



The Relationship Between Silicone Layer Thickness, Free Silicone Oil and Protein Aggregation In Prefilled Syringes

Markus Lankers
rap-ID Particle Systems GmbH

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Conference

1. Motivation
2. Siliconization chracterization
3. Interaction of water / silicone
4. Experimental techniques
5. Experimental results
6. Conclusion

1. Is there a high silicone content connected to higher tendency of protein aggregation ?
2. What is the impact of transportation on the formation of particles
3. Does the reduction of silicone lead to lower particle counts
4. Further develop analytical toolbox

- Manufacturing process – Lubrication process
 - Direct application of silicone oil on barrel surface
 - Spray-on of silicone oil with high viscosity (≥ 1000 cSt)
 - For glass barrels both processes are possible,
for plastic barrels only direct application is possible

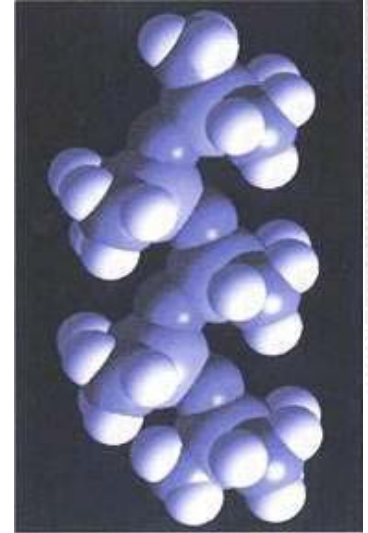


Mixture of water and emulsifying agents
(e.g. Tween, SDS) 1-3% PDMS- proportion

- a homogeneous distribution has to be forced by constant stirring
- when going below the critical minimum percentage of emulsifier, an even „formation of droplets“ is no longer ensured, breaking (non-homogeneity) of the emulsion is possible
- emulsions have a low viscosity before curing the layers will flow like water and can easily drop out
- additives (water and emulsifying agents) are partly removed by heat

Curing

- Curing at temperatures of typ. 250°C – 300°C
- covalent bindings (Si-O-Si) are generated
- 1-2 layers are “fixated” onto the glass surface
- Most of the silicone is still a “free silicone”



1. Layer

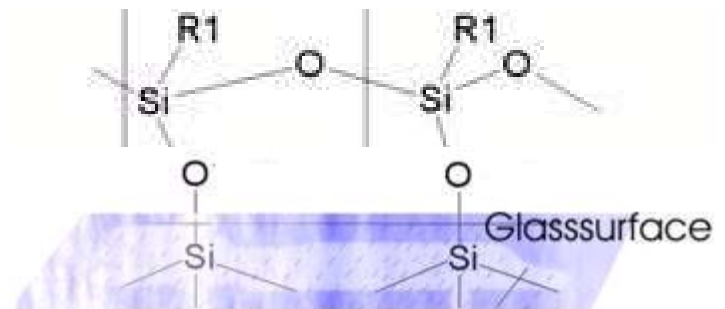
Chemical bonded Silicone oil, not extractable by solvents ~ 1-2 monolayer, *only baked on process*



2. Layer

Depends on the amount of applied silicone emulsion

Extractable by solvents

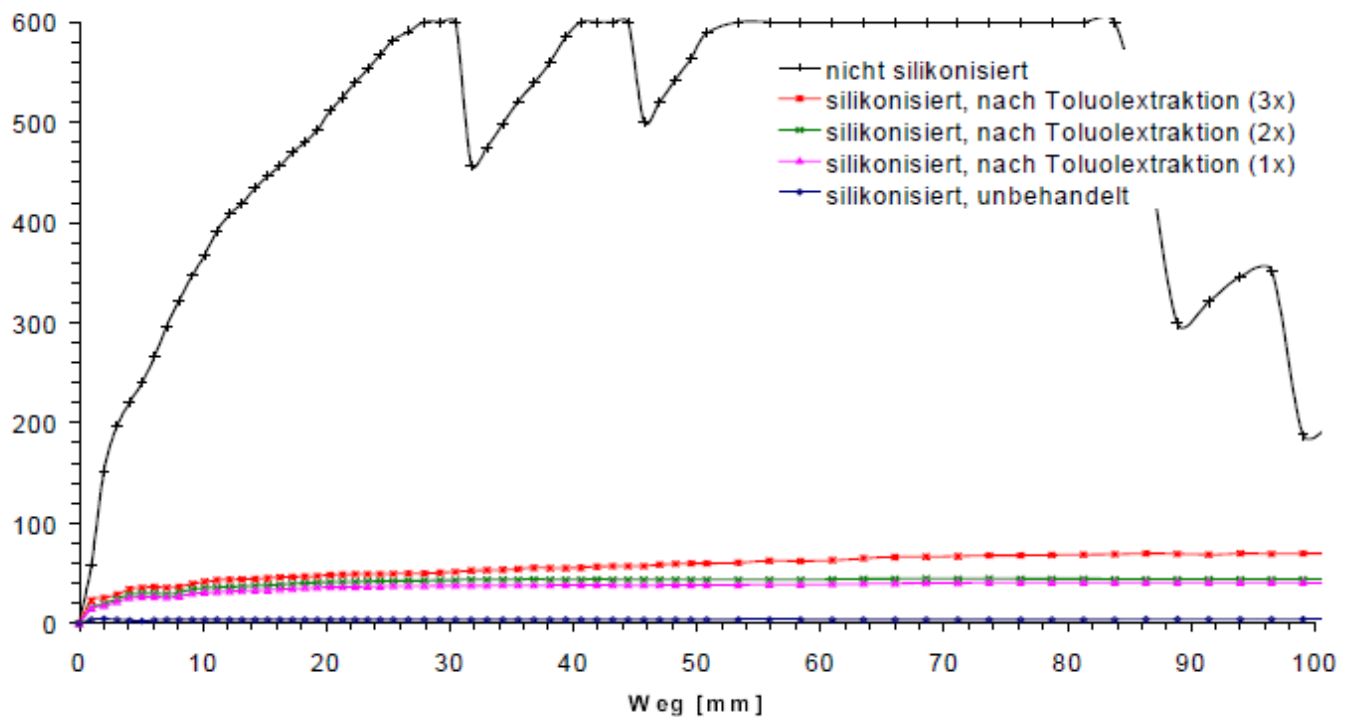


1. Poor understanding of the chemical process
2. No objective tool to characterize degree of homogeneity of silicone layer

Load displacement

7

e.g. DEKA melab



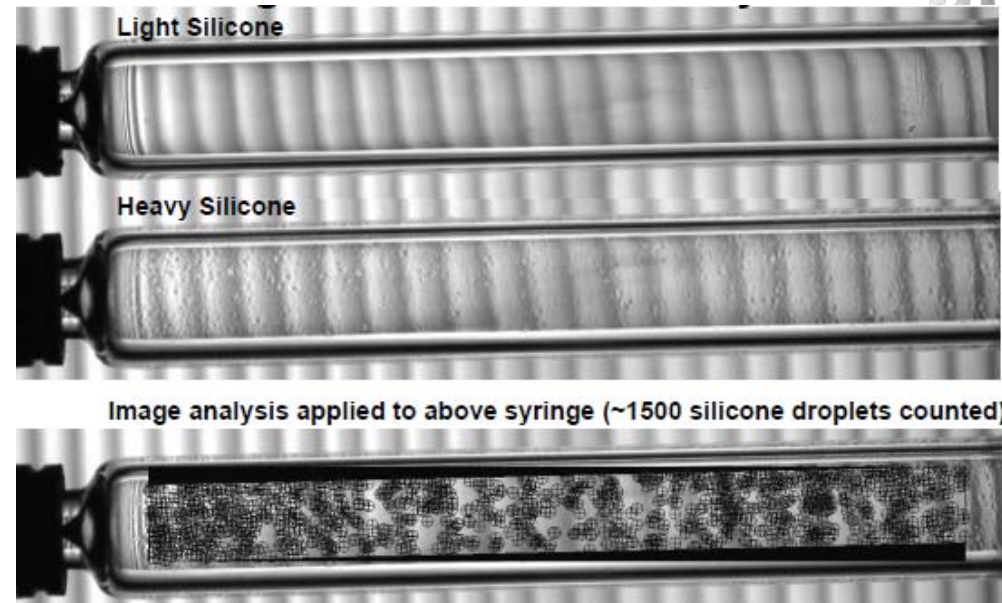
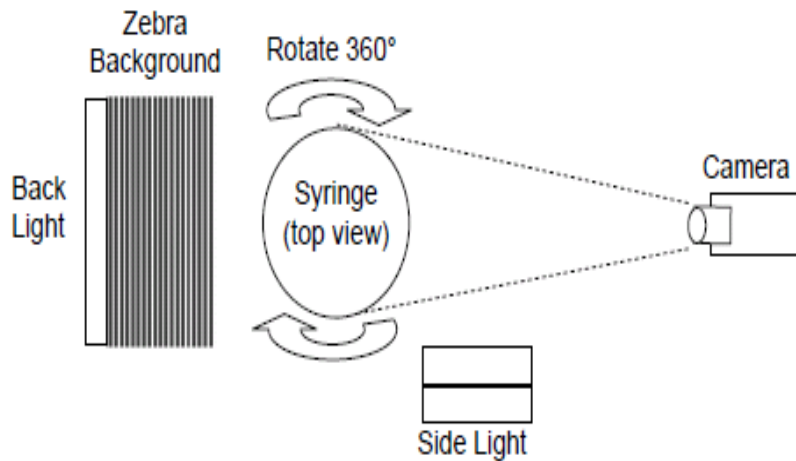
Dust Test

8



1. Zebra Inc.

System Setup: Illuminate zebra background with back or side light
(patent pending)



Layer Explorer

10



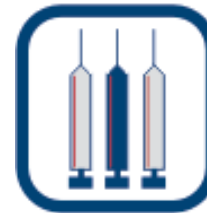
Homogeneity



Layer Thickness



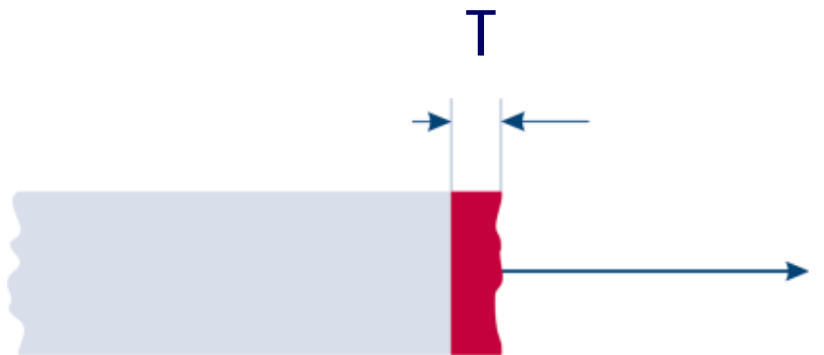
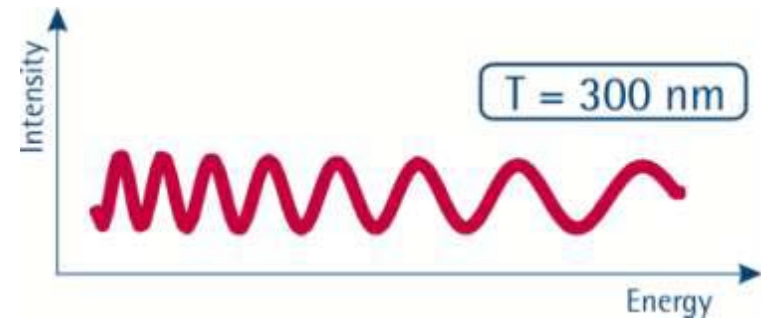
Identity



