Particle Characterization and Identification

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483 Observations

Root cause, particle characterization

Investigation ….. regarding the metal particulate contamination in lots ….. was inadequate …..The atypical contamination found in these lots was metal, however, the batches were not rejected. Additionally, there was no investigation conducted to determine the cause of the black metal particulates found in these lots

"reported a particle identified in a vial during an AQL inspection. There was no documentation on the identity of the particle and whether it was inherent or foreign (black debris, fiber, glass fragments, etc.)."

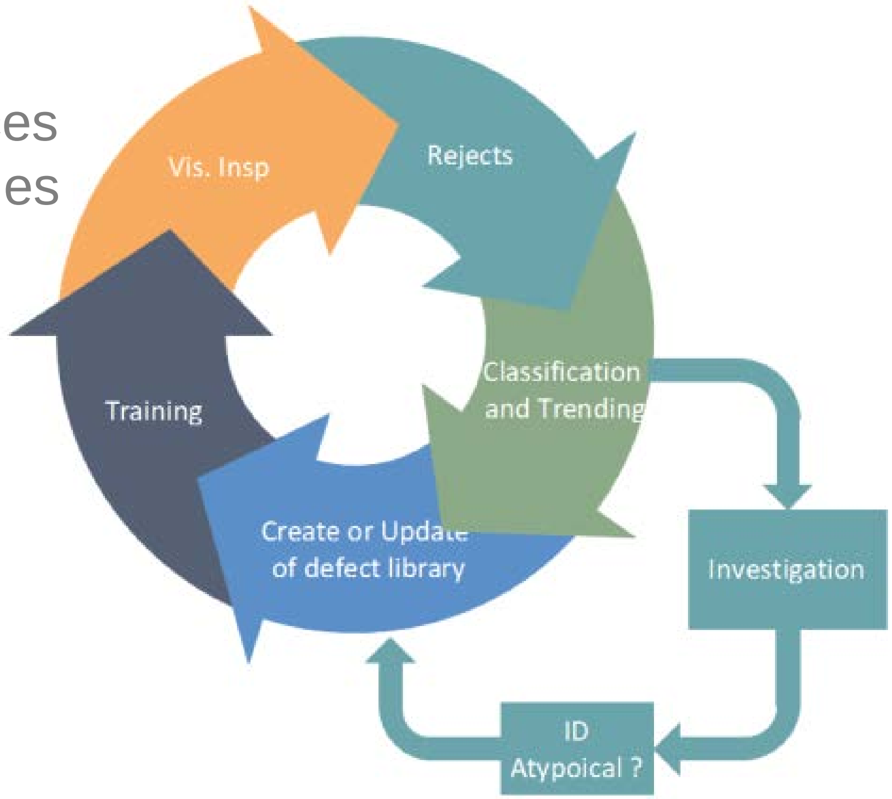
2015

Reasons for Particle ID

1. Classifcation and Trending
2. Root cause analysis
3. Manufacturing Process
   Continuous Process Improvement

Visual Inspection Lifecycle

- Use the Trending Data from Reject Characterization and Monitoring
- Review the various particulate sources for Process Improvement opportunities
- Focus on the most predominant particle types
- Repeat the Cycle of Monitoring, Trending, Corrective actions and follow-up Monitoring



Classification and Trending

Quality Control Methods
Particulate Characterization/ID Levels

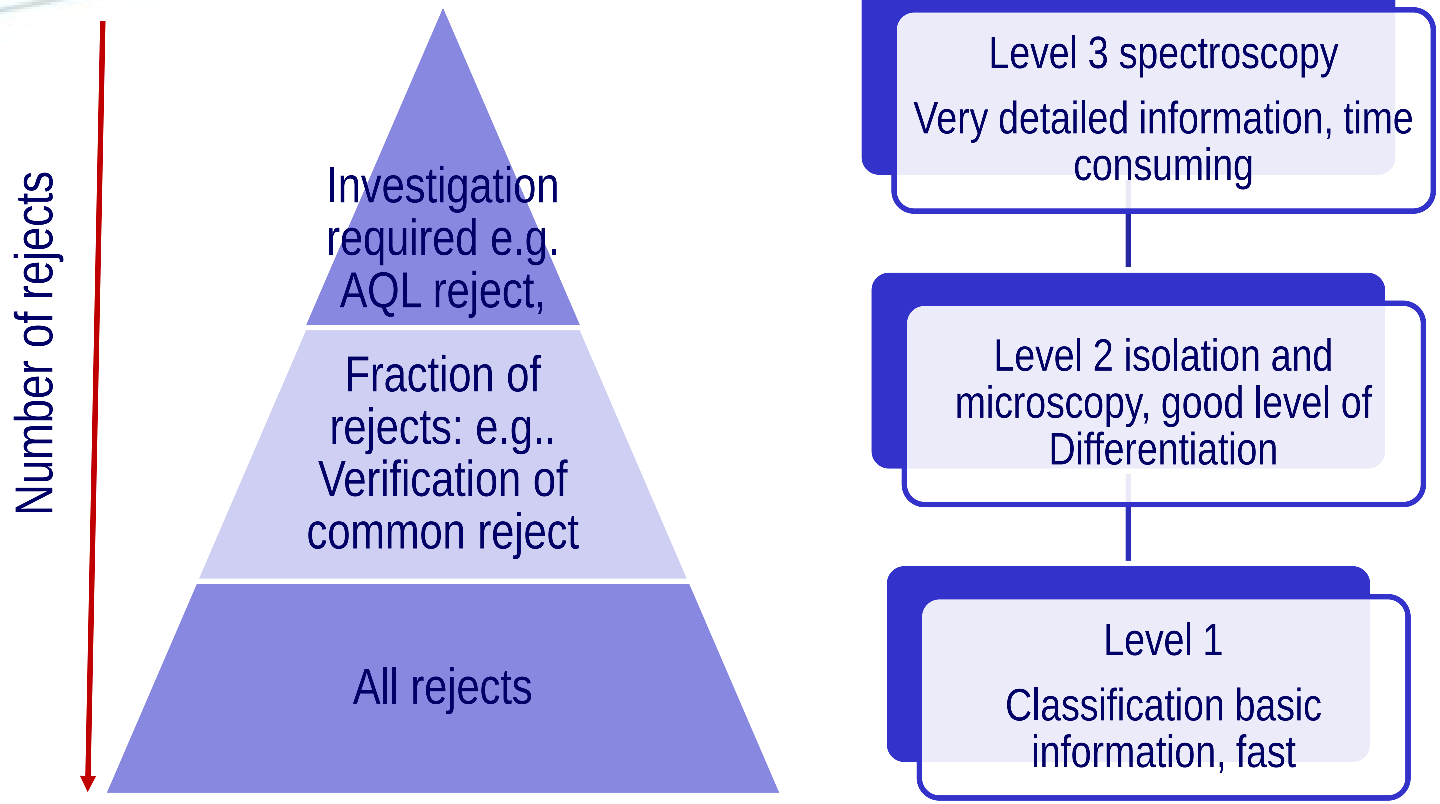
Level One: Visual classification (in-Situ)
• Nondestructive, as seen during manual inspection
• Light, dark, sinking, floating, color, shape, etc.

Level Two: Macroscopic and Microscopic
• Rapid characterization to specific material categories
• Metallic, glass, rubber, plastic, fiber (natural or synthetic), silicone lubricant, inherent particles, etc.

Level Three: Spectroscopic or other fingerprint ID
• FTIR, Raman, Elemental, Mass Spec, etc.

Roy Cherris Visual Inspection Forum 2013, Bethesda

When do I need which kind of information ?



Comparison of Characterization Level

Level		Cost	time/particle
1	light microscopy	Invest: 2T€ €	15 min
2	Isolation, Polarized Light microscopy	Invest: 60 T€	30 min
3	SEM / Raman/ IR	Invest: 70 T€ (IR), 150 T€ (Raman), 180 T€ (SEM)	30 min

Classification Level 1

1. Classification is based on basic observations Defined by trajectory, shape, density

2. Classification could be done by a experienced operator probably trained for special tools

3. Reason to go on with level 2 characterization could be statistics, uncertainty about nature of the particle

Categories / Attributes

Categories

Category		Category	
Glass-Like	[ ]	Polymeric-like	[ ]
Metallic-like	[ ]	Dark Particle	[ ]
Fiber-like	[ ]	Light Particle	[ ]

Attributes for further description

Shape	Colour	Location	Density	Size
Spherical	Light	Body	Floater	
Irregular	Dark	Bottom	Fixed	
Elongated	Transparent	Shoulder		

Microscopic investigation – Level 2

Level 1 characterization groups e.g. dark particle, light particles, fiber-like might be sampled by a basic universal sampling plan like $\sqrt{N}+1$

Isolation is required for further investigation
Clean area mandatory:

- clean room, clean bench, ultra cleaned glassware, requires trained personnel

Various tools for isolation:

- Capillary, tungsten needles, filtration

Microscope helps to give further details:

- Rubber, metal, synthetic vs natural fiber, crystal shape, color

After isolation particle can be easily transferred to level three

PLM – Level 2 characterization

Olympus CX31-P Microscope

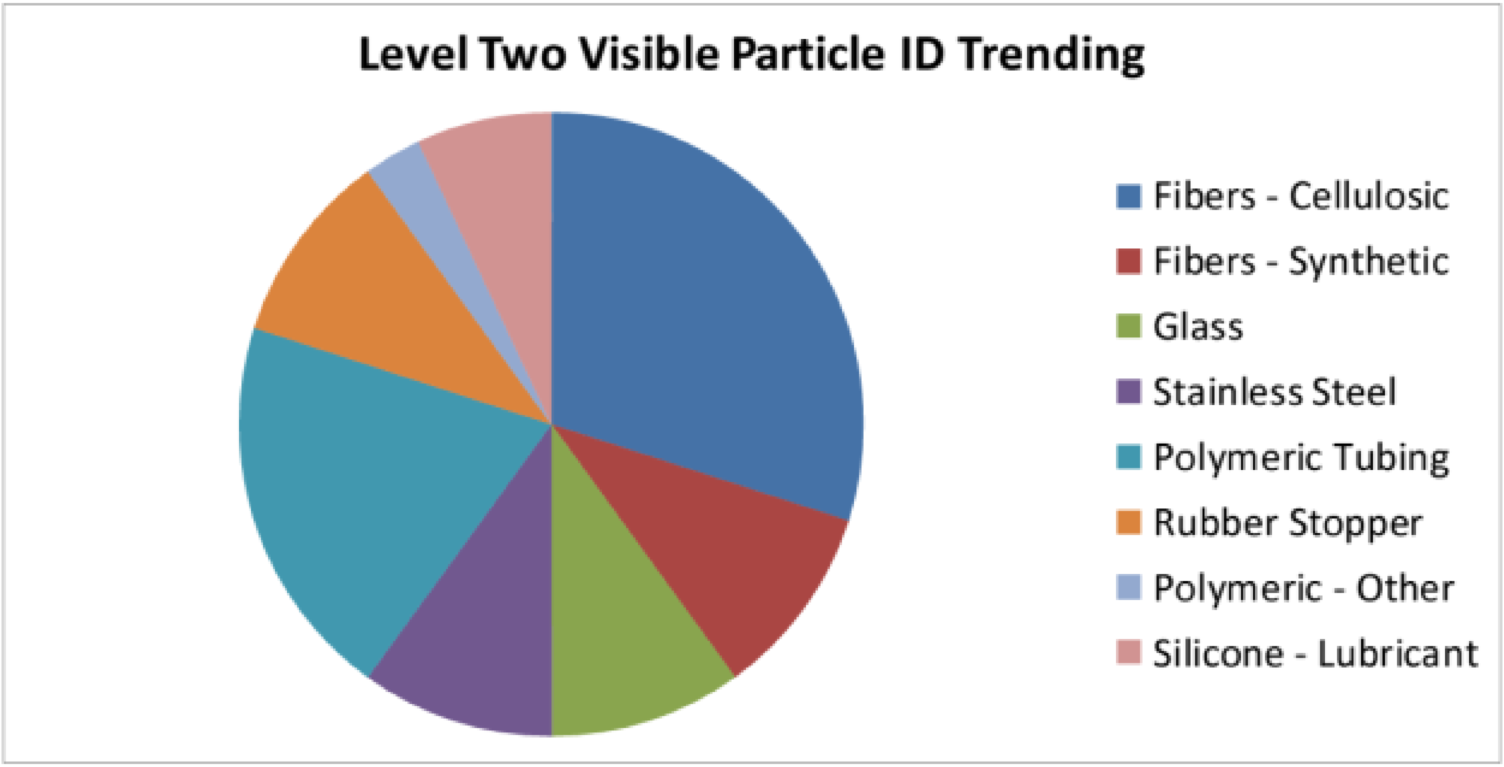
Figure 1

Microscopic information – Level 2

Incident Light	Select	Transmitted Light	Select
Clear	[ ]	Transparent	[ ]
Opaque	[ ]	Opaque	[ ]
Reflective	[ ]	Crystalline	[ ]
Physical	**Select**	**Crossed Polars**	**Select**
Crystalline	[ ]	Isotropic	[ ]
Shaving	[ ]	Anisotropic	[ ]
Resilient	[ ]	Pseudo-Birefringence	[ ]
Shard	[ ]	Isotropic Rod	[ ]
Size Length (um)		Uniform fiber	[ ]
Size Width (um)		Irregular frayed fiber	[ ]

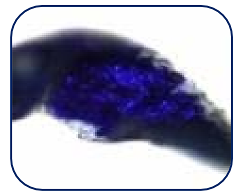
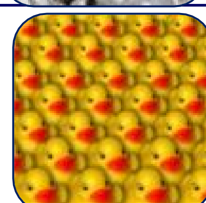
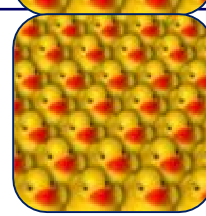
Level II Category	Select	Level II Category	Select
Glass	[ ]	Polymeric	[ ]
Metallic	[ ]	Rubber Stopper	[ ]
Fiber	[ ]	Semi-Solid - Silicone	[ ]
Fiber - Natural	[ ]	Possible Inherent API	[ ]
Fiber - Synthetic	[ ]	Possible Extrinsic	[ ]

Trending



Level Two Visible Particle ID Trending
Fibers - Cellulosic
Fibers - Synthetic
Glass
Stainless Steel
Polymeric Tubing
Rubber Stopper
Polymeric - Other
Silicone - Lubricant

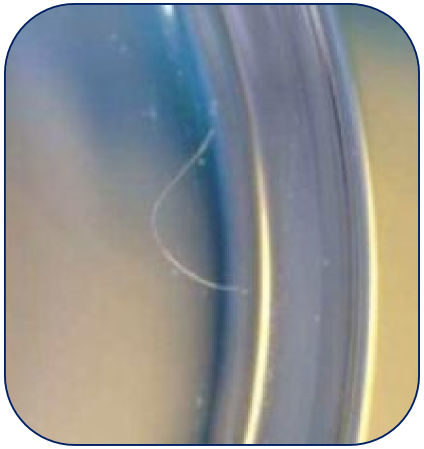
Spectroscopy Level 3

method	meaning	time/particle
PLM (polarized light microscopy)	color + shape e.g.: black fibres 	1-5 min
SEM/EDS analysis	> 5µm Elements  	20-180 min
IR – microscopy	> 50 µm Structure  	20-180 min
RAMAN - microscopy	> 0.5 µm Structure  	20-180 min

Fiber – Level 1

Category	*Select*
Glass-Like	[ ]
Metallic-like	[ ]
Fiber-like	[x]

