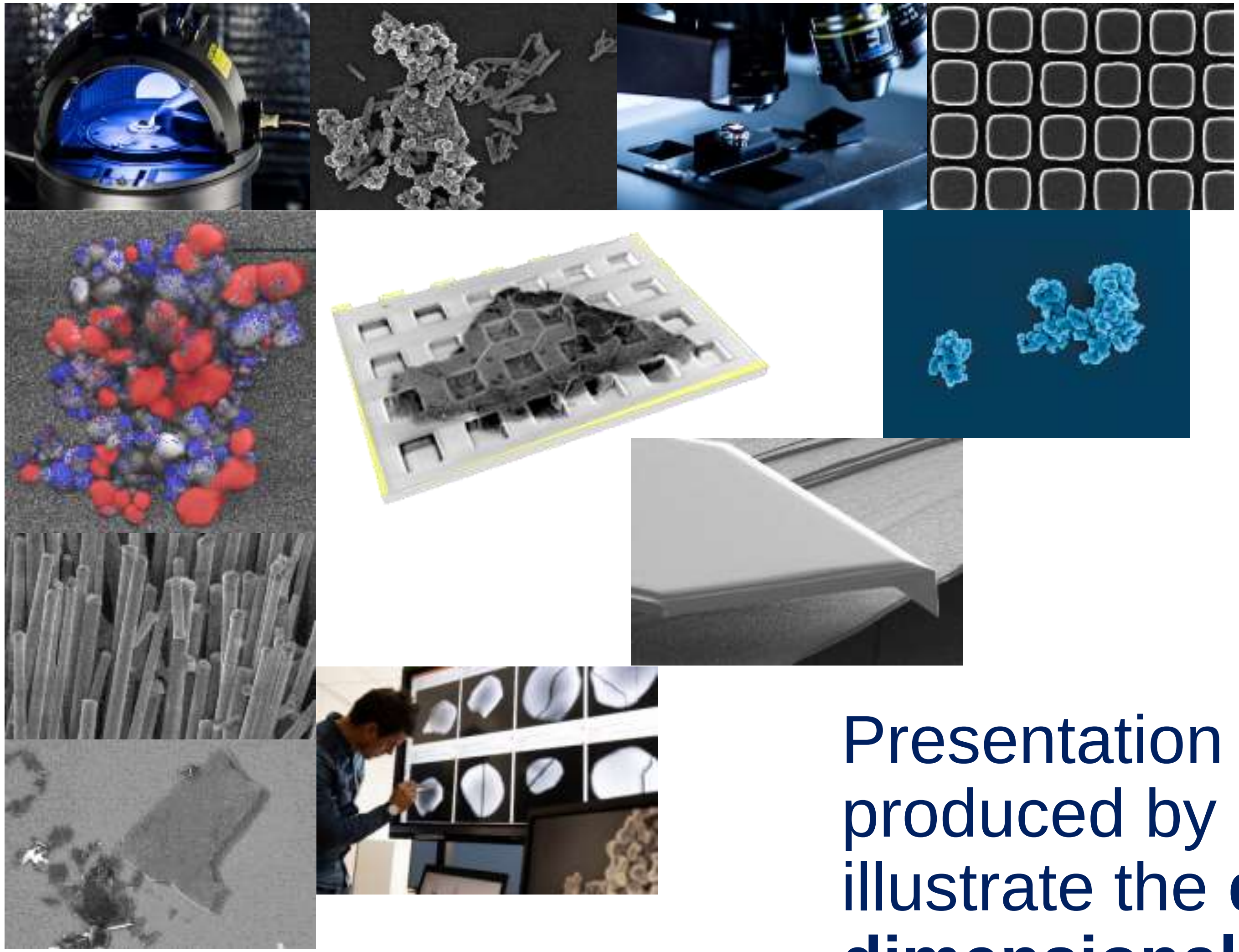


Presentation of a guide by example, produced by **NanoMesureFrance**, to illustrate the **difficulties encountered in dimensional analysis** by <u>electron and atomic force microscopy</u> of **particles**.

Valérie GODEFERT
LNE & NanoMesureFrance

François-Xavier OUF, PhD
LNE & NanoMesureFrance

USE OF NANOMATERIALS: *CHALLENGES*

https://euon.echa.europa.eu/fr/uses

Safe & sustainable development*

REGULATORY FRAMEWORK

TRUST

https://euon.echa.europa.eu/documents/2435000/3268573/nano_perception_study_en.pdf

NEED FOR RELIABLE & COMPARABLE **TEST DATA**

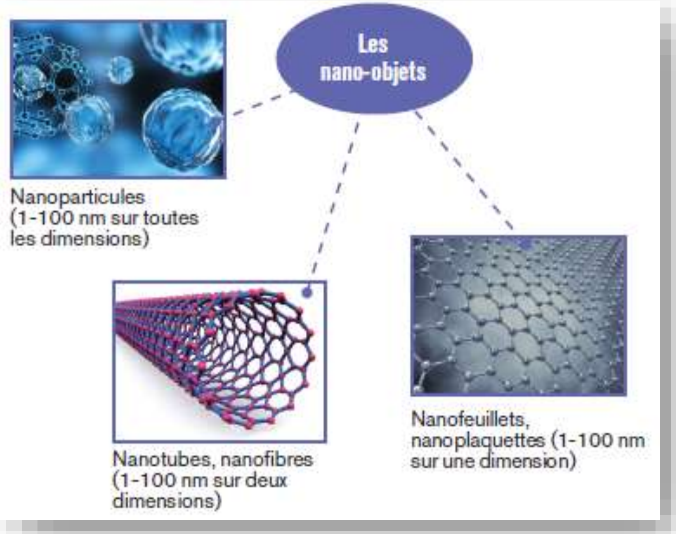
GOVERNANCE

*https://joint-research-centre.ec.europa.eu/jrc-news-and-updates/contributing-greener-eu-safe-and-sustainable-nanomaterials-design-stage-2021-04-19_en

NANOMATERIALS & NANOPARTICLES

Scientific Concept

NANO-OBJECT / NANOPARTICLE



XP ISO/TS 80004-1

≠

Regulatory Concept

NANOMATERIALS

HAS ADOPTED THIS RECOMMENDATION:

1. 'Nanomaterial' means a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions:

   (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm;

   (b) the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;

   (c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm.

   In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 μm need not be considered.

   However, a material with a specific surface area by volume of < 6 m^2/cm^3 shall not be considered a nanomaterial.

2022/C229/01

NANOMATERIALS & NANOPARTICLES

Scientific Concept

NANO-OBJECT
/
NANOPARTICLE

≠

Regulatory Concept

NANOMATERIALS

Discrepancies between standard definitions or **recommendations** in different **regulatory contexts**

Knowledge of the **external dimensions** of <u>nano-objects</u> is key to <u>identifying</u> & <u>assessing the performance</u> of **nanomaterials**

What **guides are available** recommanding the <u>best analytical methods</u>?

AVAILABLE GUIDES: *FRENCH LEVEL*

ANSES GUIDE

- **FR experts** under ANSES coordination
- Comprehensive guide **for experts** to produce data on key properties expected for REACh
- **Partially operational** and in need of revision to take account of the new EU recommendation

anses

Revue des méthodes analytiques disponibles pour la caractérisation des nano-objets, de leurs agrégats et agglomérats en vue de répondre aux exigences réglementaires

RAPPORT d'appui scientifique et technique

https://www.anses.fr/system/files/AP2018SA0168Ra.pdf

METHODOLOGICAL NOTE SCL

- **Expertise of** Service Commun des Laboratoires (DGCCRF, DGDDI)
- **Electron Microscopy** as a reference, supported by screening methods (spICP-MS, DLS…)
- Importance of sample preparation & assessments of laboratory skills

MINISTÈRE DE L'ÉCONOMIE DES FINANCES ET DE LA SOUVERAINETÉ INDUSTRIELLE ET NUMÉRIQUE

Service commun des laboratoires DGCCRF - DGDDI

https://www.economie.gouv.fr/files/files/directions_services/dgccrf/securite/produits_non_alim/note-methodologique-scl-anomateriaux.pdf?v=1678964736

AVAILABLE GUIDES: *EUROPEAN LEVEL*

EUROPEAN PROJECTS

- **Development of reference** or **representative testing materials**
- **Validation of methods** *(TEM, SEM, AFM, SAXS, PTA, DLS, aerosol…)* & **test protocols:** sample preparation, count number size distribution analysis, analysis rules, inter-laboratory comparison
- **Methods applicable to specific nanomaterials** & qualified on the basis of samples that are sometimes far from « real life » ones

https://publications.jrc.ec.europa.eu/repository/handle/JRC117501

JRC GUIDES (**EU LAB**)

- **Key terms & concepts** related to the European recommendation of definition of nanomaterials
- **Recommandations** for selecting the **most appropriate analytical methods / strategies**

https://publications.jrc.ec.europa.eu/repository/handle/JRC113469

https://publications.jrc.ec.europa.eu/repository/handle/JRC132102

AVAILABLE GUIDES: *INTERNATIONAL LEVEL*

OECD TEST GUIDELINES

- Available free of charge on the OECD website
- Documents including preparation protocols and recommended materials for method validation

TG N°125: NANOMATERIAL PARTICLE SIZE AND SIZE DISTRIBUTION OF NANOMATERIALS

- Inter-laboratories comparison on analytical techniques: *AFM, CLS, DLS, DMAS, PTA/NTA, SAXS, SEM, TEM*
- Electron Microscopy: sample preparation + counting rules

https://www.oecd-ilibrary.org/environment/test-no-125-nanomaterial-particle-size-and-size-distribution-of-nanomaterials_af5f9bda-en

Nano Mesure France

AVAILABLE GUIDES: *LESSONS LEARNED*

ELECTRON MICROSCOPY (EM) AS A « REFERENCE » BUT:

- **Number of standards*** or **guidelines** is too limited & often « theoretical »
- **Available FR, EU & INT guides** are still at a general level and may lead to interpretations
- **Level of applicability** of EM to « real-life » nanomaterials is poorly documented

EM ANALYTICAL PROTOCOLS NEED TO BE **DEVELOPED, VALIDATED & HARMONISED**

NEED OT KNOW ABOUT:

- Difficulties & limitations, of **electron microscopy techniques**, encountered in the dimensional analysis of **« real-life » particulate materials**

***ISO 19749:2021 Nanotechnologies** — *Measurements of particle size and shape distributions by scanning electron microscopy (SEM)*
***ISO 21363:2020 Nanotechnologies** — *Measurements of particle size and shape distributions by transmission electron microscopy (TEM)*

NANOMESUREFRANCE: *A STRUCTURING RESPONSE*

PIPAME
Pôle interministériel de prospective et d'anticipation des mutations économiques

2018 - 2020
Discussions with various stakeholders (> 100)

Nano Mesure France

September 2022
Creation of
NANOMESUREFRANCE


ASSOCIATION « NANOMESUREFRANCE »

STATUTS DE L'ASSOCIATION

Non profit ASSOCIATION

3 founding members

FRANCE CHIMIE	**PRESIDENT**
FEBEA FÉDÉRATION DES ENTREPRISES DE LA BEAUTÉ	**VICE-PRÉSIDENT**
LNE	**TREASURER & SECRETARY**

Financial support from the Ile-de-France region and the French government (09/2021 - 09/2024)

PRÉFET DE LA RÉGION D'ÎLE-DE-FRANCE · Région ÎledeFrance · bpifrance

NANOMESUREFRANCE: *MAIN CHALLENGES*

Identify

nanomaterials in support of increased traceability of their use in value chains

Characterise

their key physical and chemical properties and their possible changes during the life of the substance with regard to regulatory requirements

Evaluate

emissions of nano-objects at different key stages of the product life cycle as part of the risk analyses to be conducted

- IDENTIFYING NEEDS & COORDINATING FRENCH EFFORTS IN THE FIELD OF **PRE-STANDARDIZATION OF TEST METHODS**
- DEFINE **PRIORITIES** AND BUILD **COLLECTIVE ROADMAP & SOLUTIONS**

NANOMESUREFRANCE: *OUR MEMBERS IN 2025*

Materials & Chemistry

- FRANCE CHIMIE
- BASF — We create chemistry
- MERCK
- Baikowski
- UGAL
- TRONOX
- IMERYS Talc
- SENSIENT — COSMETIC TECHNOLOGIES
- Carbon Waters — ENDLESS HORIZON OF MATERIALS

Cosmetics

- FEBEA — Fédération des entreprises de la beauté
- COSMED
- COSME BIO
- L'ORÉAL
- CHANEL — PARFUMS BEAUTÉ
- LVMH RECHERCHE — Parfums & Cosmétiques
- Pierre Fabre
- sisley PARIS
- INSTITUT NAOS — des sciences de la vie
- CATALYONS

Others

- IPC
- MICHELIN
- MERCK
- IFP Energies nouvelles
- CSTB — le futur en construction
- SARPI VEOLIA
- ASNR
- DANONE

Service providers / Analytical Platforms

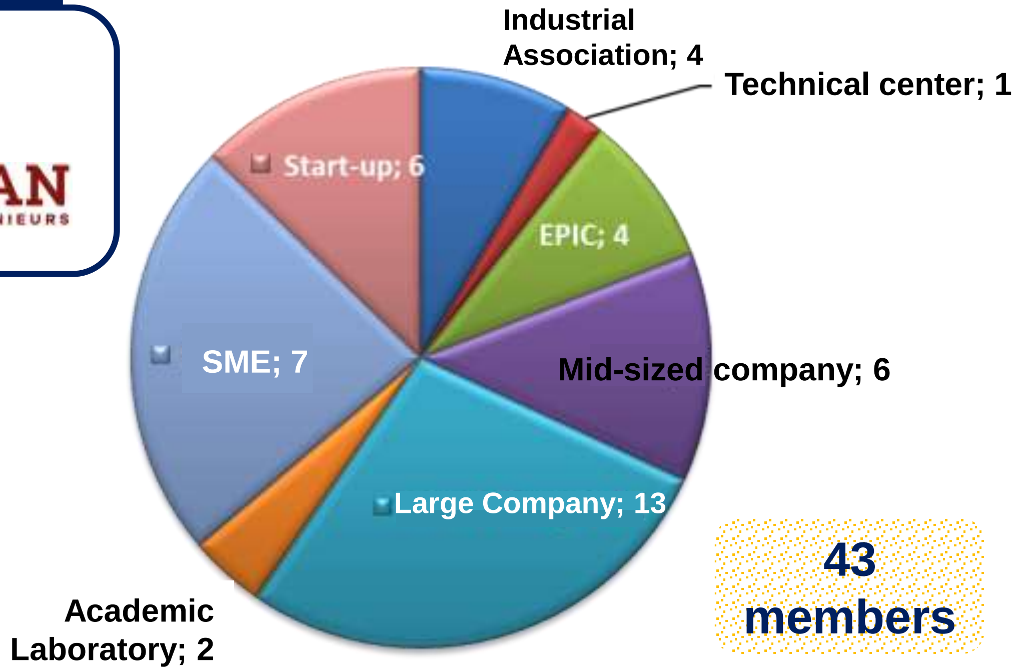
- LNE
- eurofins
- FILAB — Laboratoire de chimie
- Institut Jean Lamour
- ILV — Institut Lavoisier de Versailles — UVSQ — CNRS
- PURPAN — École d'ingénieurs

Instrumentation

- ThermoFisher SCIENTIFIC
- Nanoscale Metrix — Ultra-small size distribution
- Digital Surf
- HORIBA
- Quantum Design France
- JEOL
- Park SYSTEMS

Nanomedecine

- NANOBIOTIX — EXPANDING LIFE
- inside Tx
- SUPERBRANCHE



43 members

NANOMESUREFRANCE: *ADVISORY BOARD*

- **Communicating on the actions** carried out by NanoMesureFrance
- Express the **views, expectations and recommendations of key external bodies**
- To shed light on **regulatory issues**
- Provide information on **initiatives undertaken** by other national, european or international bodies

ASSOCIATION REPRESENTATIVES

Founding Members

- France Chimie
- FEBEA
- LNE

WG Representatives

EXTERNAL EXPERTS

OECD

cnrs

GDR NaMasTE

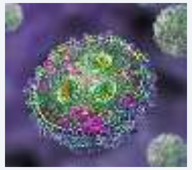
DGE DIRECTION GÉNÉRALE DES ENTREPRISES

JRC EUROPEAN COMMISSION

afnor NORMALISATION

NANOMESUREFRANCE : *NMs IDENTIFICATION*






1. SHARE GOOD PRACTICES WITH ALL MEMBERS

- **Inter-working groups meetings**
- **NanoMesureFrance Website / events** (technical days “Cosmetics”, “Identification”, “REACh” or workshops “Nanomedecine”)

