

**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

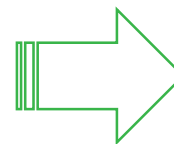


Physical NM characterization

New Instrument



Physical NM characterization
+ Chemical NM characterization



Physical NM characterization
+ Chemical NM characterization
+ Fully automated

Conventional TEM: Physical NM characterization

Methodology

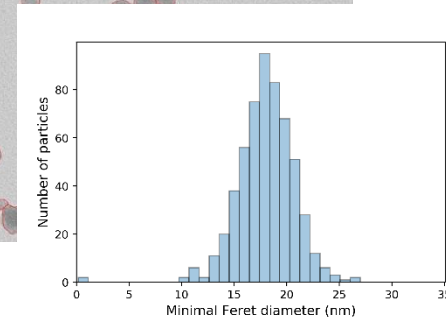
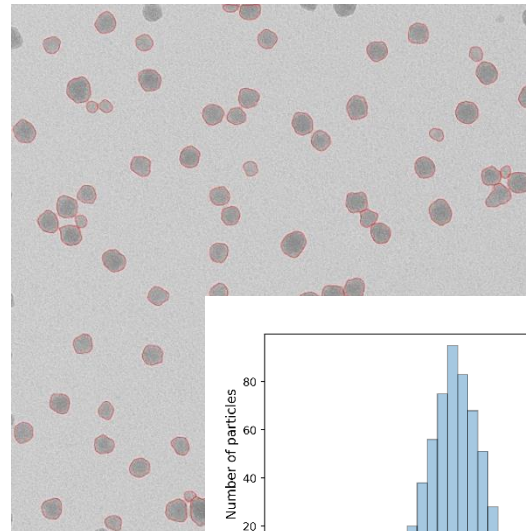
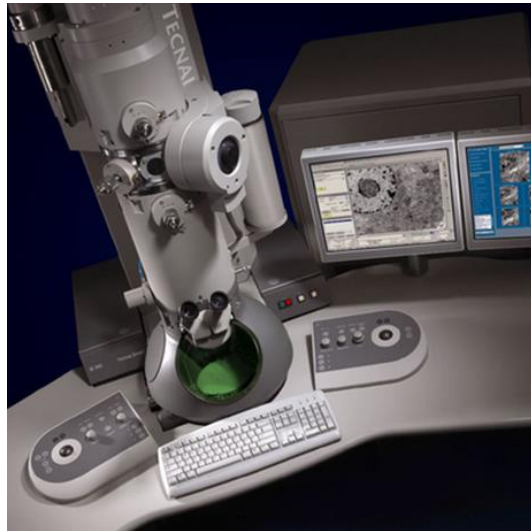
Sample
preparation

TEM
imaging

Image
analysis

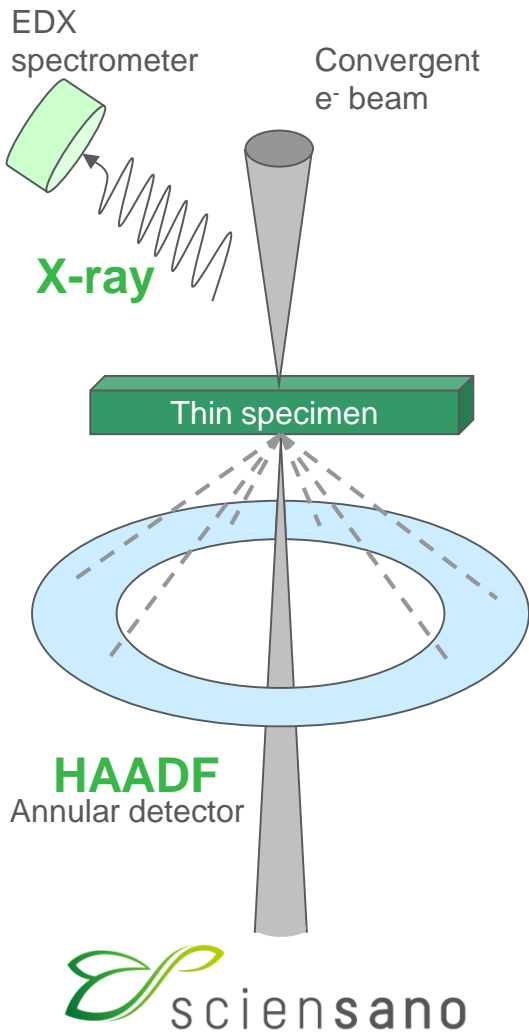
Size
distribution

Median
value

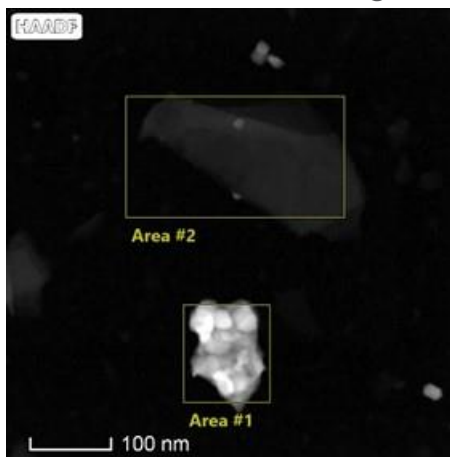


Developed for implementation of EC Recommendation on the definition of a nanomaterial (2011/696/EU)

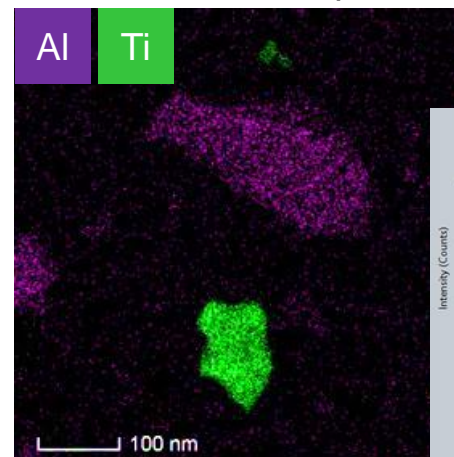
Analytical TEM: Physical + Chemical NM characterization



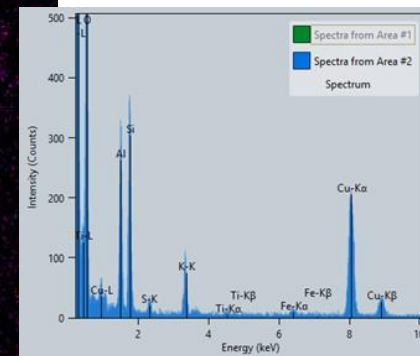
HAADF-STEM image



Elemental maps



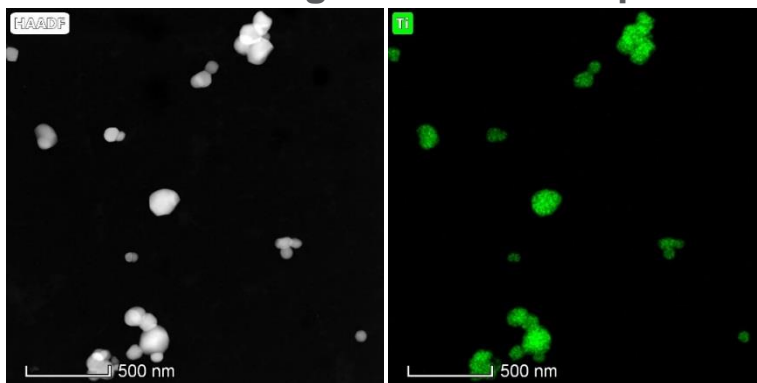
Characteristic X-rays



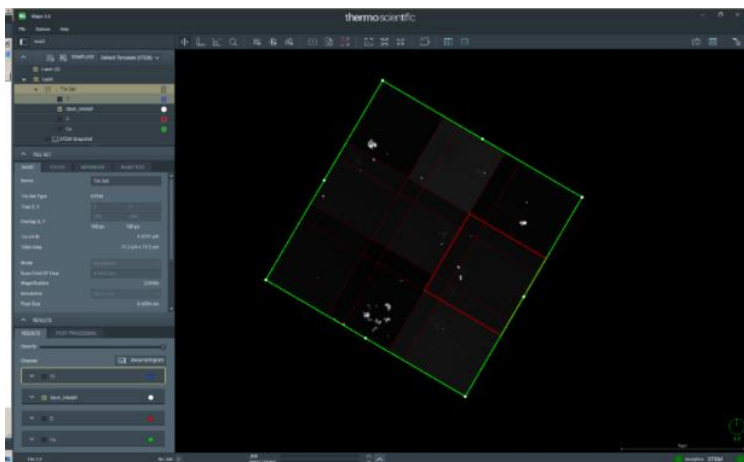
Automated TEM



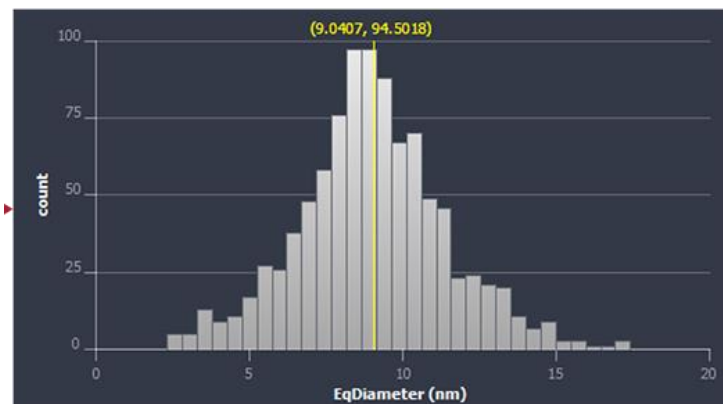
VELOX: STEM images and EDX maps recording