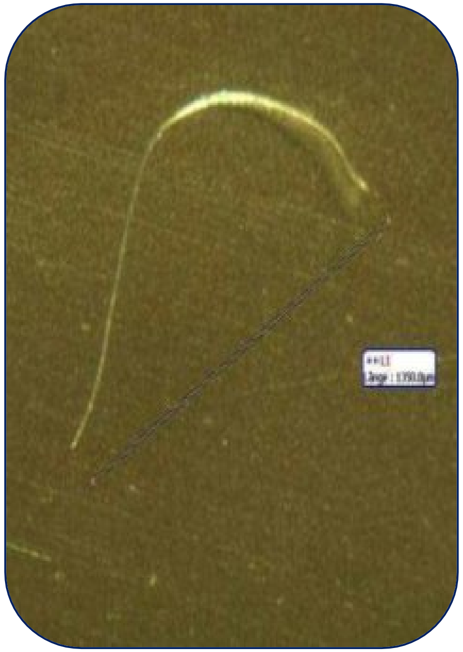
Category	*Select*
Polymeric-like	[ ]
Dark Particle	[ ]
Light Particle	[x]



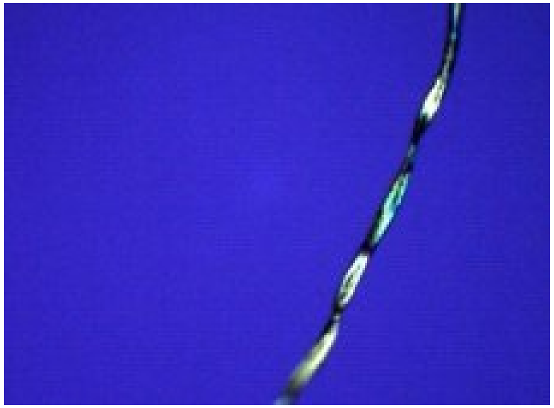
- Fibers can be easily classified. Might be sufficient for trending
- Further classification of fibers can be preformed in situ with an inverted microscope due to morphology and texture

Fiber-Level 2: Isolation Microscopy

- Microscopy of isolated fiber gives further information (cotton, protein based fiber, synthetic)
- Spectroscopy can give a very specific fingerprint for root cause or kind if synthetic fiber



Level 2

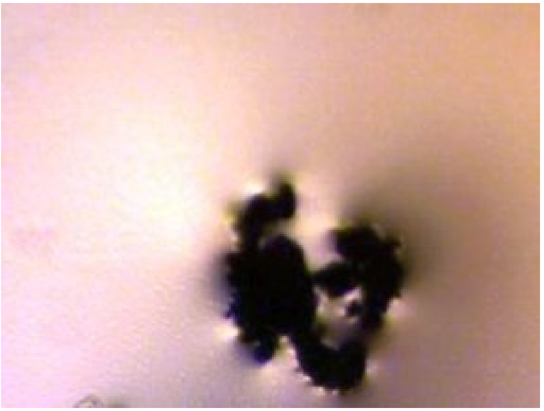


Level 2

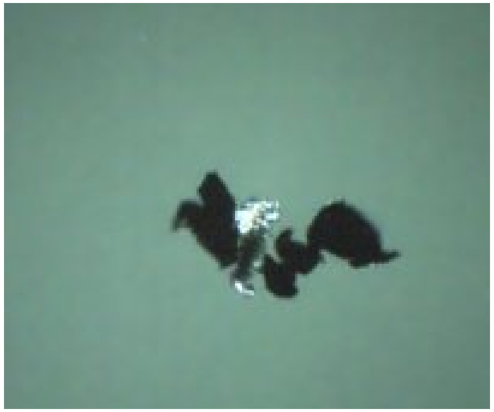
Level 1

Metal particle Level 1 and Level 2

- Characterized density and reflectivity
- Sufficient for trending
- Hard to observe while swirling
- Usually easy to find at the bottom



Level 1



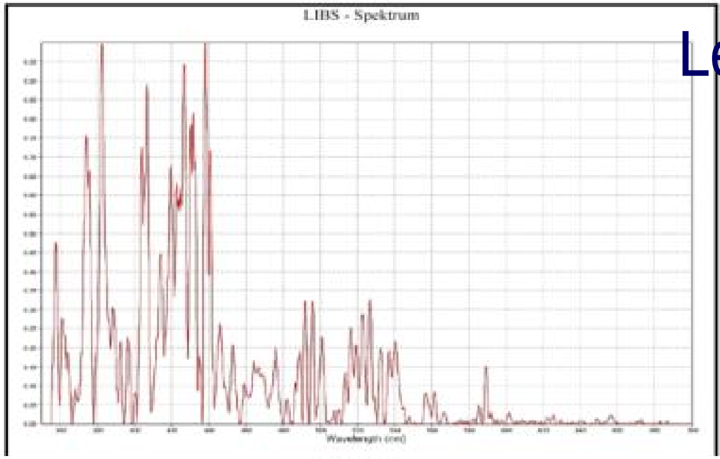
Level 2

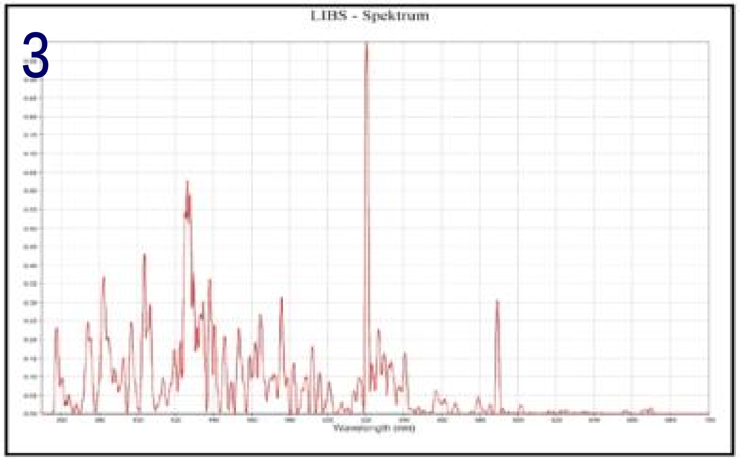
Level 2

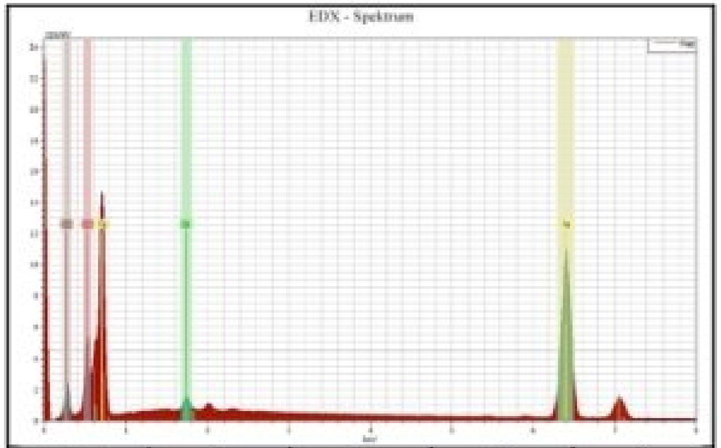
Spectroscopy on metals – Level 3

Spectroscopy gives more detailed information on the kind of steel e.g. low alloyed vs high alloyed steel which might be needed for root cause investigation

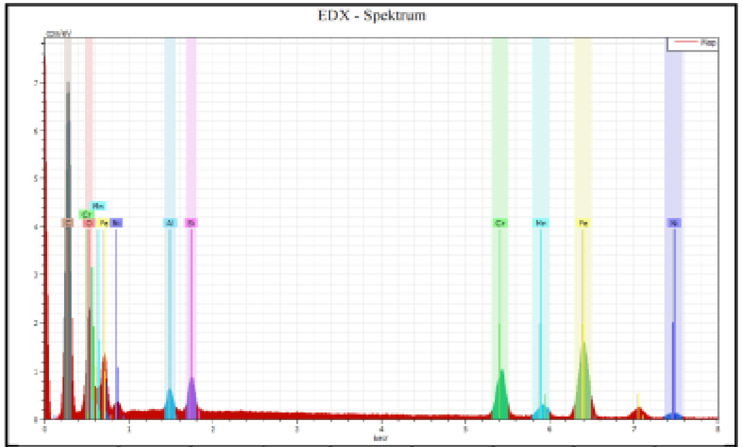
Level 3







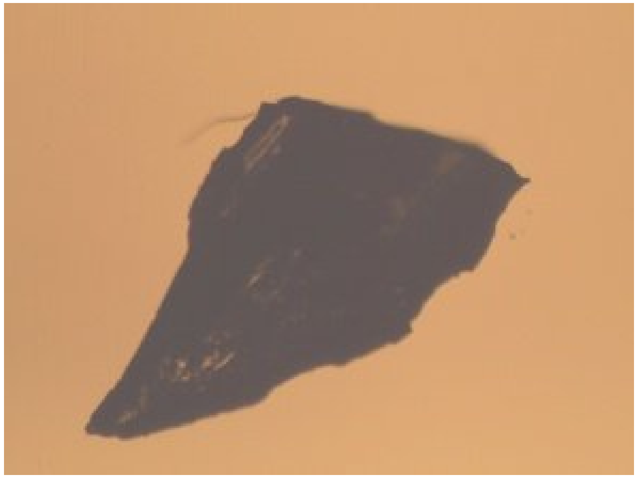
Element	Fe	C	O	Si
nor. [Gew-%]	73,8	13,0	12,1	1,0



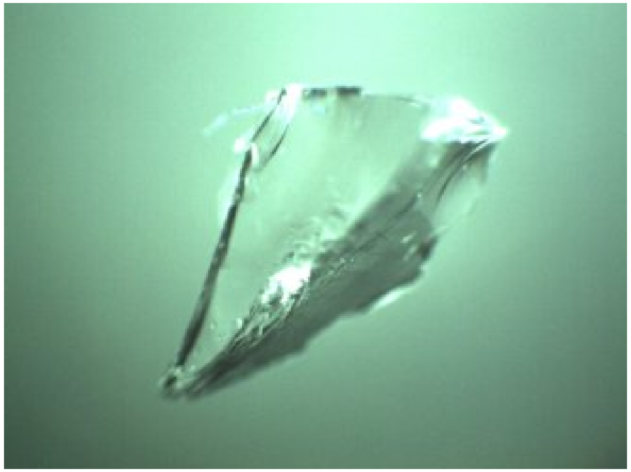
Element	C	Fe	O	Cr	Ni	Si	Mn	Al
nor. [Gew-%]	41,2	26,4	15,4	9,2	2,9	2,1	2,0	0,9

Glass particle - Level 1 and Level 2

- Glass has a very characteristic shape which is sufficient for classification
- Further characterization for root cause investigation: element specific methods e.g. SEM or LIBS favorable

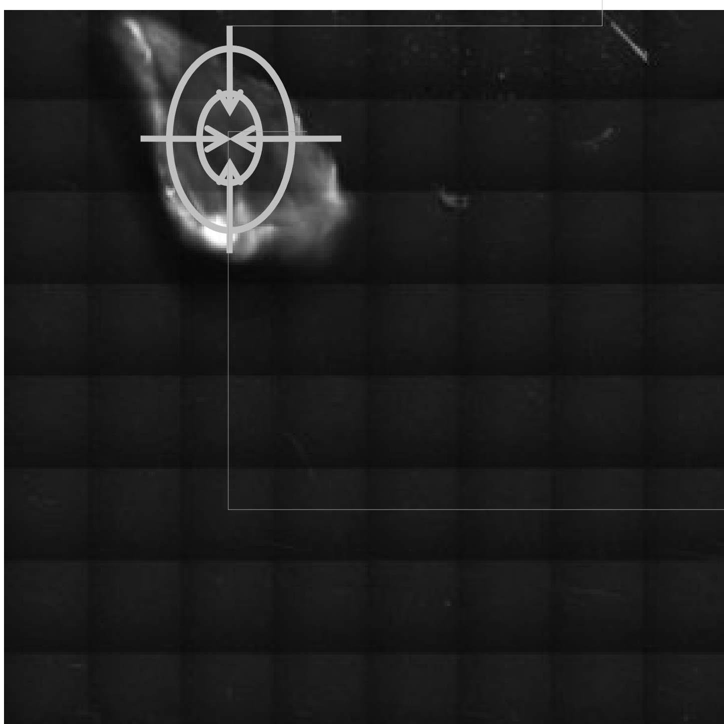


Level 1

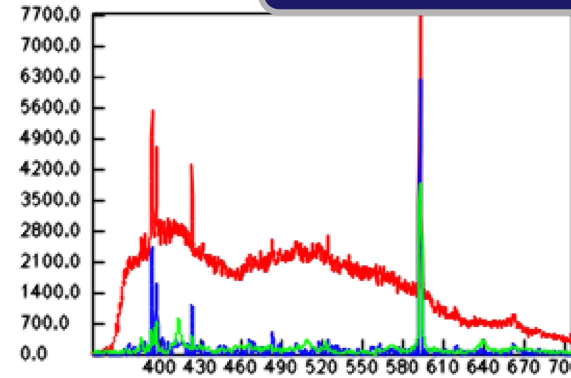


Level 2

Glass – Level 3

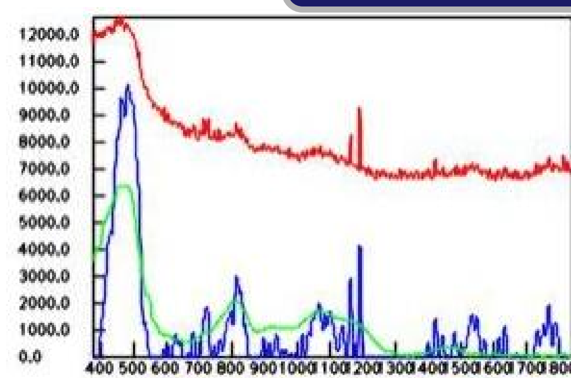


LIBS



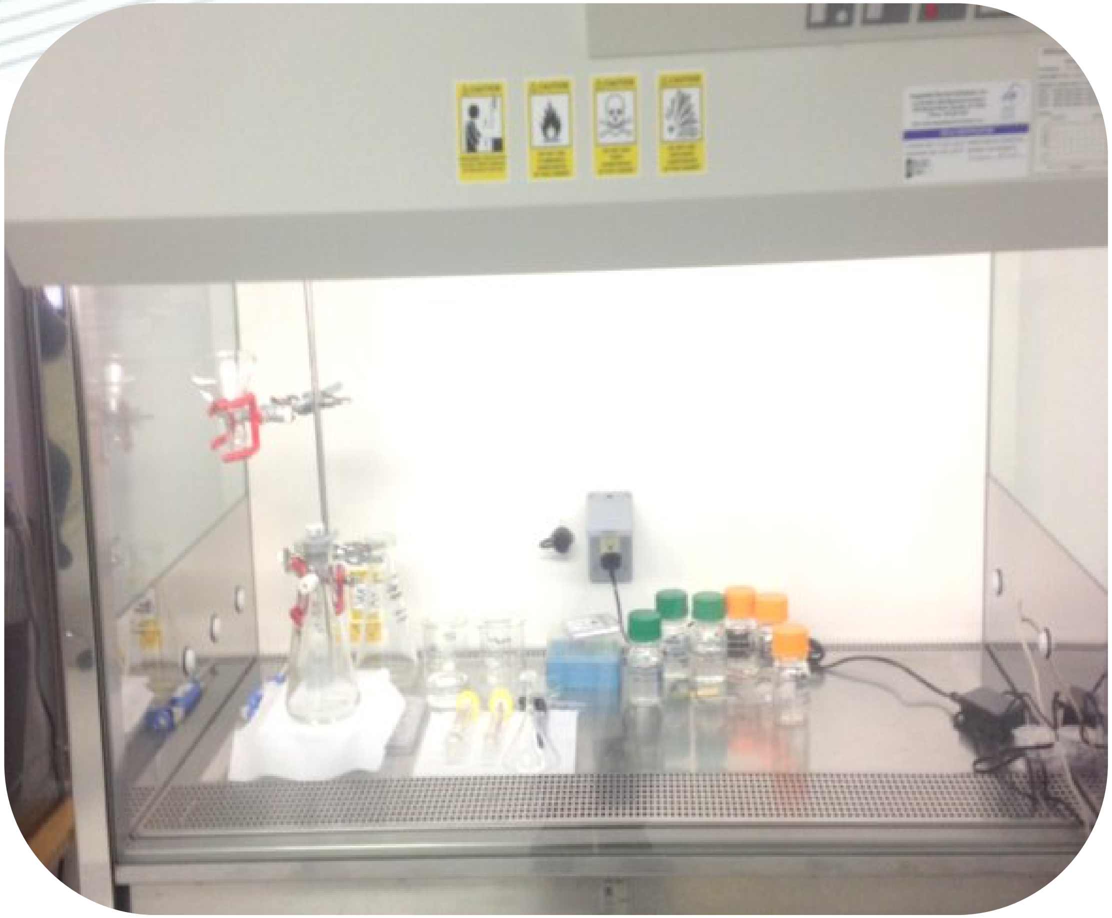
Si, Na, Al (Fiolax)

