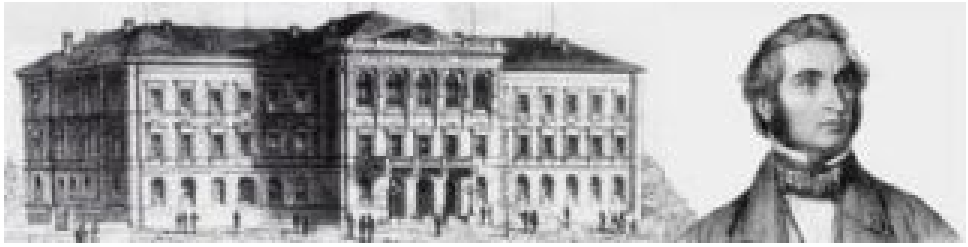
JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN

400 Jahre
UNIVERSITÄT GIESSEN
1607–2007

Gefahrstofflaboratorien Chemie und Physik am Institut für Arbeits- und Sozialmedizin

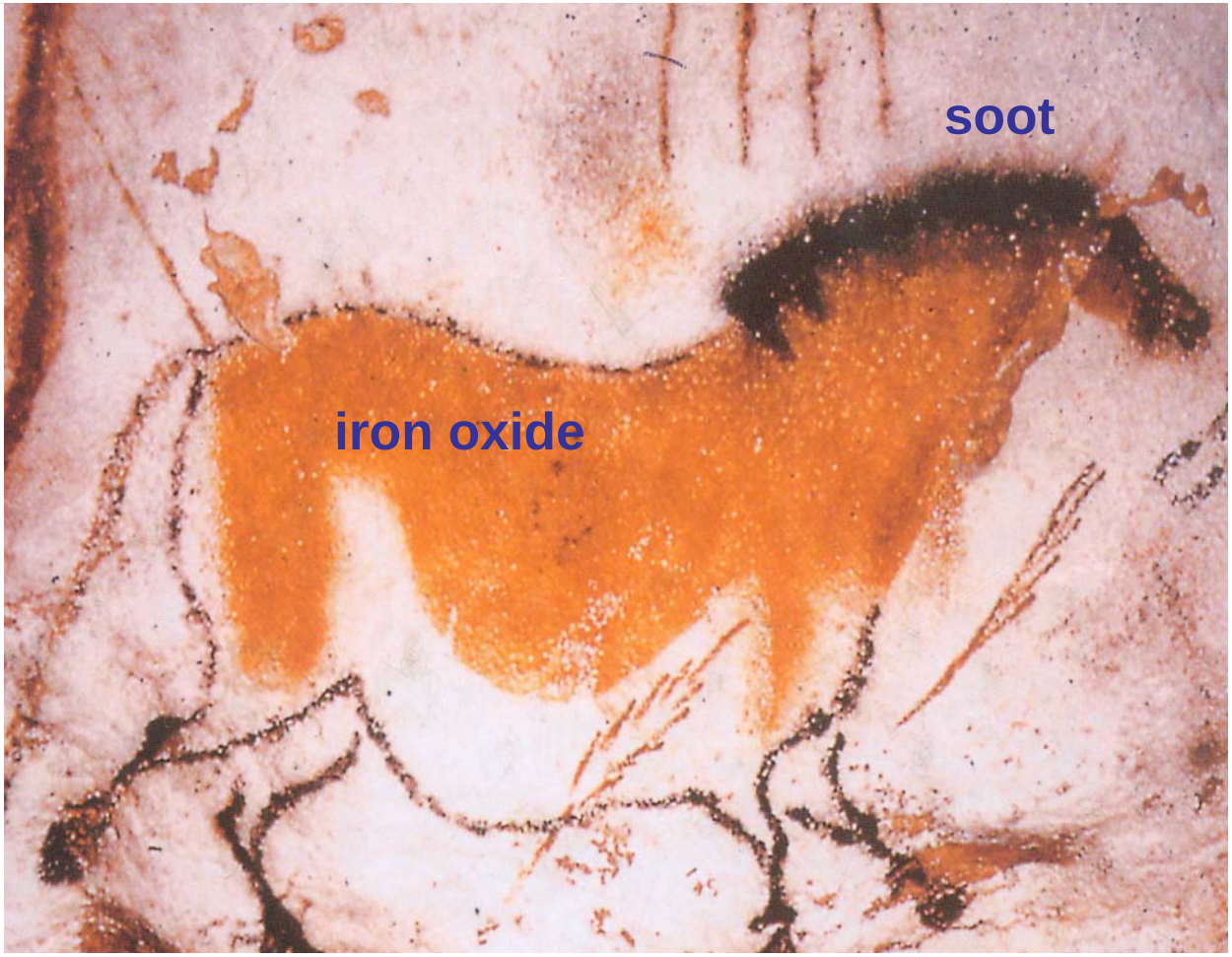
der Justus-Liebig-Universität Gießen

Nanoparticles – A problem of characterization and toxicity



Sofia – 10.06.2013

Prehistoric cave paintings



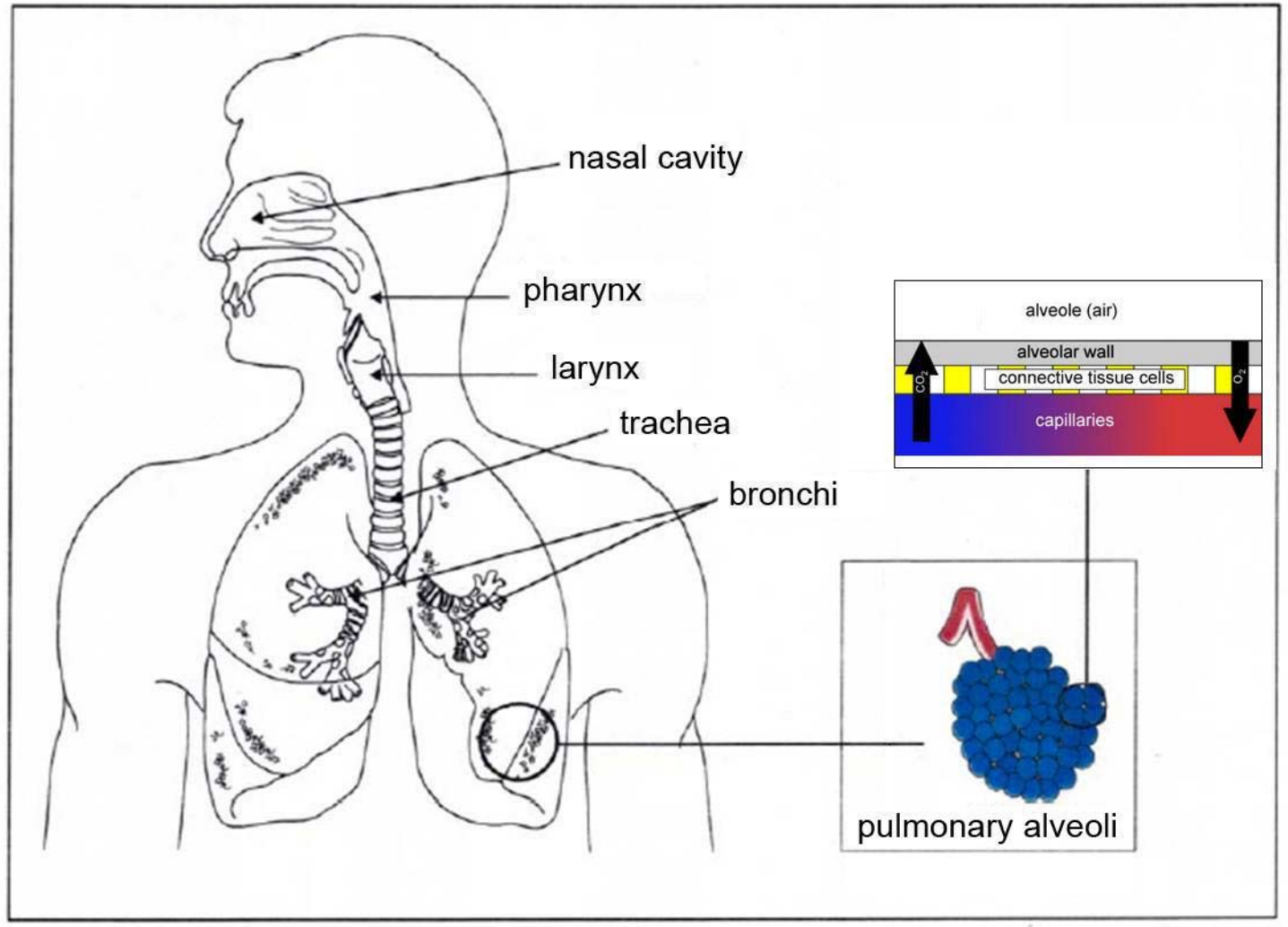
Cave art in Altamira (Spain, Province Santander)

“Historical knowledge on the toxicity”

