# Particle Characterization and Identification

Markus Lankers, PhD
May 2019

# 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***

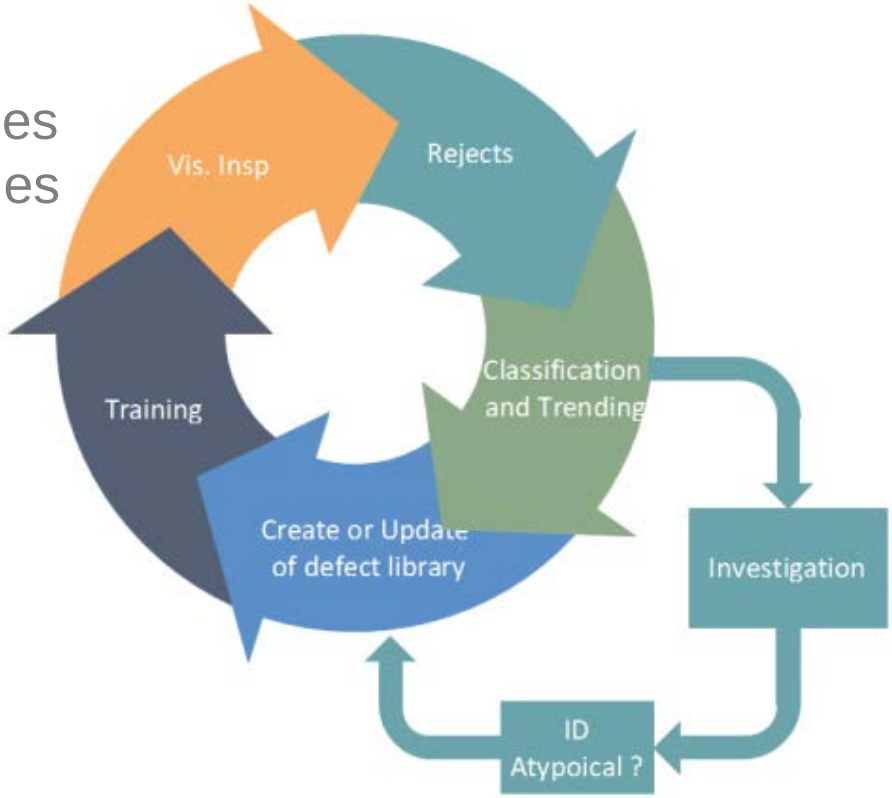
# Reasons for Particle ID



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.

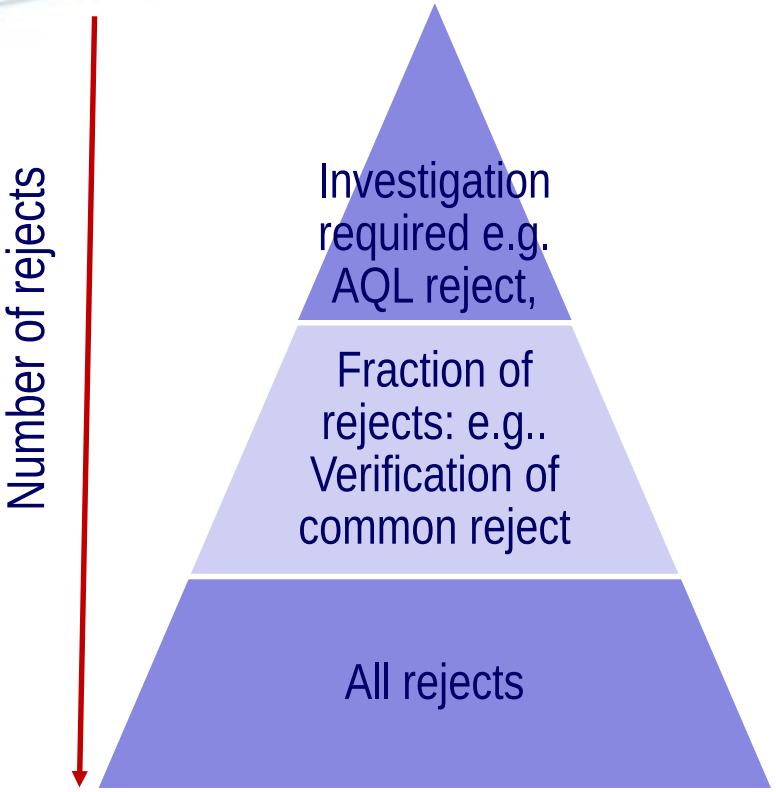
## Level Three: Spectroscopic or other fingerprint ID

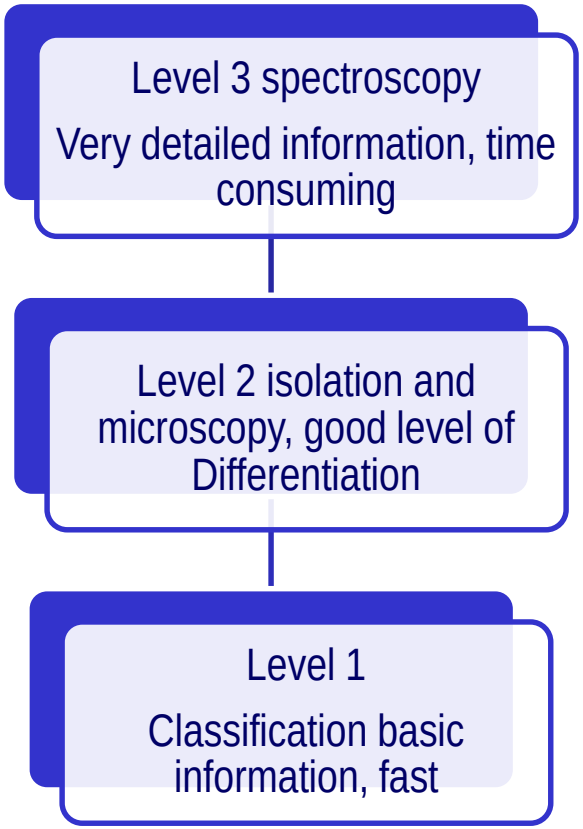
- FTIR, Raman, Elemental, Mass Spec, etc.

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



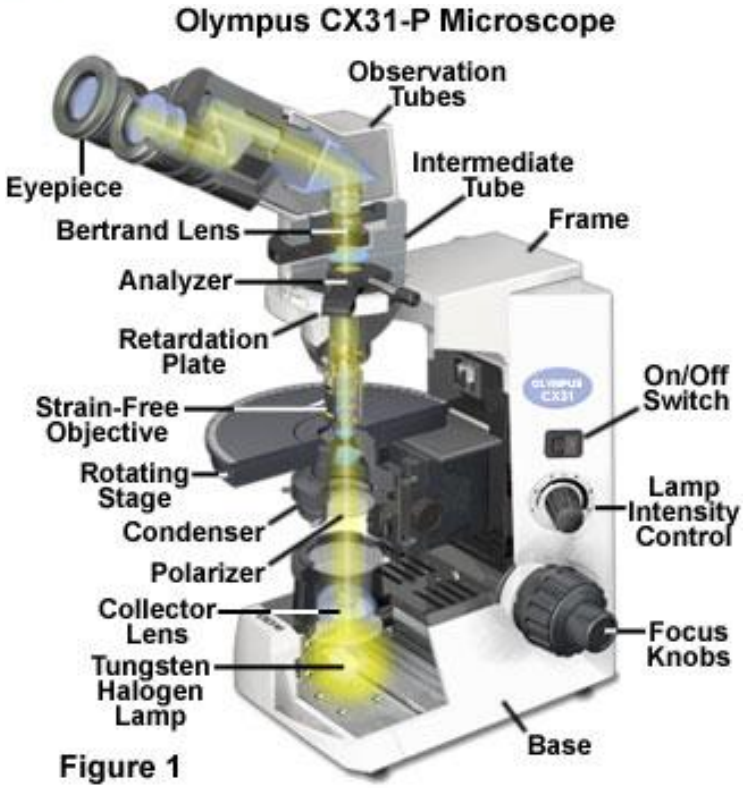



Figure 1

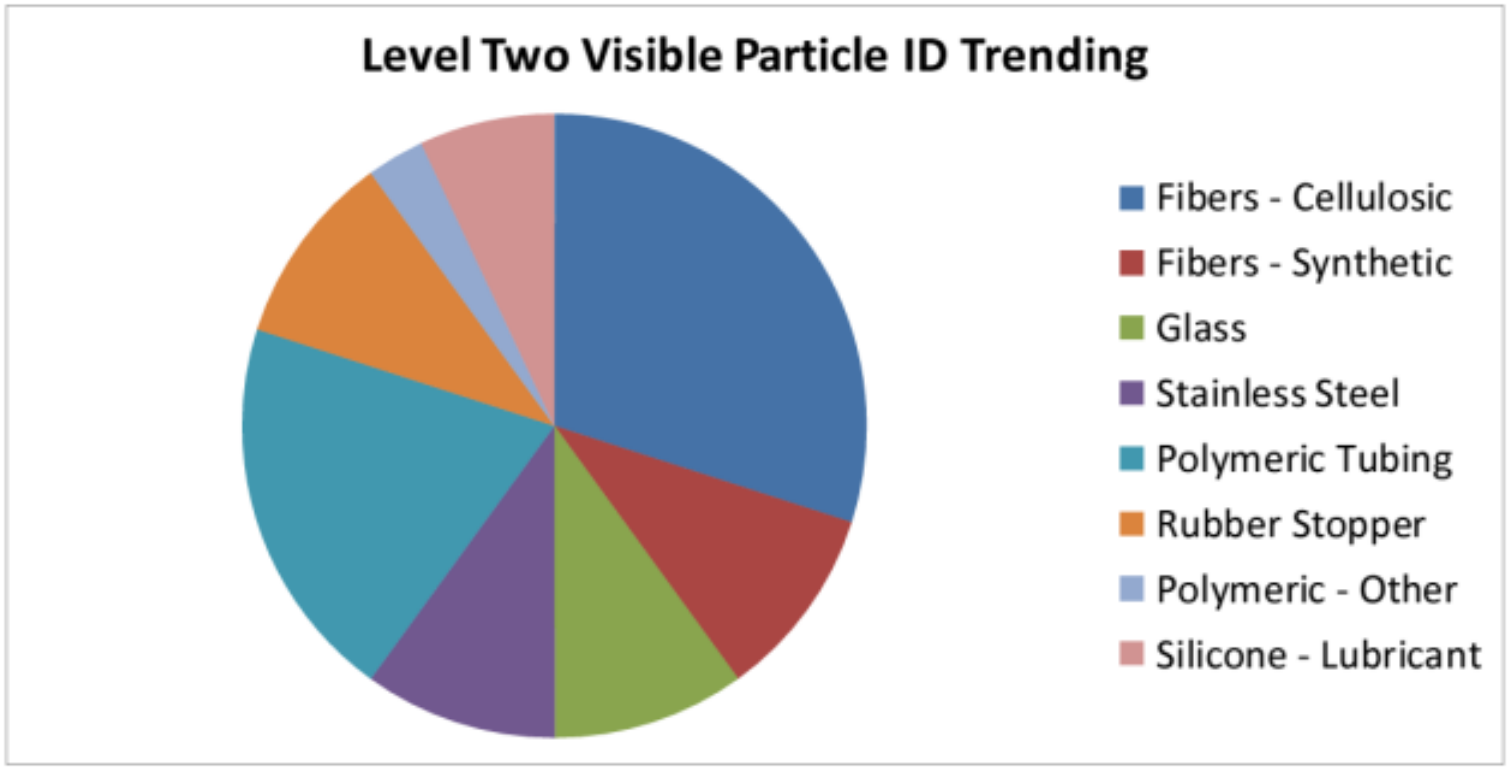
# Microscopic information – Level 2



| 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 | [ ] |

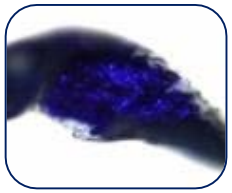
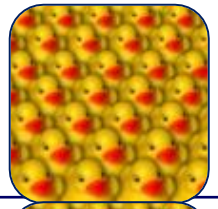
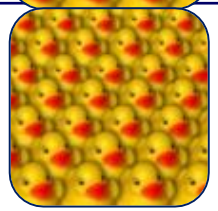
| Level II Category | Select | Level II Category | Select |
|---|---|---|---|
| Glass | [ ] | Polymeric | [ ] |
| Metallic | [ ] | Rubber Stopper | [ ] |
| Fiber | [ ] | Semi-Solid - Silicone | [ ] |
| Fiber - Natural | [ ] | Possible Inherent API | [ ] |
| Fiber - Synthetic | [ ] | Possible Extrinsic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

# 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



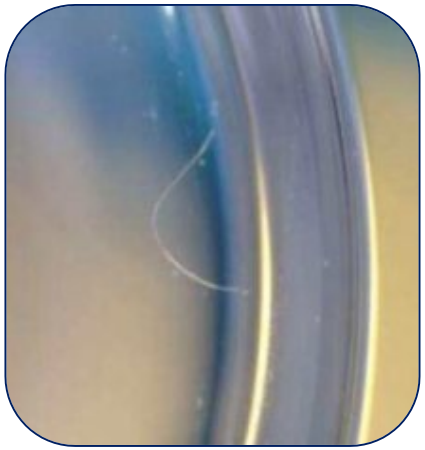
| method | meaning | time/particle |
|---|---|---|
| PLM (polarized light microscopy) | color + shape e.g.: black fibres  | 1-5 min |
| SEM/EDS analysis | > 5µm Elements   | 20-180 min |
| IR – microscopy | > 50 µm Structure   | 20-180 min |
| RAMAN - microscopy | > 0.5 µm Structure   | 20-180 min |

# Fiber – Level 1



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

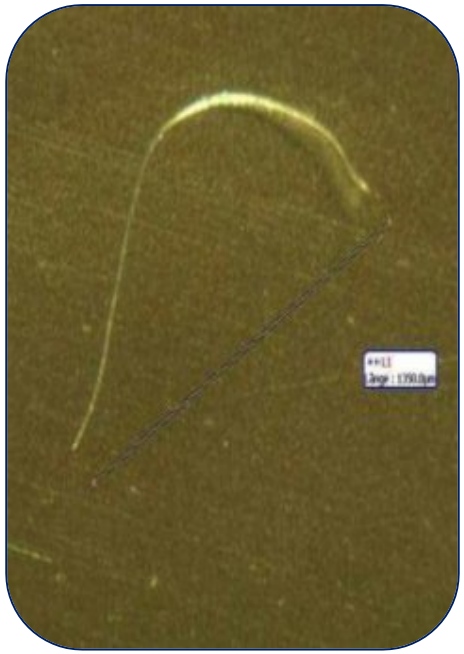
| Category | *Select* |
|---|---|
| Polymeric-like | [ ] |
| Dark Particle | [ ] |
| Light Particle | [ x ] |