Non Destructive



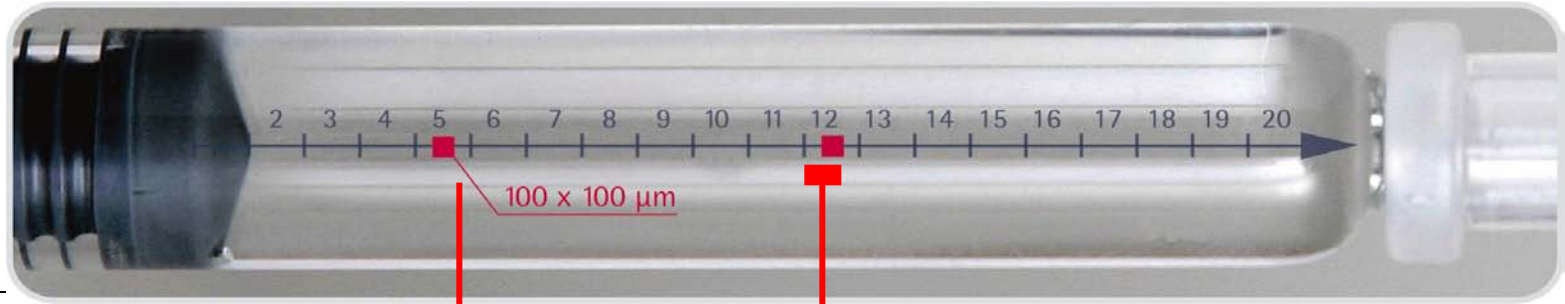
Reflectometry \rightarrow Thickness





Thickness - Result

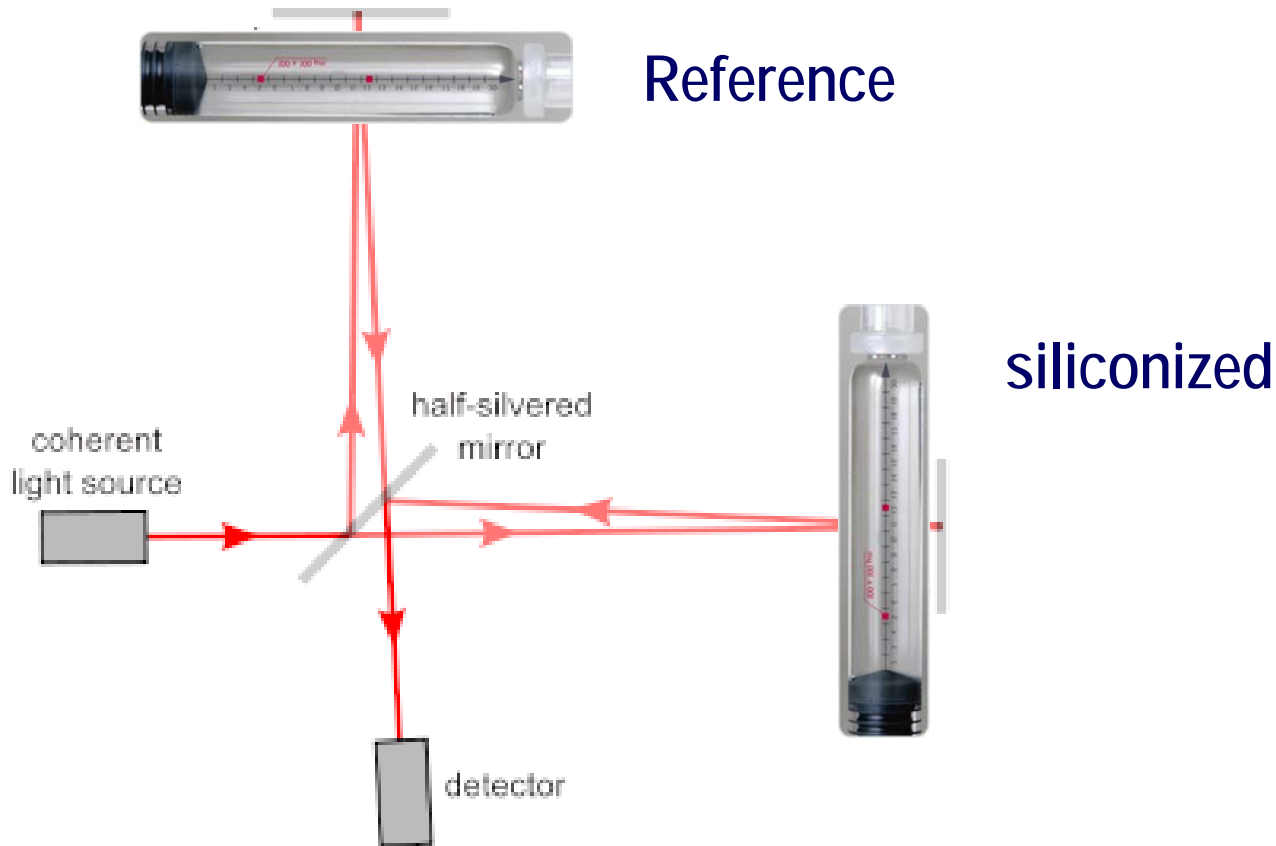
12



100 x 100 μm



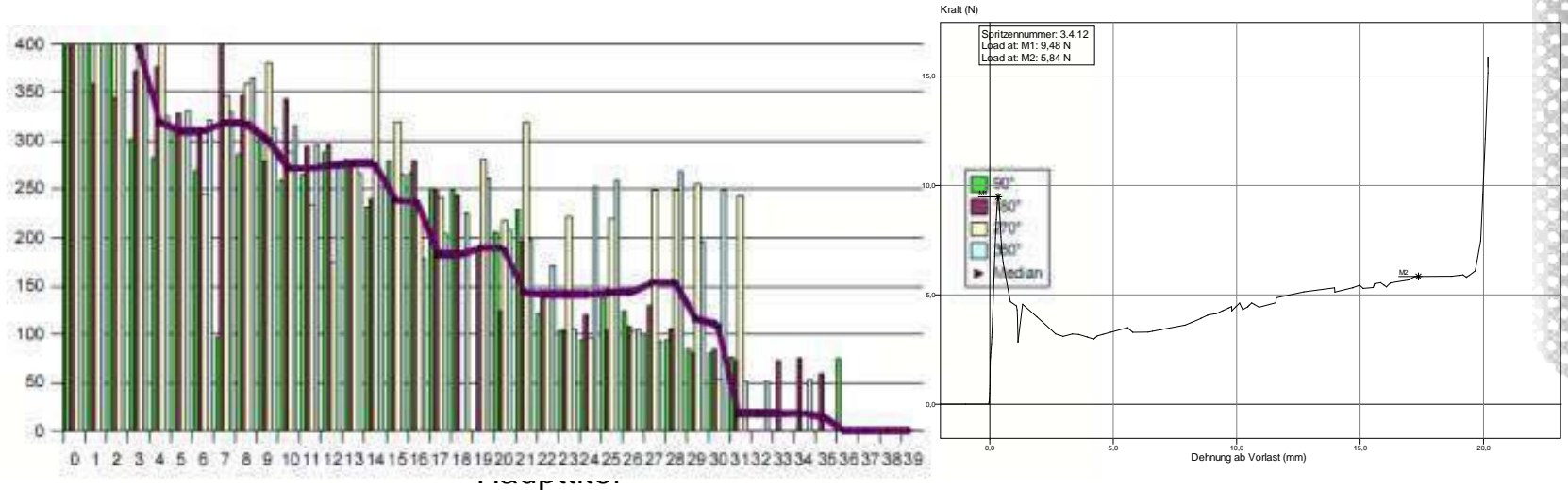
Interferometry additional approach



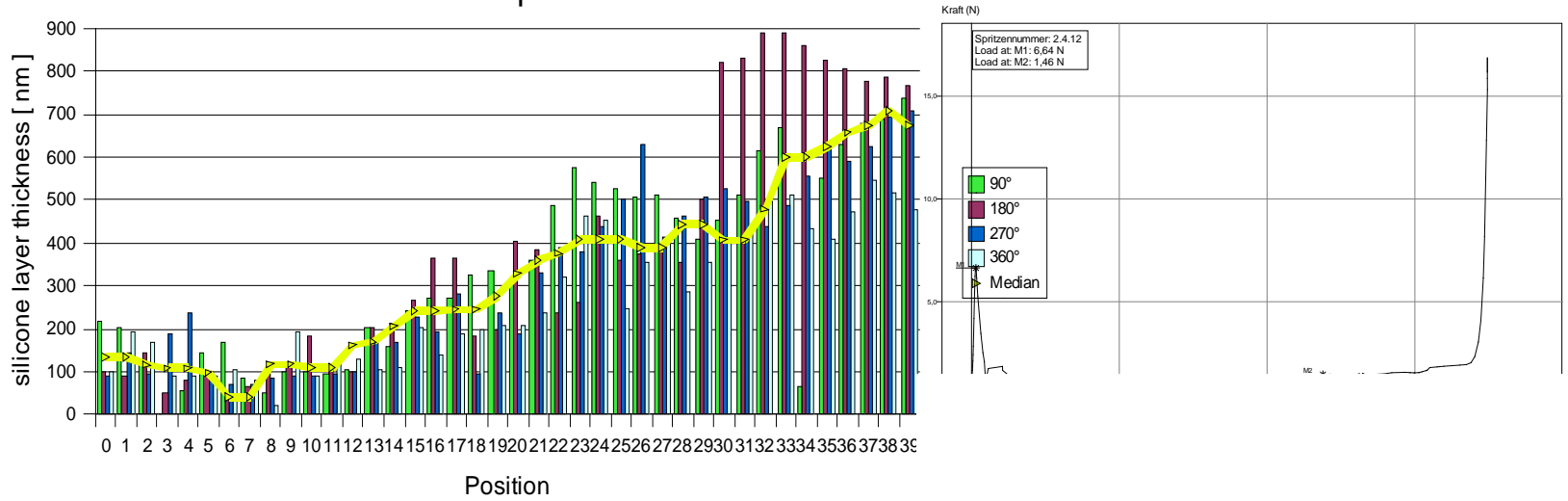
1. Development
 - Siliconization profile
 - Silicone migration
 - Stability
2. Process control
 - Control of nozzles
3. Process understanding
 - How to achieve the thinnest possible layer

Siliconization Optimization

Static

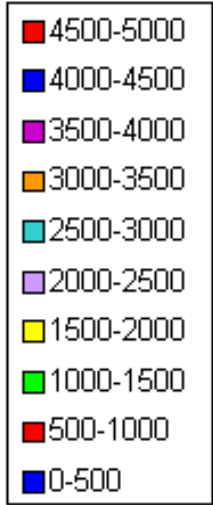
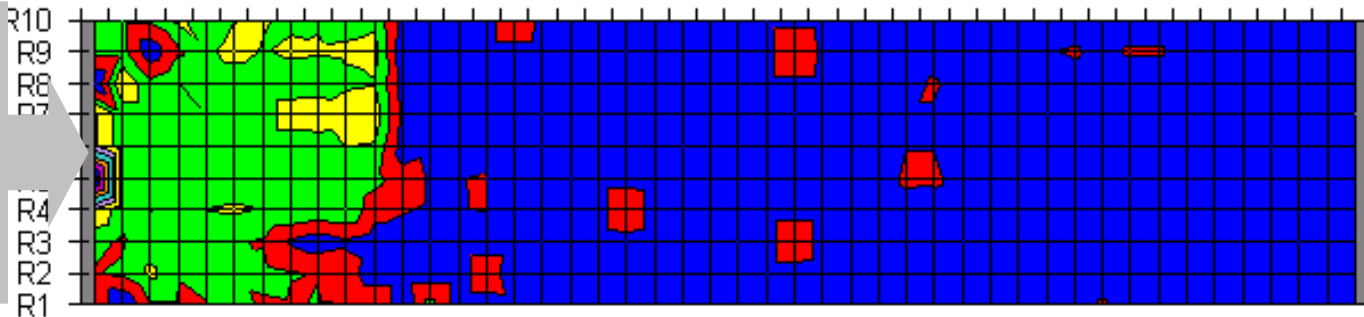


Dynamic



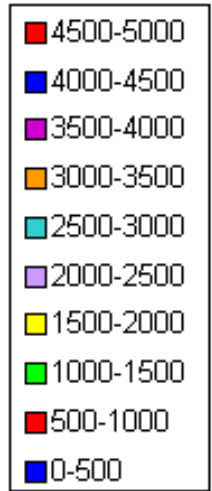
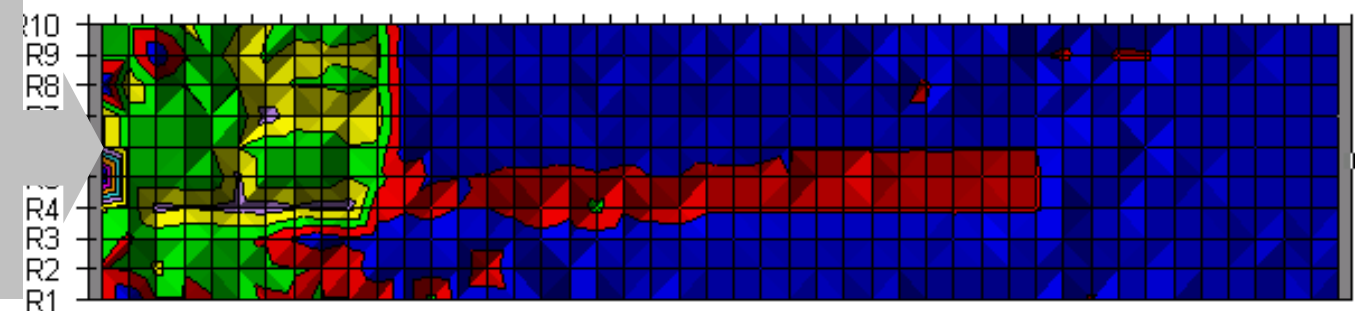
Day 0