Raman



Glass

Particle Isolation



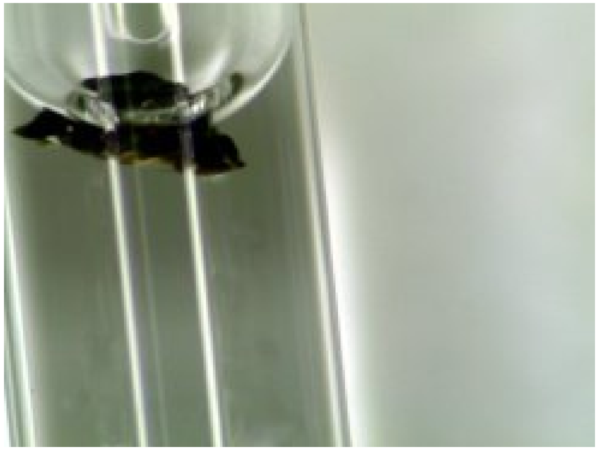
Isolation

Environmental Considerations

- Class 100 clean bench is essential
- „Ball-park“ clean rooms would be beneficial
- Cleaning is essential and system suitability tests (blanks) have to be taken
- Training and control is essential
- Benches, coats, sleeves, microscopes, equipment and water should be clean and non-shedding

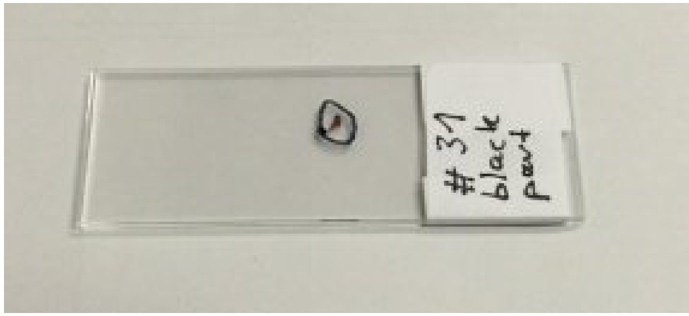
Isolation and transportation

Capillary trapping



Tungsten needles
for particle picking

Sending particles to a lab
between 2 slides



Particle Sources

Particulate Matter vs. Foreign Matter

Inherent

Particulate made entirely of components of the formulated product, arising from the product itself. These particulates are related to the product formulation: API

Intrinsic

Particulate related to the production process of components of the formulated product, arising from the product itself. Processing Equipment, Primary Package, Active and other ingredients

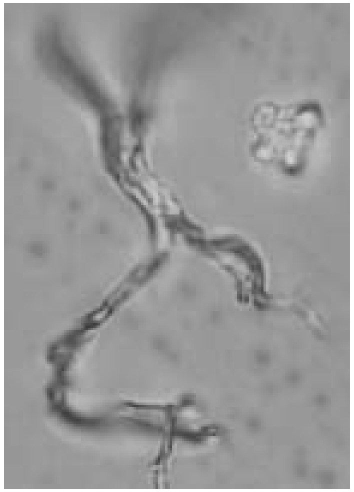
Extrinsic (Foreign)

Environmental Contaminants
insect parts, hair, fibers, paint, rust

Sources for particulate matter ?

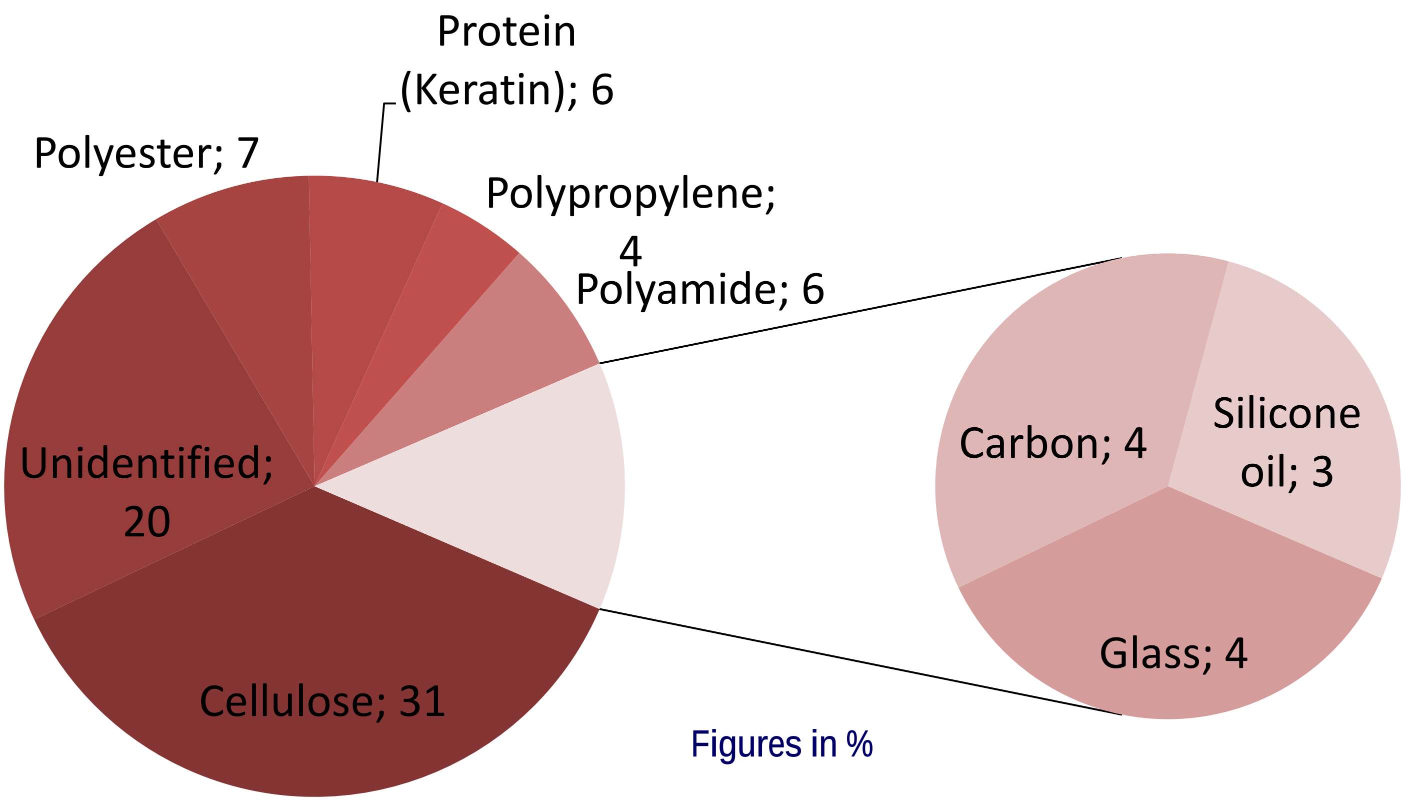
- personnel

- Garnement
- Water
- container



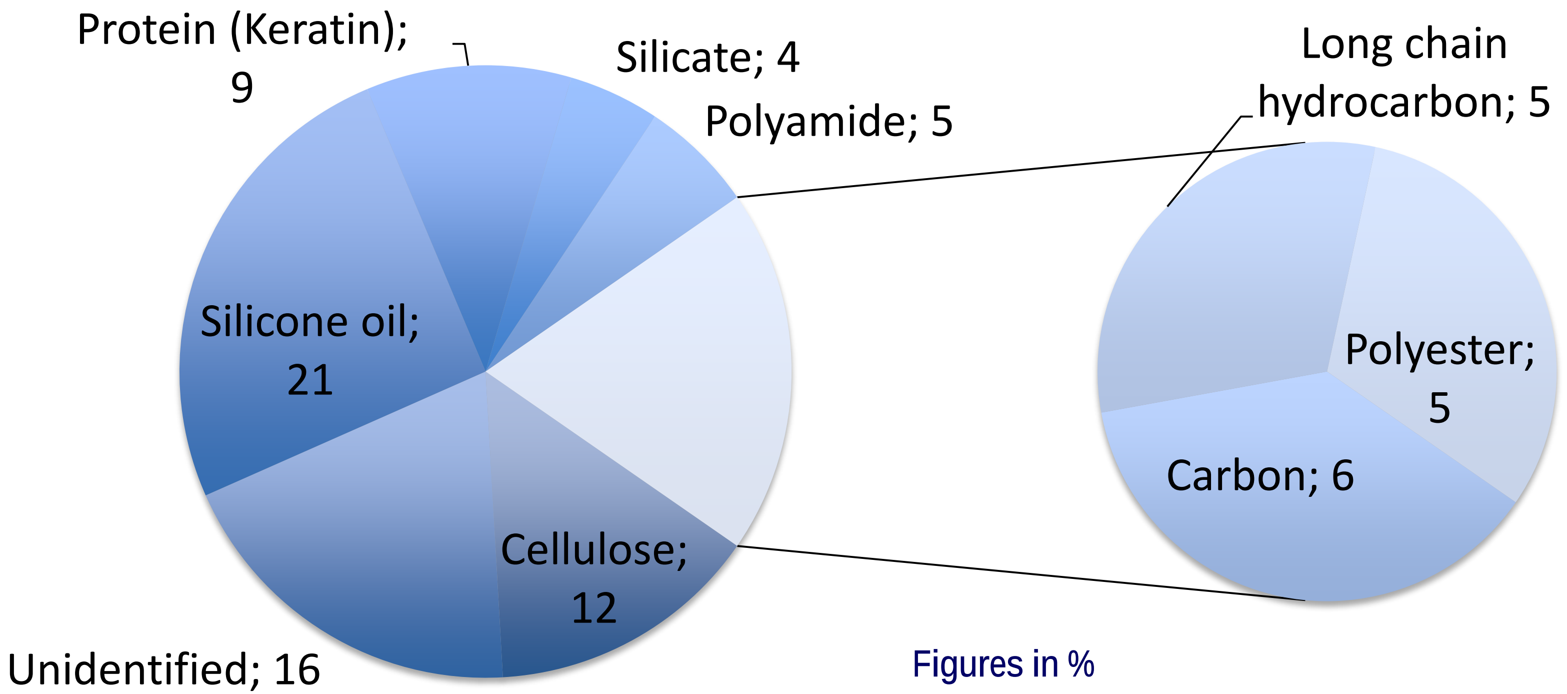
- Process / Production Equipment e.g.: rubber
- Cleaning process

Visible



Cellulose, Polyester and Protein/Polyamide particles are major contributions to particulate contamination.

Sub-visible



Figures in %

Silicone oil, Protein, Cellulose particles are the most often found contaminants

Top Ten in more detail

- Cellulose: mostly fibres
  - source: clothes, **towels**, wipers, autoclave paper

- Longchain hydrocarbon
  - source: rubber (stopper), PE (bottles)

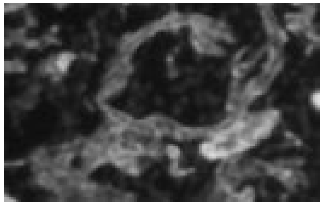
Top Ten in more detail

- Glass: fibres and particles
  - Source: Primary packaging
  - But also glassfibers and hollow glass fibres (filter material)

- Carbon: particles
  - Usally black particles contain high content of carbon:
  - Sealings rubber material filled with carbon
  - Burned material

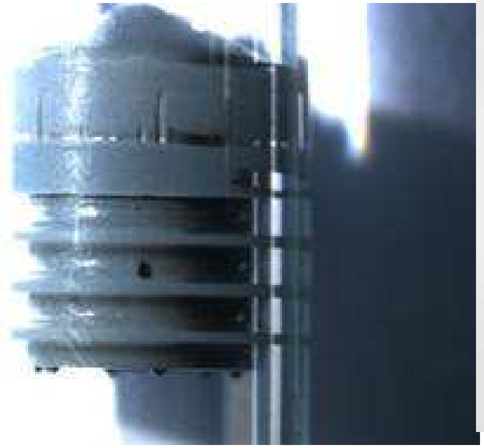
Top Ten in more detail

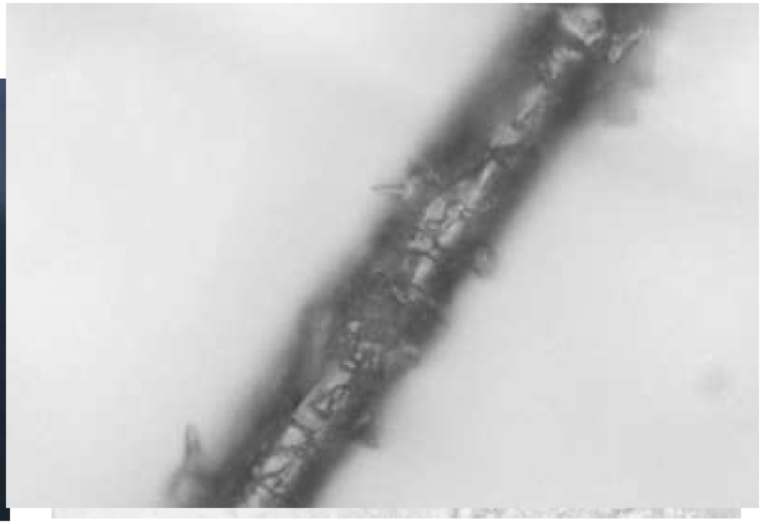
- Polyester: fibres and particles
  - Source: Cleanroom clothes and defect filter
- Protein: mostly flakes
  - Source human dust, protein particles from protein solution
- Silicone oil: compact particles
  - Source: sealings, siliconisation