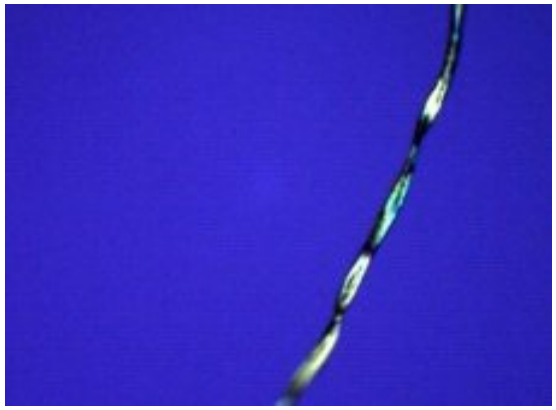
- Fibers can be easily classified. Might be sufficient for trending
- Further classification of fibers can be preformed in situ with an inverted microscope due to morphology and texture

# Fiber-Level 2: Isolation Microscopy[17]

- 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



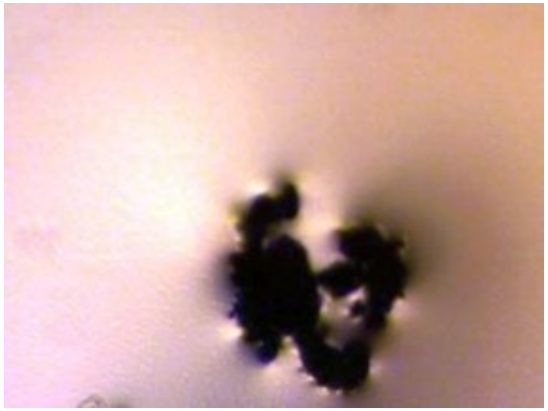
Level 2

Level 1

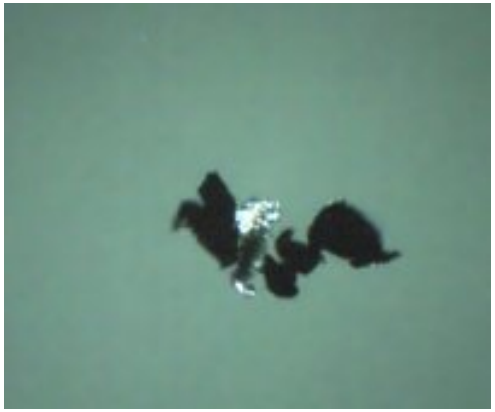
# Metal particle Level 1 and Level 2



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



Level 1



Level 2

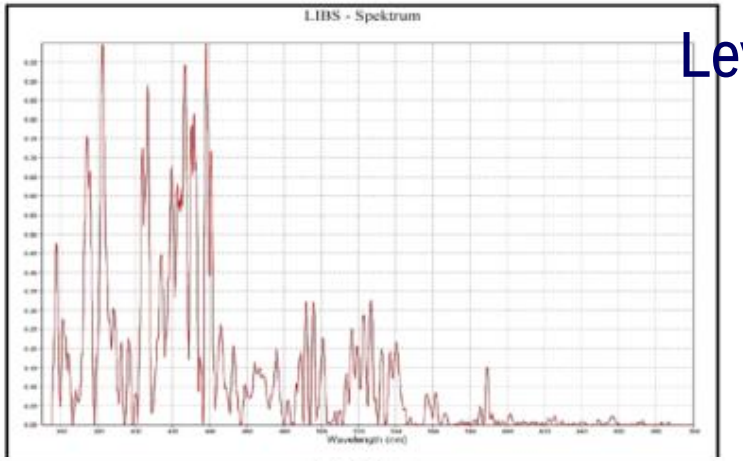
Level 2

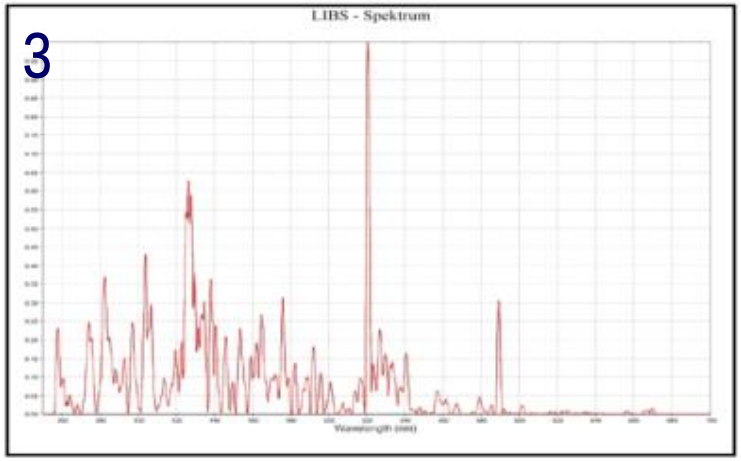
# Spectroscopy on metals – Level 3

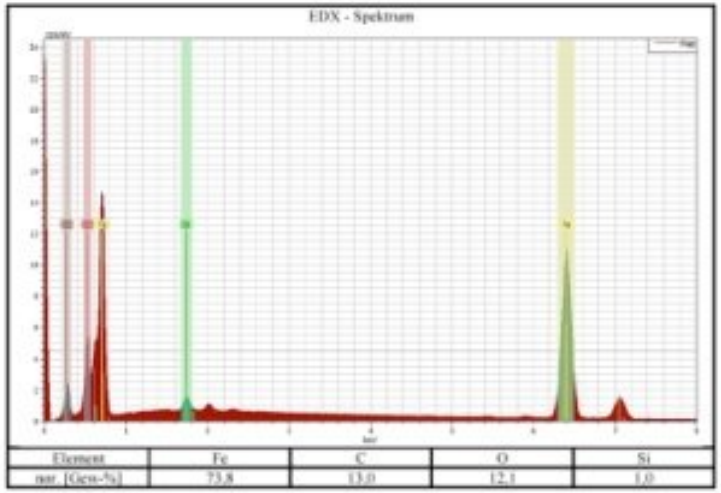


Spectroscopy gives more detailed information on the kind of steel e.g. low alloyed vs high alloyed steel which might be needed for root cause investigation

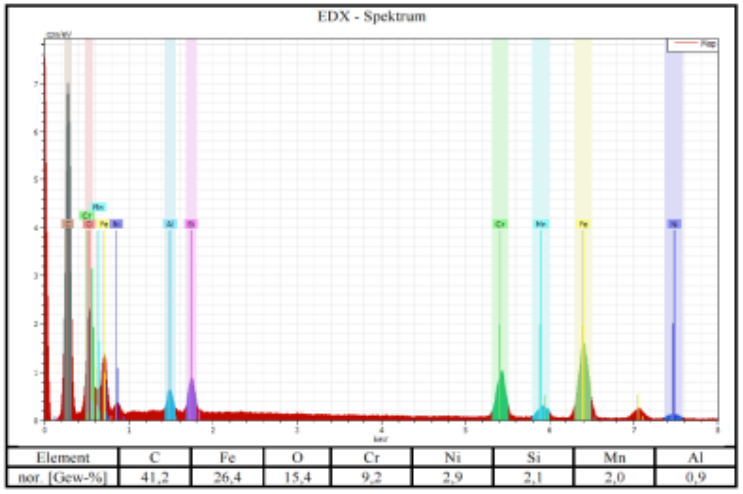
Level 3



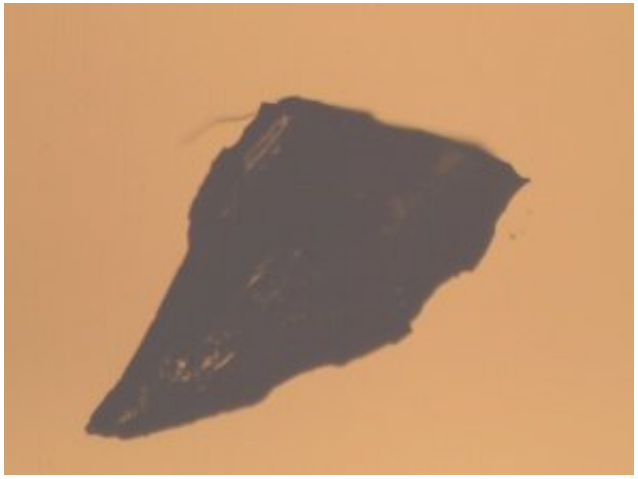

| Element | Fe | C | O | Si |
|---|---|---|---|---|
| nor. [Gew-%] | 73,8 | 13,0 | 12,1 | 1,0 |



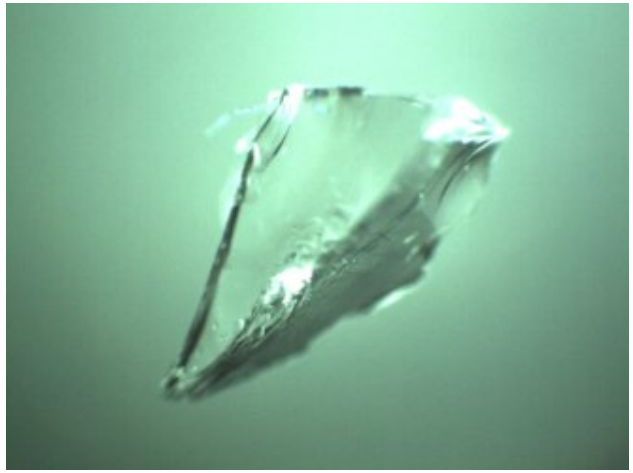

| Element | C | Fe | O | Cr | Ni | Si | Mn | Al |
|---|---|---|---|---|---|---|---|---|
| nor. [Gew-%] | 41,2 | 26,4 | 15,4 | 9,2 | 2,9 | 2,1 | 2,0 | 0,9 |

# 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



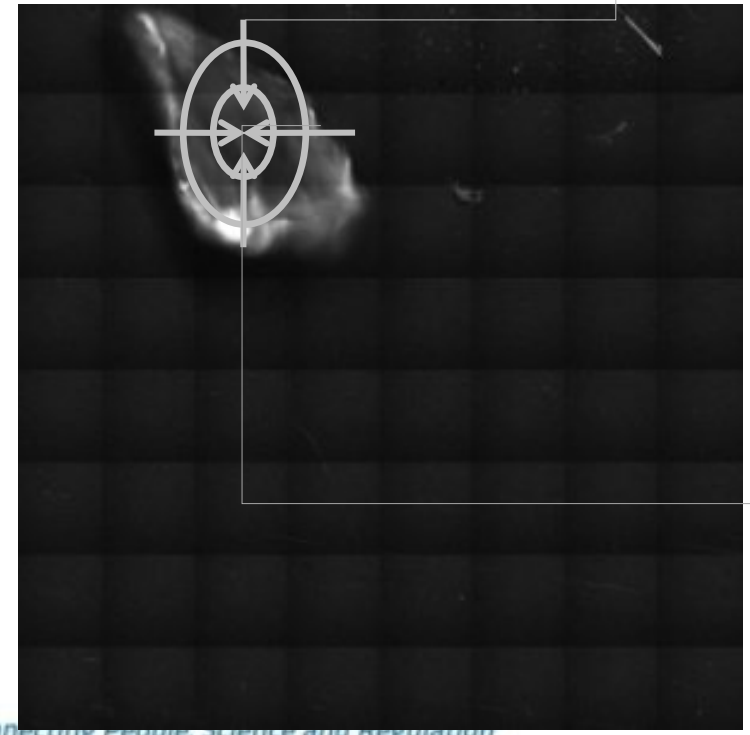
Level 1



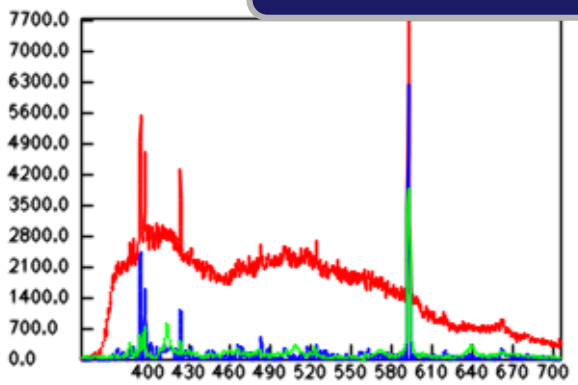
Level 2

# Glass – Level 3



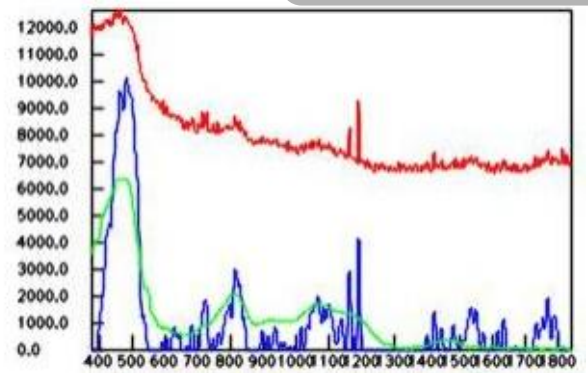


LIBS



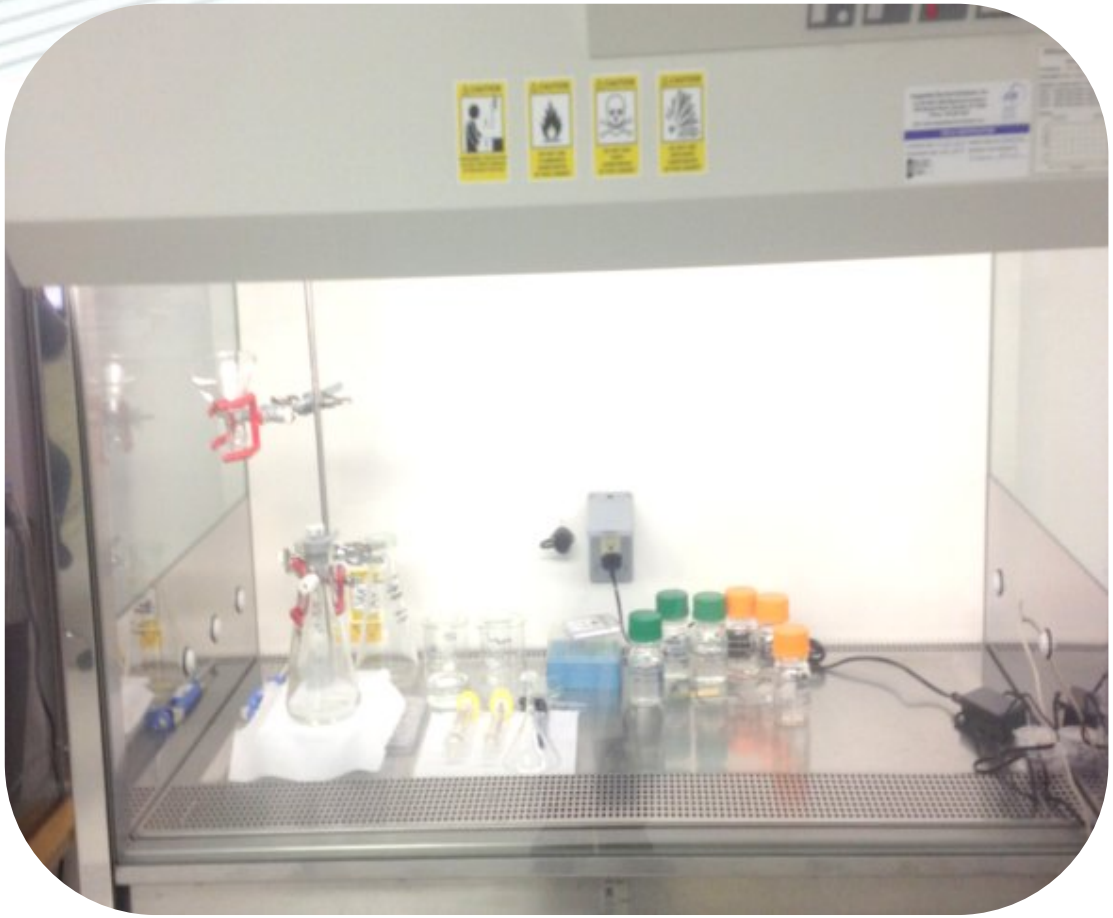

Si, Na, Al (Fiolax)