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45

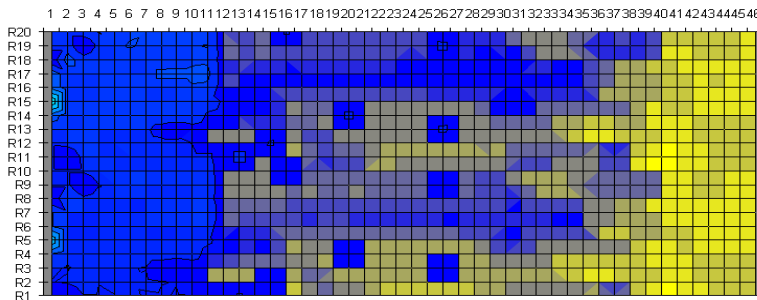
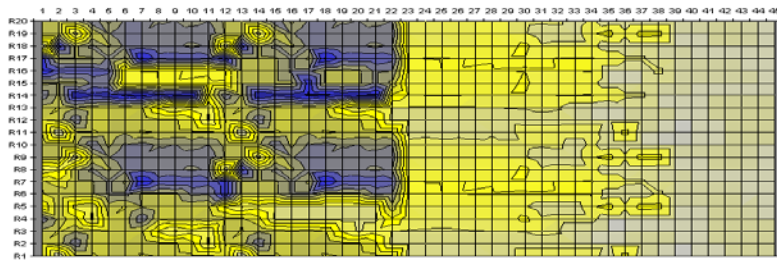
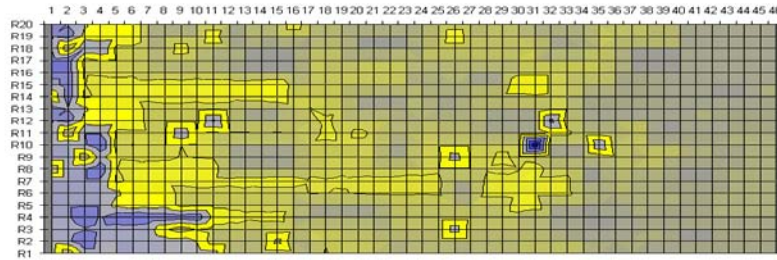


Day 4

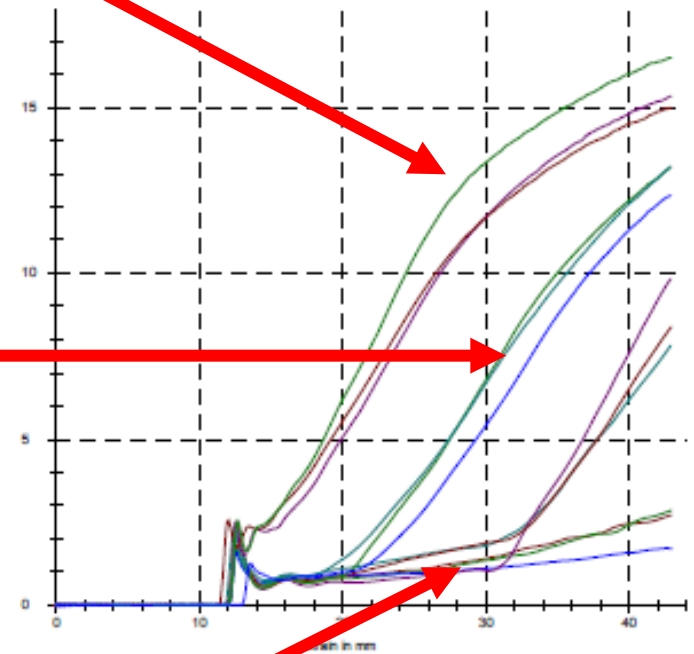
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45



Homogeneity

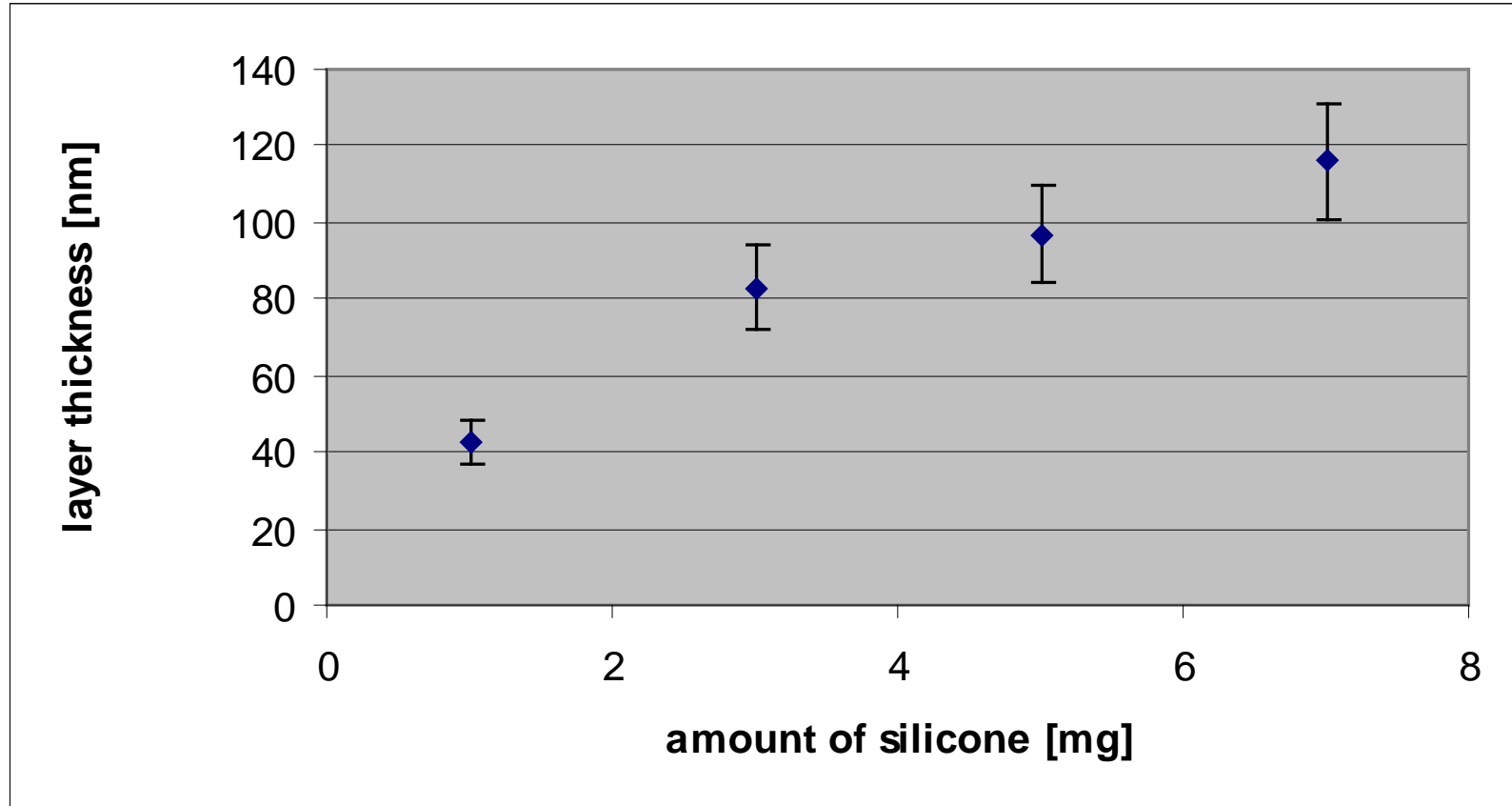


Empty Syringes



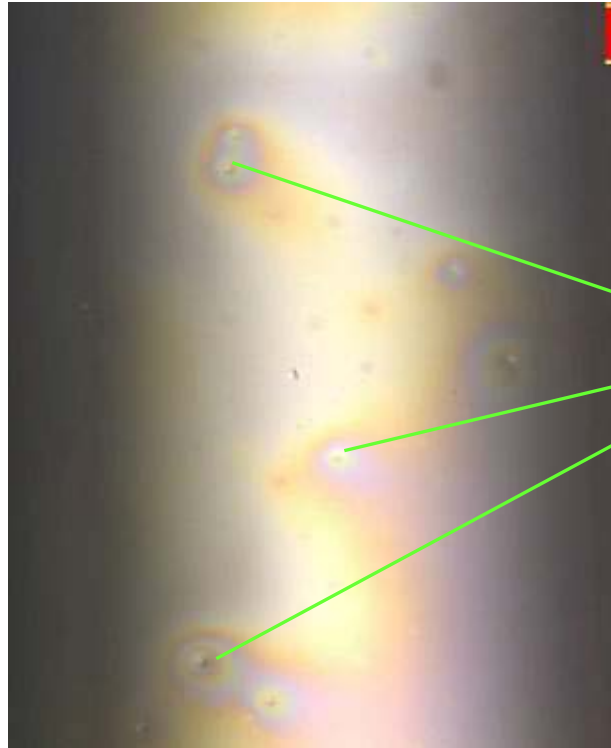
- 100 nm
- 50 nm
- <20 nm

Baked on siliconization

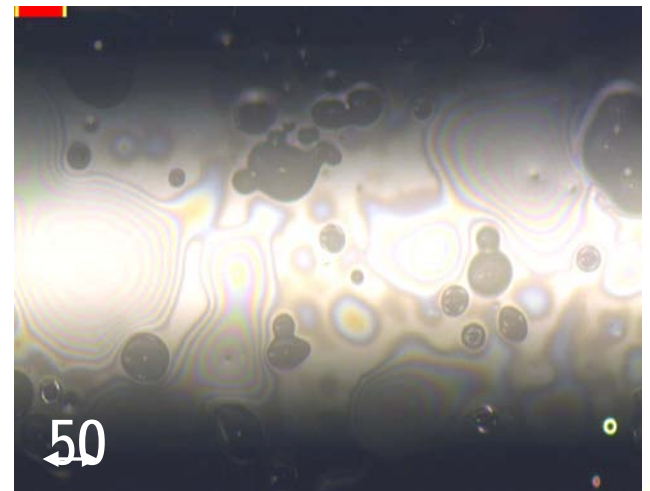


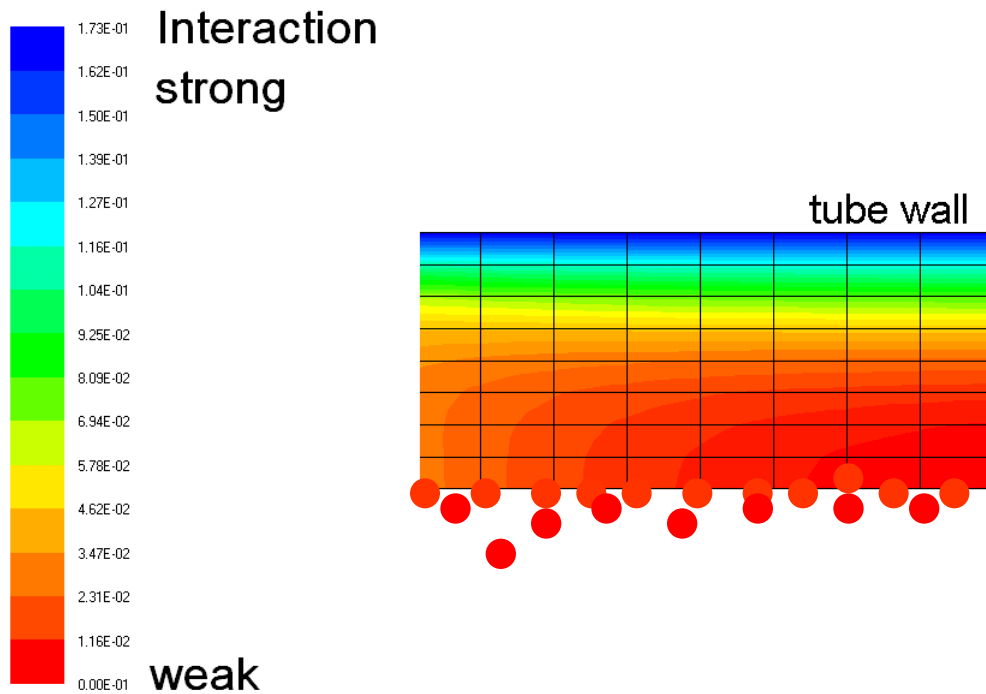
More silicone => increase of layer thickness

