

# **INNOVATIVE TEM-BASED APPLICATIONS FOR CHARACTERIZATION OF NANOPARTICLES IN FOOD IN A REGULATORY CONTEXT**

## **EM-unit**

### **SD Chemical and physical health risks**

# Introduction

- Rapid development of nanotechnology → **innovation** in many industrial sectors
  - agricultural production,
  - animal feed and treatment,
  - food additives and food processing,
  - food contact materials,
  - cosmetics,
  - textiles,
  - medical devices,
  - sensory applications,
  - biocides,
  - ...
- May pose a **risk to human health and the environment** → due to specific NM properties and potential widespread use and exposure.
  - NM size, VSSA and DOS is different from bulk
  - NM carry chemicals including metals and hydrocarbons.
  - Into body through the lungs, skin and digestive system
  - Concern that once nanoparticles are in the bloodstream, they are able to cross the blood-brain barrier.
- In the EU, safe use of NM applications is ensured by **specific legislation and dedicated (non-binding) recommendations and guidances**



# Electron microscopy unit activities

- We characterize in a regulatory context the **physico-chemical properties of engineered NM** by TEM,
- We make images of particles at **nanometer scale**.
  - ➔ assess the size, morphology, agglomeration state, elemental composition and crystallinity of the particles.



- Focus on NM in the food chain, cosmetics, medical devices and environment.
- **NRL** for characterization of engineered nanomaterials in the food chain and food contact materials, and appointed as representative Belgian laboratory in the 'Nano in Food' project for the EC.

# Methodologies

## Old Instrument

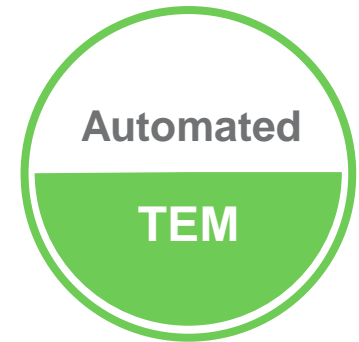
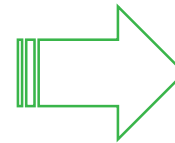


Physical NM characterization

## New Instrument



Physical NM characterization  
+ Chemical NM characterization



Physical NM characterization  
+ Chemical NM characterization  
+ Fully automated



# Conventional TEM: Physical NM characterization

## Methodology

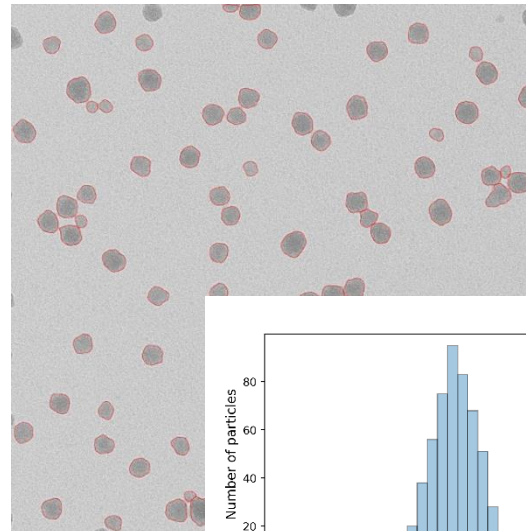
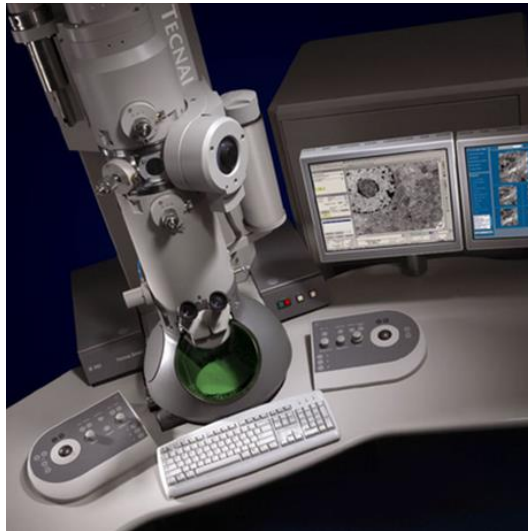
Sample  
preparation

TEM  
imaging

Image  
analysis

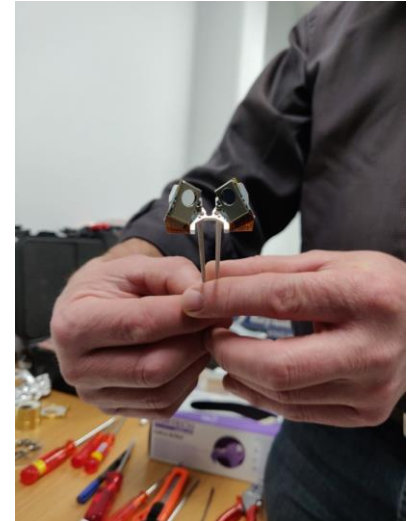
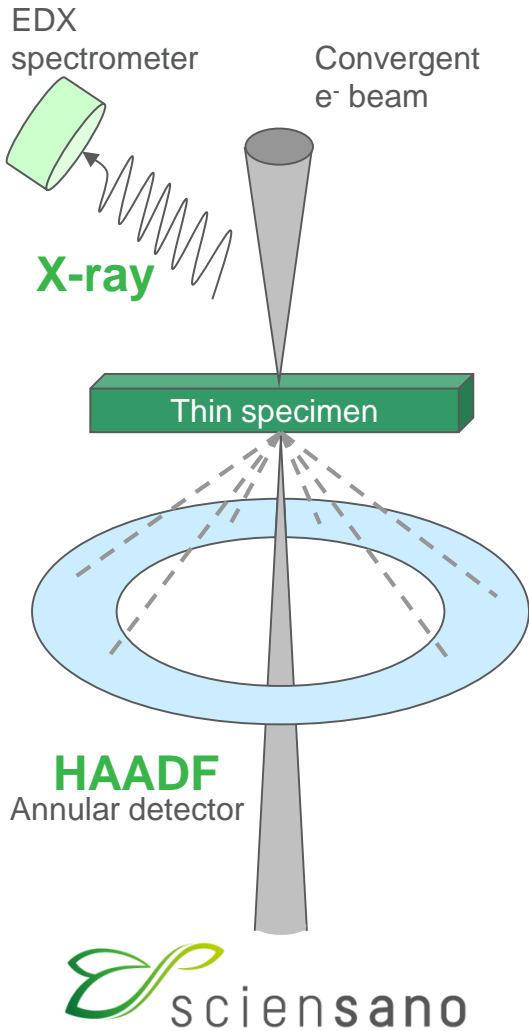
Size  
distribution

Median  
value

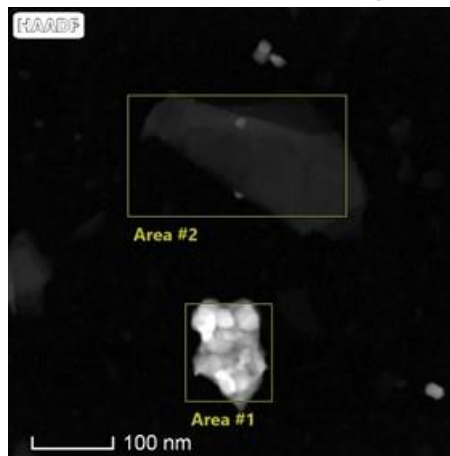


Developed for implementation of EC Recommendation on the definition of a nanomaterial (2011/696/EU)

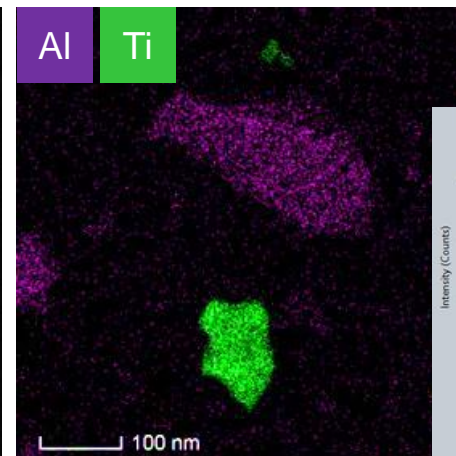
# Analytical TEM: Physical + Chemical NM characterization



