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Biopharmaceutical Research and Development Service

Micro-Flow Imaging and resonant mass measurements (Archimedes) evaluated for a quantitative differentiation of protein particles and silicone oil droplets

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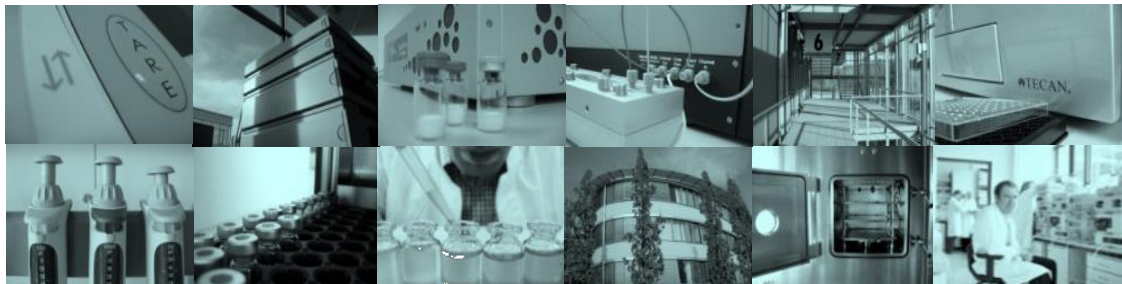
EIP Symposium - Munich

Outstanding solutions for biopharmaceuticals



Requirements & trends for particle characterization

- authorities require more than data for particles > 10 µm and
  > 25 µm (light obscuration) and visual inspection:
  Quantification: 2 -10 µm (type and amount)
  Characterization: 0.1 and 1 µm

- increasing need for particle identification for all size classes:
  e.g. differentiation **proteinaceous particles ↔ silicone oil droplets**
  is highly relevant for products in DCCs or pre-filled syringes

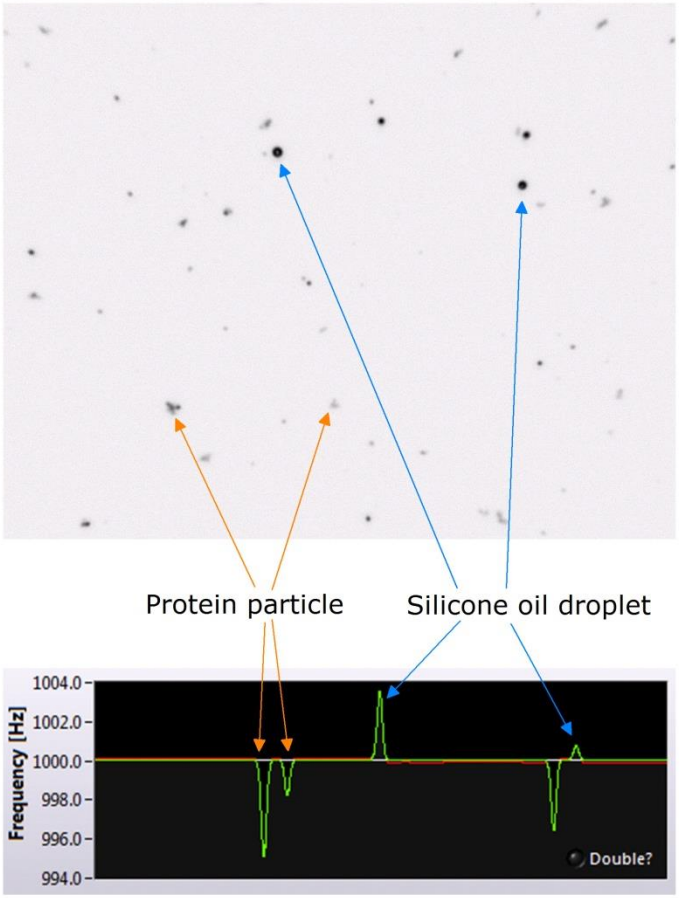
Methods for silicone oil droplet ↔ protein particle