2. ASSESS THE APPLICABILITY OF THE RECOMMENDED METHODS

- Subjectivity of particles counting rules
- Limitations of electron microscopy inherent in the physchem specificities of “real-life” particulate materials

3. IDENTIFY ANALYTICAL SOLUTIONS AND THE MEANS TO IMPLEMENT THEM IN RESPONSE TO DIFFICULTIES AND LIMITATIONS

Nano Mesure France

SHARE GOOD PRACTICES: *JRC GUIDANCE*

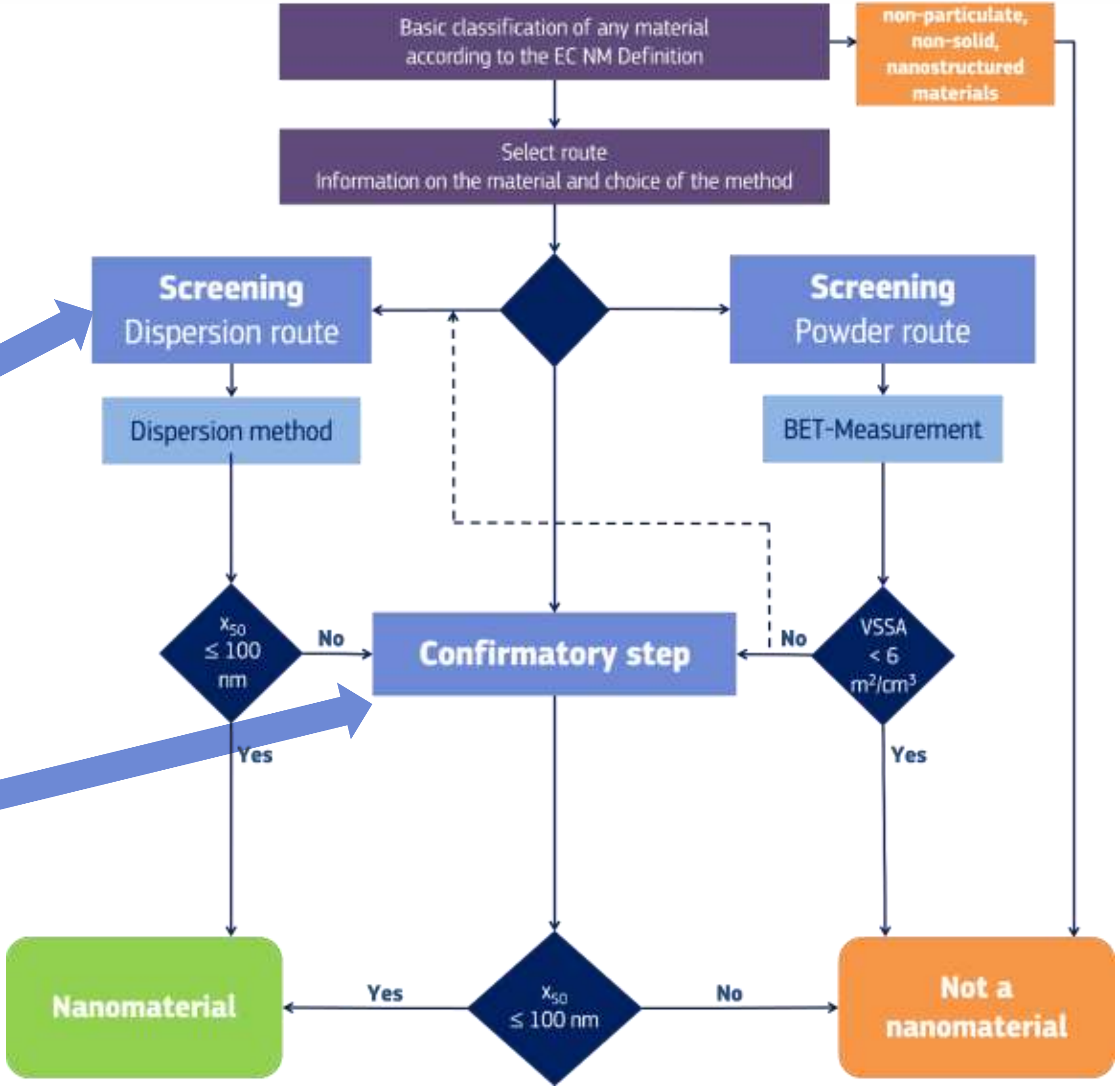
https://publications.jrc.ec.europa.eu/repository/handle/JRC132102

METHODOLIGICAL DECISION TREE

Screening:
DLS, spICP-MS, CPS…

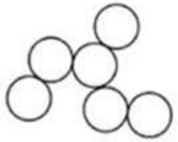
Confirmation:
Electron microscopy or atomic force microscopy

What are the recommandations?



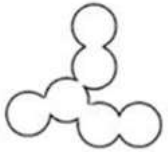
SHARE GOOD PRACTICES: *WHICH OBJECTS?*

Constituent particles



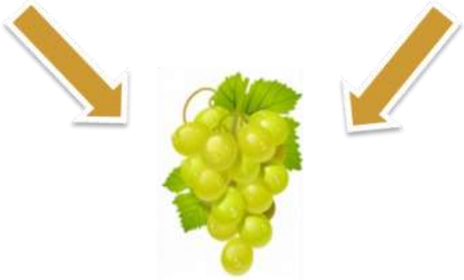
Agglomerate

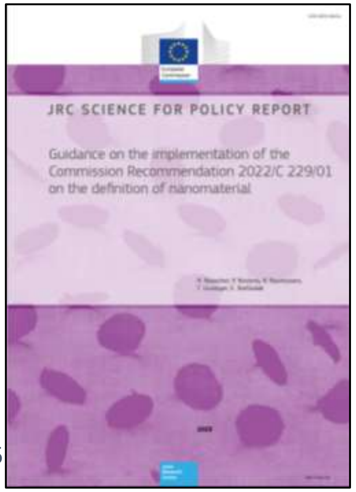
Constituent particles linked together by weak forces



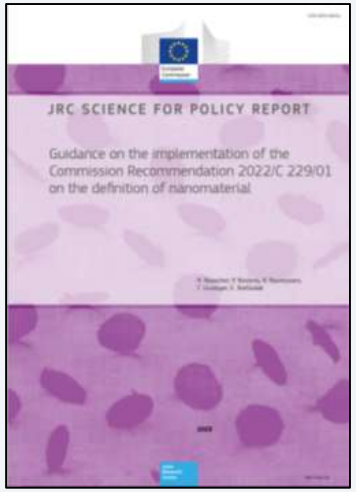
Aggregate

Constituent particles linked together by strong forces





Agglomerates and aggregates are thus secondary structures that are made up of smaller particles. In the context of the EC NM definition, the smallest indivisible unit of an agglomerate/aggregate is called a 'constituent particle'[b]. Agglomerates can consist of a mix of weakly bound constituent particles and smaller aggregates. To be able to include the constituent particles in the number of particles identified in the material, they must be (morphologically) identifiable in order to determine their external dimensions.



change, especially in agglomerates. This is the main reason why the EC NM definition is based on the external dimensions of the constituent particles, which is a more stable feature, even if the constituent particles may sometimes be difficult to measure. Therefore, while the implementation of the EC NM definition does not require distinguishing between aggregates and agglomerates, the difference between aggregates and agglomerates can influence the selection of suitable measurement methods.

- Screening techniques **CANNOT** directly measure the size of constituent particles of aggregates and agglomerates.

- **ELECTRON MICROSCOPY** is one of the techniques capable of producing the **data needed to measure the size of the constituent particles**, whether or not they are isolated.

SHARE GOOD PRACTICES: *COUNTING RULES*

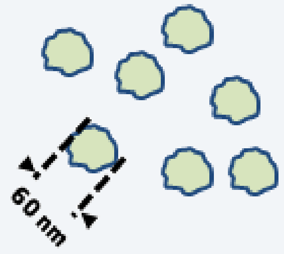
PARTICLES COUNTING RULES FOR ELECTRON MICROSCOPY

- Simple case: isolated particles
- Complex cases: aggregates and agglomerates with **identifiable** constituent particles

INHERENT SUBJECTIVITY

- Identification of constituent particles by operators
- The **choice of laboratories with regard to the expected resolution** of EM images used to identify constituent particles

a) Particles present on their own



ASSESS THE APPLICABILITY: *METHODOLOGY ADOPTED*

Considering the confirmation methods (**EM & AFM**) and recommended analysis rules (**JRC & OECD guides**)

FEEDBACK from NanoMesureFrance members on materials for which they are unable to establish the number size distribution and to conclude on « nanomaterial » status

Identify **specific features** & **group** materials into complexity classes

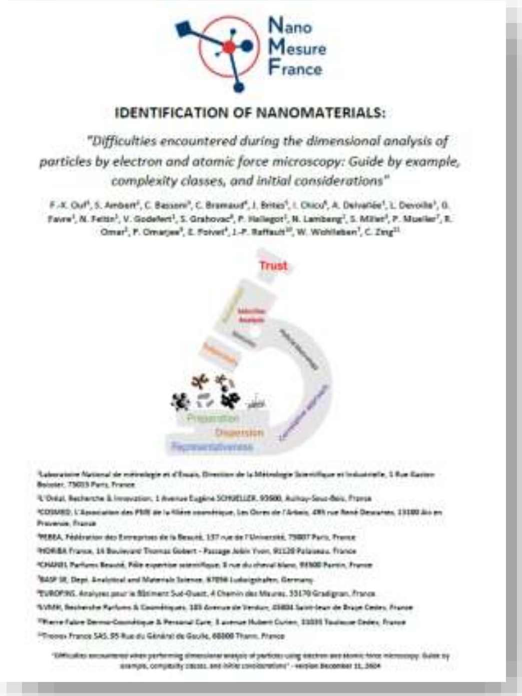
Identify the **limitations** of SEM, TEM & AFM techniques

Propose **first analytical solutions**

NanoMesureFrance guide outlining the **limitations of SEM, TEM & AFM techniques** for characterising the size of **some commonly encountered particulate materials**

GUIDE BY EXAMPLE: *DIFFERENT LEVELS OF READING*

Full version
(~ 50 pages with tables & appendices)


Nano Mesure France

IDENTIFICATION OF NANOMATERIALS:

"Difficulties encountered during the dimensional analysis of particles by electron and atomic force microscopy: Guide by example, complexity classes, and initial considerations"

Summarised version
(~ 10 pages with glossary)


Nano Mesure France

Identification of nanomaterials: Anticipating and addressing the main difficulties during dimensional analysis by microscopy of particulate substances

Documents available in French and English

1. Supporting **analytical experts** when implementing microscopy techniques
2. Assisting **stakeholders** in the process of <u>identification of « ***Nanomaterials*** »</u> in a regulatory framework

1. Sharing key information with **non-experts** in order <u>to anticipate possible identification difficulties</u>
2. Inform the **regulatory authorities** so that <u>they are aware of the problems encountered</u>

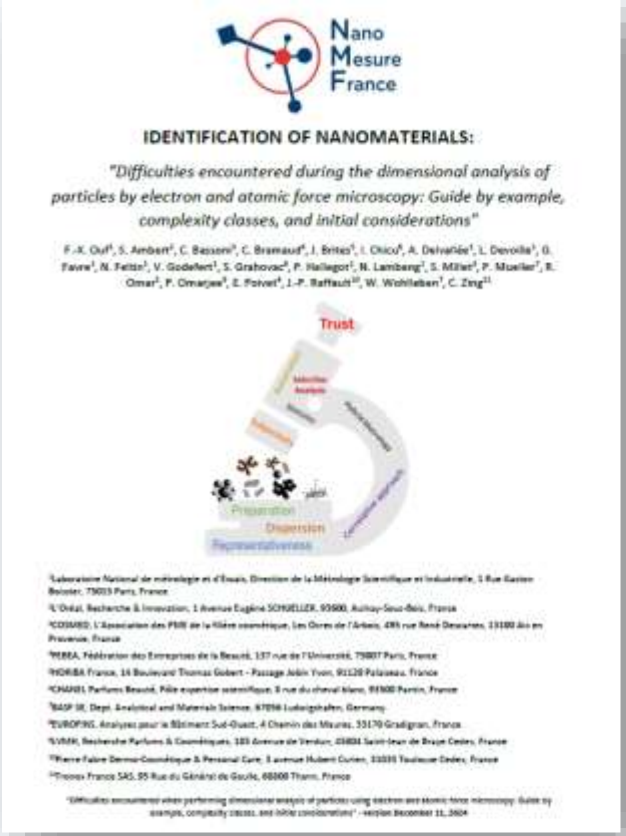
GUIDE BY EXAMPLE: *OVERVIEW*

IDENTIFICATION OF NANOMATERIALS:

- **Difficulties encountered** during the **dimensional analysis of particles** by **electron and atomic force microscopy**: *Guide by example, complexity classes and initial considerations*

A COLLECTIVE WORK:

- **15 co-authors**, members of NanoMesureFrance
- **Co-authors' profiles:** analytical experts, regulatory experts, non-experts stakeholders
- **Sectors:** multi-sectoral, including ➔ manufacturers and users of nanomaterials / instrument manufacturers / service providers



Diversity of profiles & sectors of co-authors to include the <u>expectations & needs of as many stakeholders as possible</u>

GUIDE BY EXAMPLE: *OVERVIEW*

OBJECTIVES:

- **To define complexity classes** useful for establishing complementary decision trees
- To highlight the **difficulties associated with using** electron/atomic force microscopy for these classes
- **Illustrate these difficulties** with "real-life" examples
- **Take stock** of the technologies available
- Introduce **possible technological solutions and areas of development** to be prioritised

This guide focuses on the **difficulties encountered** when using **microscopy techniques** to characterize the size of particulate materials

It does not cover the problems associated with the **extraction phase from finished products** of these materials

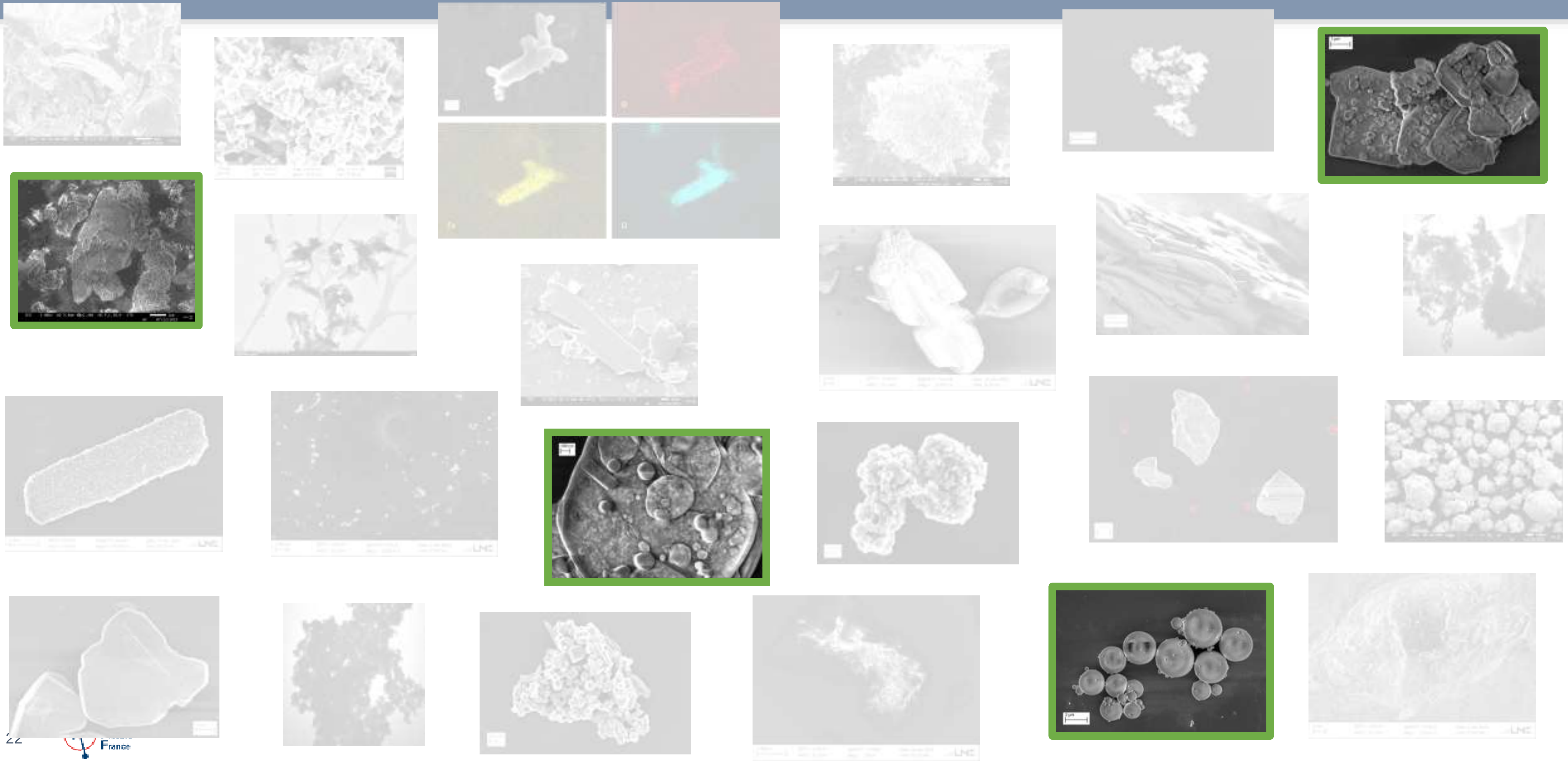
This guide is not intended to provide detailed solutions for the analysis conditions to be implemented