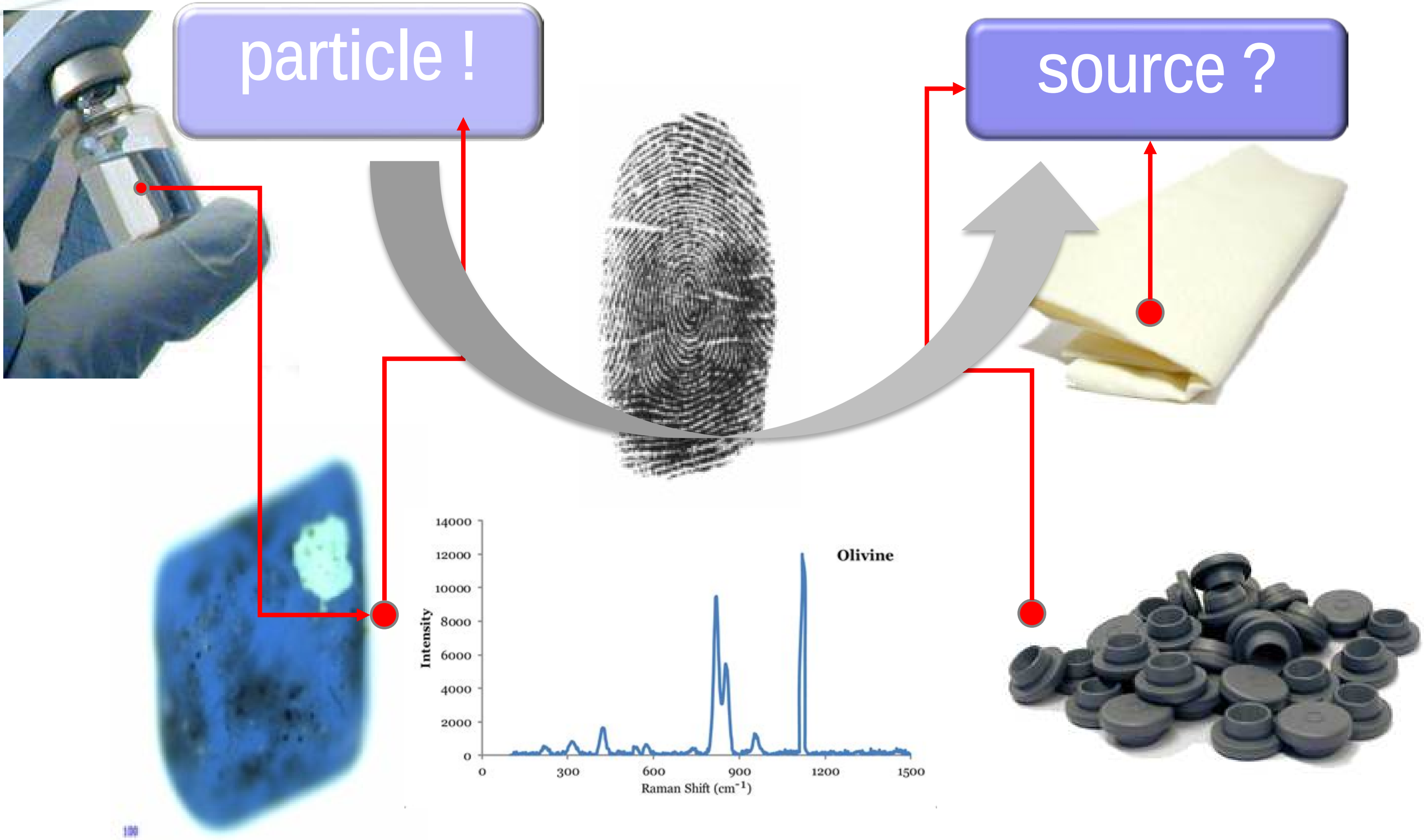
Plunger/stopper related defects

- White or black spots on/between lips
  – Foreign material trapped between plunger and glass wall Glass bits
  – Rubber chunks
  – Fibres
  – Hair





Root cause analysis

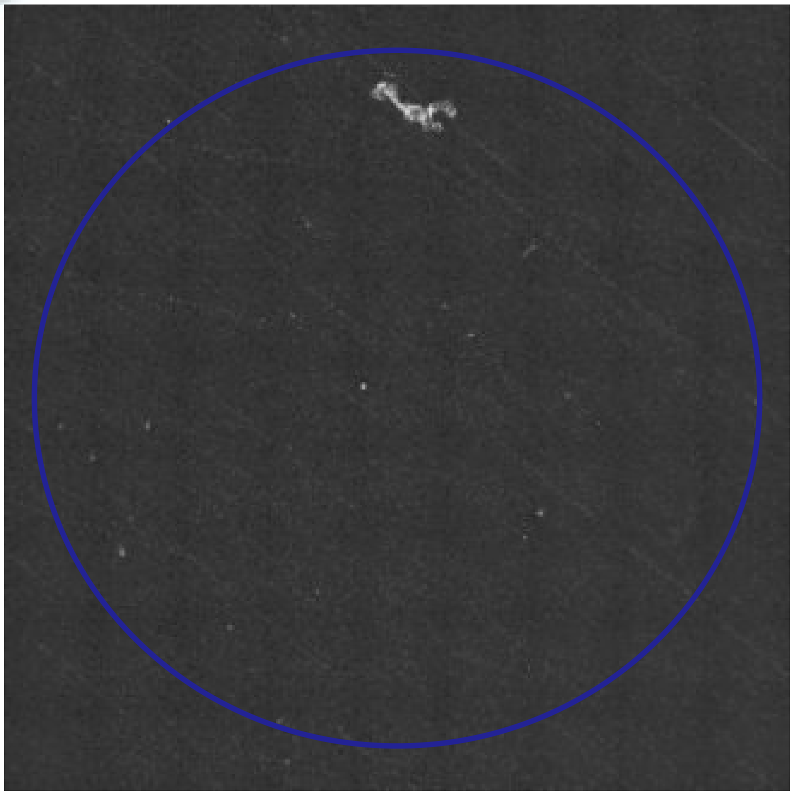


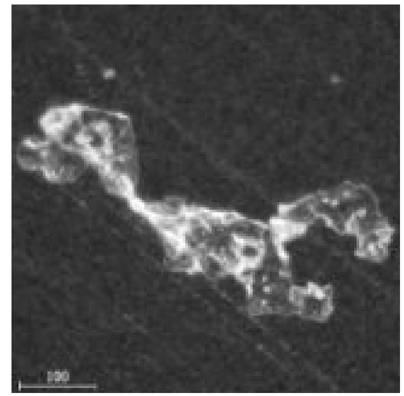
Particle investigation

1. Documentation of the defect →in-situ (in the closed container)
2. Filtration and documentation of the sample on the membrane filter
3. Documentation of the analysis and the identification of the reject by Raman spectroscopy
4. Identification of sub-visible to gather further information
5. Verification of the findings (particle observed by visible inspection) with FT-IR or LIBS, EDX