Raman




Glass

# Particle Isolation



# Isolation

# Environmental Considerations

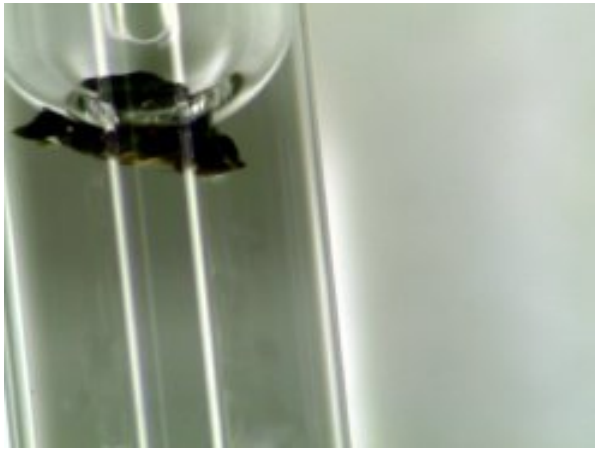
- Class 100 clean bench is essential
- „Ball-park“ clean rooms would be beneficial
- Cleaning is essential and system suitability tests (blanks) have to be taken
- Training and control is essential
- Benches, coats, sleeves, microscopes, equipment and  water should be clean and non-shedding

# Isolation and transportation

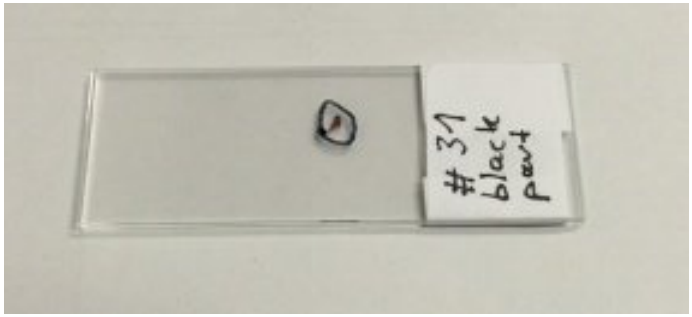


Tungsten needles for particle picking

Capillary trapping



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 ?

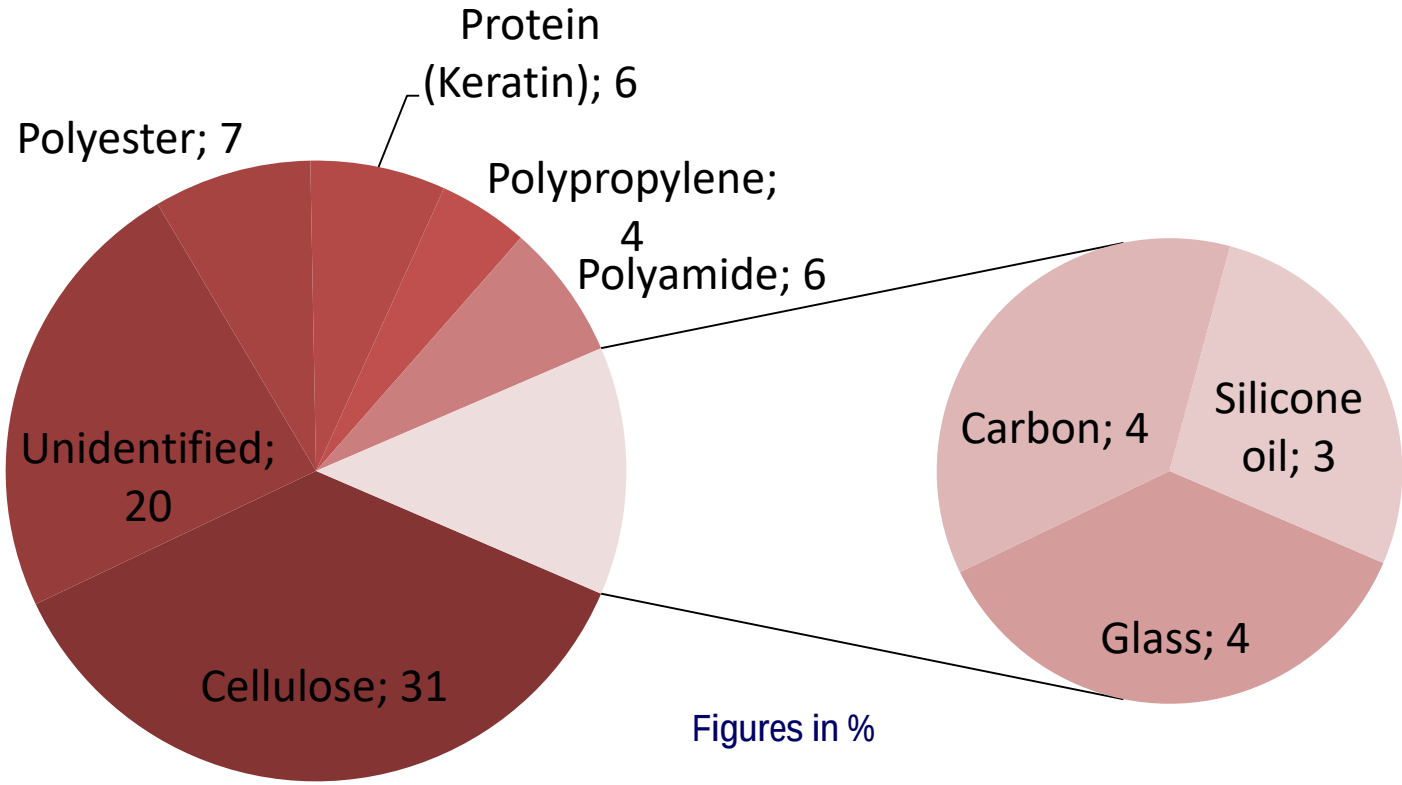


- personnel

- Garnement
- Water
- container

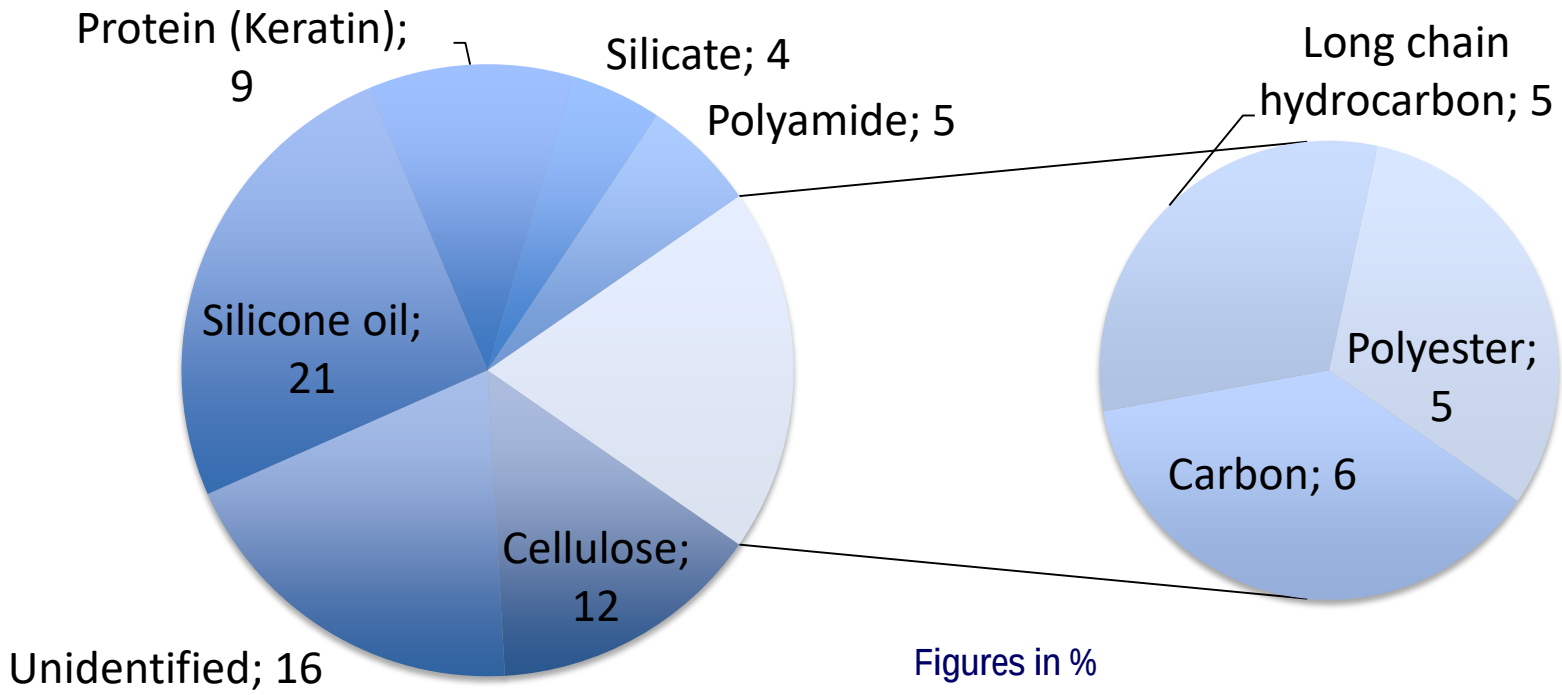
- Process / Production Equipment e.g.: rubber
- Cleaning process

# Visible




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

# Sub-visible




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



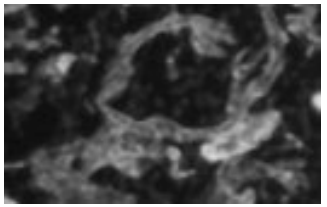
- Glass: fibres and particles
  - Source: Primary packaging
  - But also glassfibers and hollow glass fibres (filter material)

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

# Top Ten in more detail



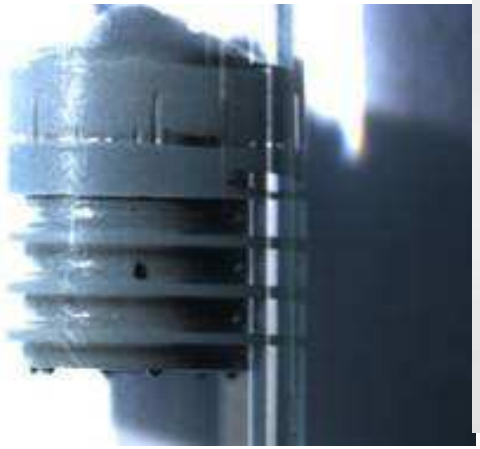
- Polyester: fibres and particles
  - Source: Cleanroom clothes and defect filter
- Protein: mostly flakes
  - Source human dust, protein particles from protein solution
- Silicone oil: compact particles
  - Source: sealings, siliconisation

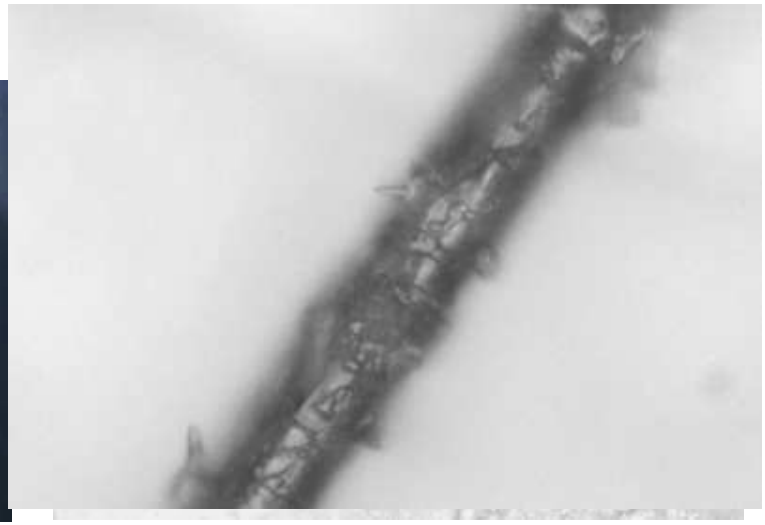


# Plunger/stopper related defects



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



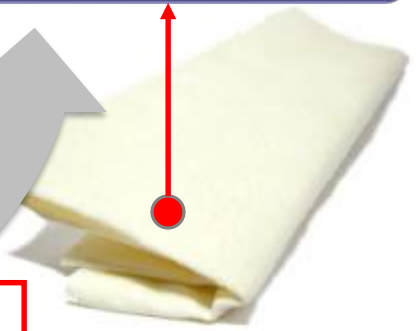


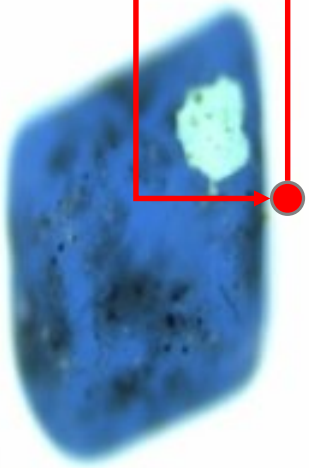
# Root cause analysis

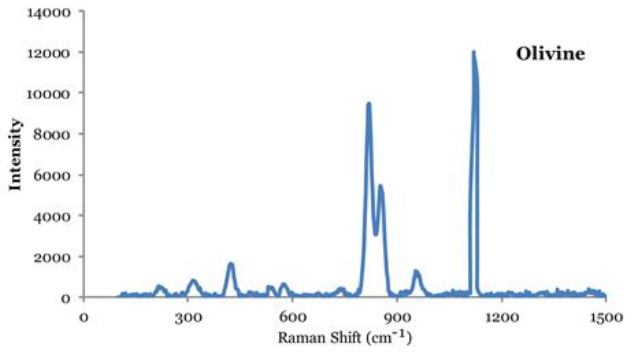


particle !

source ?