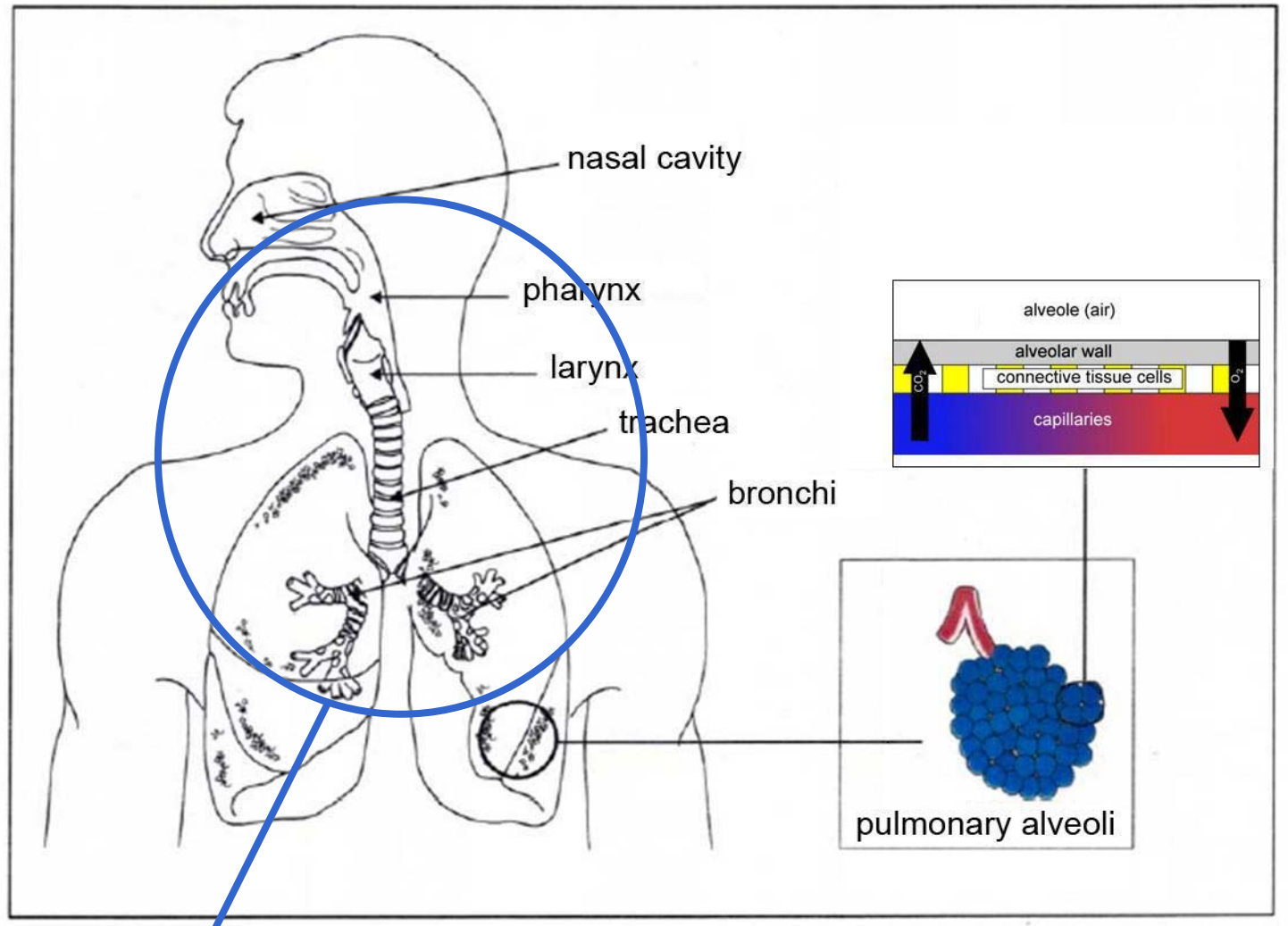
Scheele`s green $CuH(AsO_3)$

Napoleons tapestry on Elba

Respiratory tract

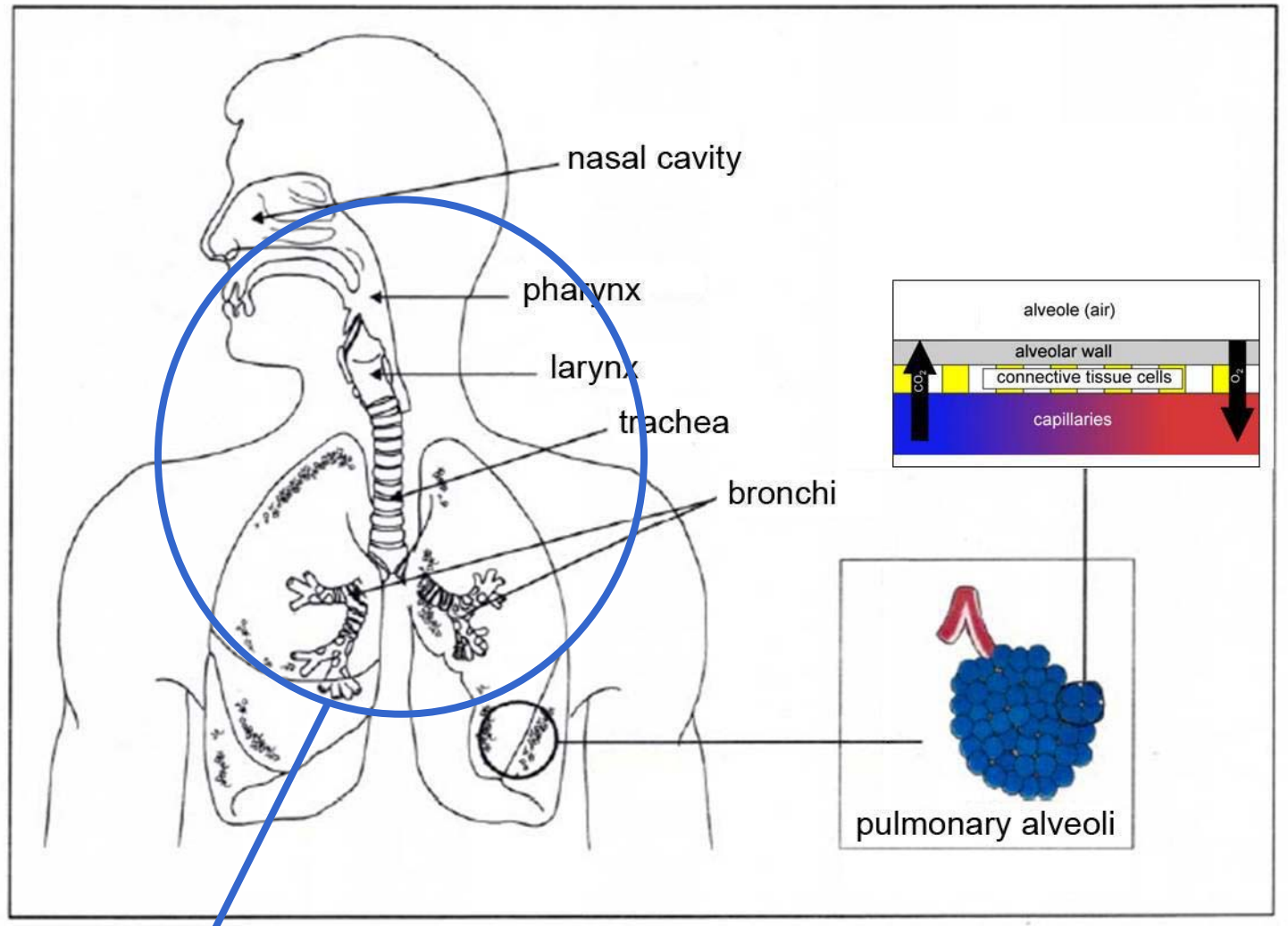


Respiratory tract



respirable dust

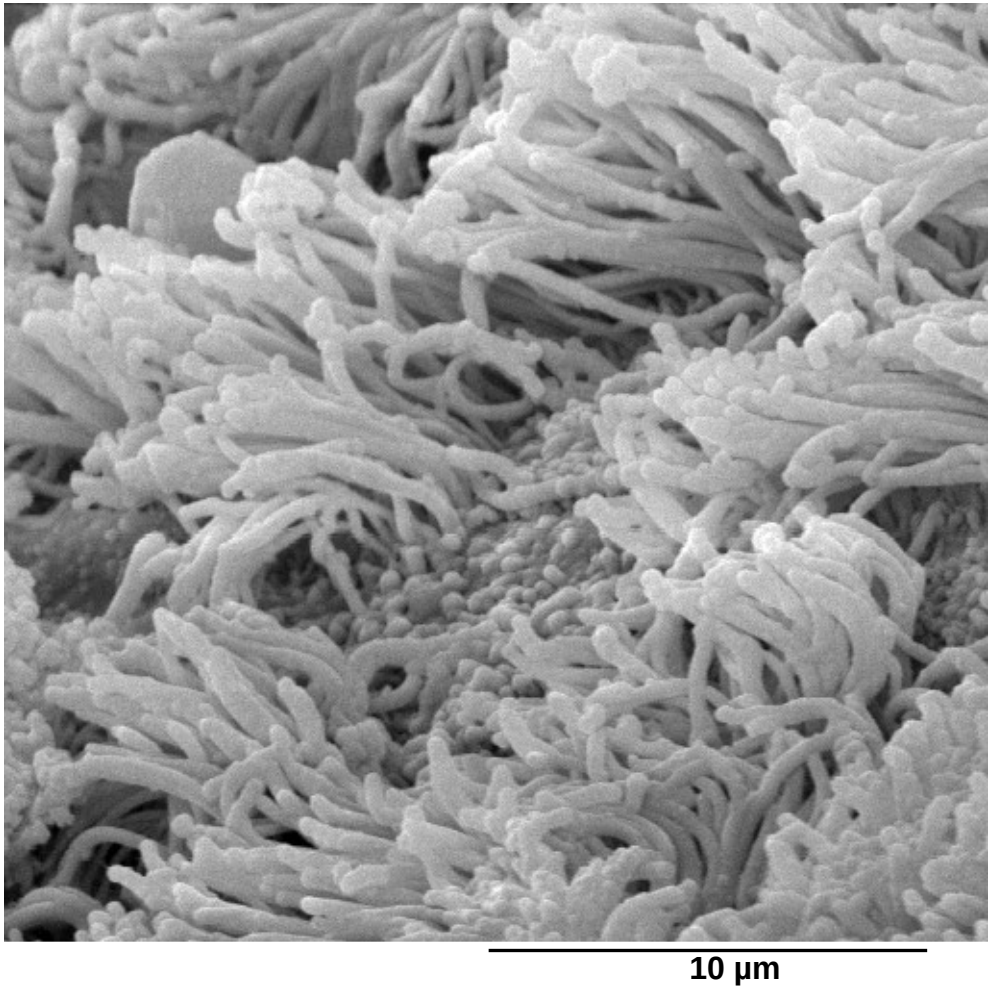
Respiratory tract



cleaning mechanism

respirable dust

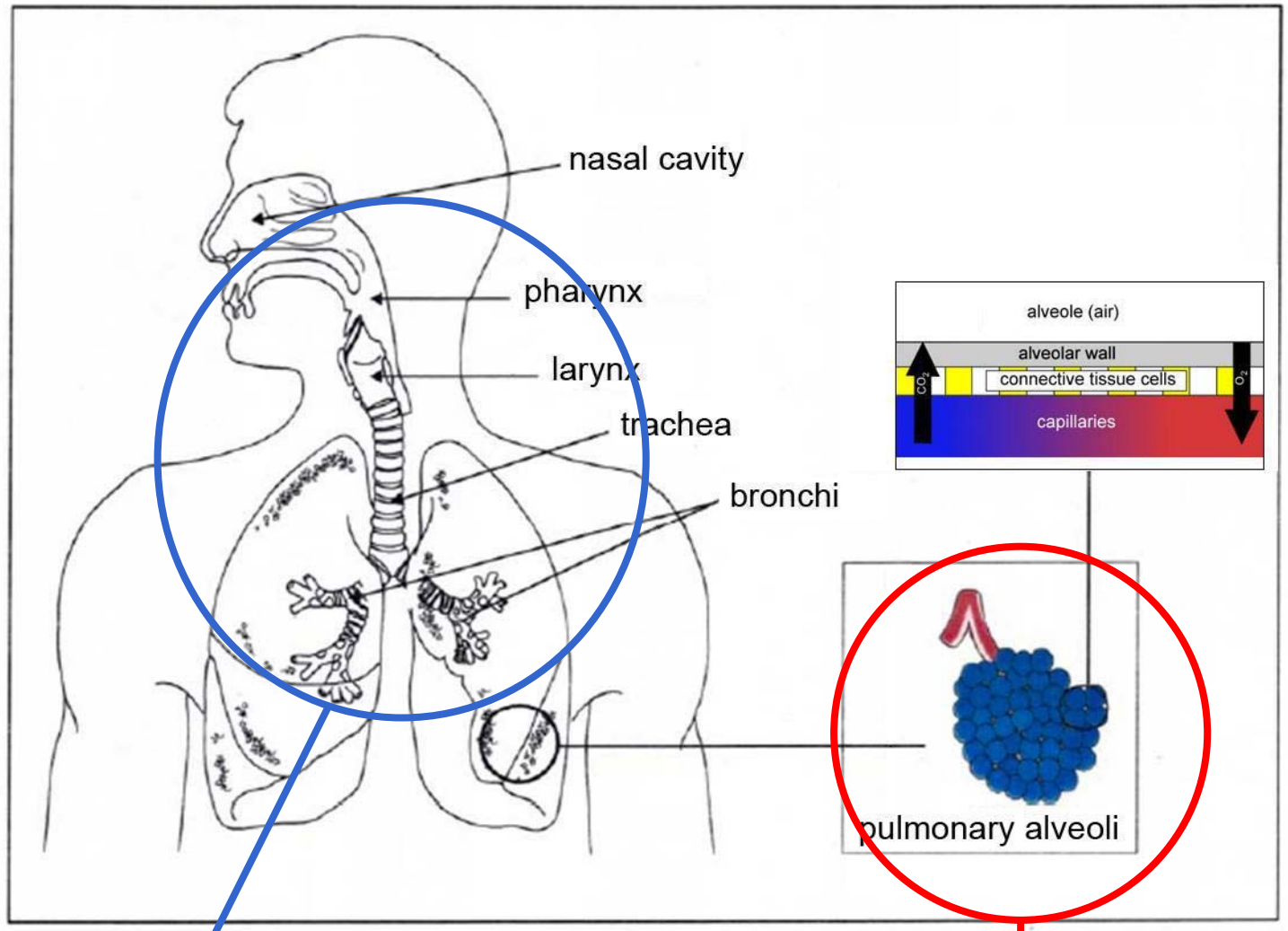
Mucociliary clearence



Small hairs (*cilium*) remove the dust to the nose-pharynx-larynx region

(REM image. Magnification, 5000-fold)

Respiratory tract

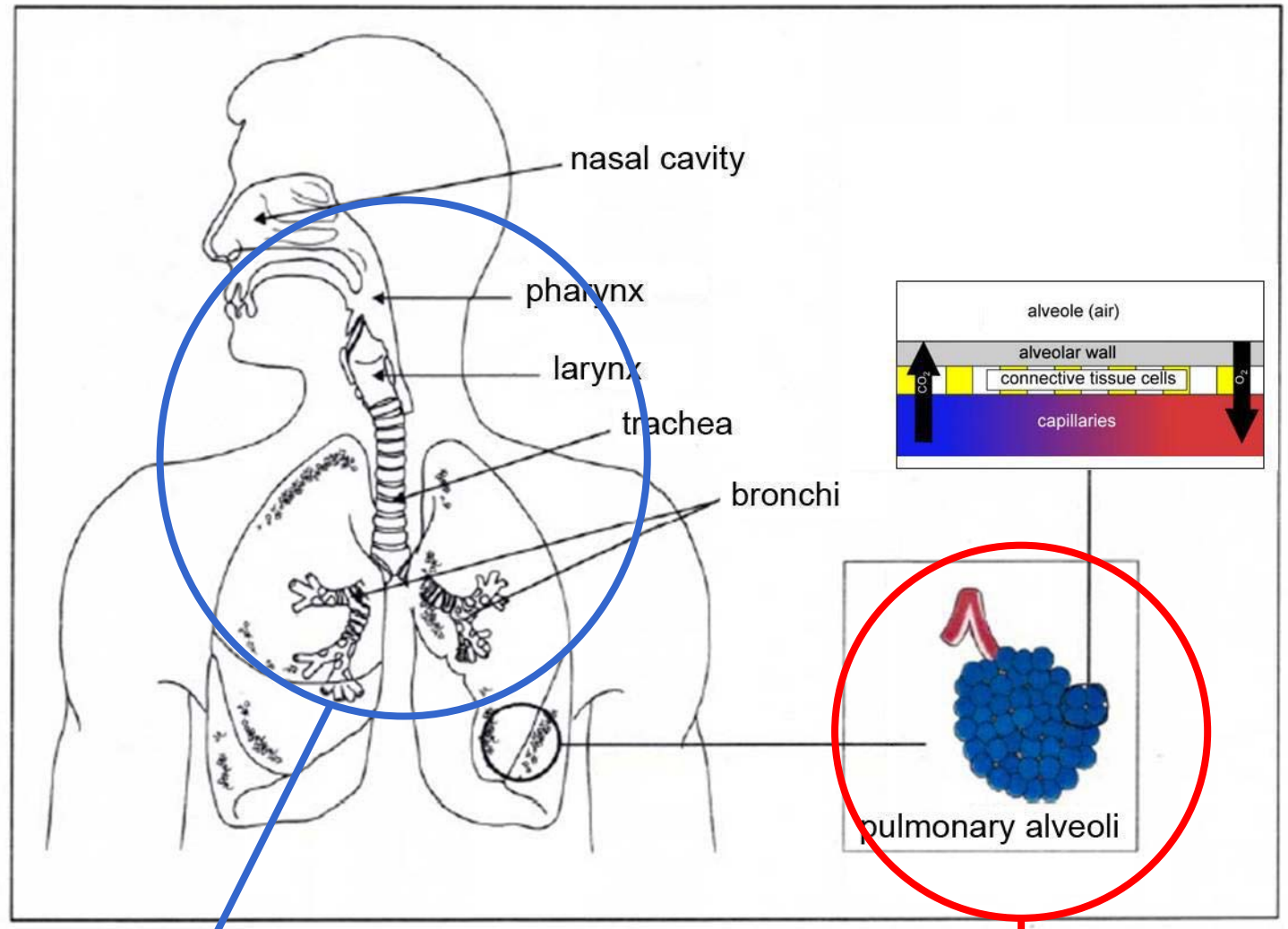


cleaning mechanism

respirable dust

“nano” dust

Respiratory tract



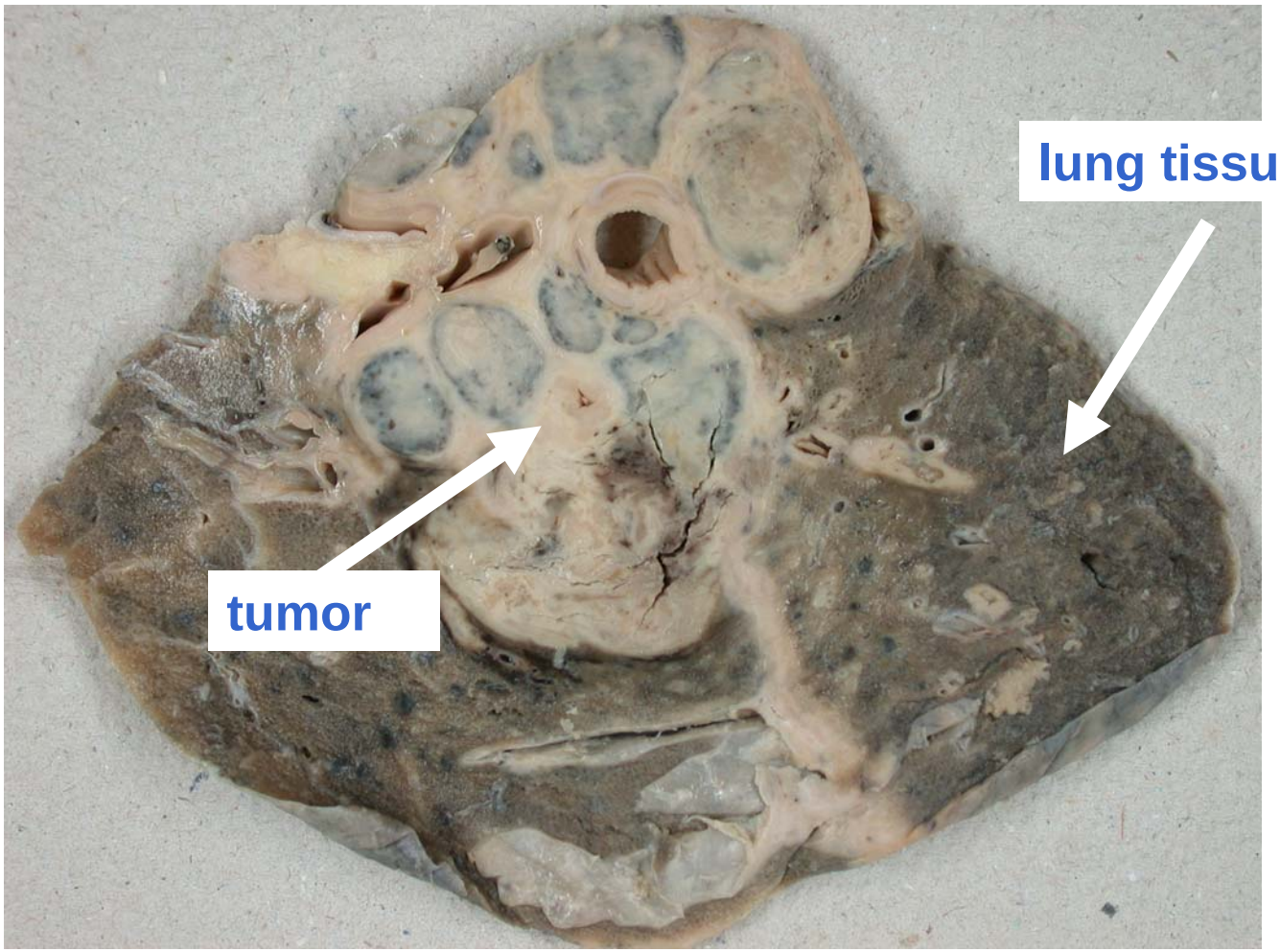
cleaning mechanism

deposition

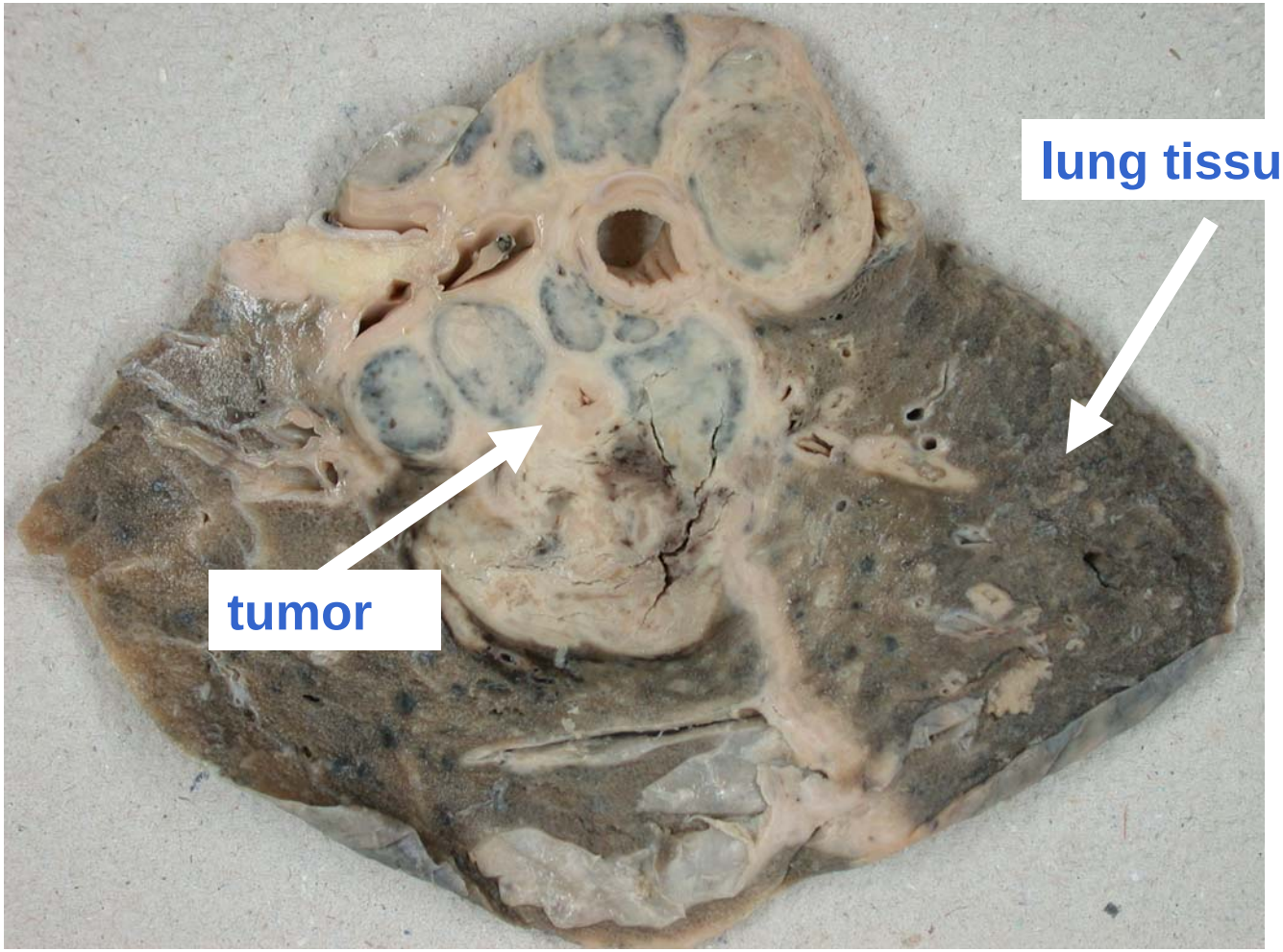
respirable dust

“nano” dust

Lung cancer

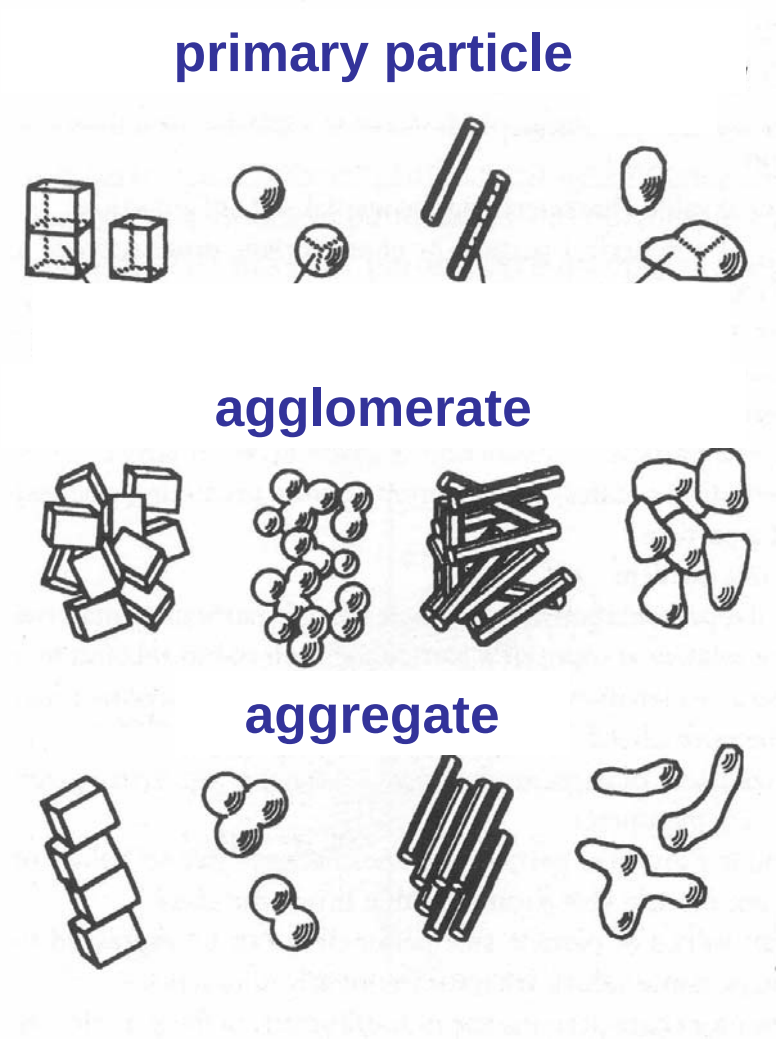


Lung cancer



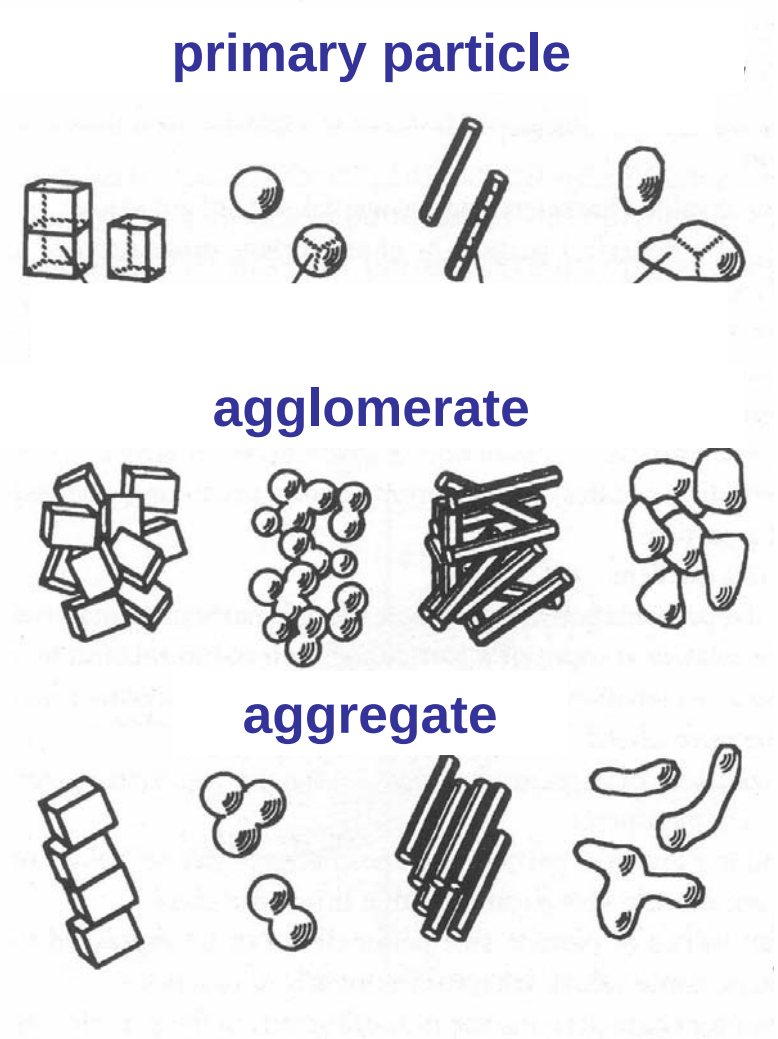
Worst case!

