Characterizing Nanoparticles in Sunscreen Samples

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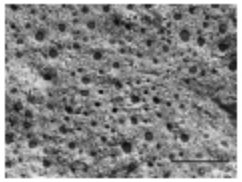
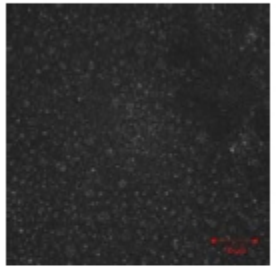
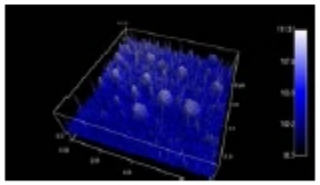
Food and Drug Administration

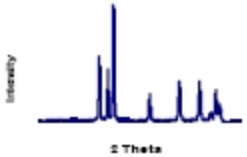
Presentation Outline

- Definitions
- Nanotechnology
- Characterization Techniques
  - Electron microscopy
  - Optical microscopy
  - Scanning probe microscopy
  - Scattering methods
- Proposed FDA approach for Analysis









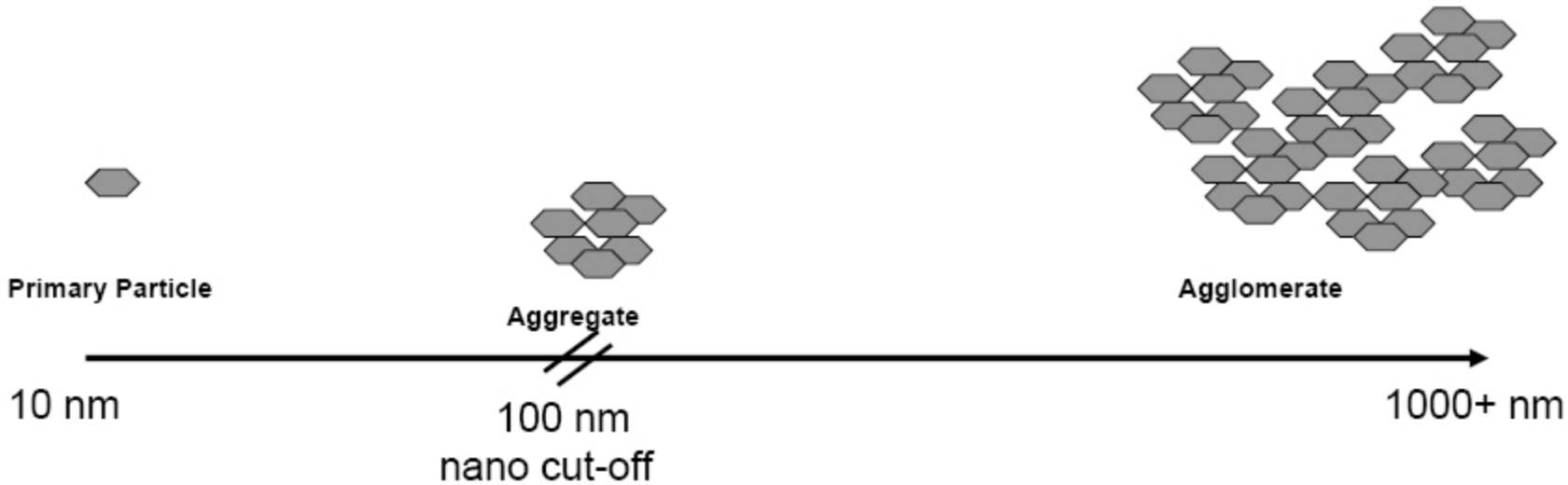
Acryonyms

ADME	Absorption, Distribution, Metabolism, Excretion
AFM	Atomic Force Microscopy
ASTM	American Society for Testing and Materials
BSE	Backscattered Electrons
DLS	Dynamic Light Scattering
EDS	Energy Dispersive X-ray Analysis
EM	Electron Microscopy
ESEM	Environmental Scanning Electron Microscopy
ESF	European Science Foundation
FDA	Food and Drug Administration
FIB	Focused Ion Beam
FWHM	Full Width Half Maximum
LSCM	Laser Scanning Confocal Microscopy
NNI	National Nanotechnology Initiative
OM	Optical Microscopy
PCS	Photon Correlation Spectroscopy
PPS	Primary Particle Size
PSD	Particle Size Distribution
RI	Refractive Index
SE	Secondary Electrons
SEM	Scanning Electron Microscopy
SPF	Sun Protection Factor
SPM	Scanning Probe Microscopy
TEM	Transmission Electron Microscopy
UV	Ultra-Violet
VPSEM	Variable Pressure Scanning Electron Microscopy
XRD	X-Ray Diffraction

Definitions - Nanotechnology

- Multiple definitions exist;
  - NNI, ASTM, Royal Society, etc.
- Most focus on size/function between 1-100 nm
  - Some definitions >> 100 nm
  - For this presentation - 100 nm or less will be used for “nanoparticle”

Definitions - Nanotechnology



Primary Particle
•Individual crystals/particles
•Surface area↑ as diameter↓
•Huge surface areas result in surface dominated effects
•Aggregation/agglomeration forces increase

Aggregate
•Formed from primary particles during synthesis/conditions
•Van der Waals forces, electrostatic forces, H bonding/ water bridging, covalent bonds
•Overall surface area is usually reduced
•Will not revert to primary particles

Agglomerate
•Weak interactions between aggregates and primary particles
•Can be broken up by mild forces
•Size changes due to conditions

Nanotechnology - Preclinical Characterization

Pre-clinical characterization of nanomaterials is fundamental for understanding safety issues.

- Nanomaterials in life-sciences area are most likely supramolecular aggregates of active and non-active atoms/molecules
- Overall particulate size may be between 1-100 nm (but in some cases more than 100 nm).
- Due to the increased surface area of nanoparticules, nanomaterials may have unique physical and chmical properties compared with larger particulate aggregates of the same materials
- ***Nanoparticles may differ substantially from larger aggregated particles during biodistribution.***

Nanotechnology - Current FDA Outlook

The nano-material should be characterized in the form to which the end-user will be exposed

- True for all drugs the FDA reviews (crystal phase, carrier form, etc.)
- Example; If you characterize the primary particle of the active ingredient, but the formulation results in aggregation or agglomeration - end-user could be exposed to different properties of the active ingredient.
  - ADME profiles could be different
  - Difficulties in analyzing results
- Methods for analysis of nano-materials are still evolving
  - Particles in suspensions have well-defined characterization routes
- There are no specific characterization methods for unmodified samples

Nanotechnology – Current FDA Outlook

- Characterization
  - What is it
    - Chemical composition, purity
    - Reactivity, surface effects
  - How big is it?
    - Particularly relevant for those claiming “nano” effects
    - Size, size distribution, aggregation, agglomeration, bulk dispersion
- Unmodified samples
  - The samples are not subject to any conditions that the formulation would not be subject to in the normal course of use
    - No dilution, separation of components, excessive heating, (excessive stress/strain, drying), etc.
    - Exposure during regular usage

Nanotechnology – Current FDA Outlook

Currently marketed nano-drugs

Product	Type of nanoparticle	Indication	Particle size
Magenvist	Gadolinium dimeglumine	MRI contrast agent	<1 nm
Feridex	Superparamagnetic Iron Oxide	MRI contrast agent	120-180 nm
Rapamune	Nanocrystal/Sirolimus	Immunosuppressant	100-1000nm
Emend	Nanocrystal/Aprepitant	Antiemitic	100-1000 nm
TriCor	Nanocrystal/Fenofibrate	Hypolipidemic	
Megace ES	Nanocrystal/Megesterol Acetate	Appetite enhancer	
Doxil	Liposome/Doxorubicin	Antineoplastic	~ 100 nm
AmBisome	Liposome/Amphotericin	Antifungal	
Diprivan	Liposome/Propofol	Anesthetic	
Abraxane	Albumin-coated nanoparticles	Antineoplastic	~ 130 nm
Definity	Lipid coated gas particles	Echography contrast agent	

Nanotechnology and Sunscreens

- 2006 Citizen's Petition to the FDA to evaluate nano-sunscreens
  - TiO_2
  - Wide-range of FDA-initiated projects to address safety/toxicity issues
  - Characterization of the raw nanomaterials as the as-formulated materials (commercial and in-house) is an integral part of these studies
- Sunscreens are complex, multi-component formulations
  - Often opaque
  - Often can not be dried down (oily)
  - Often contain other nano-domains
  - Useful system for comparing characterization techniques