GUIDE BY EXAMPLE: *FEEDBACK*

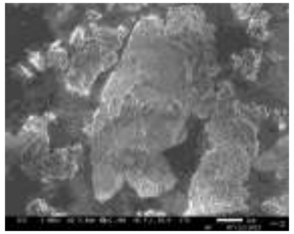
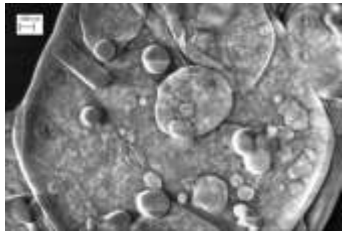
STEP 1: Nearly **50 materials** proposed by NanoMesureFrance members for which microscopy techniques **do not** (*or only with difficulty*) **allow conclusions to be drawn**

STEP 2: Group these materials into **complexity classes**: *families of materials with similar physico-chemical properties or specificities*

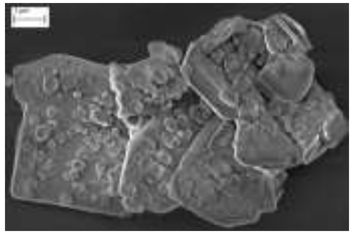
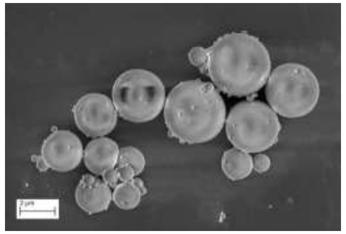
GUIDE BY EXAMPLE: *SIMILARITIES*



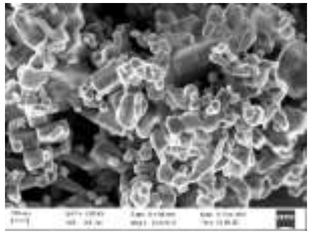
GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