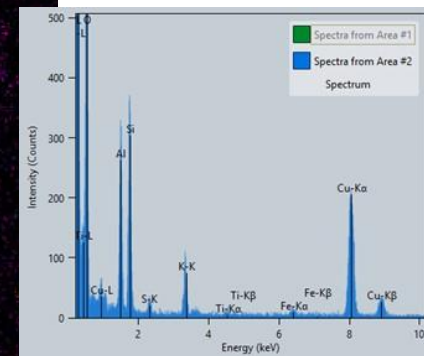
HAADF-STEM image



Elemental maps



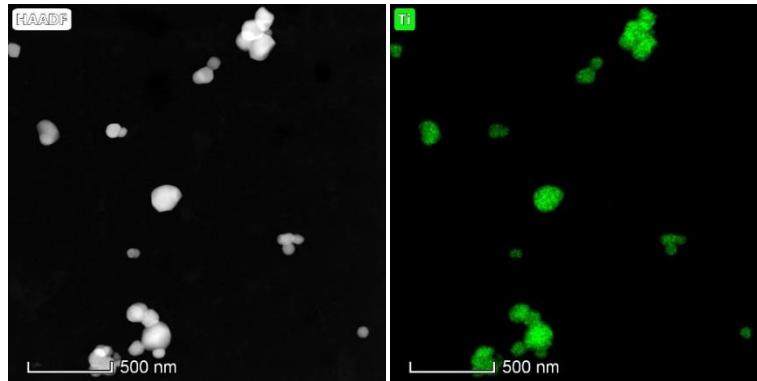
Characteristic X-rays



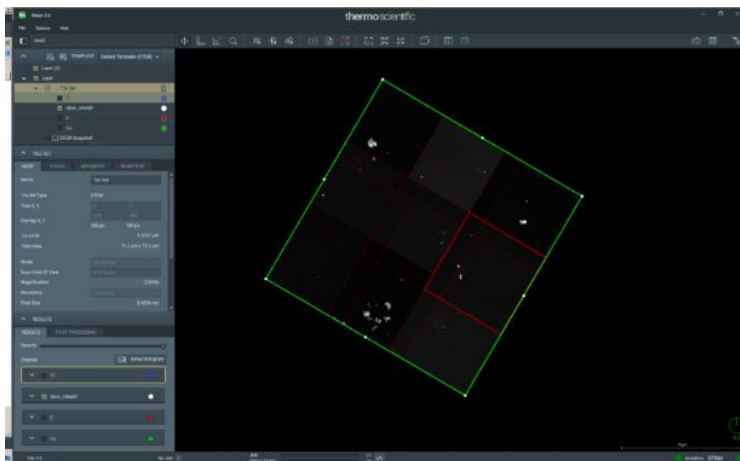
# Automated TEM



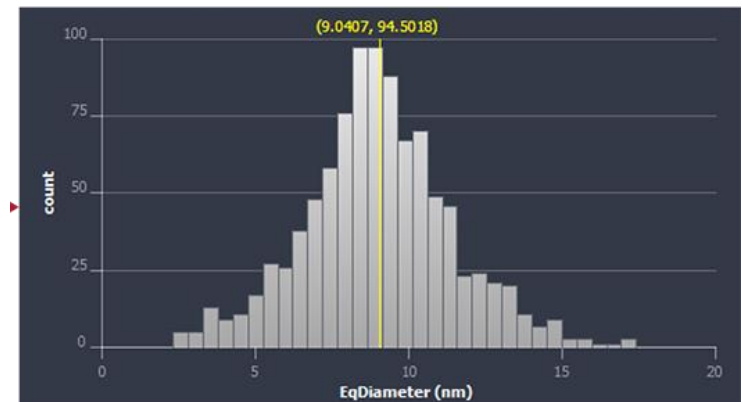
## VELOX: STEM images and EDX maps recording



## MAPS: Automated grid scanning



## AVIZO: online particle measurement



# Applications

## Applications

Analysis of pristine food additives

Analysis of food additives in food products

Analysis of FCM

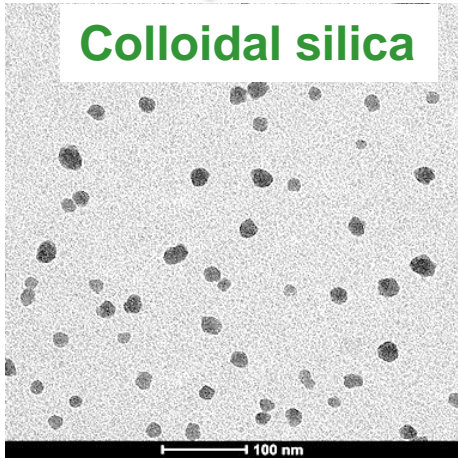
In vitro testing of cellular uptake of food additives



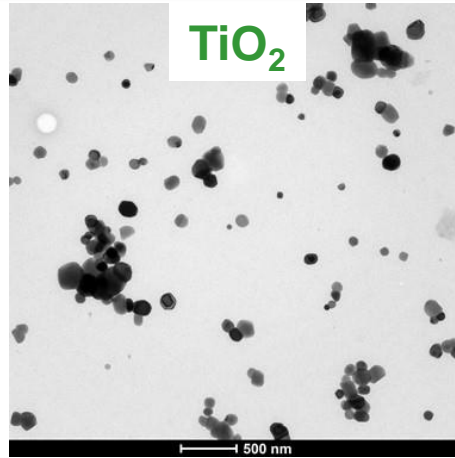
# Physical characterization of pristine NM