Silicone water interaction



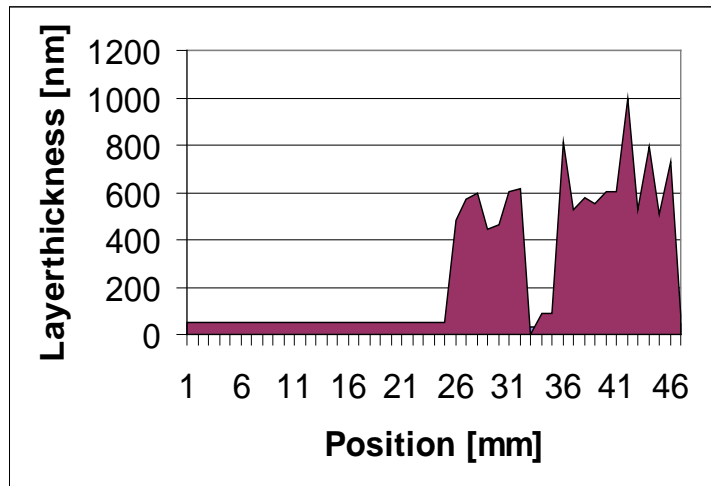
Silicon droplets up to 50 μm in diameter and several μm thick



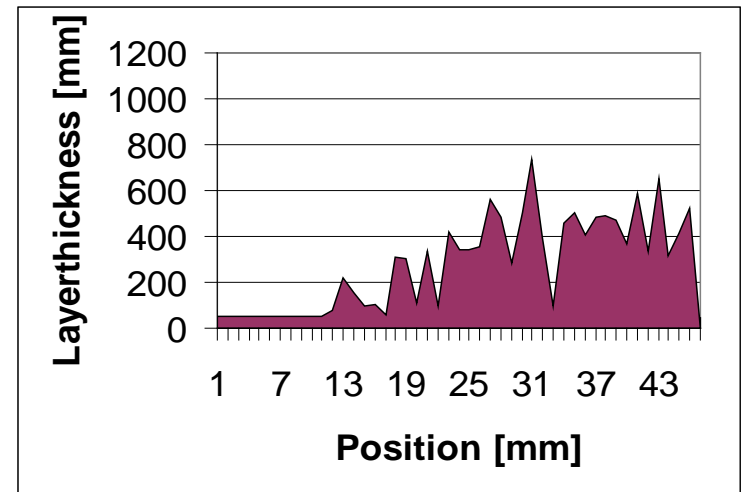
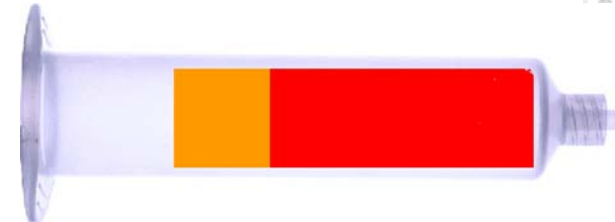


1. Droplet structures result of siliconization and silicon water interaction
2. Release needs some energy
3. Formation of releasable droplets is easier for thick layer

Syringe half siliconized

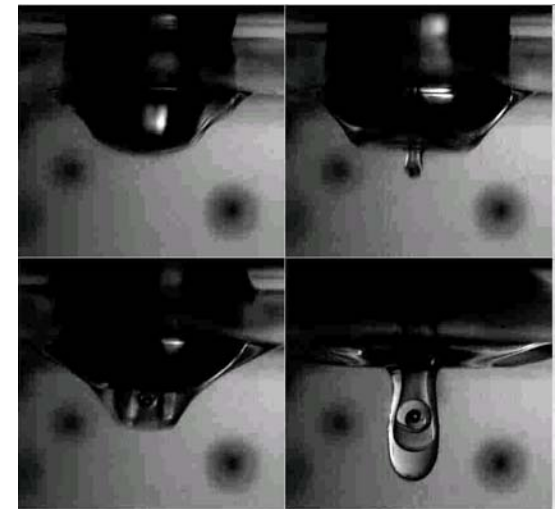


Syringe after filling



Silicone oil layer distribution

1. Protein aggregation can be induced by silicone
2. Aggregation might be proportional to interaction surface
3. Silicone droplets enlarge interaction surface dramatically
 - Droplet structure formation after filling due to water silicone interaction
 - Largest droplets out of thick silicone layer
 - Release by vibration e.g. transportation



1. Storage
 - Temperature (8°C, 20°C, 40°C)
2. Time points
 - 0, 4 W, 12 W
3. Transport simulation (shaking)
4. Analytics
 - Turbidity
 - Image Analysis
 - Particle ID
5. Lysozyme, HSA, BSA
6. 1 ml long; 3 different amounts of silicone oil:
0.4, 0.7 and 1 mg as well as 1 baked on siliconized syringe

1. To simulate transportation syringes were shaken after 8 weeks for 8 h. For a more realistic scenario the syringes were stored another 4 weeks.
2. Prior to sample preparation all samples were gently shaken for ~ 1 min.

method	meaning	time/analysis
Chromatography	Amount of silicone	60-180
ICP/AAS	Amount of Si	60-180 min
Flow imaging	Amount of silicone particles by shape	Few minutes
IR -microscopy	Amount of Si	40 min
RAMAN - microscopy	ID of silicone	40 min

filtr.AID



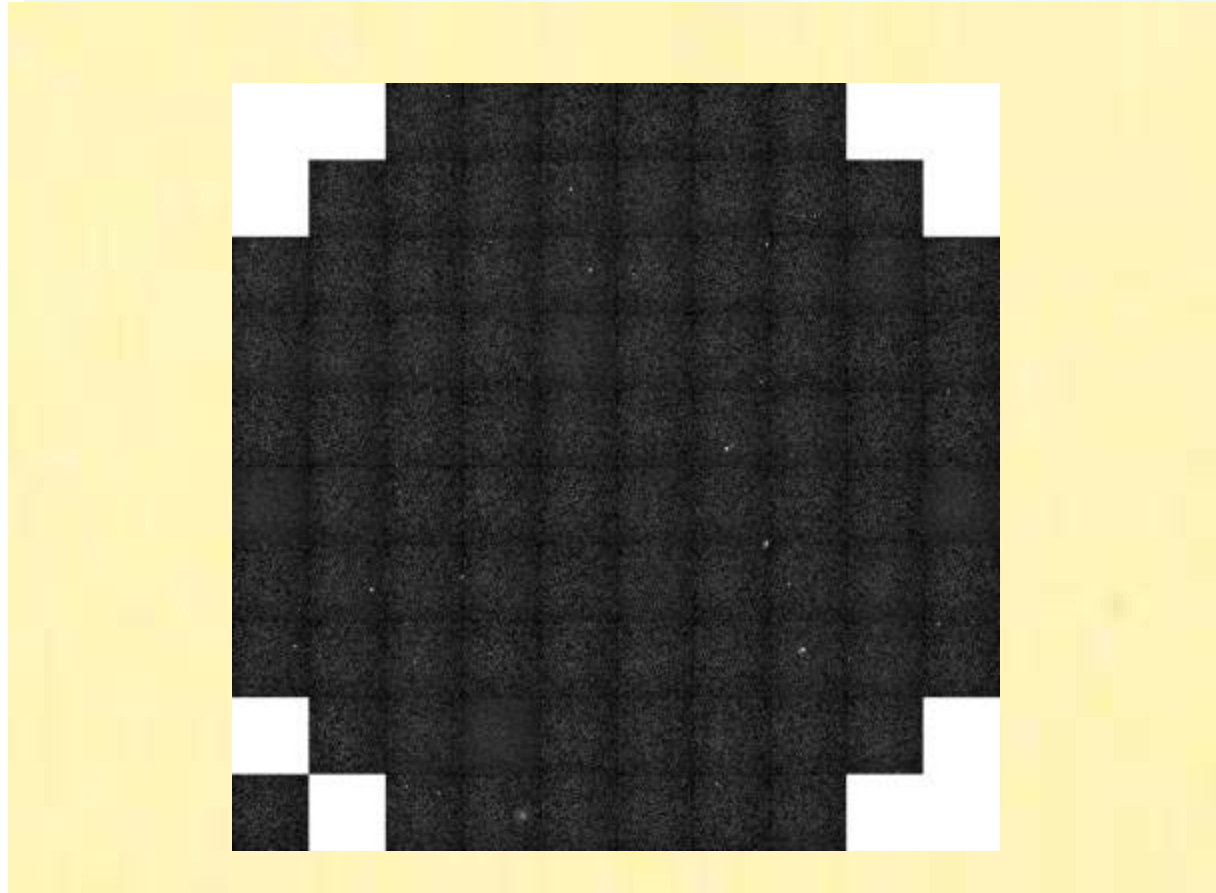
- Low blanks
- Cleanroom manufactured
- System integrated
- High sensitivity
- No background signal for ID



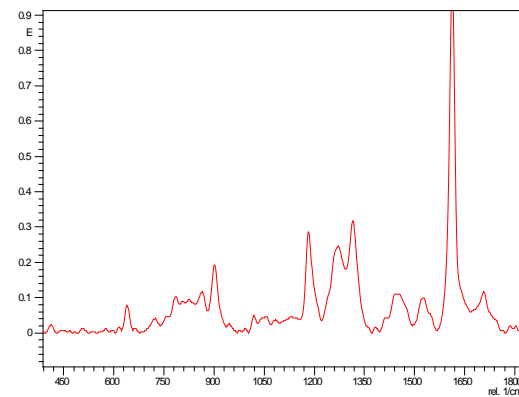
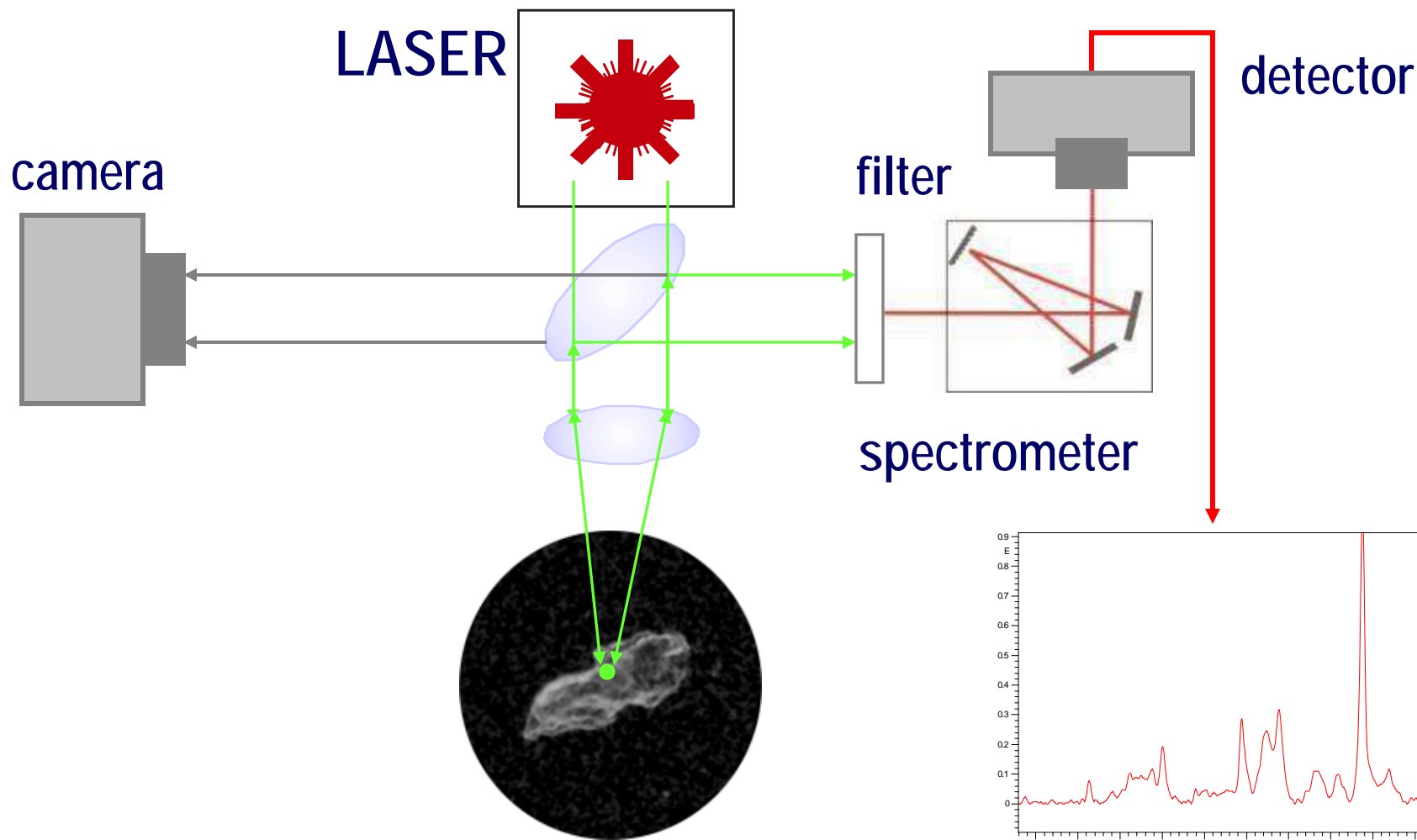
Filter → Scan Fields