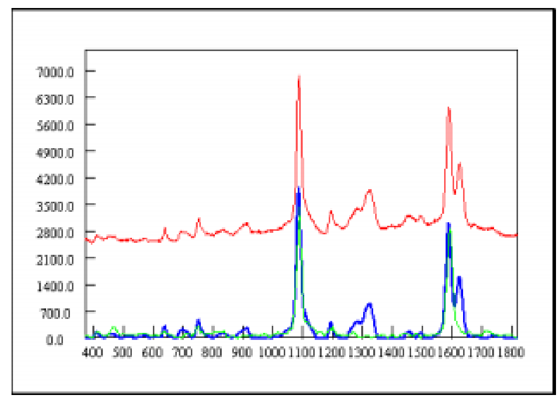
1. Particle in the vial

3. Particle Imaging + raman.ID





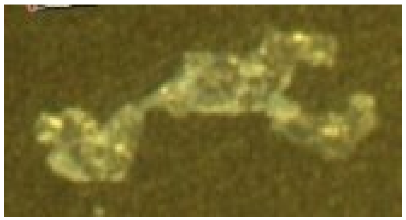
L=505.0 µm
w=202.6 µm
E=2.49
R=0.3071

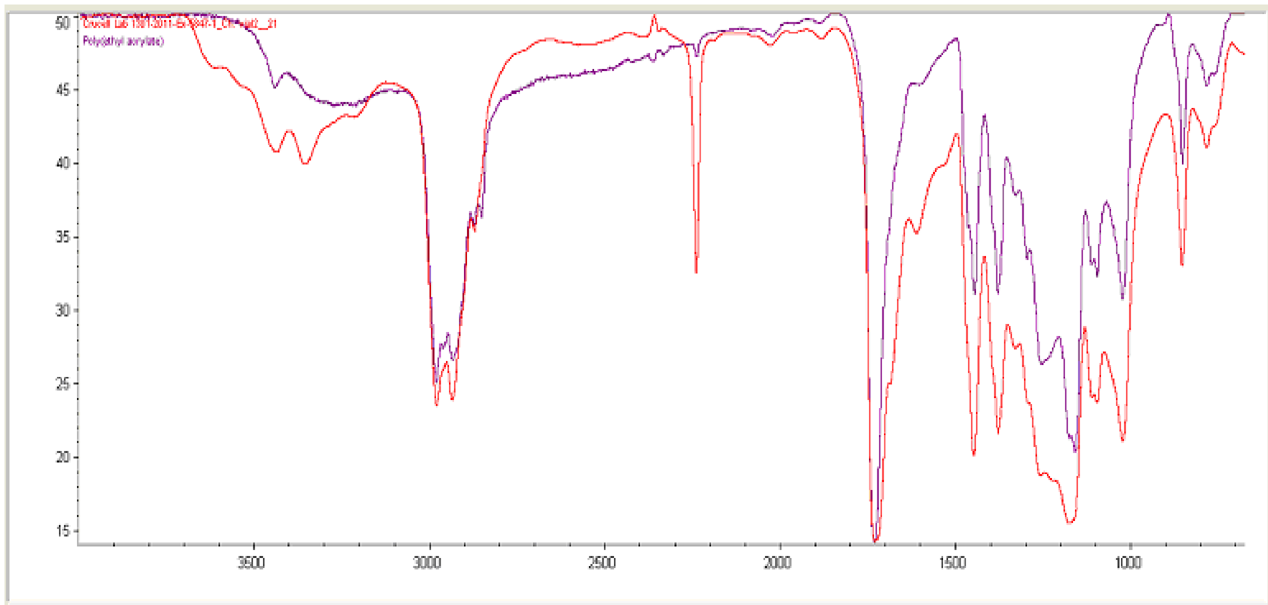


Raman.ID: Polyethylene-terephtalate, PET

Rank: 887

4. Verification FT-IR → PET

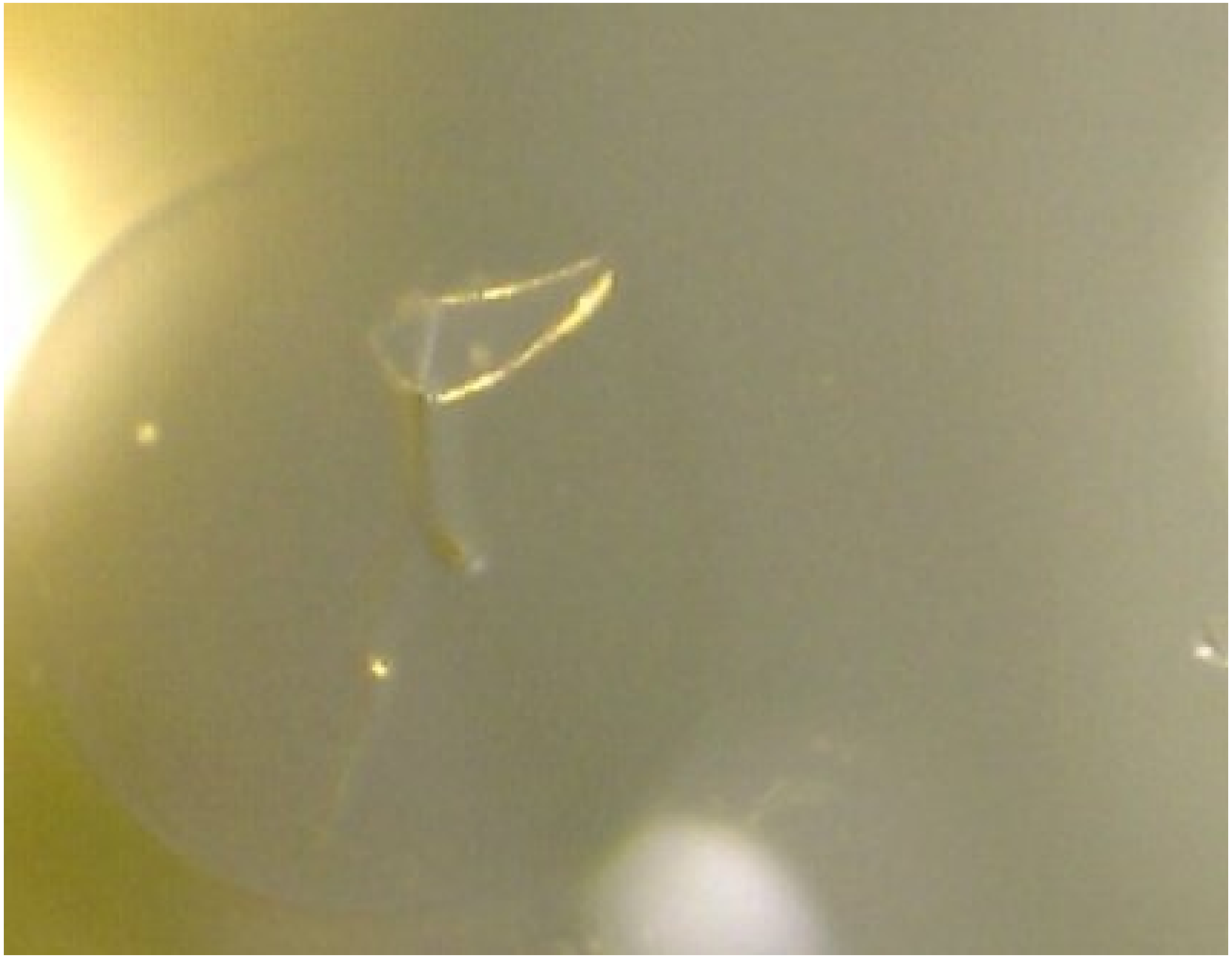




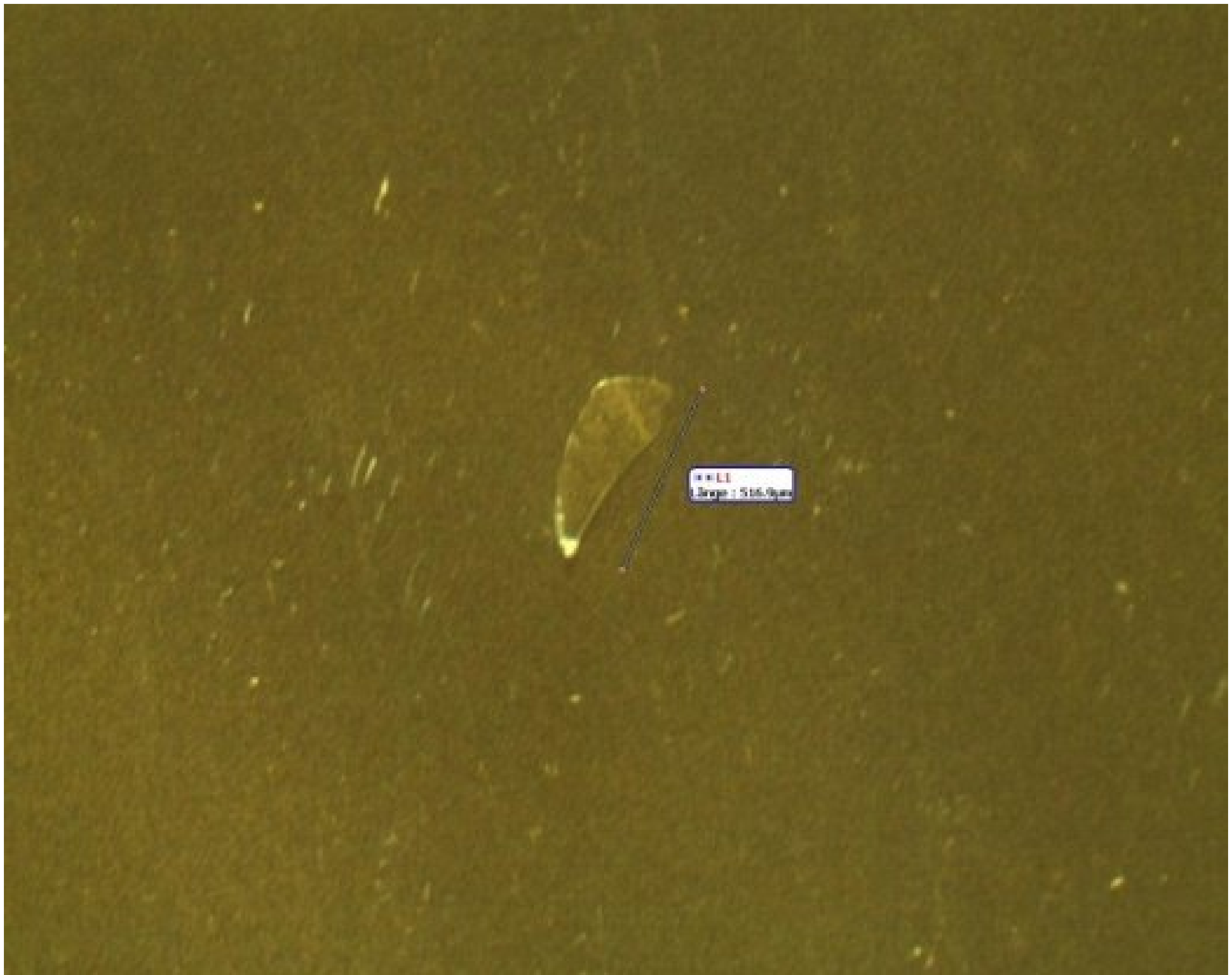
Polyethylene Terephtalate, PET

76.16% matching

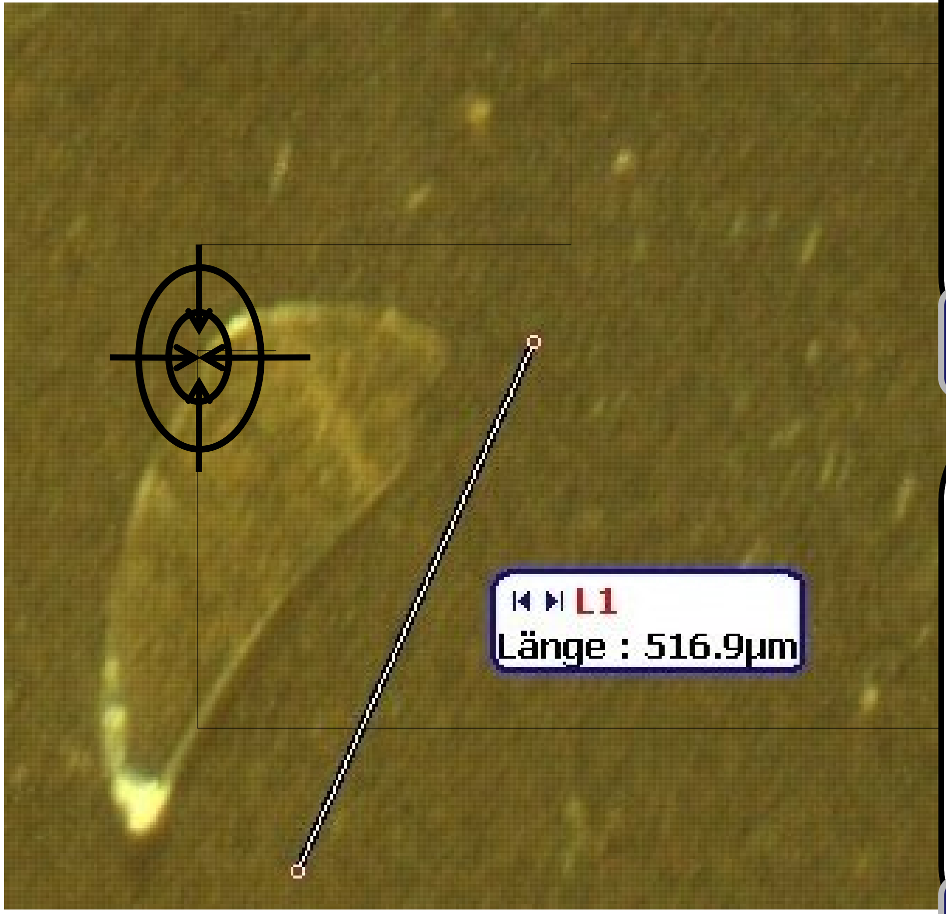
Visible Inspection: Particle Reject II



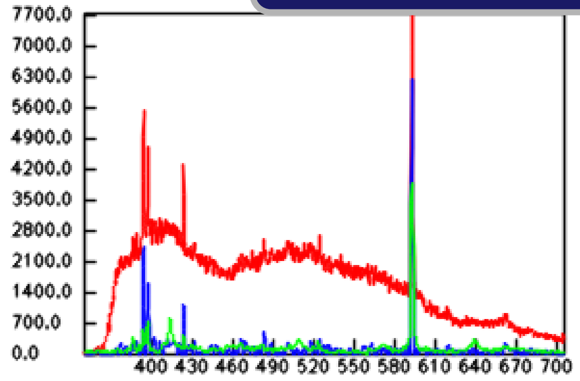
Sample prep. + Documentation



Glass Particles

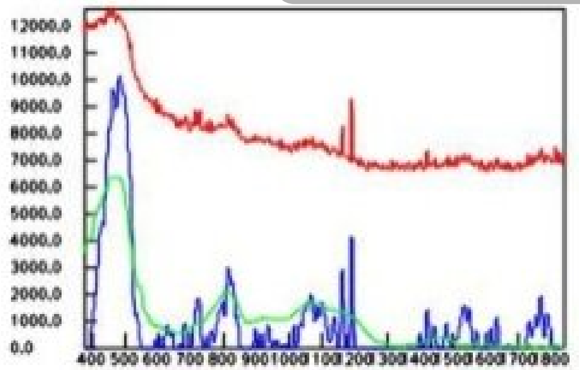


LIBS



Si, Na, Al (Fiolax)

Raman



Glass

Report II - Summary

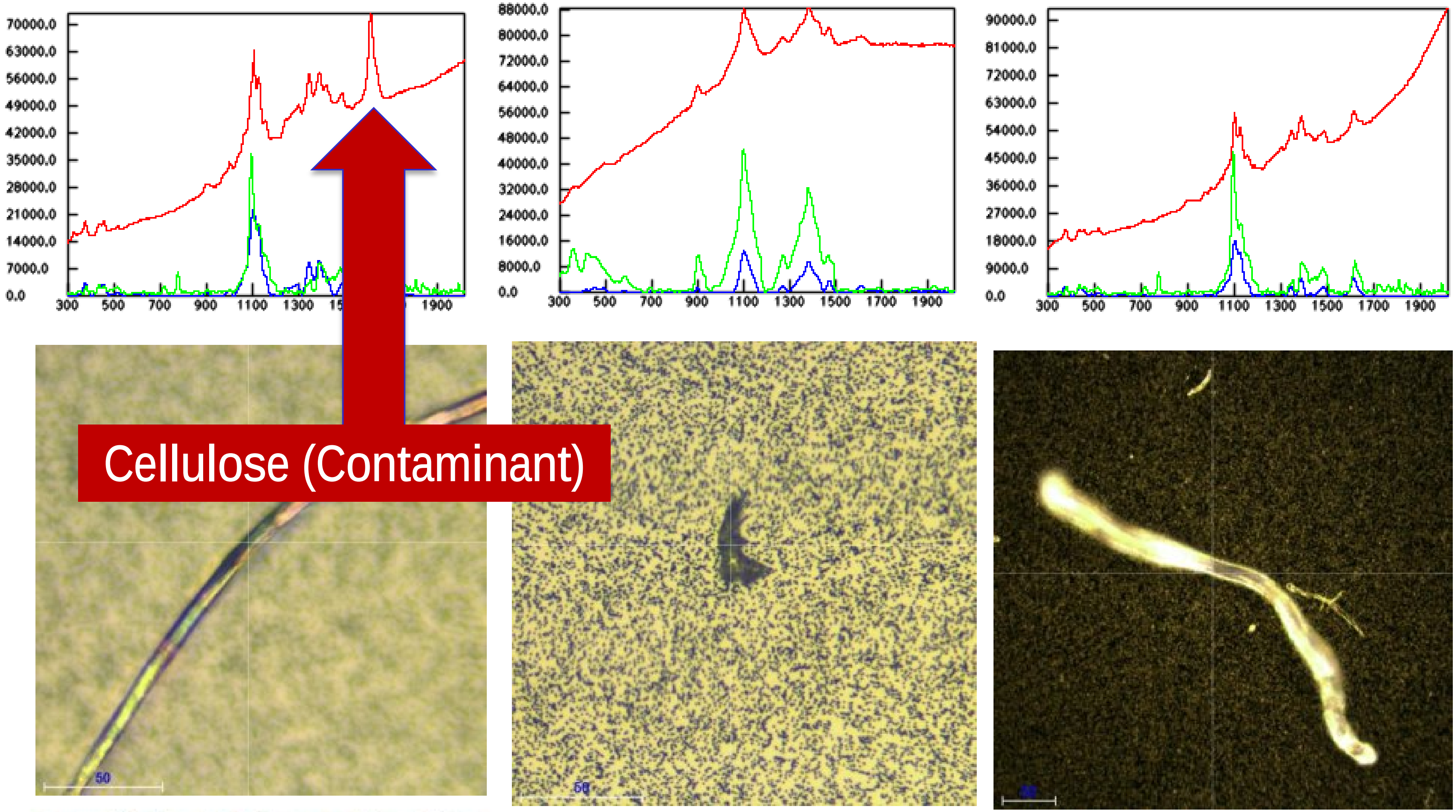
1. The largest Particle we observed in the vial (516 µm) was identified by means of Raman as well as FT-IR spectroscopy as glass.
2. This finding was also confirmed by LIBS Laser induced brakdown spectroscopy. The particle matched with Fiolax (Al is a marker).

CELLULOSE SOURCE

Example Cellulose Source

1. 4 batches failed in a row
2. 3 samples of each of the failed batches and one of the good batches were investigated
3. Soon it became clear that the problem was cellulose related….

Several cellulose fibers were found

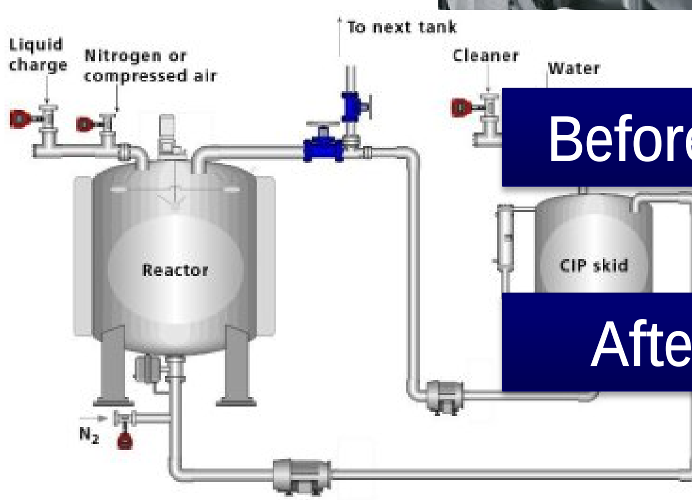


Samples from the filling were taken

Stopper Sample

Before Tubing Rinse Sample

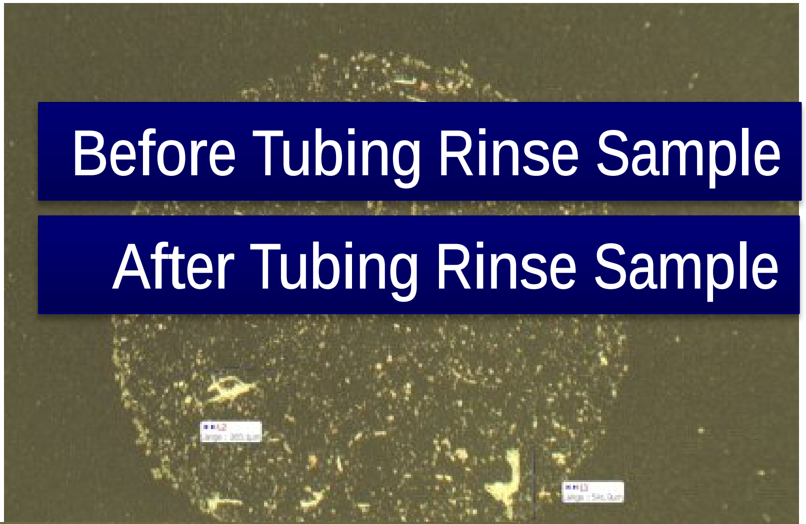
After Tubing Rinse Sample

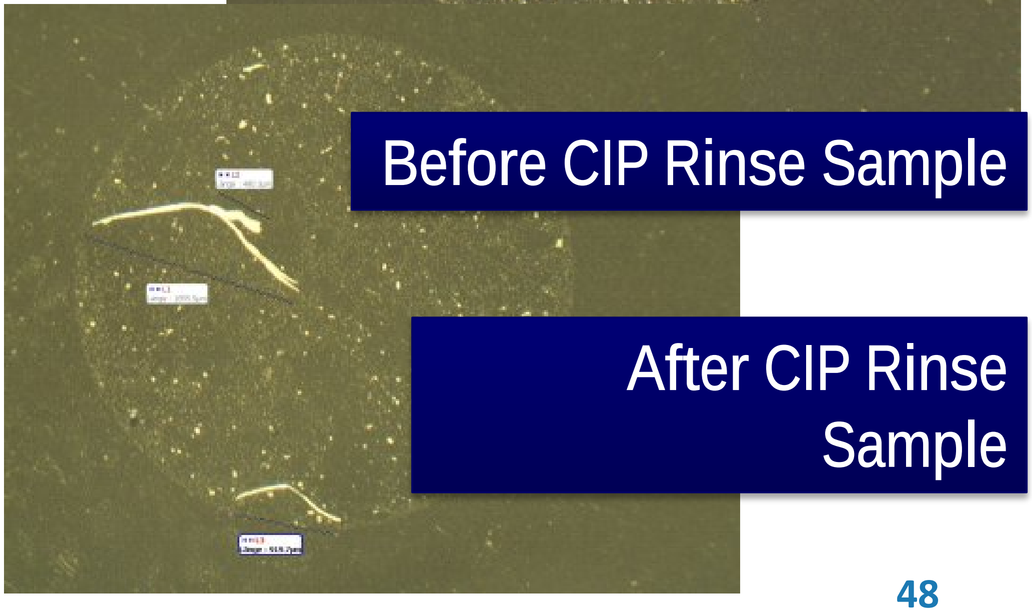


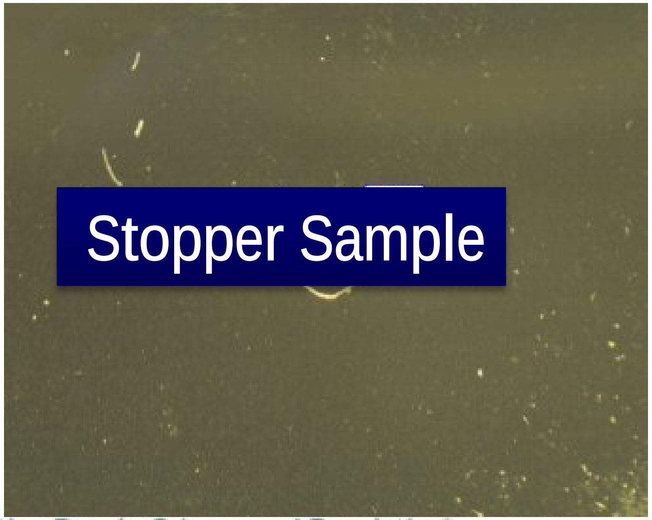
Before CIP Rinse Sample

After CIP Rinse Sample

Samples from the process were taken

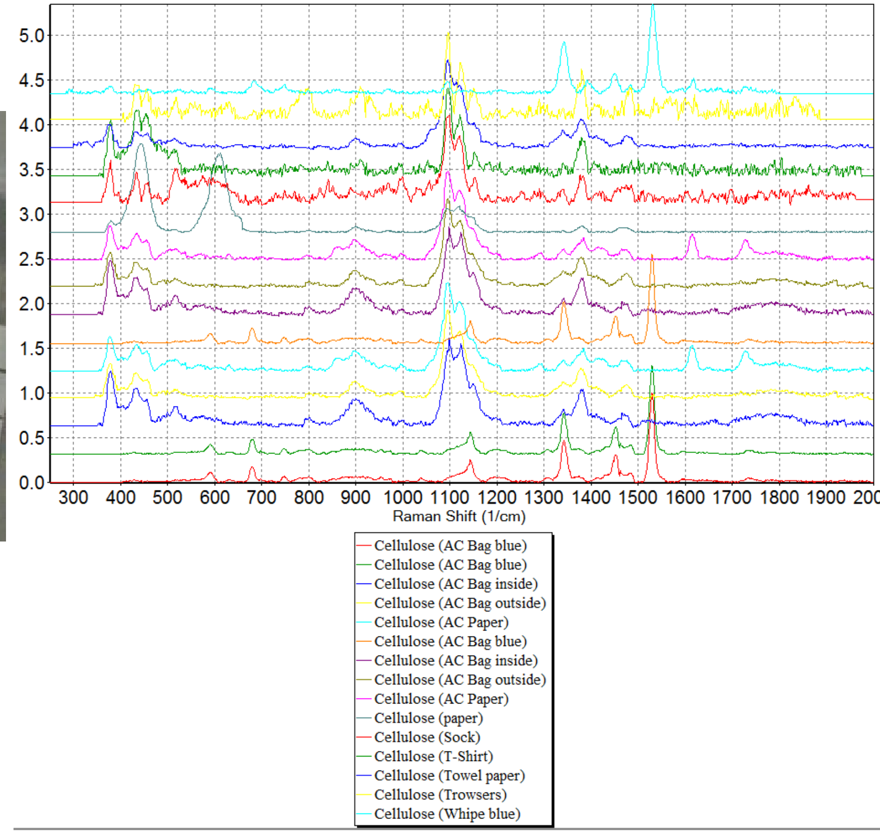






Database with filling line related materials was built

1. N



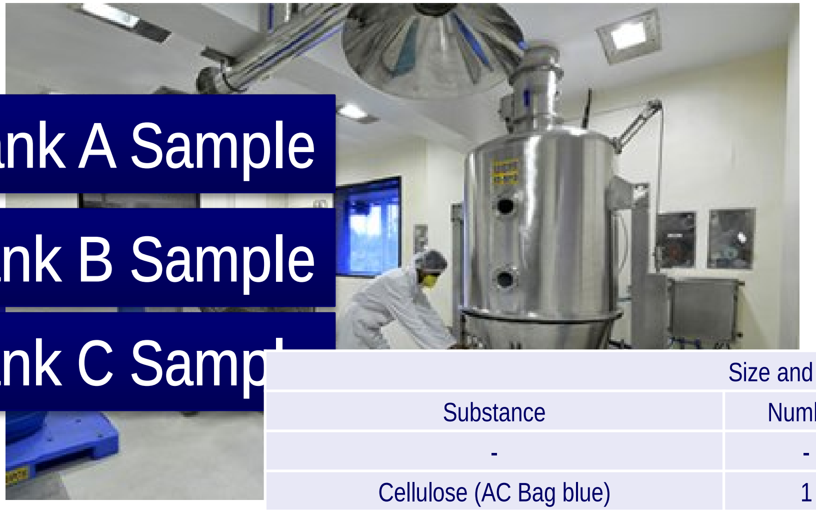
No Cellulose (Contaminant) !