Nanotechnology and Sunscreens

Size and Effect

1. Primary Particle Size (PPS)

   Aggregate size, surface reactivity, SPF

2. Aggregate

   Visible transmittance

3. Agglomeration

   Application to skin

4. Particle Size Distribution (PSD)

   UV attenuation spectrum

5. Dispersion

   Application to skin

Nanotechnology and Sunscreens

(RED) PRODUCTS CONTAIN MANUFACTURED NANOPARTICLES

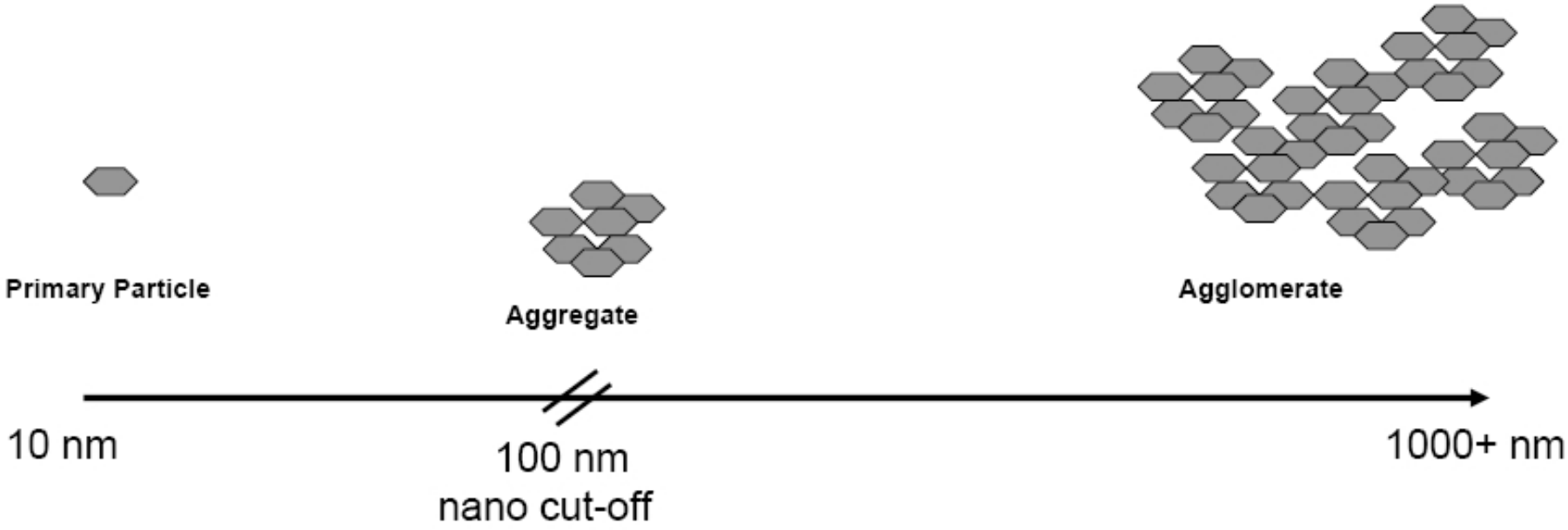
PRODUCT	MANUFACTURER	NANO CONTENT CLAIM
Applied Therapeutics™	Applied Therapeutics™	Others claim nano content
Bebe/Enfant High Protection SPF 50	Mustela	Others claim nano content
Blue Lizard® BABY	Crown Laboratories, Inc.	Manufacturers claim nano content
Blue Lizard® Regular	Crown Laboratories, Inc.	Others claim nano content
Blue Lizard® Sensitive	Crown Laboratories, Inc.	Manufacturers claim nano content
Chemical-Free Sunscreen SPF 15	Burt's Bees® Inc.	Manufacturers claim nano content
Cotz SPF 58	Fallene	Others claim nano content

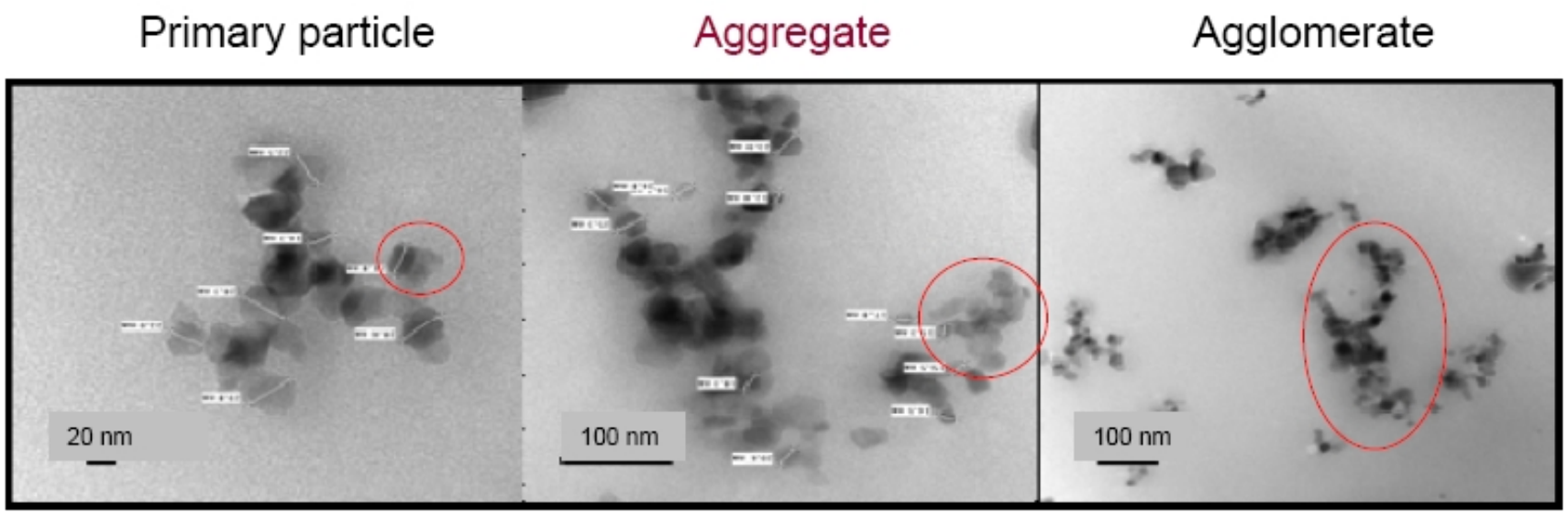
Nanotechnology and Sunscreens

Materials

COMMERCIAL PRODUCT	PARTICLE	CLAIMED AMOUNT
1	TiO_2	9.1%
2	TiO_2	2.4%
3	TiO_2	not listed
4	ZnO	6.0%
5	ZnO, TiO_2	5%, not listed
6	ZnO	8.0%

Formulations were chosen based upon product labeling (possible “nano”product)
Some formulations contained additional inorganic particles





TEM image courtesy of the Electron Microscopy Facility Image Analysis Laboratory, NCI Frederick, in collaboration with the Nanotechnology Characterization Laboratory

Nanotechnology and Sunscreens

Composition

1. Chemical composition

   TiO_2, ZnO, other formulation additives

2. Purity
3. Crystal phase

   Anatase/rutile

4. Surface coating
5. Reactivity

   Photoreactivity

Many of these parameters would not be expected to change during formulation for TiO_2 or ZnO

Nanotechnology and Sunscreens

Methods

ELECTRON MICROSCOPY

Scanning electron microscopy (SEM)

Variable pressure SEM with energy dispersive x-ray analysis (VPSEM with EDS)

Focused ion beam (FIB)

Cryogenic transmission electron microscopy

SURFACE PROBE MICROSCOPY

Atomic force microscopy (AFM)

Near-field scanning optical microscopy (NSOM)

OPTICAL MICROSCOPY

Laser scanning confocal microscopy (LSCM)

Two photon scanning confocal microscopy

Cytoviva Ultra Resolution imaging

SCATTERING METHODS

X-ray diffraction (XRD)

Small angle neutron scattering (SANS)

Tri-laser angular light scattering

Dynamic light scattering/PCS

Laser diffraction

Photon cross-correlation spectroscopy (PCCS)