29

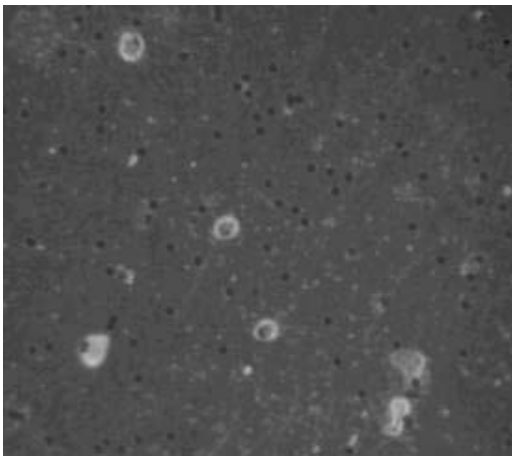
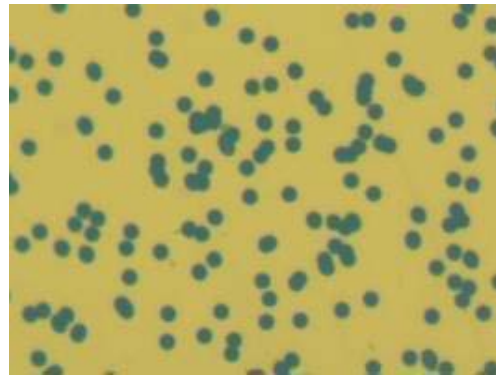
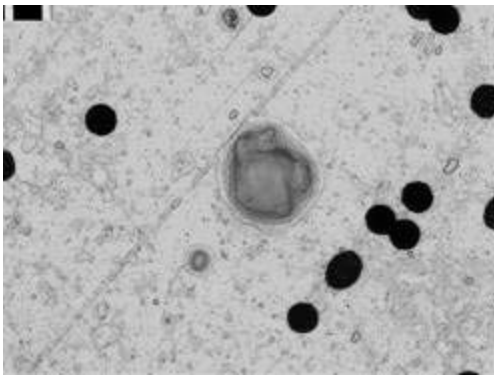
- Membrane (0.8 μm , 3 μm) RAMAN inactive



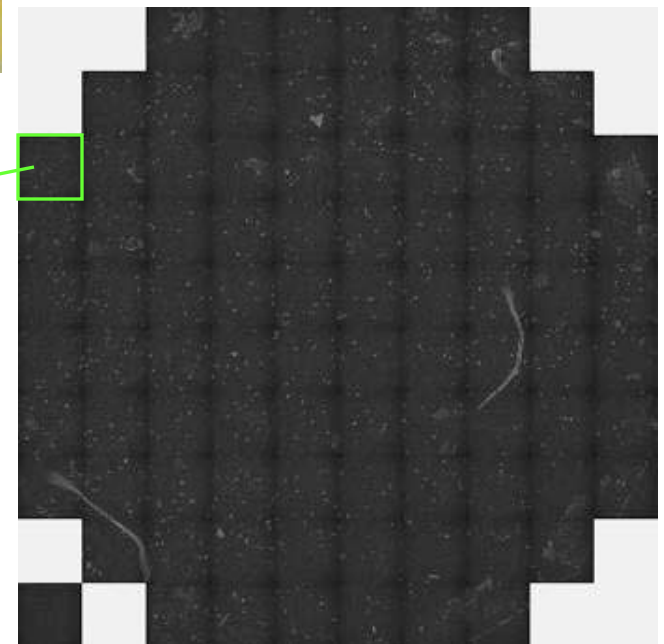
Chemical information



Use of Polycarbonat or Polyester membrane filter (Osmonics, Millipore) is essential



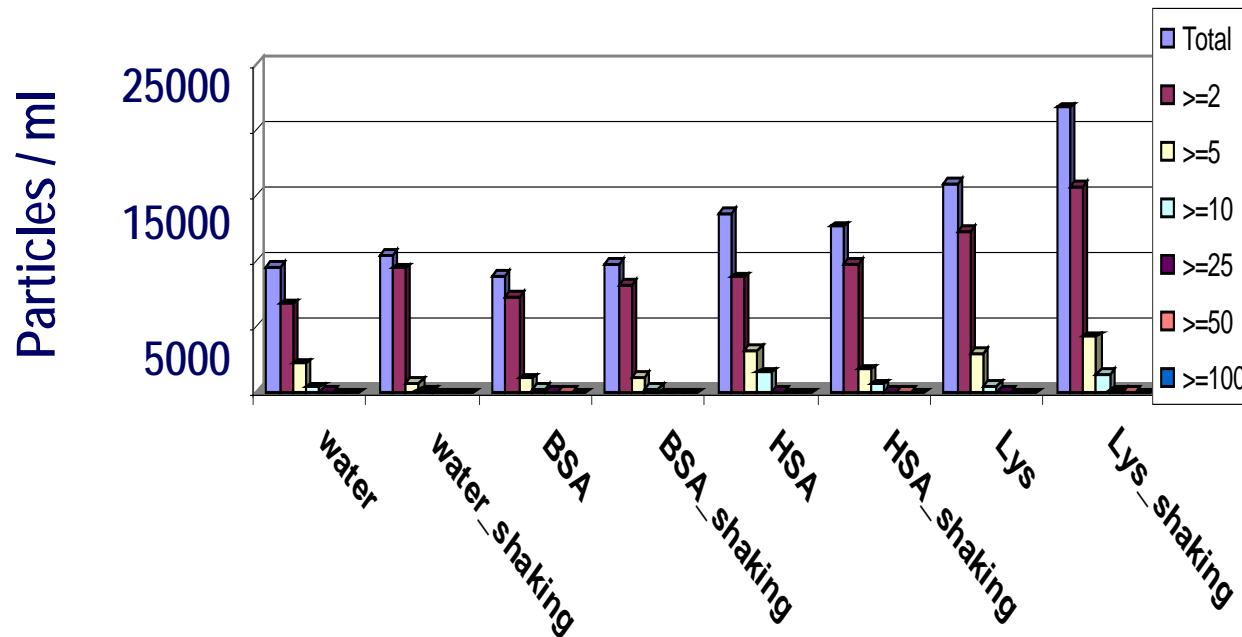
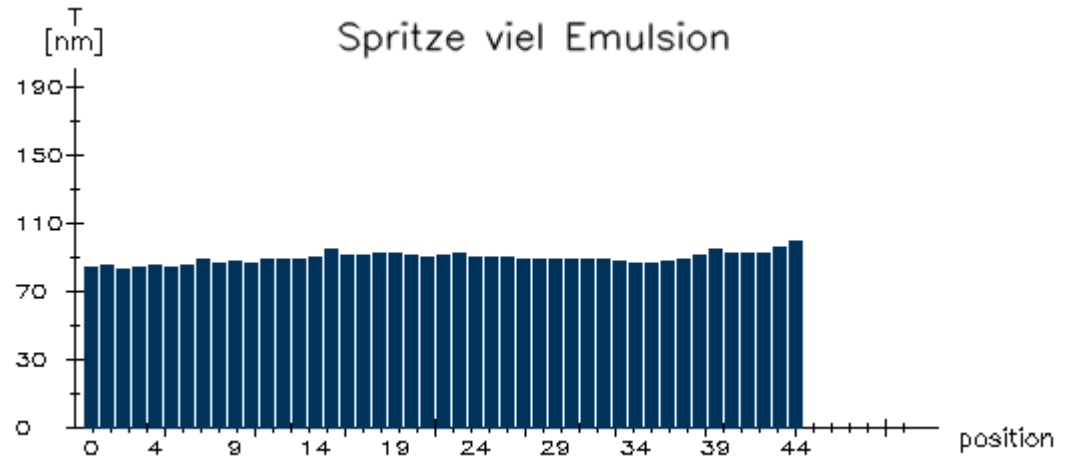
973 silicone particles/droplets > 25µm



Results

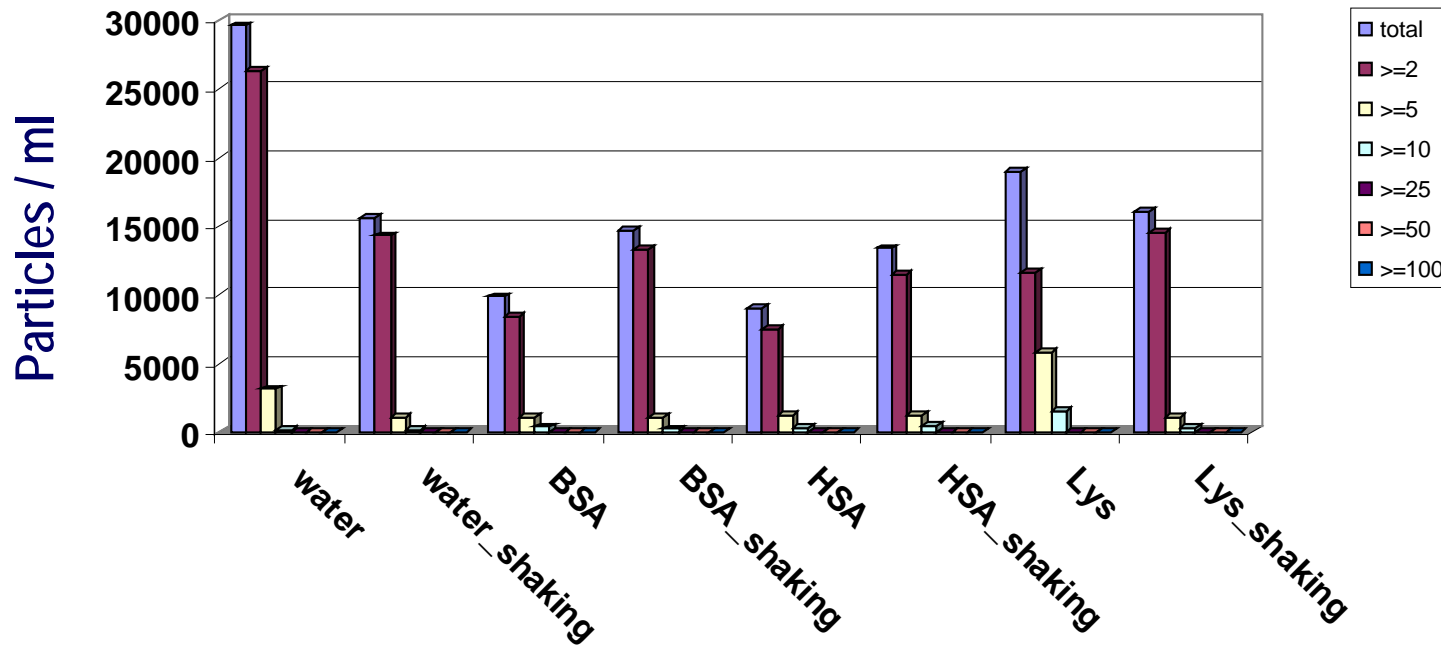
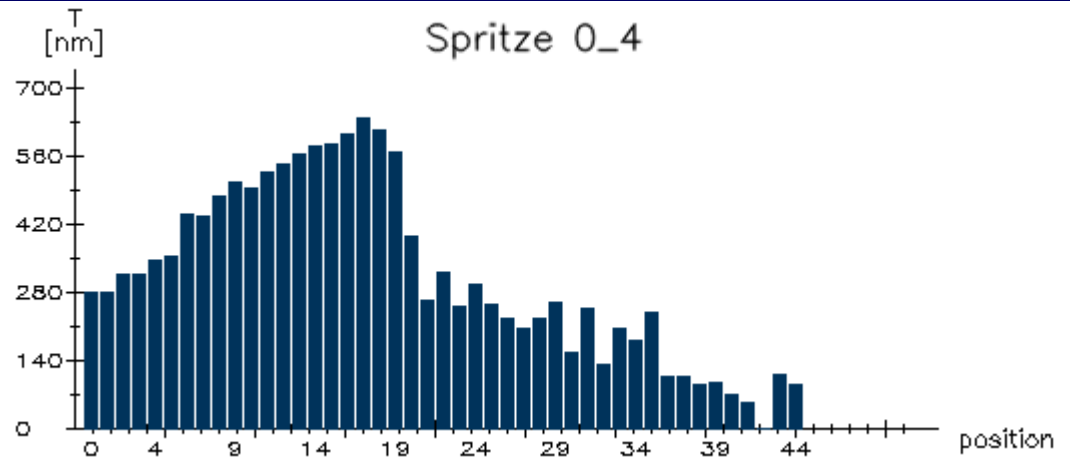
Particle counts