Tube rinse result

Size and Substance Distribution of Measured Particles						
Substance	Number	Size Distribution [µm]				
-	-	>=5	>=10	>=25	>=50	>=100
Cellulose (AC Bag blue)	5	0	0	0	1	4
Cellulose w. Polyester (Papertowel II)	1	0	0	0	1	0
Ethyl Cellulose	1	0	0	0	0	1
Cellulose (AC Bag inside)	19	0	0	0	6	13
Pigment, Indian Yellow	1	0	0	0	0	1
Other Particles	143	0	0	5	38	100
beta-Carotene	50	0	0	3	19	28
Skipped particles	2283	889	808	432	137	17
All particles	2503	889	808	440	202	164

No Cellulose (Contaminant) !

Closer look into the API production (site in Italy)



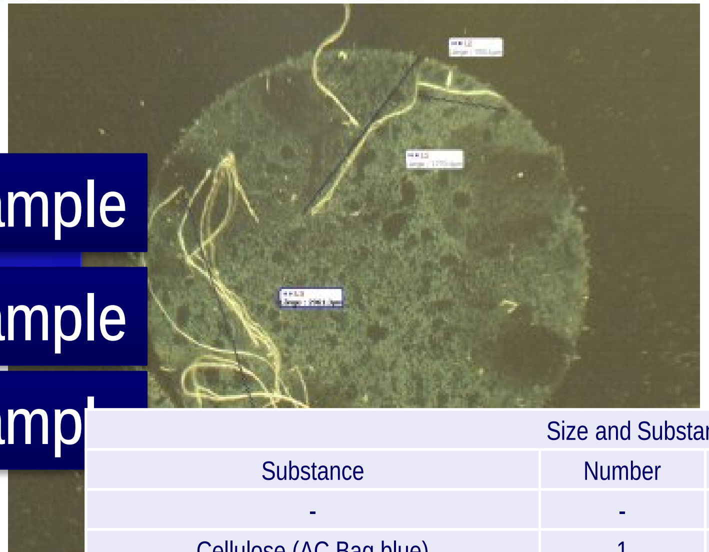
Tank A Sample

Tank B Sample

Tank C Sampl

Size and Substance Distribution of Measured Particles						
Substance	Number	Size Distribution [µm]				
-	-	>=5	>=10	>=25	>=50	>=100
Cellulose (AC Bag blue)	1	0	0	0	1	0
Labcoat	1	0	0	1	0	0
Fluorescence	1	0	0	1	0	0
Carbon	4	0	0	3	1	0
Cellulose	1	0	0	0	1	0
Indanthrene Blue	1	0	0	1	0	0
Cellulose (Contaminant)	31	0	0	8	18	5
Pigment, Indian Yellow	3	0	0	3	0	0
Polysulfone	5	0	0	1	2	2
Cellulose (Towel paper)	5	0	0	5	0	0
Other Particles	28	0	0	22	1	5
Skipped particles	1716	1353	362	1	0	0
All particles	1797	1353	362	46	24	12

Samples from API tanks and tubings showed this type of fiber.



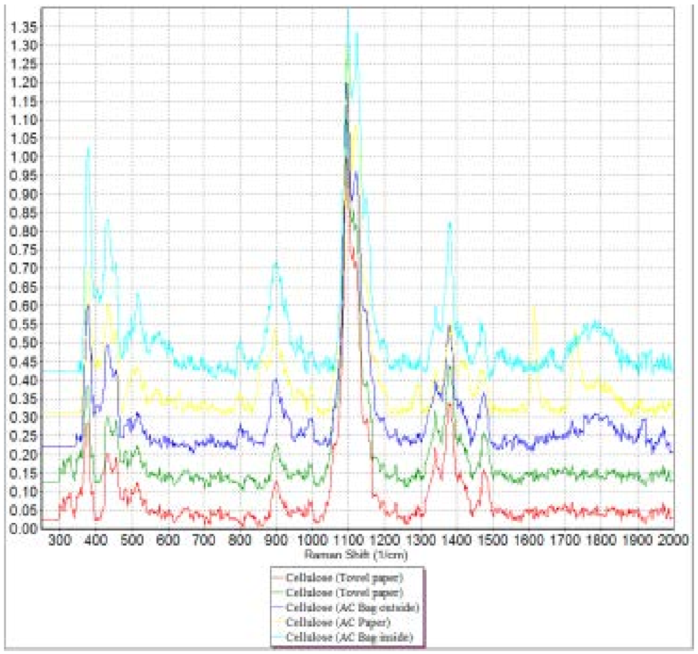
Tank A Sample

Tank B Sample

Tank C Sampl

Size and Substance Distribution of Measured Particles						
Substance	Number	Size Distribution [µm]				
-	-	>=5	>=10	>=25	>=50	>=100
Cellulose (AC Bag blue)	1	0	0	0	1	0
Labcoat	1	0	0	1	0	0
Fluorescence	1	0	0	1	0	0
Carbon	4	0	0	3	1	0
Cellulose	1	0	0	0	1	0
Indanthrene Blue	1	0	0	1	0	0
Cellulose (Contaminant)	31	0	0	8	18	5
Pigment, Indian Yellow	3	0	0	3	0	0
Polysulfone	5	0	0	1	2	2
Cellulose (Towel paper)	5	0	0	5	0	0
Other Particles	28	0	0	22	1	5
Skipped particles	1716	1353	362	1	0	0
All particles	1797	1353	362	46	24	12

Update of the library with towels used in API production



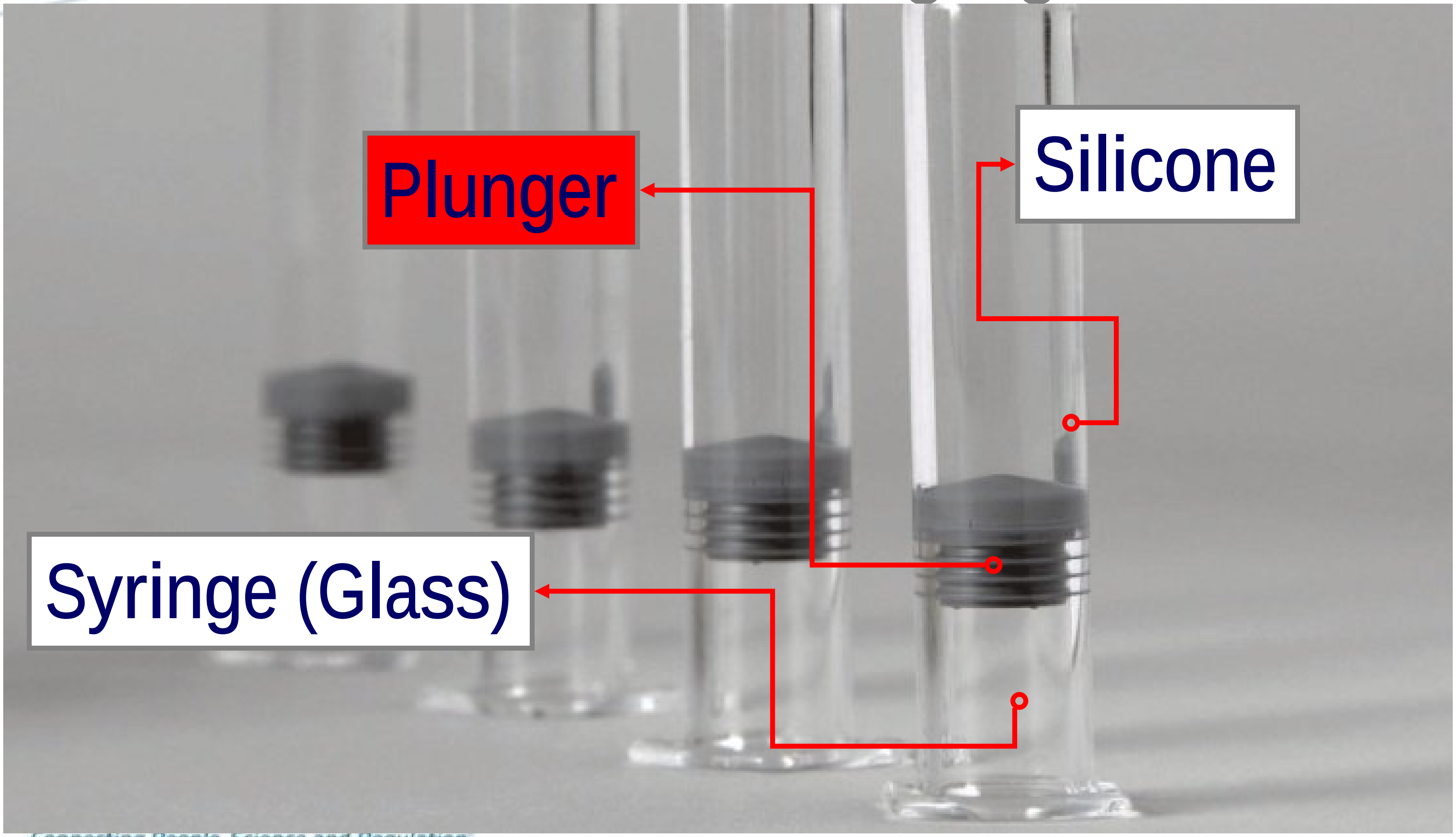
Cellulose (Contaminant)

Conclusion Cellulose Example

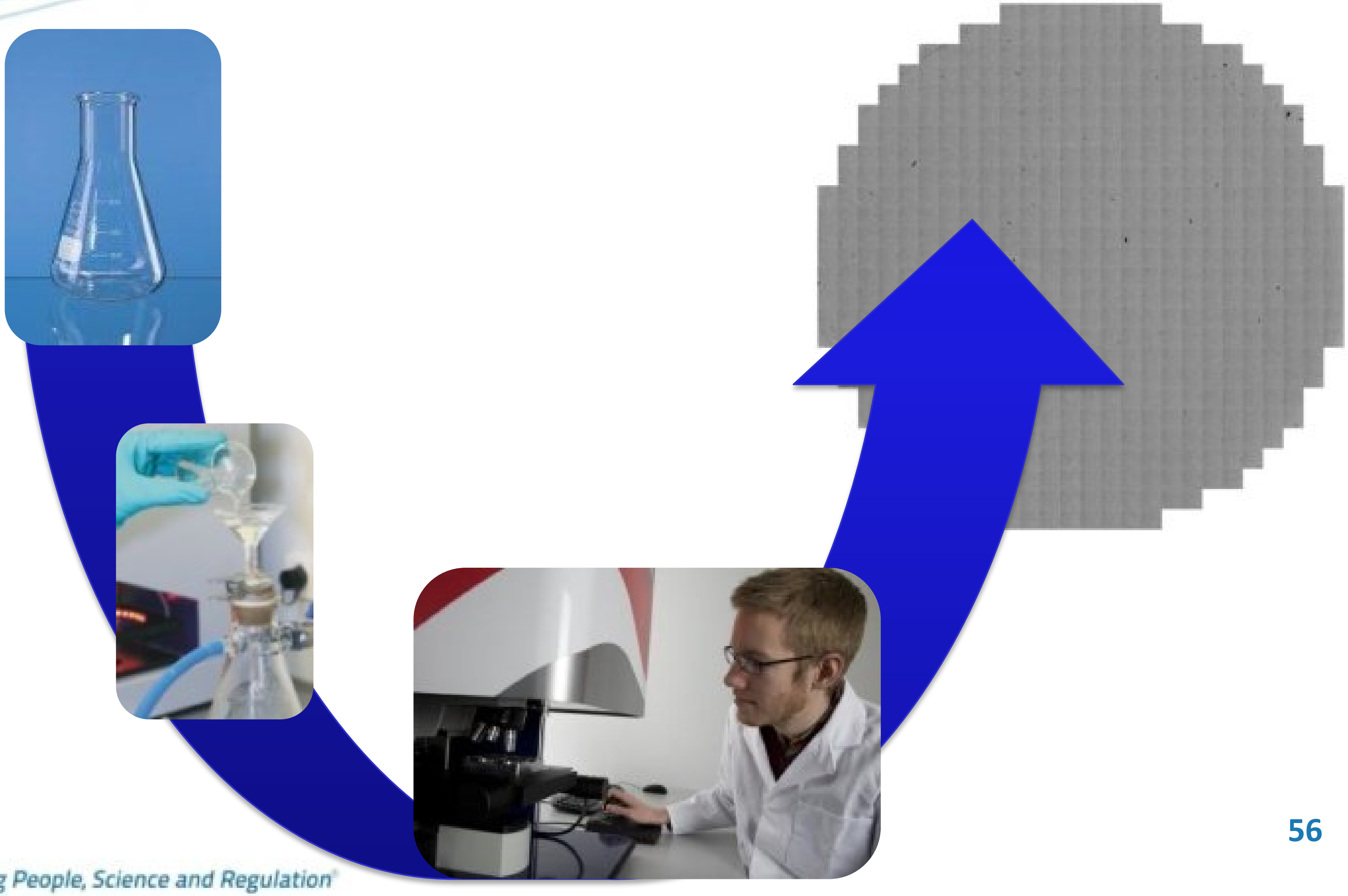
1. One special type of cellulose could be identified by the typical peak @ 1600
2. Database was built with suspect cellulose samples used in production
3. These Cellulose (contamination) fibers were found in smaller concentration in CIP rinses no fibers …were found in the process prior to filling!
4. Samples from API tanks and tubings showed this type of fiber.