Primary particle, agglomerate and aggregate



Primary particle, agglomerate and aggregate

adhesion

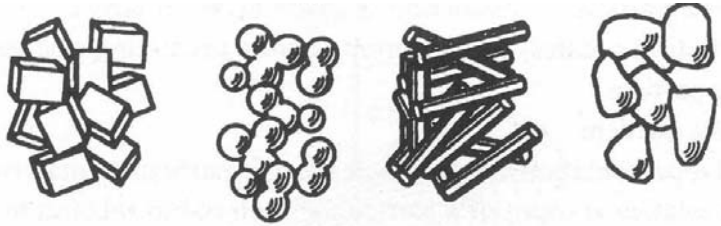


Primary particle, agglomerate and aggregate

primary particle

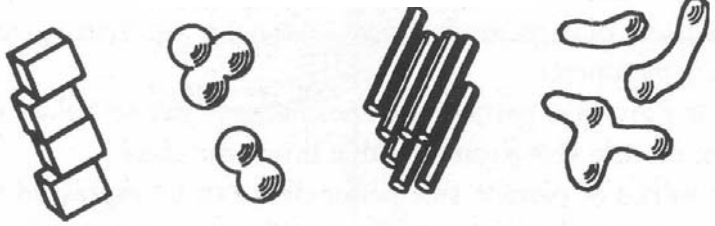
agglomerate

adhesion

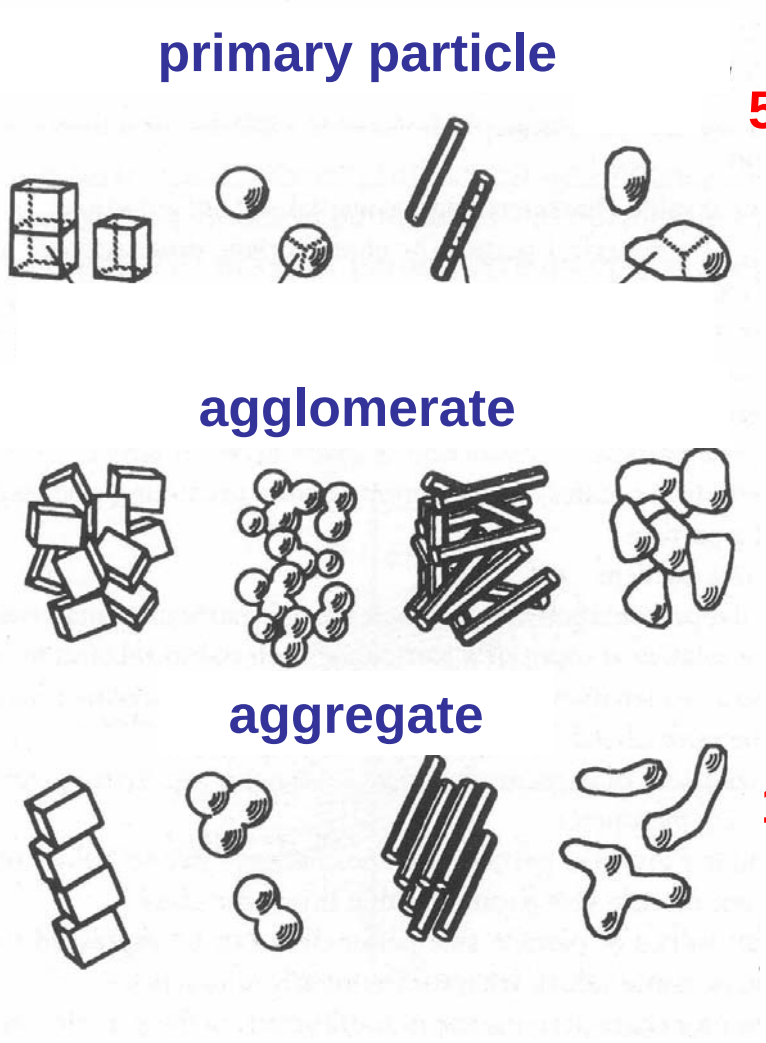


aggregate

sintered body
(crystal structure)



Primary particle, agglomerate and aggregate



adhesion

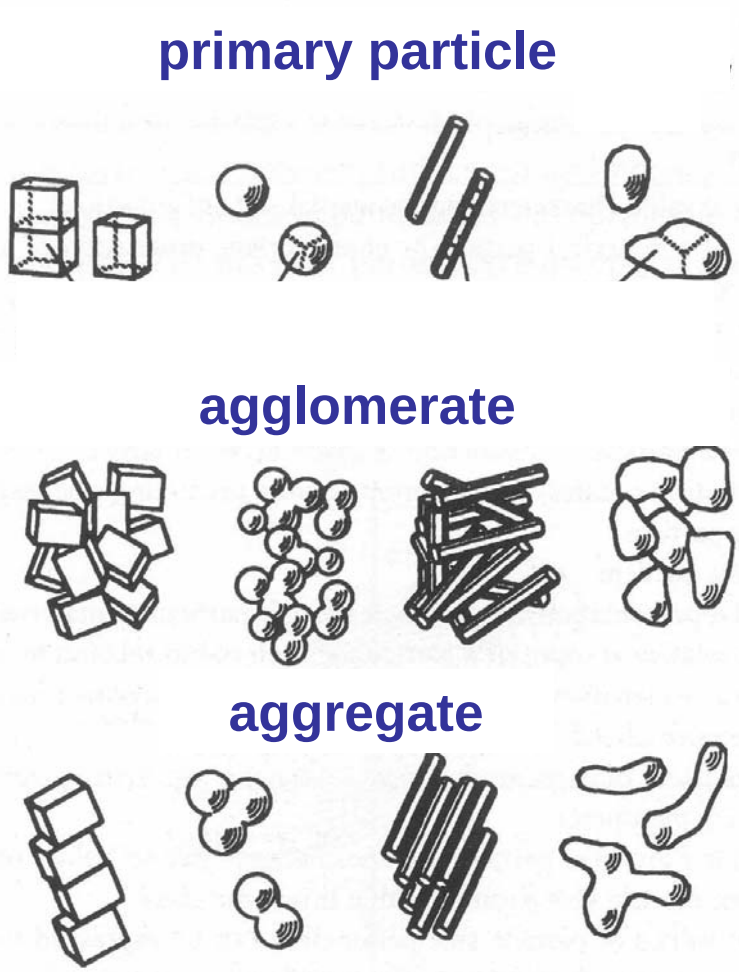
sintered body
(crystal structure)

e.g.
5 primary particles

5 agglomeration by physical interactions of primary particles (agglomerate)

1 new formed particle (aggregate)

Primary particle, agglomerate and aggregate



e.g.

5 primary particles

5 agglomeration by physical interactions of primary particles (agglomerate)

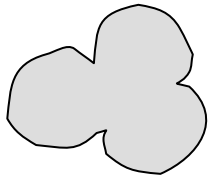
1 new formed particle (aggregate)

adhesion

sintered body (crystal structure)

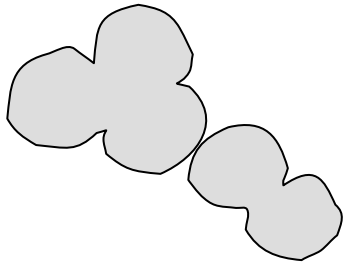
The specific surface area of an aggregate is smaller than the sum of ist original primary particles!

Primary particle, agglomerate and aggregate



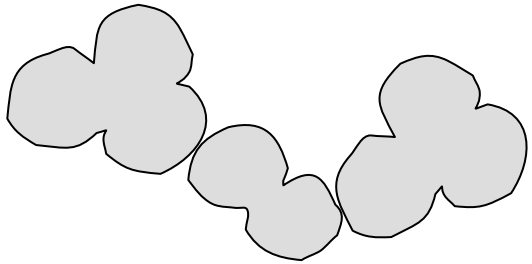
Aggregates

Primary particle, agglomerate and aggregate



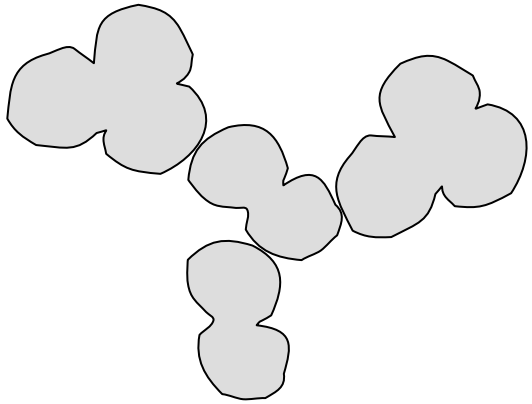
Aggregates are

Primary particle, agglomerate and aggregate



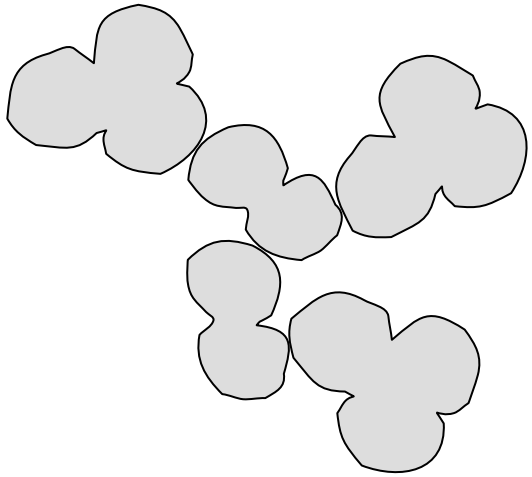
Aggregates are able

Primary particle, agglomerate and aggregate



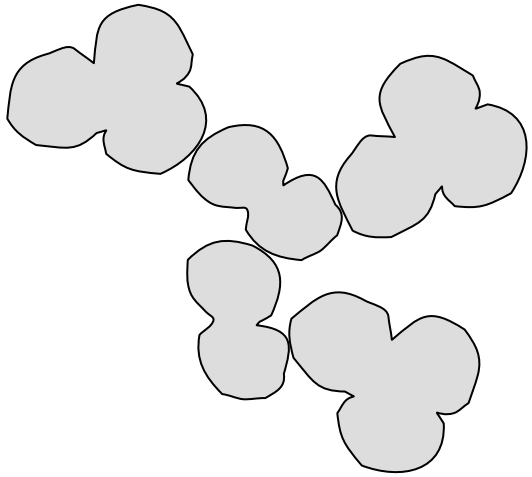
Aggregates are able to form

Primary particle, agglomerate and aggregate



Aggregates are able to form agglomerates to!

Primary particle, agglomerate and aggregate



Agglomerates disagglomerate in lung!

Aggregates are able to form agglomerates to!

Preparation of nanoparticles

“top down” (mechanical stress)

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„crystal lattice" - small single crystals or polycrystalline samples of a (mostly) thermodynamic product

Preparation of nanoparticles

“top down” (mechanical stress)

„crystal lattice" - small single crystals or polycrystalline samples of a (mostly) thermodynamic product

“bottom up” (formation of gas- or liquid-phase reactions)

Preparation of nanoparticles

“top down” (mechanical stress)

„crystal lattice" - small single crystals or polycrystalline samples of a (mostly) “thermodynamic product“

“bottom up” (formation of gas- or liquid-phase reactions)

„lattice defects" - small particles from crystalline areas that do not correspond to the ideal lattice (defect structure). These structures which are difficult to describe, are typical of “kinetic products“