MFI

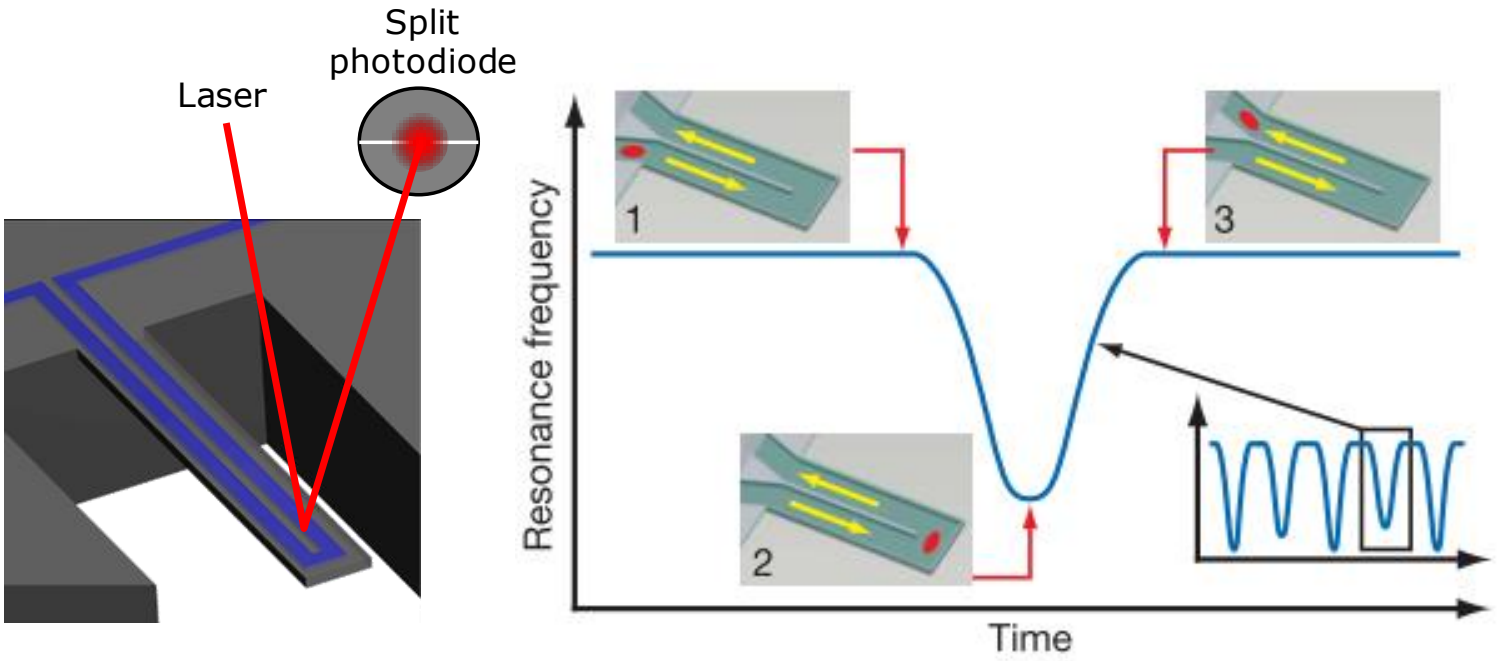
- → flow microscopy combined with digital image analysis
- → size range: about 1 – 70 µm
- → differentiation of particles (silicone oil and protein) based on image analysis

RMM

- → particle size determination by mass measurement
- → size range: 100 nm – 1 µm (Nanosensor)
  300 nm – 5 µm (Microsensor)
- → differentiation of particles based on density (above or below formulation density)



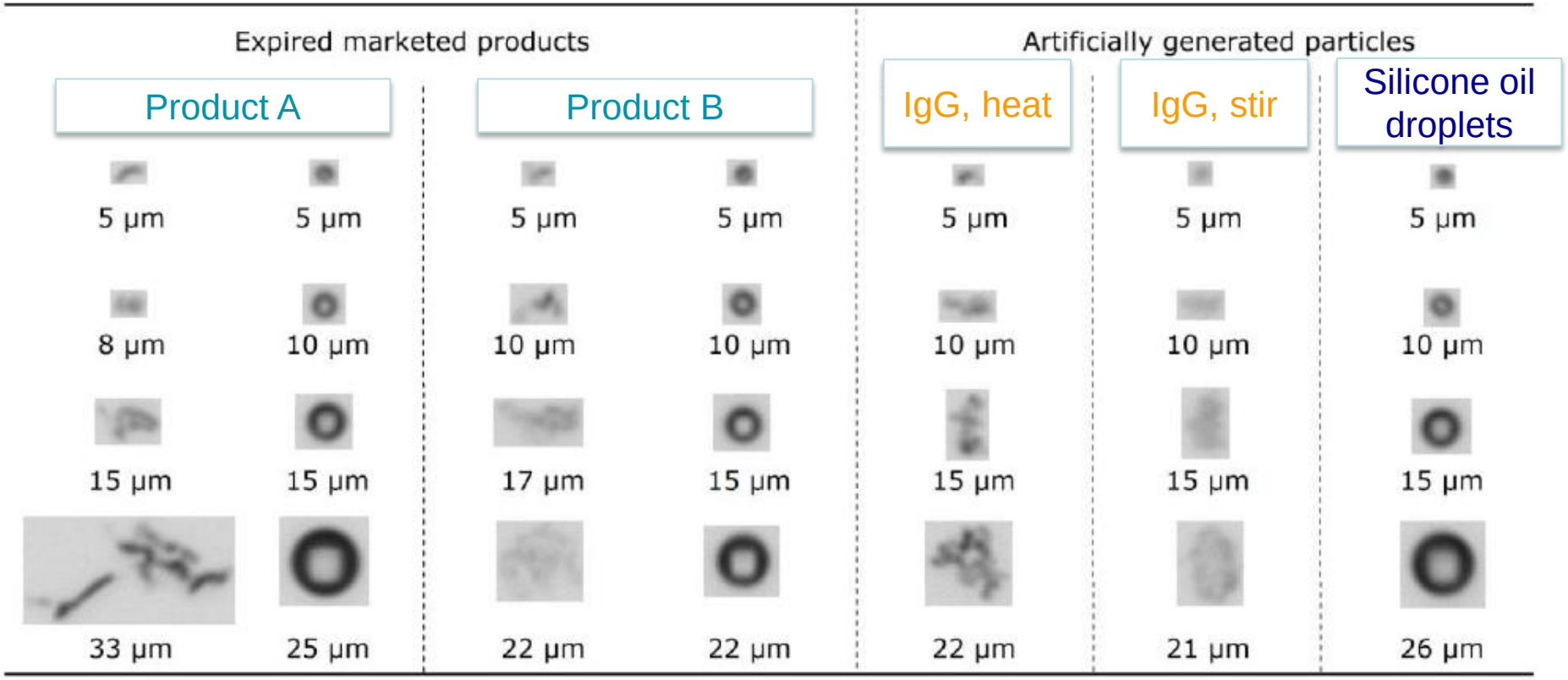
Resonant mass measurements (*ARCHIMEDES*)