Near-spherical

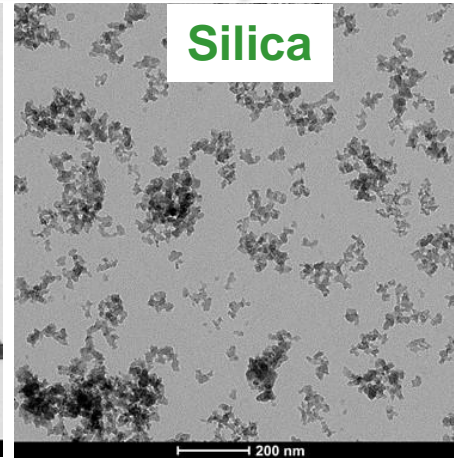
Colloidal silica



TiO<sub>2</sub>

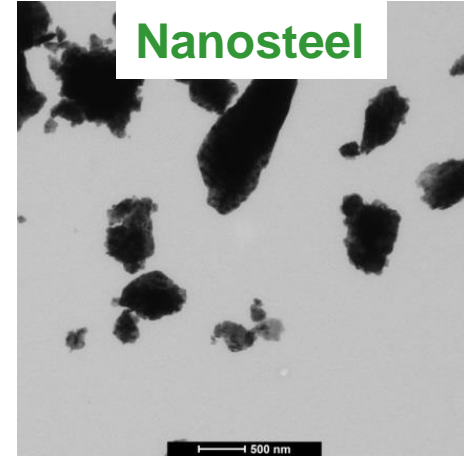


Silica



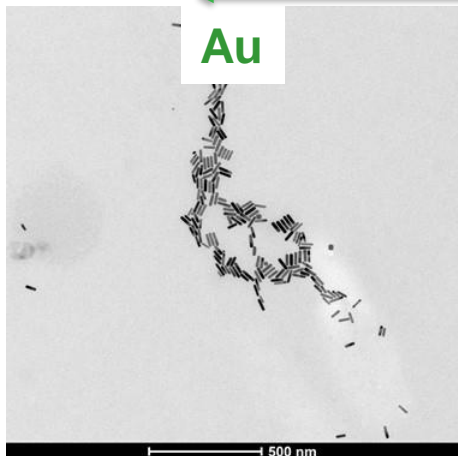
Platelets

Nanosteel

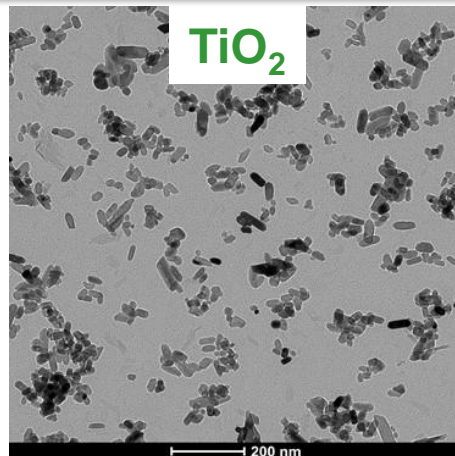


Rod-like

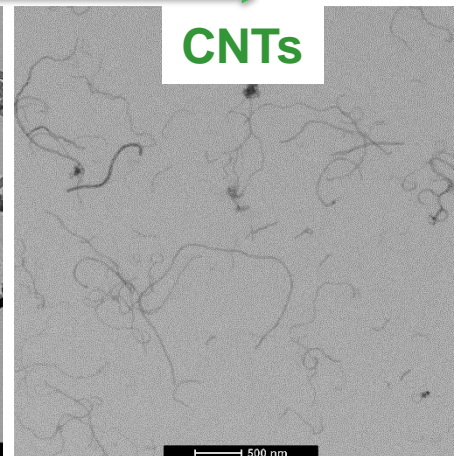
Au



TiO<sub>2</sub>

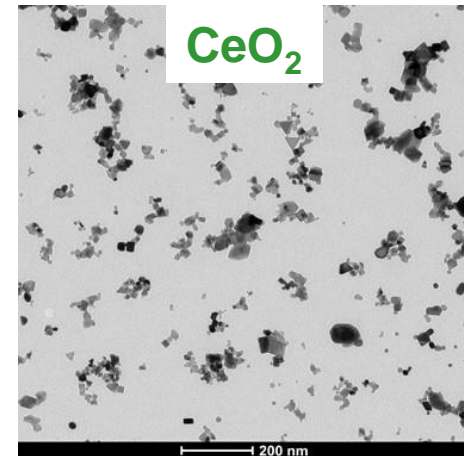


CNTs

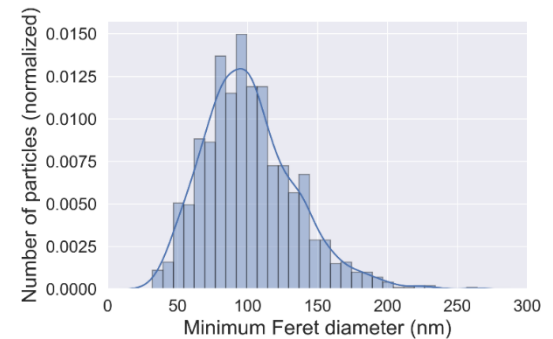
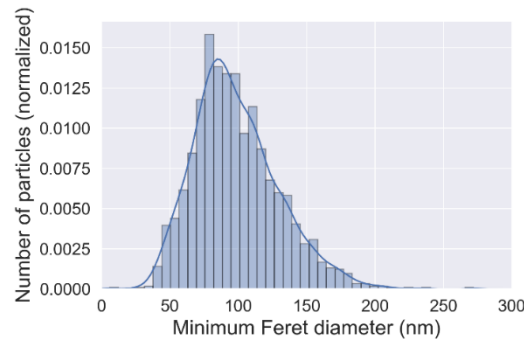
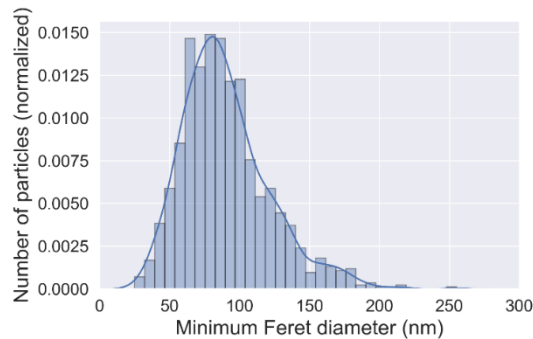
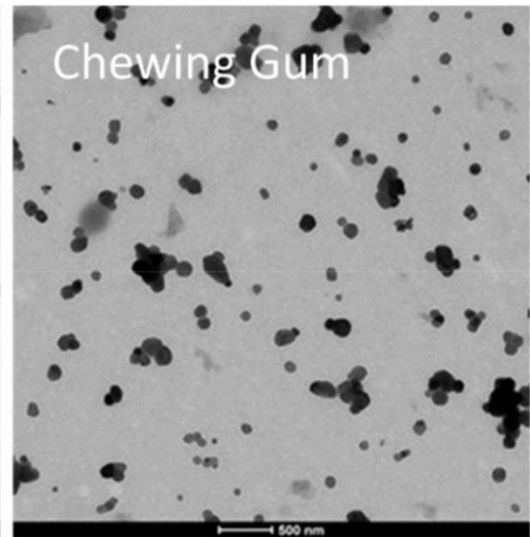
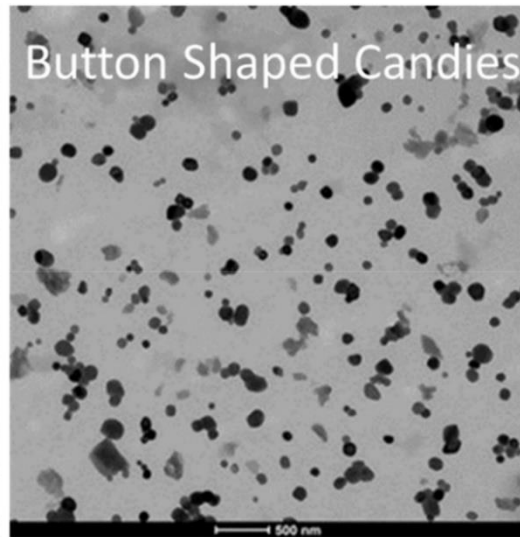
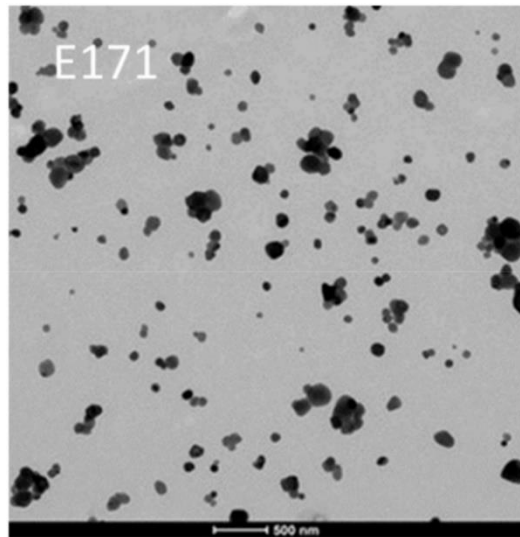


Irregular

CeO<sub>2</sub>



# Particles in a food matrix - simple models

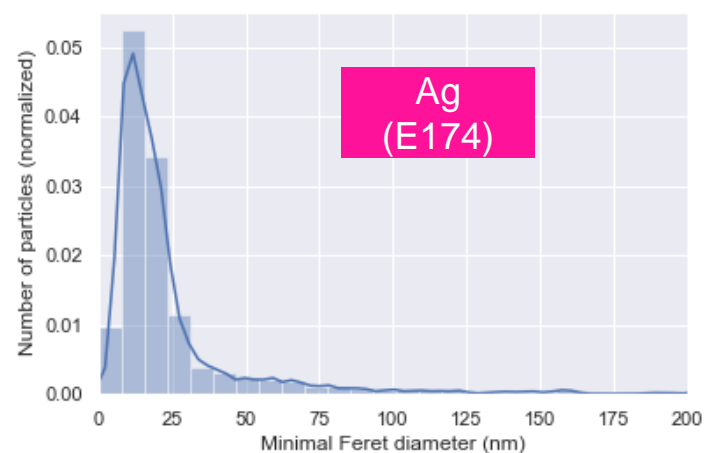
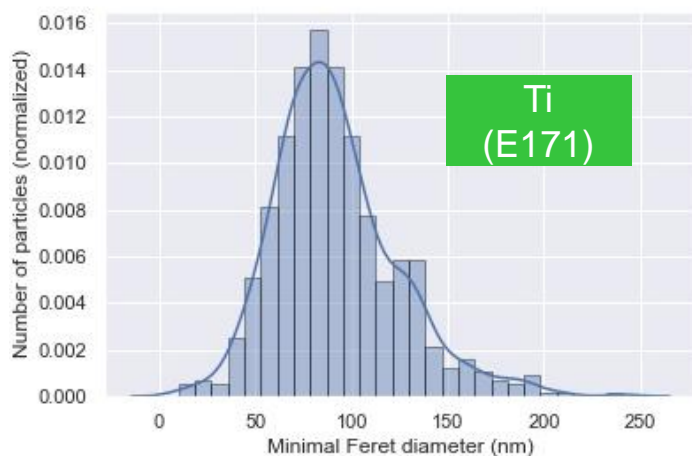
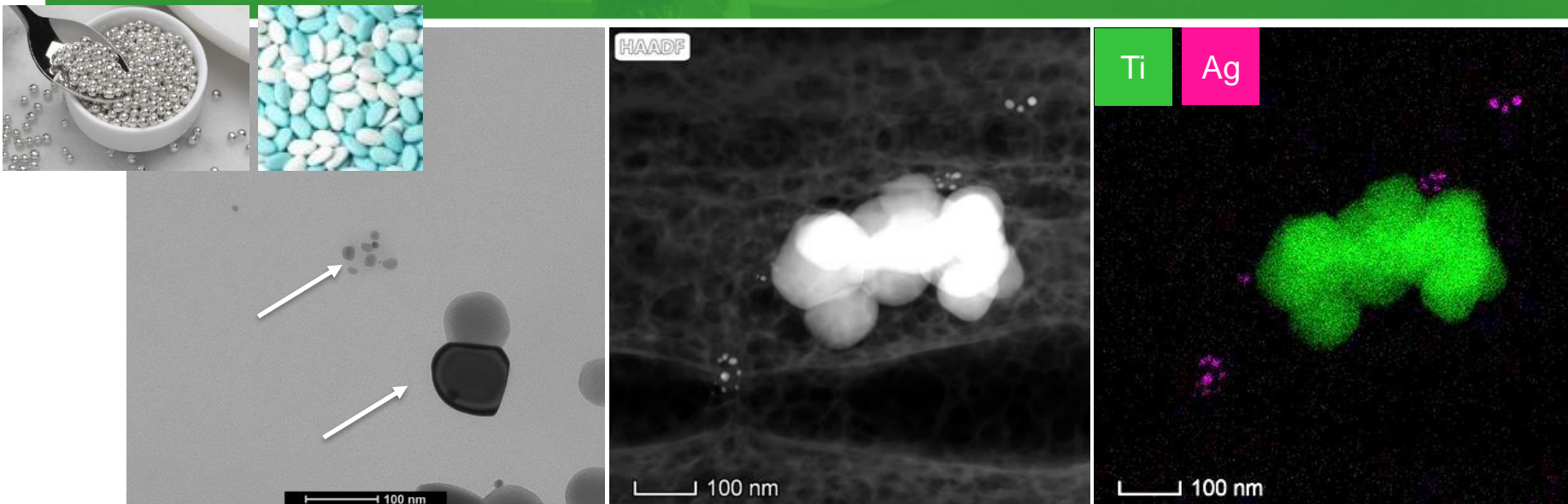


- Optimised sample prep
- Limited matrix interference → EDX not really necessary
- Characterization results are published: Geiss et al. 2020: Particle size analysis of pristine food-grade titanium dioxide and E 171 in confectionery products: Interlaboratory testing of a single-particle inductively coupled plasma mass spectrometry screening method and confirmation with transmission electron microscopy



