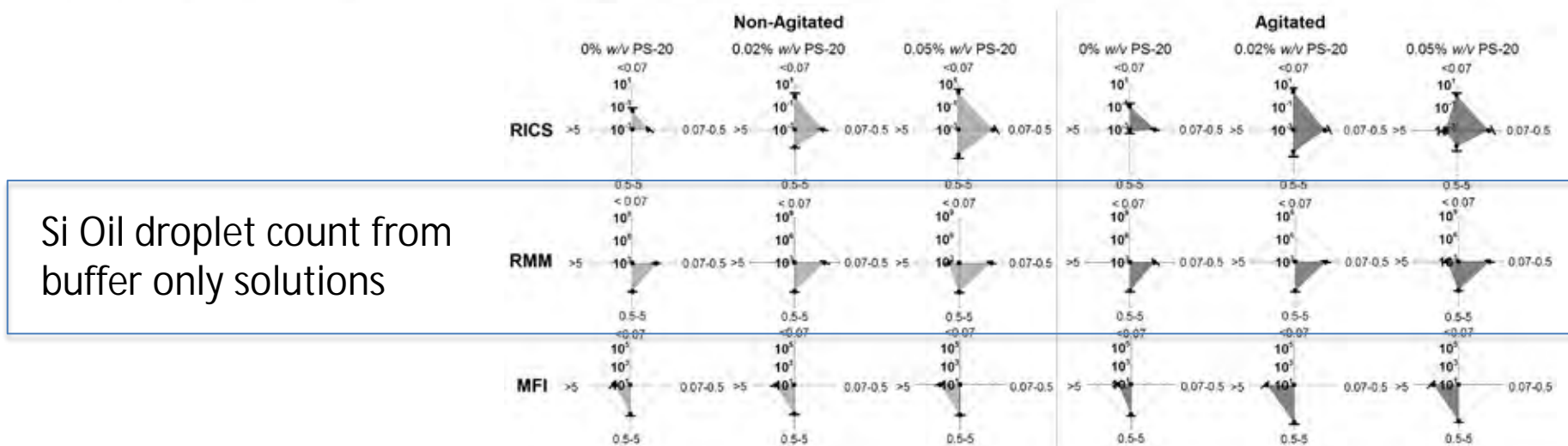
Shah et al. Int J Pharm (2017), 519, 58-66

# Move towards real formulations (PFS)

Differentiation between proteinaceous and non-proteinaceous sub visible particles



Si Oil droplet count from mAbs solutions



Si Oil droplet count from buffer only solutions



# Conclusion

- Image analyses are useful tools
- RICS has been benchmarked and covers a wider range than RMM
- RICS is able to distinguish between proteinaceous and non-proteinaceous aggregates
- PS contributes to Silicone oil droplets presence
- Move towards flow to increase sampling
- Use of SP8 allows for kinetics (aggregates formation) as dataset obtained for each image

# Thank you for listening

## Acknowledgments

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# Are imaging tools novel?

- Imaging techniques and associated analyses in use
- Examples:

- Microflow imaging

- Size and conformation
- Complementary to Archemides (RMM)

(Weinbuch et al., J Pharm Sci, 2013)



- Nanoparticle tracking

- Evanescent wave
- Multiple particle tracking

Advantages/disadvantages:  
MFI: particle range  
NTA: dilution



## A non exhaustive list of current techniques

Analytical method	Aggregate size ( $\mu\text{m}$ )	Advantages	Disadvantages
DLS	0.001-5	Sensitive to large aggregates broad analyte concentration	Semi-quantitative, bias towards large aggregates, dust, unsuitable for polydisperse/complex samples
Analytical ultracentrifugation	0.001-0.1	High resolution, absolute concentration, size shape	Low sample throughput, sample dilution, complex data analysis, susceptible to excipients, expert operator needed
Asymmetric field flow fractionation	0.001-100	Low concentration sample analysis	Sample dilution, qualitative for large aggregates, potential interaction with membrane

Adapted from Hamrang et al., TRENDS in Biotechnology, 2013

A more structured and complete list is proposed in Hawe et al. (J Pharm Sci 2012)