MAPS: Automated grid scanning



AVIZO: online particle measurement



Applications

Applications

Analysis of pristine food additives

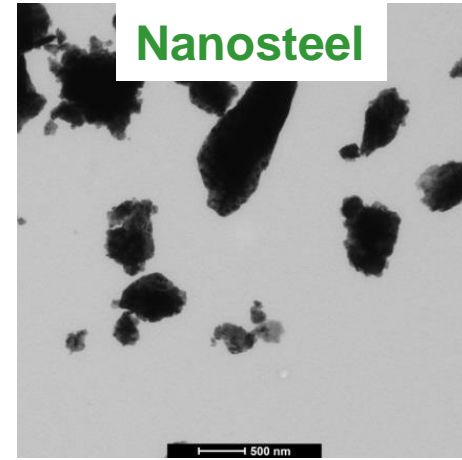
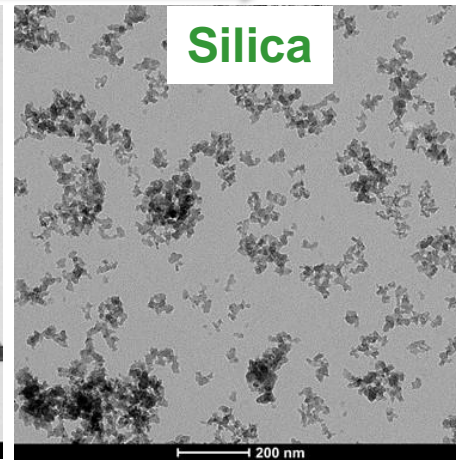
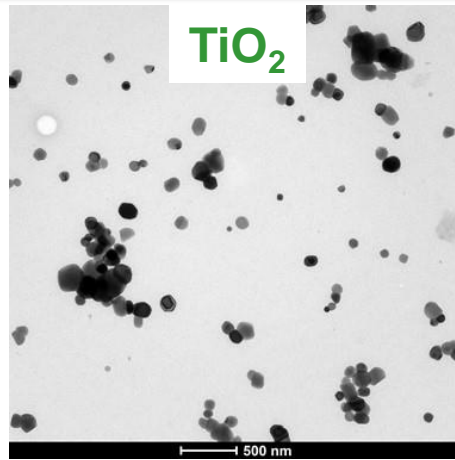
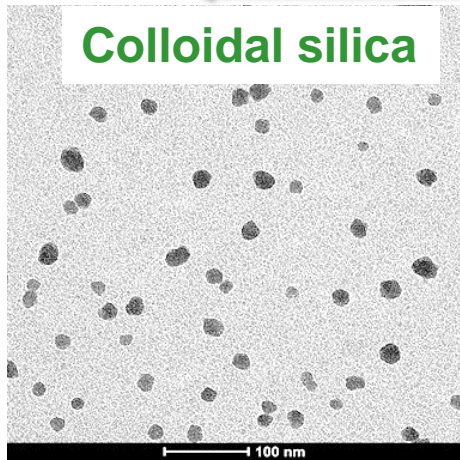
Analysis of food additives in food products

Analysis of FCM

In vitro testing of cellular uptake of food additives

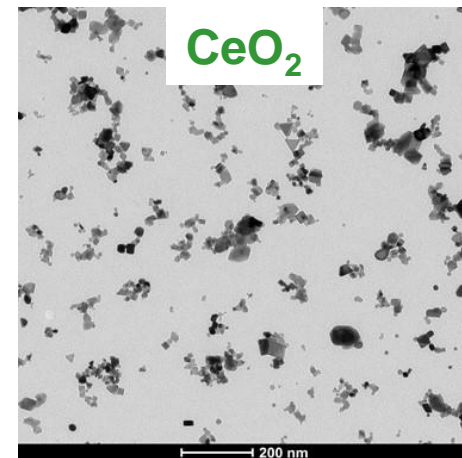
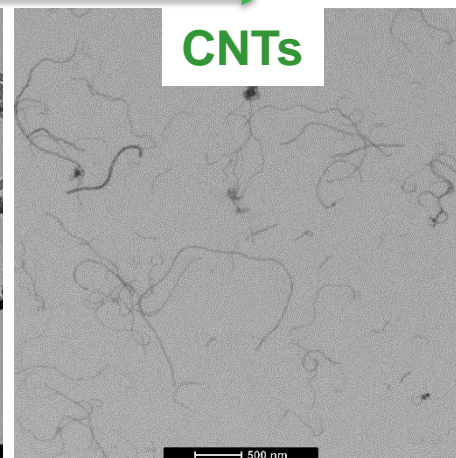
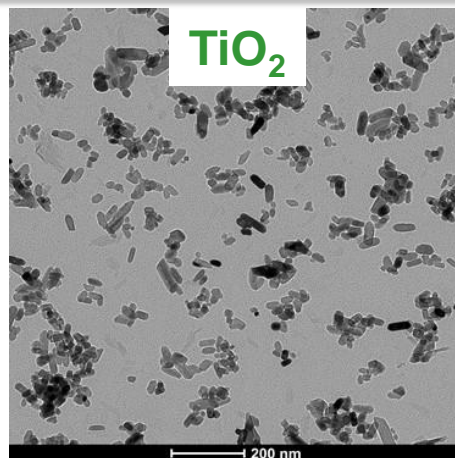
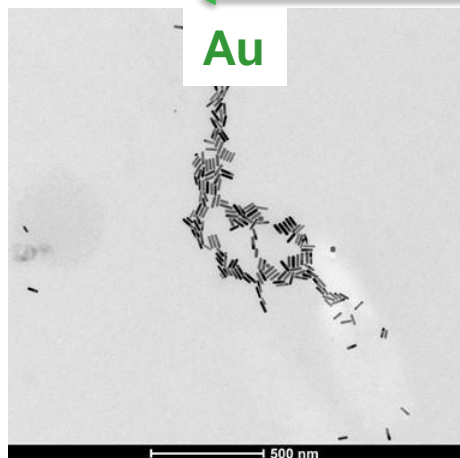
Physical characterization of pristine NM