- individual particles are weighed in a mechanically resonating microfluidic channel

  → frequency changes depending on particle mass
- calculation of particle size based on assumed density

Protein particles – silicone oil droplets in product



→ artificially generated protein particles and silicone droplets are representative for particles in marketed products

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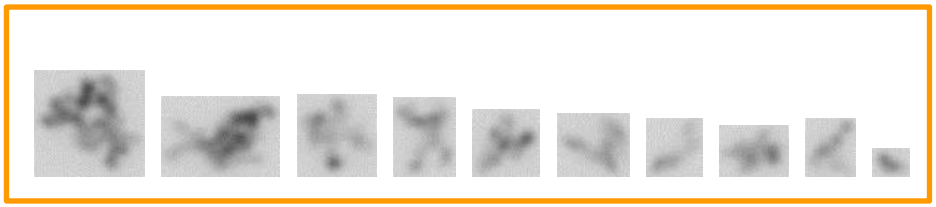
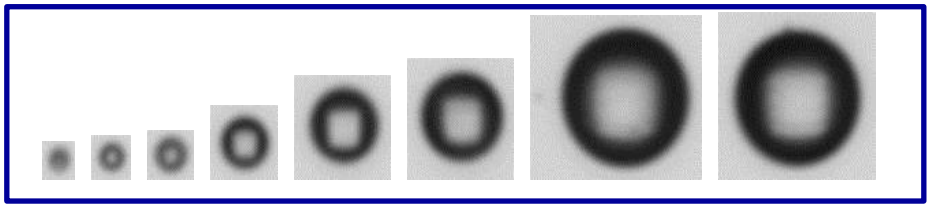
Zölls / Weinbuch et al. (2012), submitted for publication

Set-up of the study

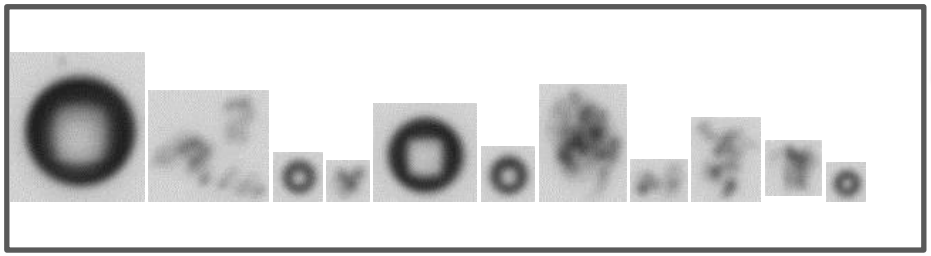
Heat stressed IgG



Silicone oil droplets



Preparation of various spiking ratios



Analysis

Micro-Flow Imaging (MFI)
resonant mass measurements (RMM)

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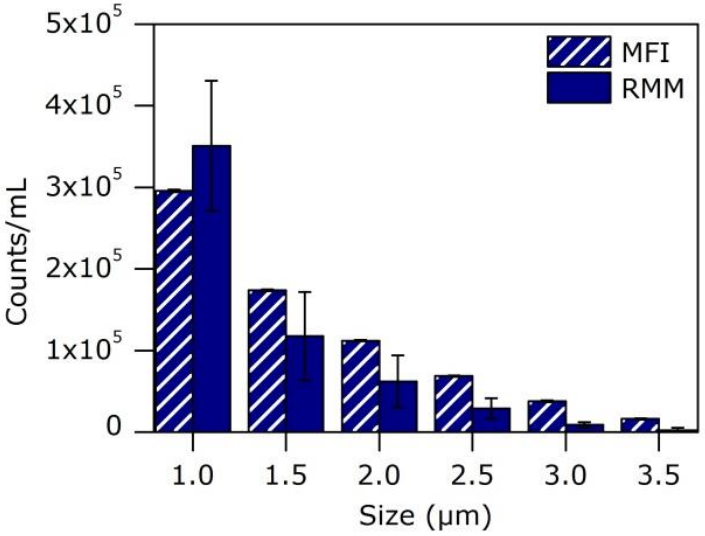
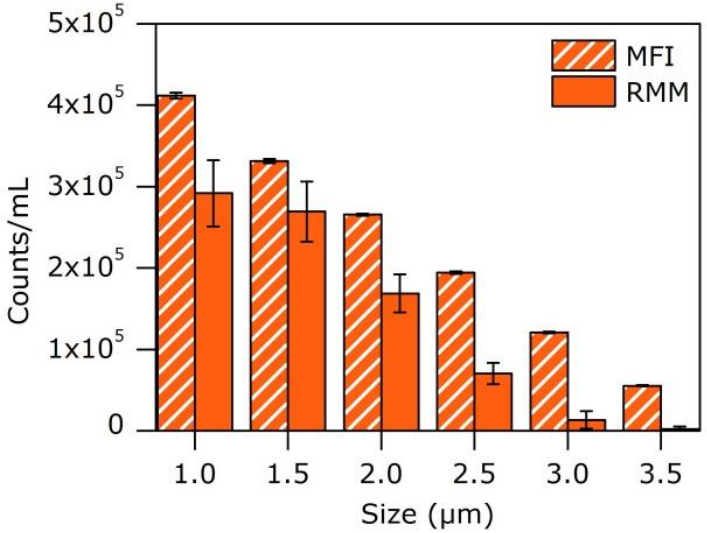
Analysis of individual samples