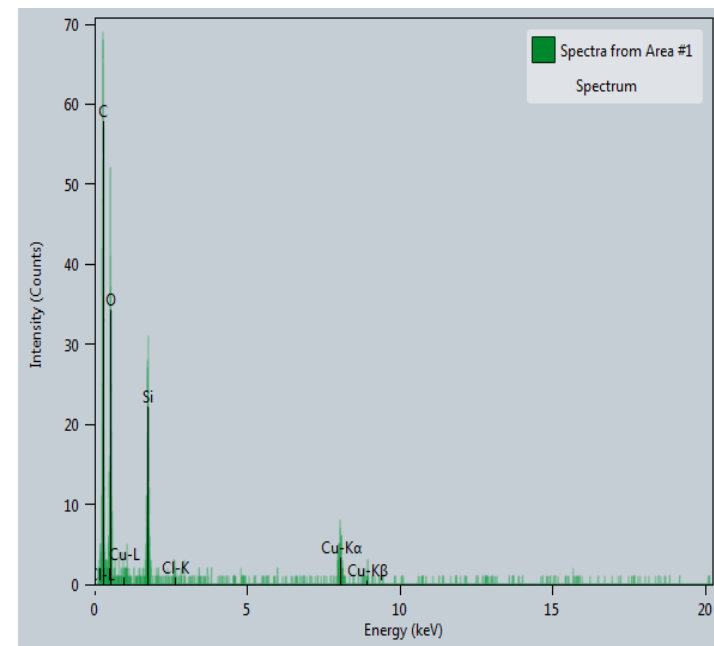
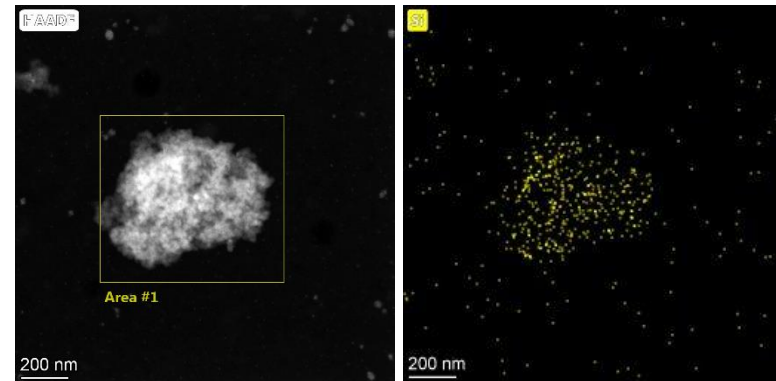
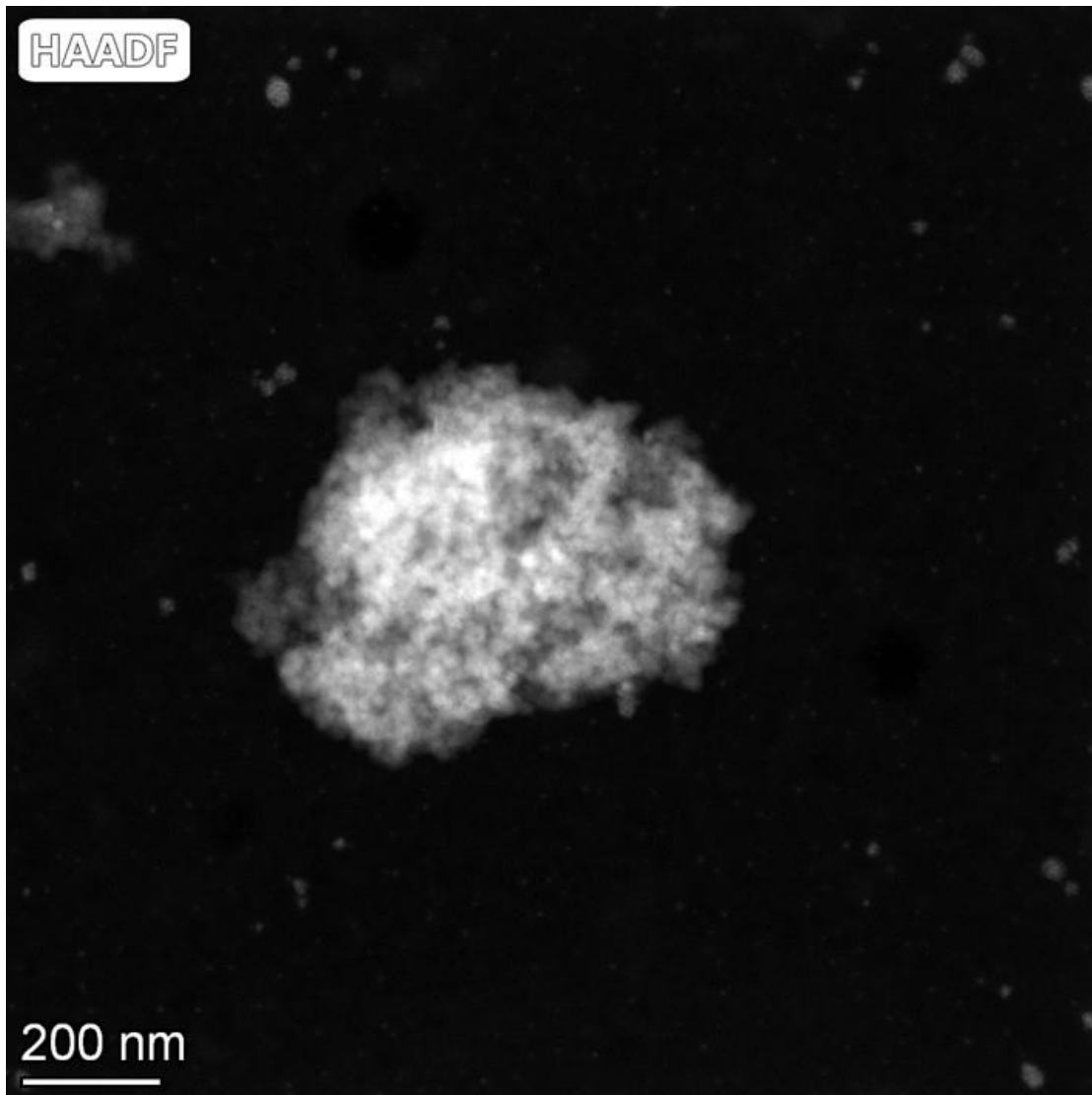
# Particles in a food matrix: complex models

Food additives E171 and E174 in a food product



# Particles in a food matrix: complex models

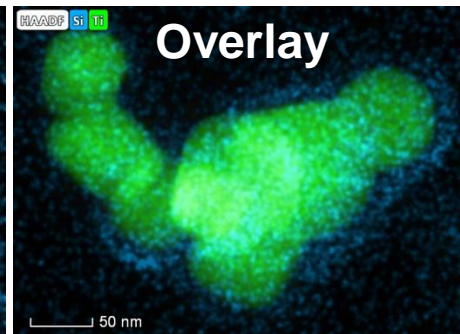
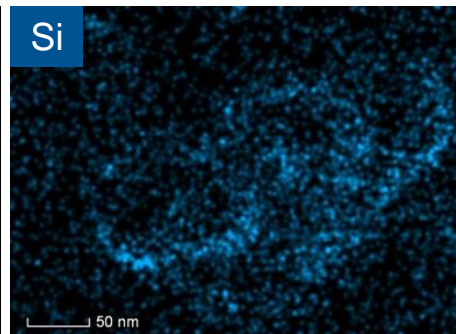
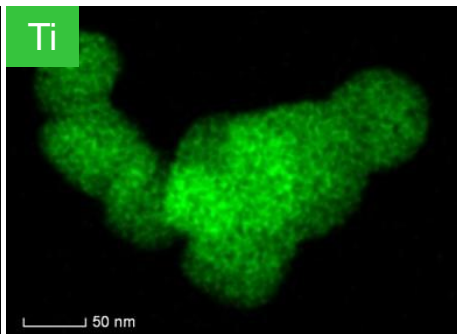
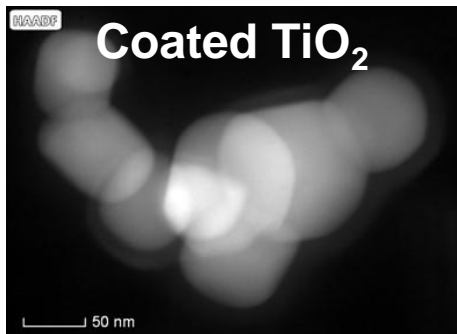
Food additive E551 (silica) in food products