Near-spherical



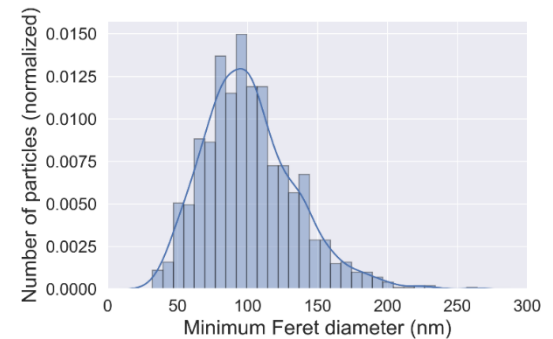
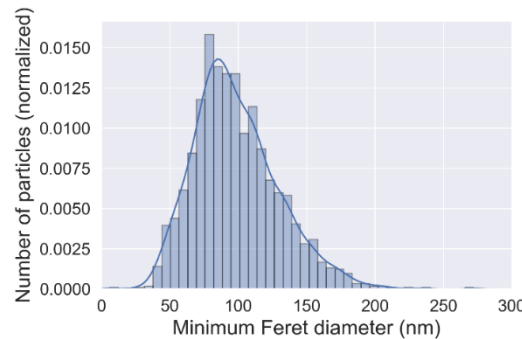
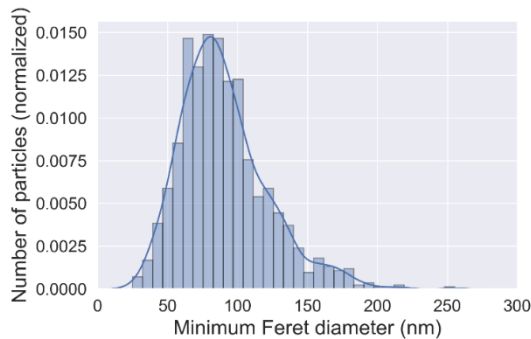
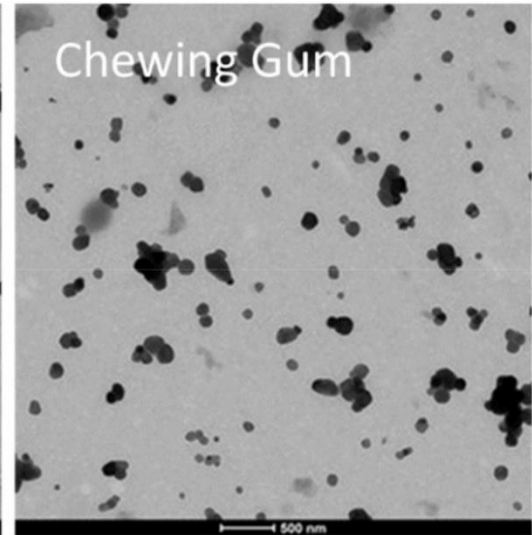
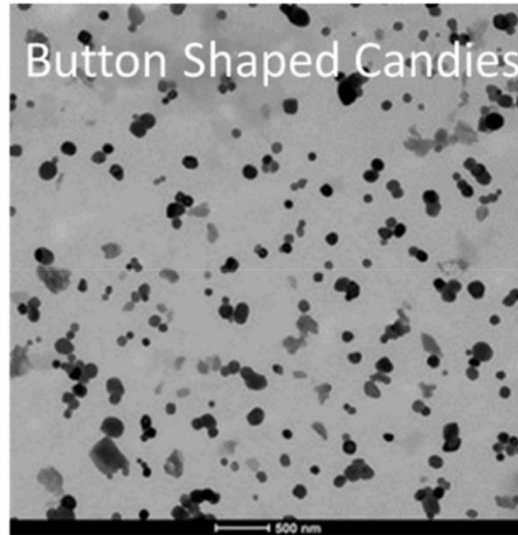
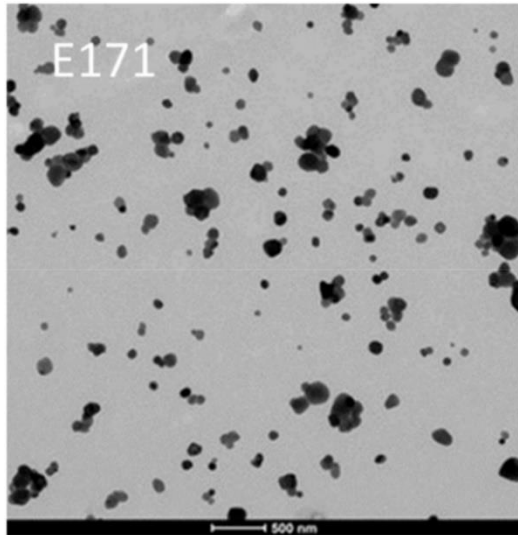
Platelets

Rod-like



Irregular

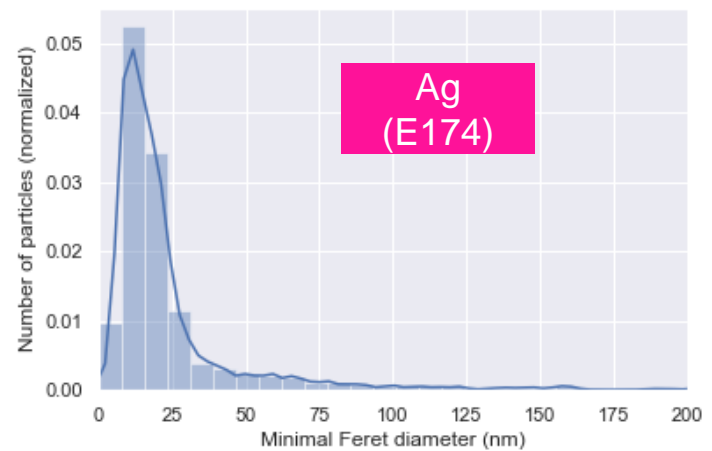
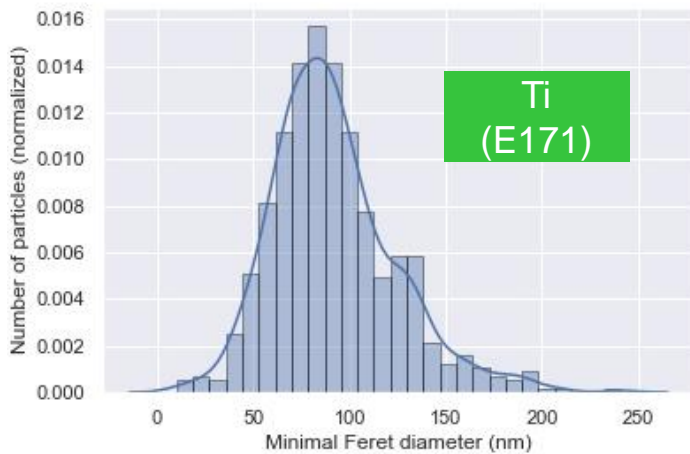
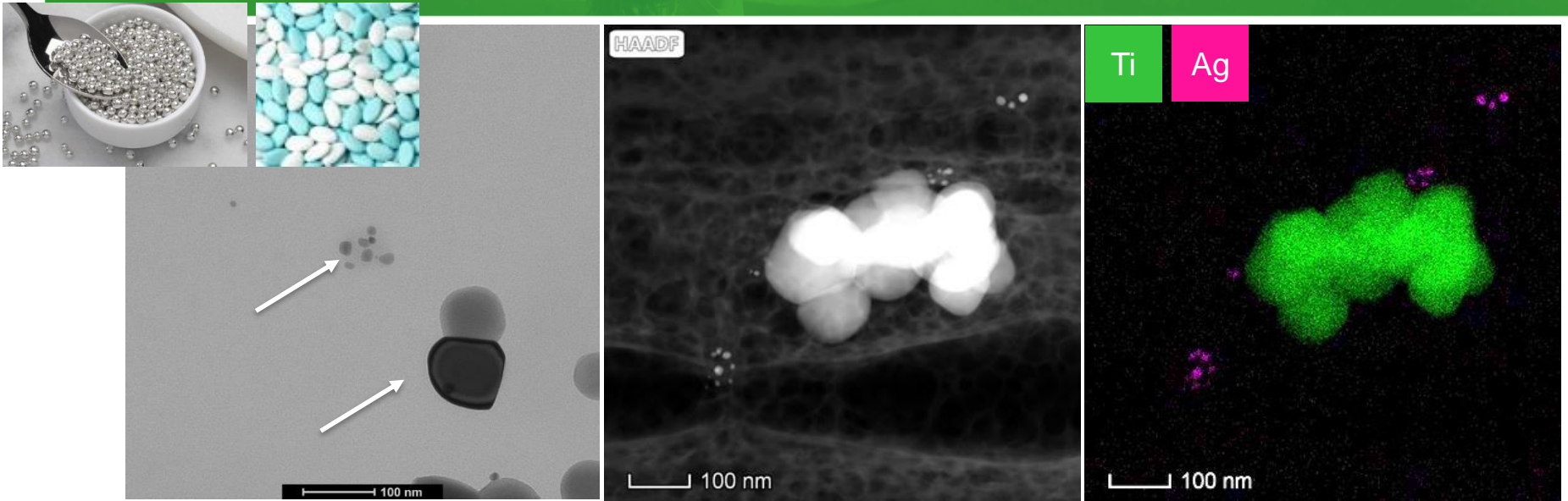
Particles in a food matrix - simple models