Preparation of nanoparticles

kinetic product

defect chemistry

Preparation of nanoparticles

kinetic product

defect chemistry

real structure

Preparation of nanoparticles

kinetic product

defect chemistry

real structure

classification

0-dimensional defects

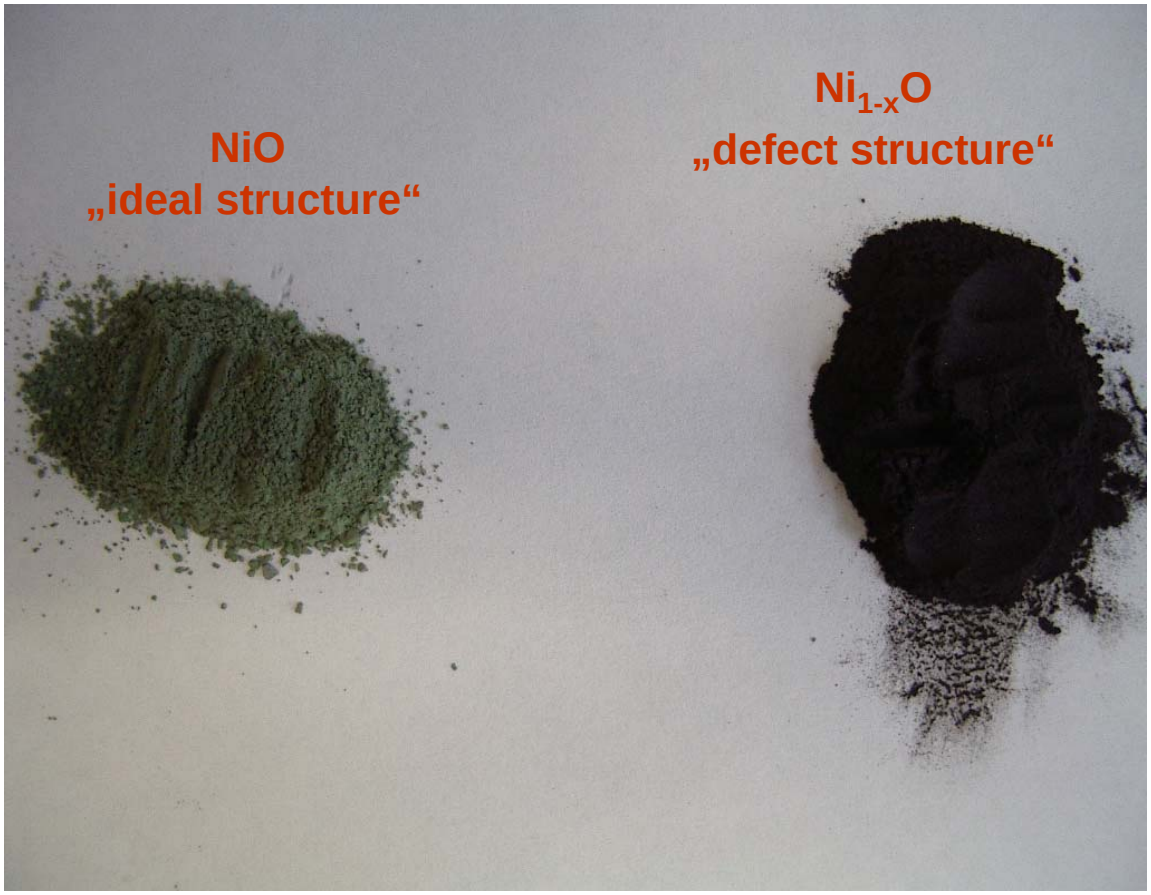
1-dimensional defects

2-dimensional defects

3-dimensional defects

Real structure

0-dimensional: nonstoichiometry



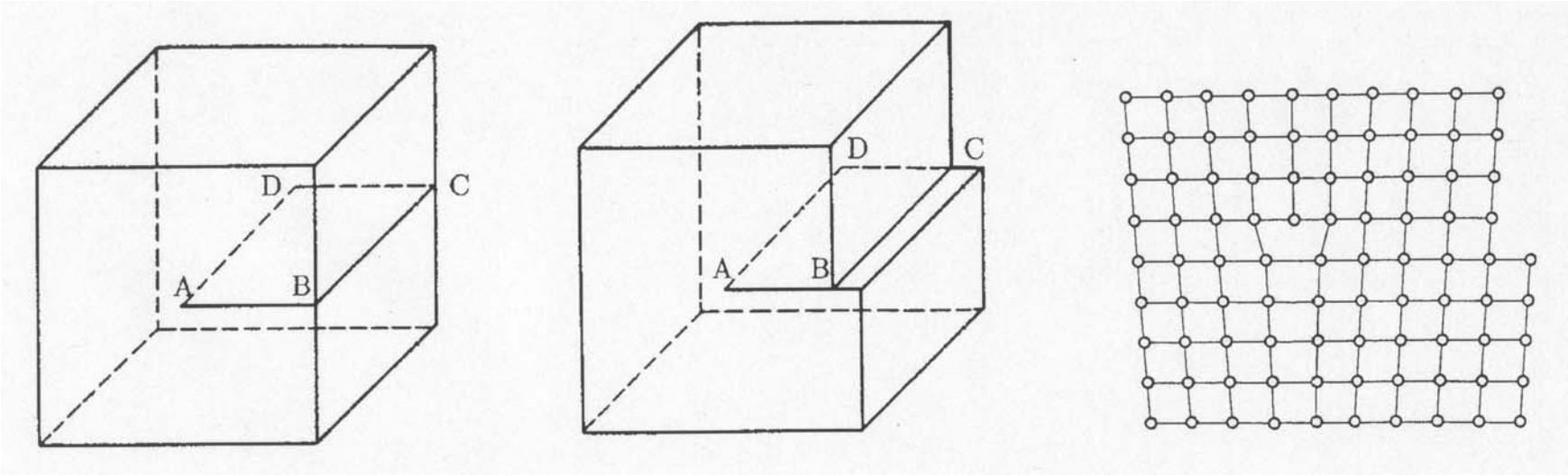
from alkaline carbonate

from nickel and oxygen

Real structure

classification of defects

1-dimensional: edge disclocations

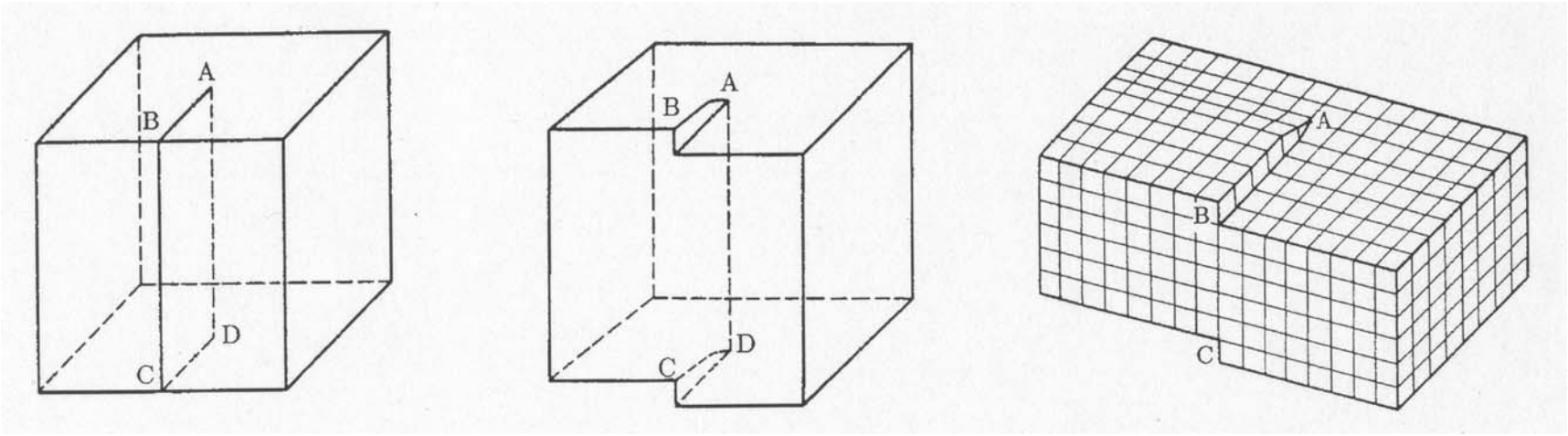


separation ABCD compression of top crystal plane atomic illustration

Real structure

classification of defects

1-dimensional: screw dislocations

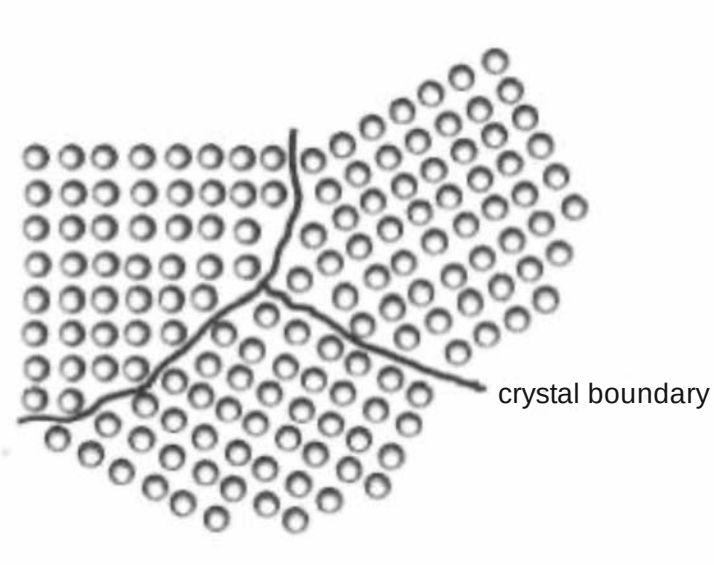


separation ABCD shearing BC atomic illustration

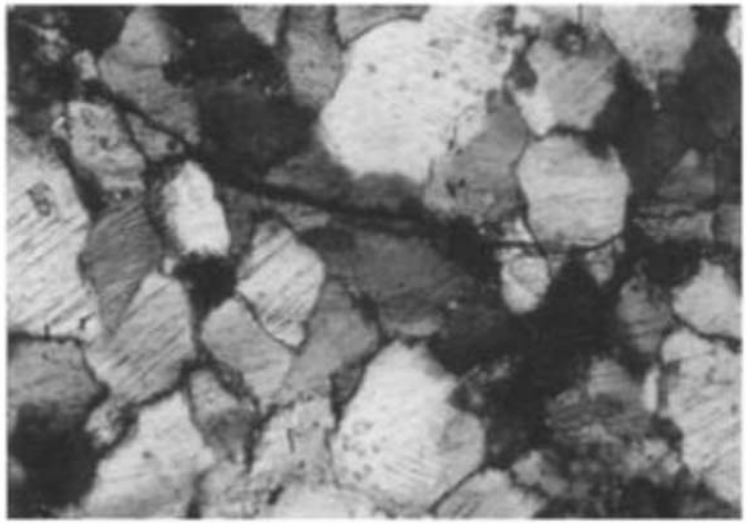
Real structure

classification of defects

2-dimensional: grain boundaries



schematic illustration

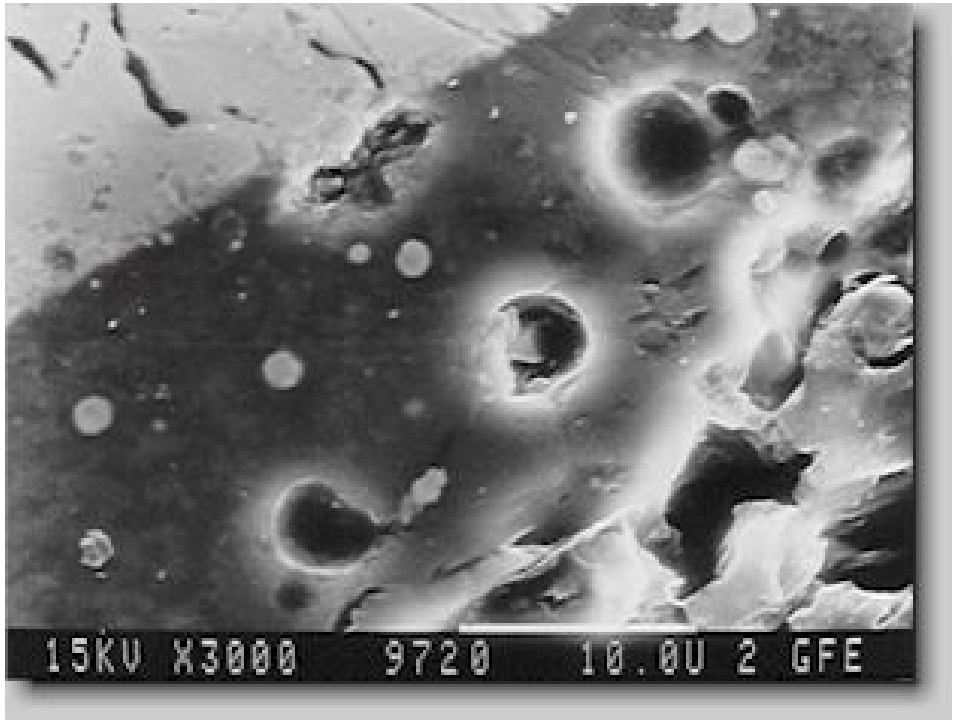


polycrystalline quartz,
thin-section image

Real structure

classification of defects

3-dimensional: pores



metal oxide,
REM image

Preparation of nanoparticles

“top down” (mechanical stress)

“bottom up” (formation of gas- or liquid-phase reactions)

Particles with
altered physical and
chemical properties are
formed!

Preparation of nanoparticles

“bottom up” methods can also form molecules

Preparation of nanoparticles

“bottom up” methods can also form molecules

“cluster”

Preparation of nanoparticles

“bottom up” methods can also form molecules

“cluster”

cluster <50 - 100 atoms have molecular character
cluster >50 - 100 atoms have metalic (solid state) character

Preparation of nanoparticles

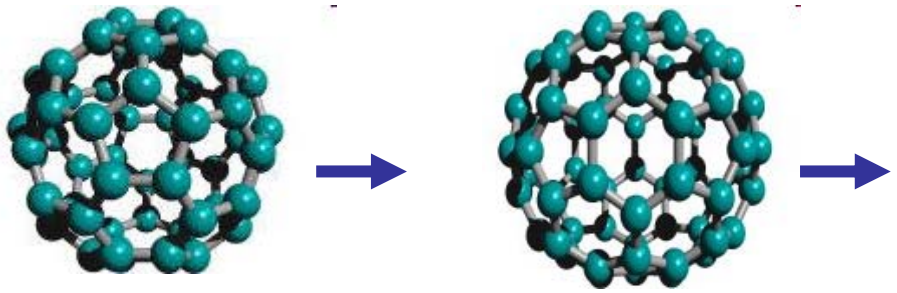
example: carbon

C 60

molecule (fullerene)

Preparation of nanoparticles

example: carbon

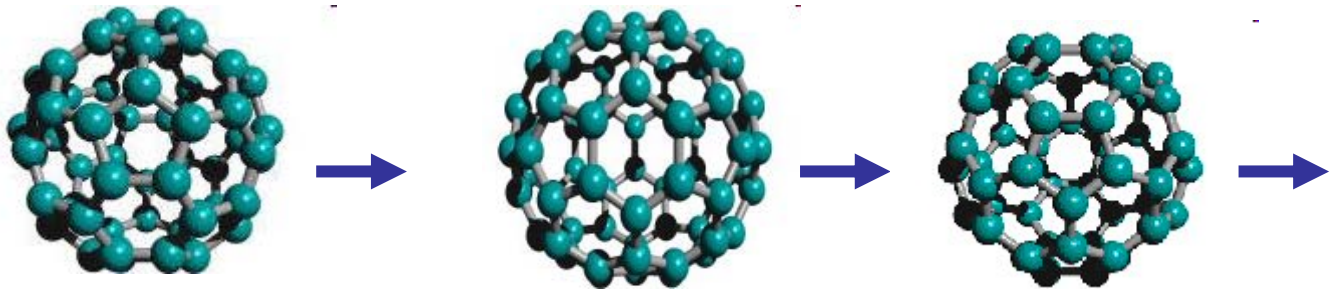


C 60 C 70

molecule (fullerene)

Preparation of nanoparticles

example: carbon

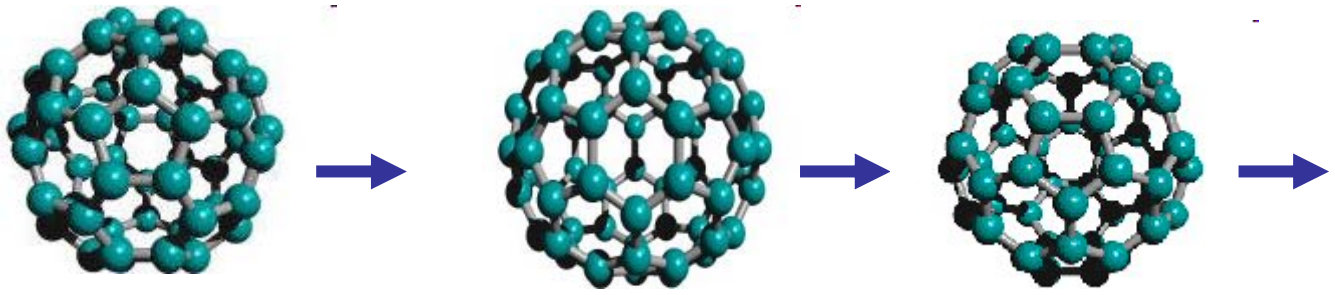


C 60 C 70 C 84

molecule (fullerene)

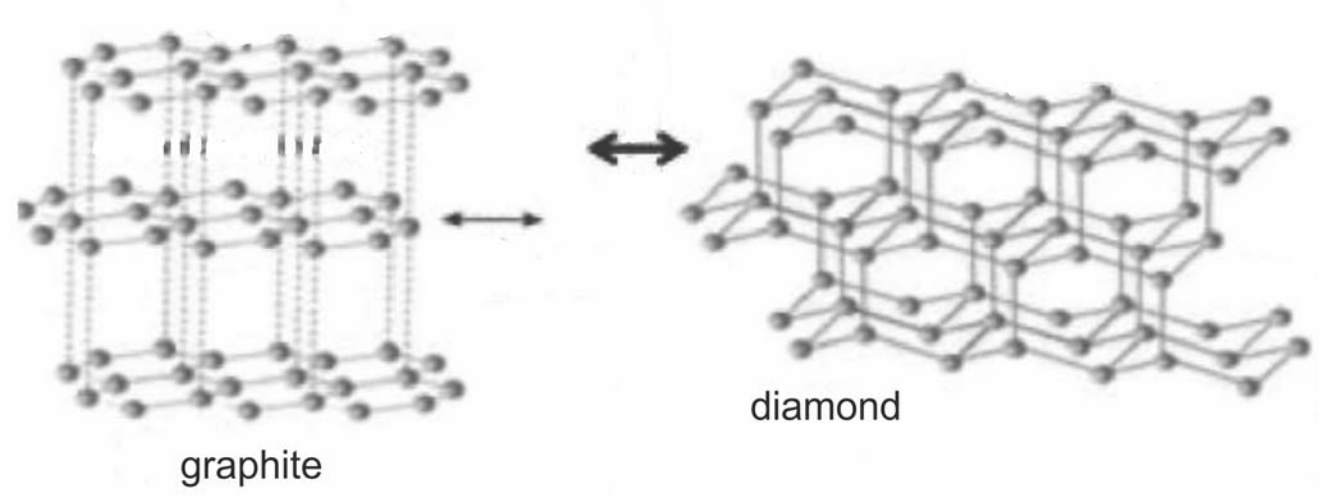
Preparation of nanoparticles

example: carbon



C 60 C 70 C 84

molecule (fullerene)



crystal lattice

Determination of nanoparticle concentrations

particle counter

or

particle sizer

Determination of nanoparticle concentrations

particle counter

or

particle sizer

disadvantage:

no differentation between various substances

no diffentation between primary particles and agglomerates

Determination of nanoparticle concentrations

particle counter

or

particle sizer

disadvantage:

no distinction between different substances

no distinction between primary particles and agglomerates

↓

a more detailed characterization of nanoparticles is necessary

Characterization of nanoparticles

- X-ray powder diffraction (XRD) (unsuitable)

Characterization of nanoparticles

- **X-ray powder diffraction (XRD) (unsuitable)**
- ***synchrotron powder diffraction (XRD) (suitable)***

Characterization of nanoparticles

- **X-ray powder diffraction (XRD) (unsuitable)**
- ***synchrotron powder diffraction (XRD) (suitable)***
- **determination of the spec. surface area (BET) (uns*uitable*)**

Characterization of nanoparticles

- X-ray powder diffraction (XRD) (unsuitable)
- *synchrotron powder diffraction (XRD) (suitable)*
- determination of the spec. surface area (BET) (un*suitable*)
- *electron microscopy (TEM + EDX)* (*suitable*)

Characterization of nanoparticles

- X-ray powder diffraction (XRD) (unsuitable)
- *synchrotron powder diffraction (XRD) (suitable)*
- determination of the spec. surface area (BET) (un*suitable*)
- *electron microscopy (TEM + EDX)* (*suitable*)
- *thermal analysis (TG-FTIR, TG-MS) (suitable)*

Determination of the specific surface

BET adsorption

$$\frac{p}{n(p^* - p)} = \frac{1}{n_m b} + \frac{b - 1}{n_m b}\,\frac{p}{p^*}$$

p	equilibrium pressure at an occupancy n
p*	saturation vapor pressure of pure adsortives at a temperature T
b	$= e^{(q_1 - q_2)/RT}$
n	number of adsorbed molecules
n_m	number of adsorbed molecules in monomolecular layer

Brunauer, S.; Emmet, P. H.; Teller, E. J.: J. Am. Chem. Soc. **60** (1938) 309-319

Determination of the specific surface

geometry (REM)  **spec. surface** (BET)

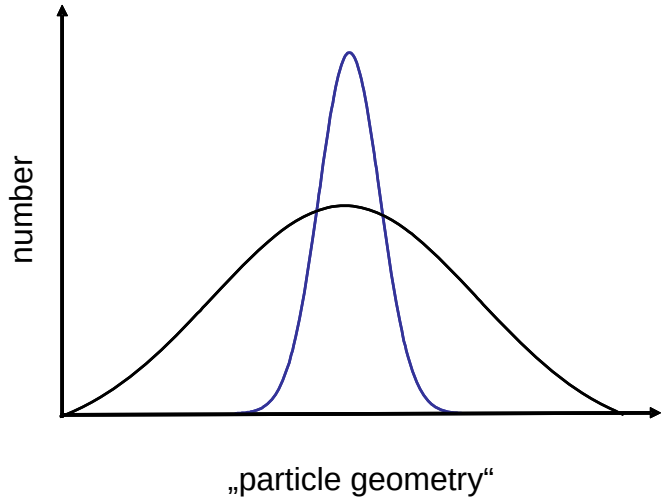
synonym: **spec. surface [m^2/g] is used for particle size (geometry)**

Determination of the specific surface

geometry (SEM) ⟷ **spec. surface** (BET)

synonym: **spec. surface [m^2/g] is used for particle size (geometry)**

↓ **assumption**

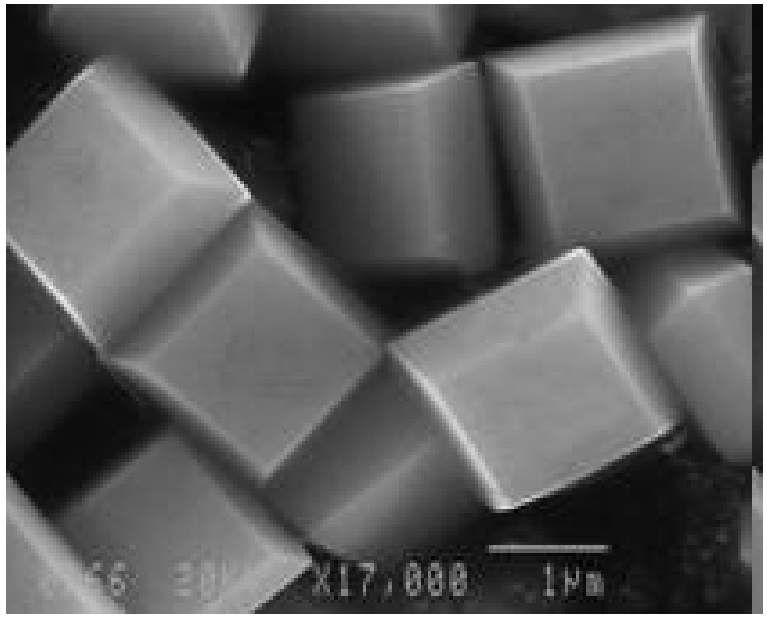


particles of identical size and geometry!

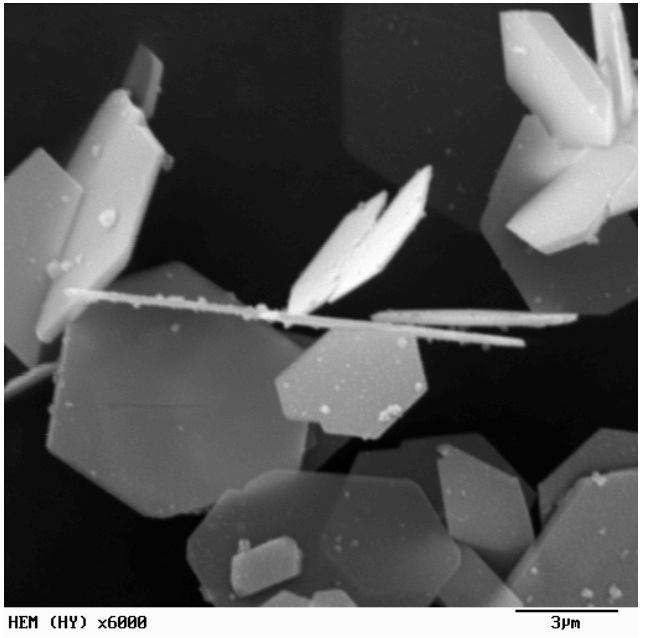
synthetic pigments with an even surface morphology sufficently meet these requirements

Determination of the specific surface

Synthetic pigments with an even surface morphology sufficently meet these requirements



TiO_2

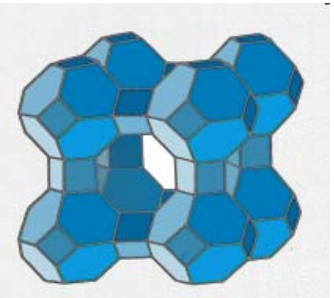


Fe_2O_3

Determination of the specific surface

Particles with a fissured surface morphology and/or pore structure (inner surface) no longer readily fulfil this requirement