→ API manufacturer used paper towels and introduced cellulose into the process

Control Your Packaging Material

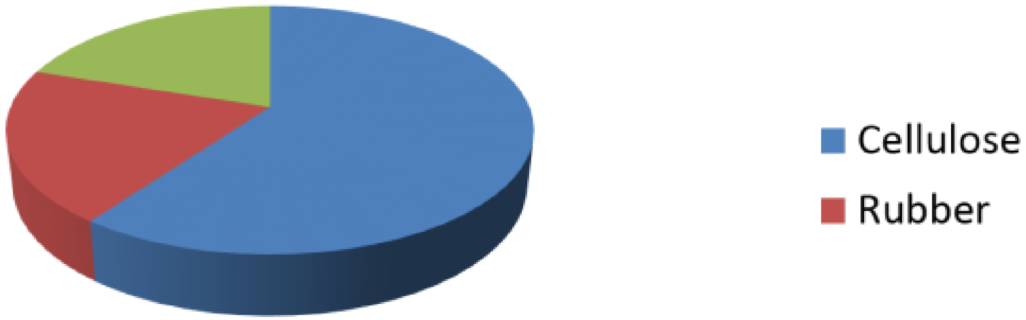


ISO 8871-3



Fibers and particles on rubber

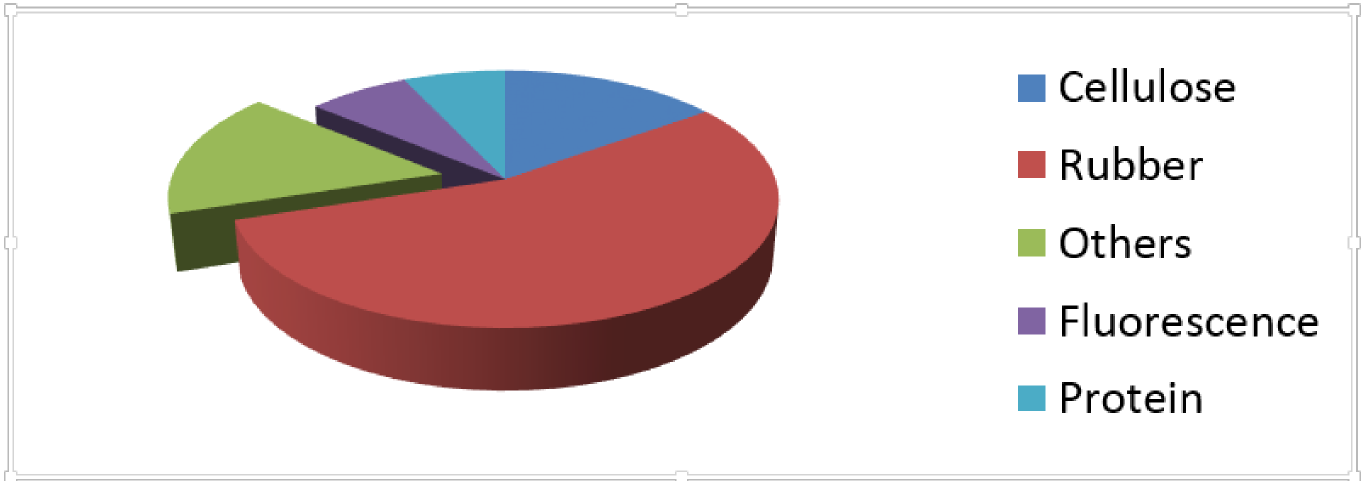
- 10 stoppers contaminated with fiber
  Cleaning following ISO 8871
- 51 particles > 50 µm found



Large scattering in particle number and composition can be observed in one batch and different bags

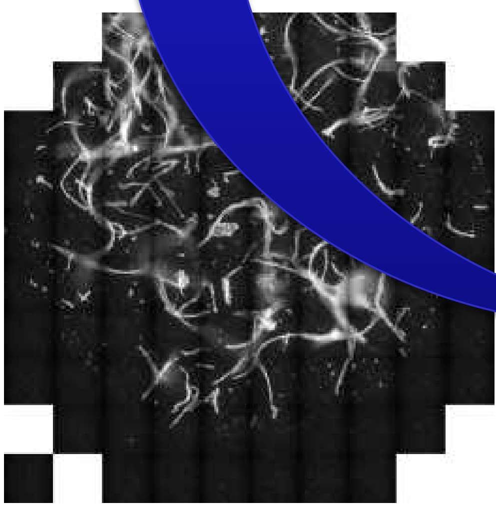
Fibers and particles on rubber

- 10 stoppers contaminated with particles Cleaning following ISO 8871
- 144 particles found > 50 µm

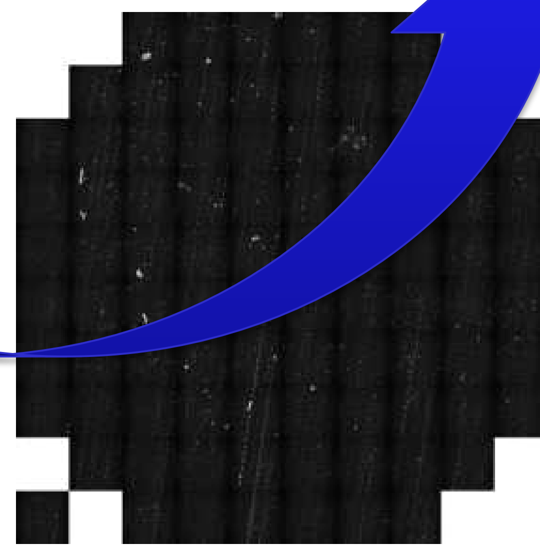


Bags

Stopper Bags have an impact or reflect stopper quality



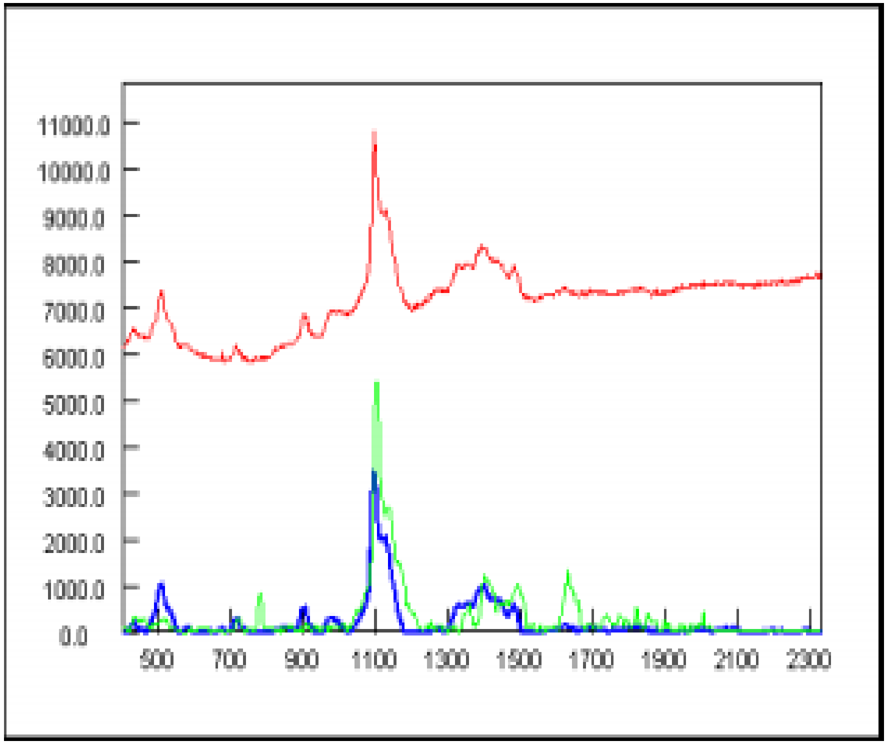
Fibres collected from one bag; 375 particles > 25 µm



Fibres collected from one bag; 45 particles > 25 µm

Test Procedure: Bag rinsed with 250 ml water / SDS, filtration, counting

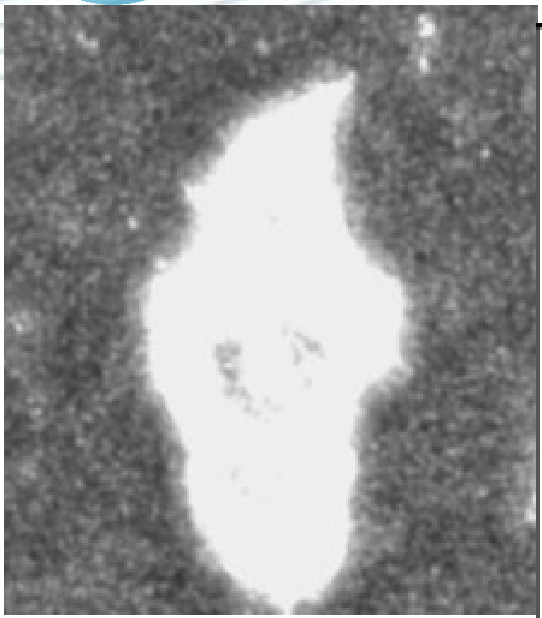
Fiber → Paper

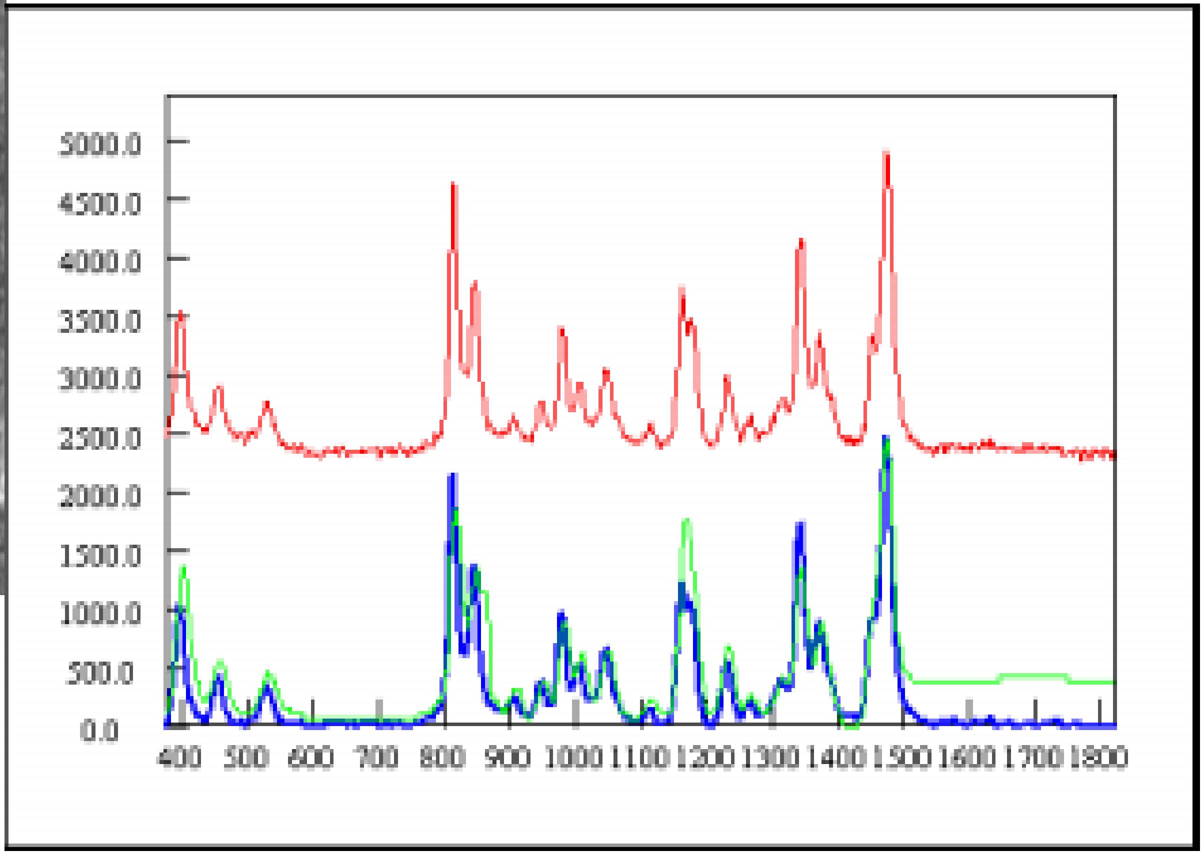


RESULT: **Cellulose [Paper]**

RANK: 882, S/N: 39.2

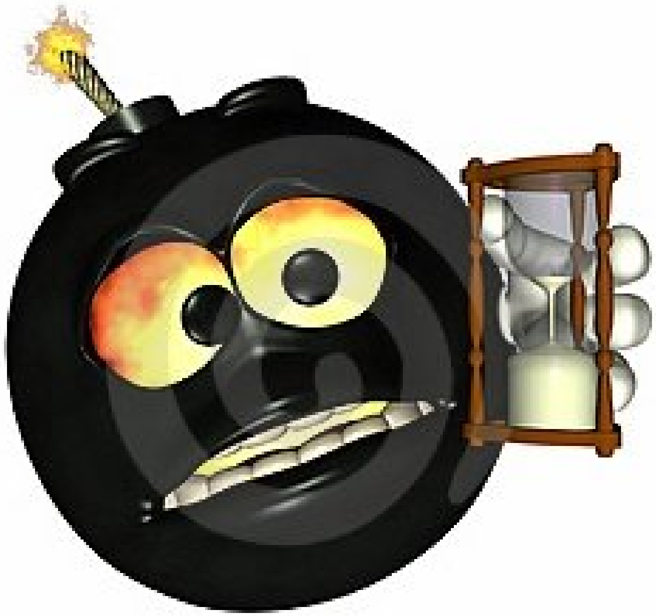
Particles → Rubber





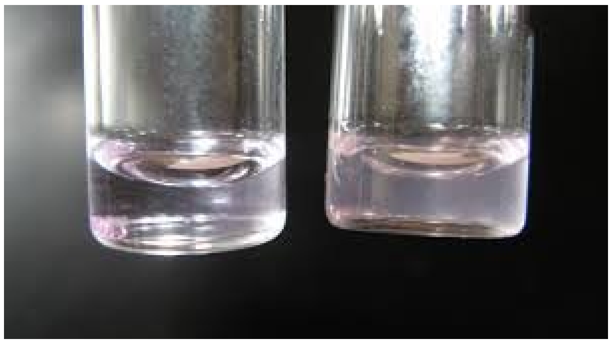
RESULT: **Rubber,** RANK: **974**, S/N: 30.3

Time bombs

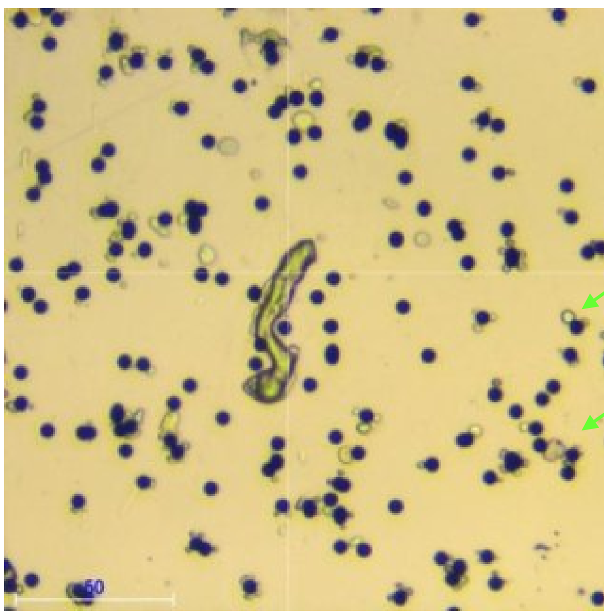


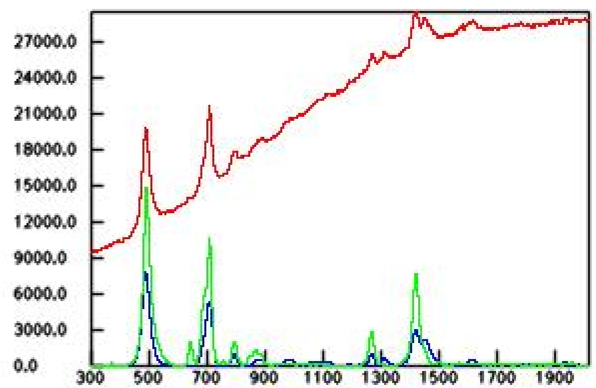
1. Increase of rejects with time
2. Chemical reactions taking some time
   - Silicone oil on stoppers: Agglomeration of Proteins
   - Coatings
   - Glass delamination