- 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

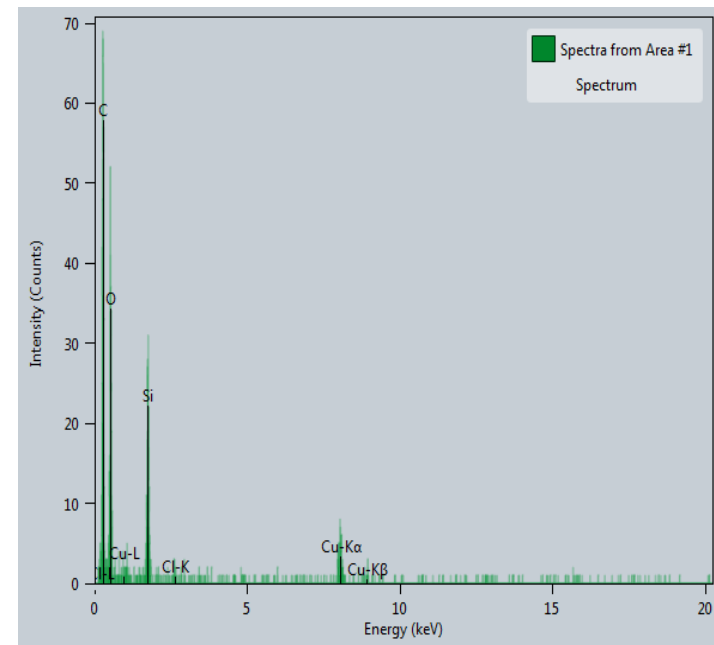
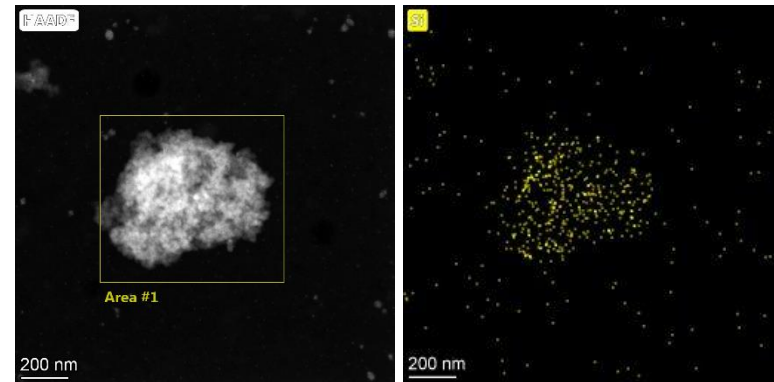
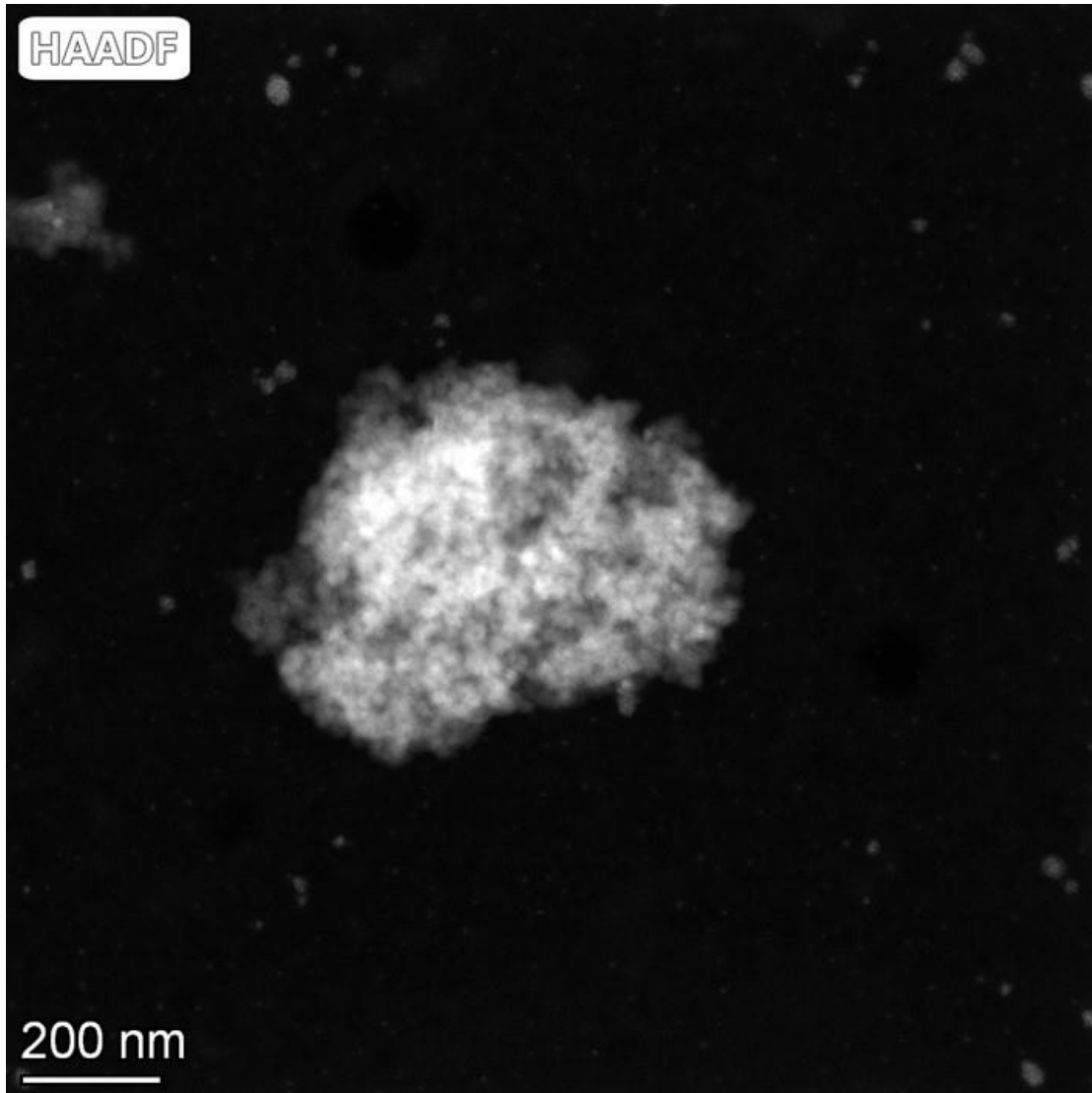
Particles in a food matrix: complex models

Food additives E171 and E174 in a food product



Particles in a food matrix: complex models

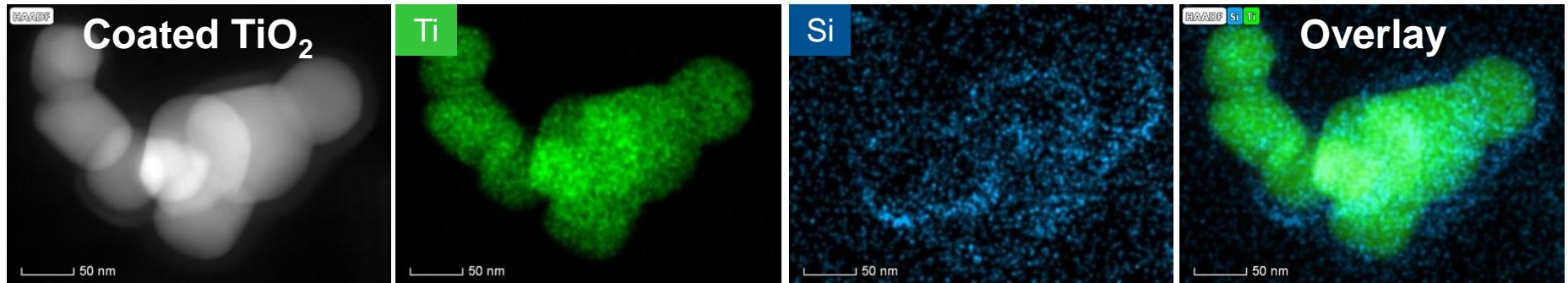
Food additive E551 (silica) in food products