Number Silicone containing
Particles : Baked on
siliconisation 12 W



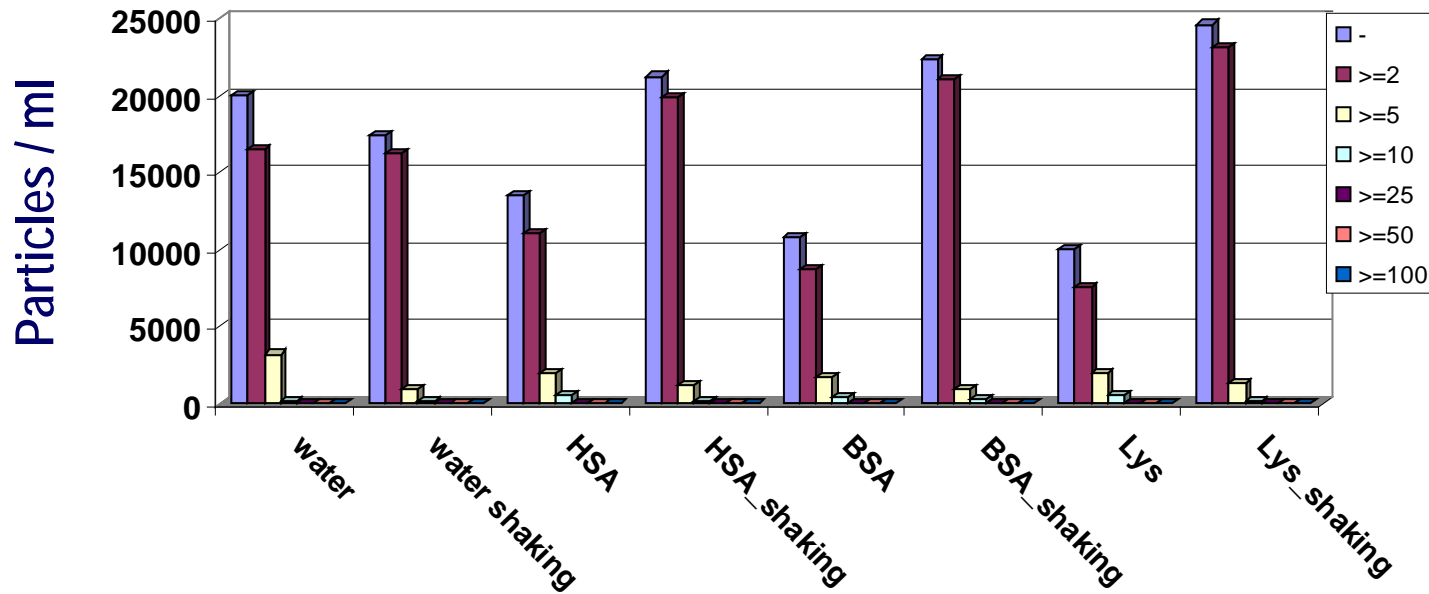
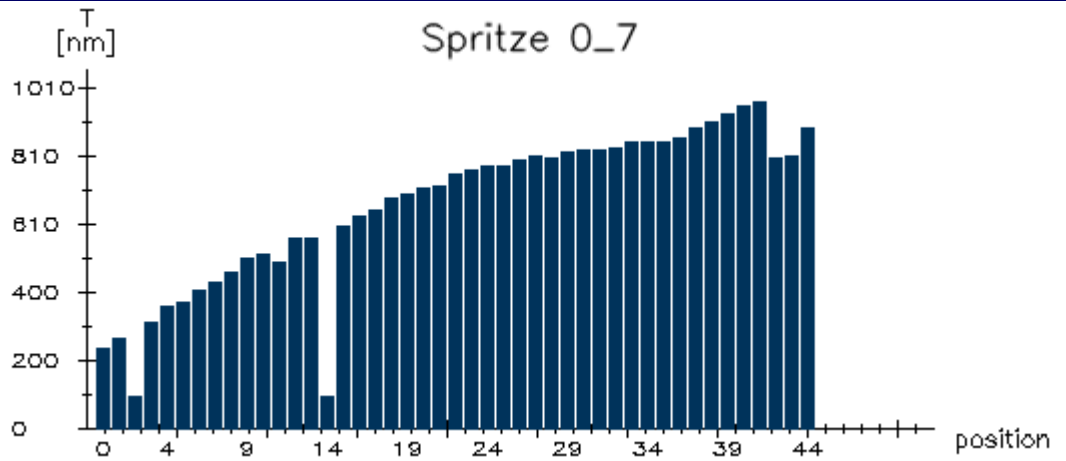
Particle counts

Number Silicone containing
Particles : 0.4 mg 12 W



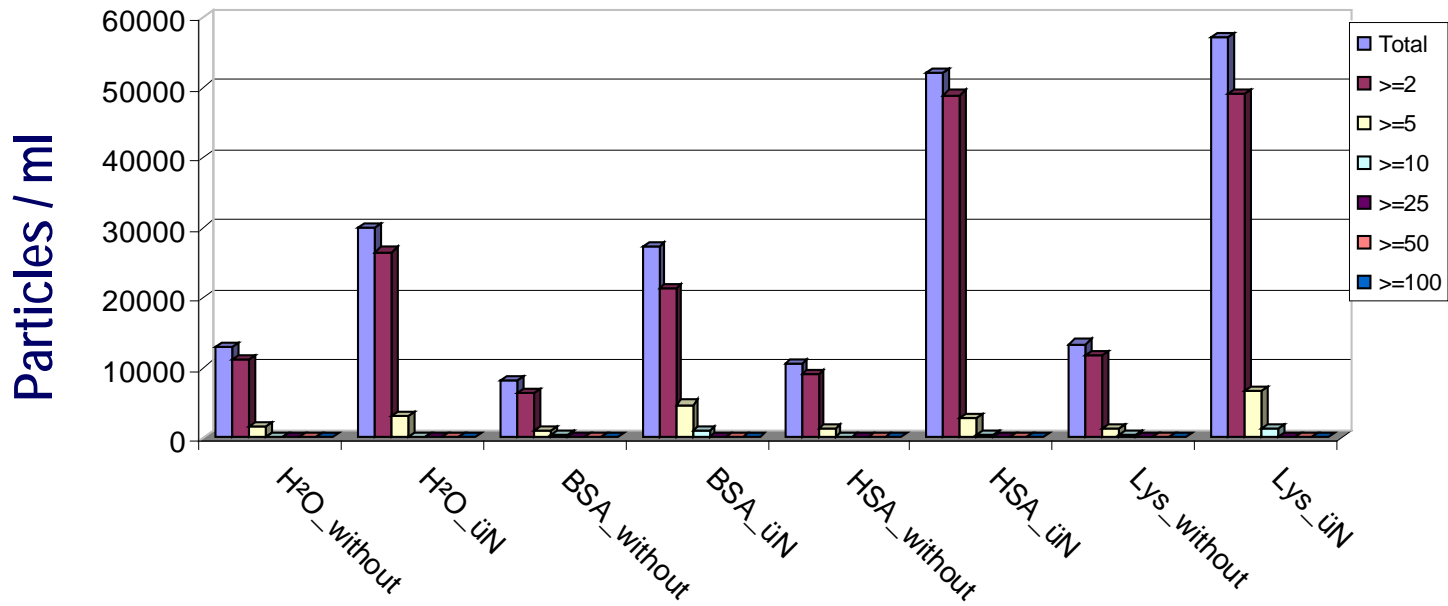
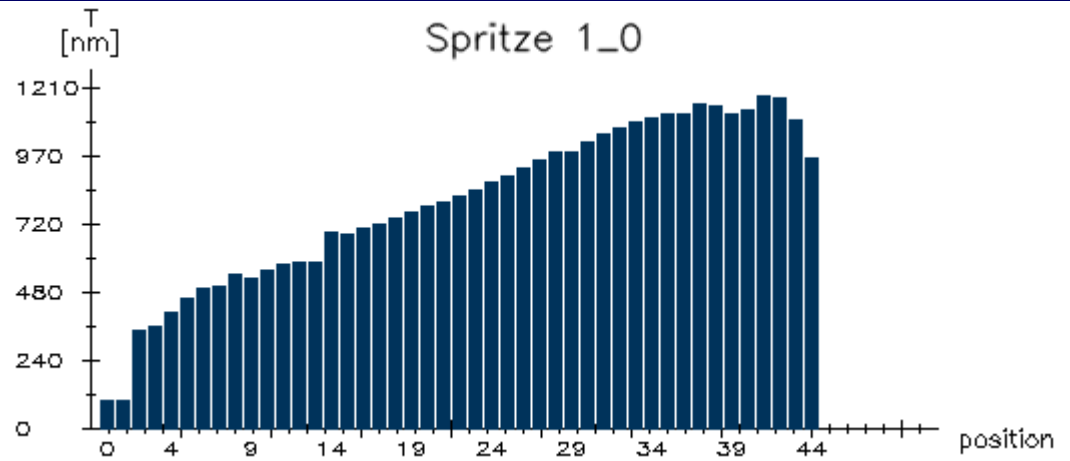
Particle counts