Silicone oil droplets: more in RMM



Protein particles: more in MFI



Possible reasons for differences between RMM and MFI:

(i) fragmentation of larger silicone oil droplets within RMM to form smaller ones → higher silicone oil numbers in RMM than in MFI

(ii) possible blockage of RMM by larger protein particles → underestimation of protein particle numbers

(iii) different measurement principle

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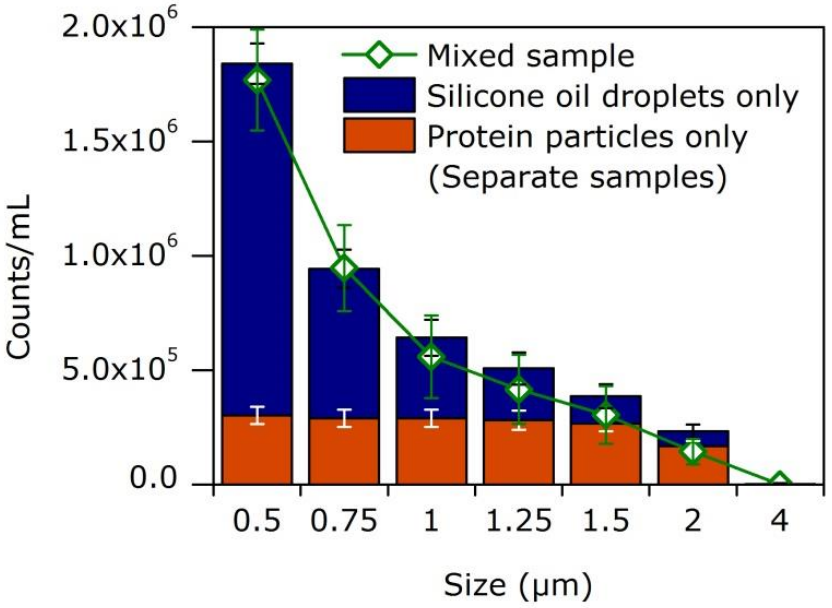
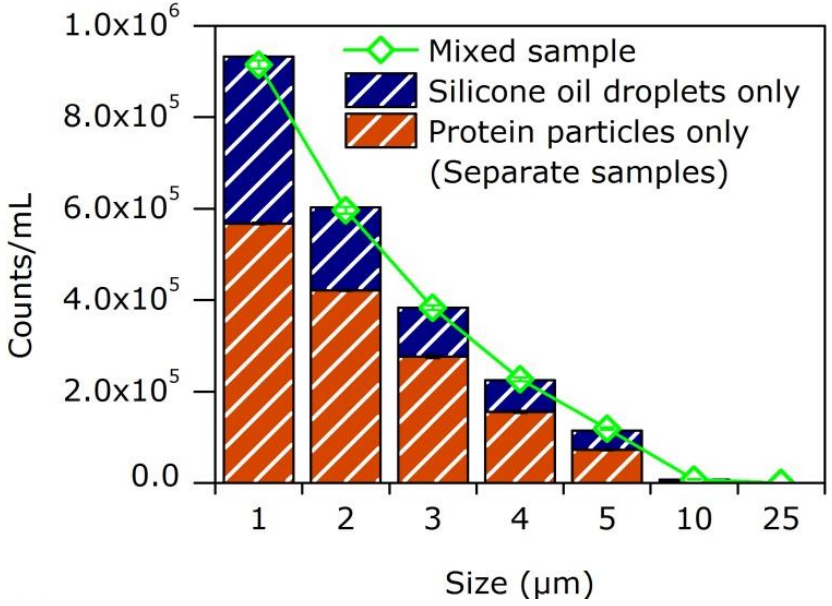
MFI and RMM: separate samples ↔ mixture

RMM



MFI



→ study design was controlled by analyzing separate samples (=only silicone oil or protein particles) compared to the mixture of both

→ measured concentration for mixed samples = theoretical sum of silicone oil droplets + protein particles

→ individual samples can be used for the theoretical concentration in the mixed samples