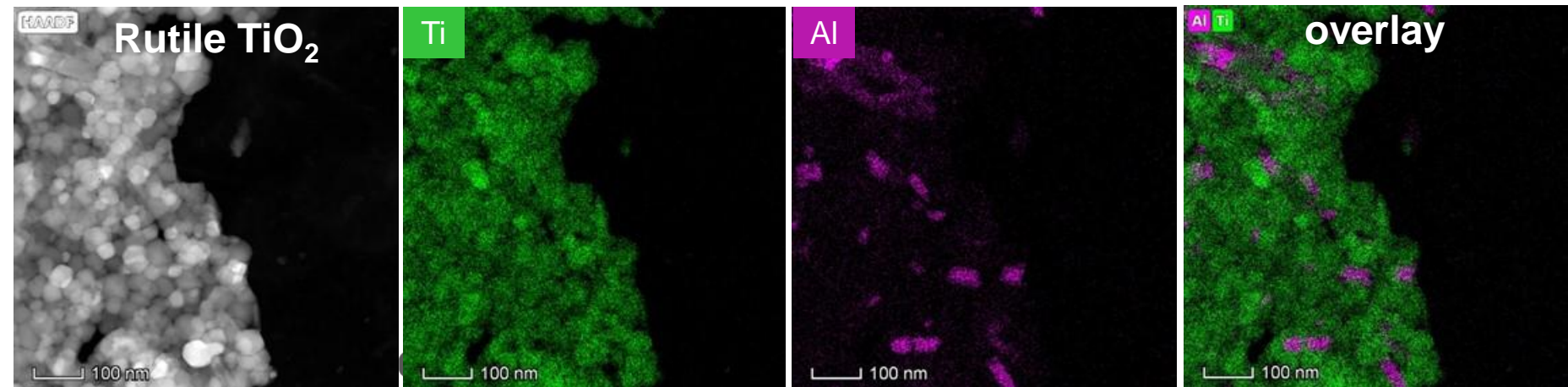
Analysis of coatings and impurities

Food additive E171

Visualization + composition of coatings

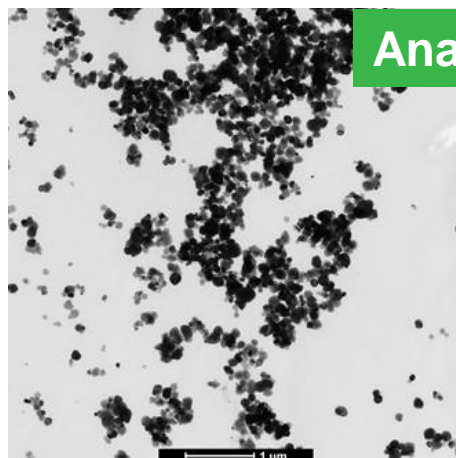
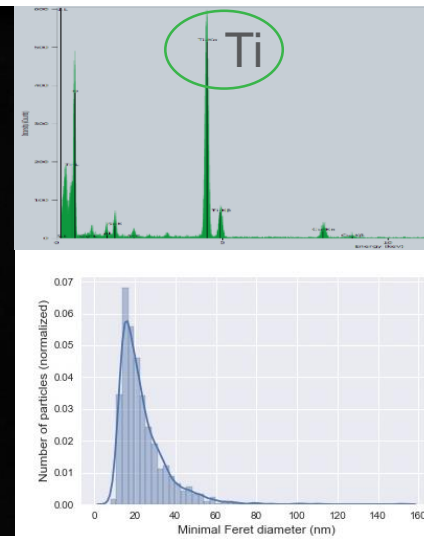
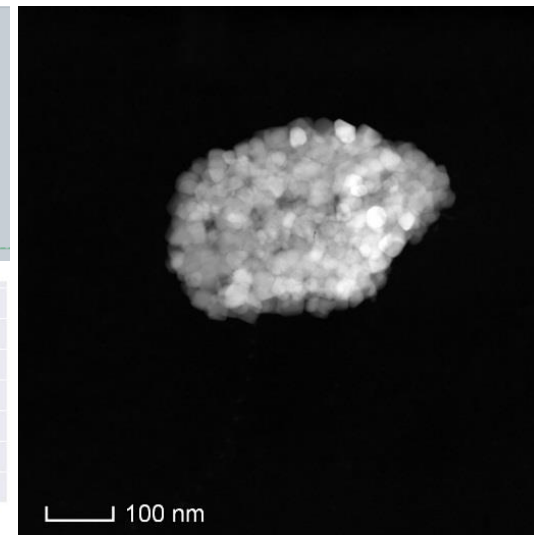
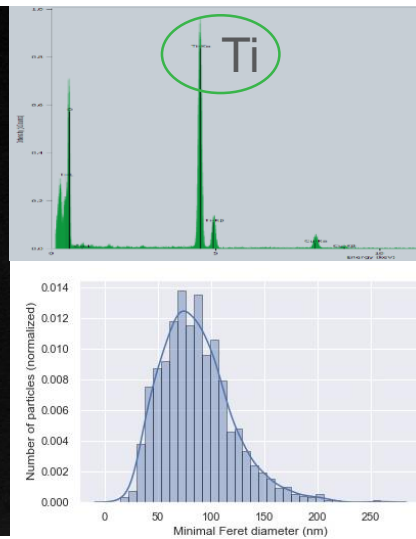
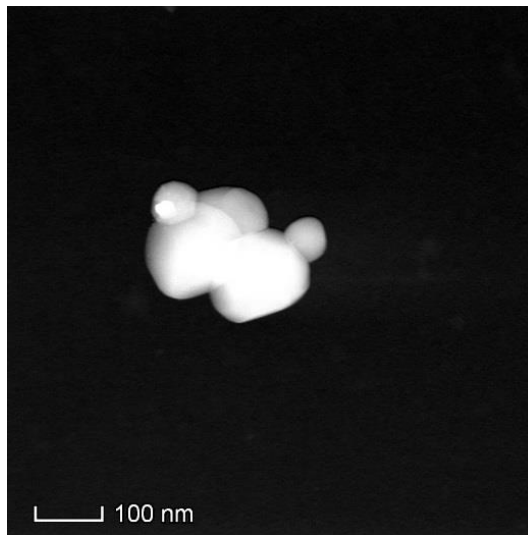


Visualization + composition of impurities

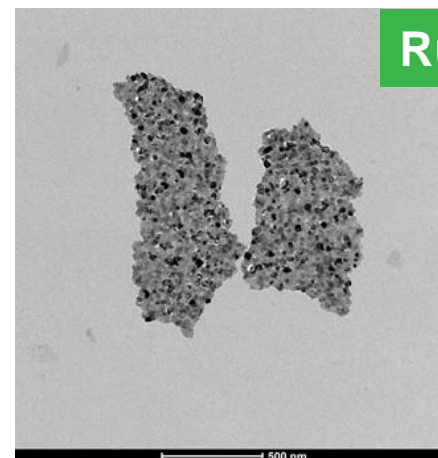
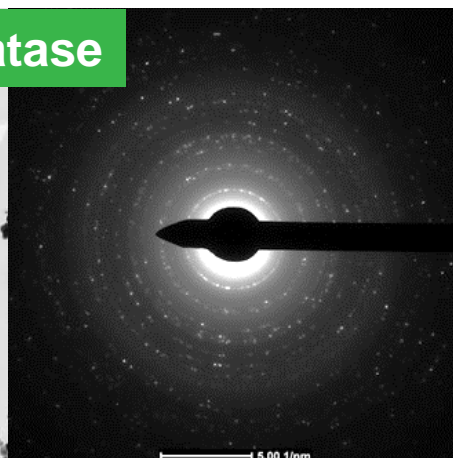


Food – cosmetics: Phase determination - crystallography

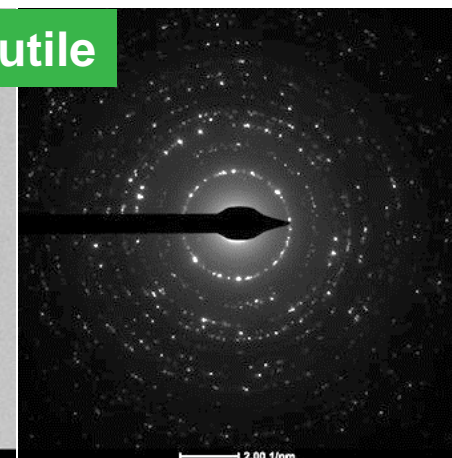
Anatase TiO_2 vs pearlescent pigments



Anatase



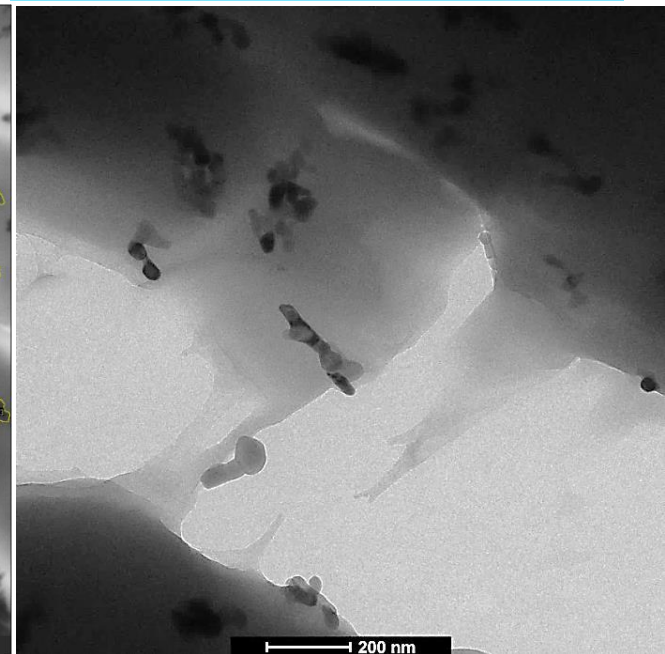
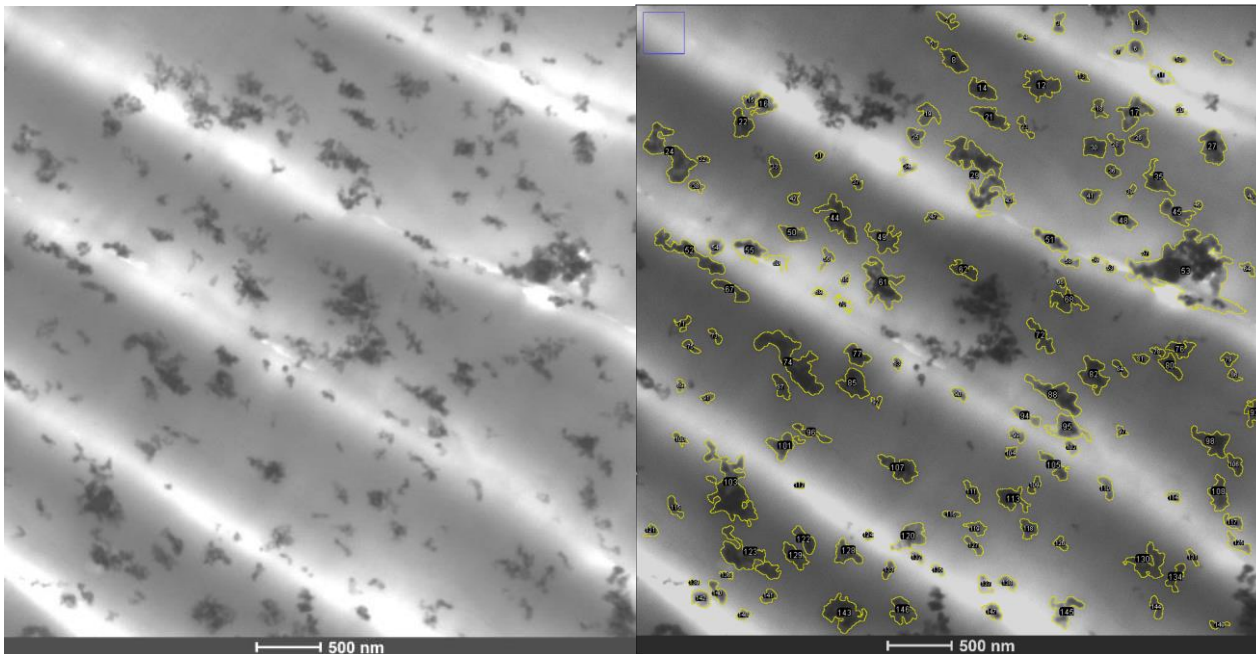
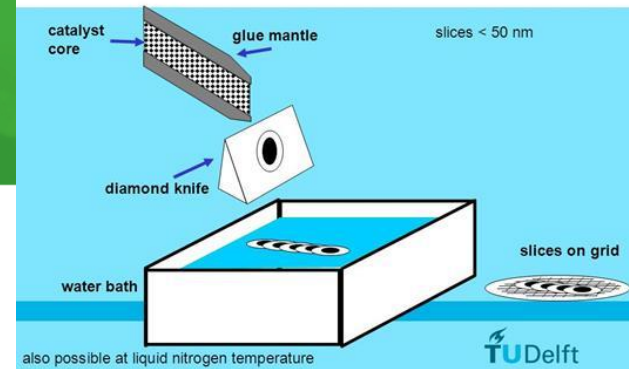
Rutile