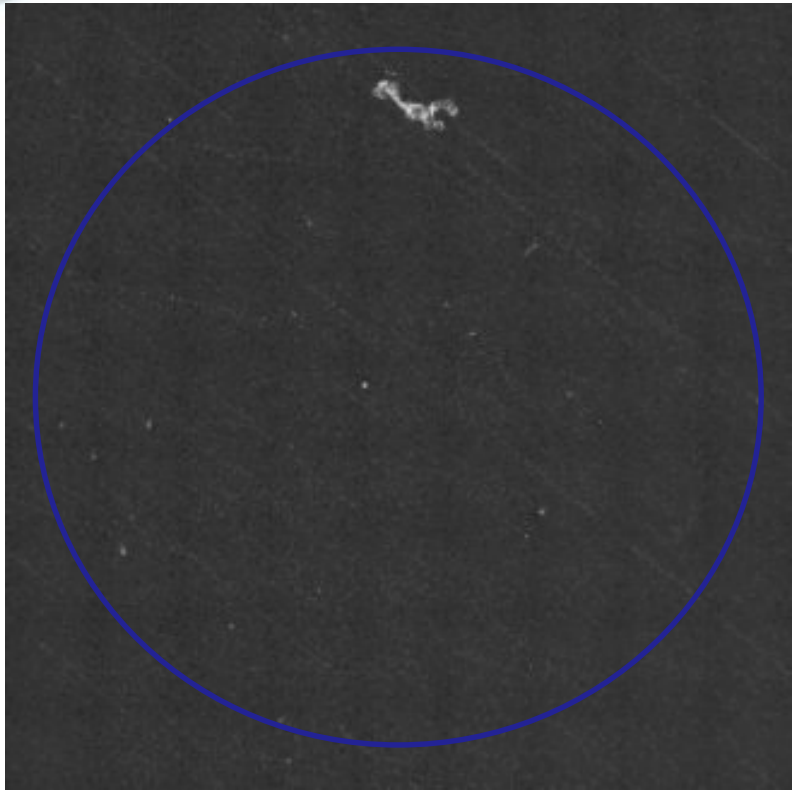


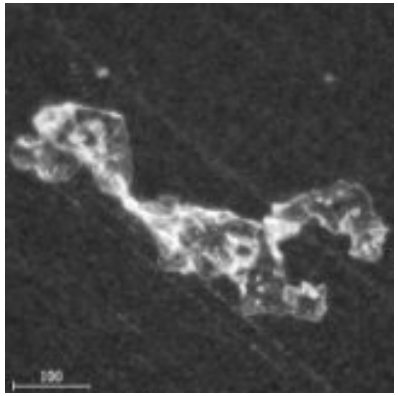

# Particle investigation

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

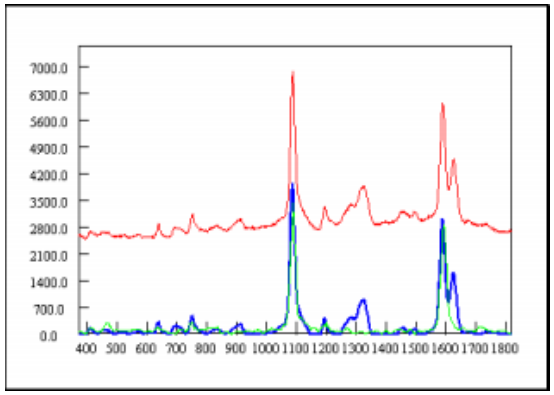
# 1. Particle in the vial

# 3. Particle Imaging + raman.ID





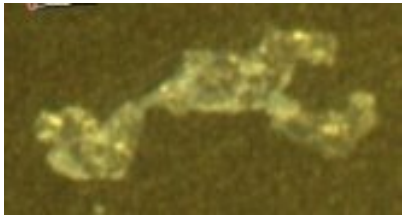
L=505.0 µm
w=202.6 µm
E=2.49
R=0.3071

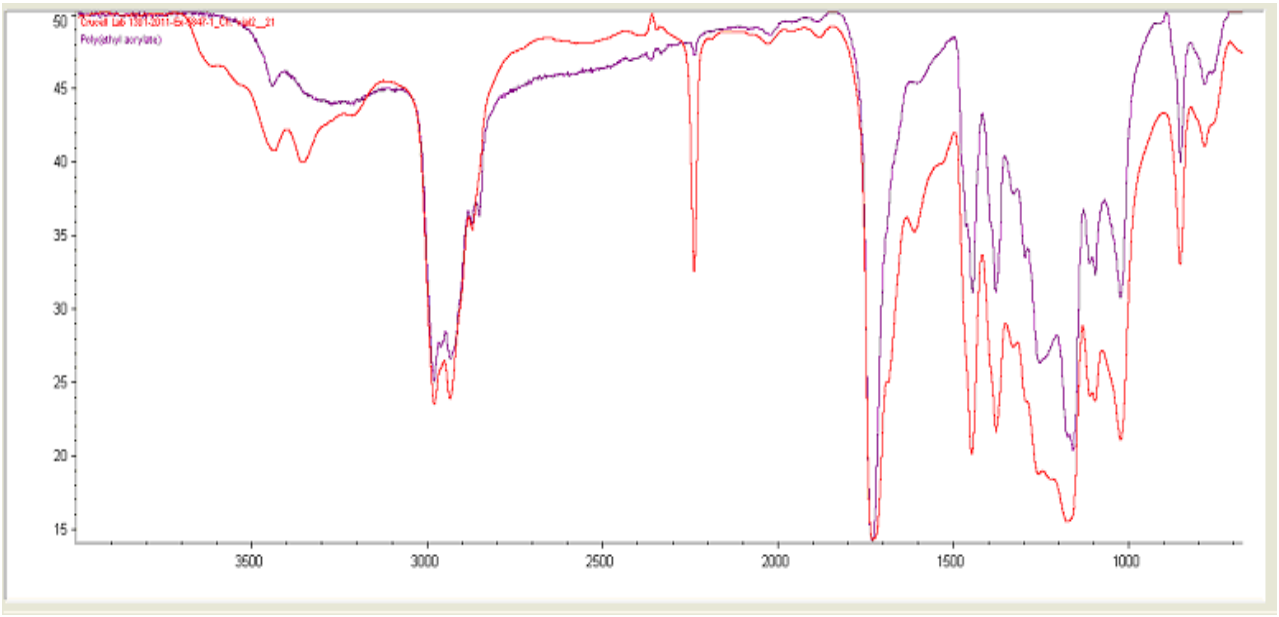



Raman.ID: Polyethylene-terephtalate, PET

Rank: 887

# 4. Verification FT-IR → PET

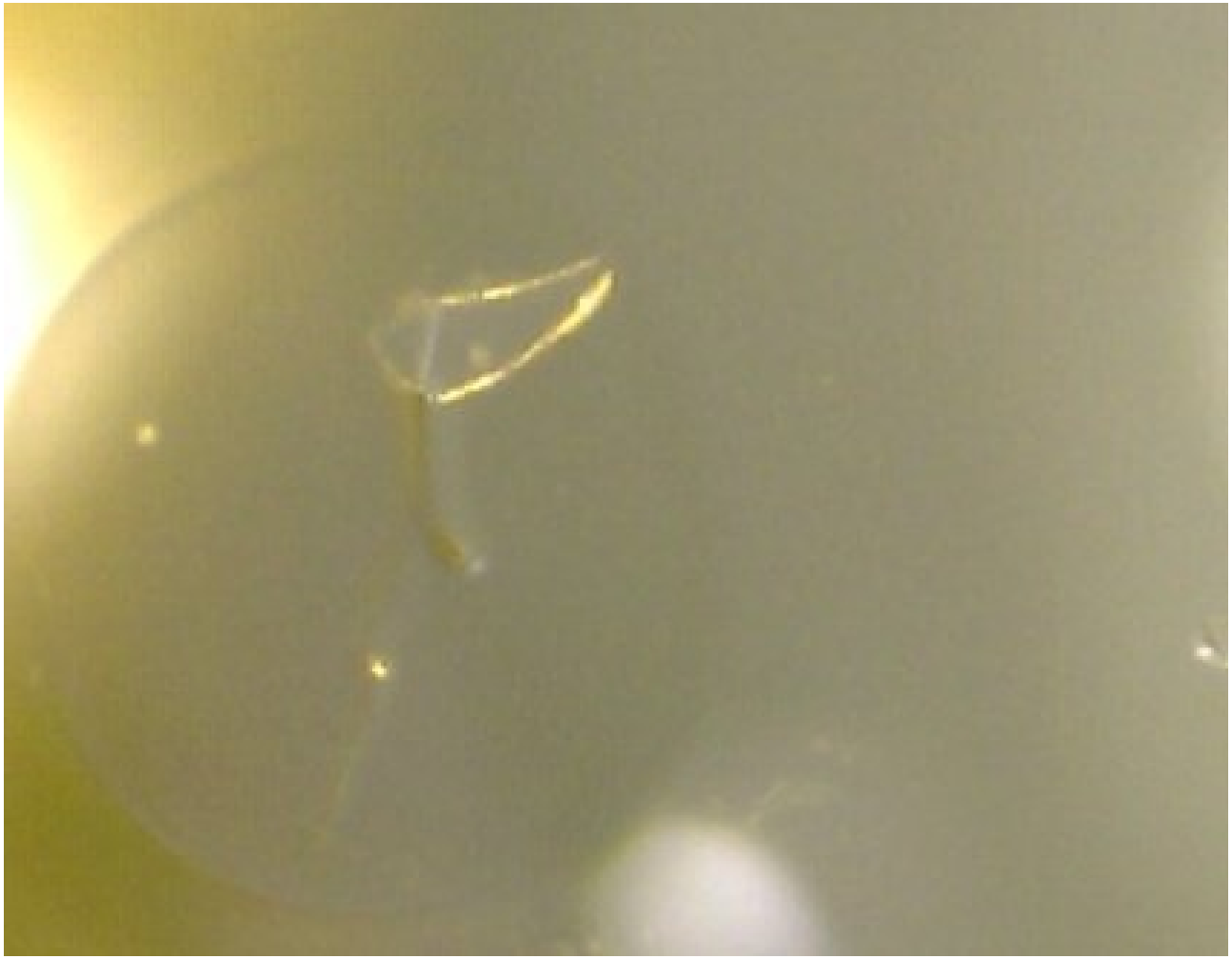




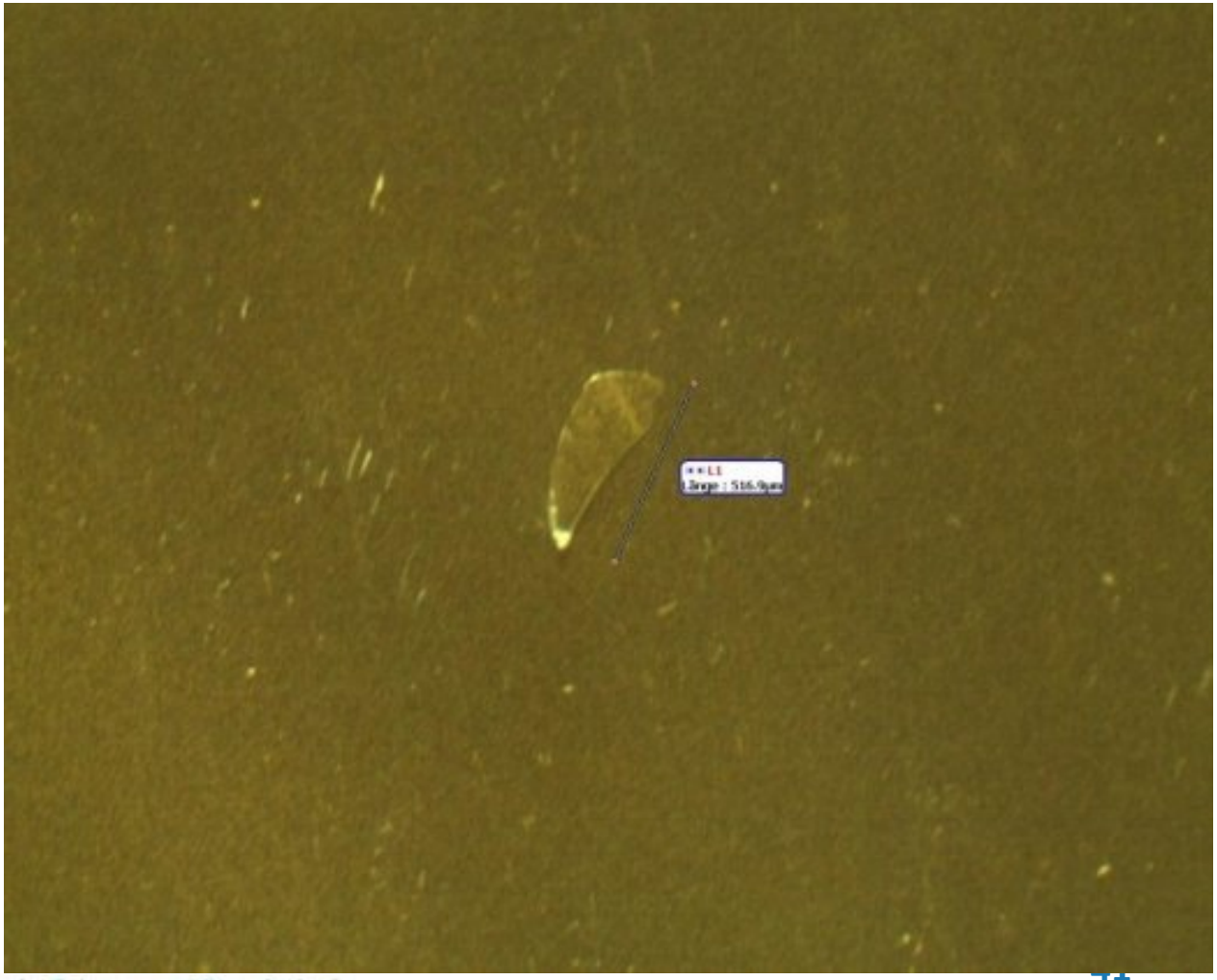

**Polyethylene Terephtalate, PET**

**76.16% matching**

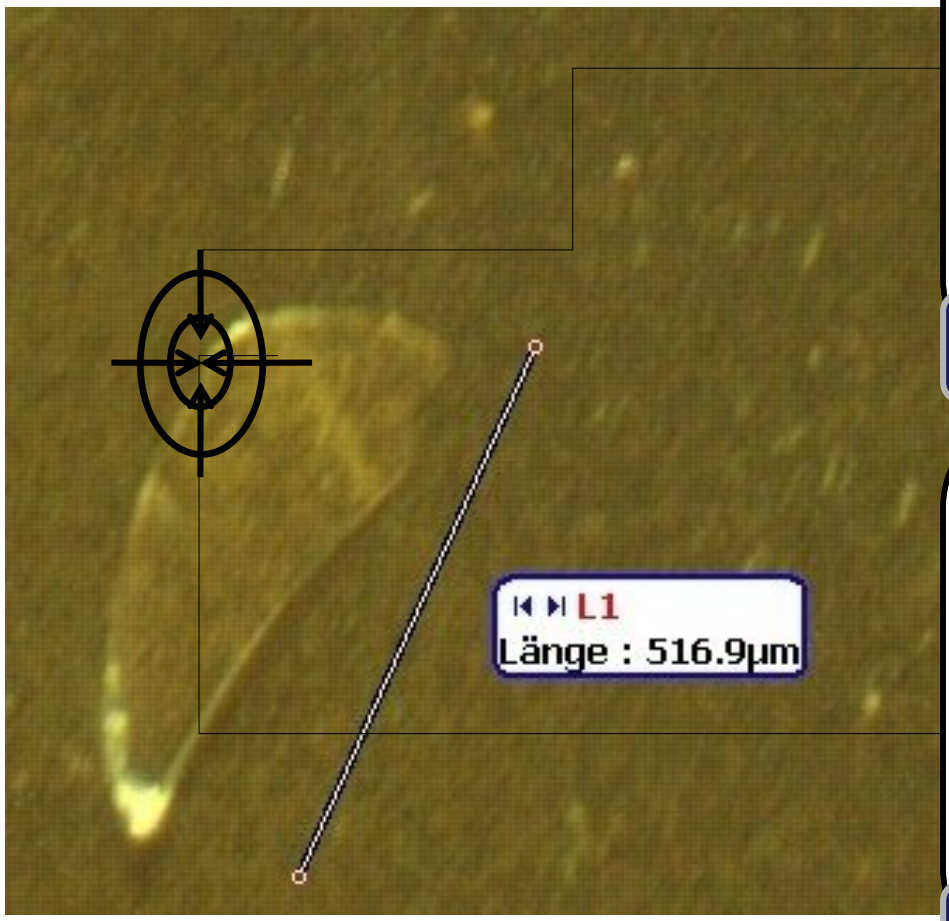
# Visible Inspection: Particle Reject II



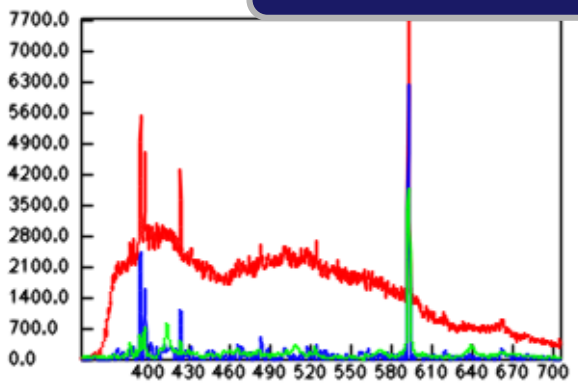
# Sample prep. + Documentation



# Glass particles

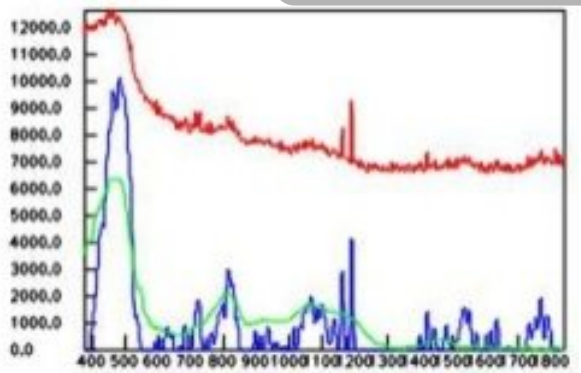