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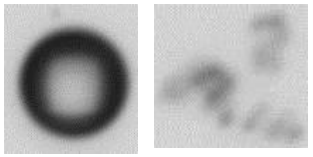
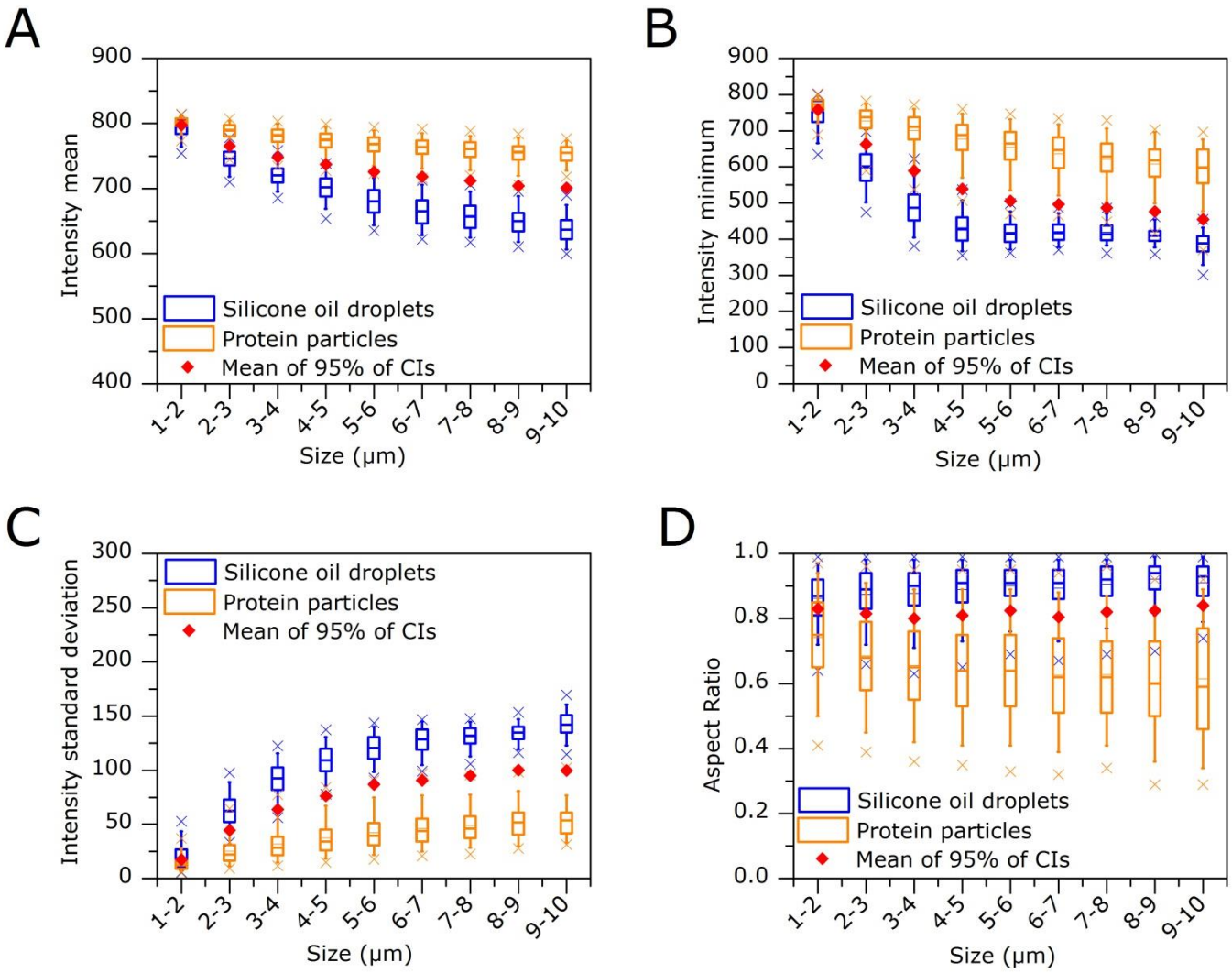
Customized filter for MFI data



- Samples used for customize filter development
  - silicone oil droplets
  - heat-stressed IgG
- Parameters included into the filter
  - intensity mean = mean intensity value over all pixels within one particle
  - intensity minimum = the intensity of the darkest pixel of a particle
  - intensity standard deviation = differences between higher and lower intensity values within the same particle
  - aspect ratio = shape parameter (1 = spherical; 0”= a needle with an infinite length)
- Cut-offs defined at the mean value of 95% confidence intervals between two populations for sizes 2-9 µm based on polynomal fit
- If all four cut-offs were fulfilled → particle marked as silicone oil