Sections: Food contact materials

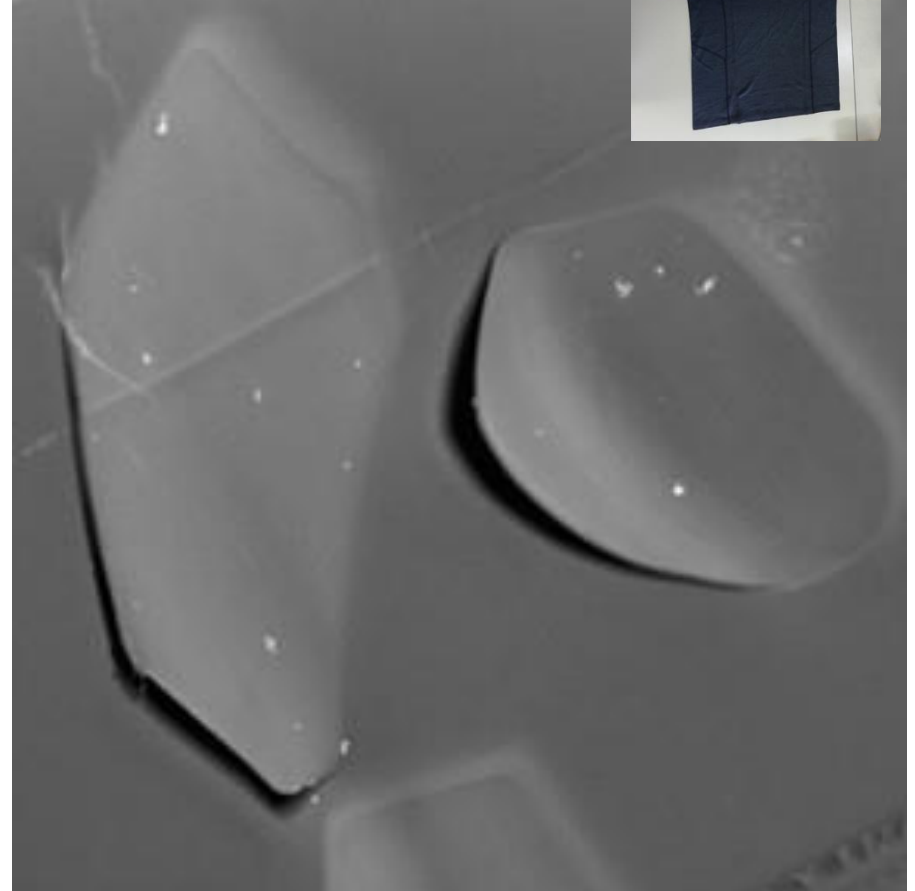
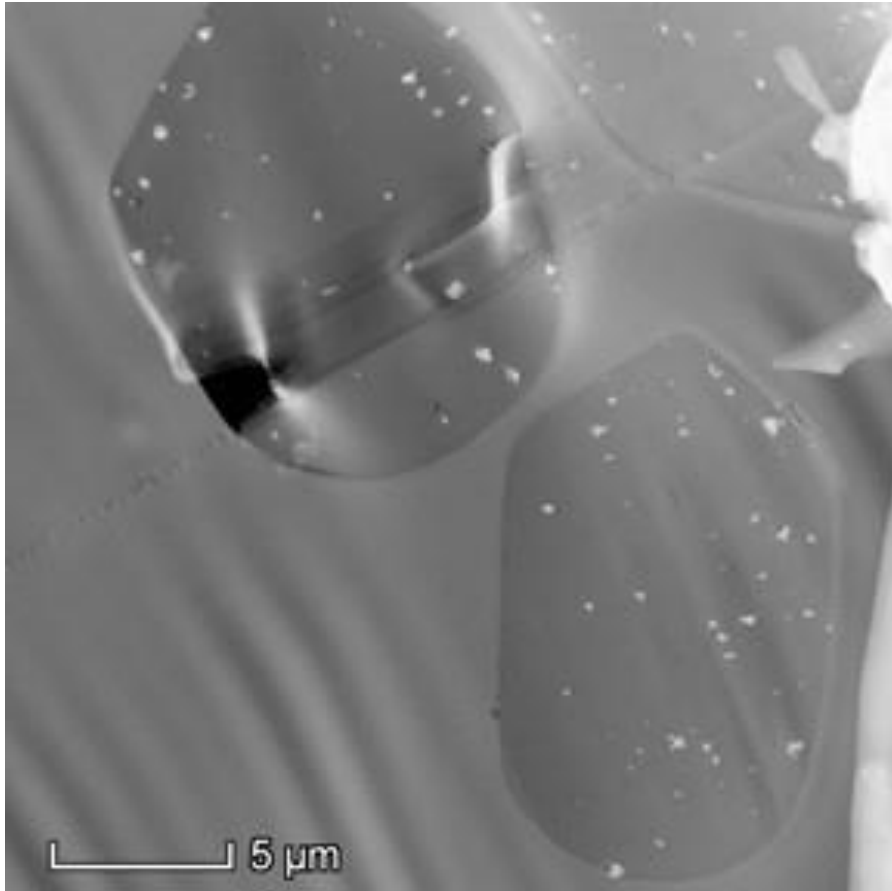
Detection of Fe_2O_3 in PE