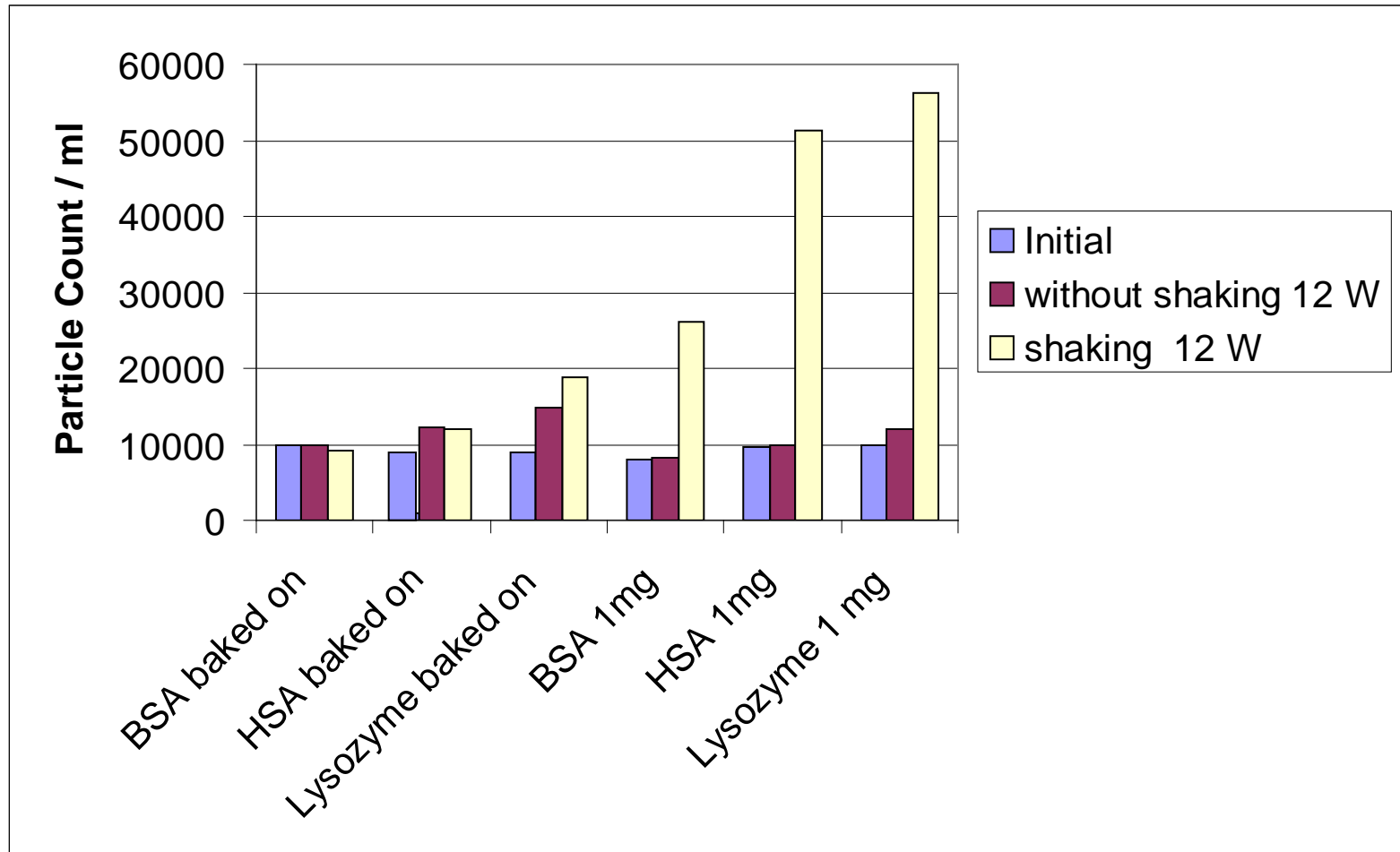
Number Silicone containing
Particles : 0.7 mg 12 W



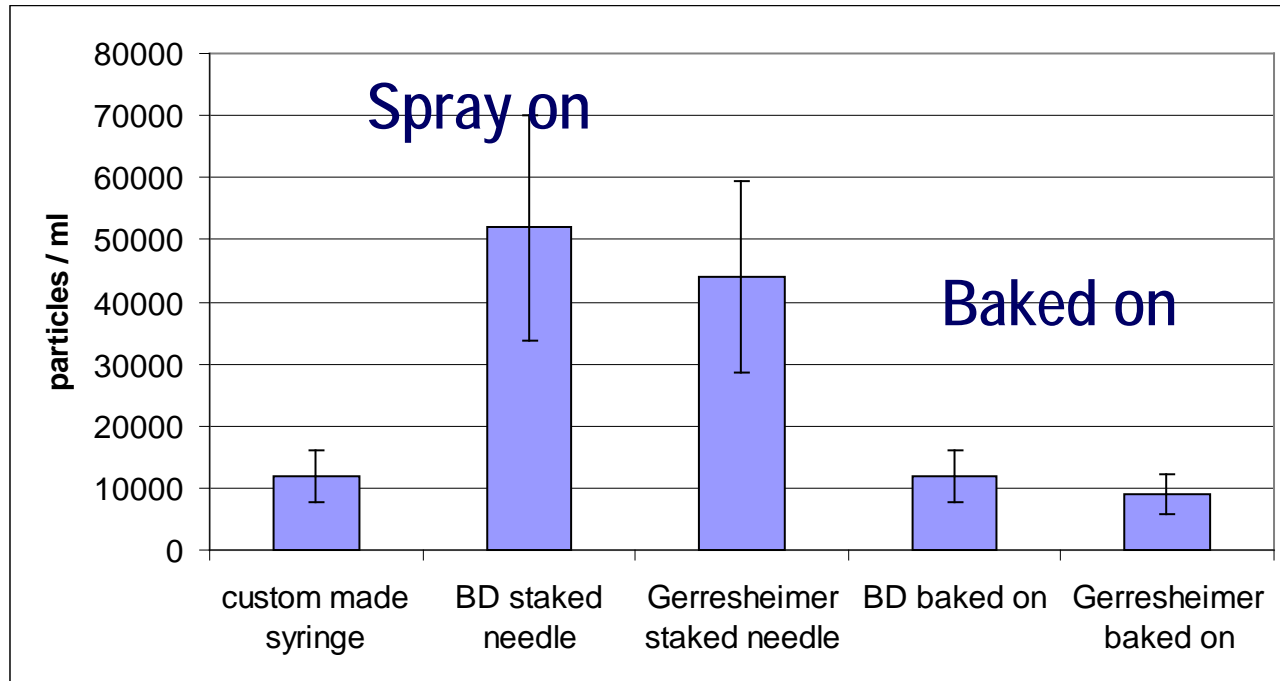
Particle counts

Number Silicone containing
Particles : 1.0 mg 12W





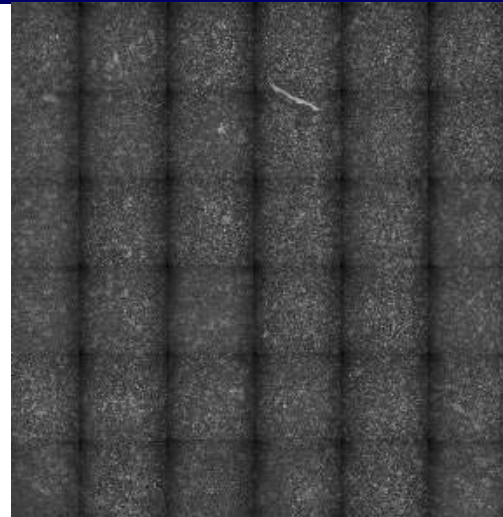
Shaking has a big influence on particle formation for heavy siliconized syringes



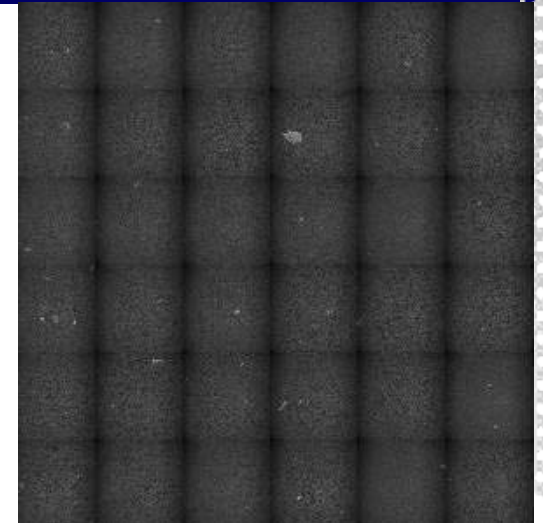
Large amount of silicone droplets in syringes with large variability between syringes

Correlation between Silicone content and Silicone particles

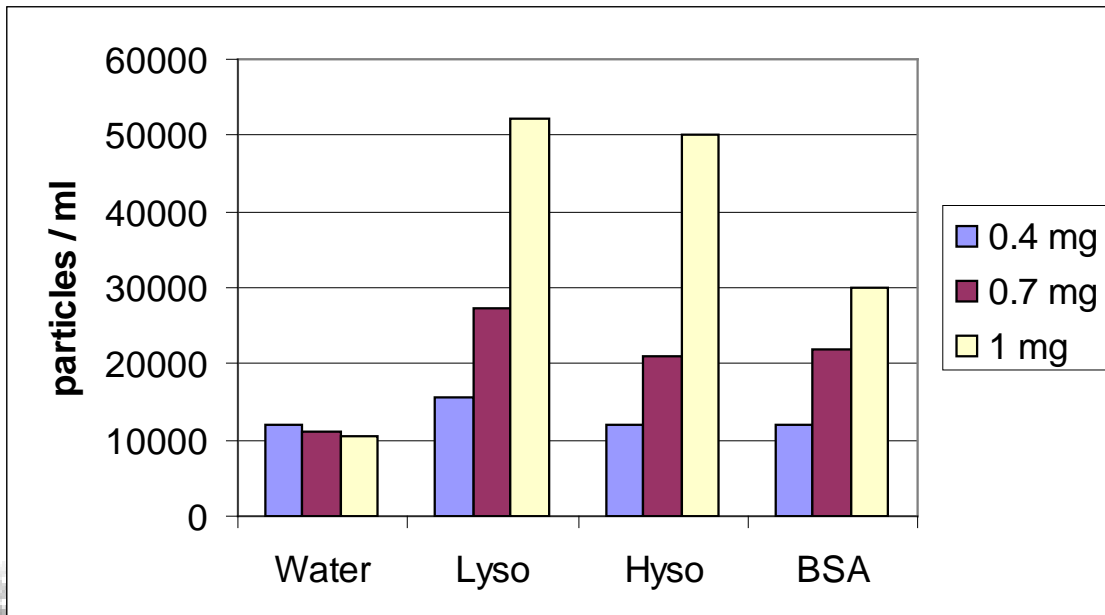
39



1 mg Silicone

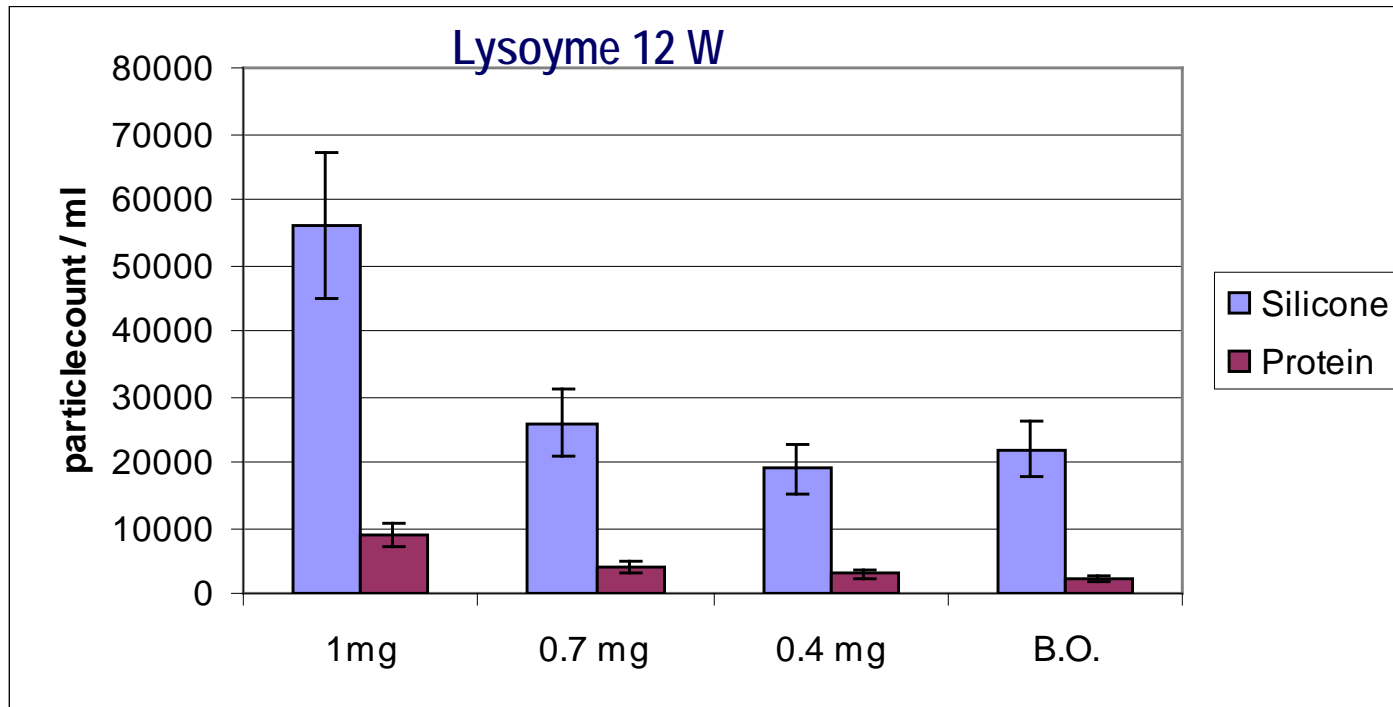


0.4 mg Silicone



Details : Lysozyme 12 W shaking (100 μ l sample)