LIBS




Si, Na, Al (Fiolax)

Raman




Glass

# Report II - Summary

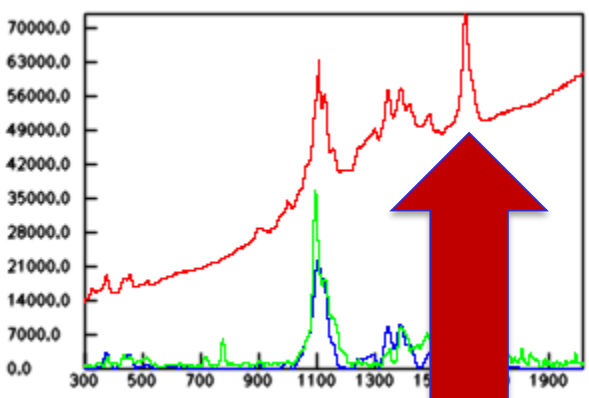
1. The largest Particle we observed in the vial (516 µm) was identified by means of Raman as well as FT-IR spectroscopy as glass.
2. This finding was also confirmed by LIBS Laser induced brakdown spectroscopy. The particle matched with Fiolax (Al is a marker).

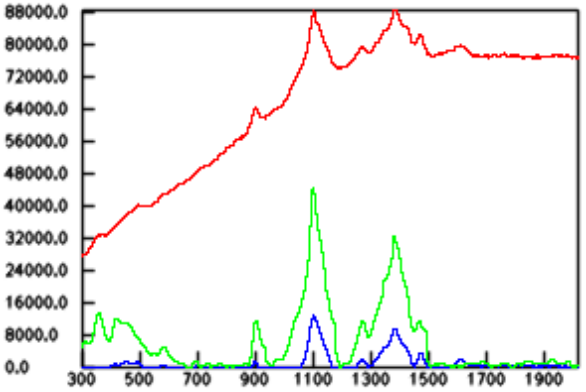
# CELLULOSE SOURCE

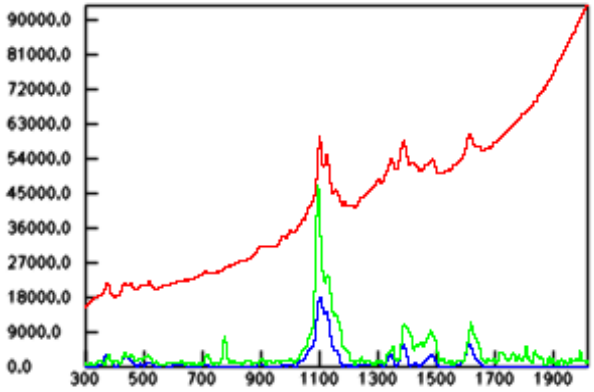
# Example Cellulose Source

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….

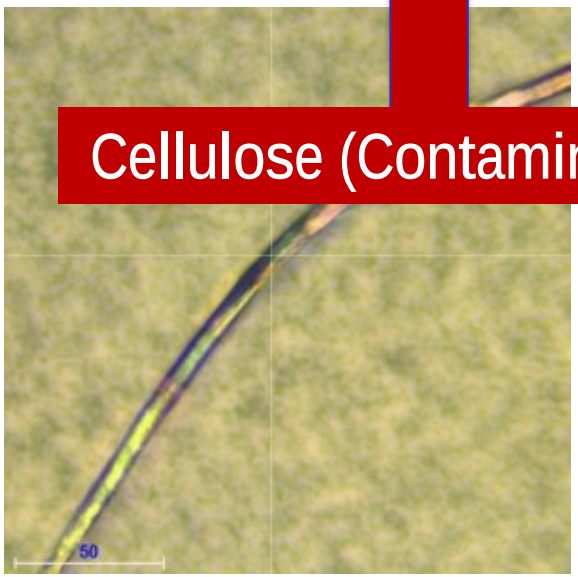
# Several cellulose fibers were found

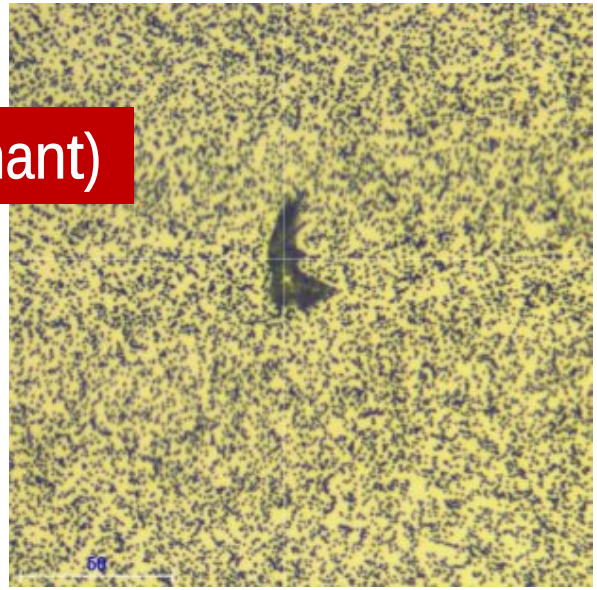


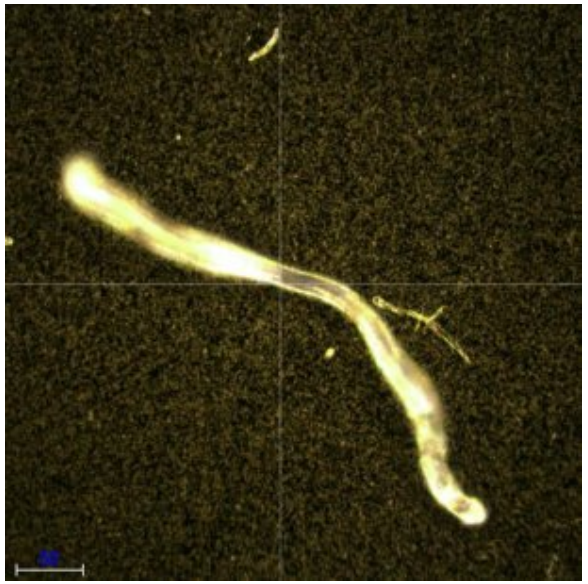




Cellulose (Contaminant)