Sample preparation: ultramicrotomy

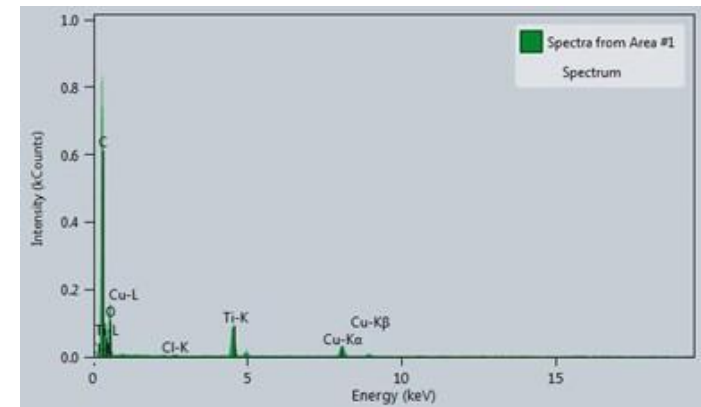
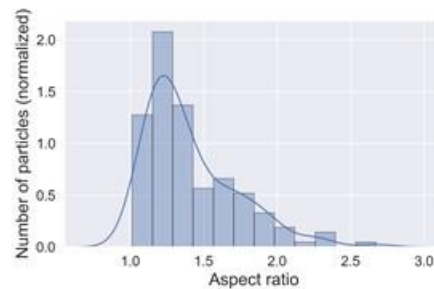
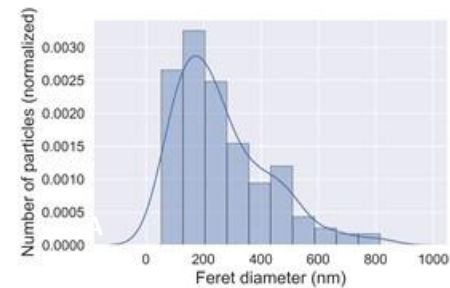
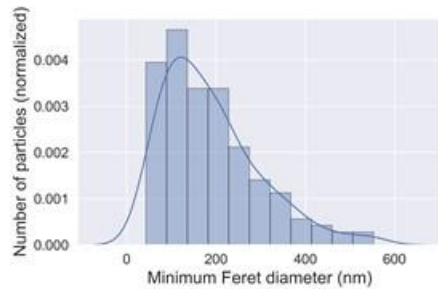
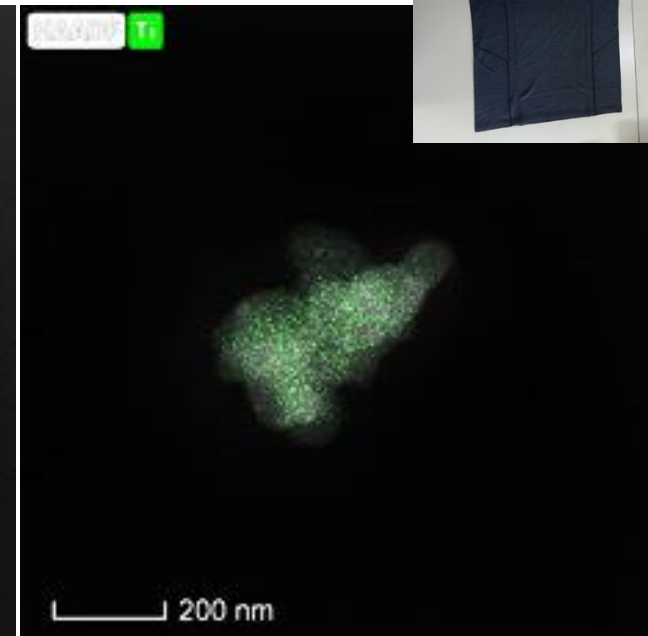
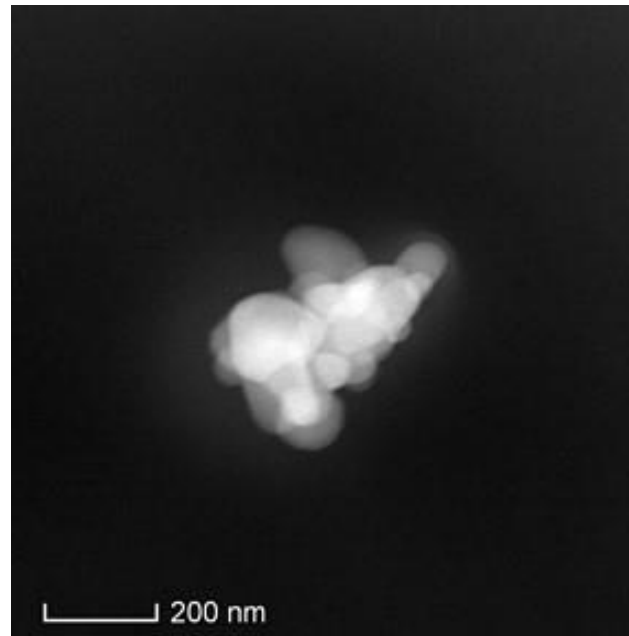
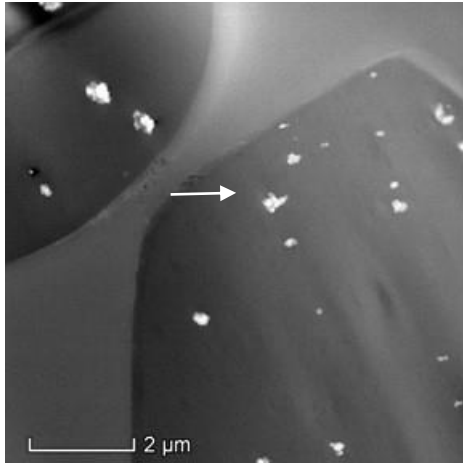


- Sections have a thickness of about 80-150nm
- Aggregates are easily detected
- Constituent particle detection is more difficult but not impossible

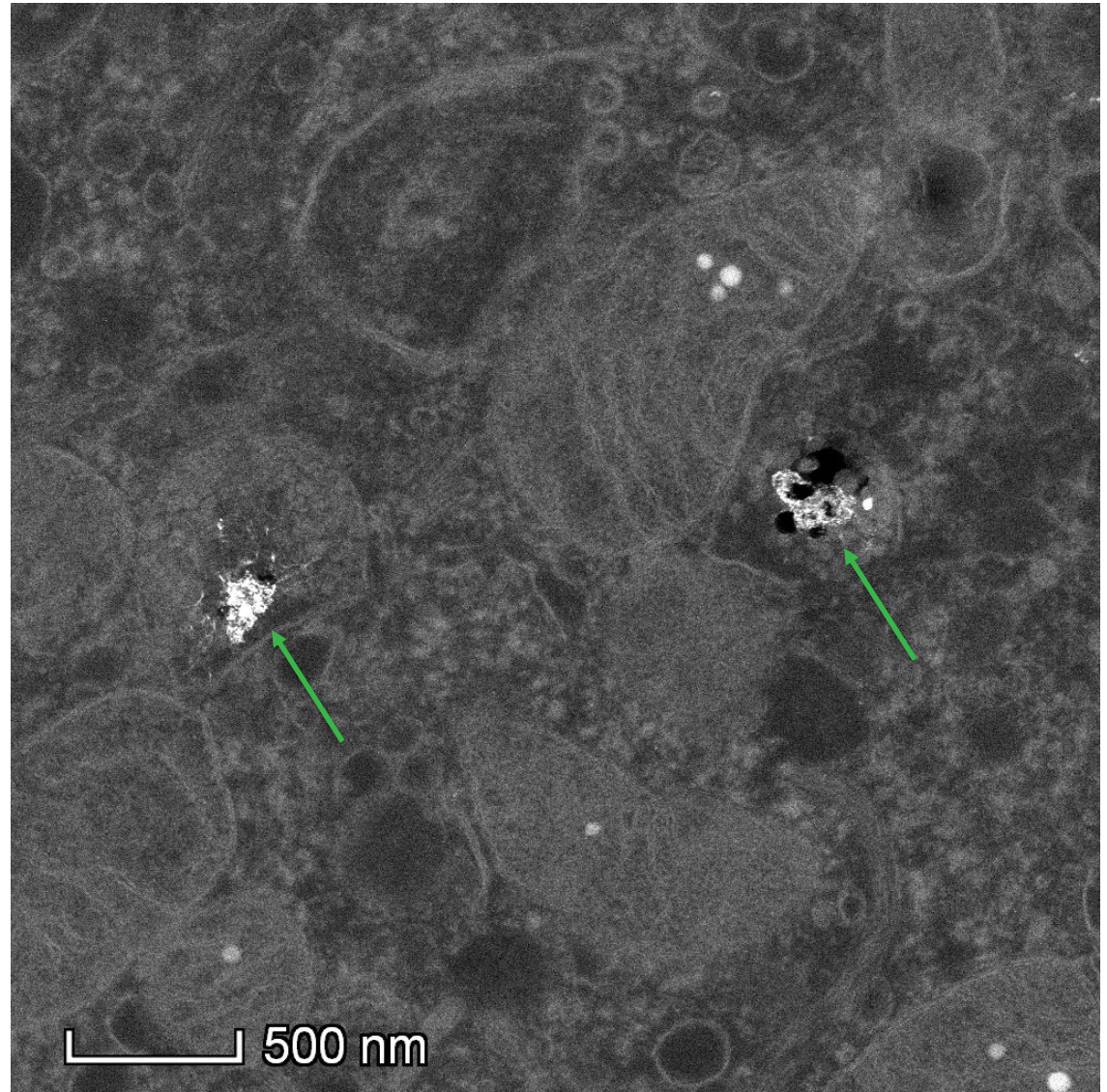
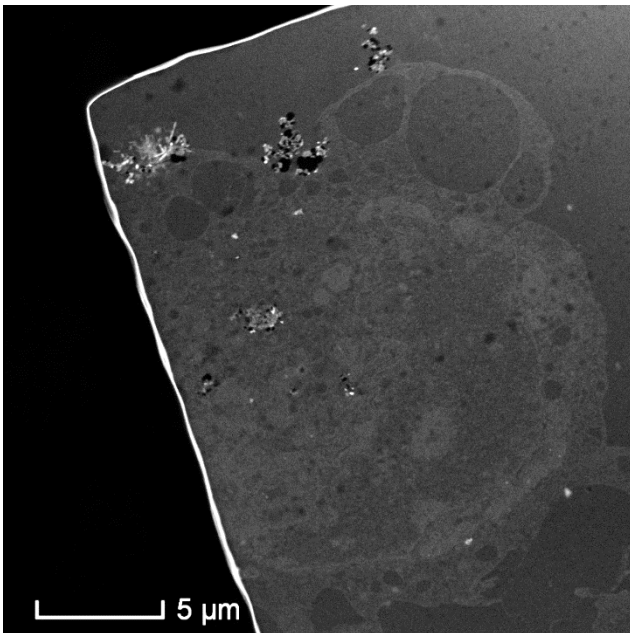
Detection of nanoparticles in fibers