Turbidity / Haziness

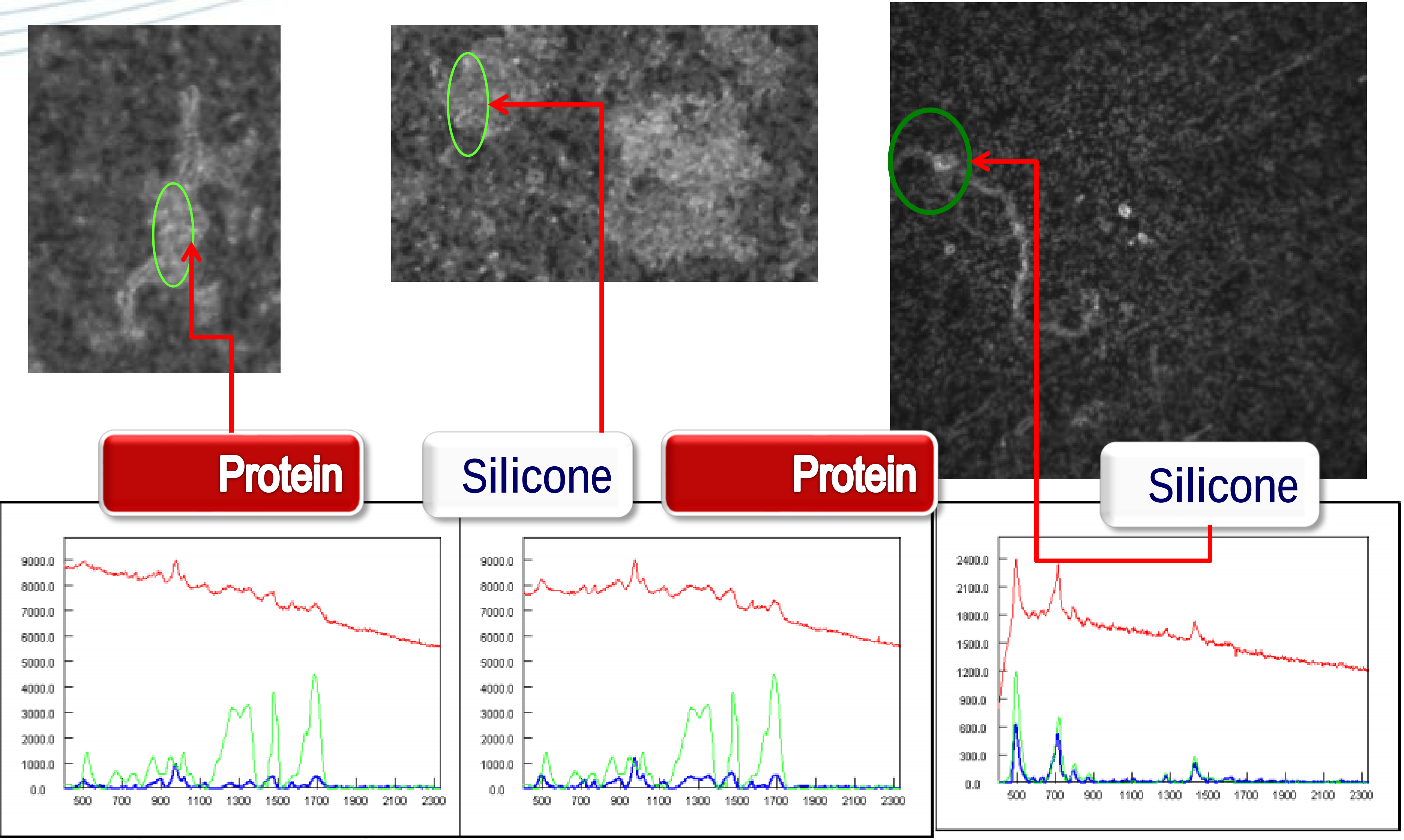


Observation of haziness and aggregates in a new a new batch after slight process change

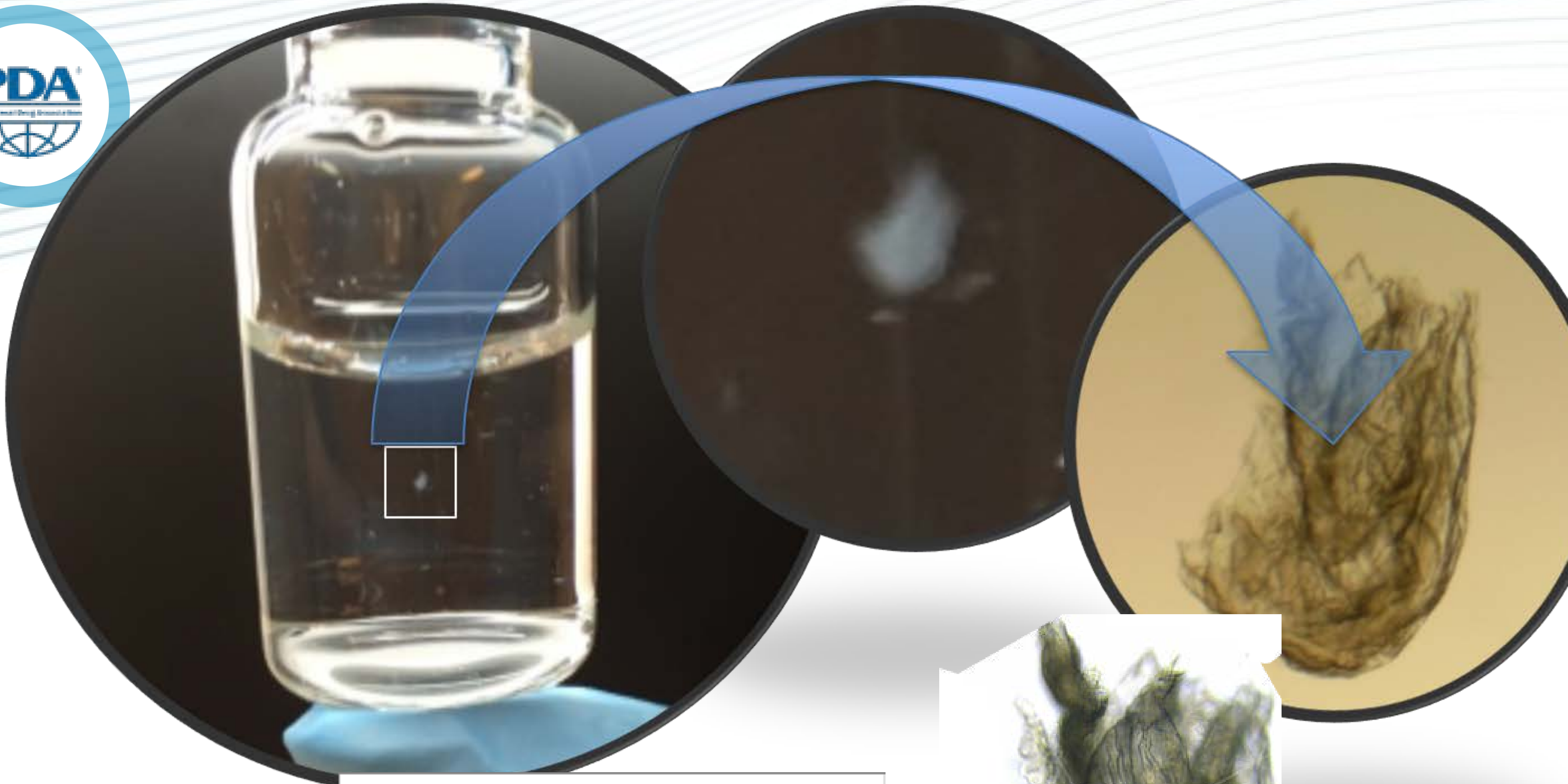


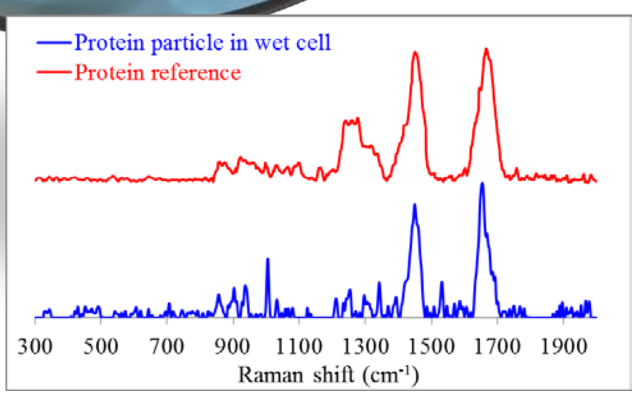


Protein and Silicone



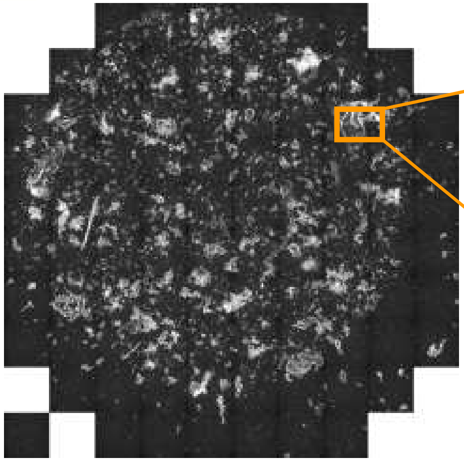
VISIBLE INHERENT PARTICLE

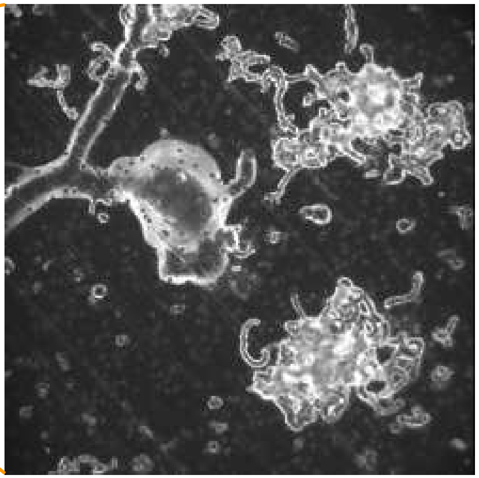



Protein particle in wet cell
Protein reference
300 500 700 900 1100 1300 1500 1700 1900
Raman shift (cm⁻¹)

Coating

Increasing number of rejects in visual inspection with time





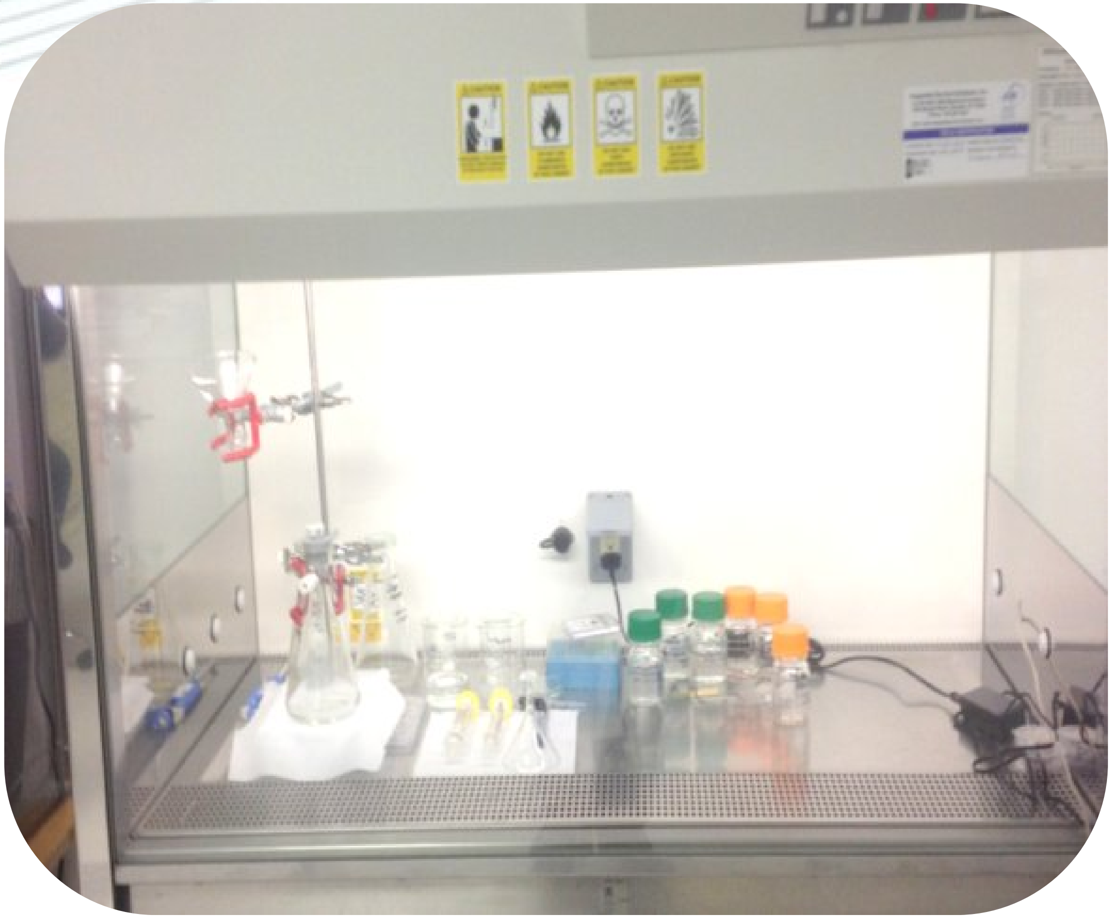
Size and Substance Distribution of Measured Particles					
Substance	Number	Size Distribution [µm]			
-	-	>=10	>=25	>=50	>=100
Proteine	6	0	0	1	5
Fluorescence	18	0	0	1	17
Coating	185	23	44	32	86
Skipped particles	3058	2142	657	232	27
All particles	3267	2165	701	266	135

Supplemental Testing or Inspection

Destructive reconstitution, dilution, transfer, clearing, solubilizing, filtration, screening, or sieving thatmallows a product to be visually examined or evaluated microscopically to determine the presence, type,and size of foreign particulate contamination present within the product, container, or device.

Destructive Inspection and Test Methods

- Reconstitution
- Filtration
- Clarification
- Transfer Dilution
- Sieve/Mesh
- Panning
- Rinse/Flush and Filtration



Isolation

Environmental Considerations

- Class 100 clean bench is essential
- „Ball-park“ clean rooms would be beneficial
- Cleaning is essential and system suitability tests (blanks) have to be taken
- Training and control is essential
- Benches, coats, sleeves, microscopes, equipment and water should be clean and non-shedding

Supplemental Testing or Inspection

5.3 DIP Product Formulations

Common inspection or testing approaches for DIP product formulations are listed in **Table 5.3-1.**

Table 5.3-1 Common Inspection or Testing Approaches for DIP Product Formulations

DIP Formulation Type	Common Destructive Methods Applied	Method #
Deeply colored solutions (opaque)	Filtration and microscopic exam in sub-visible and/or visible ranges	2
	Transfer and dilution (if required) in a verified clean transparent container followed by visual inspection	4
Emulsions	Clarification and visual Inspection	3
	Clarification → Filtration and microscopic exam in sub-visible and/or visible ranges	3
	Sieving	5
	Additional considerations: – Inspection of settled product with observation of bottom layer for dispersion of dense (sinking) metallic or glass particles	
Gels	Direct visual inspection (USP <790> with modifications, if needed, for increased illumination and dwell time)	USP790
	Dilution → Filtration and microscopic exam in sub-visible and/or visible ranges	4
Lyophilized (freeze-dried product)	Reconstitution and visual inspection	1
	Reconstitution → Filtration and microscopic exam in sub-visible and/or visible ranges	2
	<1 ml Small volumes reconstitution and pooling	4
Powders, API	Reconstitution and visual inspection	1

thank you for your attention !