MISC. METHODS

Raman chemical imaging**OPTICAL**

Centrifugal separation analysisLaser

X-ray disc centrifugeTwo

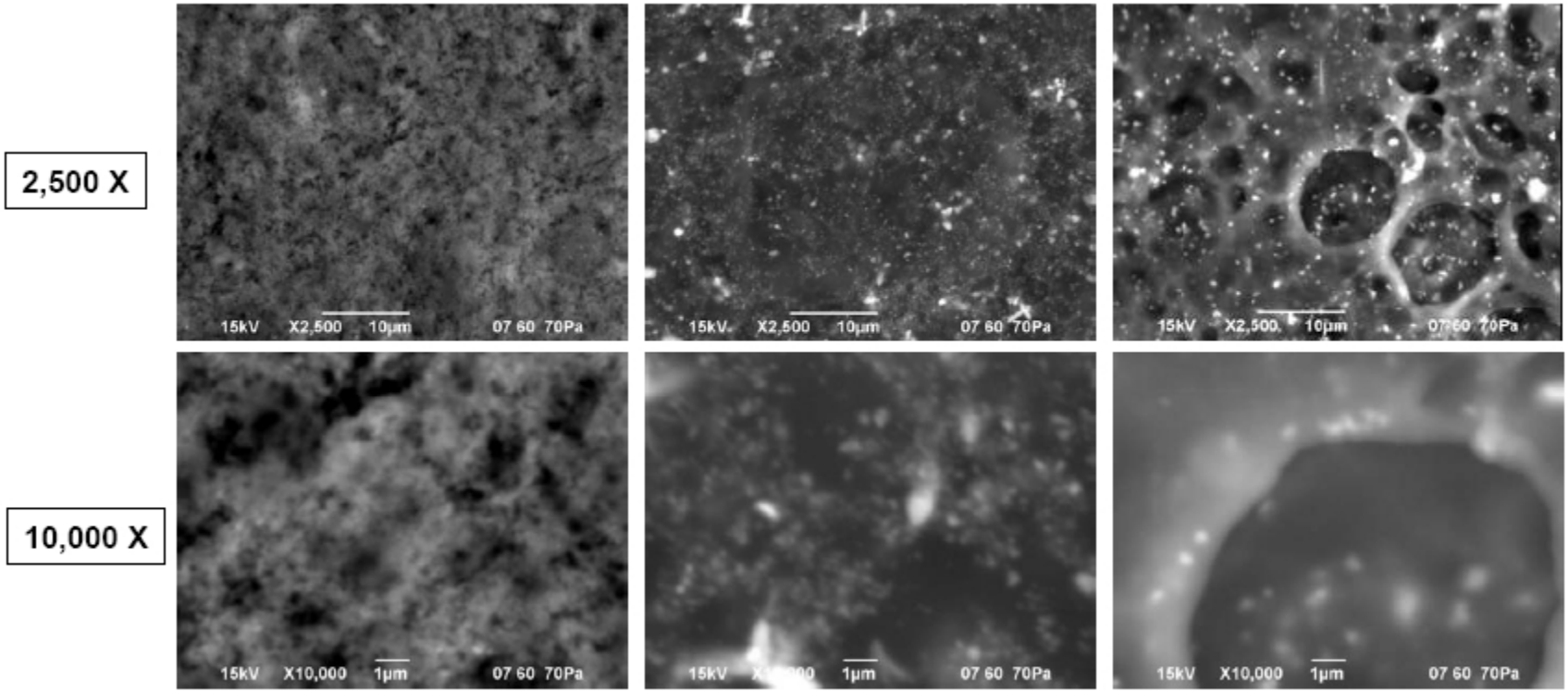
Acoustic attenuation spectroscopyCytoviva

Acoustic spectrometry

Sunscreens were tested directly out of the container

Electron Microscopy

VPSEM

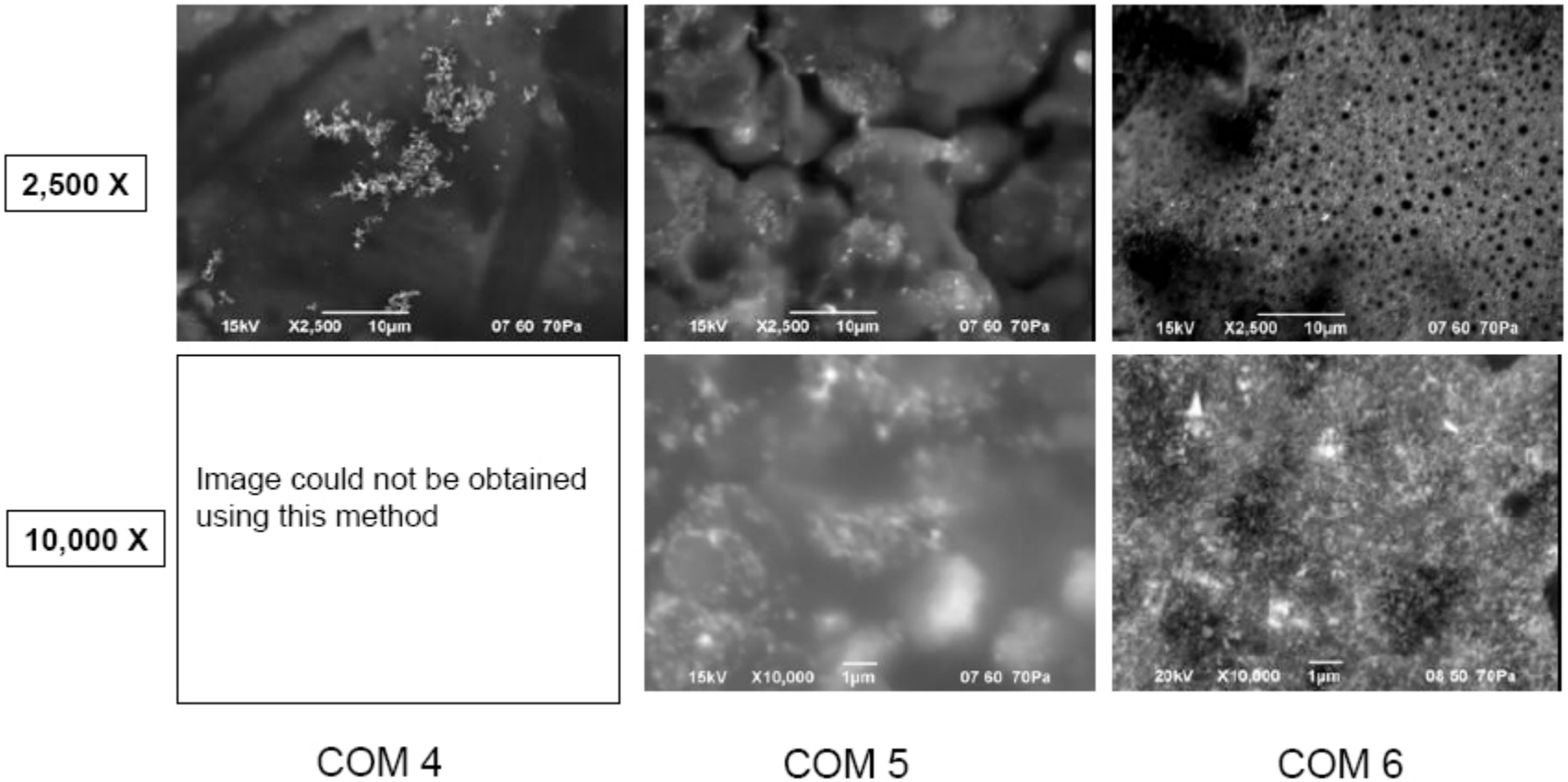


COM 1 COM 2 COM 3

Aggregate size, agglomerate size, 2D distribution may be determined
Charging, degradation of sample, loss of resolution can be observed