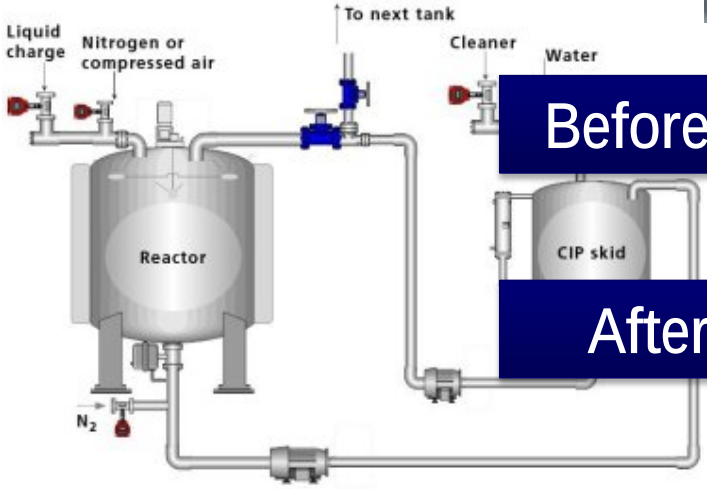



# Samples from the filling were taken

Stopper Sample

Before Tubing Rinse Sample

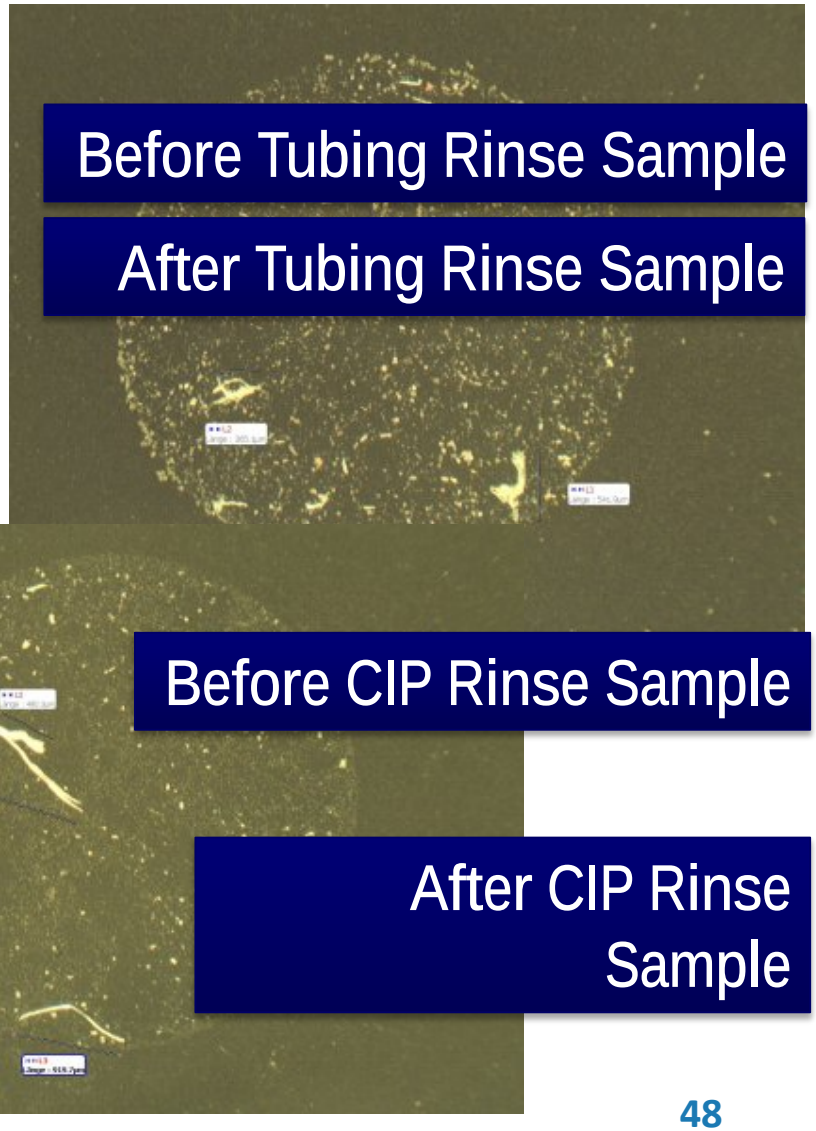
After Tubing Rinse Sample




Before CIP Rinse Sample

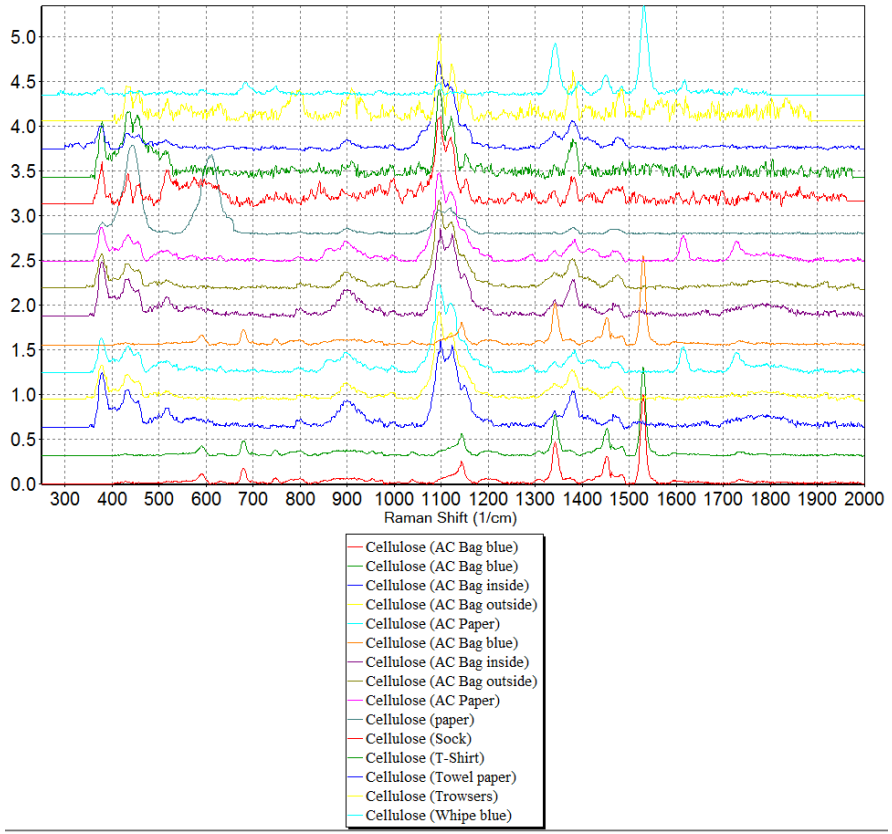
After CIP Rinse Sample

# Samples from the process were taken




# Database with filling line related materials was built

1.



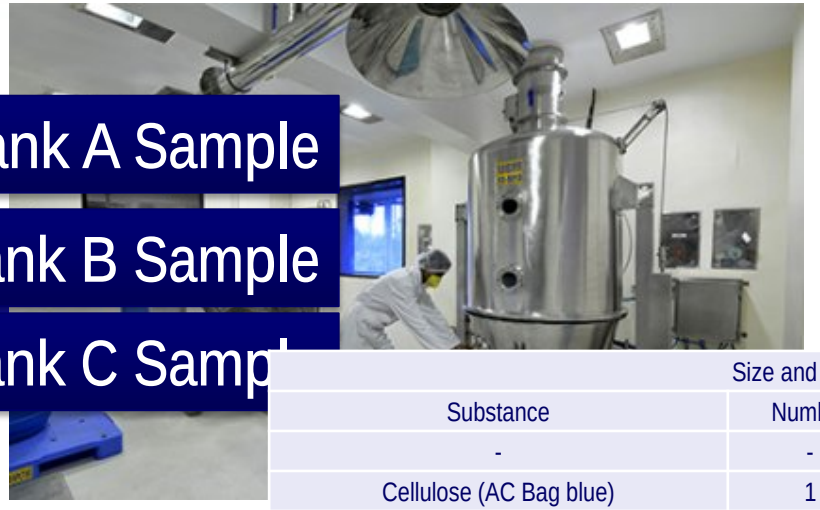

No Cellulose (Contaminant) !

# 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)



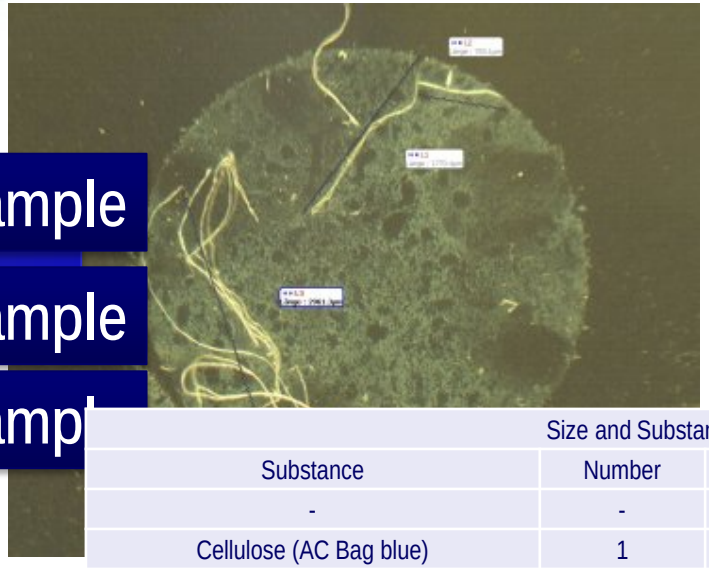
Tank A Sample

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.



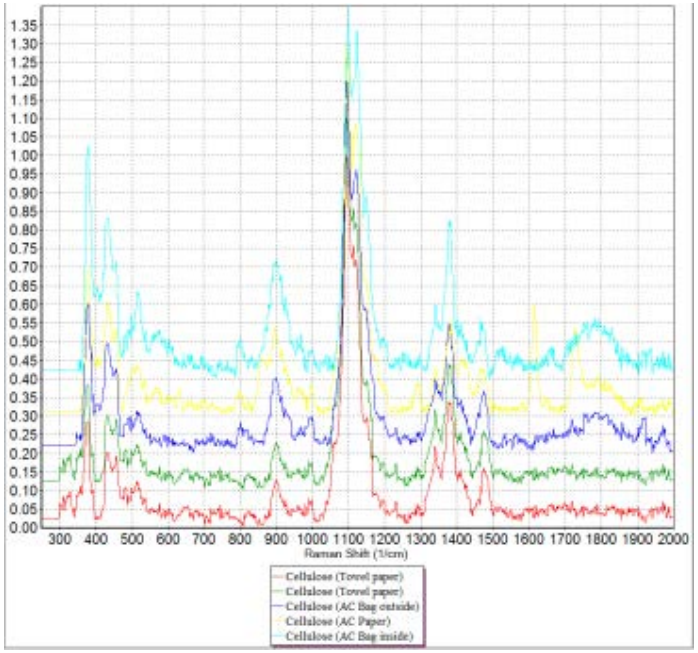
Tank A Sample

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



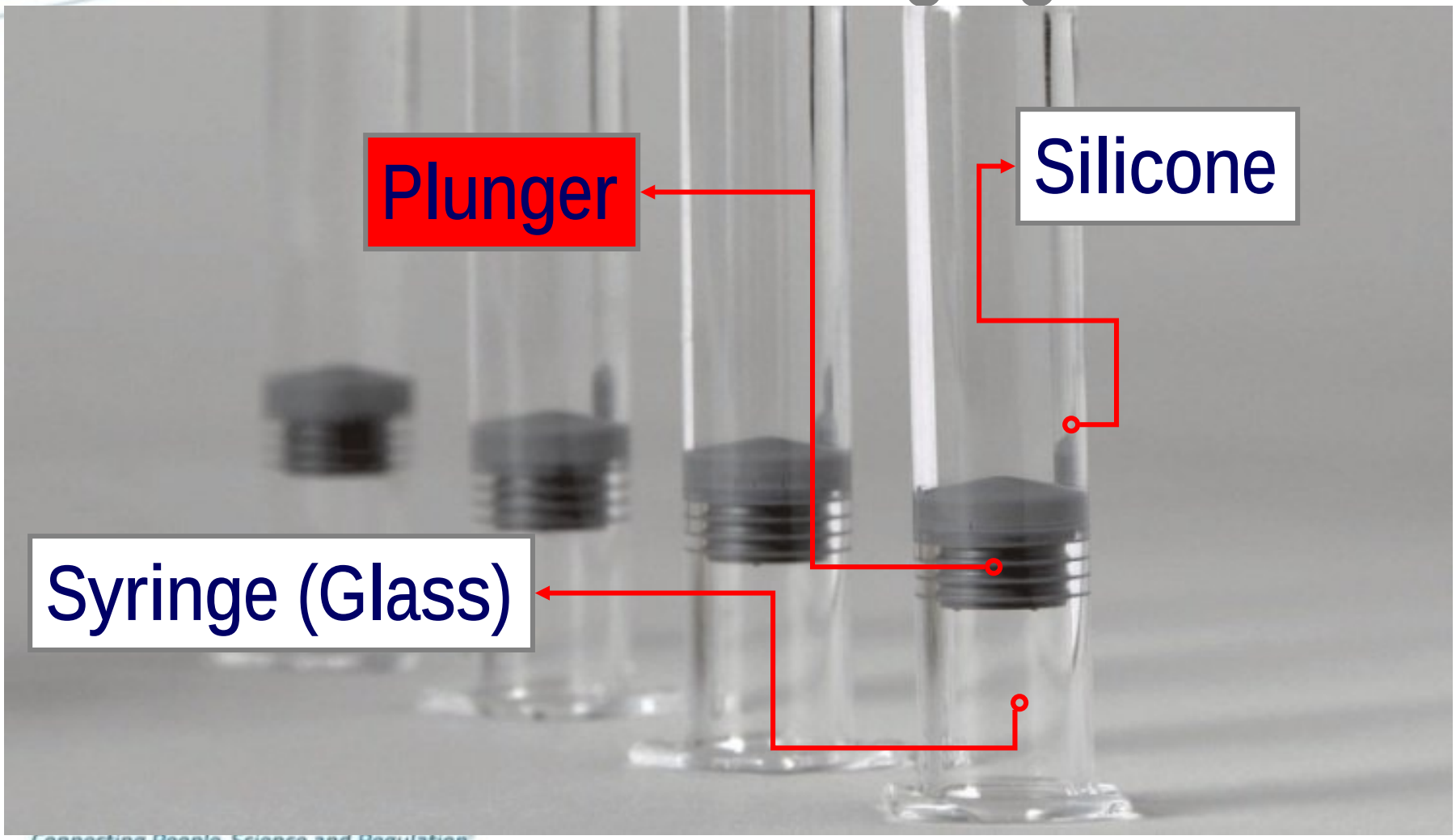

**Cellulose (Contaminant)**

# Conclusion Cellulose Example

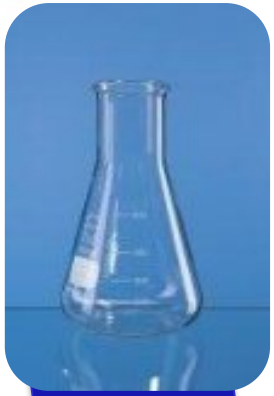
1. One special type of cellulose could be identified by the typical peak @ 1600
2. Database was built with suspect cellulose samples used in production
3. These Cellulose (contamination) fibers were found in smaller concentration in CIP rinses  no fibers …were found in the process prior to filling!
4.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

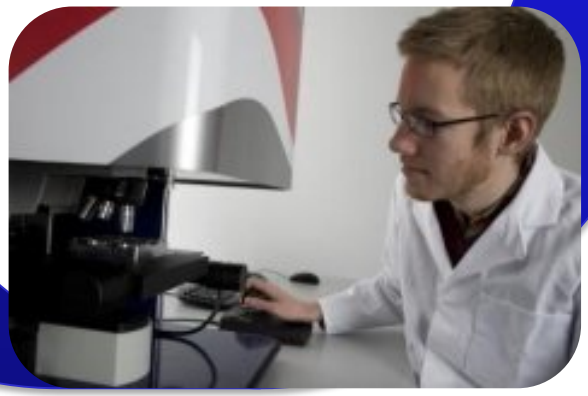
→ API manufacturer used paper towels and introduced cellulose into the process