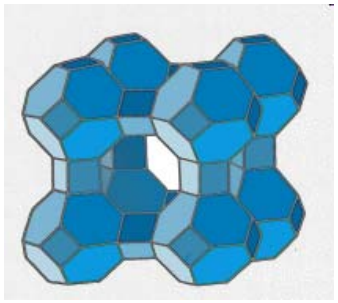
zeolithe A

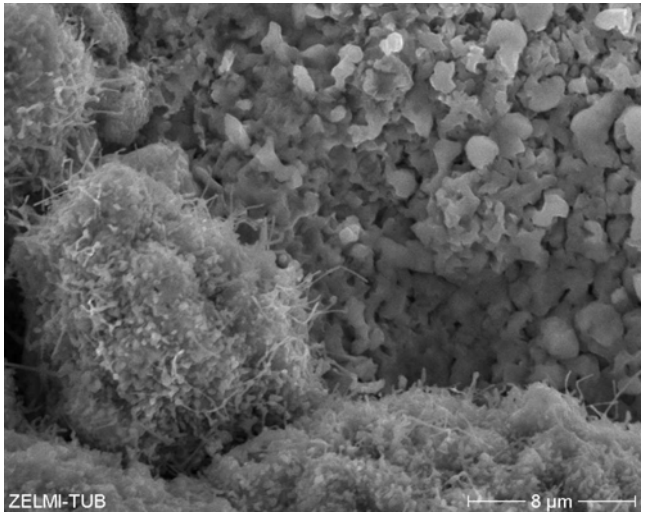


Determination of the specific surface

Particles with a fissured surface morphology and/or pore structure (inner surface) no longer readily fulfil this requirement

zeolithe A



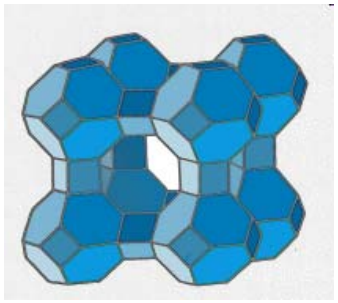


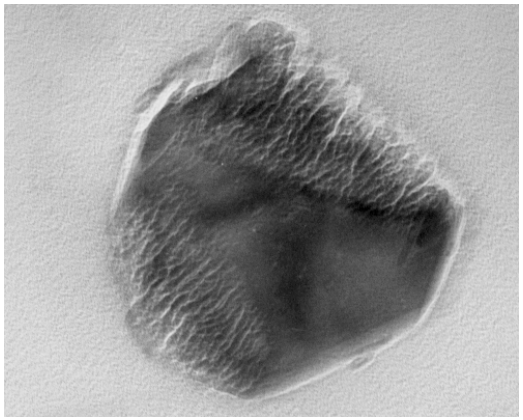
modified SiC

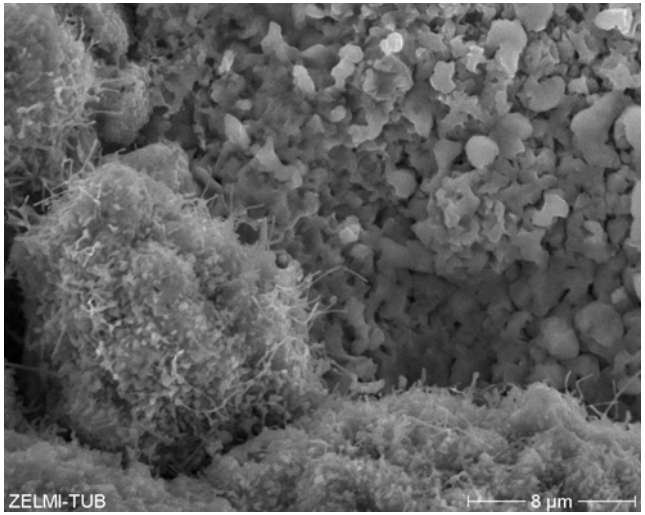
Determination of the specific surface

Particles with a fissured surface morphology and/or pore structure (inner surface) no longer readily fulfil this requirement

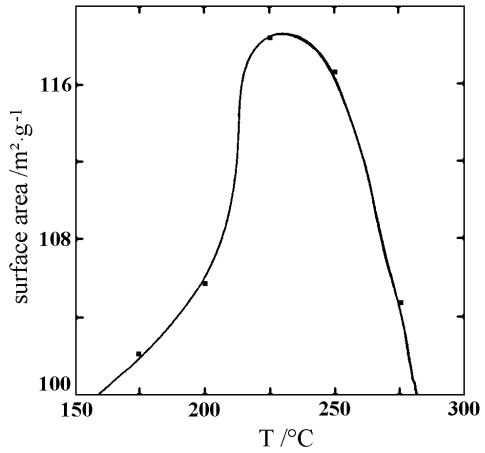
zeolithe A







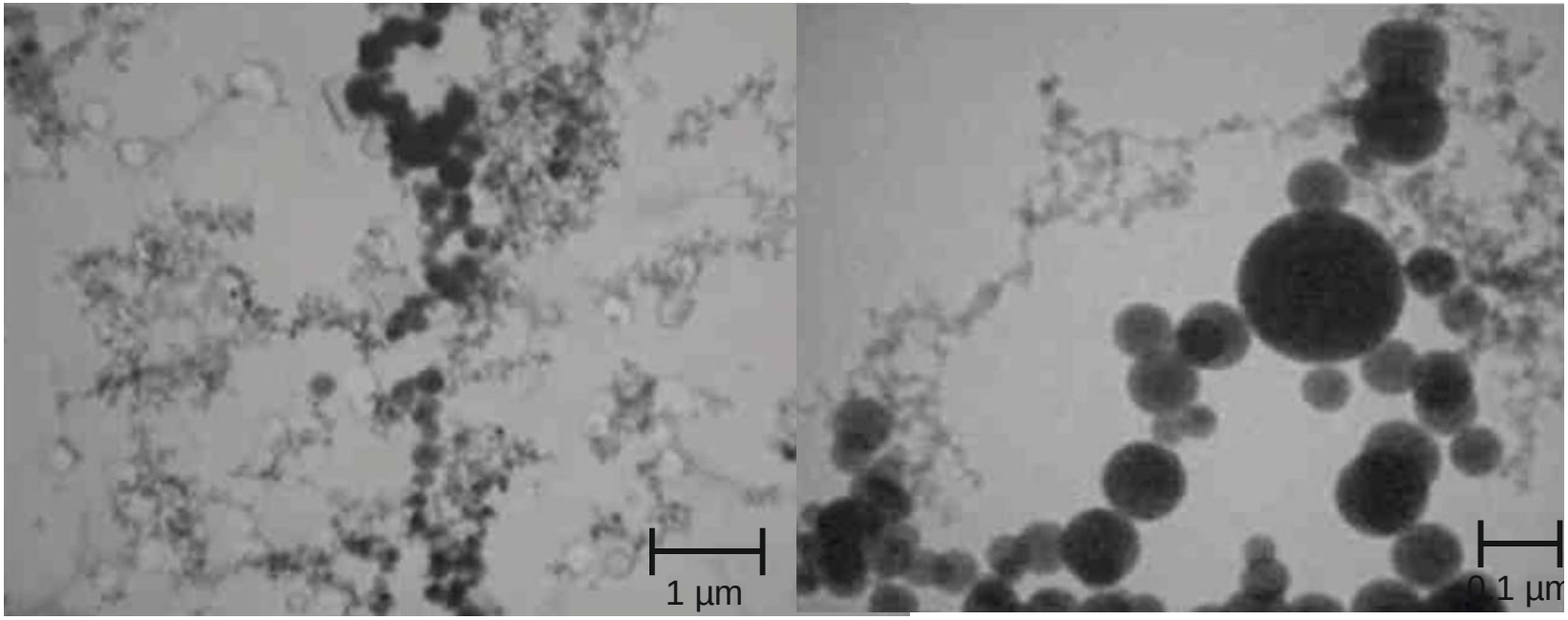
modified SiC



goethite/hematite-sample

Determination of the specific surface

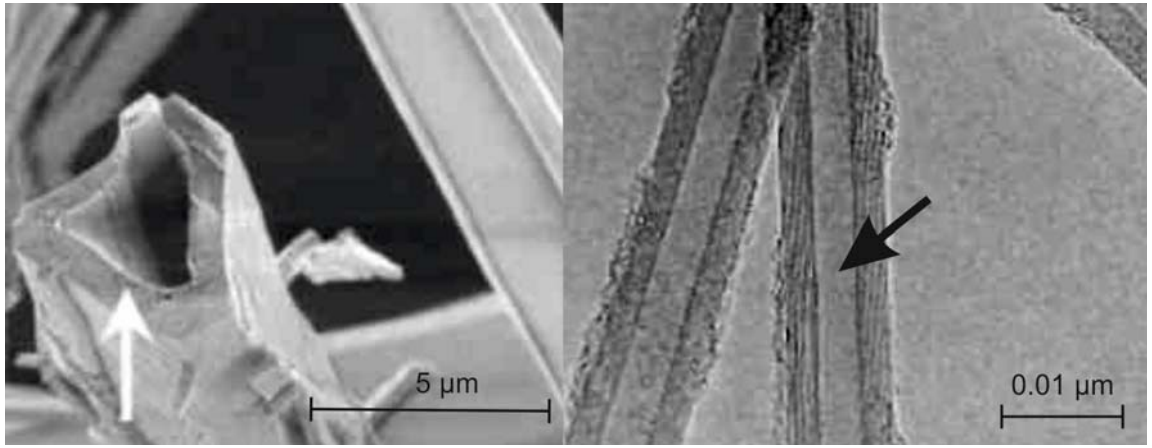
Particles of different particle sizes can not be characterized by simple BET measurements!



Welding fume sample consisting of primary particles, agglomerates and aggregates of different particle sizes

Determination of the specific surface

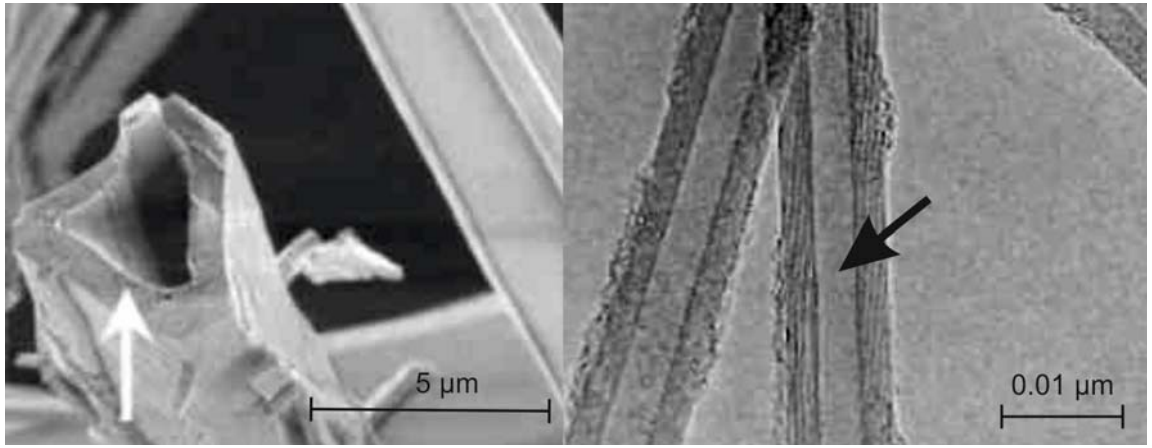
Toxicological relevance of the inner surface (eg macrophages)



yes **no!**

Determination of the specific surface

Toxicological relevance of the inner surface (eg macrophages)



yes **no!**

Don’t trust published spec. surface data (nanoparticles) without further particle characterization

TiO_2 – application (nanosized) of titanium dioxide pigments

UV-absorber

small TiO_2 particles (10 - 50 nm) absorb UV radiation in a wavelength range of 280 - 400 nm:

$\Rightarrow$ cosmetic products (sunscreen)

$\Rightarrow$ polymers (protection from UV radiation)

paper

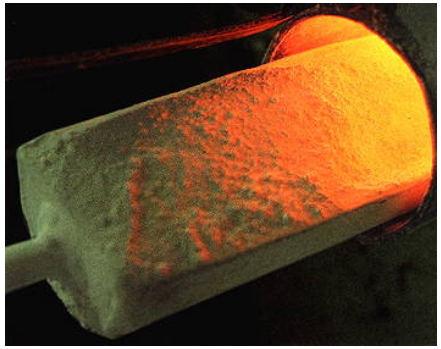
High quality paper containing titanium dioxide pigments as filler. Glossy paper is coated with titanium dioxide pigments.

catalysts

TiO_2 pigments are used in heterogeneous catalysis either directly or as support material.

TiO_2 – technical production (“surface impurities”)

modificatios: rutile, anatase und brookite



Production of titanium dioxide

sulfate process

⇩

+ hot sulfuric acid

⇩ SO_2

ironsulfate/titanium sulfate

⇩

+ water
crystallisation

⇩ iron sulfate

water steam
(hydration)

⇩ dilute acid (H_2SO_4)

titanium oxid hydrate

⇩

calcination

⇩

titanium dioxide

chloride process

⇩

+ chlorine (1000°C)

⇩ CO_2

titanium chloride (gaseous)
iron chloride

⇩

washing out

⇩ iron chloride
hydrochloric acic

condensation
distillation
condensation

⇩

titanium chloride (purified)

⇩

+ oxygen

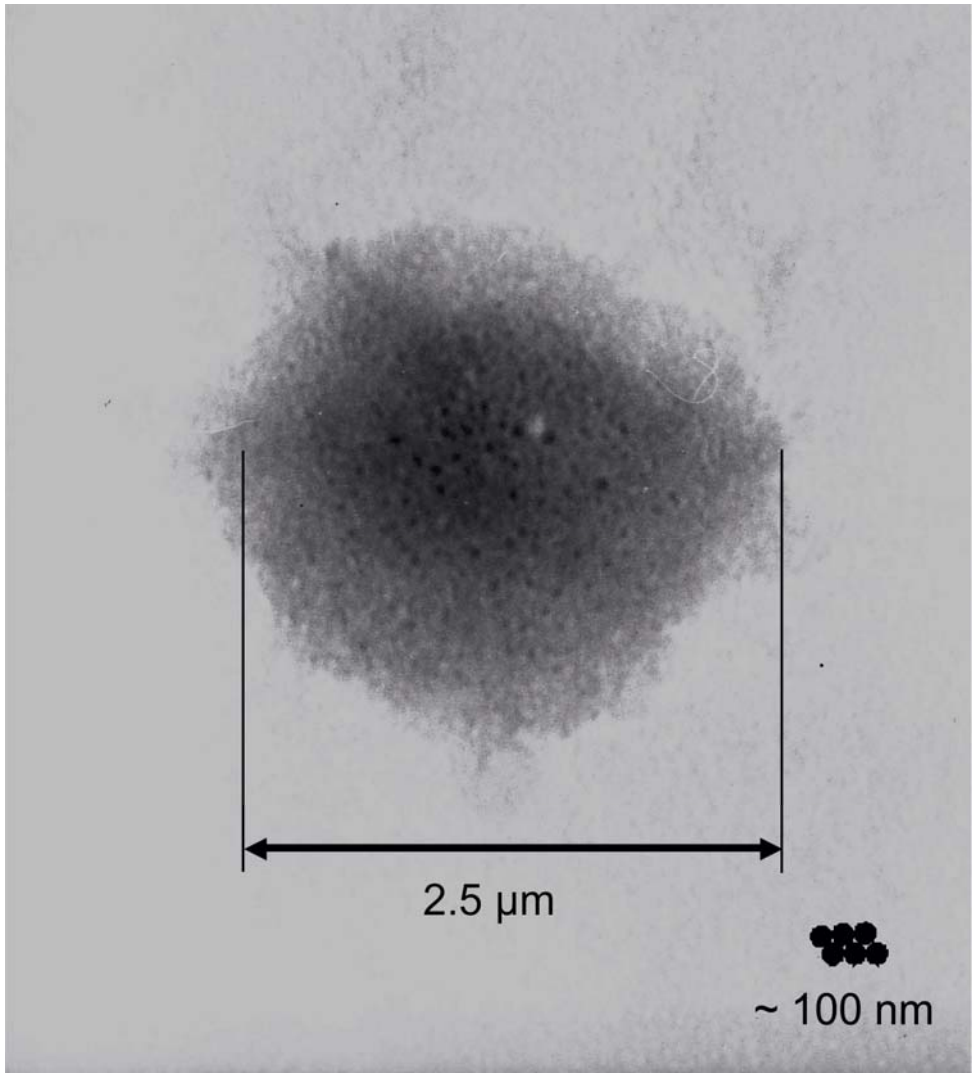
⇩

titanium dioxide

$SO_{2(sf)}$?

$Cl^-_{(sf)}$?

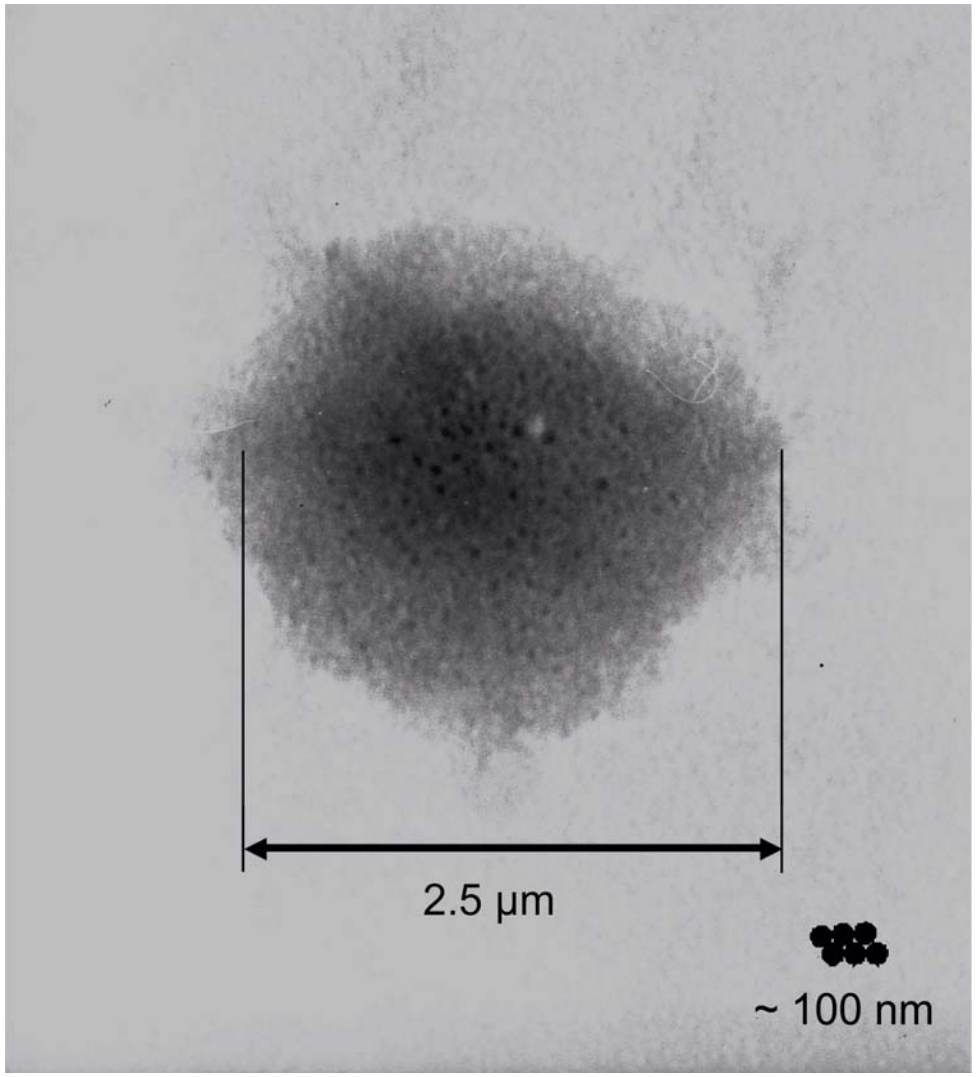
Primary particles - agglomerates



TEM image, 53 500x

TiO_2 agglomerat

Primary particles - agglomerates



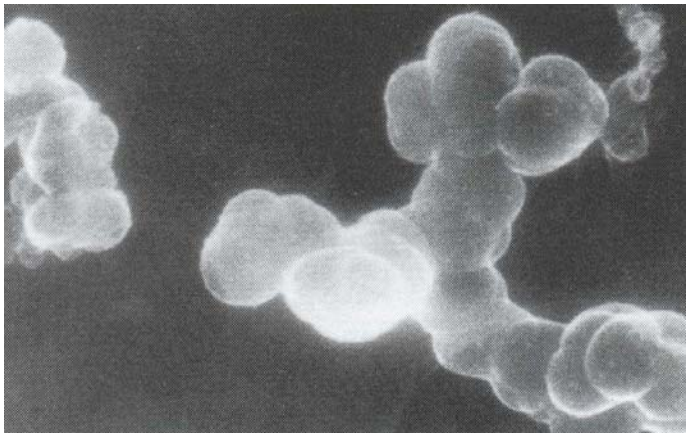
TiO_2 agglomerat

TEM image, 53 500x

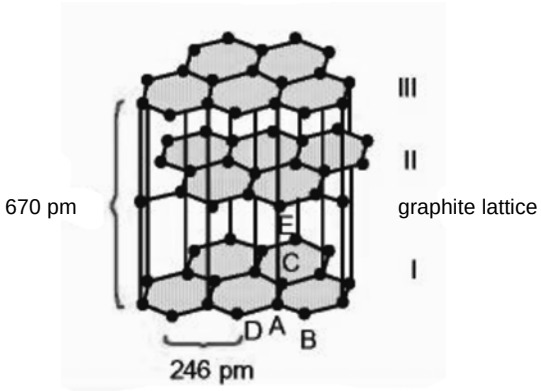
primary particle
d ~ 5 nm

Carbon black (industrial soot)

Soot is a manifestation of carbon appearing in the complete combustion of carbon-containing substances



formation of aggregates (“Carbon black structure”) consisting of spherical primary particles with a diameter of 5 - 500 nm



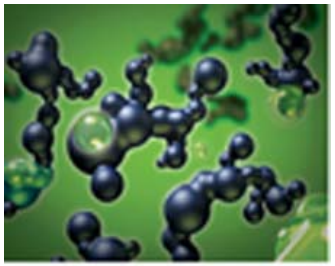
primary particles are composed of six-membered carbon ring layers like graphite, but the distances between the layers are greater than in the graphite lattice

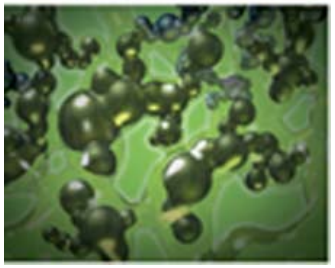
Application of Carbon black

Rubber industry (85% worldwide)

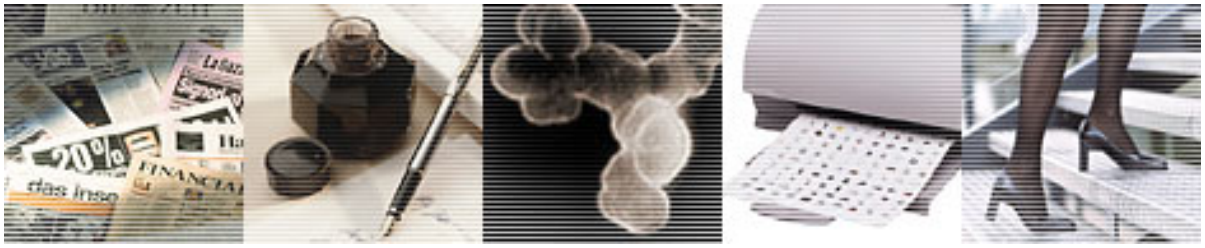
Carbon black optimize the properties of rubber compounds for tires

Elasticity, soil adhesion, abrasion, etc. depend on particle size, surface and agglomerate state!