Electron Microscopy

VPSEM

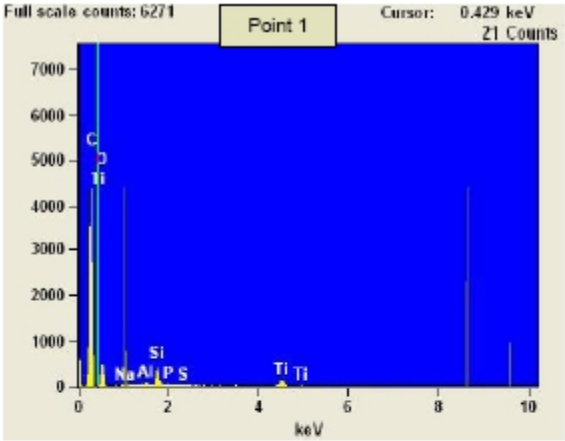


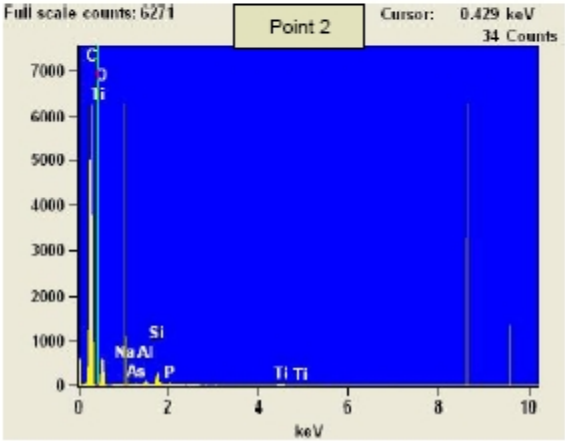
A wide range of particle sizes and dispersions can be observed for the sunscreens
In some instances, particles could not be accurately sized due to loss of resolution

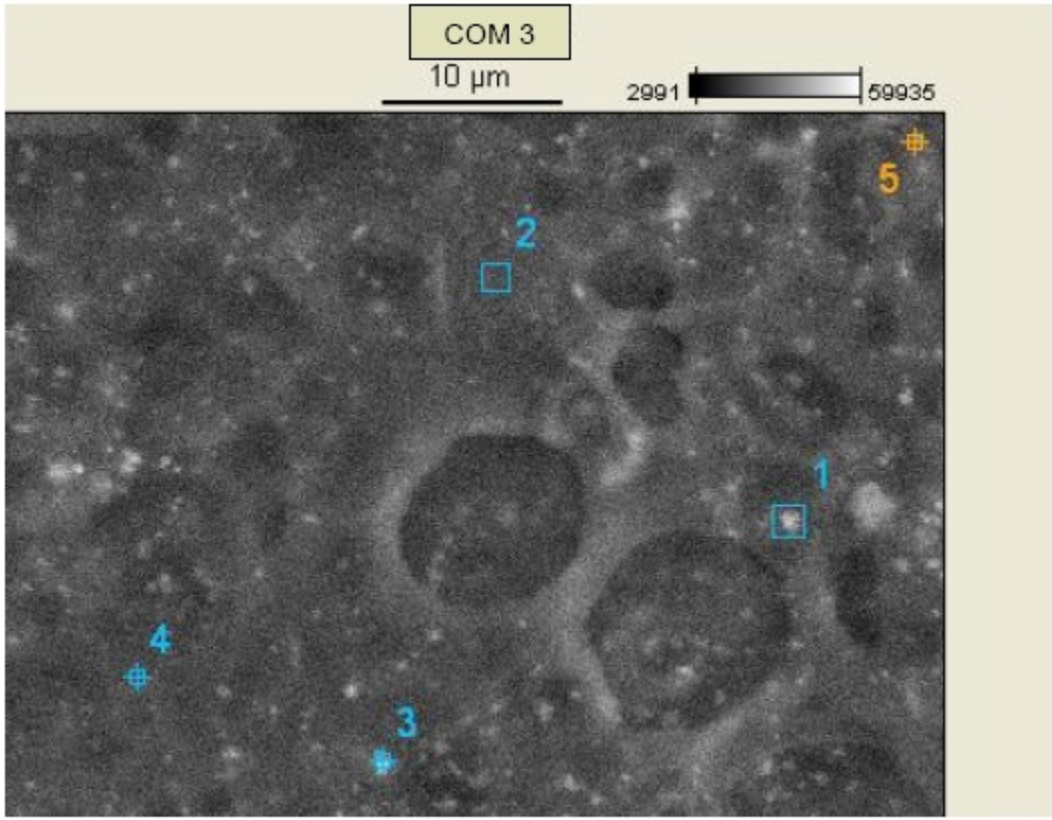
Electron Microscopy

EDS

ELEMENT IDENTIFICATION





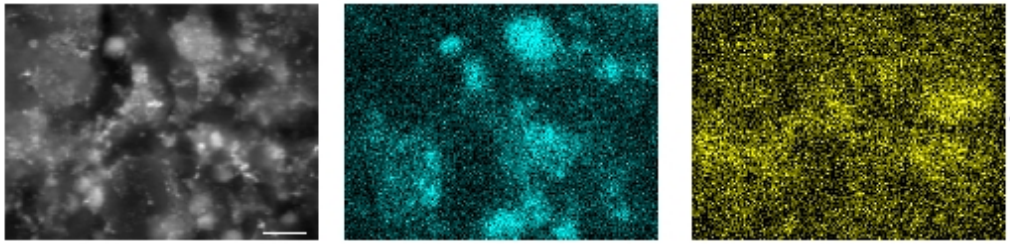


Nanoparticles containing Ti can be identified

Electron Microscopy

EDS

ELEMENT IDENTIFICATION

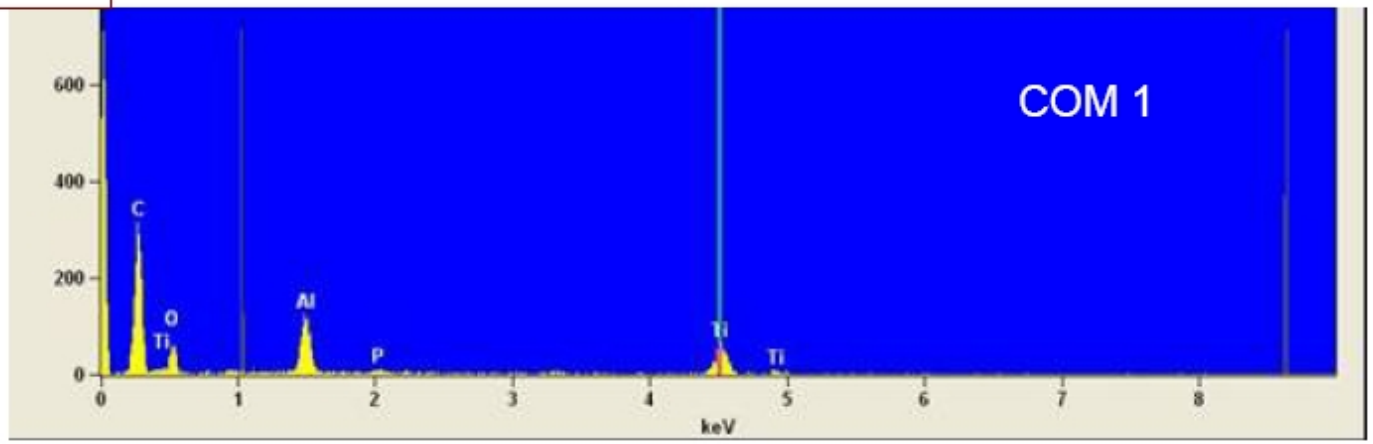


COM 5 Ti Zn

ZnO particles are evenly distributed throughout the sample

The TiO_2 particle was coated with aluminum hydroxide

SURFACE COATING



Electron Microscopy

VPSEM-Summary

SAMPLE	PARTICLE SIZE*	PSD	DISTRIBUTION (2D)	ELEMENTS	NANO?
COM 1	100 nm or less	Small	Even	Ti, Al (surface?)	YES
COM 2	200 nm or more	Large	Uneven	Ti	NO
COM 3	200 nm or more	Large	Even	Ti, Si	NO
COM 4	200 nm (?)	?	Uneven	Zn, Si	?**
COM 5	100-200 nm	Large	Uneven	Ti, Zn, Si	YES
COM 6	100 nm or less	Small	Even	Zn, Si	YES