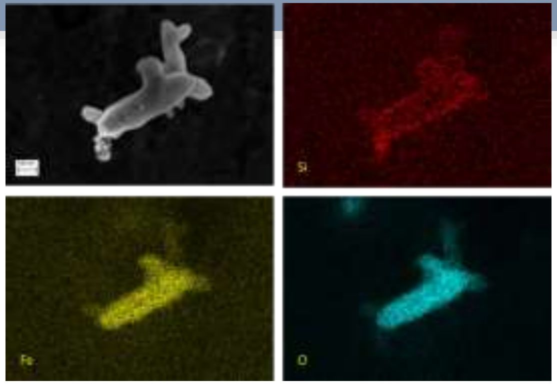



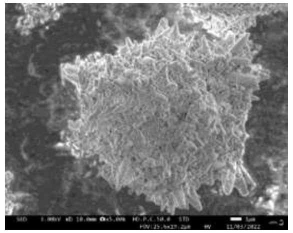
High size polydispersity

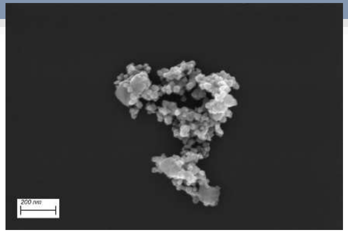



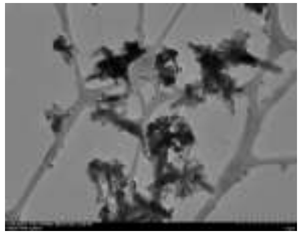
GUIDE BY EXAMPLE: *FEEDBACK*

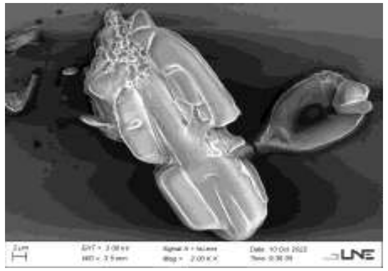


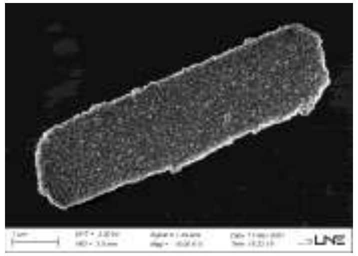


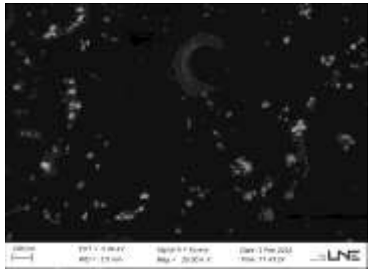


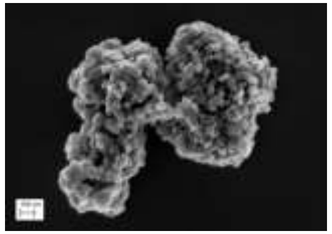


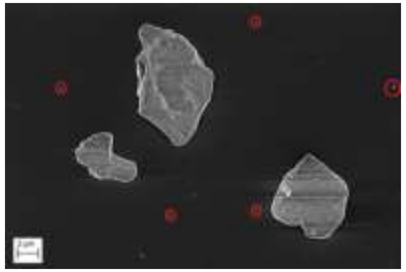


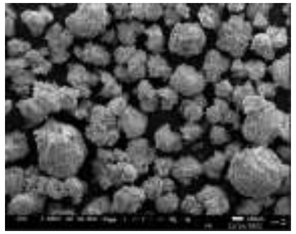


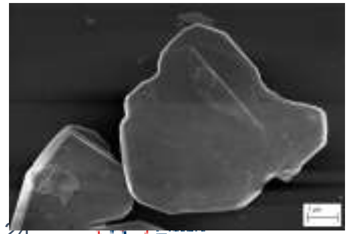


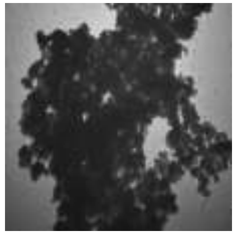


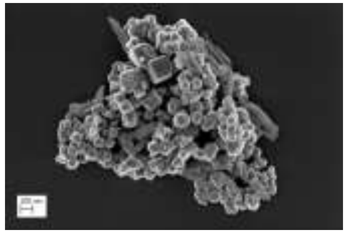


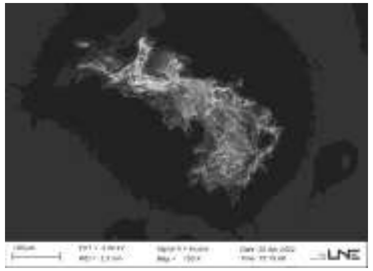


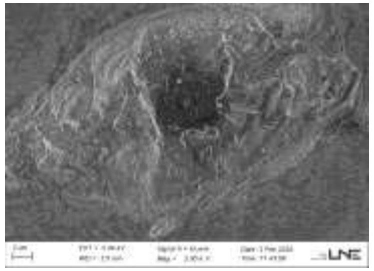




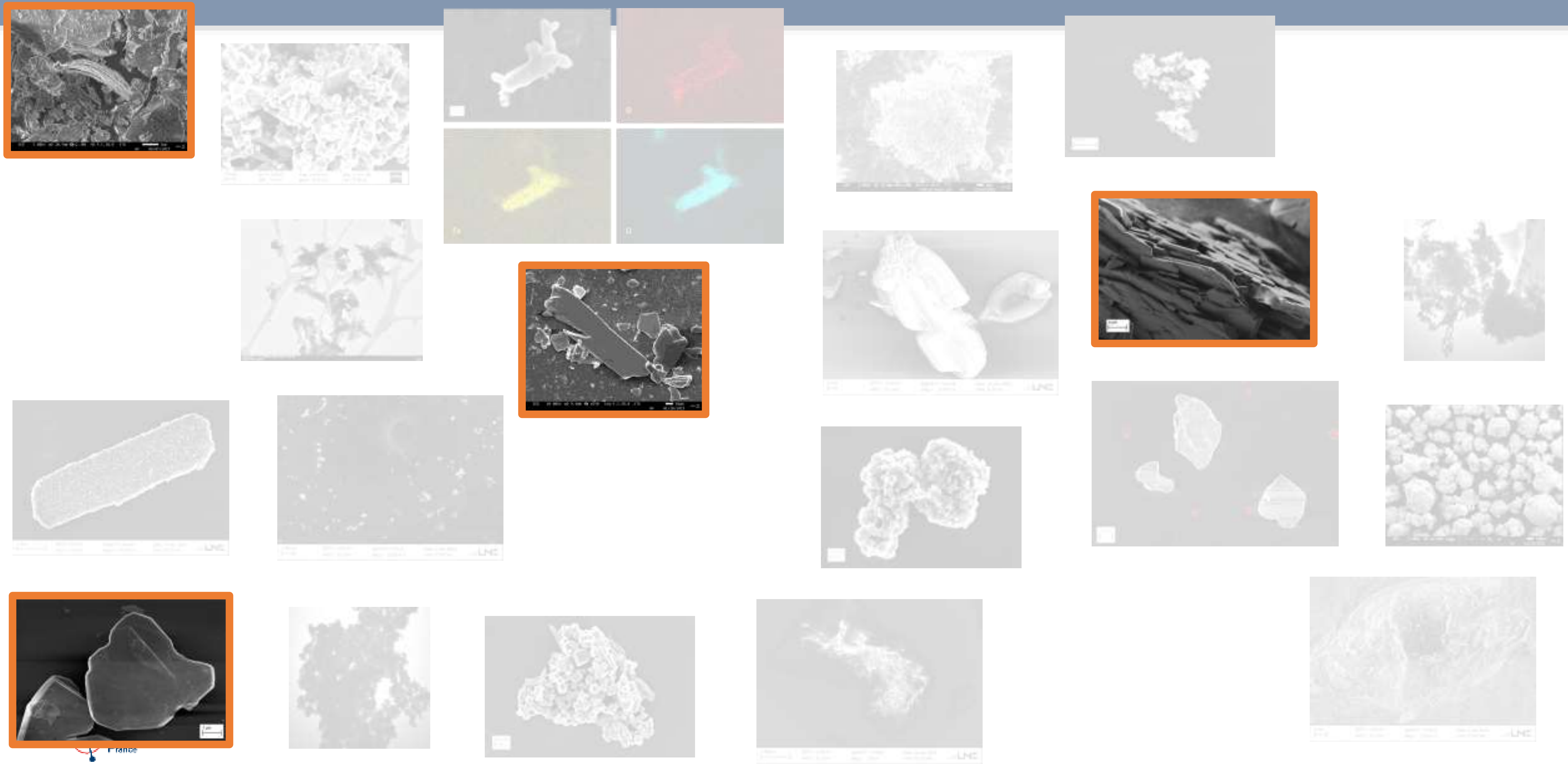




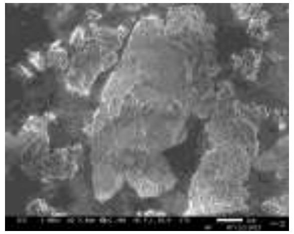


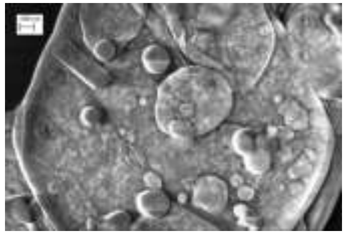


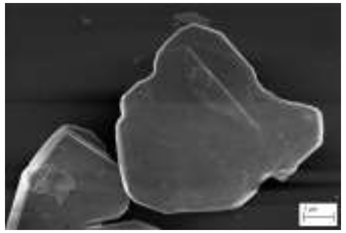
GUIDE BY EXAMPLE: *SIMILARITIES*



GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

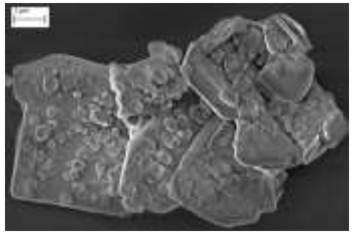


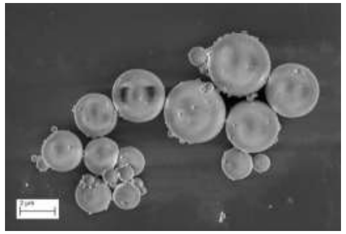




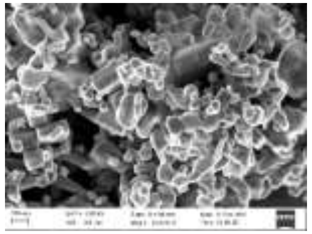
High size polydispersity

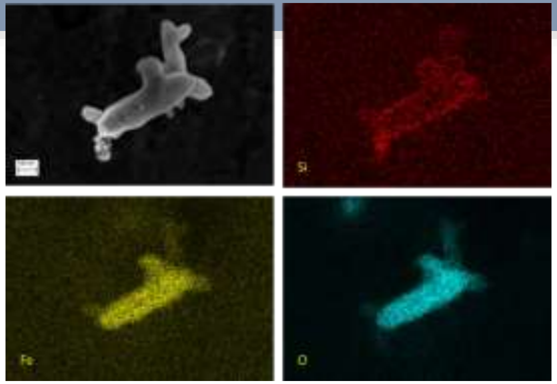
Platelet-type

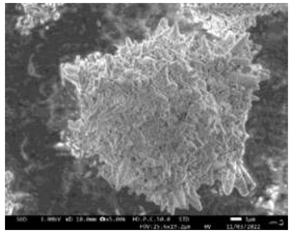


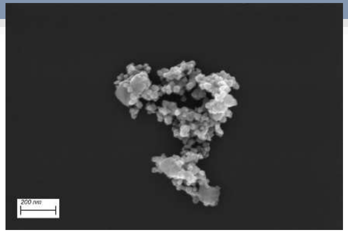


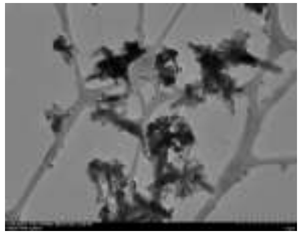
GUIDE BY EXAMPLE: *FEEDBACK*

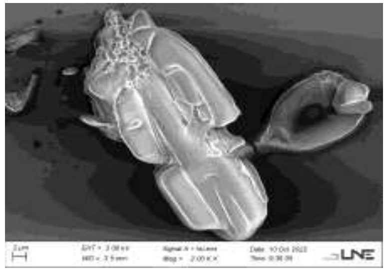


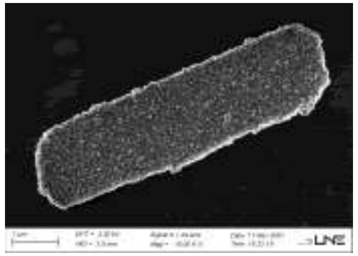


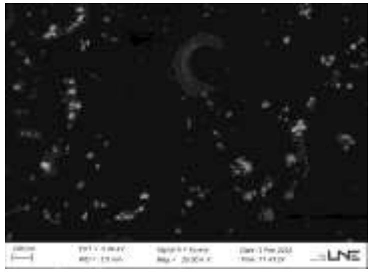


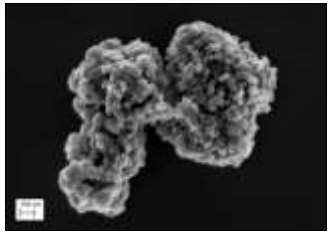


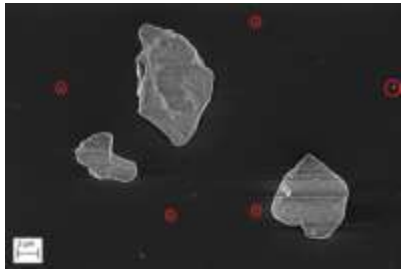


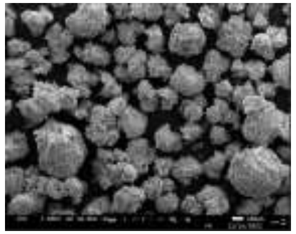


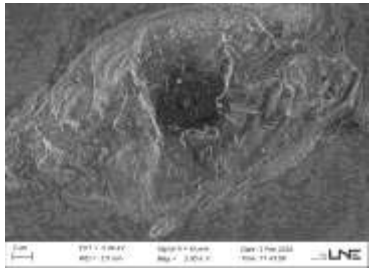


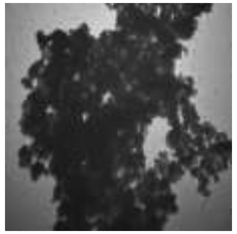


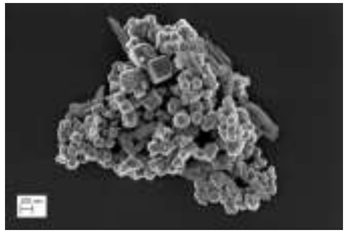


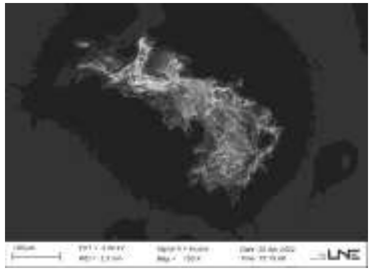




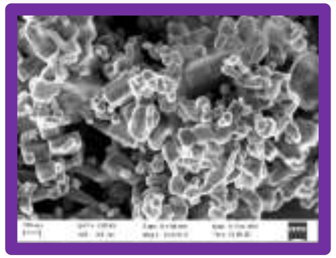
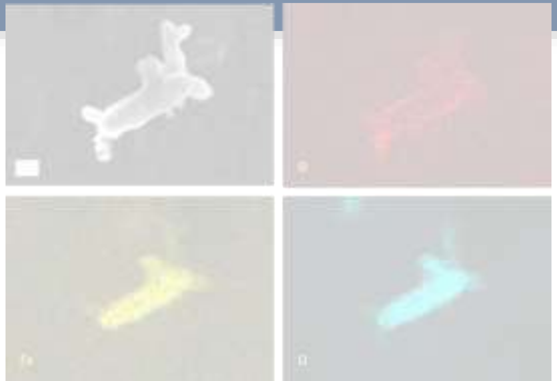
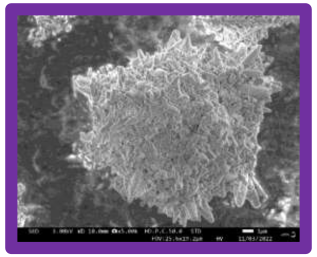


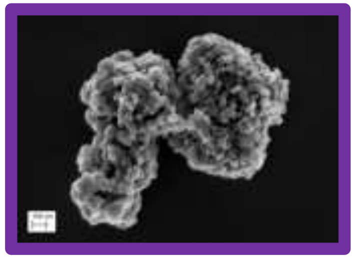


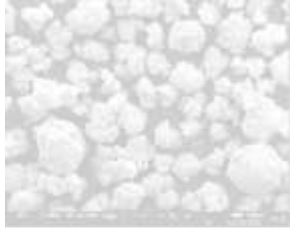
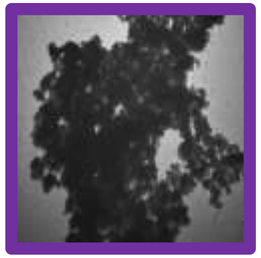




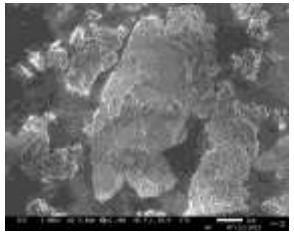
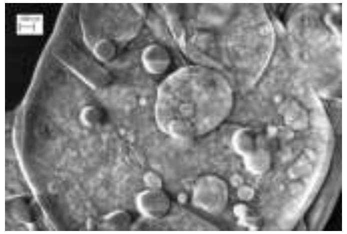
GUIDE BY EXAMPLE: *SIMILARITIES*

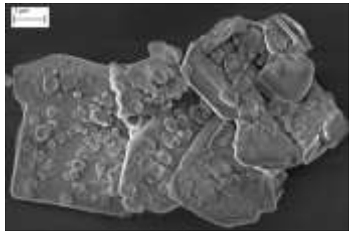
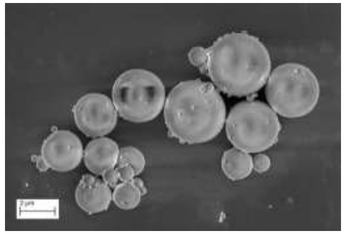


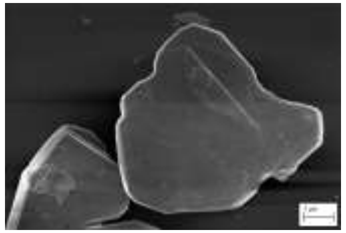




GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

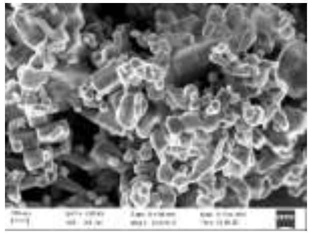
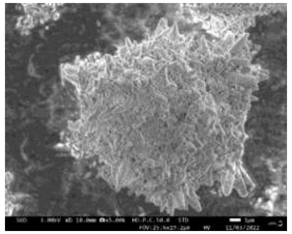



High size polydispersity

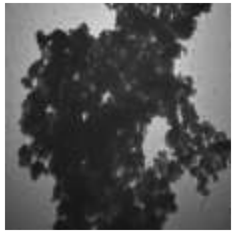
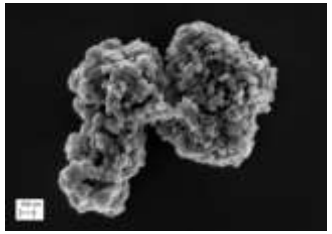



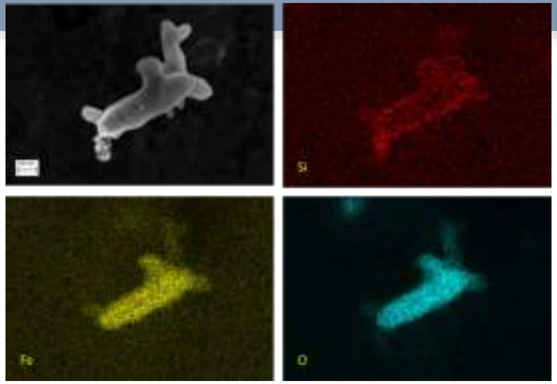
Platelet-type

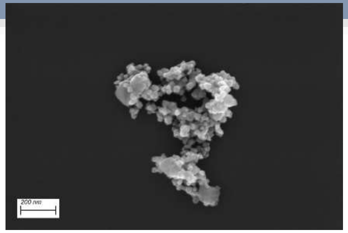




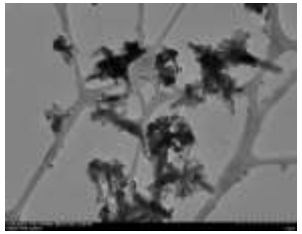
Aggregates

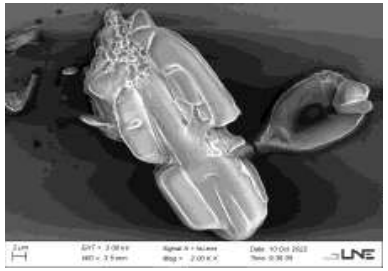



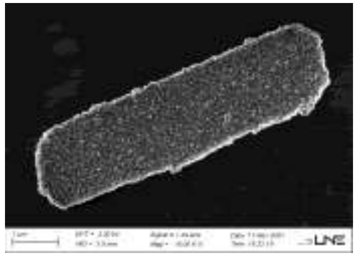
GUIDE BY EXAMPLE: *FEEDBACK*

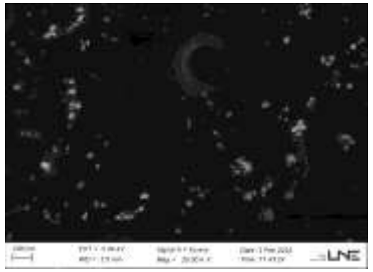


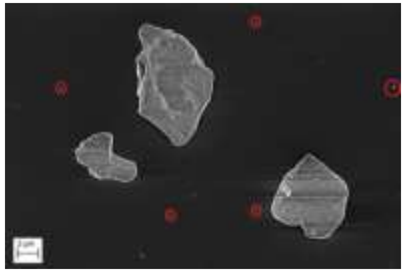


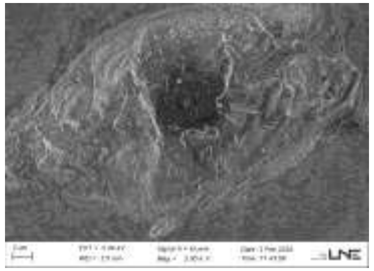


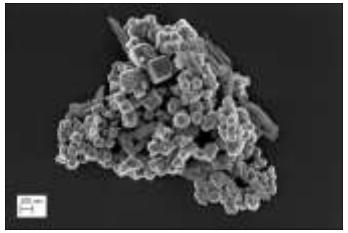


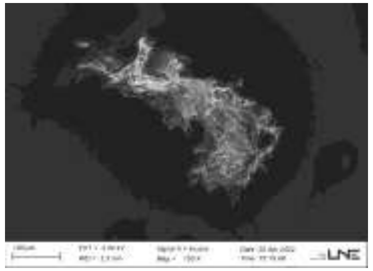




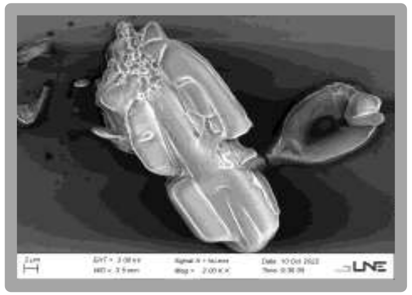


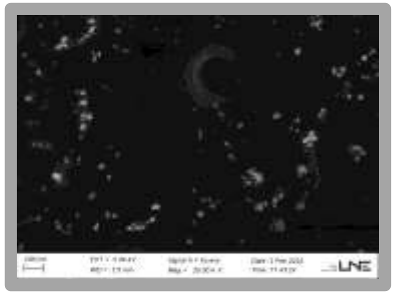


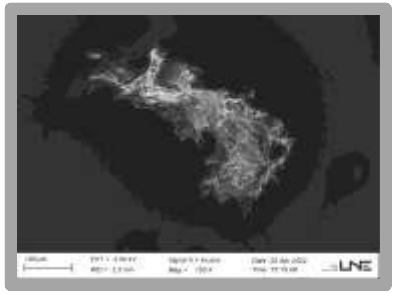


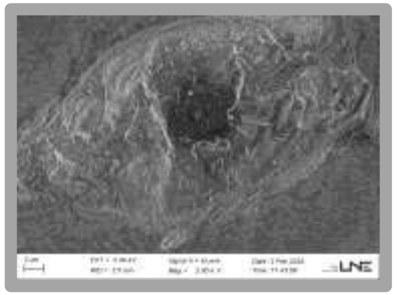


GUIDE BY EXAMPLE: *SIMILARITIES*

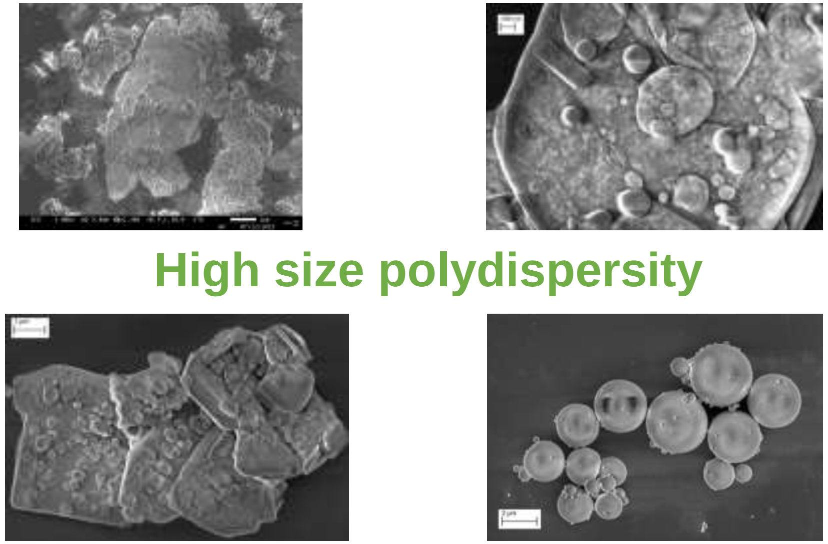




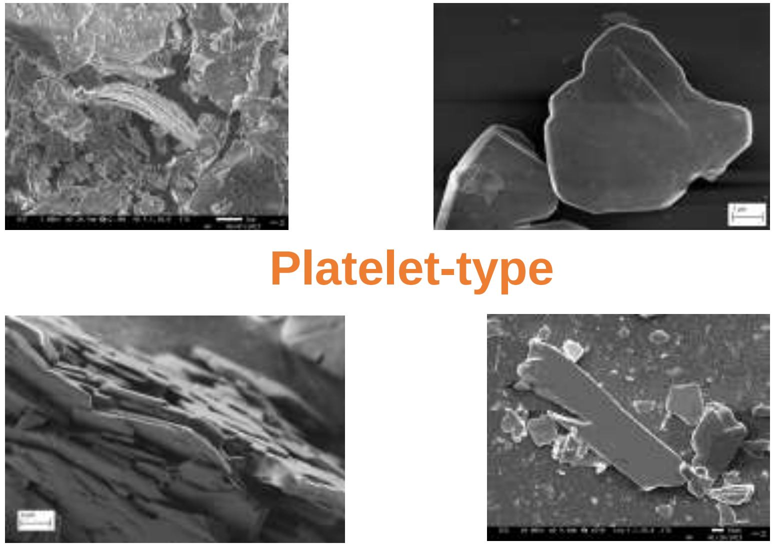




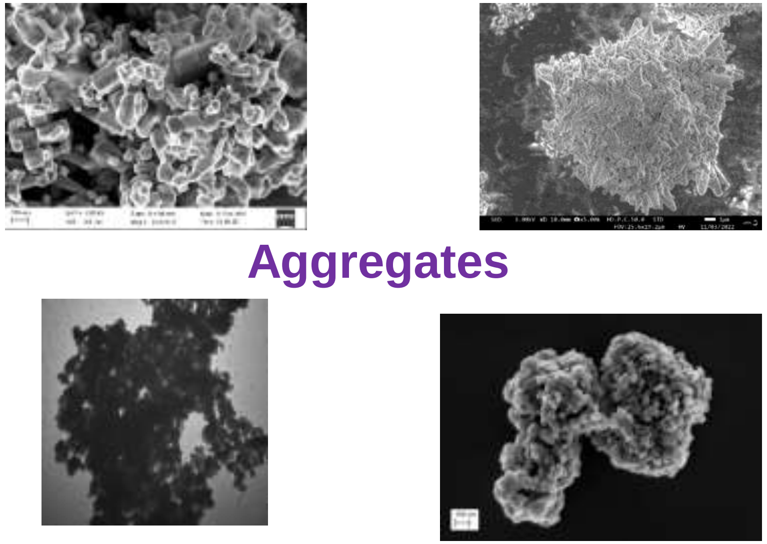
GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*