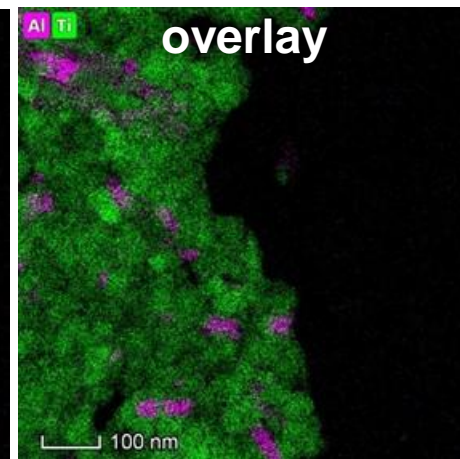
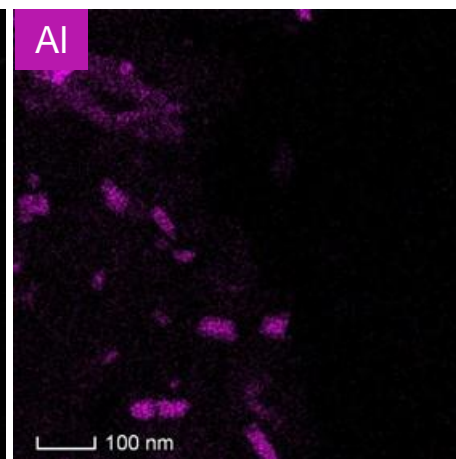
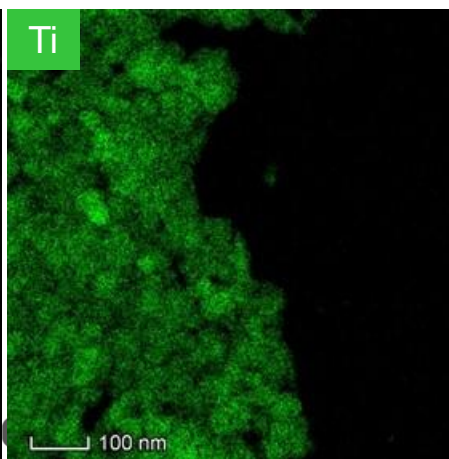
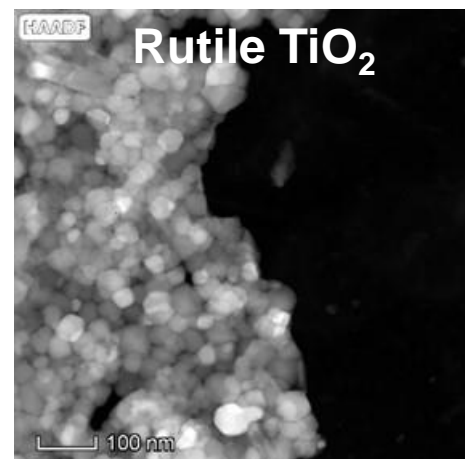
# Analysis of coatings and impurities

Food additive E171

Visualization + composition of coatings



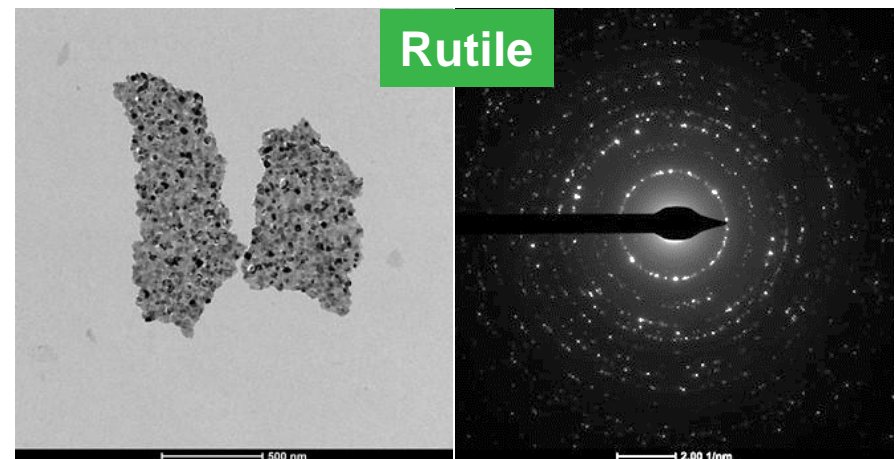
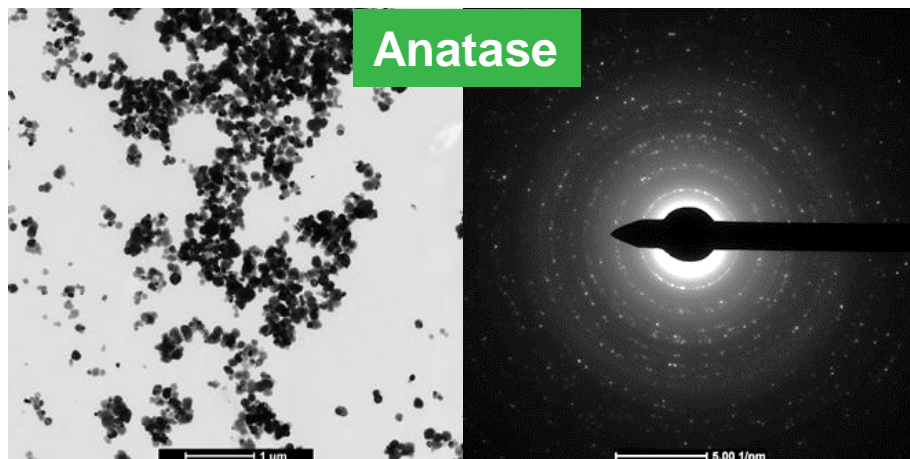
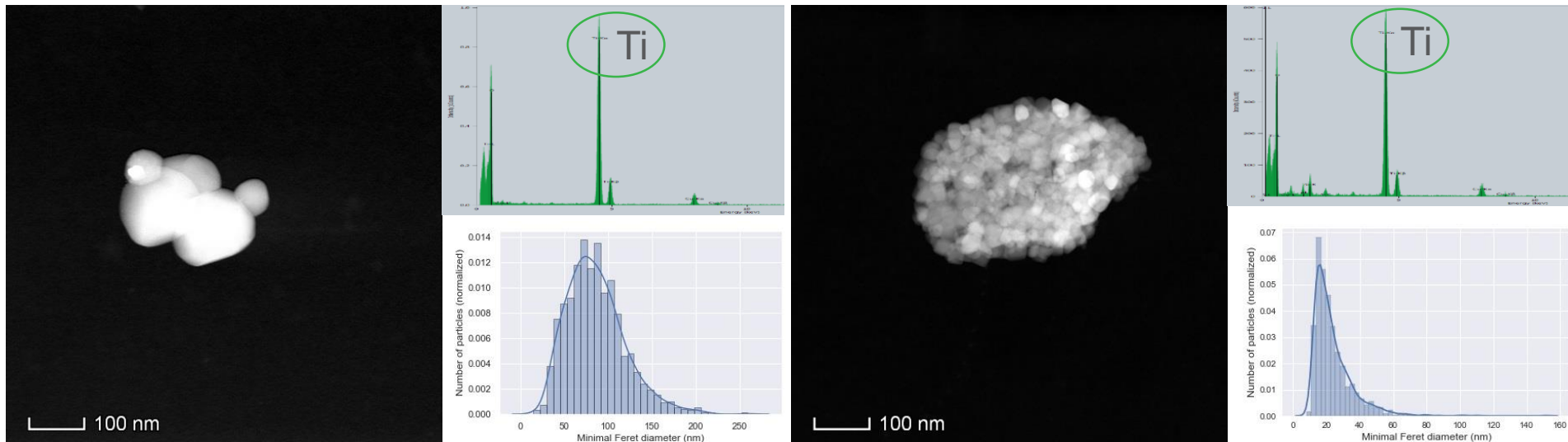
Visualization + composition of impurities





# Food – cosmetics: Phase determination - crystallography

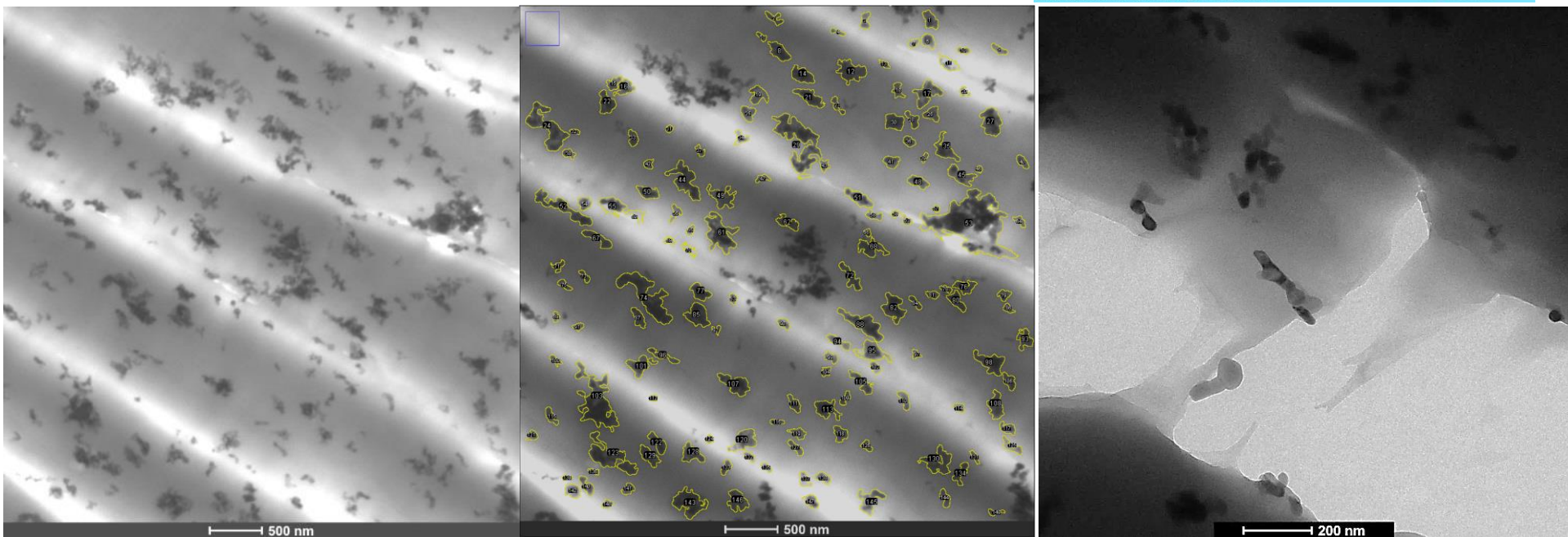
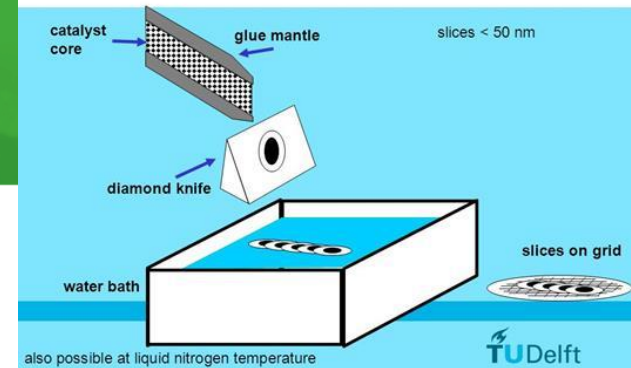
Anatase  $\text{TiO}_2$  vs pearlescent pigments