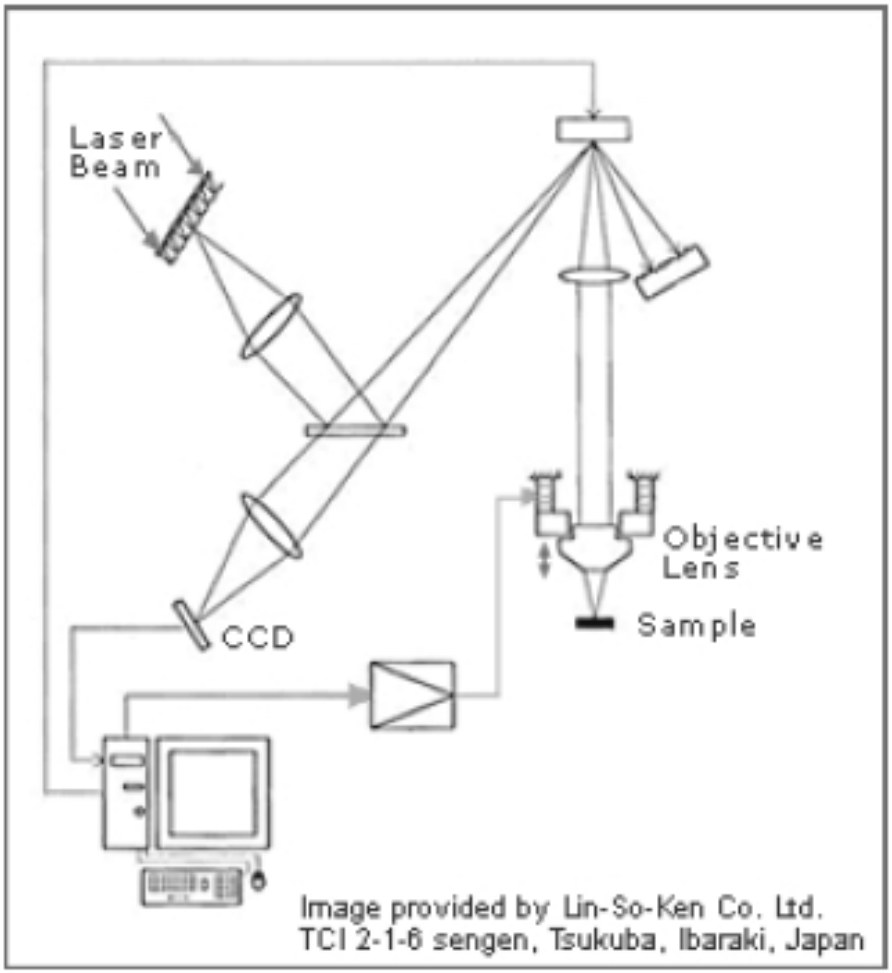
Nanoparticle size and distribution could be determined
Elemental identification and distribution could be detected

* Particle size is the dominant particle size (aggregate)
** Based on label information, starting material was nano-sized

Optical Microscopy

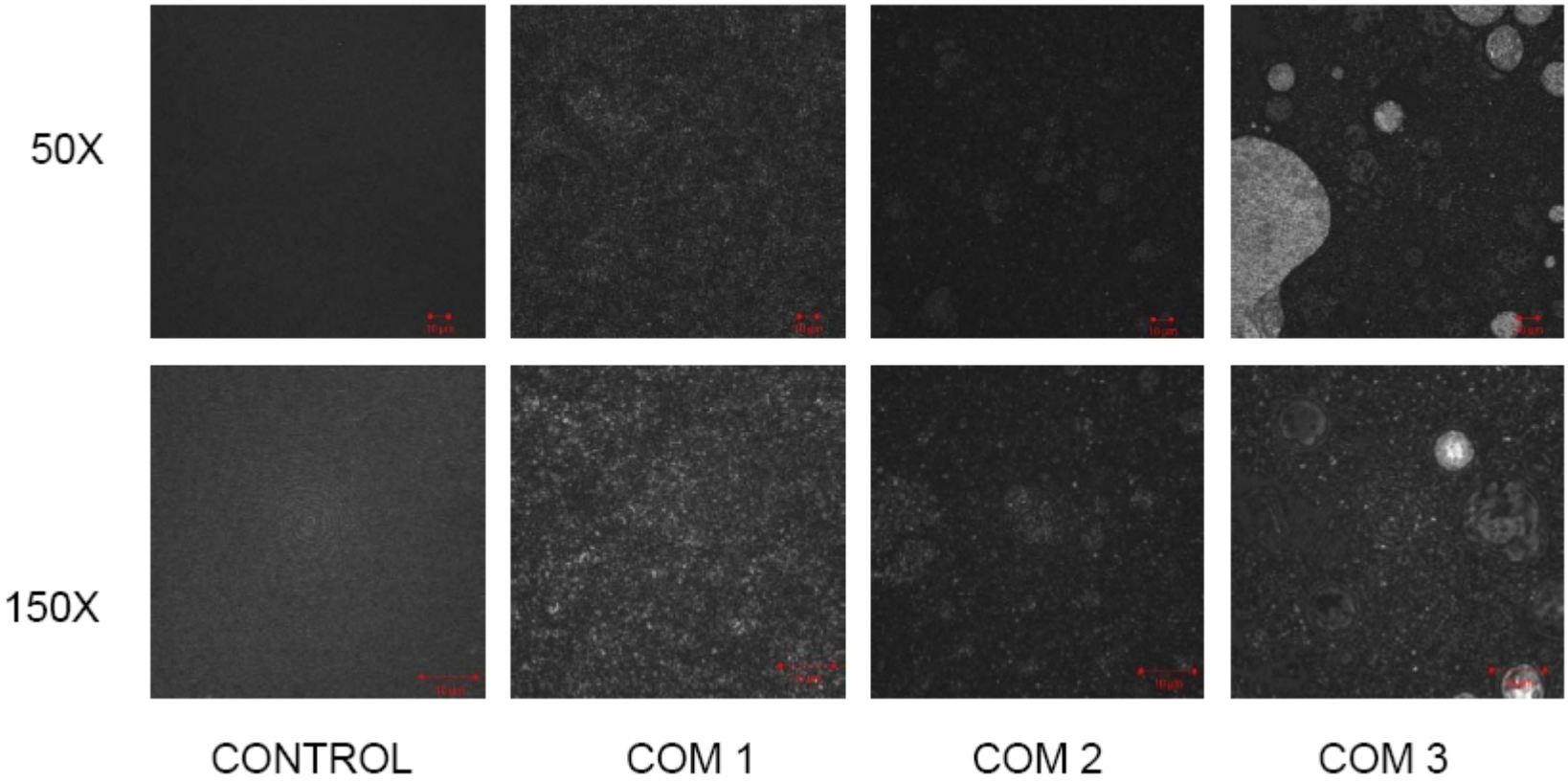
LSCM

- **Most optical methods will not resolve nanoparticles**
- LSCM in reflection mode
  - Confocal microscopy makes use of pinhole geometry to selectively focus on a single optical plane allowing for 3D observations.
  - Contrast is based on the reflection of laser light at the interfaces between media of different RI
  - TiO_2 and ZnO (~2-3), rest of sunscreen formulation (~<1.6)
- Any substrate can be used
  - Glass microscope slide
- Samples can be analyzed with no modification
- Resolution
  - Due to optical resolution limits, the nanoparticles can be detected, but not necessarily sized
  - Standard sampled may be used to aid in nanoparticle observations
  - TiO_2 nanoparticles in water (!100 nm with a sharp PSD as measured through DLS)
- Additional notes
  - Dispersion can be analyzed in 3D
  - Difference phases may be detected, not just particles
  - Quick observations and analysis allow for a wide variety of tests over a wide region
  - 2D projections