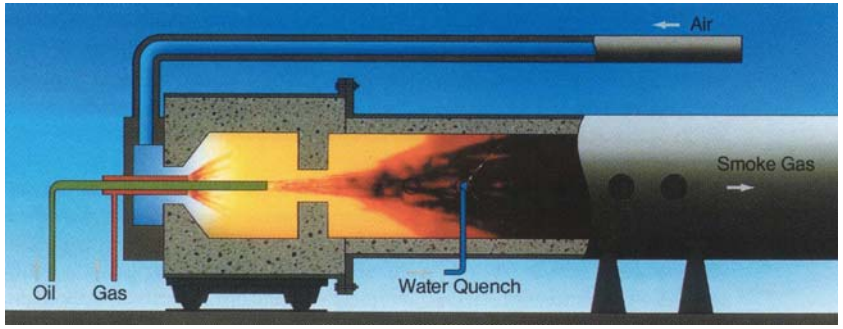
Other applications (globally 15%)



Printer paint, ink, staining of polymers, etc.

Carbon black – production

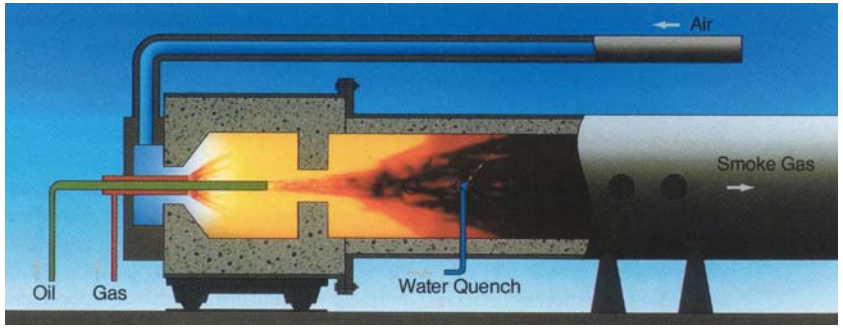
furnace black process



Continuous process by burning oil vapor resulted in specific surface areas of between 20 - present 1000 $m^2 \cdot g^{-1}$

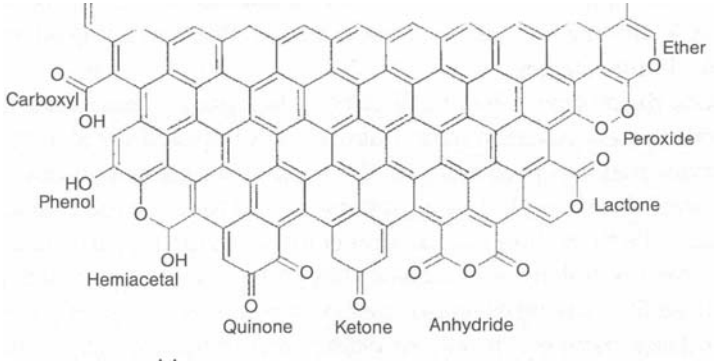
Carbon black – production

furnace black process



Continuous process by burning oil vapor resulted in specific surface areas of between 20 - present 1000 $m^2 \cdot g^{-1}$

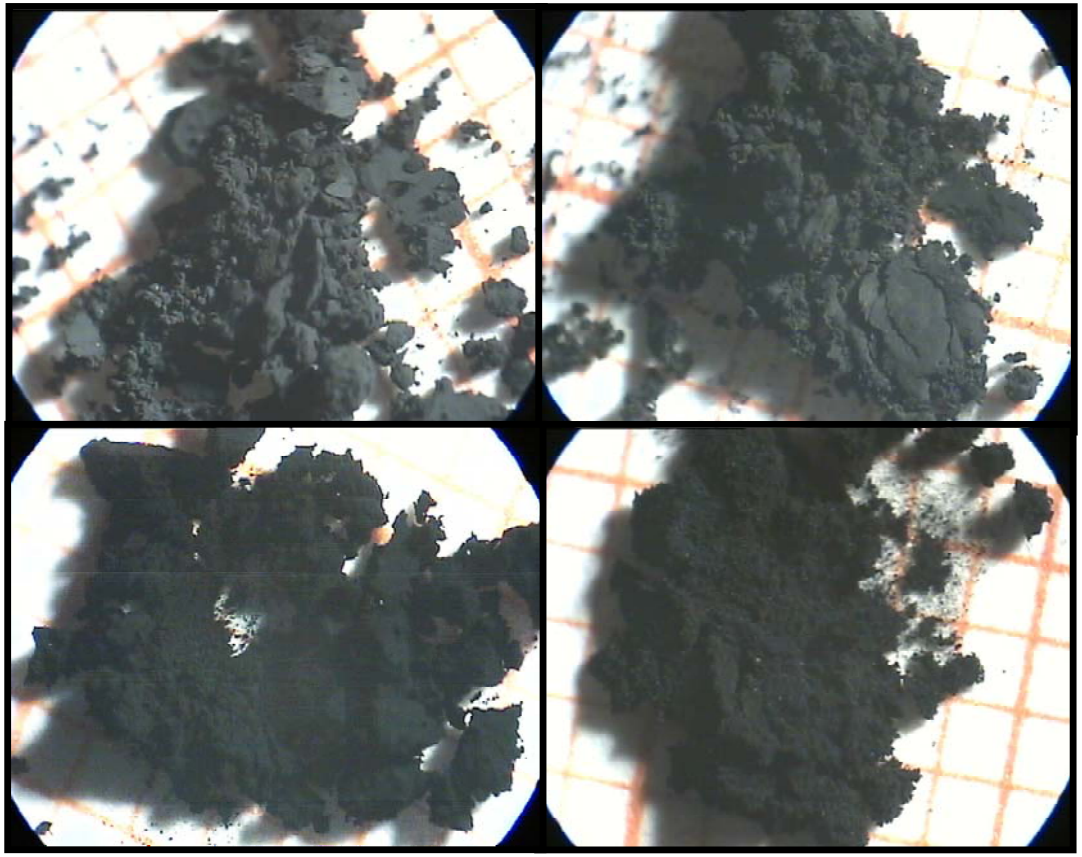
Problem of modification



"functional groups" can be formed at the edges of the carbon ring layers

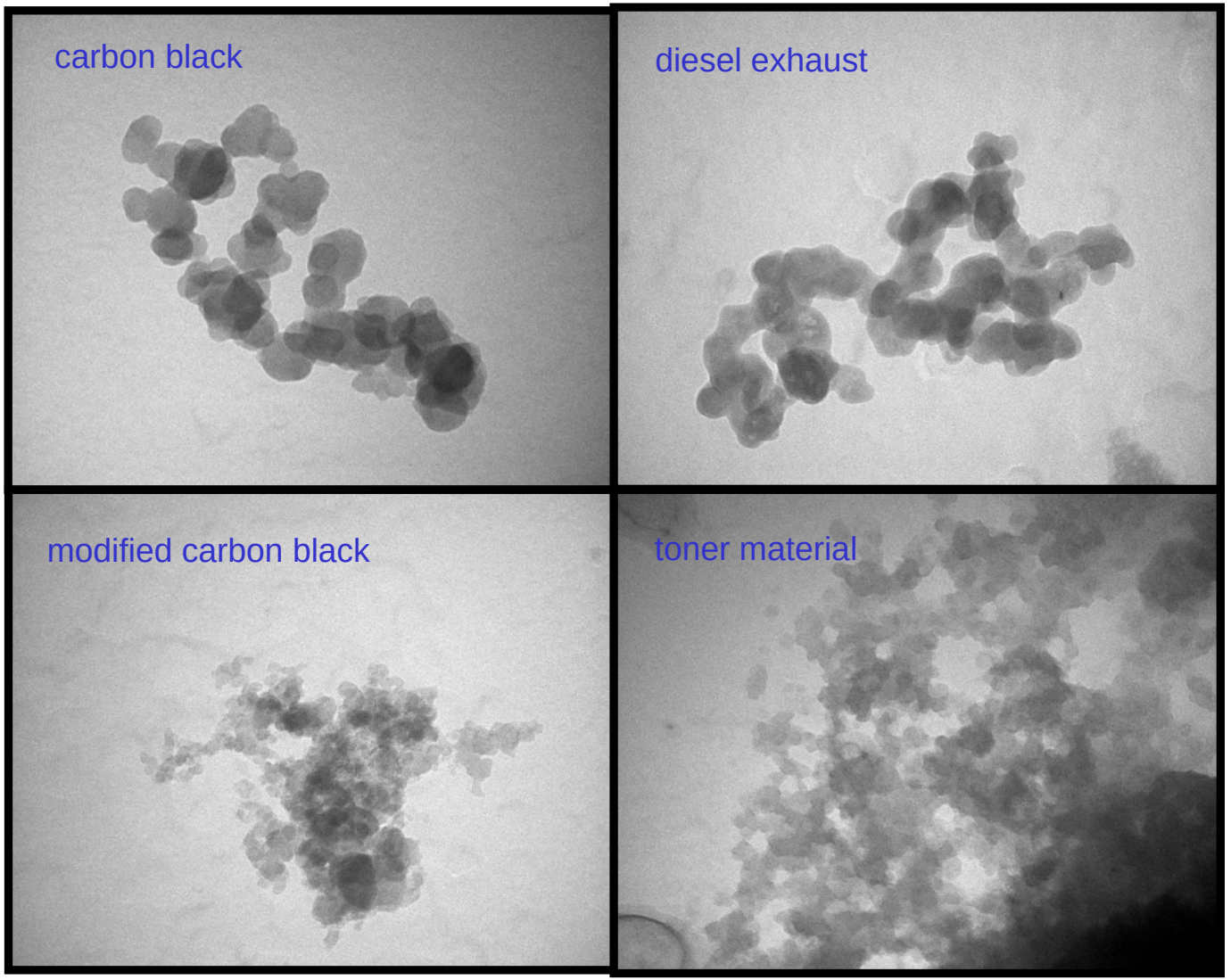
⇒ formation of PAH

Characterization of „Carbon“-samples



Characterized dust samples (light microscope, magnification: 20 - fold): Carbon black (top left), diesel exhaust (top right), a modified Carbon black (Printex 90) (bottom left) and toner material (bottom right)