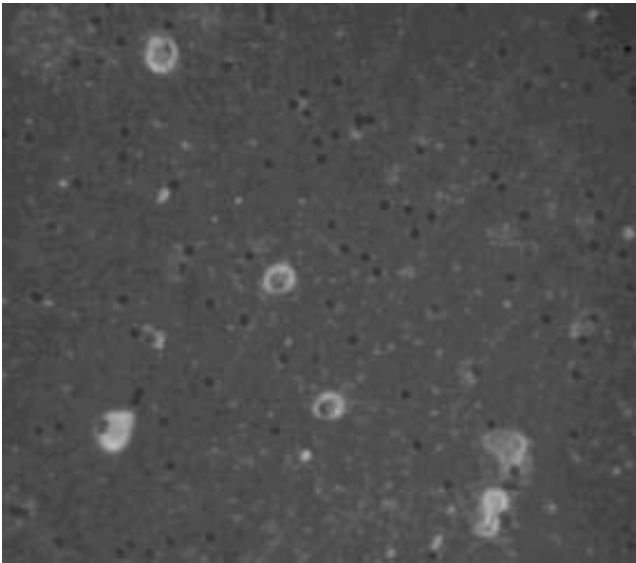
Substance	Number	Size Distribution [urn]					
		≥ 2	≥ 5	≥ 10	≥ 25	≥ 50	≥ 100
–	–						
Silicon carbide	1	0	0	1	0	0	0
Starch, hvdrolvzate	21	21	0	0	0	0	0
Indanthrene Blue	1	0	0	1	0	0	0
Graphite	45	0	0	1	0	44	0
Titaniumoxide Anatase	191	128	51	12	0	0	0
Fluorescence	58	54	0	2	1	1	0
Unidentified substance	82	21	55	5	0	0	1
Protein	852	534	221	81	16	0	0
Silicone / Protein mix	1775	1421	262	92	0	0	0
Silicone Oil	3764	3312	323	95	22	12	0
Analyzed Particles	6790	5491	912	290	39	57	1



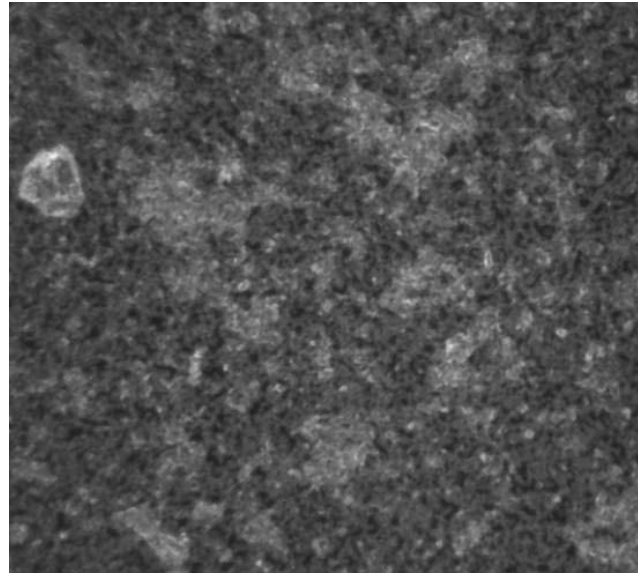
More Silicone particles lead to more protein particles

However, silicone and particles containing mostly silicone

Calibration of database spectra would lead to a better picture

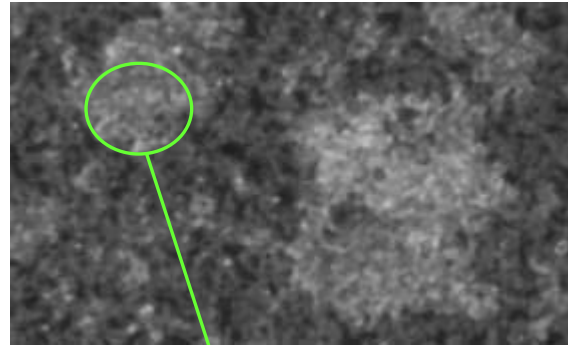
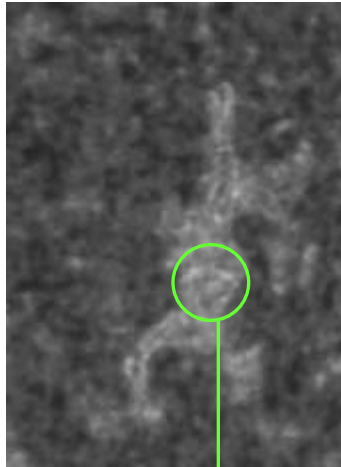


Silicone oil droplet morphology. Only water and silicone oil

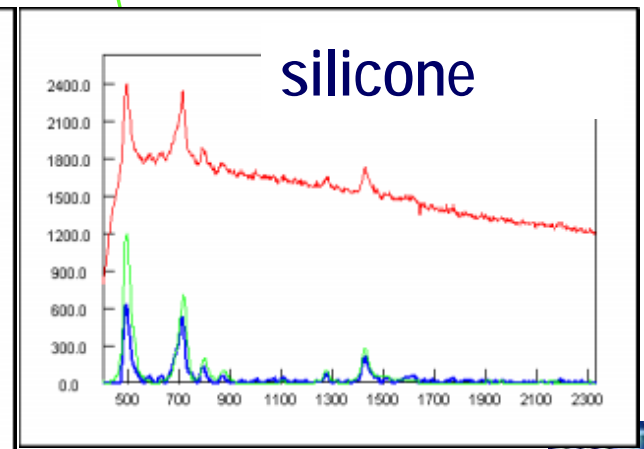
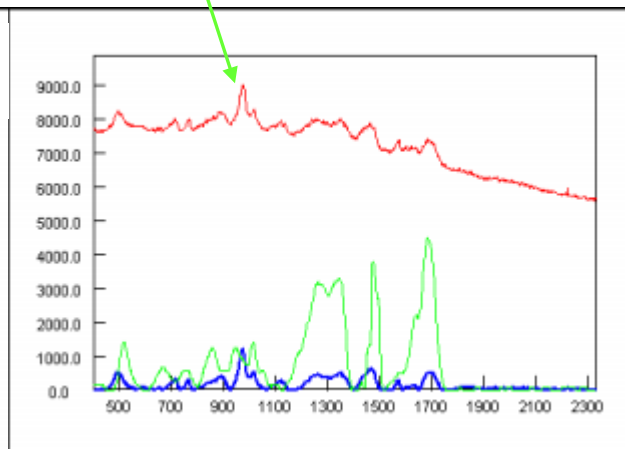
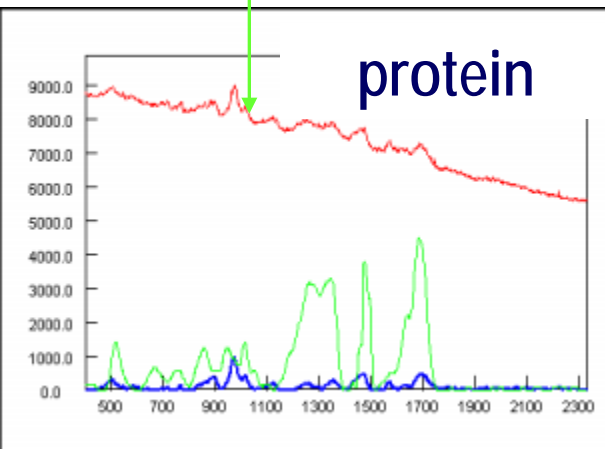
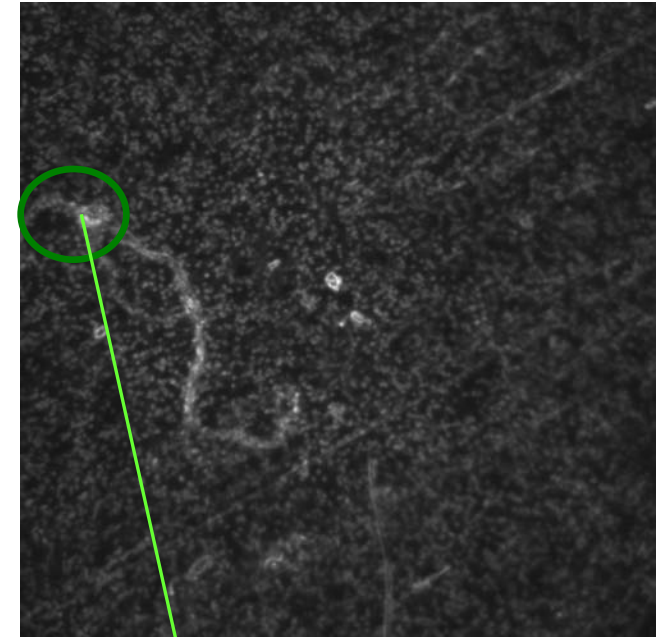


Silicone oil droplet and protein mixtures. Not only droplets show silicone signature morphology.

Darkfield Image



Darkfield Image



1. Transport (Agitation) influences the particle count
2. Syringe to syringe variations are in the same range
3. Increasing silicone oil content increases count of silicone droplets
4. Protein particles, silicone droplets and mixtures can be differentiated by vibrational spectroscopy
5. If you know your protein is sensitive against silicone. Use only the necessary amount of silicone to avoid more interaction surface for Protein agglomeration

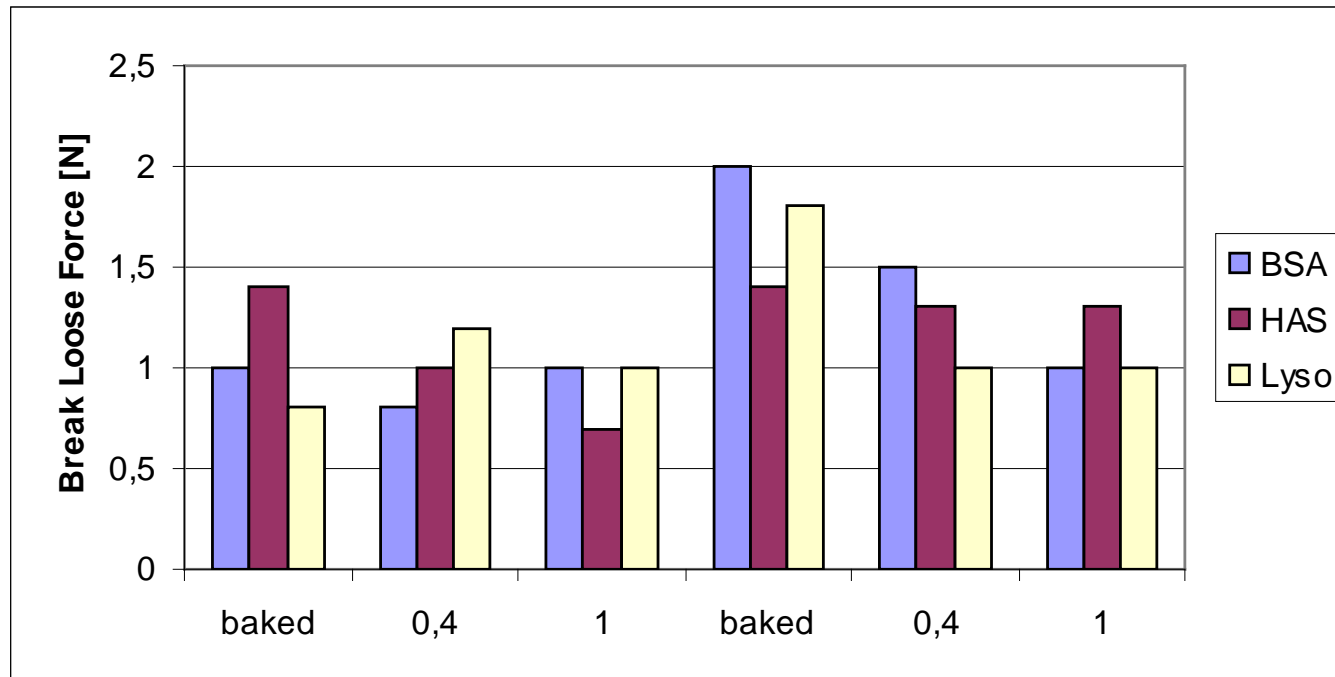
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attention !

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No real difference detectable