# Sections: Food contact materials

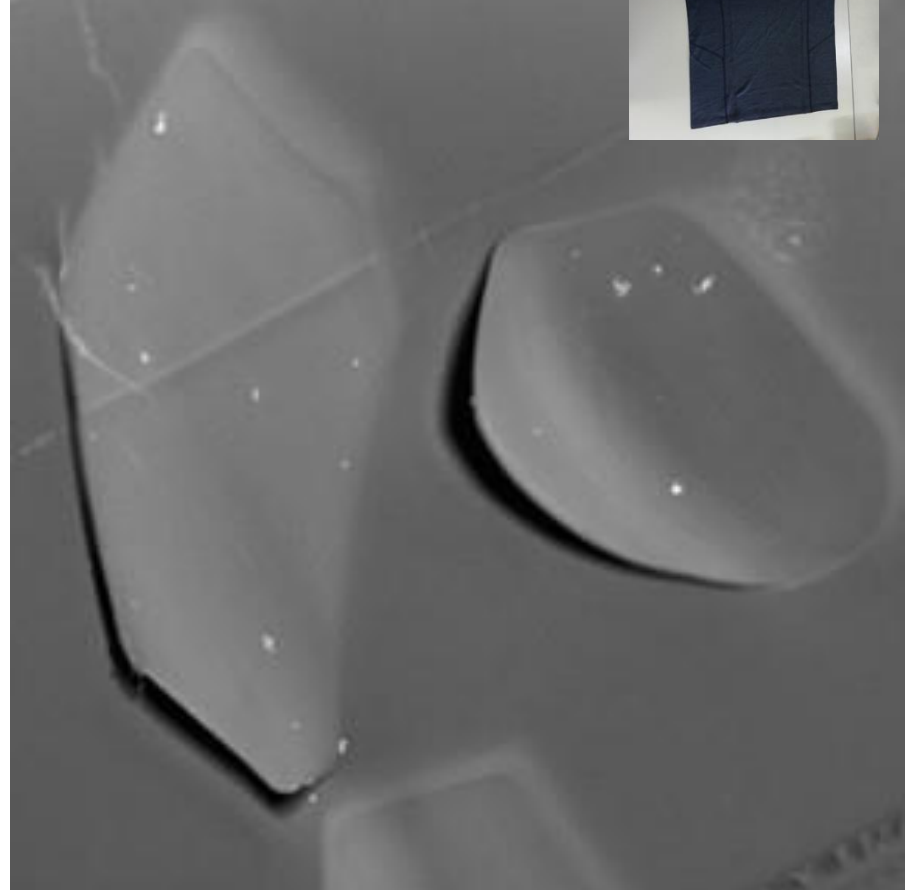
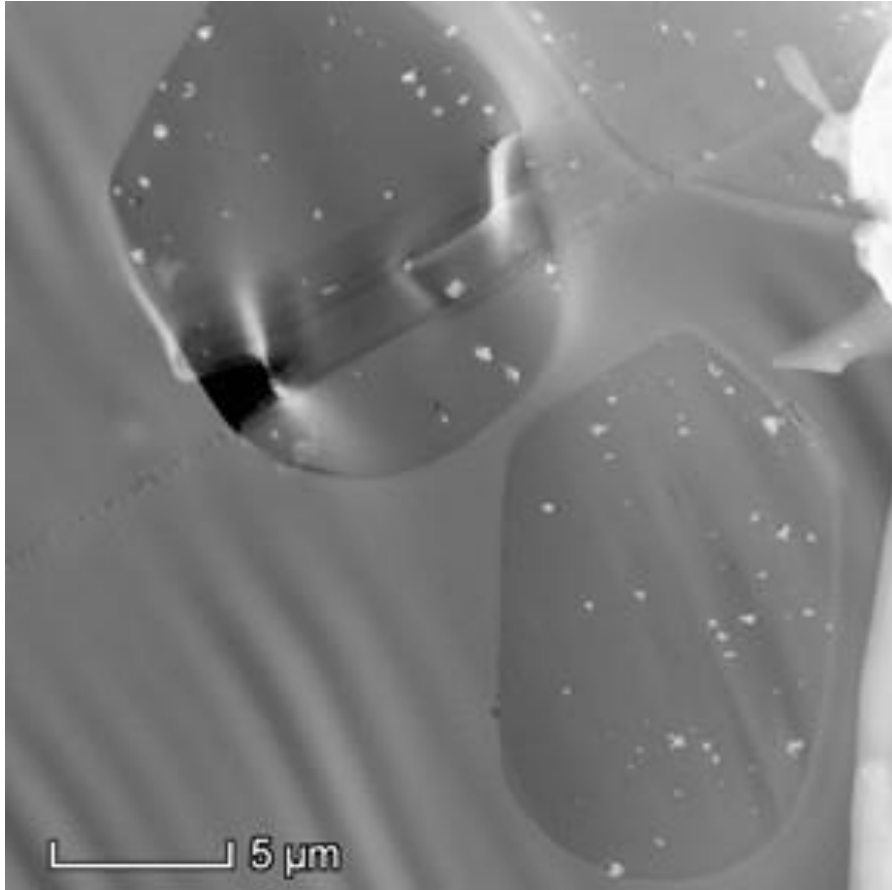
## Detection of $\text{Fe}_2\text{O}_3$ in PE