Customized filter for MFI data



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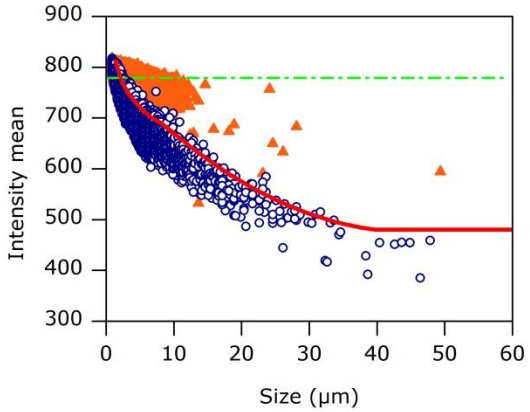
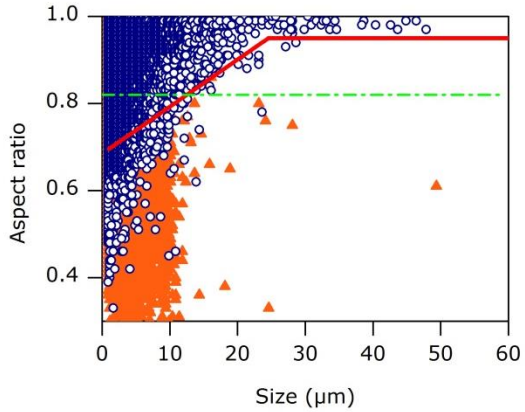
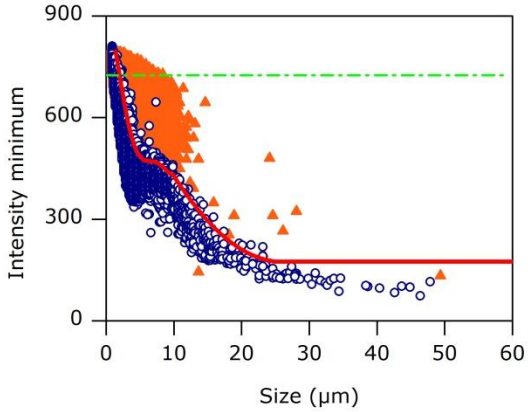
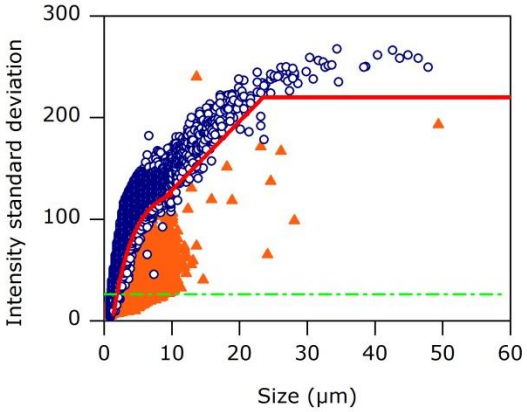
Customized filter for MFI data

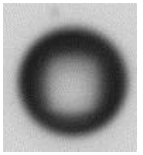








silicone oil droplets



protein particles

customized data filter

"find similar" filter

→ customized filter combines several particle properties and enables more specific cut-offs

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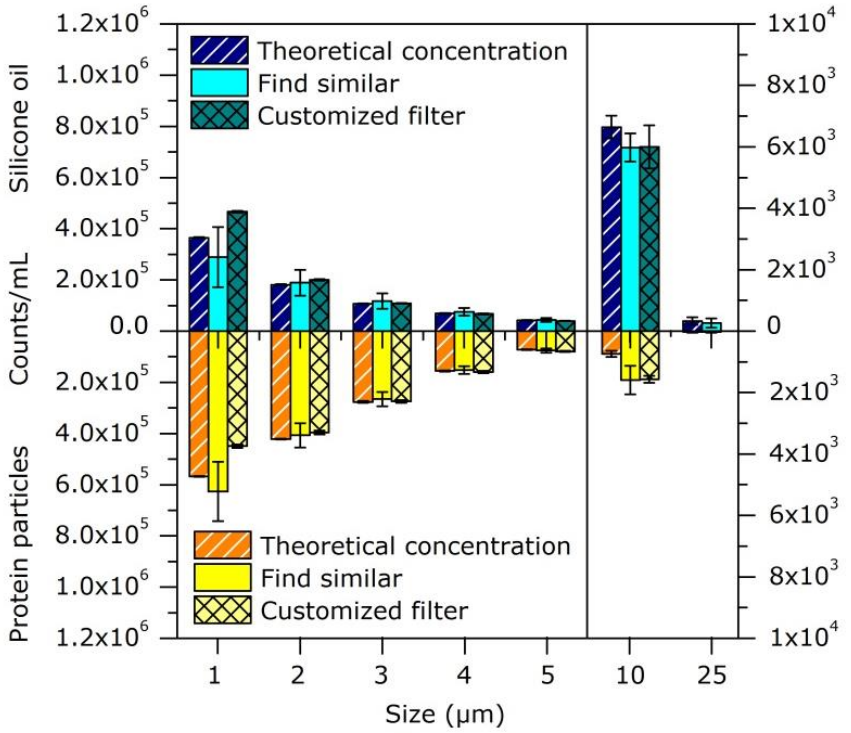
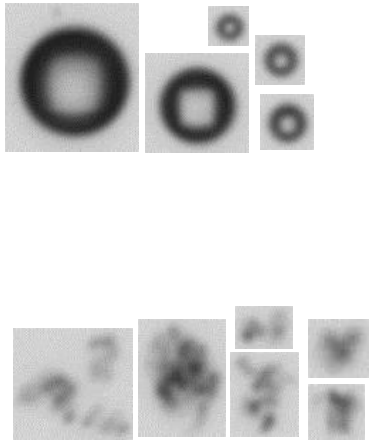
Zölls / Weinbuch et al. (2012), submitted for publication

MFI: protein ↔ silicone oil

Mixing ratio of 40:60 (protein particles : silicone oil droplets)