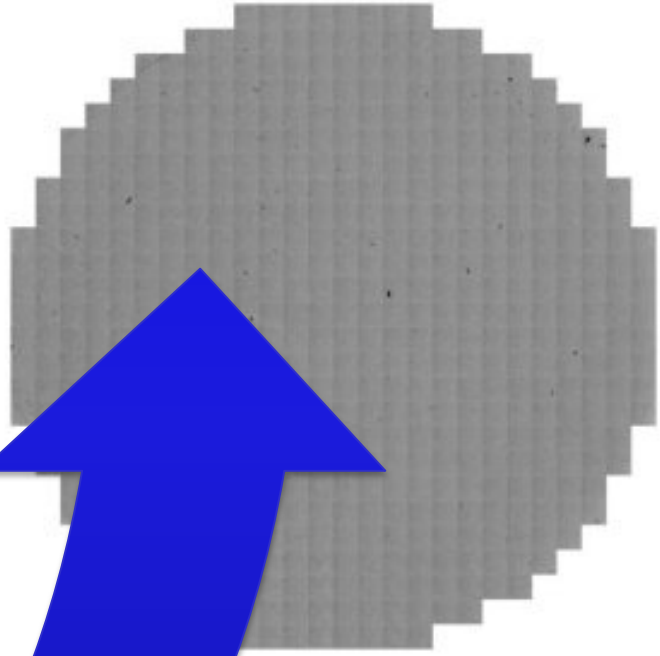
# Control Your Packaging Material



# ISO 8871-3

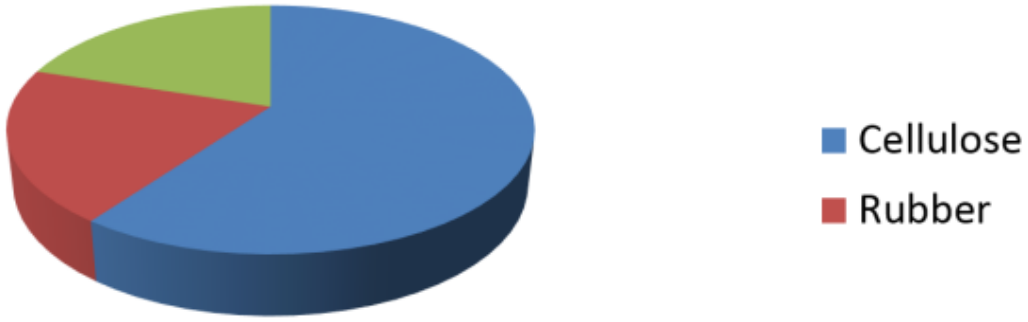






# Fibers and particles on rubber

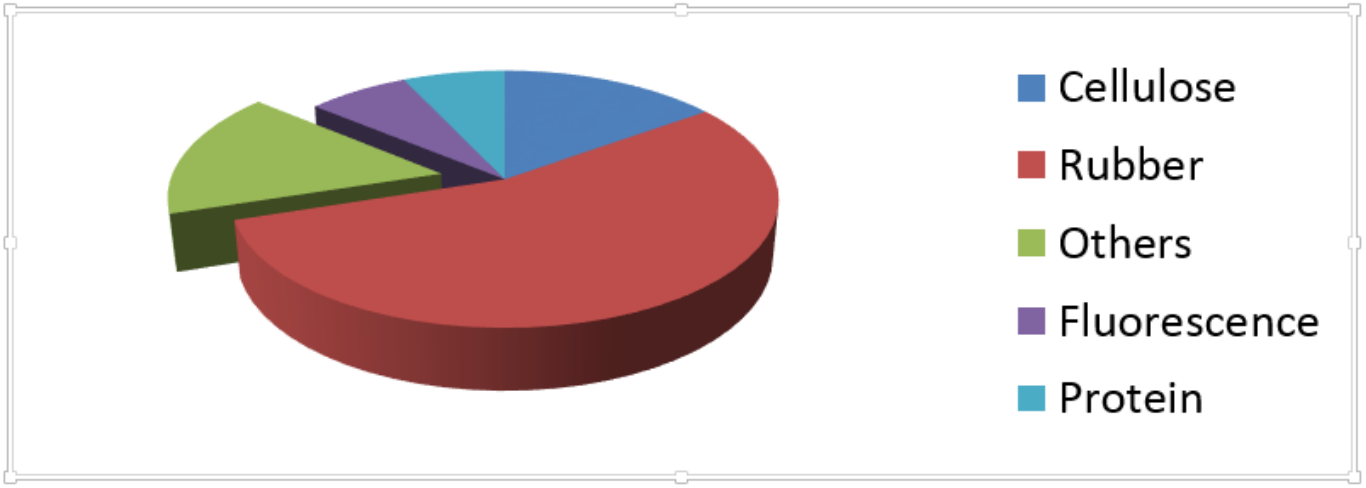
- 10 stoppers contaminated with fiber
  Cleaning following ISO 8871
- 51 particles > 50 µm found




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

# Fibers and particles on rubber

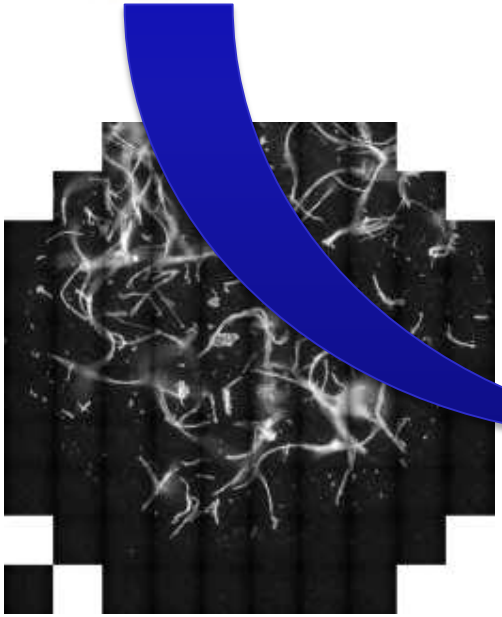
- 10 stoppers contaminated with particles Cleaning following ISO 8871
- 144 particles found > 50 µm

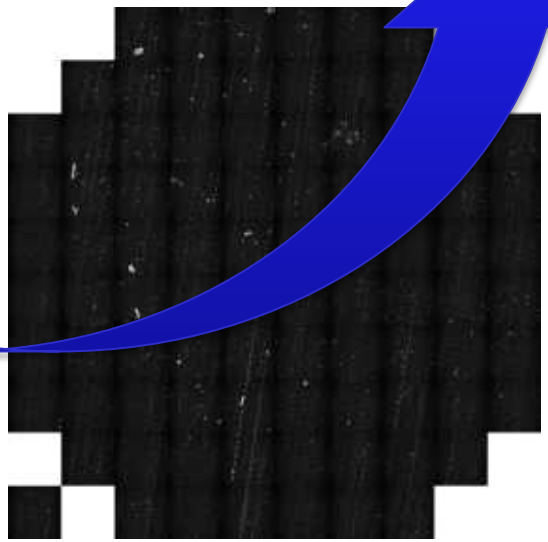


# Bags



Stopper Bags have an impact or reflect stopper quality



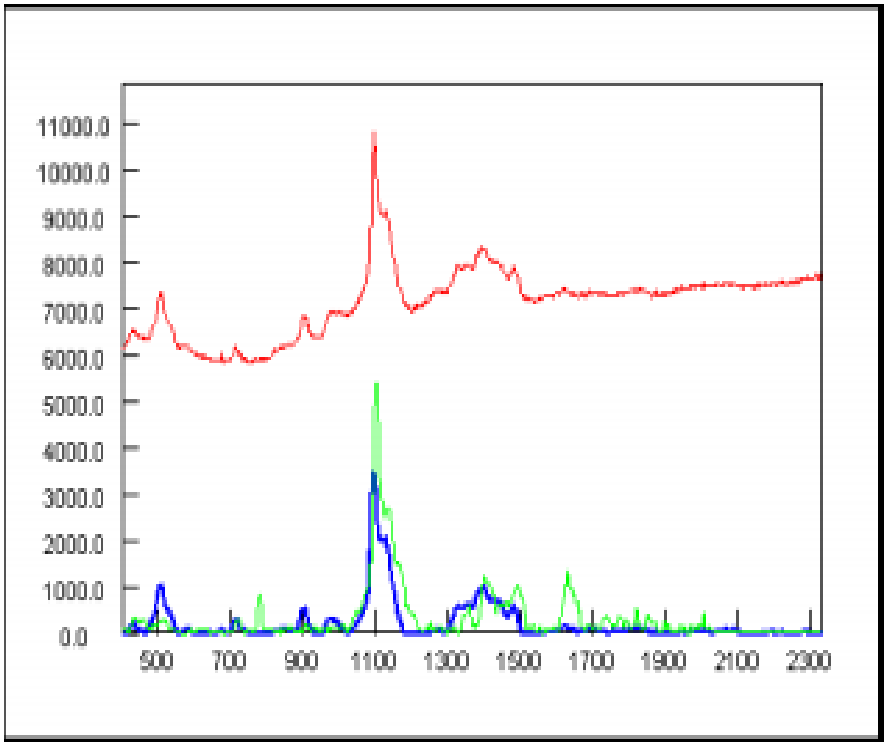


Test Procedure: Bag rinsed with 250 ml water / SDS, filtration, counting

Fibres collected from one bag; 375 particles > 25 µm

Fibres collected from one bag; 45 particles > 25 µm

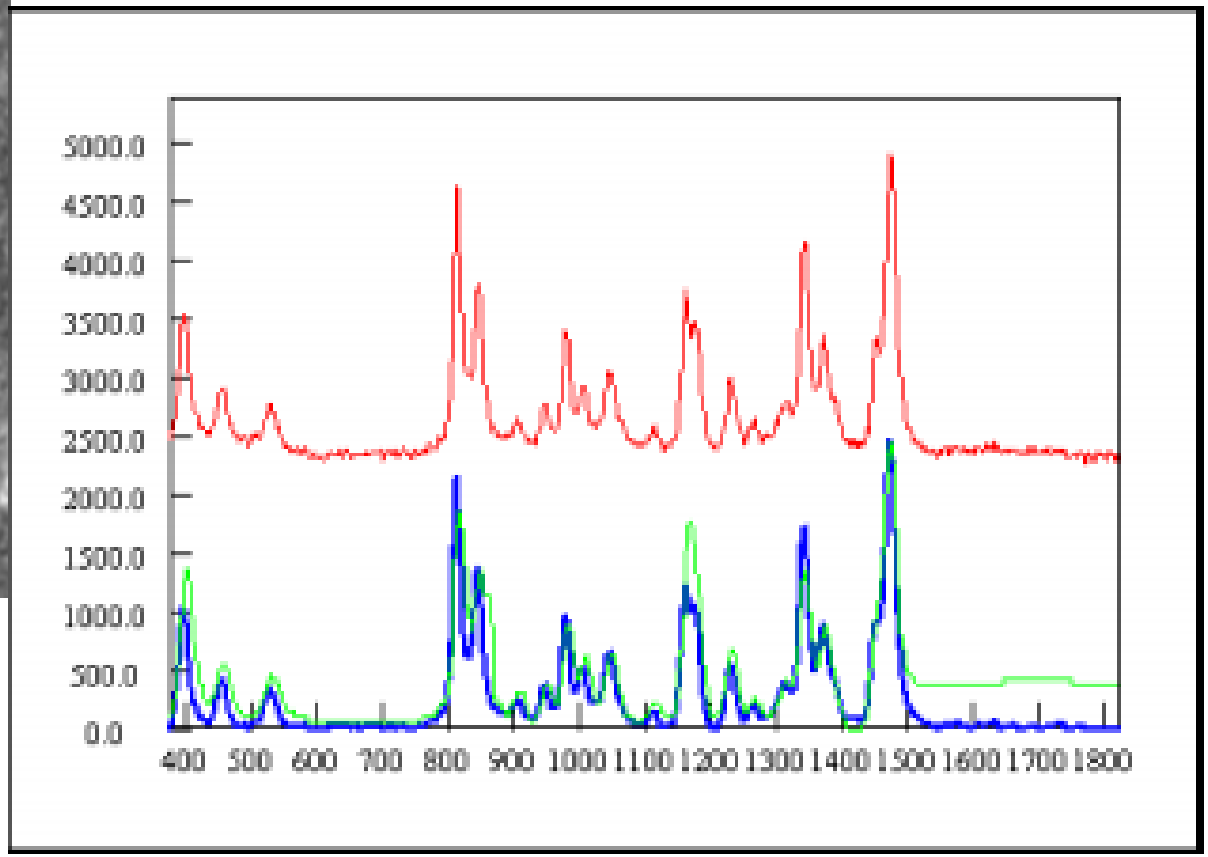
# Fiber → Paper




RESULT: **Cellulose [Paper]**

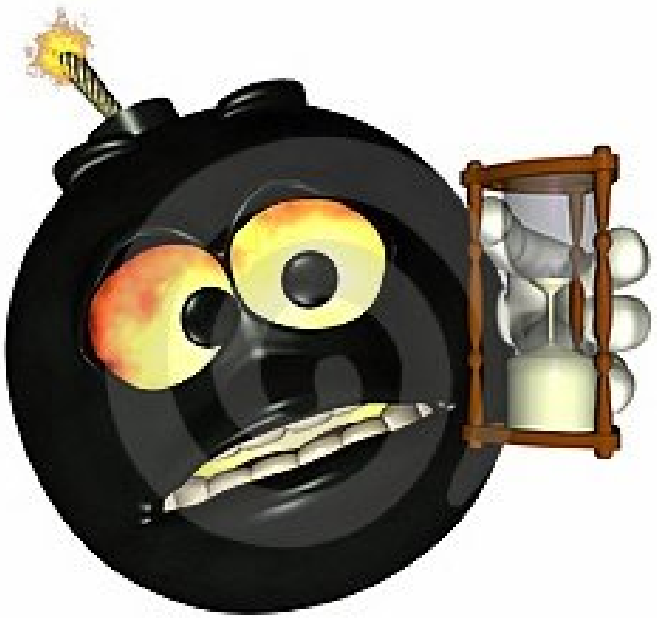
**RANK: 882, S/N: 39.2**

# Particles → Rubber




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

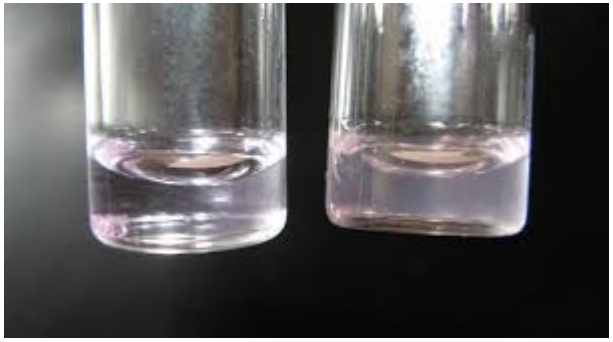
# Time bombs



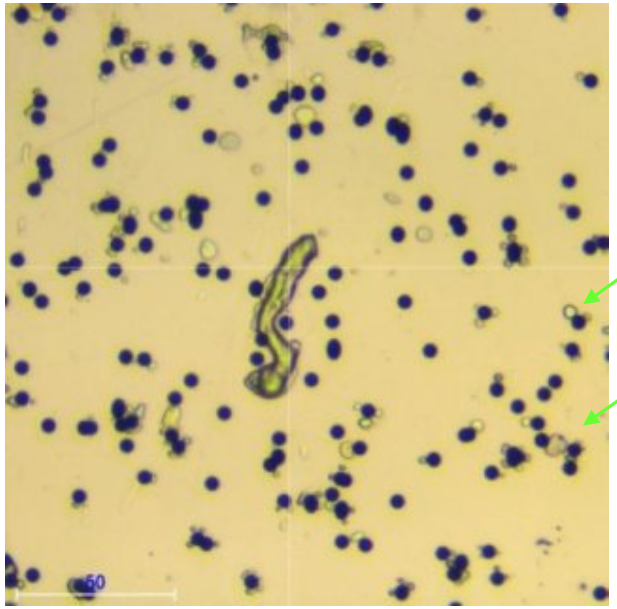
1. Increase of rejects with time
2. Chemical reactions taking some time
   - Silicone oil on stoppers: Agglomeration of Proteins
   - Coatings
   - Glass delamination