Sample preparation: ultramicrotomy



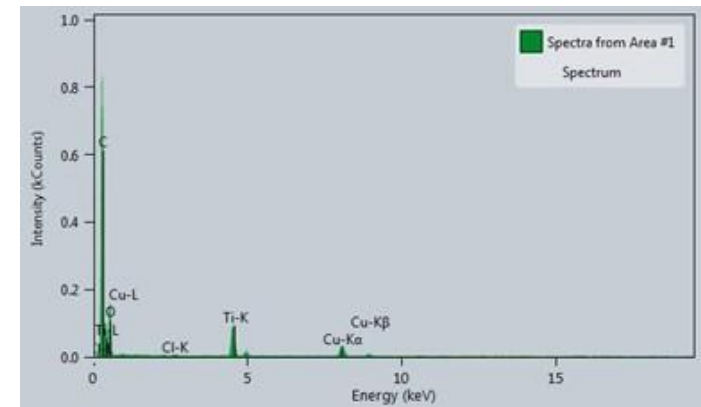
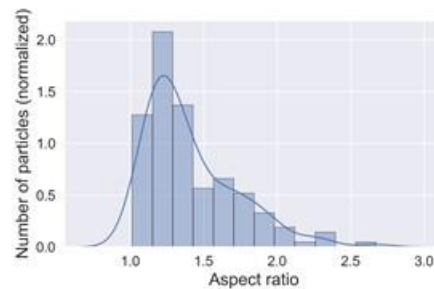
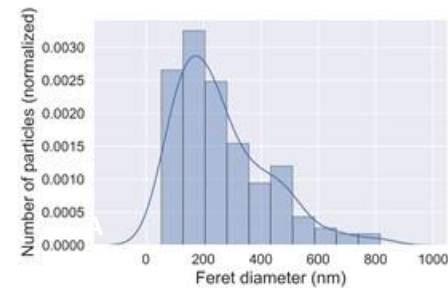
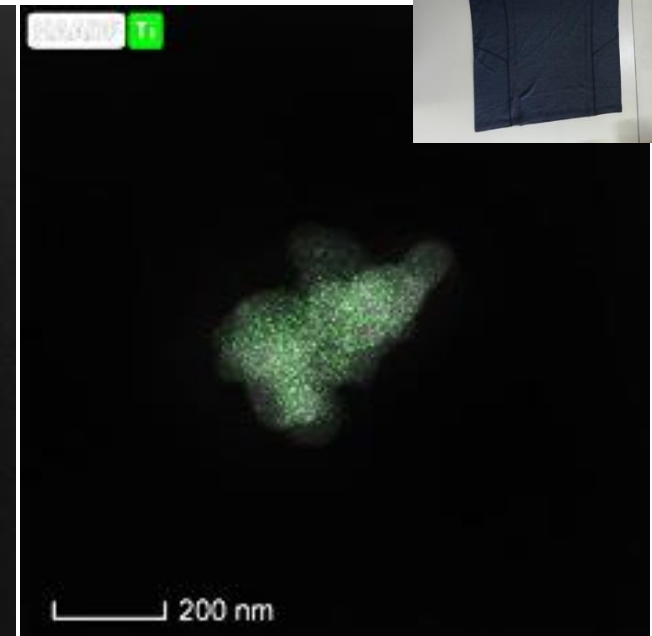
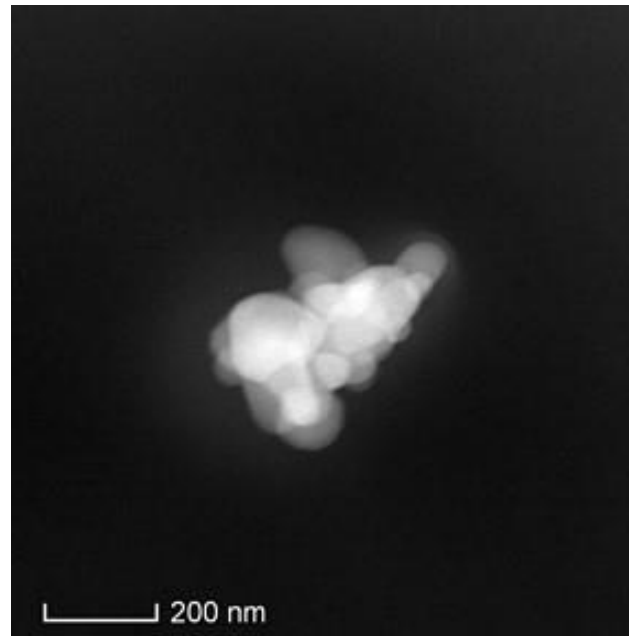
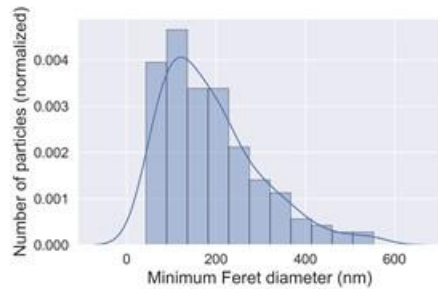
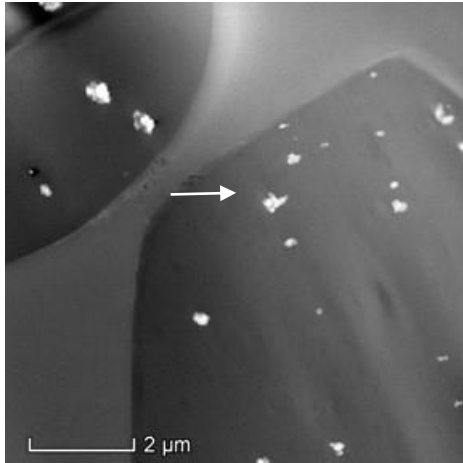
- Sections have a thickness of about 80-150nm
- Aggregates are easily detected
- Constituent particle detection is more difficult but not impossible

# Detection of nanoparticles in fibers

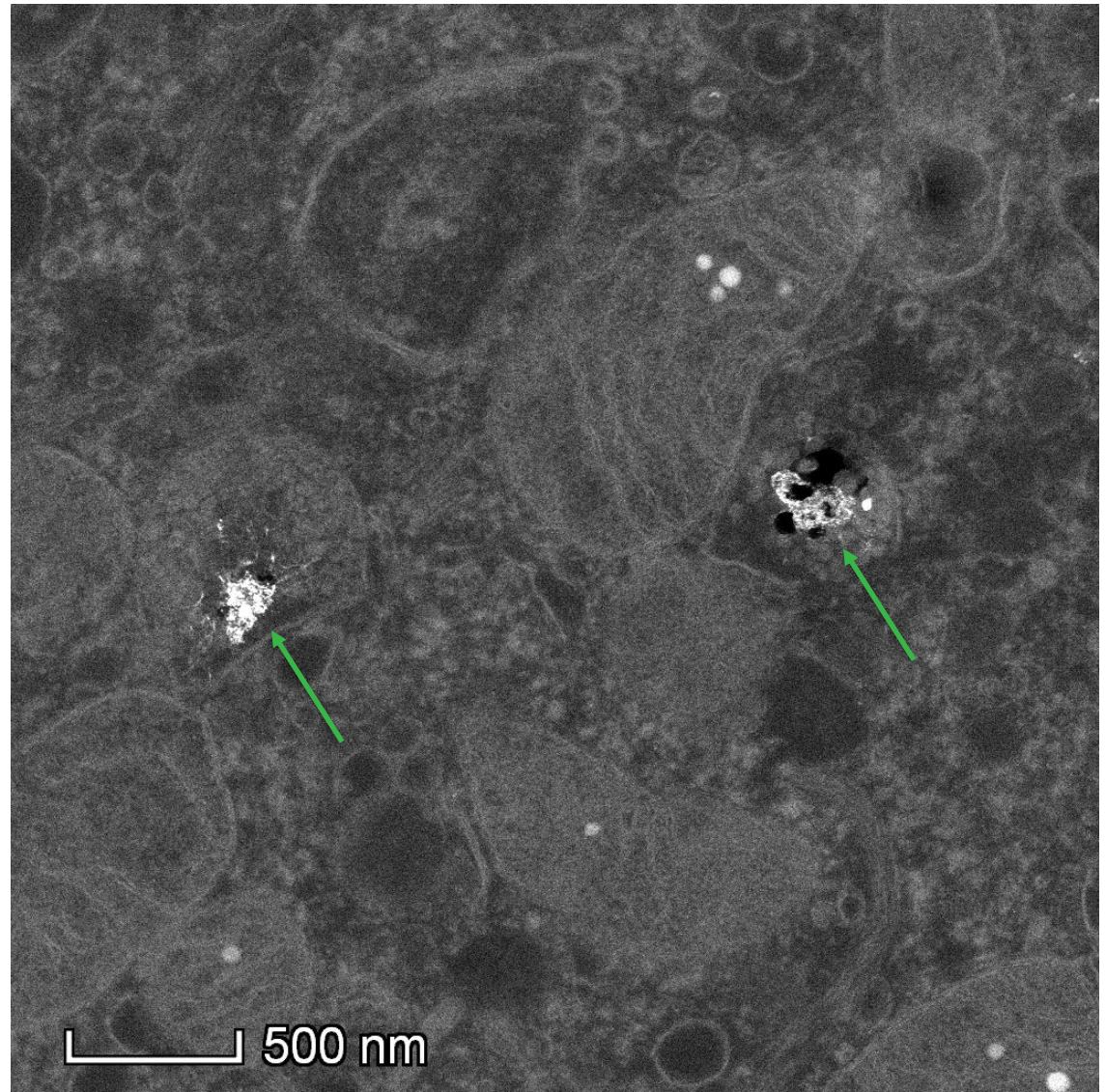
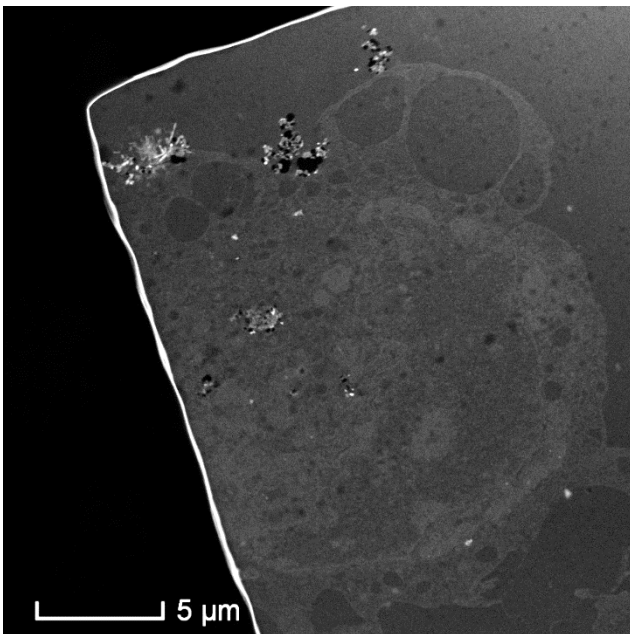