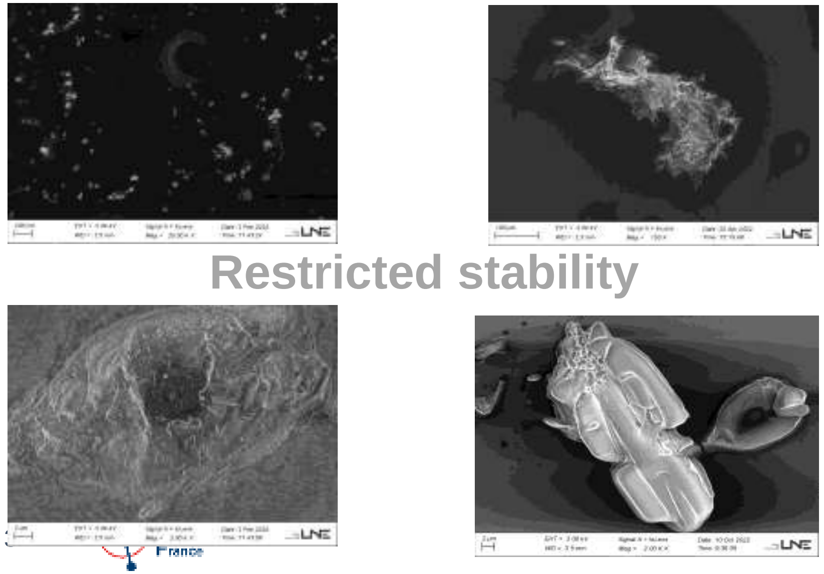
High size polydispersity



Platelet-type

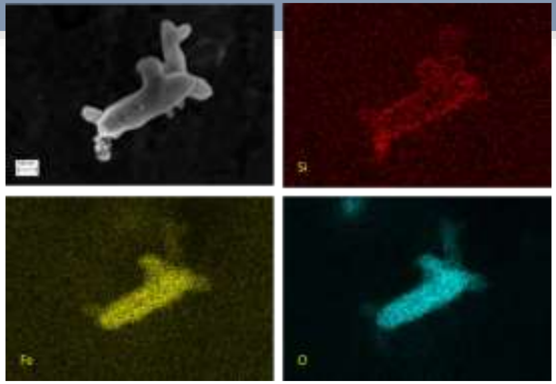


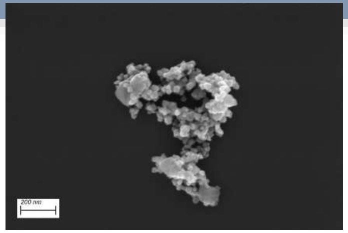
Aggregates

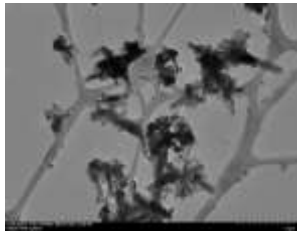


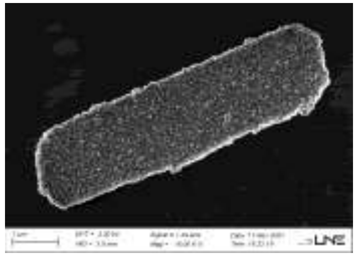
Restricted stability

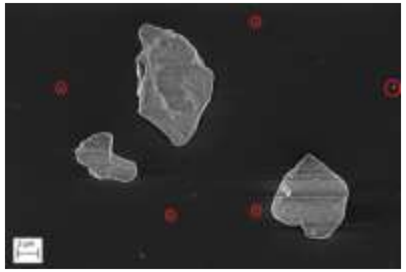
GUIDE BY EXAMPLE: *FEEDBACK*

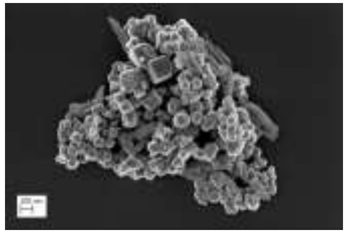




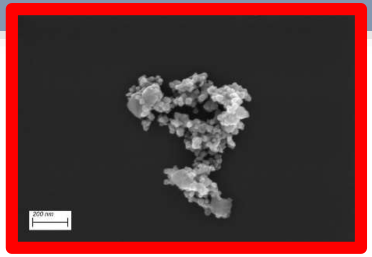


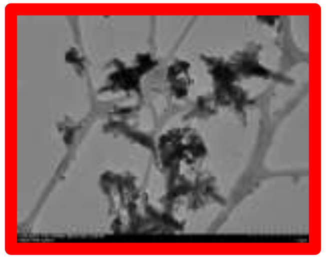


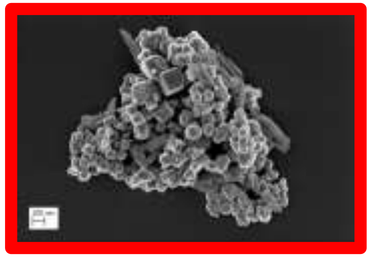




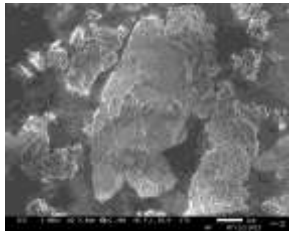
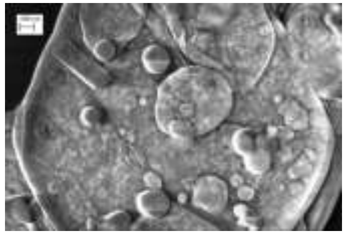
GUIDE BY EXAMPLE: *SIMILARITIES*



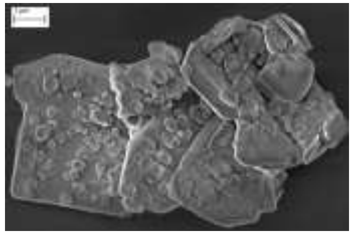
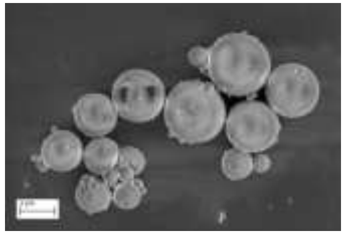


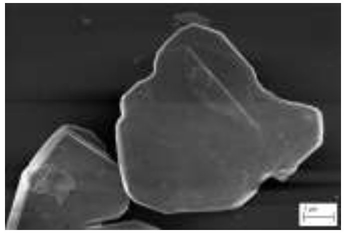


GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

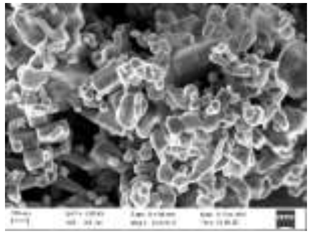
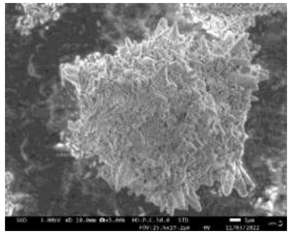



High size polydispersity

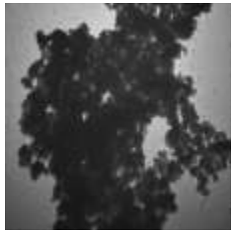
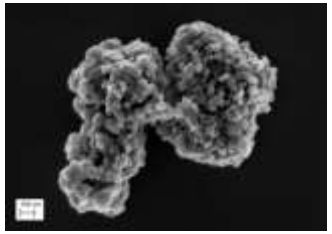



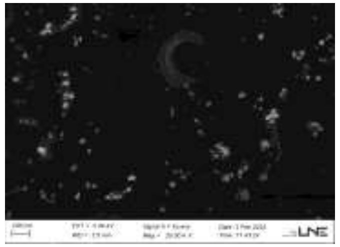
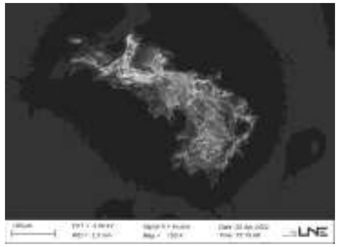



Platelet-type

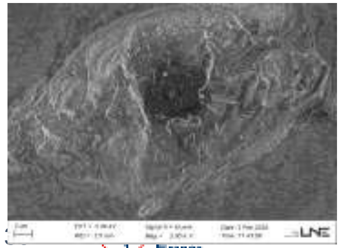
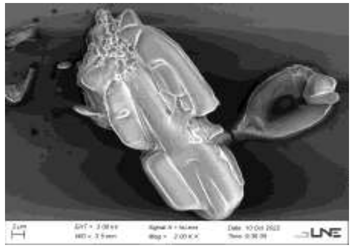




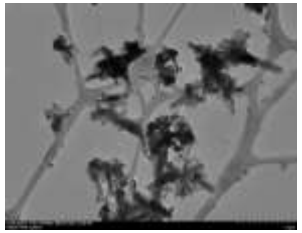
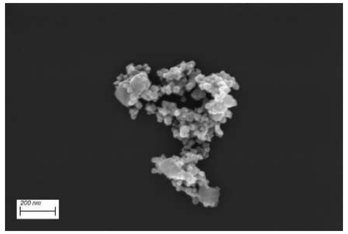
Aggregates

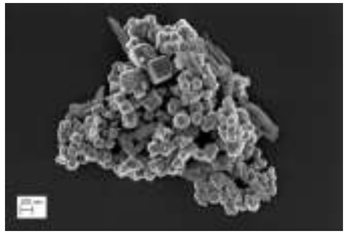



Restricted stability

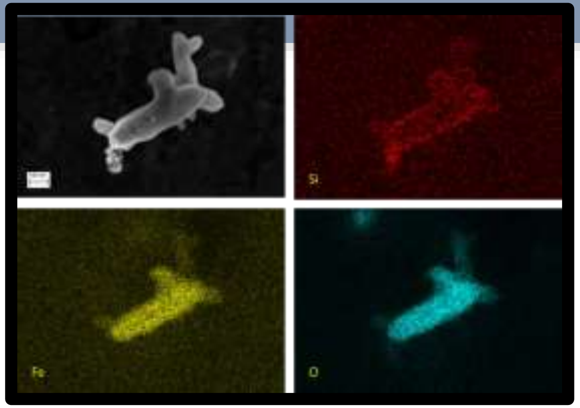



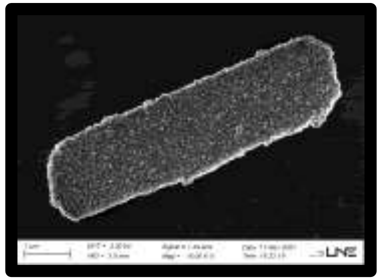



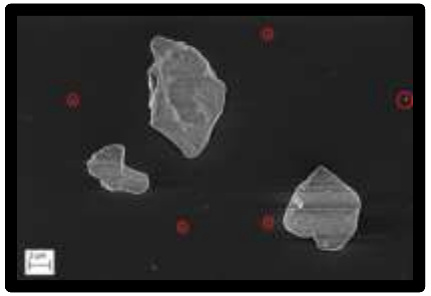
Mixtures of materials

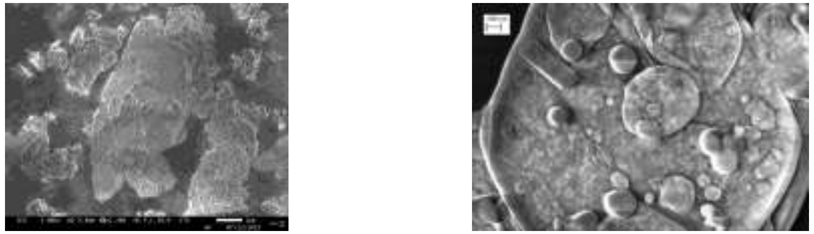
GUIDE BY EXAMPLE: *SIMILIARITIES*



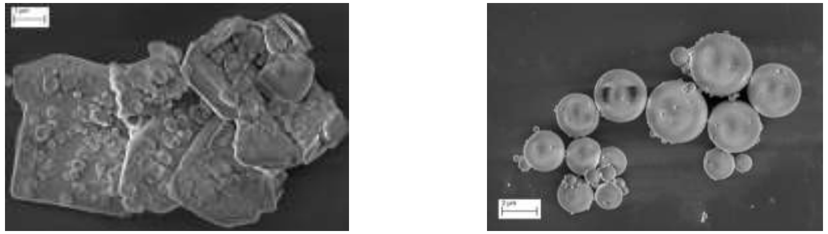


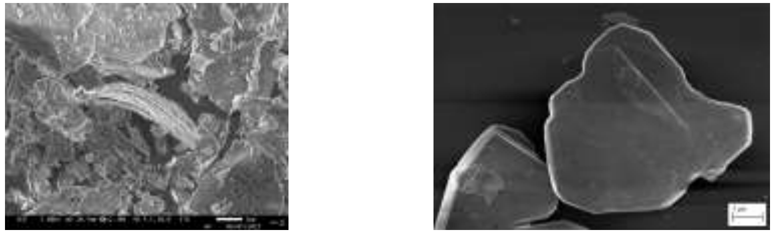


GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

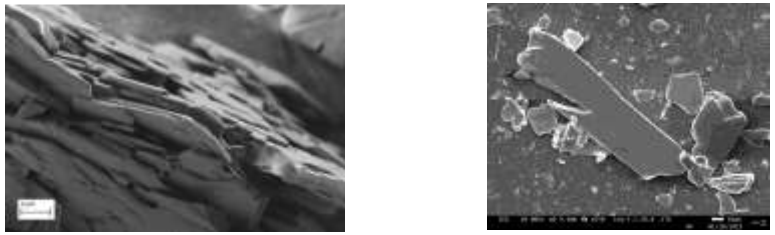


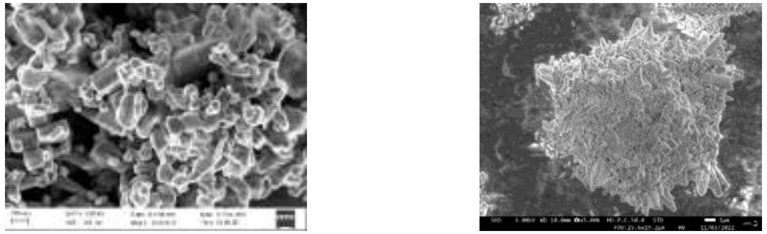
High size polydispersity



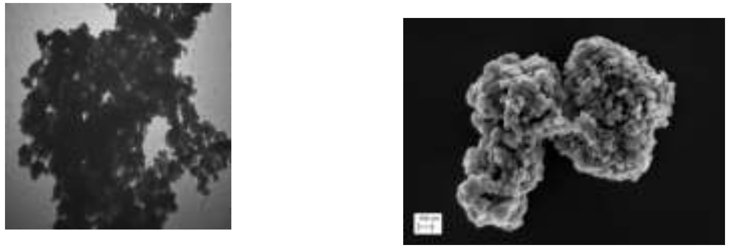


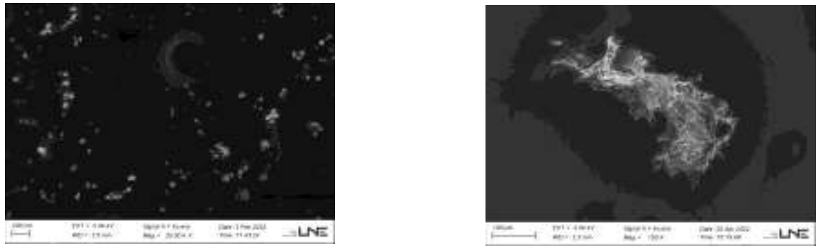
Platelet-type



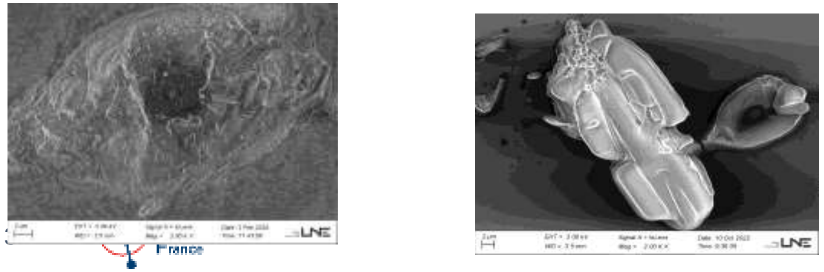


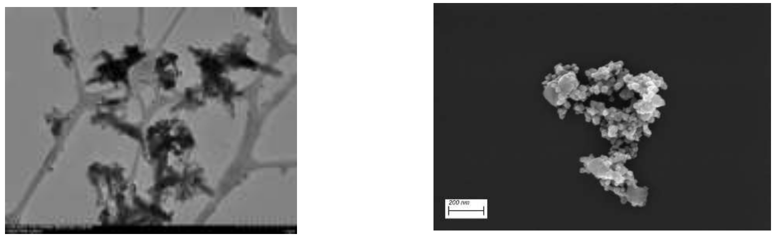
Aggregates



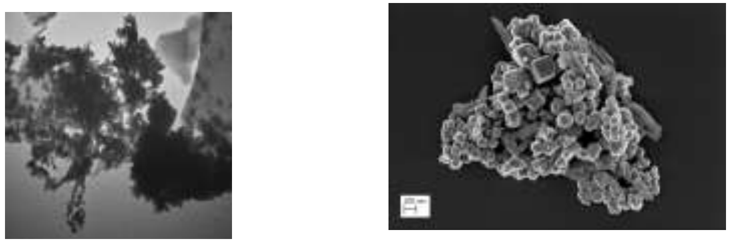


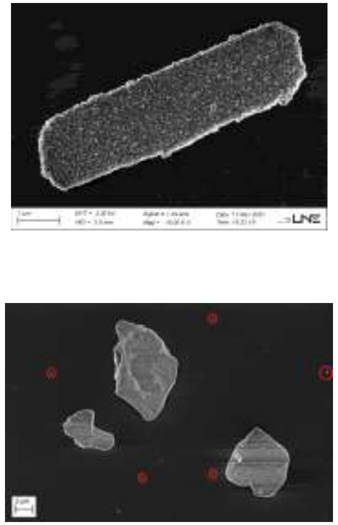
Restricted stability

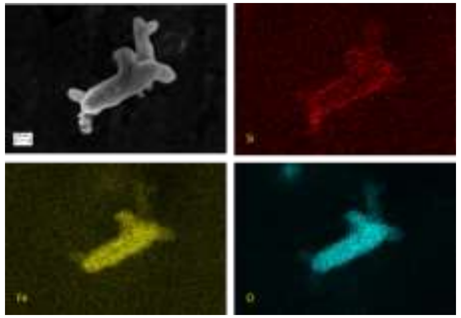




Mixtures of materials







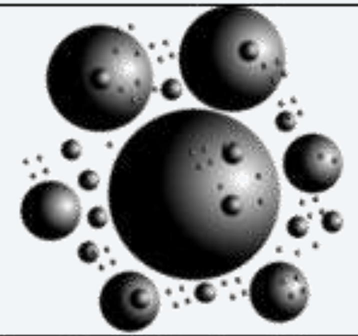
Composite materials

GUIDE BY EXAMPLE: *COMPLEXITY CLASSES*

This list **is not intended to cover all the difficulties encountered** when using EM or AFM techniques to characterise the size of particulate materials