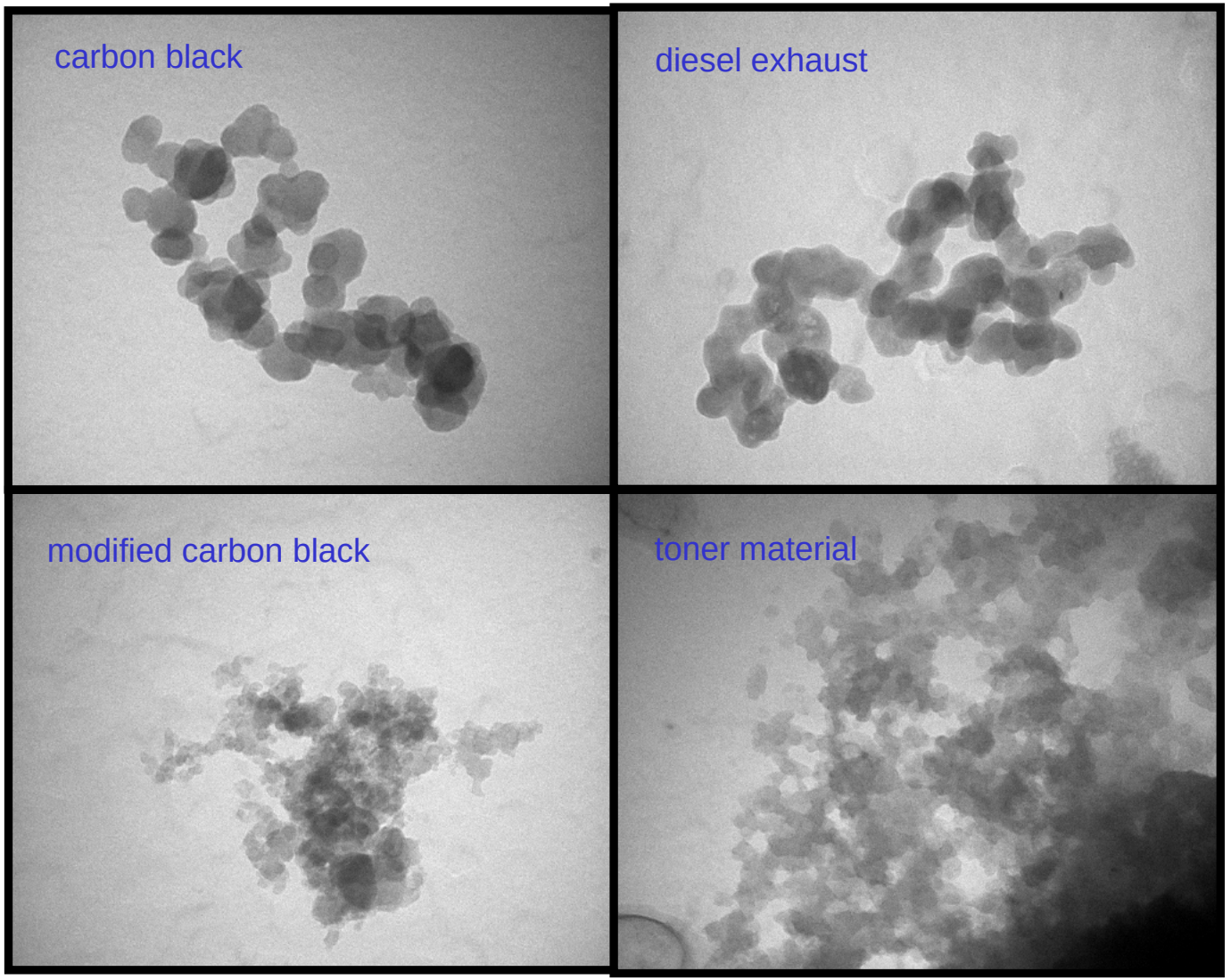
Characterization of „Carbon“-samples



TEM images, 100 000x

Characterization of „Carbon“-samples

primary particles
d ~ 50 nm



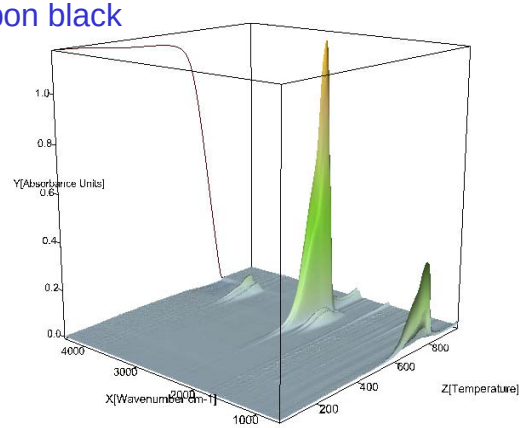
primary particles
d ~ 5 nm

primary particles
d ~ 10 nm

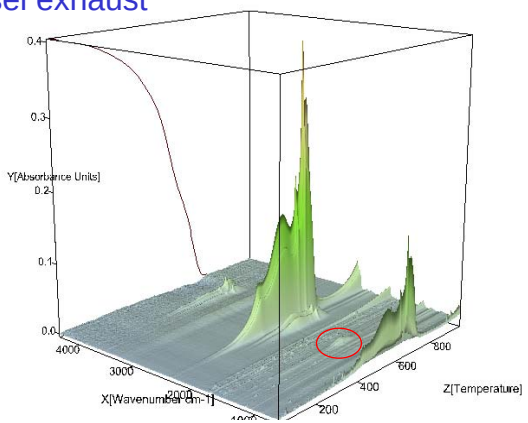
TEM images, 100 000x

Characterization of „Carbon“-samples

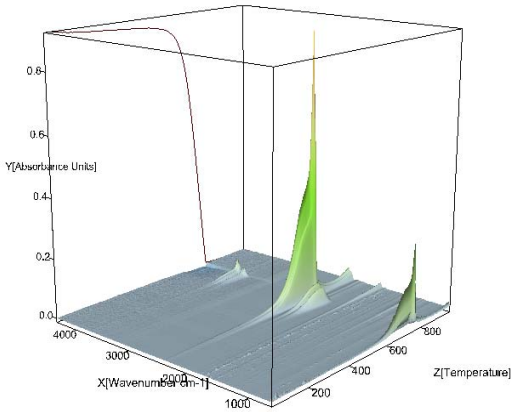
carbon black



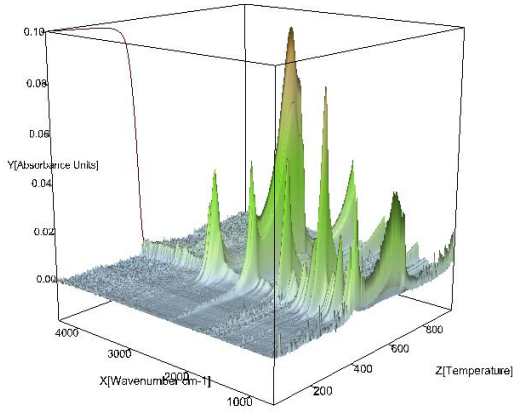
diesel exhaust



modified carbon black

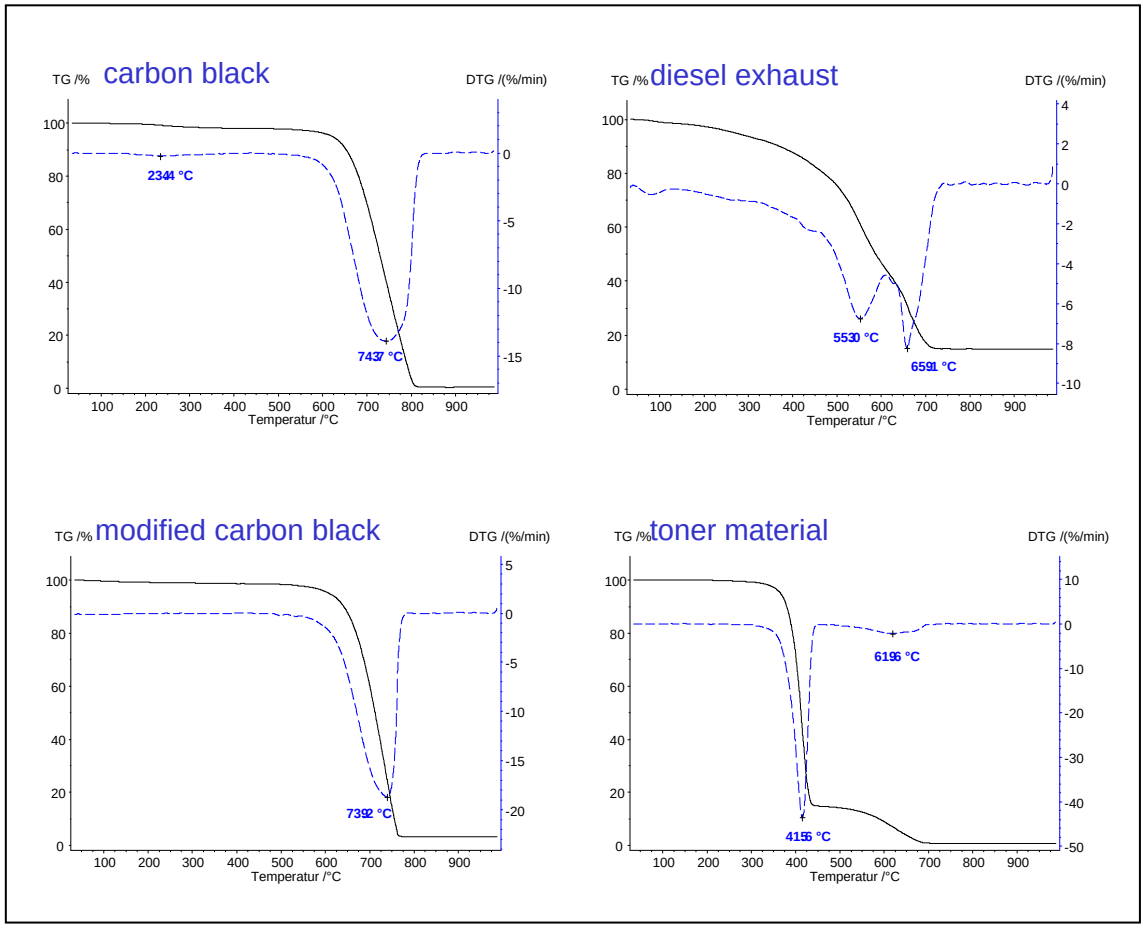


toner material



Results of coupled TG-FTIR measurements, heating rate 20 K/min

Characterization of „Carbon“-samples



Results TG measurements, heating rate 5 K/min

Nanoparticle concentrations – particle counter versus TEM

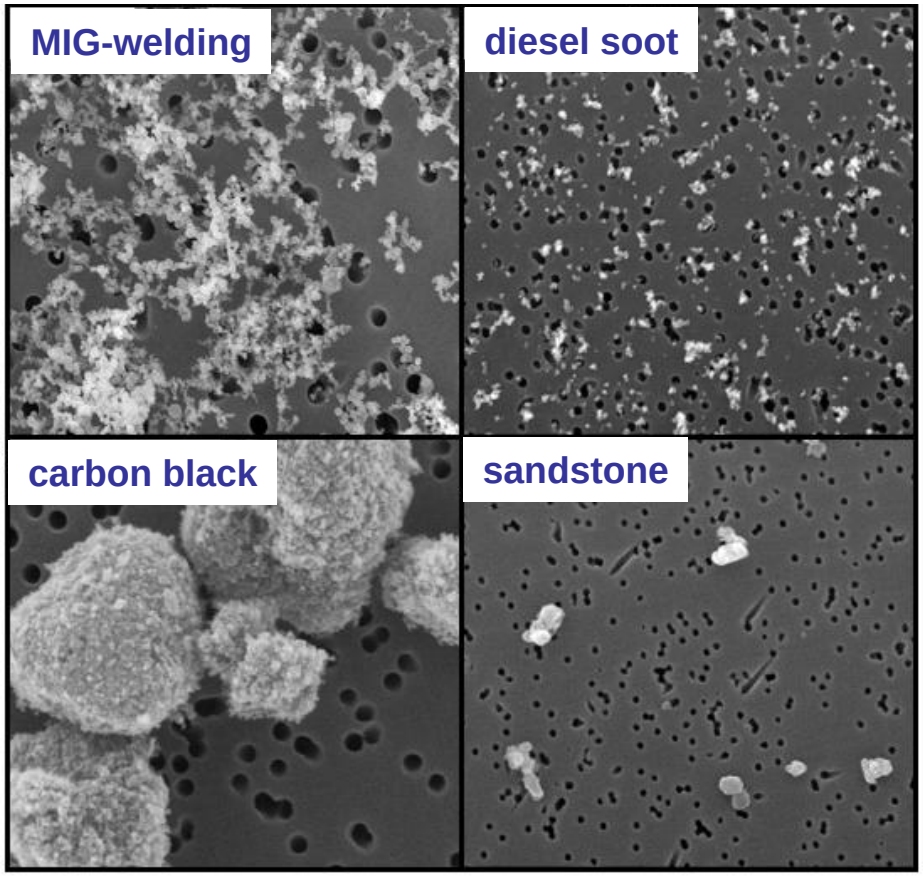
Results (particle counter)

Various workplaces

carbon black	$0{,}7 \cdot 10^9$ particles/mg
sandstone	$1{,}3 \cdot 10^9$ particles/mg
MIG-welding	$15 \cdot 10^9$ particles/mg
MMA-welding	$62 \cdot 10^9$ particles/mg
diesel soot	$740 \cdot 10^9$ particles/mg

Determination of nanoparticle concentrations

Results (SEM)



Comparison of different dust samples by SEM.
Magnification, 10 000x

Determination of nanoparticle concentrations

Results (TEM)

mean number of primary particles per agglomerate

Various workplaces

sandstone	3	primary particles/agglomerate
diesel soot	20	primary particles/agglomerate
MMA-welding	20	primary particles/agglomerate
MIG-welding	300	primary particles/agglomerate
carbon black	10000	primary particles/agglomerate

Determination of nanoparticle concentrations

Number of toxicological relevant particles (primary particles) by electron microscopy (*worst case*)?

Workplace	results (particle counter)	results (TEM)
carbon black	$0{,}7 \cdot 10^9$ particles/mg	$7 \cdot 10^{12}$ (tr)particles/mg
sandstone	$1{,}3 \cdot 10^9$ particles/mg	$3{,}9 \cdot 10^9$ (tr)particles/mg
MIG-welding	$15 \cdot 10^9$ particles/mg	$4{,}5 \cdot 10^{12}$ (tr)particles/mg
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Determination of nanoparticle concentrations

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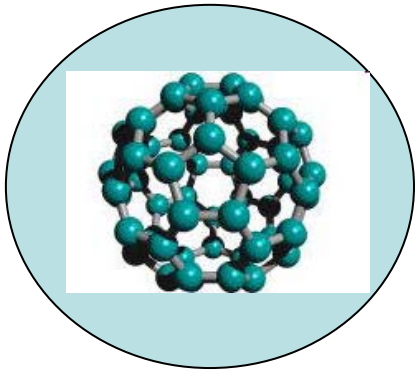
diesel soot	$740 \cdot 10^9$ particles/mg	$1{,}5 \cdot 10^{13}$ (tr)particles/mg

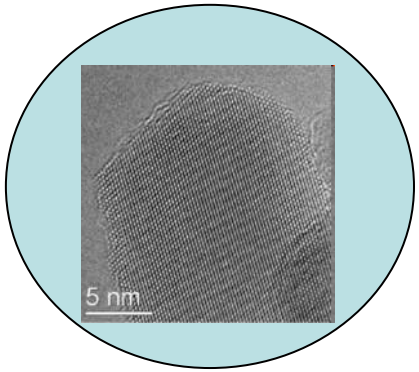
Difference between particle counter and EM caused by agglomeration $\Rightarrow$ “nano” particles

Primary particle

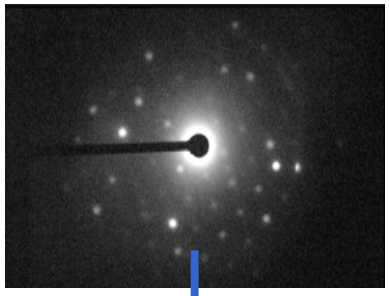


molecule

Primary particle

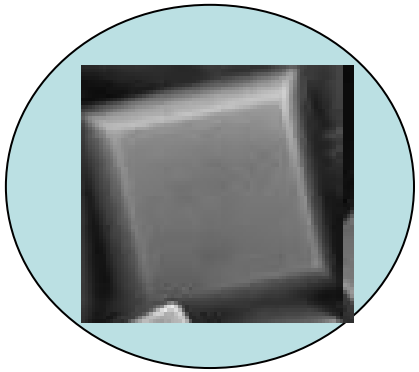


perfect crystal structure



electron diffraction pattern

Primary particle



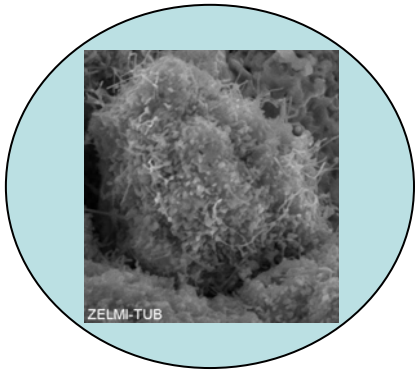
real

crystal structure

defects

homogeneous,
compact surface

Primary particle



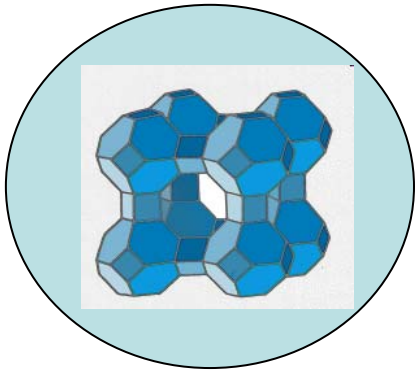
real

crystal structure

defects

fissured, porous surface

Primary particle

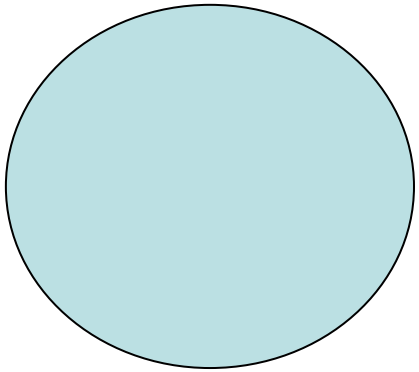


real
crystal structure
defects

pores and / or
inner surface

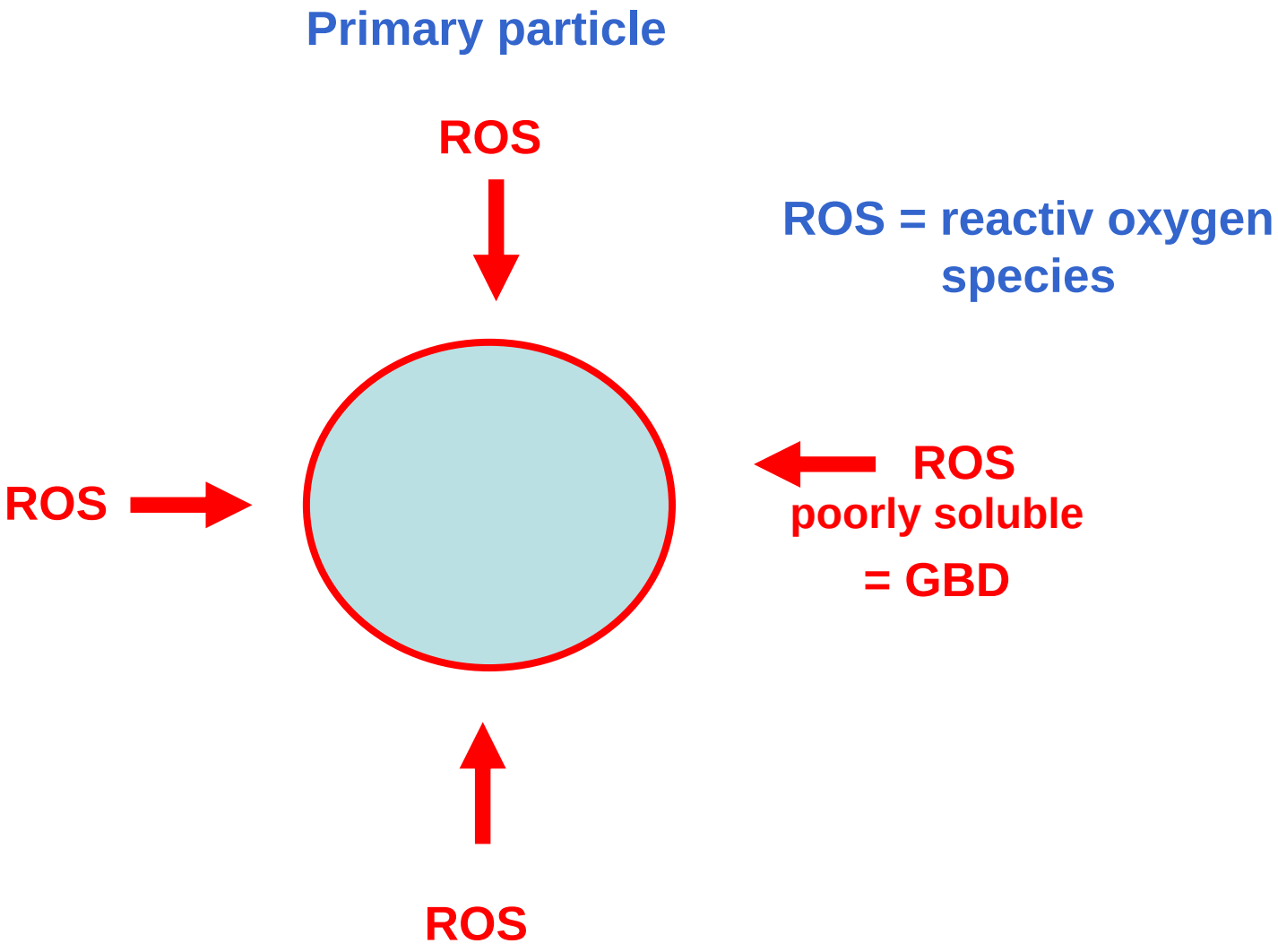
Primary particle

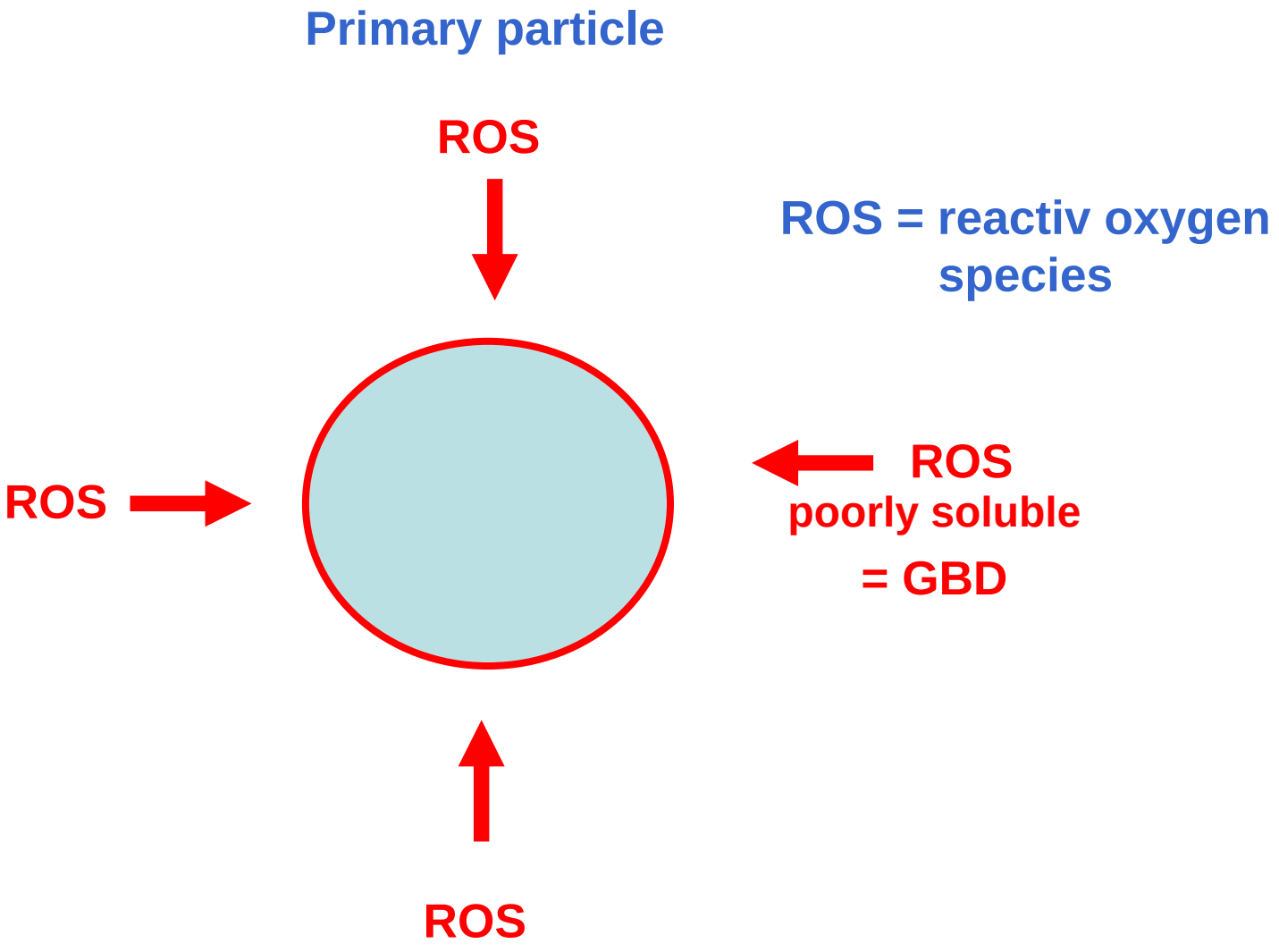
GBD = granular biopersistent dust



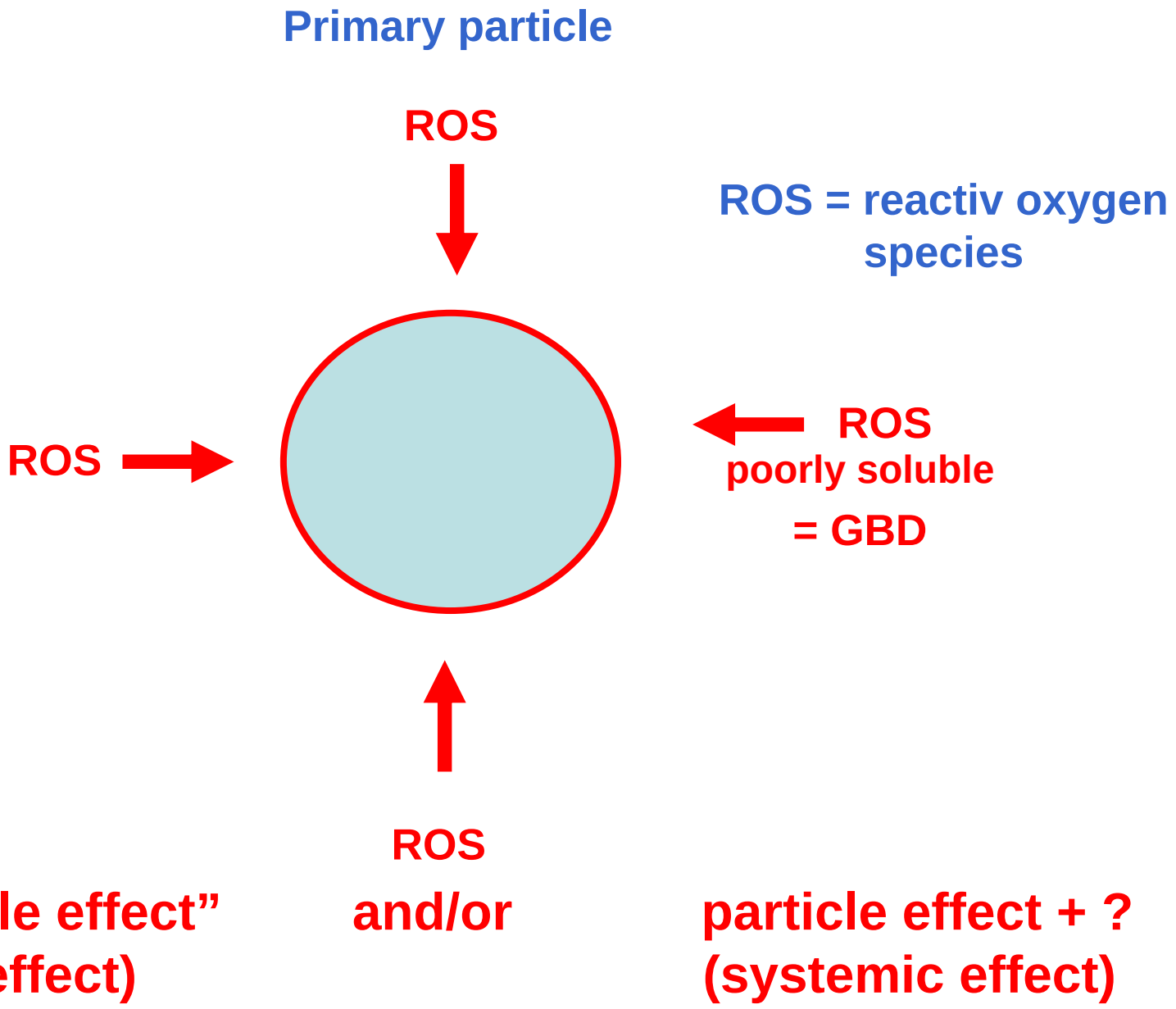
poorly soluble

= GBD

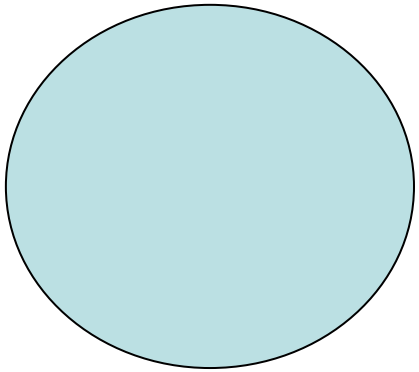

Primary particle
ROS
ROS = reactiv oxygen species
ROS
ROS
poorly soluble
= GBD
ROS