# Detection of nanoparticles in fibers

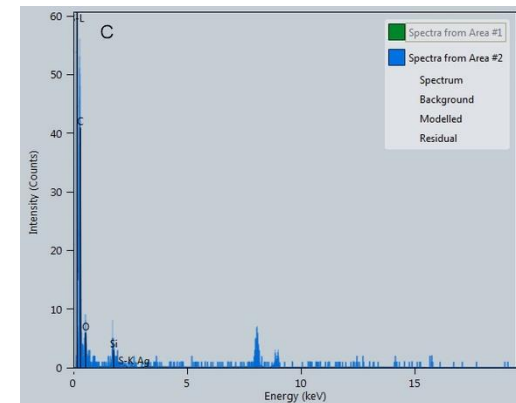
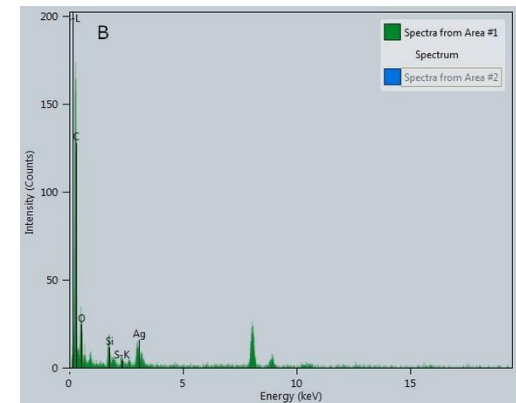
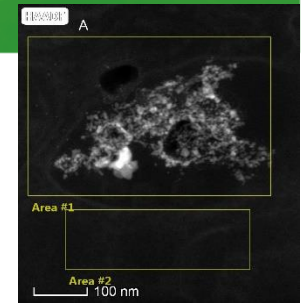
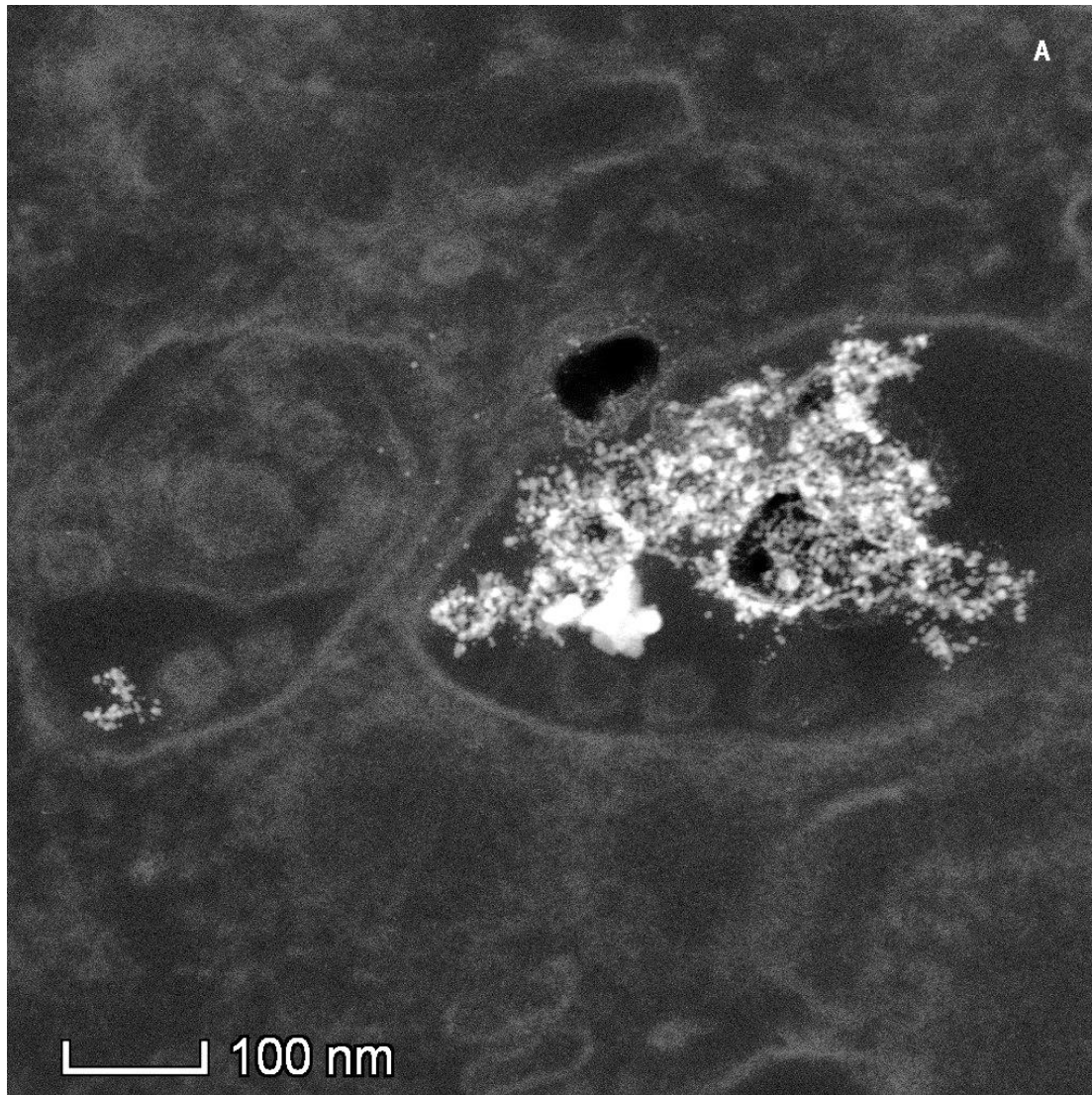


# Sections: Localisation of transformed silver nanoparticles in cells

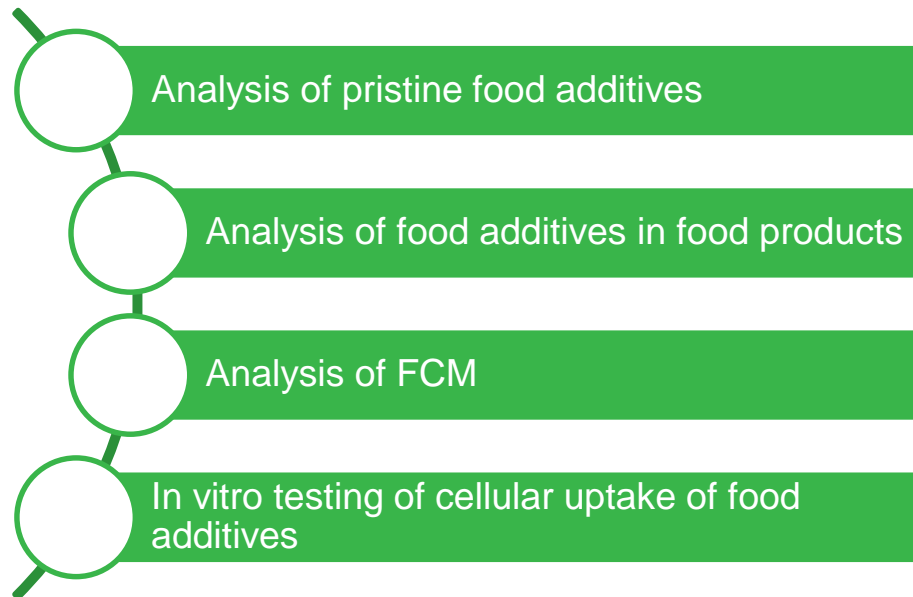
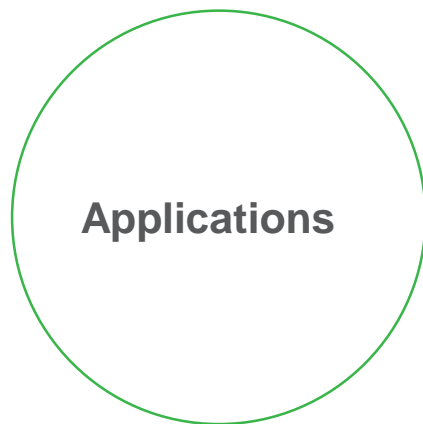




# Sections: Localisation of transformed silver nanoparticles in cells



# Conclusion



- Our methodologies are essential for our stakeholders and external clients to implement nano-specific regulations and legislation
- EFSA, ECHA, JRC, Nanoregister, FPS, DG4, FAVV, FAGG, Belgian and European companies and research institutions
- The methodologies are directly applicable in several sectors

# Webinar Workshop

## Scope:

- Provide **training on the physicochemical characterization** of nano-sized particles in food additives and in food products
- Focus on **TEM** and **single particle ICP-MS**.
- Demonstrate the **analytical capacities** of state-of-the-art methodologies to implement guidance and legislation based on **(automated) analytical TEM**.
- Method validation

**Provisional date:** beginning 2021

## How to subscribe:

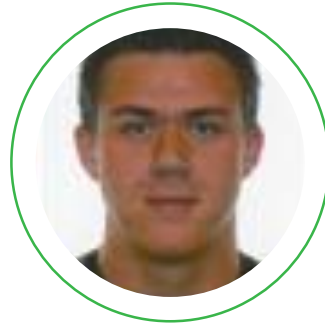
- mail to **EMgrp@sciensano.be**



# The EM-Team



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Technician



Frédéric Brassinne  
Lab Manager



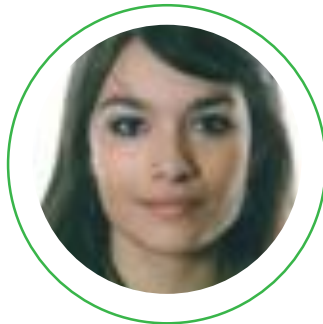
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Scientist  
Head of EM unit



Jan Mast  
Scientist  
Head of service



Frederic Van Steen  
Technician



Sandra De Vos  
PhD student



Stella Mathioudaki  
Scientist





Happy Holidays!!!  
from  
The Electron Microscopy unit  
Sciensano

Section of a textile fiber (polyester) containing  $\text{TiO}_2$  nanoparticles