Optical Microscopy

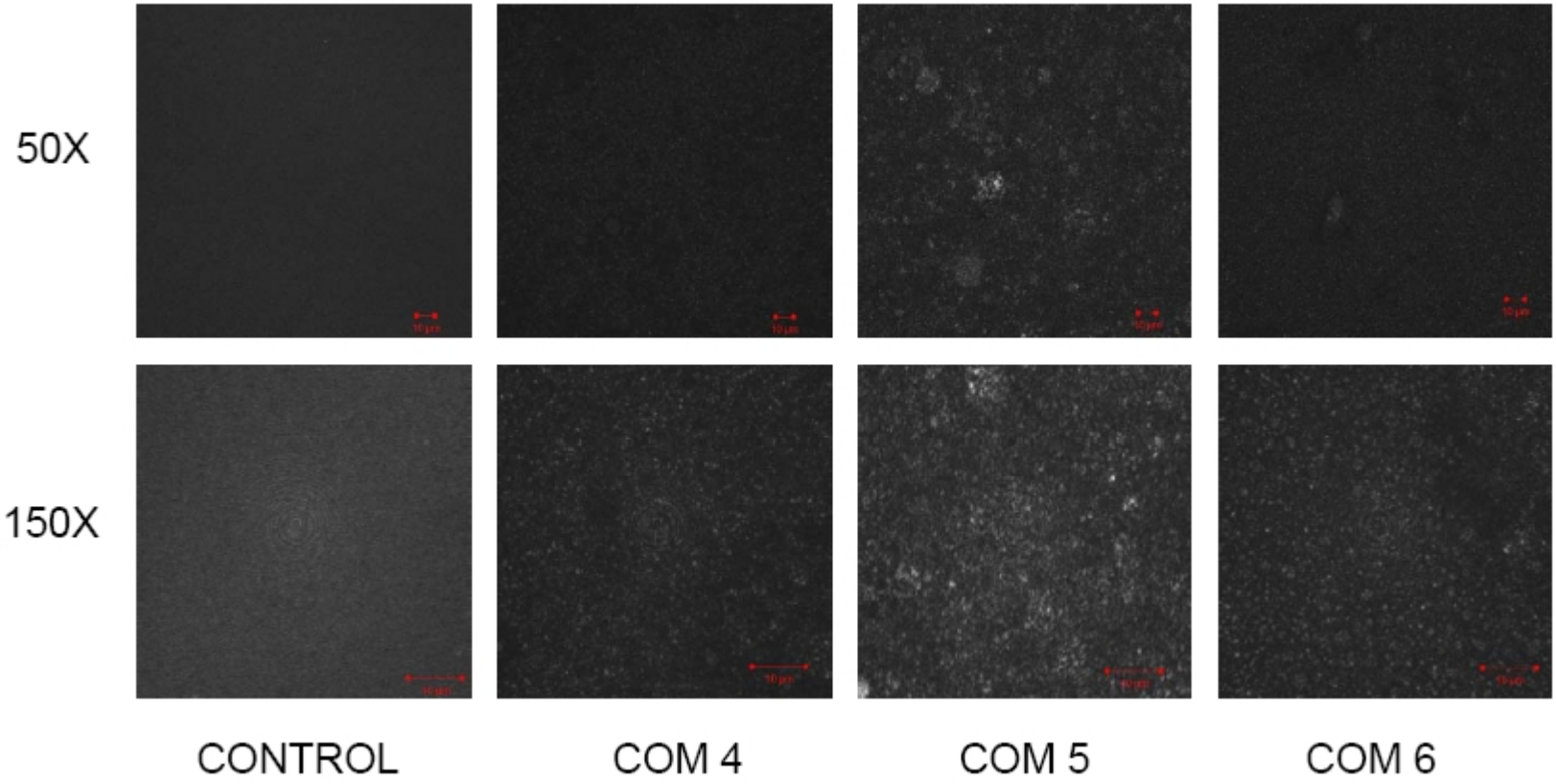
LSCM



A wide range of particle sizes and dispersions may be observed for the sunscreens

Optical Microscopy

LSCM



Most particles appear agglomerated, although small individual particles may be observed

Optical Microscopy

LSCM - Summary

SAMPLE	PSD	DISTRIBUTION (3D)	NANO?
COM 1	Small	Even	YES
COM 2	Small	Uneven	NO
COM 3	Large	Uneven*	NO
COM 4	Large	Even	YES
COM 5	Large	Uneven	YES
COM 6	Large	Even	YES

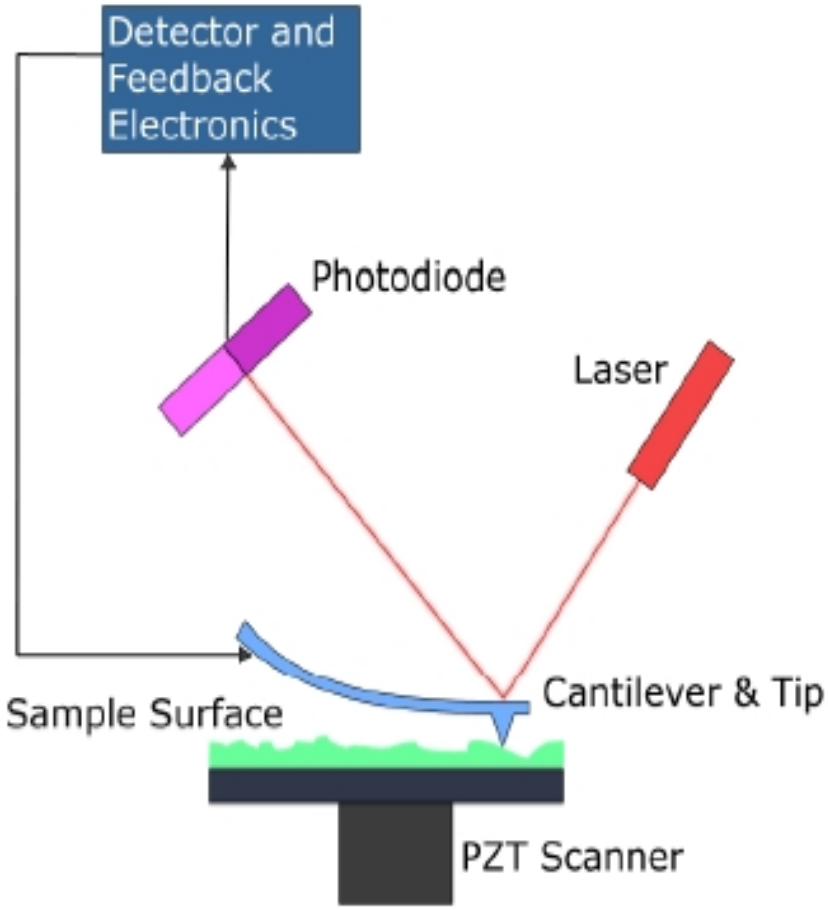
PSD, 3D distribution, and estimate of “nano” possible
Particle size “nano” estimates are similar to VPSEM
Dispersion profiles and PSD were sometimes different than VPSEM