These 6 classes **reflect the state, at the end of 2024, of the main concerns** (based on their feedback) of NanoMesureFrance members

The elements of the guide will evolve to include new solutions or future classes of complexity



A. Materials with **high size polydispersity**

Nano Mesure France

GUIDE BY EXAMPLE: *STRUCTURE*

- **Summary** and introduction of complexity class
- **Issues** (limitations of EM/AFM techniques to analyse the complexity class)
- Most « illustratives » **SEM/TEM/AFM images**
- **State of the art of existing technologies** able to respond to these difficulties
- **Recommendations and development areas**

Full guide: 4 pages per complexity class

Summarised version: **1 page** per complexity class

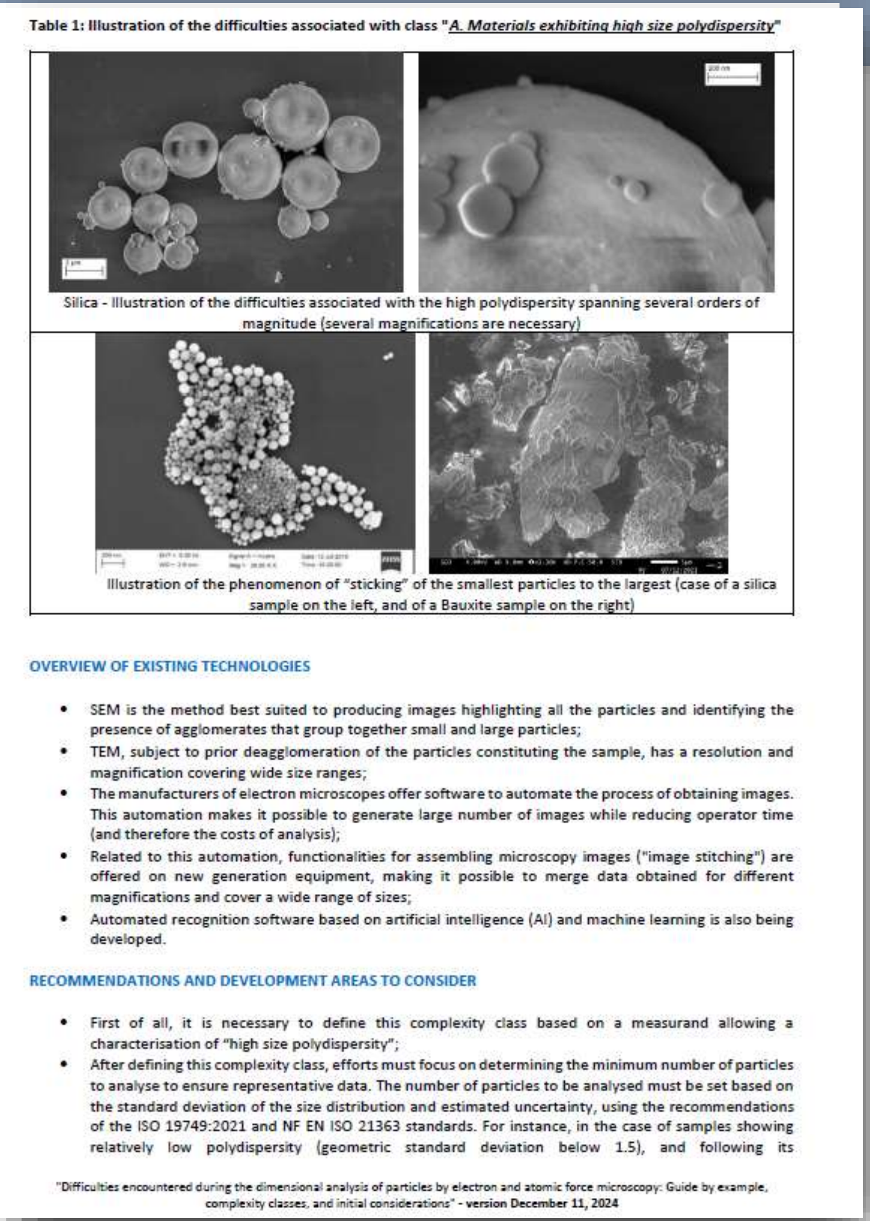


Table 1: Illustration of the difficulties associated with class "*A. Materials exhibiting high size polydispersity*"

Silica - Illustration of the difficulties associated with the high polydispersity spanning several orders of magnitude (several magnifications are necessary)

Illustration of the phenomenon of "sticking" of the smallest particles to the largest (case of a silica sample on the left, and of a Bauxite sample on the right)

OVERVIEW OF EXISTING TECHNOLOGIES

- SEM is the method best suited to producing images highlighting all the particles and identifying the presence of agglomerates that group together small and large particles;
- TEM, subject to prior deagglomeration of the particles constituting the sample, has a resolution and magnification covering wide size ranges;
- The manufacturers of electron microscopes offer software to automate the process of obtaining images. This automation makes it possible to generate large number of images while reducing operator time (and therefore the costs of analysis);
- Related to this automation, functionalities for assembling microscopy images ("image stitching") are offered on new generation equipment, making it possible to merge data obtained for different magnifications and cover a wide range of sizes;
- Automated recognition software based on artificial intelligence (AI) and machine learning is also being developed.

RECOMMENDATIONS AND DEVELOPMENT AREAS TO CONSIDER

- First of all, it is necessary to define this complexity class based on a measurand allowing a characterisation of "high size polydispersity";
- After defining this complexity class, efforts must focus on determining the minimum number of particles to analyse to ensure representative data. The number of particles to be analysed must be set based on the standard deviation of the size distribution and estimated uncertainty, using the recommendations of the ISO 19749:2021 and NF EN ISO 21363 standards. For instance, in the case of samples showing relatively low polydispersity (geometric standard deviation below 1.5), and following its

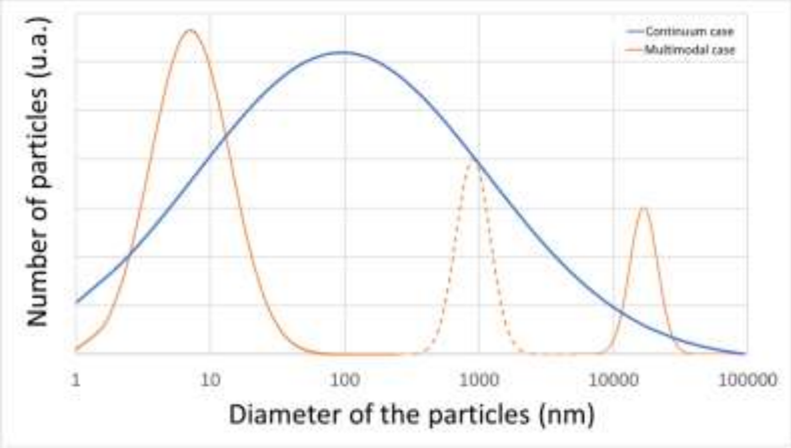
"Difficulties encountered during the dimensional analysis of particles by electron and atomic force microscopy: Guide by example, complexity classes, and initial considerations" - version **December 11, 2024**

Nano Mesure France

GUIDE BY EXAMPLE: *HIGH SIZE POLYDISPERSITY*

DEFINITION:

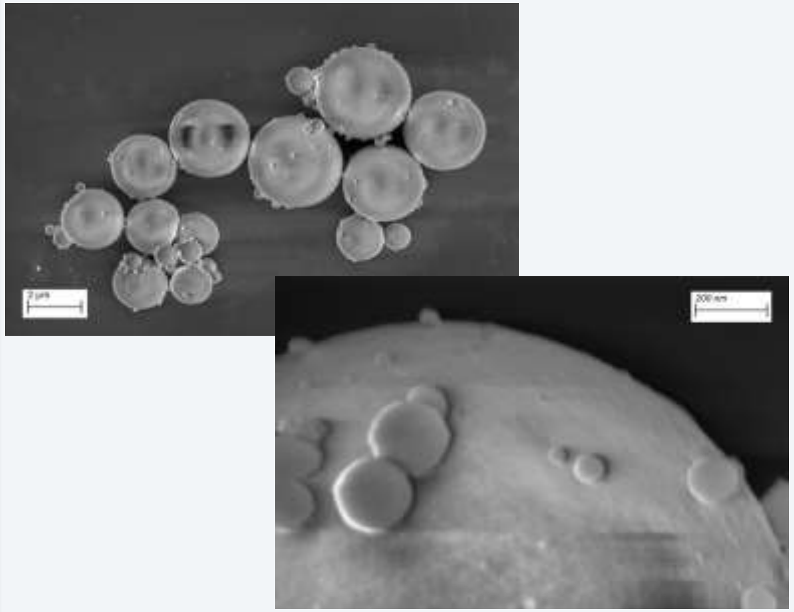
- A "material exhibiting high size polydispersity" is a material composed of particles **with a size distribution featuring several distinct peaks** (or **modes) or covers a continuous wide range spanning several orders of magnitude** (ranging from 1 nm to 100 µm)



ISSUES:

- **Obtaining** a sufficient number of **electron microscopy images** with suitable resolution to determine particle size over such a wide range of sizes (1 nm to 100 µm)

- **Sample preparation**: inhomogeneity of deposit in terms of size and « sticking » of smaller particles to larger ones



Nano Mesure France

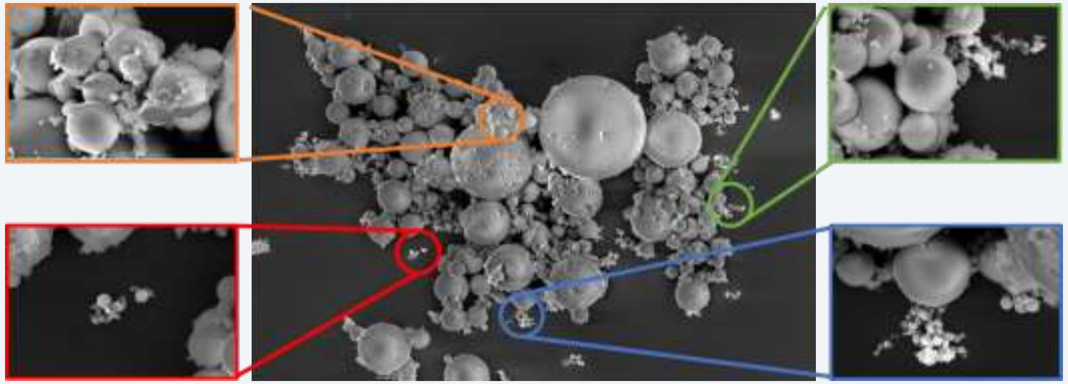
GUIDE BY EXAMPLE: *HIGH SIZE POLYDISPERSITY*

AVAILABLE TECHNOLOGIES:

- **Screening methods** not adapted
- **SEM & TEM** (after de-agglomeration) are able to cover wide size ranges
- **Automation of image** acquisition (stitching) and analysis (Machine Learning) under development

DEVELOPMENT AREAS:

- **Define a measurand** to characterize « high size polydispersity »
- **Counting statistics ➔** how many constituent particles should be considered to ensure that the analysis of the material is representative?
- **Validation of preparation and analysis methods** on representative testing materials
- **Evaluation of** fragmentation (A4F) or separation (micro and ultrafiltration) methods prior to EM analysis



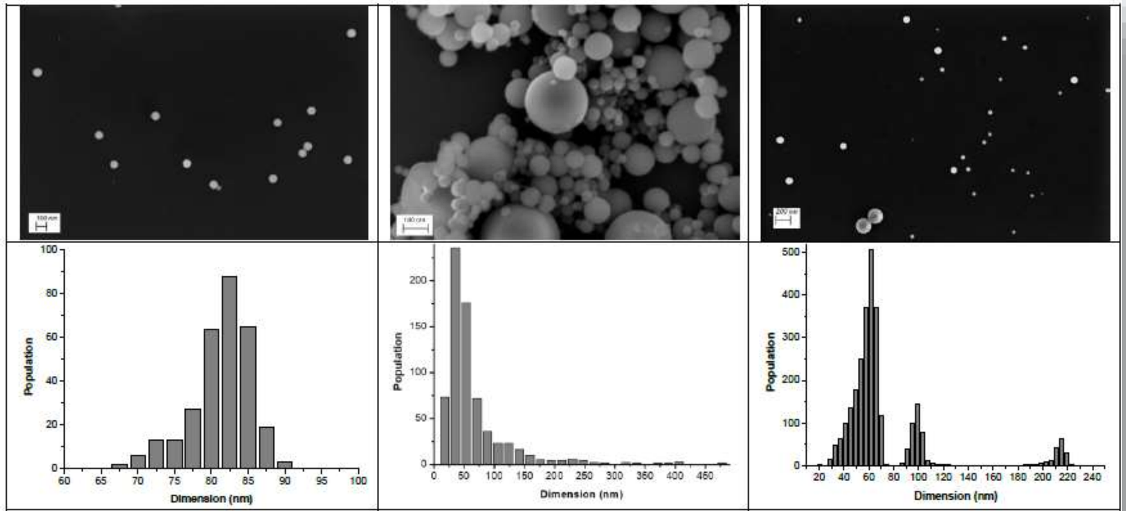
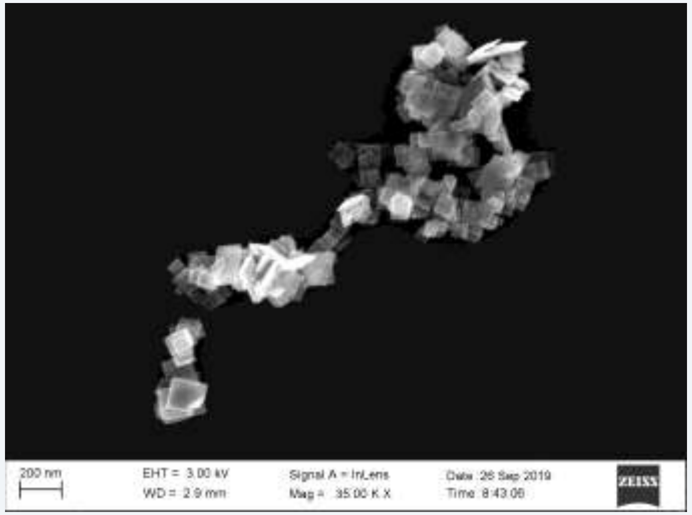


Figure 4: Illustration of the need to increase the number of particles to be counted depending on the size polydispersity of the material. Left: a sample of slightly polydispersed silica (300 particles counted), middle: a sample of silica with greater polydispersity (700 particles counted), right: a mixture of latex beads of 60, 100, and 220 nm (more than 2700 particles counted)

GUIDE BY EXAMPLE: *PLATELET-TYPE*

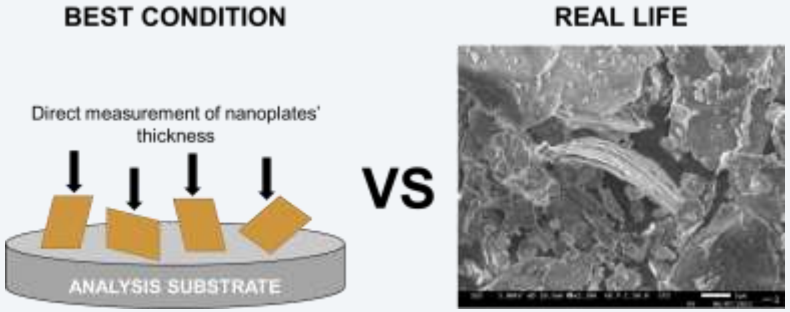
DEFINITION:

- A nanoplate is a **nano-object** that has a **thickness of less than 100 nm** and **lateral dimensions that can exceed 100 nm**. Therefore, <u>the smallest dimension to measure is the **thickness**</u>



ISSUES:

- **Sample preparation**: ensure that platelets are <u>properly dispersed to limit stacking</u>
- **To have a technique** for <u>unambiguously identifying</u> particles with a <u>platelet-like shape</u>



Nano Mesure France

GUIDE BY EXAMPLE: *PLATELET-TYPE*

AVAILABLE TECHNOLOGIES:

- **Screening methods** not adapted
- **SEM & TEM give 2D projections ➔** techniques limited by the deposition of platelets on the substrate substrat
- **In theory, only AFM can** robustly **determine the thickness of a platelet,** but it is also sensitive to sample preparation

DEVELOPMENT AREAS:

- Validation of **preparation and deposition methods**
- Definition of a **harmonised protocol for embedding** platelets/nanoplates **in a resin** to enable thickness analysis
- **Explore other methods:** EELS coupled with electron microscopy, nanotomography, etc.
- **Inter-comparison** of methods: representative materials

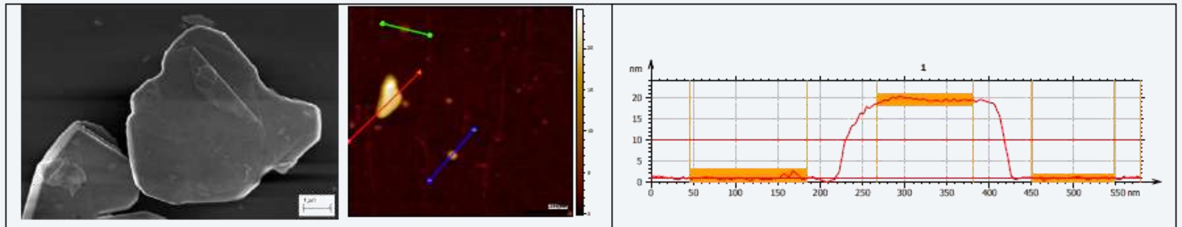


Figure 5: on the left an SEM image of particles constituting a material based on boron nitride, in the middle an AFM image of a particle of this material with the red line delineating the axis used to determine the height profile shown on the right (mean height: 18.65 nm)

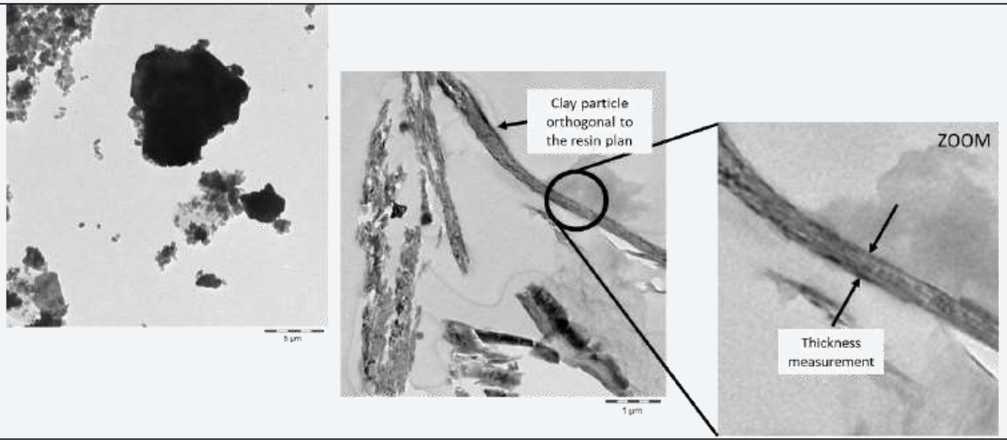


Figure 6: Illustration of the potential of the "resin embedding + ultramicrotomy" process for analysing the thickness of platelet-shaped clay particles. Left: TEM image of untreated clay particles, middle: TEM image of a cross-section prepared by ultramicrotomy of clay in platelet form embedded in a polymer. Right: enlarged view of a specific section of particles that have been cut perpendicular to the plane of the particles for thickness analysis

GUIDE BY EXAMPLE: *MIXTURES OF MATERIALS*

DEFINITION:

- Mixtures of several materials which do not interact strongly with each other:

  (i) Having different elemental chemical compositions exhibiting different particle shapes or not

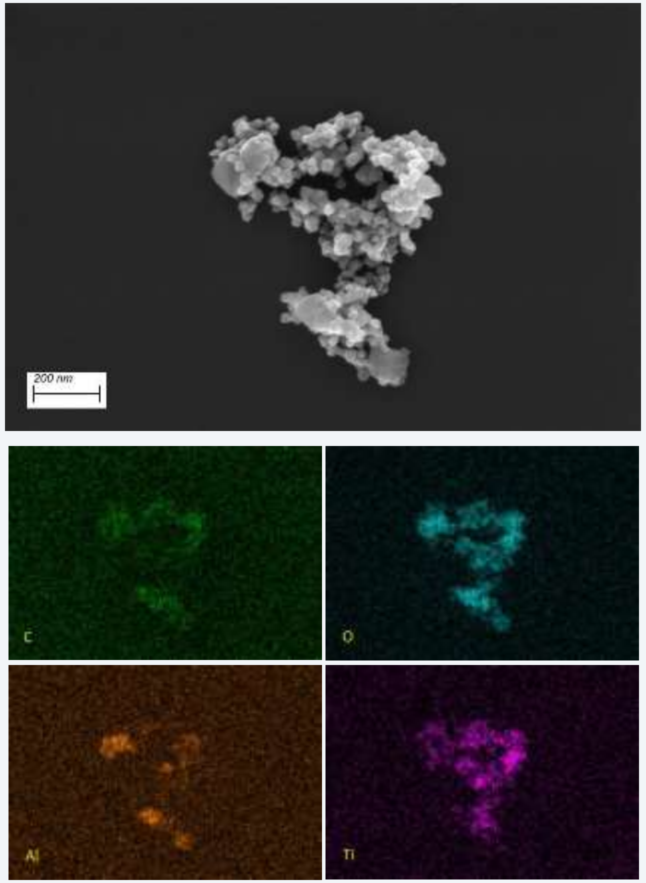
  (ii) Having the same elemental chemical compositions, but exhibiting different particle shapes

  (iii) Having the same elemental chemical compositions and the same shape, but exhibiting different chemical formulae

ISSUES:

- **Ability of currently available analytical tools** to access shape, chemical and/or crystallographic information on a particle-by-particle basis in order to be able **to establish number size distributions for each material contained in the mixture.**

- **Complex sample preparation**: selectivity if size differences are significant, choice of solvent so as not to modify the different materials



Mixture of TiO_2 and organic material - The two different morphologies of the materials are not clearly distinguishable, and only EDX elemental analysis (bottom), subject to adequate resolution, can reveal the location of the two constituents.

GUIDE BY EXAMPLE: *MIXTURES OF MATERIALS*

AVAILABLE TECHNOLOGIES:

- **Morphological discrimination possible** on the basis of SEM & TEM images
- **EM + detectors (WDS, EDS, EBSD, TKD):** mixtures of different elemental compositions or crystalline forms
- **Nano-positioning or correlative imaging systems**

DEVELOPMENT AREAS:

- **Analysis strategy:** morphological and/or correlative approaches in line with ECHA and JRC guidelines
- **Sample preparation:** anticipating the specific characteristics of the different materials making up the mixture
- **Development of correlative metrology approaches:** the challenge of having representative test materials to compare & validate methods

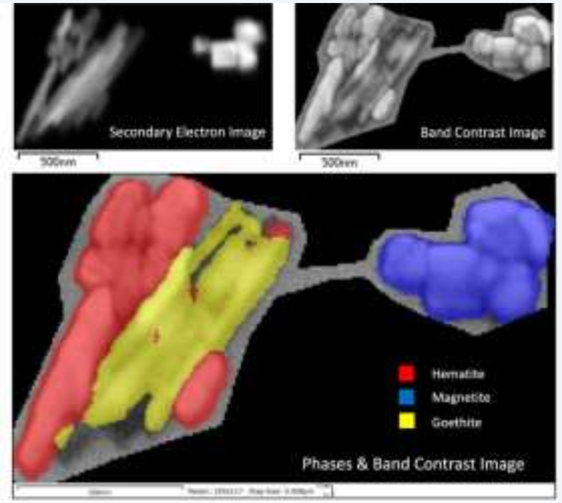
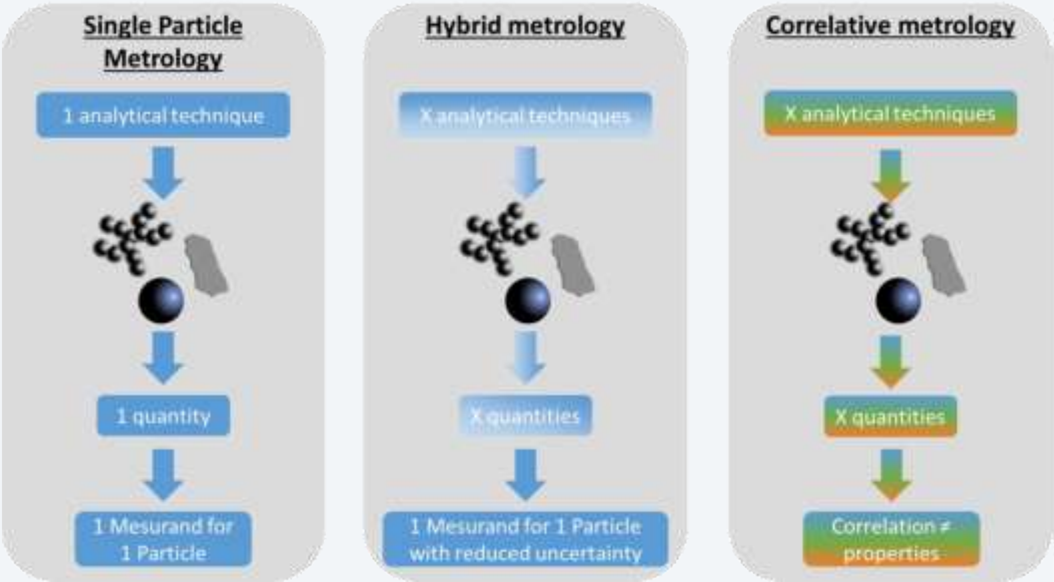


Illustration of the capacities of the TKD (transmission Kikuchi diffraction) technique **to identify different iron oxides** *(Crouzier, Feltin & Jablon, Meas. Sci. Tech. 2024)*



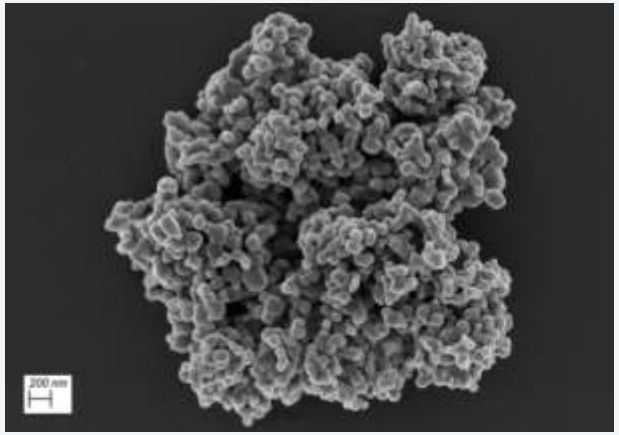
GUIDE BY EXAMPLE: *AGGREGATES*

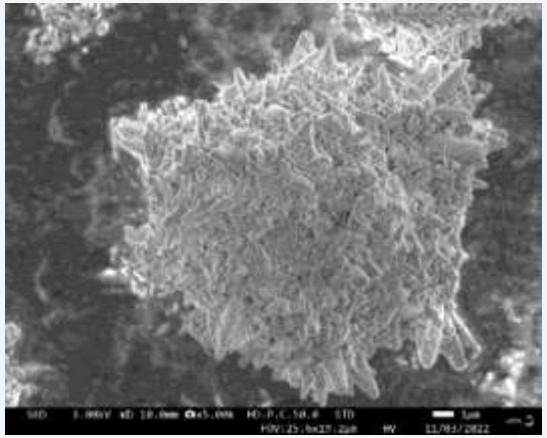
DEFINITION:

- **Nano-objects** can be found in **aggregated/agglomerated form** following synthesis

ISSUES:

- **Subjectivity of the analyst** in identifying the constituent particles
- Distinction between **aggregated particles and polycrystalline material**
- Consideration of **constituent particles at the heart of aggregates**





Precipitated calcium carbonate - mineral material that should not be interpreted as aggregates of particles but as polycrystalline particles made up of smaller grains

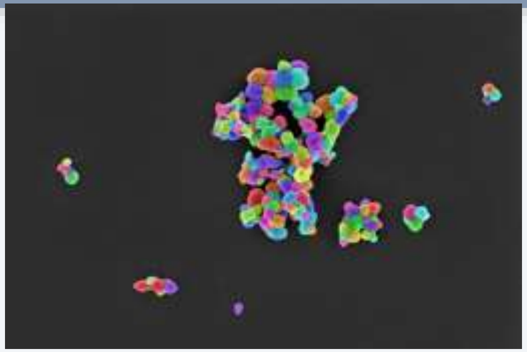
GUIDE BY EXAMPLE: *AGGREGATES*

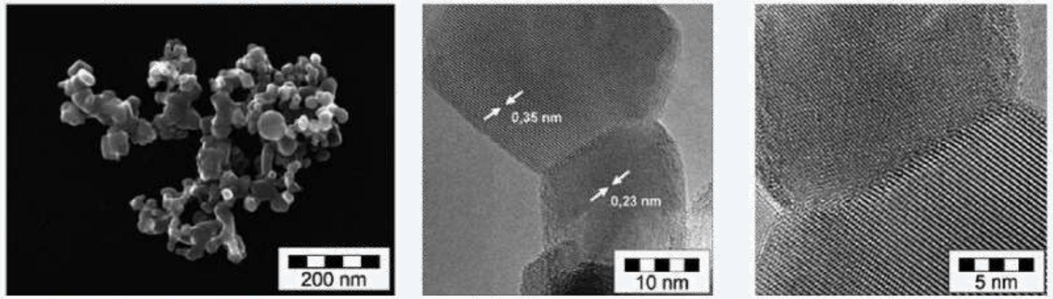
AVAILABLE TECHNOLOGIES:

- **Screening methods** not adapted
- **SEM & TEM** (after de-agglomeration) are able to image constituent particles
- **Automated analysis software** based on IA (Machine Learning) developed and qualified
- **HR-TEM** to demonstrate polycrystalline nature

DEVELOPMENT AREAS:

- **Harmonisation of counting techniques** in cases where particle identification is highly dependent on the analyst's subjectivity
- **Development of tools to complete the 'missing fraction'** (fraction of a particle's surface that may be masked by other particles)





Titanium dioxide - Illustration of the degree of resolution required for the images (the images here are made using HR-TEM high resolution transmission electron microscopy) in order to describe the bonds between the constituent particles and thus define the concepts of aggregate or agglomerate on a theoretical level (Albers et al., 2015[14)]

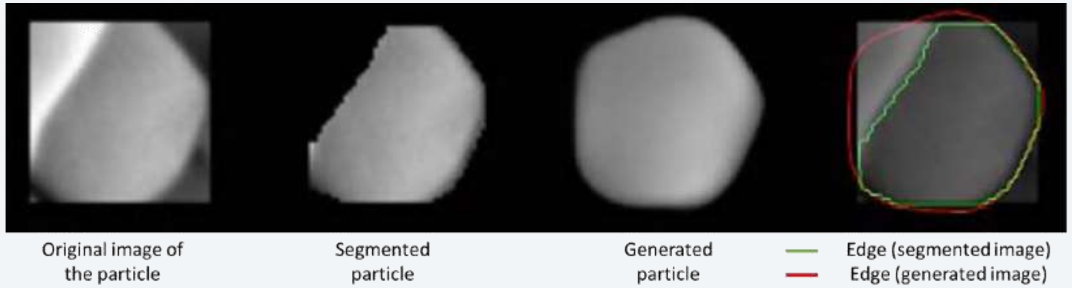


Figure 8: Illustration of the process of "completing" the missing fraction of a particle using a tool based on artificial intelligence and deep learning (adapted from Coquelin et al., 2019[16)]