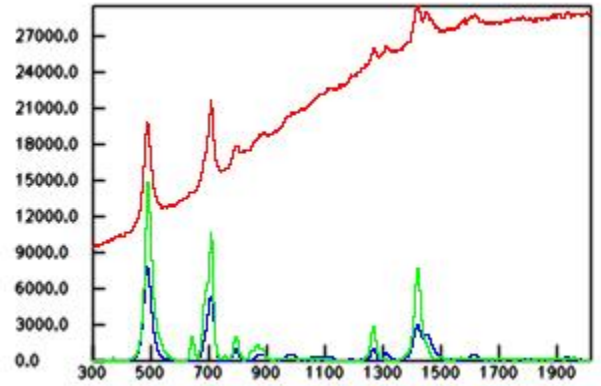
# Turbidity / Haziness



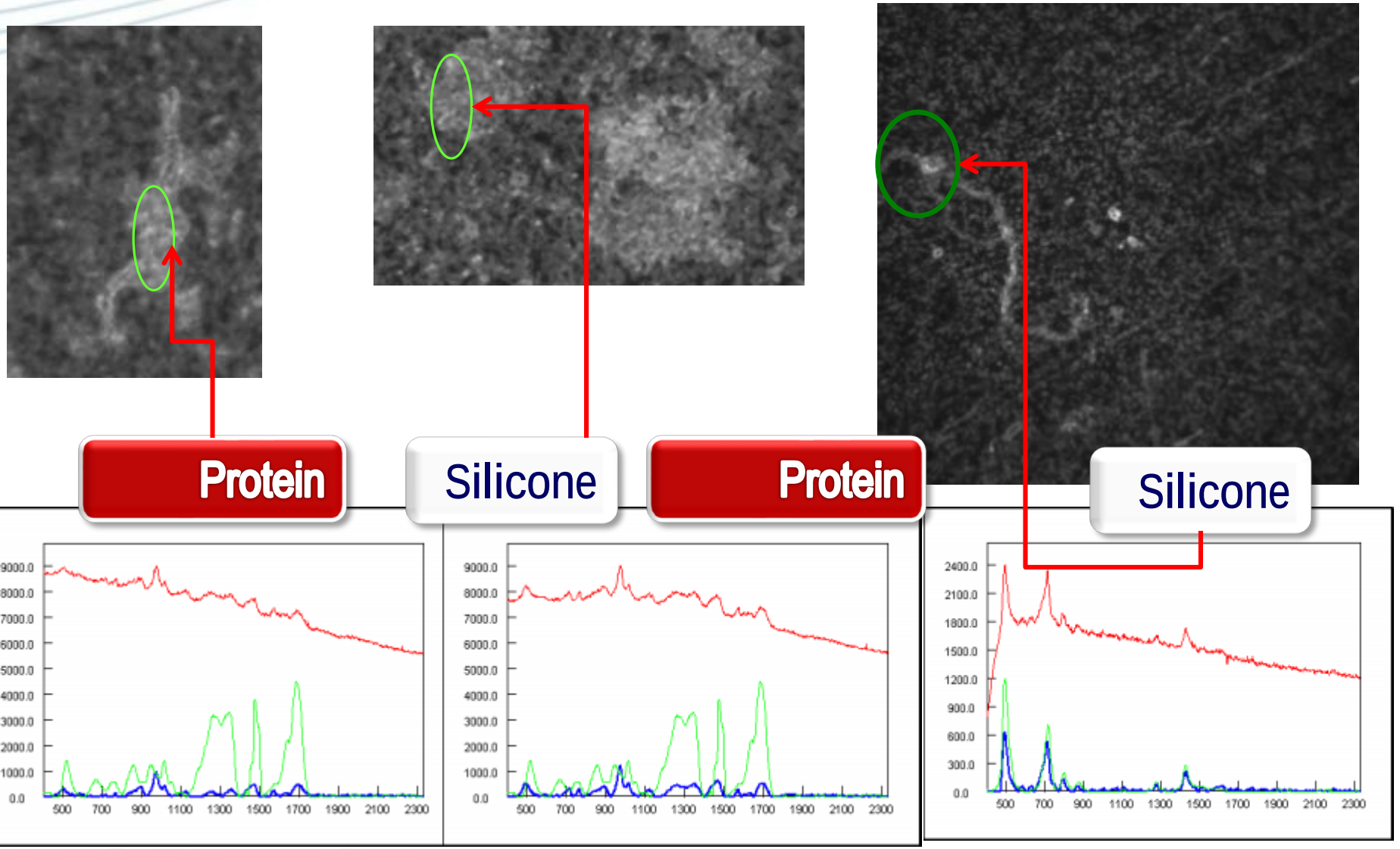


Observation of haziness and aggregates in a new a new batch after slight process change

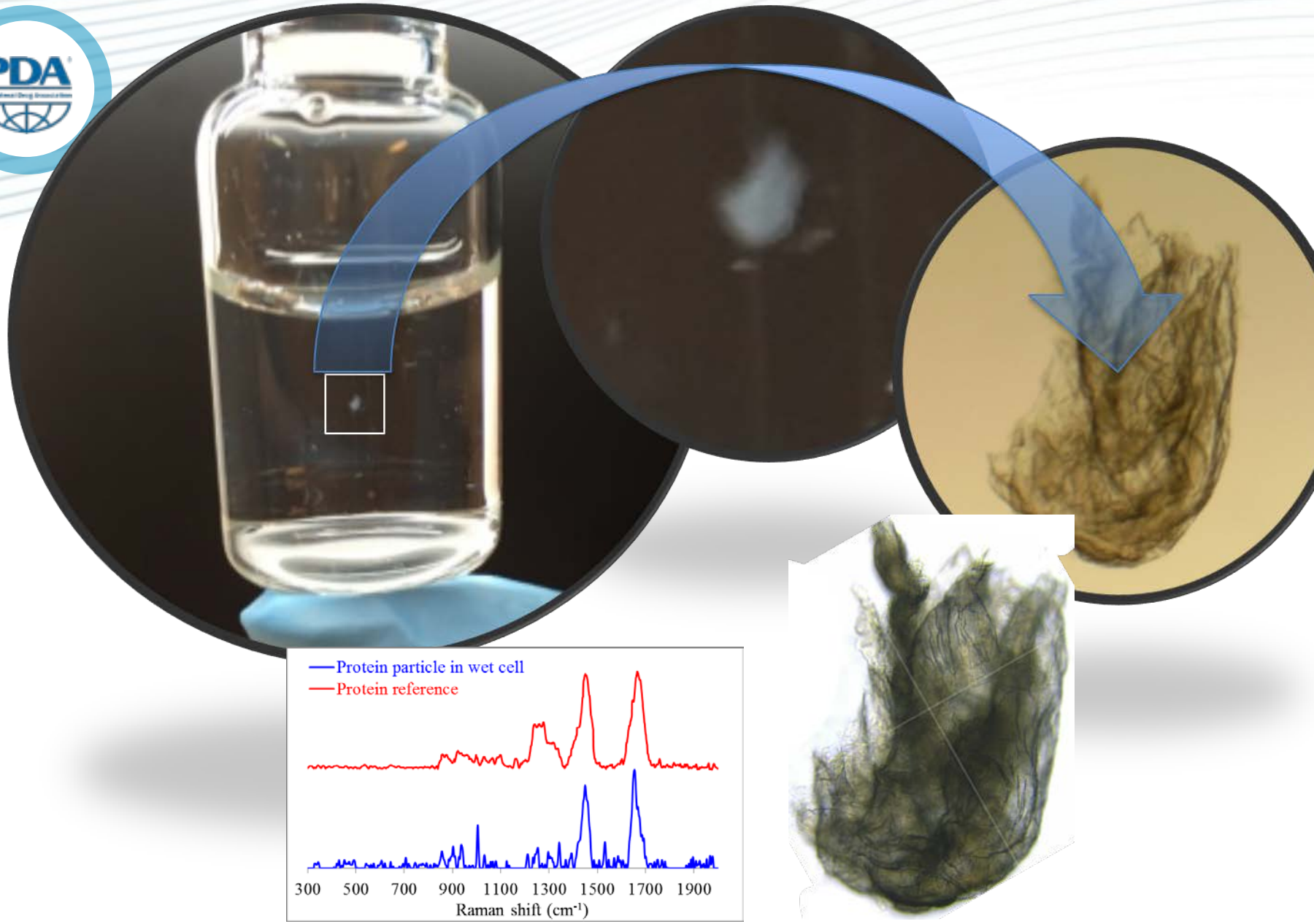




# Protein and Silicone



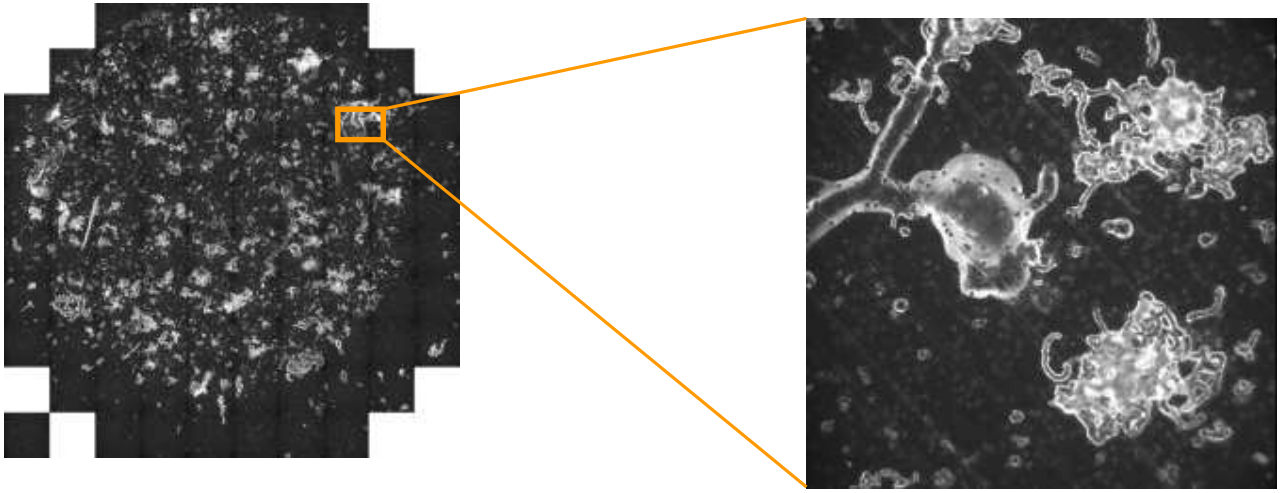
# VISIBLE INHERENT PARTICLE


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 |

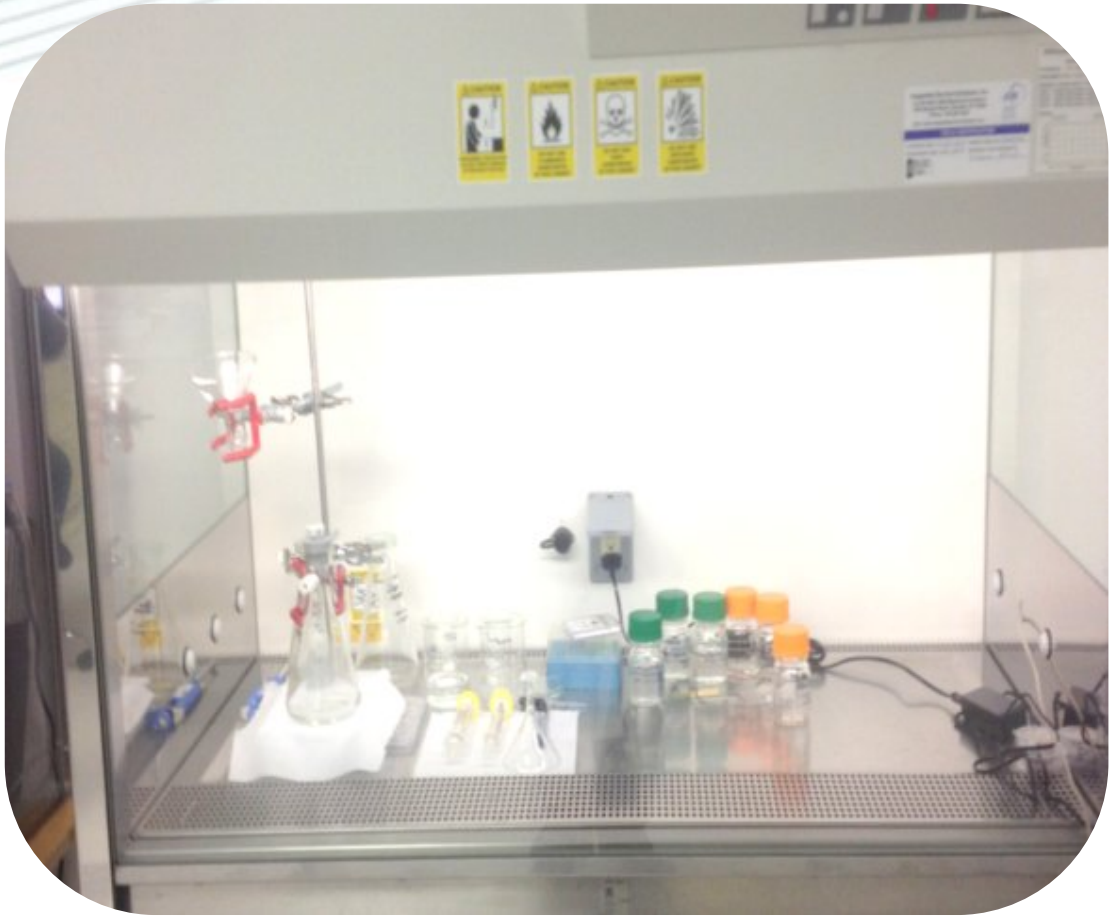
# Supplemental Testing or Inspection



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

**Technical Report No. 79**

Particulate Matter Control in Difficult to Inspect Parenterals



## 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:<br>– 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 !