* Different domains detected

Scanning Probe Microscopy

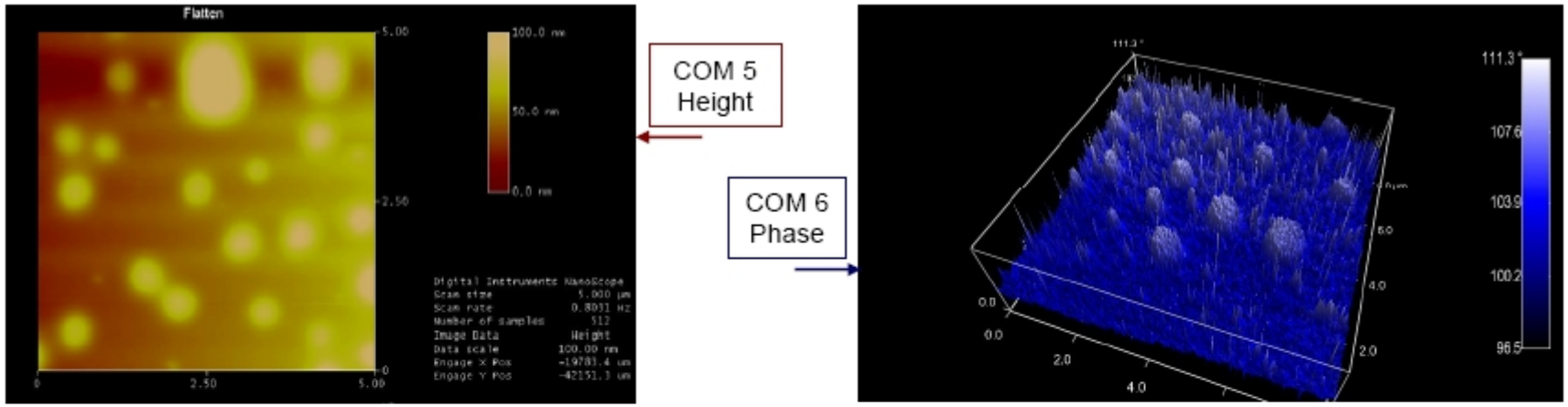
AFM

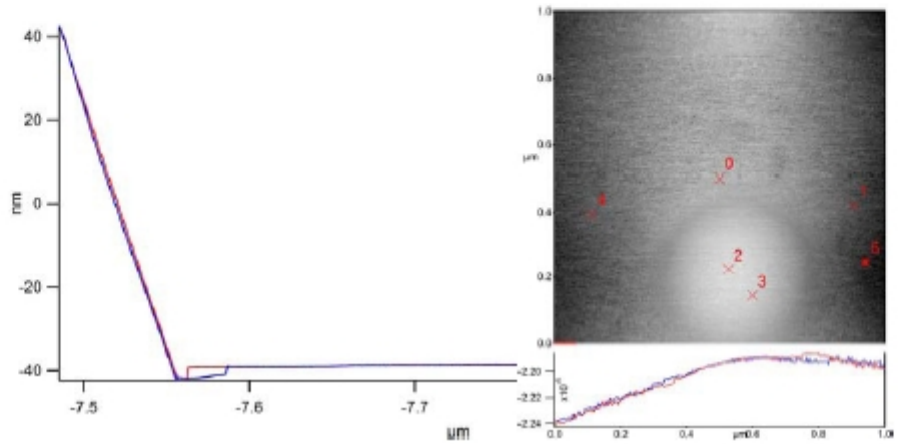
- **Most SPM methods are labor and analysis intensice**
- AFM
  - High resolution technique whereby a cantileve is used to scan the specimen surface. Depending on the conditions, different properties of the sample may be measured
  - Height, phase, forces, elemental analysis, chemical bonding, etc.
- Any substrate can be used
  - Glass microscopic slide
- Samples can be analyzed with no modification
- Resolution
  - Atomic
- Additional Notes
  - Difference phases of the sample can be analyzed (o/w emulsions)
  - Complex domain information
  - Different tips and methodologies allow for a wide range of properties and interactions to b measured
  - Labor and analysis intensive
  - Surface Method
  - Small area analyzed
  - Easy to damage tip



Scanning Probe Microscopy

AFM



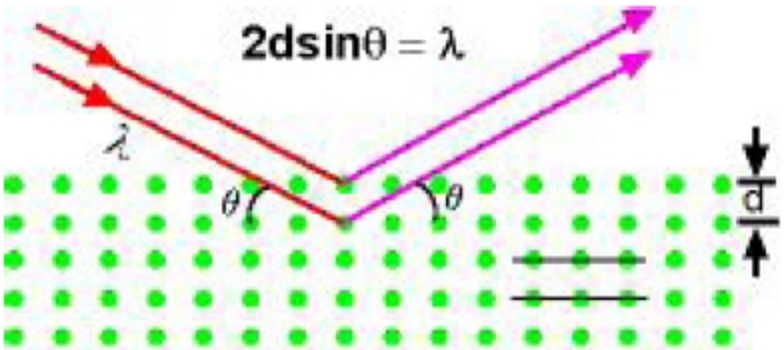


Height and phase images may be obtained for the samples. Analysis of force curves performed on the different phases can provide further information about the sample's composition.

Scattering Techniques

XRD

- Many scattering methods require dilution
- XRD (powder)
  - X-rays are diffracted from randomly oriented microcrystalsin a sample, providing information on the sample's crystal structure.
  - Phase identification, phase quantification
- Samples can be analyzed with no modification
- Size determnation
  - If the PPS is below ~200 nm
  - Particle size (PPS is will be an average of the sample
  - Can calculated PSD
- Additional Notes
  - Bulk measurement
  - Can use different substrates



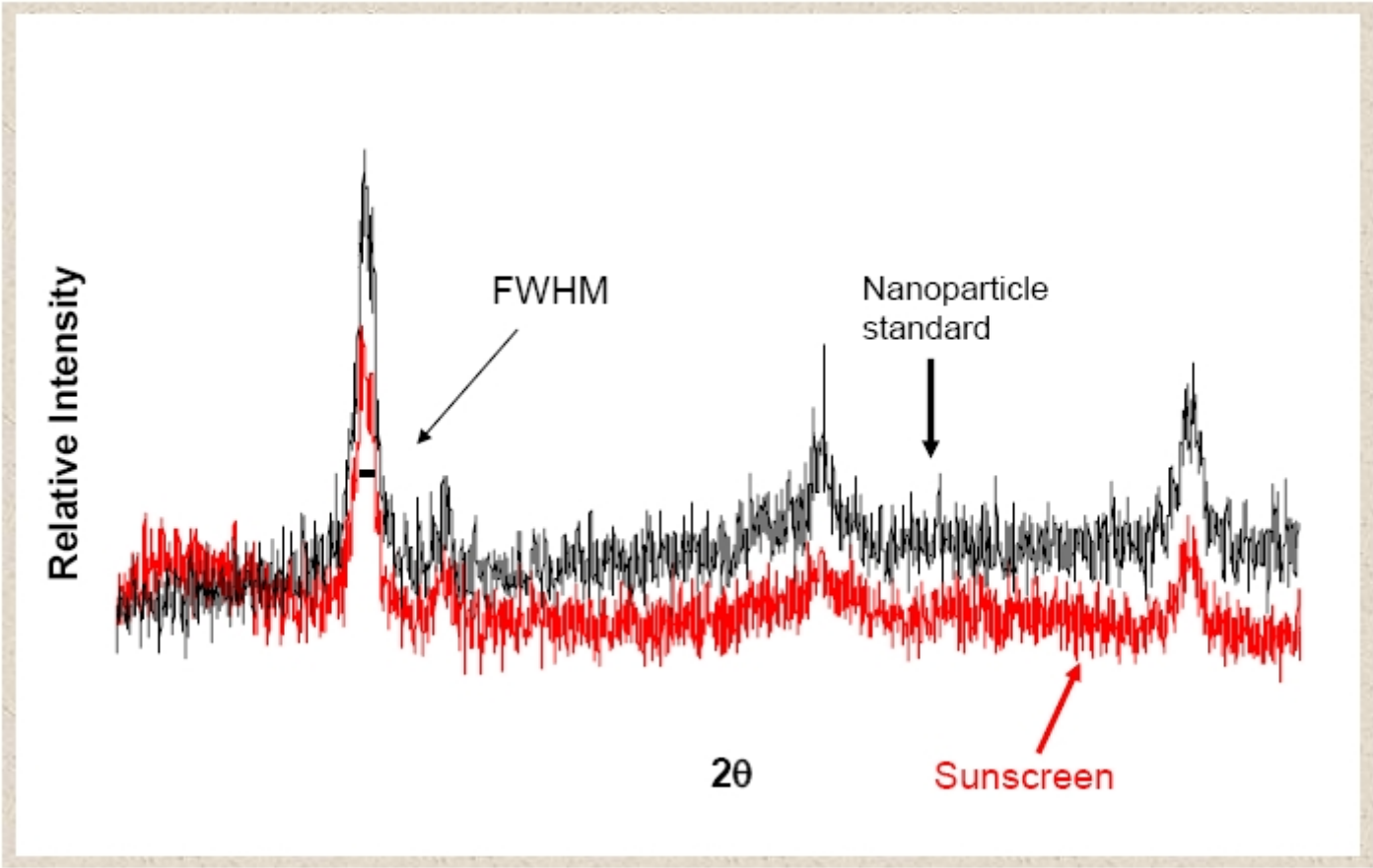
Bragg's Law

SCHERRER EQUATION:

$D = 0.9\lambda / B\cos\theta$

Scattering Techniques

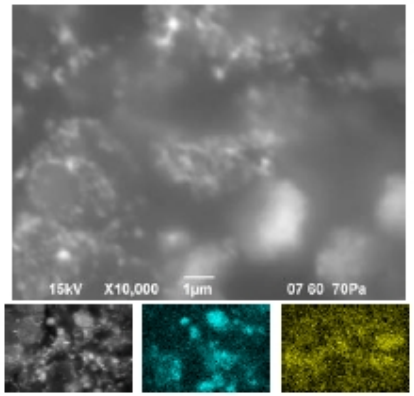
XRD



Sunscreen containing anataseTiO_2, PPS determined to be ~20 nm

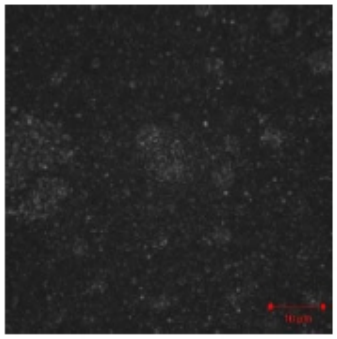
Proposal for Analysis of Sunscreens

VPSEM

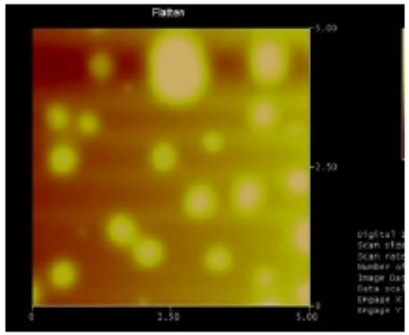


COM 5 Ti Zn

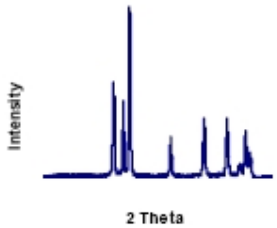
LSCM



AFM*



XRD*



SIZE
Aggregate
Agglomerate
2D distribution
PSD
CHEMICAL
Element identification
Element distribution
Surface

SIZE
Aggregate (est.)
Agglomerate
3D distribution
PSD

SIZE
Aggregate
Agglomerate
2D distribution
PSD
CHEMICAL
Surface composition
Reactivity

SIZE
PPS
PSD (PPS)

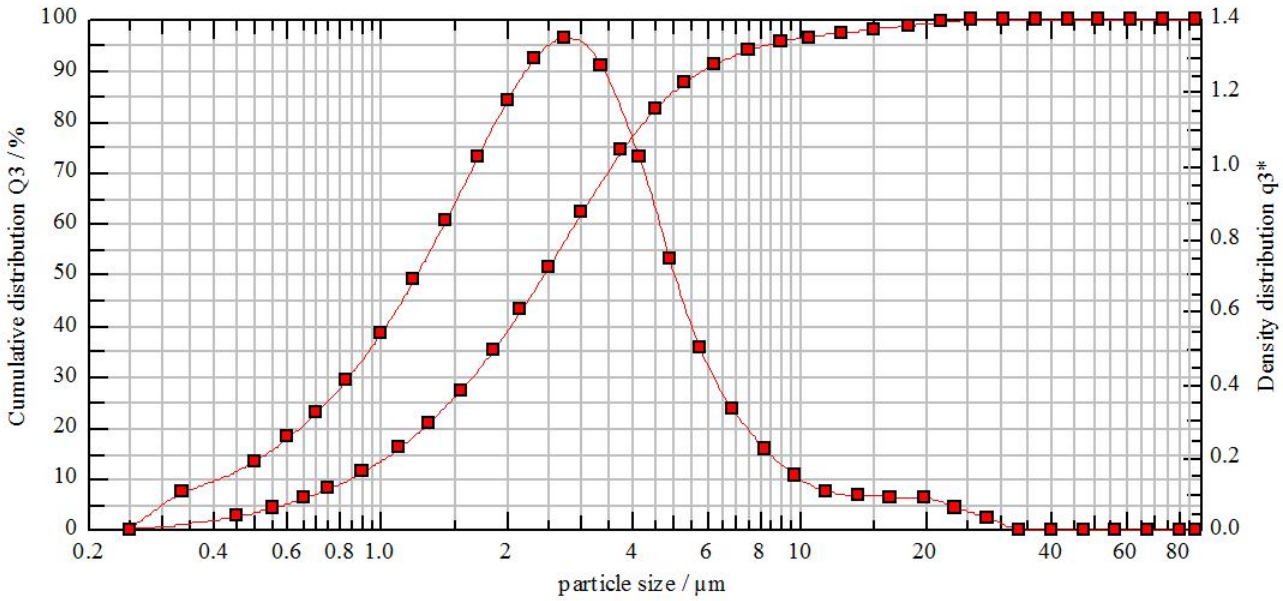
CHEMICAL
Crystal phase

Particle size of Zano® 10 and Zano® 10 Plus

As a raw material in powder form

Measured on dry powder, as supplied using laser scattering

Average particle size ~2.5µm, no particles <300 nm



materials for a better life

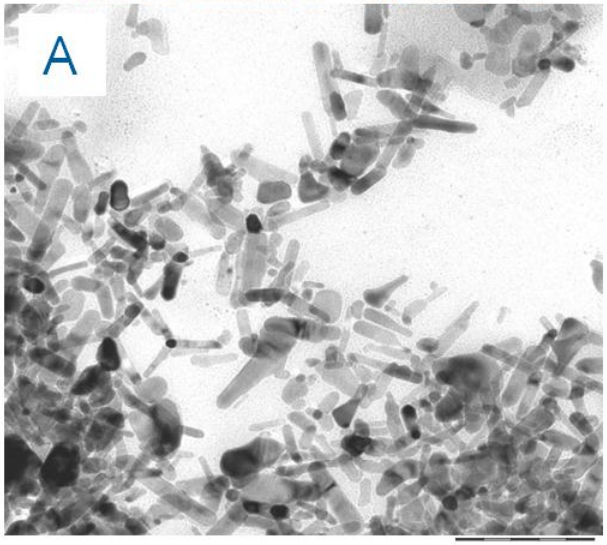
Particle size of Zano®10 and Zano®10 Plus

in a finished formulation

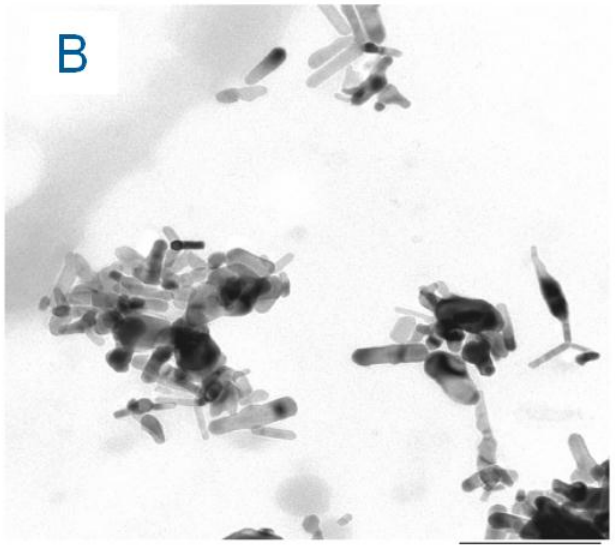
TEM analysis on unadulterated formulations containing Zano®10 and Zano®10 Plus

Average particle size of ≥ 200 nm and above

O/W formulation – 9% Zano® 10



O/W formulation – 9% Zano® 10 Plus



Proposal for Analysis of Sunscreens

In Progress and Unsuitable Methods

ELECTRON MICROSCOPY
Scanning electron microscopy (SEM)
High vacuum required
Focused ion beam (FIB)
In progress
Cryogenic transmission electron microscopy
In progress

SURFACE PROBE MICROSCOPY
Near-field scanning optical microscopy (NSOM)
Method is not suitable for application

OPTICAL MICROSCOPY
Two photon scanning confocal microscopy
In progress

Cytoviva Ultra Resolution Imaging
Particles could not be resolved from formation

SCATTERING METHODS
Small angle neutron scattering (SANS)
In progress
Tri-laser angular light scattering
Sample dilution required
Dynamic light scattering
Sample dilution required
Laser diffraction
Sample dilution required
Photon cross-correlation spectroscopy (PCCS)
Sample dilution required

MISC. METHODS
Ramen chemical imaging
In progress
Centrifugal separation analysis
Sample dilution required
X-ray disc centrifuge
Sample dilution required

Acoustic spectrometry
Method is not suitable for application

Proposal for Analysis of Sunscreens

Under Investigation

SIZE

LSCM
Aggregate/agglomerate size (estimate), 3D dispersion

XRD
PPS

↓

VPSEM
Aggregate size, PSD, agglomeration, dispersion

↓

AFM
Nano-domain sizes

↓

DESTRUCTIVE
If information can be obtained no other way

UNKNOWN SAMPLE

VPSEM/EDS
Aggregate size, PSD, agglomeration, dispersion, element identification

↙ ↘

XRD
PPS, crystal structure

LSCM
3D dispersion

↓

AFM
Additional analysis as warranted