Nano Mesure France

GUIDE BY EXAMPLE: *LESSONS LEARNED*

NANOMESUREFRANCE AS A TRUSTED FRAMEWORK:

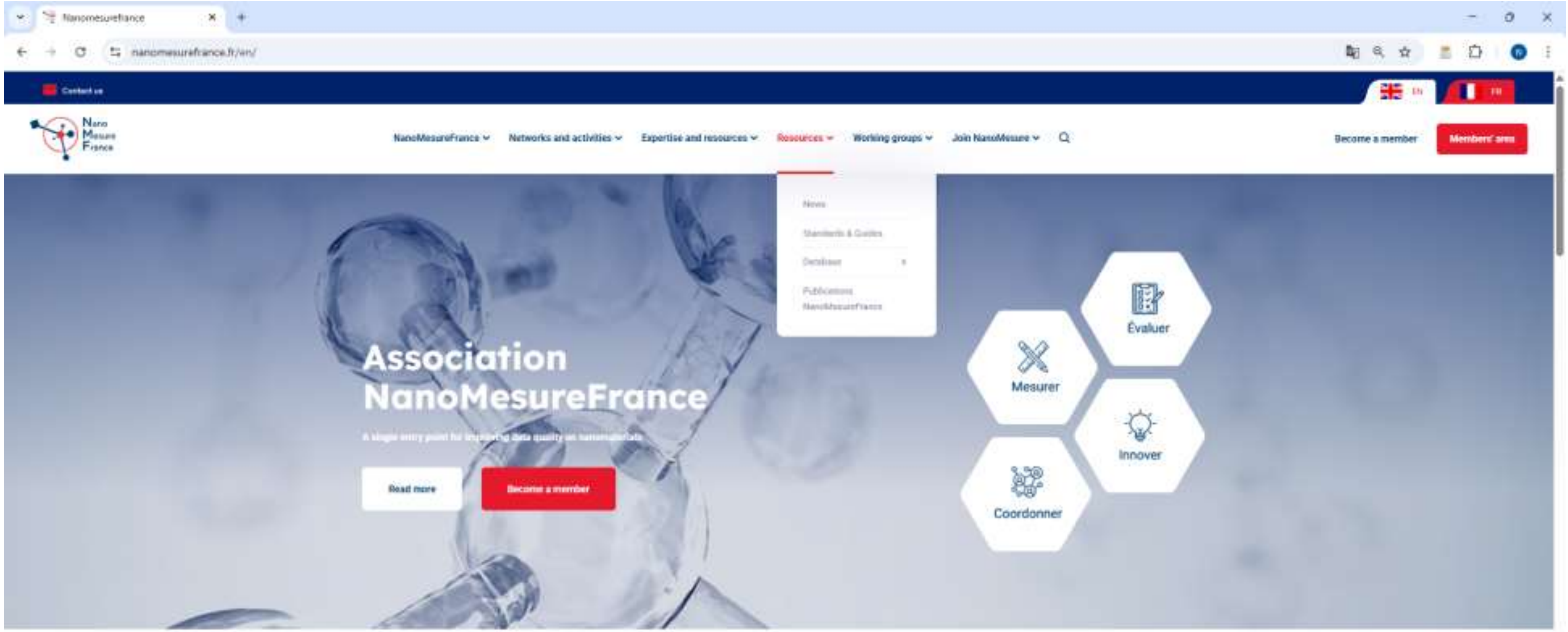
- **~ 50 materials** for which electron microscopy and atomic force microscopy methods do not (or with difficulty) **allow conclusions to be drawn** about their "nanomaterial" status
- Introduction of **complexity classes** to identify the limitations, and their origins, of the analytical techniques and protocols available to date
- Identification of **areas for development** and possible **analytical solutions**

SHARING THIS KNOWLEDGE BEYOND NANOMESUREFRANCE

- Enabling as many stakeholders as possible to **anticipate analytical difficulties**
- Testing and **improving** recommanded methods
- Build **common solutions** in response to stakeholders' expectations

GUIDE BY EXAMPLE: *AVAILABILITY*

SOON AVAILABLE ON OUR WEBSITE



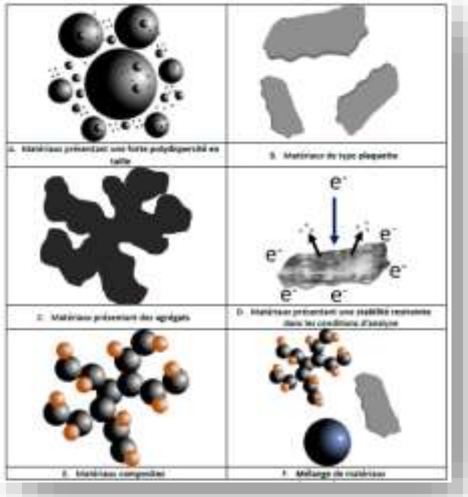
www.nanomesurefrance.fr

GUIDE BY EXAMPLE: *PROSPECTS*

BUILDING COMMON SOLUTIONS

- **Webinars, events & projects** organised by NanoMesureFrance (member-only workshop in June 2025, inter-laboratory comparison on SEM image analysis)

USE OF THE GUIDE BY ALL STAKEHOLDERS TO IDENTIFY PRIORITY COMPLEXITY CLASSES

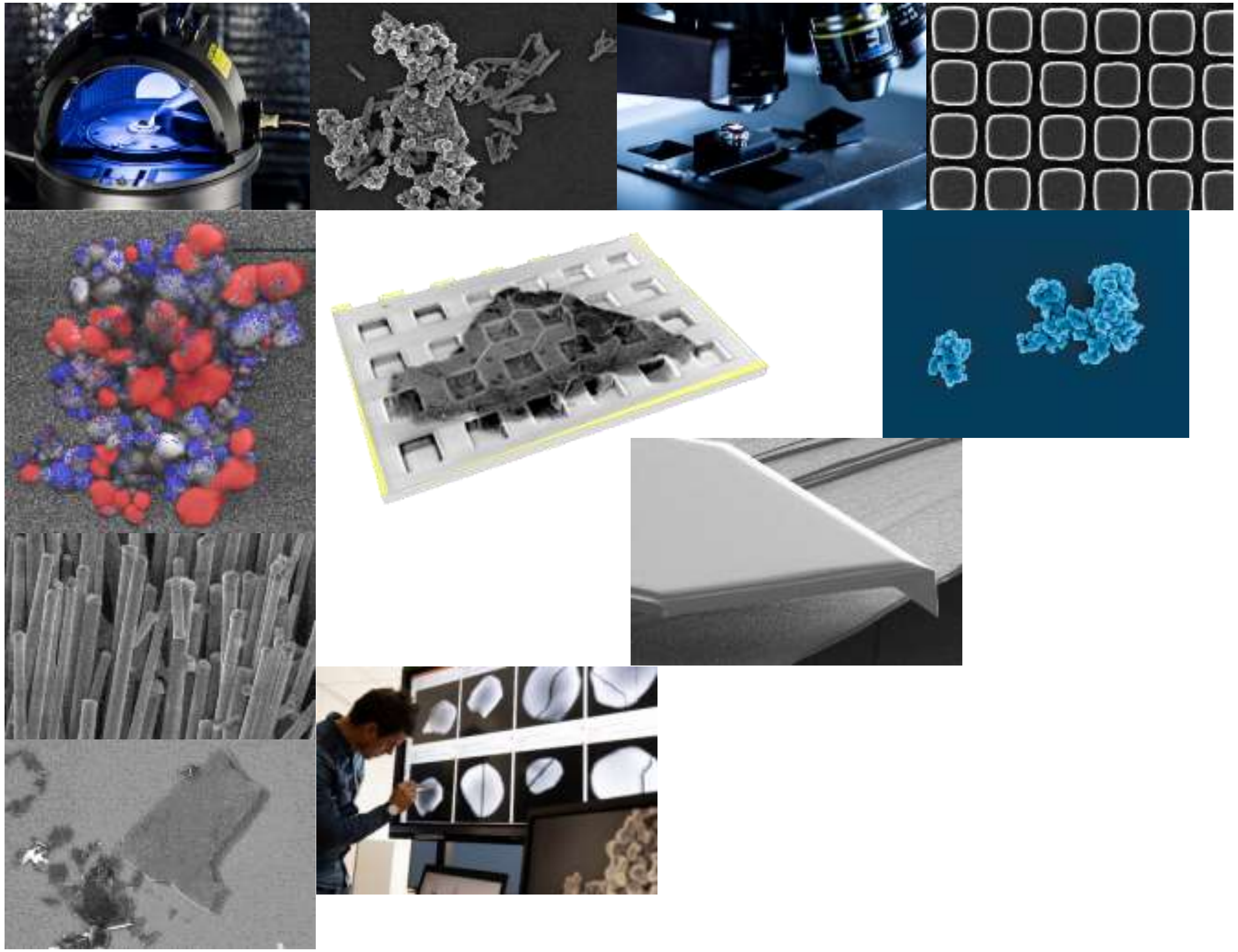


FEEDBACK from manufacturers and users

Database of materials by complexity classes

Maturity / applicability of technologies

Prioritisation of analytical development



https://www.nanomesurefrance.fr/

contact@nanomesurefrance.fr

Thank you for your attention

Nano Mesure France

Follow us on in https://www.linkedin.com/company/association-nanomesurefrance

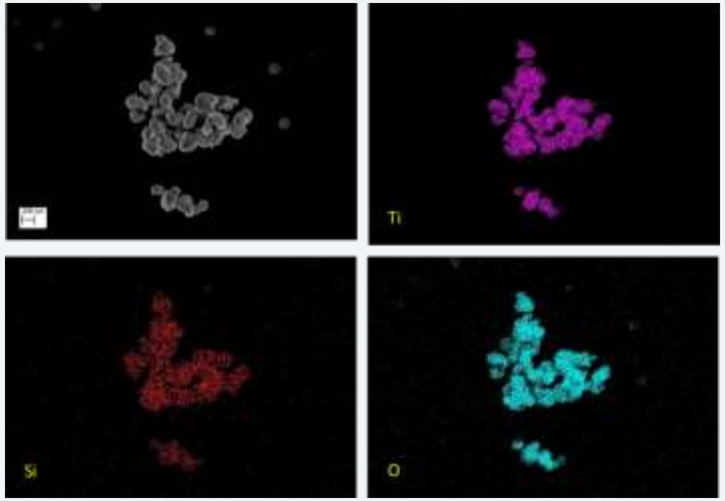
GUIDE BY EXAMPLE: *COMPOSITE MATERIALS*

DEFINITION:

- This complexity class brings together **materials composed of a substrate, sometimes on a micrometre scale, onto which a coating is bonded by strong interactions** to achieve **specific properties** (optical properties, affinity for certain matrices)
- The **chemical compositions** of the substrate and coating may be **similar** or **totally different**

ISSUES:

- Not in the measurements themselves, but in the interpretation of the data produced
- **Producing data** to demonstrate their composite nature



TiO_2 particles coated with SiO_2

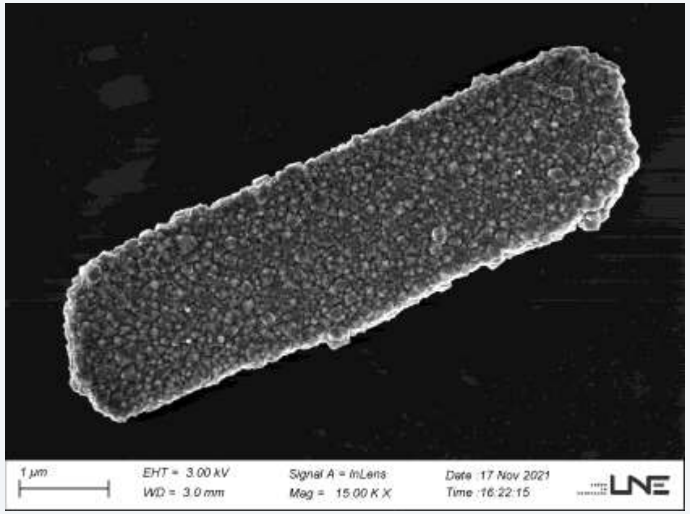
2022 Recommendation of the European Commission for the definition of a nanomaterial (2022/C229/01)

The definition should not cover large solid products or components, even when they have an internal structure or a surface structure at the nanoscale, such as coatings, certain ceramic materials and complex nanocomponents, including nanoporous and nanocomposite materials. **Some of these products or components may have been manufactured by using nanomaterials and may even still contain them.**

GUIDE BY EXAMPLE: *COMPOSITE MATERIALS*

AVAILABLE TECHNOLOGIES:

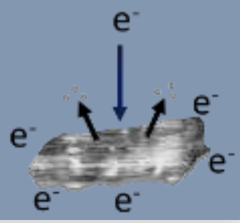
- **Screening methods** <u>not adapted</u>
- **SEM appears to be suitable**, as its <u>combination with specific detectors</u> (EDX, EBSD) enables an elemental and structural analysis of the coating/substrate pair with resolutions appropriate to their respective scales



DEVELOPMENT AREA:

- **Methodological approach under discussion** within NanoMesureFrance

GUIDE BY EXAMPLE: *RESTRICTED STABILITY*



DEFINITION:

- This complexity class brings together two cases:

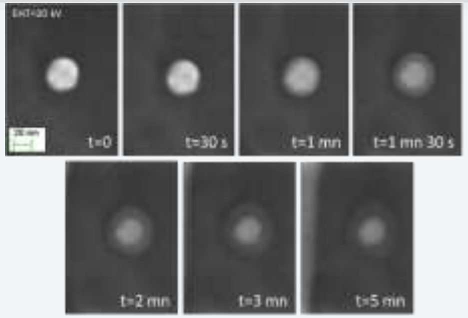
  - Materials whose **chemical properties** can cause a <u>change</u> in their **size and/or shape under the electron beam of SEM, TEM, and STEM microscopes;**

  - Materials whose **mechanical properties** can cause them to <u>deform</u>, thereby altering their **size** and **shape under the AFM tip.**

ISSUES:

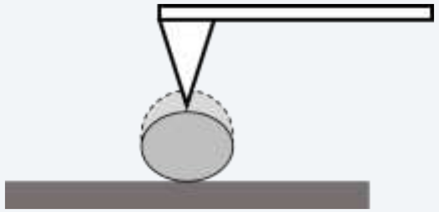
- **Lack of methods** <u>for anticipating</u> possible **changes** in the **size** and/or **shape** of the particles being tested

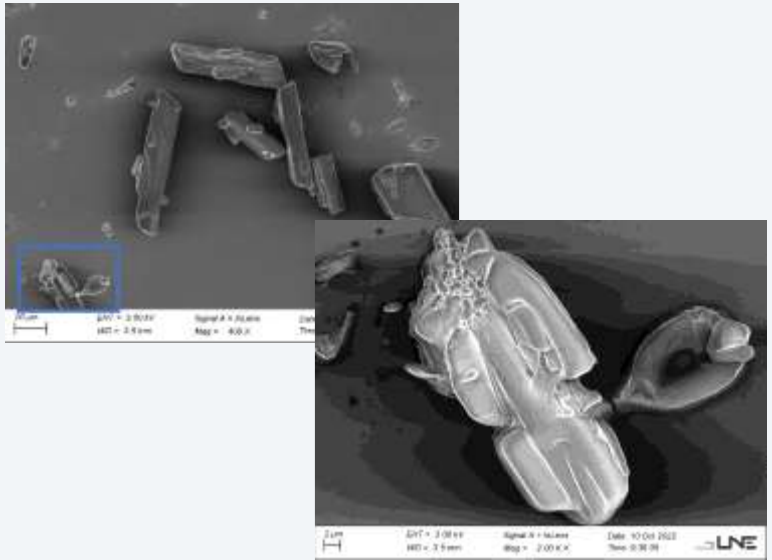
- **Sample preparation:** metallisation of samples, choice of susbtrates, etc…



Gold particles melting under the beam of an SEM (temporal evolution from left to right and from top to bottom)

Schematic illustration of the **deformation of a particle under an AFM tip**





Nano Mesure France

GUIDE BY EXAMPLE: *RESTRICTED STABILITY*

AVAILABLE TECHNOLOGIES:

- **Electron Microscopy:**
  Charge compensation devices: « *low vaccum* »
  Cooling system to limit samples' degradation
  Low acceleration voltages while maintaining resolution (nm)
- **AFM:** complementary modes for assessing mechanical properties

DEVELOPMENT AREAS:

- **Develop and harmonise analytical strategies**
- Preliminary analyses (TGA – BET) to identify the presence of a thermally unstable phase
- **Hybrid approaches (SEM-TEM/AFM)** to confirm (or refute) degradation/deformation
- Cryo-EM or environmental electron microscopy