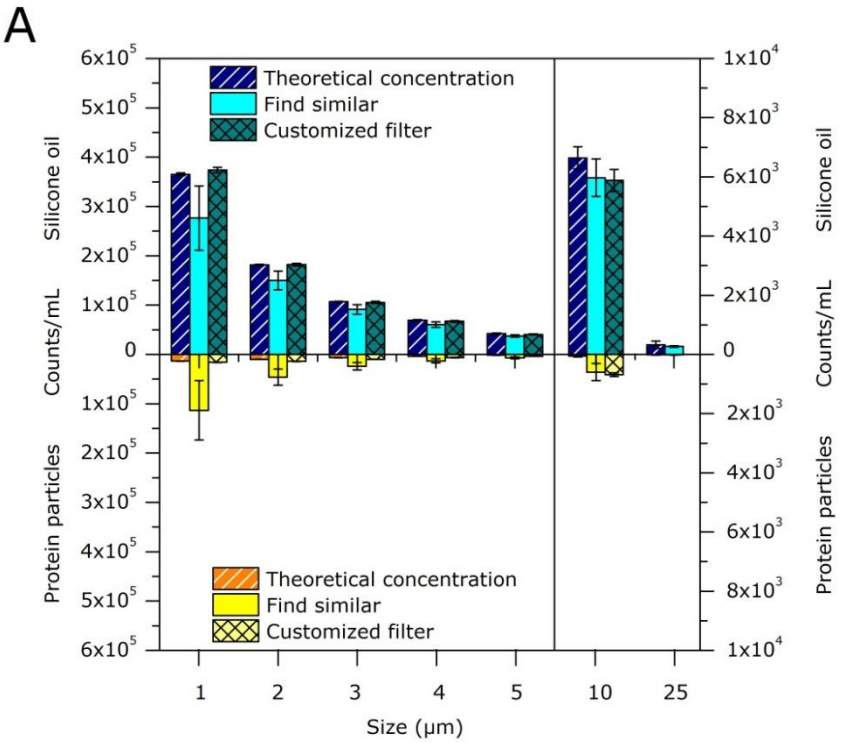


→ reliable differentiation for particles > 2 µm with both approaches

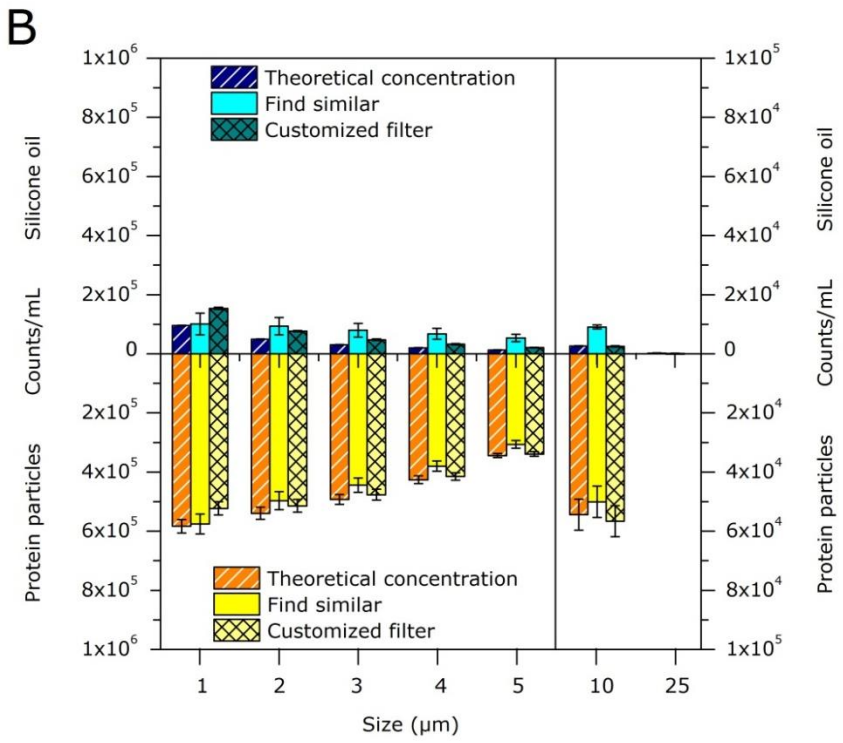
Zölls / Weinbuch et al. (2012), submitted for publication