“particle effect”
(local effect)


Primary particle
ROS
ROS = reactiv oxygen species
ROS
ROS
poorly soluble
= GBD
ROS
“particle effect”
(local effect)
and/or
particle effect + ?
(systemic effect)

Primary particle

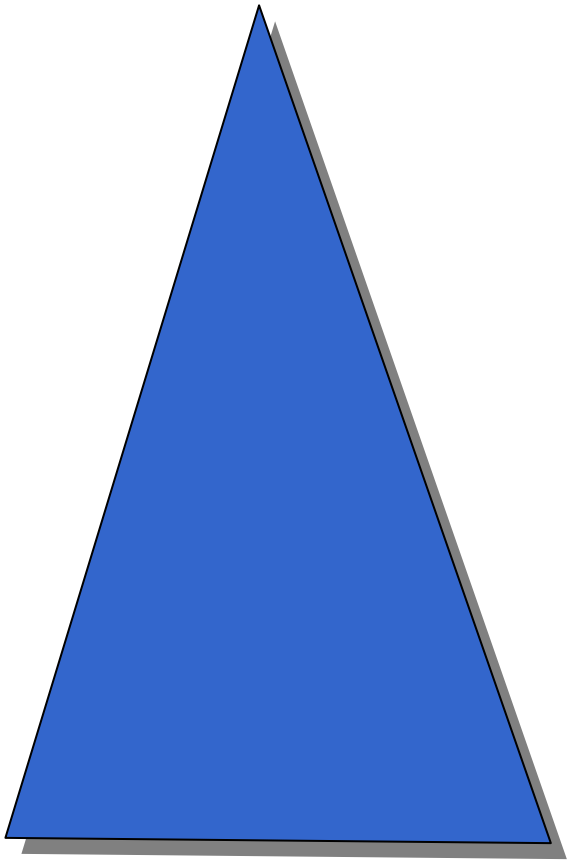


highly soluble

metal ions are locally and sytemically available

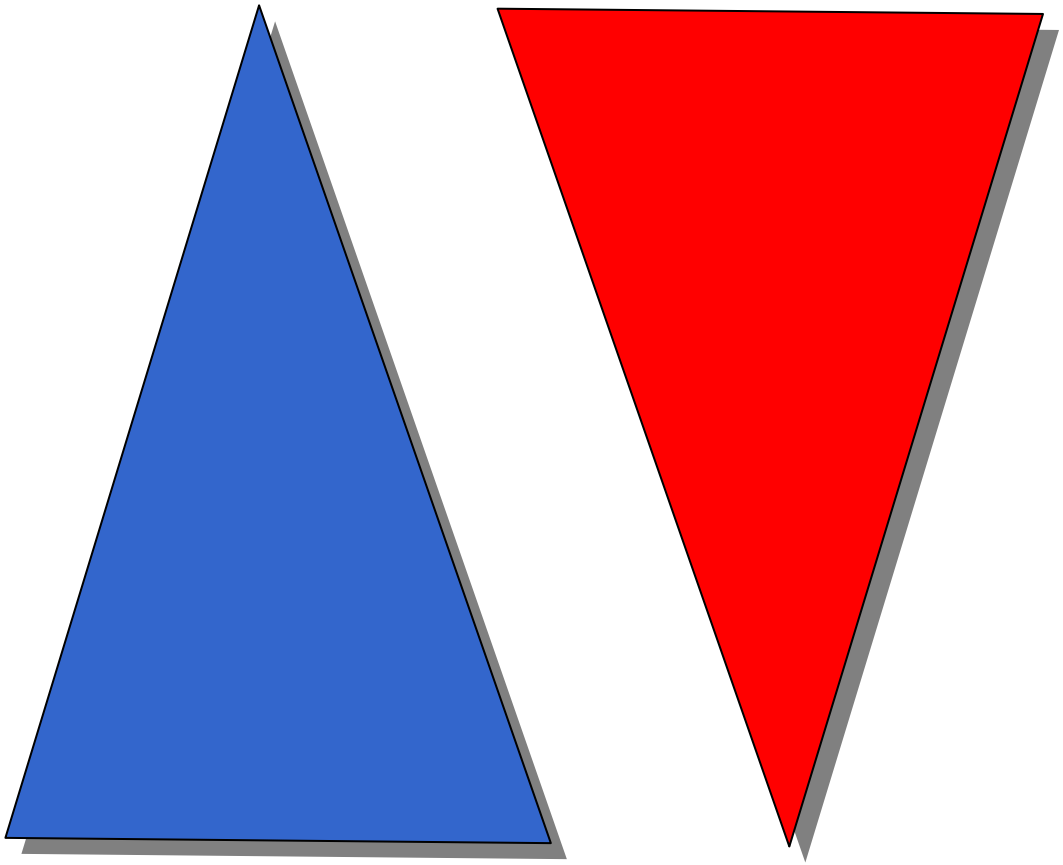
General tendencies of primary particles

particle size



General tendencies of primary particles

particle size **agglomeration**

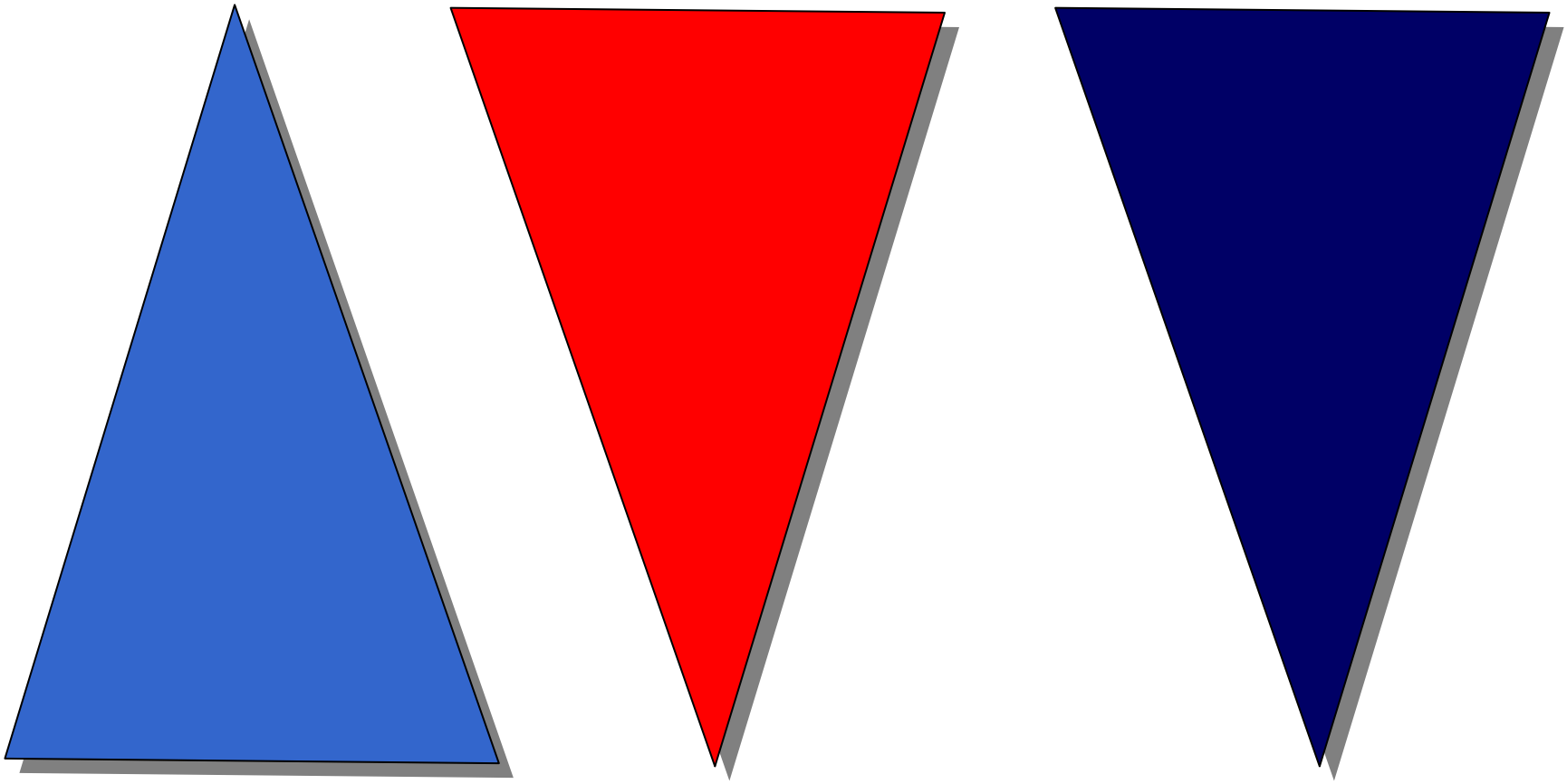


General tendencies of primary particles

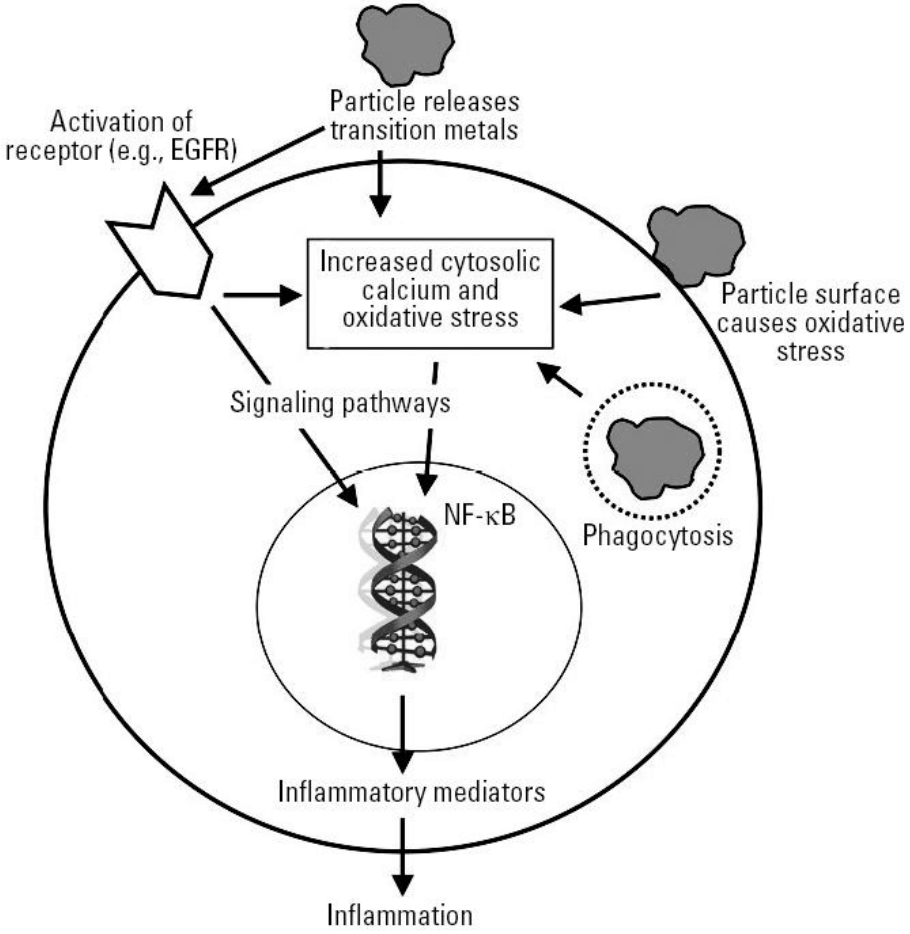
particle size

agglomeration

solubility



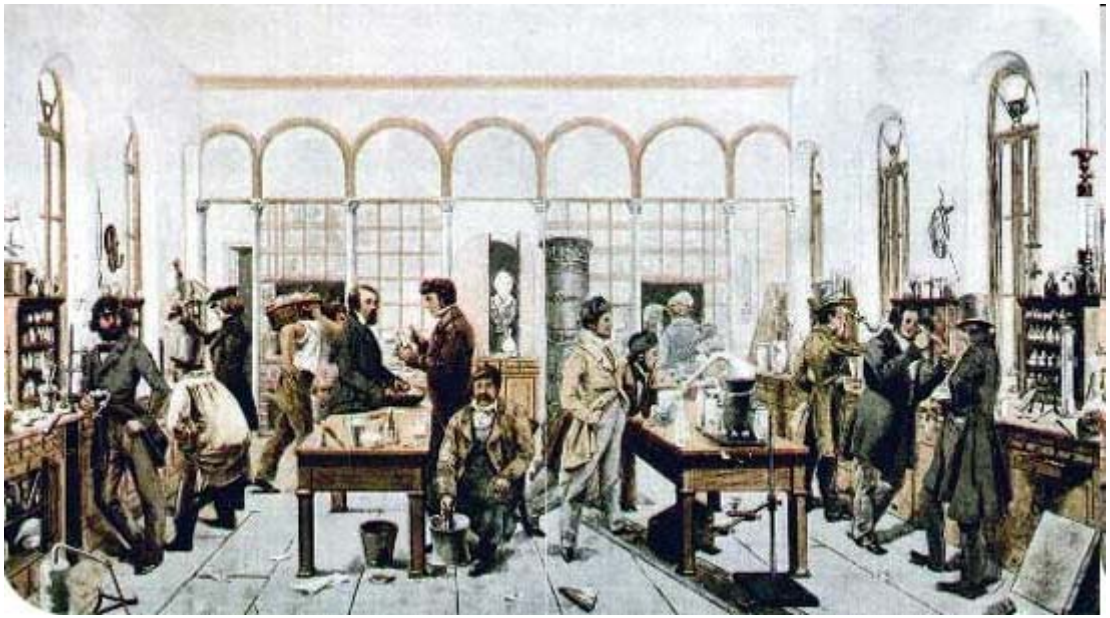
Effect of nanoparticles on the cell



Hypothetical cellular interaction of NSPs (adapted from Donaldson and Tran 2002). EGFR, epidermal growth factor receptor. Inflammation and oxidative stress can be mediated by several primary pathways: a) the particle surface causes oxidative stressresulting in increased intracellular calcium and gene activation; b) transition metals released from particles result in oxidative stress, increased intracellular calcium, and gene activation; c) cell surface receptors are activated by transition metals released from particles, resulting in subsequent gene activation; or d) intracellular distribution of NSPs to mitochondria

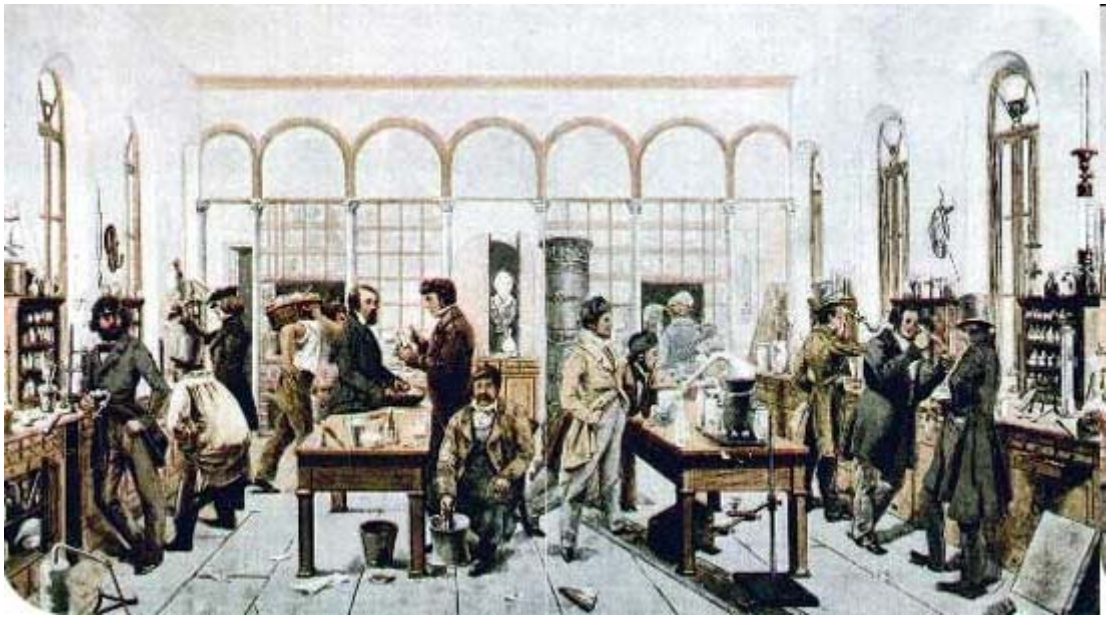
Oberdörster G, Oberdorster E, Oberdörster J (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823-839

Museum of chemistry – Liebig museum Giessen



Justus von Liebig (1803-1873)

Museum of chemistry – Liebig museum Giessen



Justus von Liebig (1803-1873)

Justus-Liebig-University Giessen

With around 24,000 students, the university is prepared to meet any challenges that the future may bring.

Thank you for your attention!

Priv.-Doz. Dr. Dr. Dirk Walter

Gefahrstofflaboratorien Chemie und Physik

am Institut für Arbeits- und Sozialmedizin

Justus-Liebig-Universität

Aulweg 129

D-35392 Giessen

dirk.walter@arbmed.med.uni-giessen.de

Preparation of dust samples by direct and indirect route

Number of agglomerates and primary particles - Comparison of different routes of preparation

MIG-welding (construction steel)

direct preparation	**$216 \cdot 10^9$ agglomerates/m^3**
indirect preparation	**$981 \cdot 10^9$ agglomerates/m^3**
indirect preparation ultrasonic	**$4670 \cdot 10^9$ agglomerates/m^3**

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well-defined area (TEM)

direct preparation	21 agglomerates + 9688 primary particles
ind. preparation ultra.	245 agglomerates + 6721 primary particles

Preparation of dust samples by direct and indirect route

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Disagglomeration of agglomerates in water!