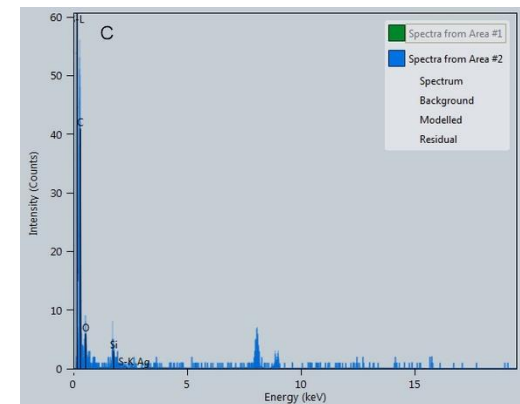
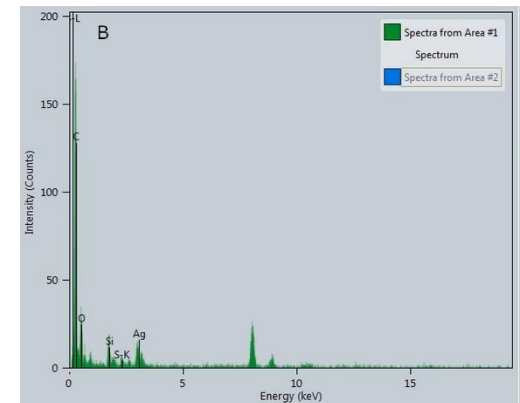
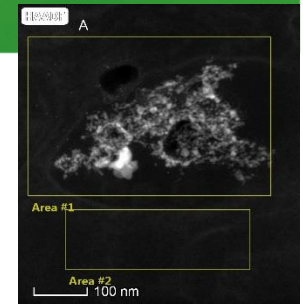
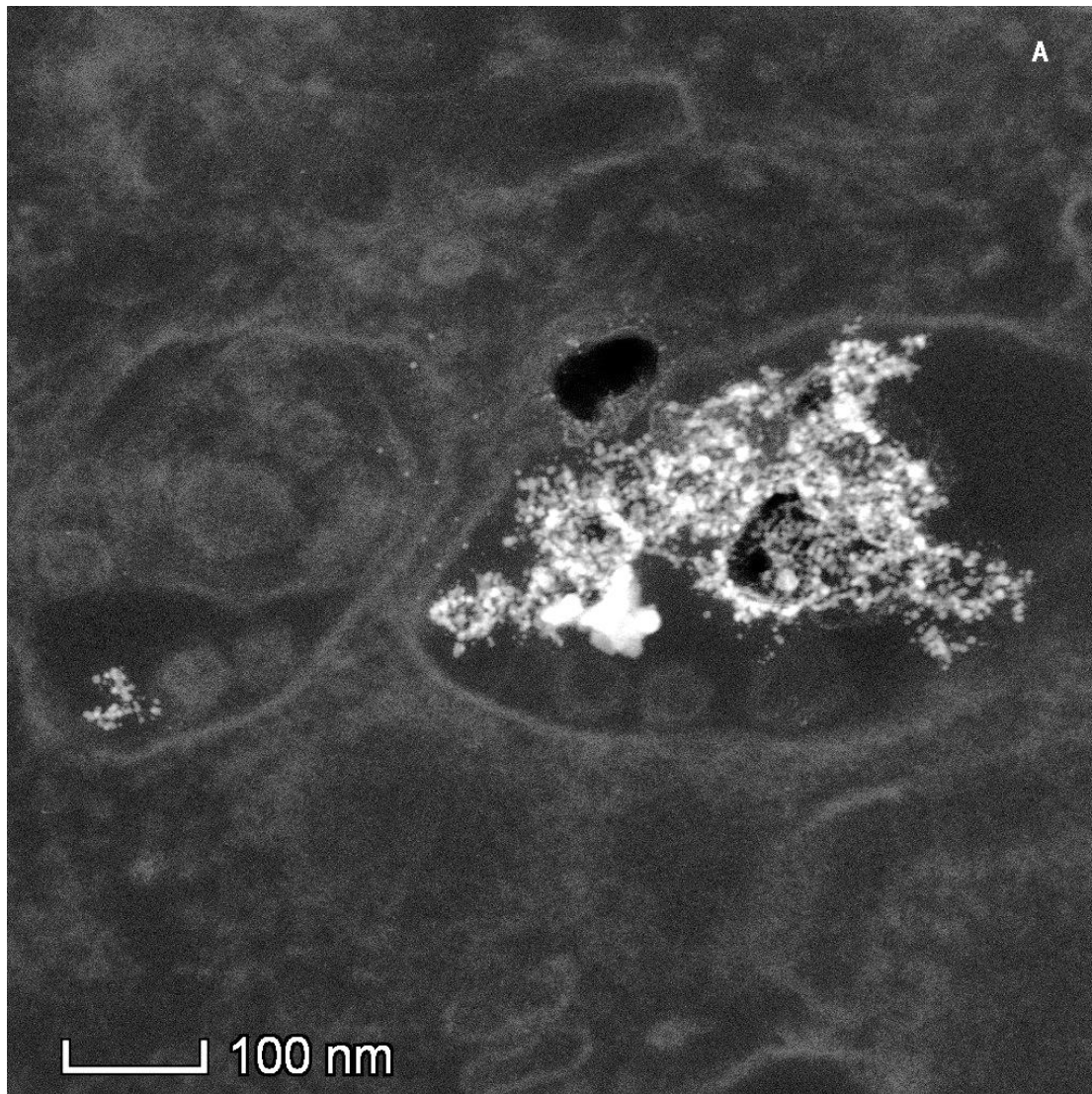
Detection of nanoparticles in fibers



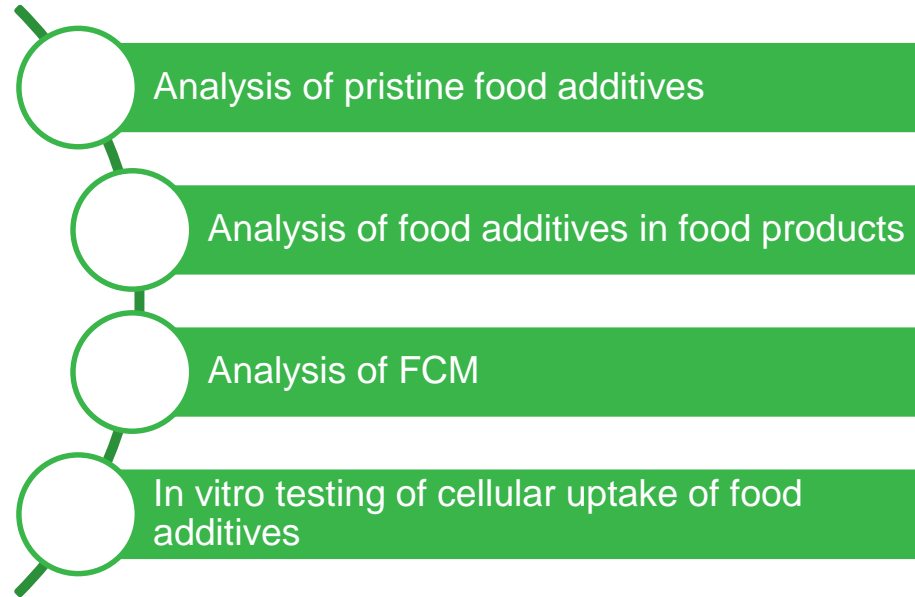
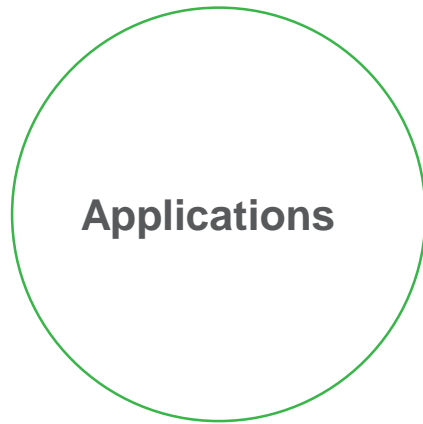
Sections: Localisation of transformed silver nanoparticles in cells



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

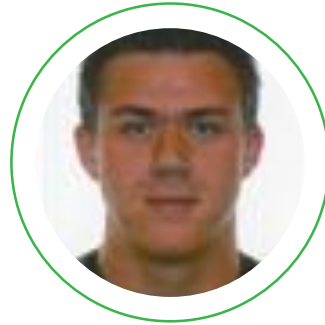
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- mail to EMgrp@sciensano.be

The EM-Team



Marina Ledecq
Technician



Frédéric Brassinne
Lab Manager



Eveline Verleysen
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
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2 μm

Section of a textile fiber (polyester) containing TiO_2 nanoparticles