MFI: protein ↔ silicone oil at extreme ratios

Mixing ratio of 95:5



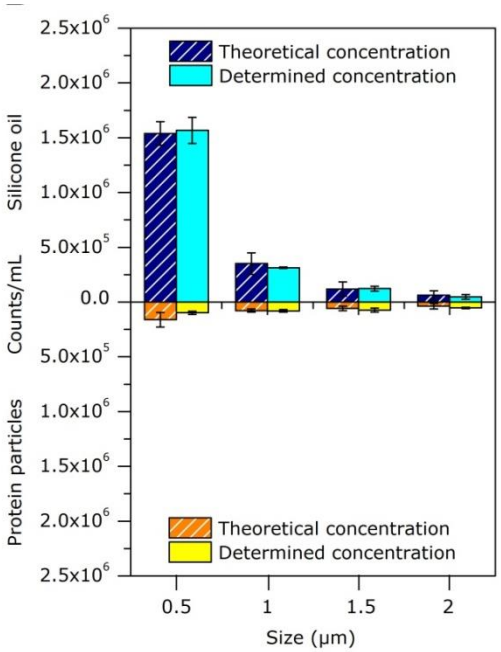
Mixing ratio of 15:85

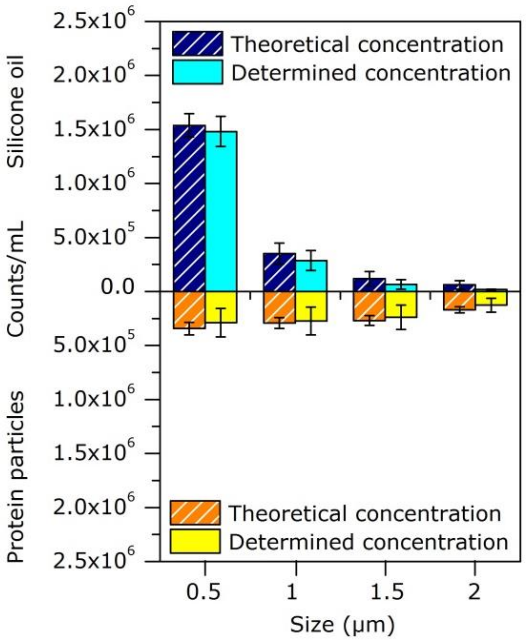


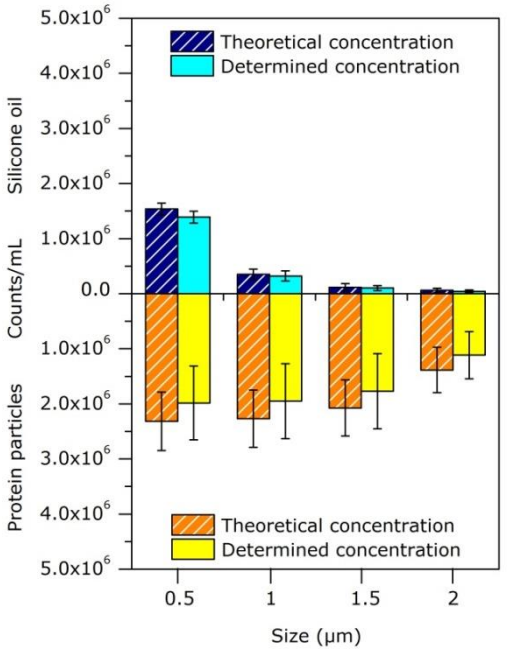
→ differentiation by customized filter slightly better for particles > 2 µm

Zölls / Weinbuch et al. (2012), submitted for publication

RMM: silicone oil ↔ protein







→ RMM discrimination is very accurate for all ratios

→ higher STDEV when low particle counts are measured or because of increased coincidence and blockage in case of high protein particle concentration

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Zölls / Weinbuch et al. (2012), submitted for publication

Comparison of MFI and RMM

- MFI and RMM allow to quantify and distinguish silicone oil droplets and protein particles using different measurement principles

- RMM → suitable for the size range of 500 nm to 2-3 µm
- MFI → image analysis reliable starting at a size of 2-4 µm to ~25 µm (find similar, customized filter)

  → discrimination by optical evaluation recommended > 25 µm

→ combination of both techniques to cover the whole size range

Acknowledgements

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- Michael Wiggenhorn (Coriolis)

- **Coriolis Pharma Team**

Coriolis' scientific Advisory Board

- Wolfgang Friess (LMU)
- Gerhard Winter (LMU)
- Wim Jiskoot (Leiden University)
- John Carpenter (University of Colorado)

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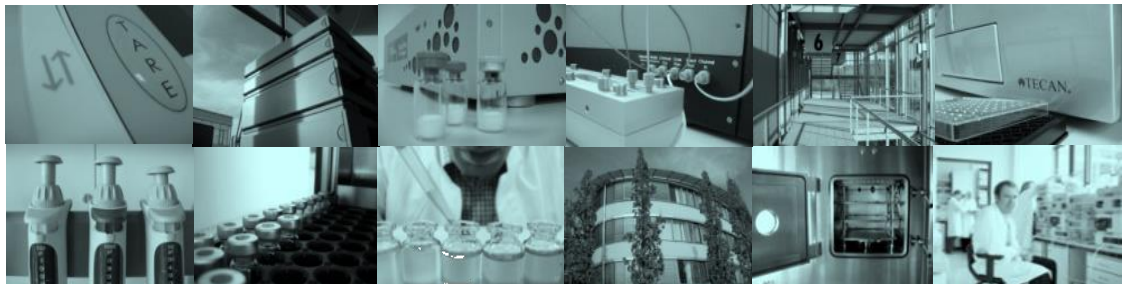

Thanks for your attention

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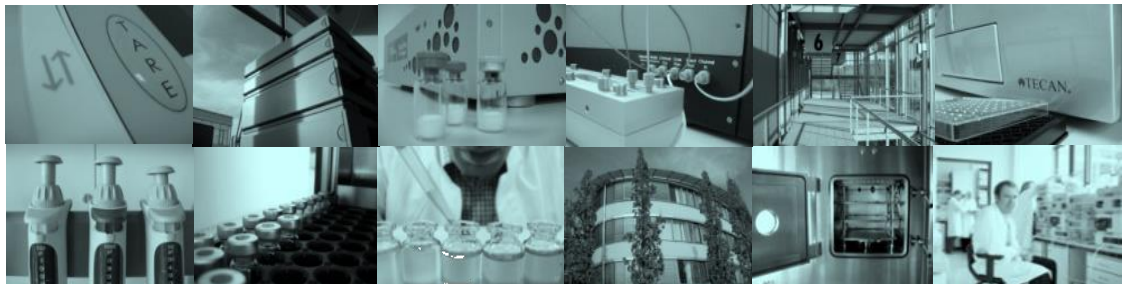

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