Applications of Selected Nano-Particles in Nano-Medicine

Nano-Biotechnology PL 2012
Malej Auli Politechniki, pl. Polytechniki 1
Warsaw, Poland
17-18 September 2012

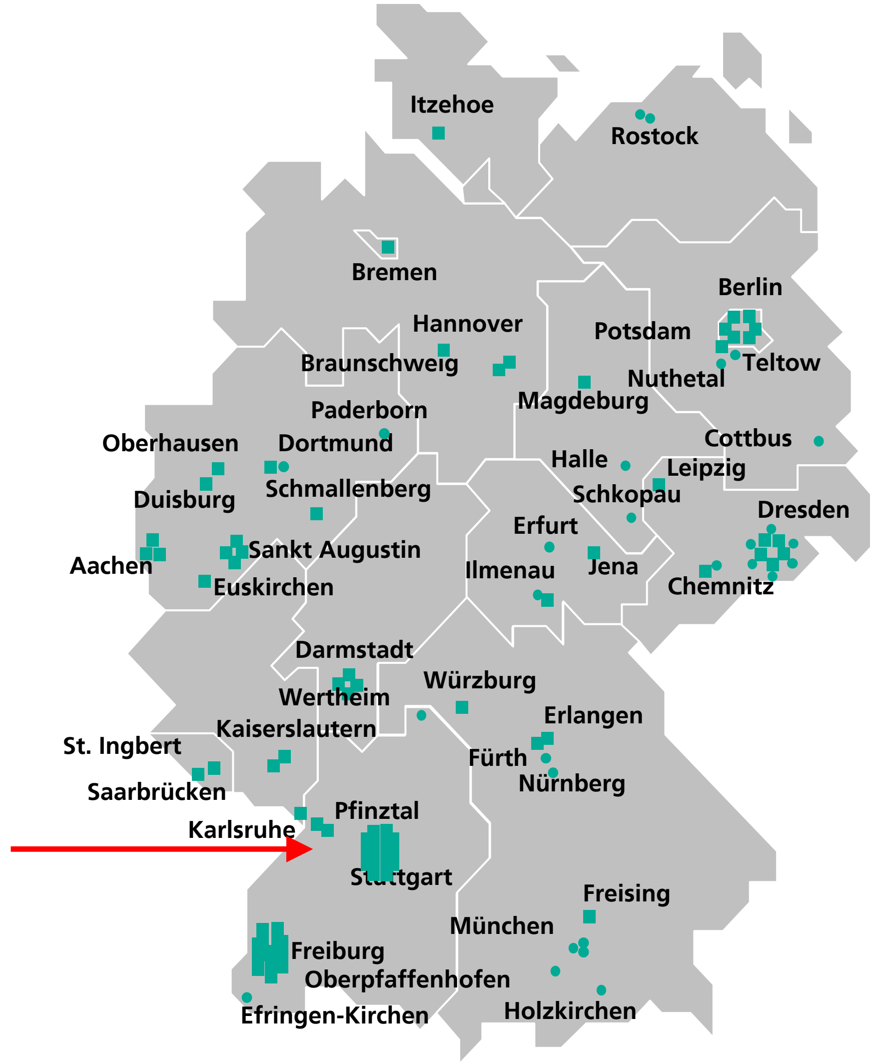
Dipl.-Chem. Helmut Schmid

Fraunhofer-Institute Chemical Technology (ICT), Box 1240, D-76318 Pfinztal, Germany
Web: www.ict.fhg.de, Phone: +49 721 4640-709, Mail: sd@ict.fhg.de

Index

Fraunhofer-Society
Locations in Germany

- 56 Institutes
- 12 770 Staff
- More than 40 locations in Germany
- International representations



FhG ICT

ICT Management
Prof. Elsner

Controlling
C. Steuerwald

Cross-Section Tasks
Dr. Hefer

Finances
Staff
Organization

Central Management
Dr. Tröster

Research planning
Communication
International activities

Energetic Materials
Dr. Krause
Dr. Keicher, Löbecke

Synthesis
Reaction technology
Formulation
Compounding
Analytics
Particle technology

Energetic Systems
W. Eckl, G. Langer

Nanotechnology
Modeling
Combustion
High speed measurement
Gas generators
Safety engineering

Applied Electrochemistry
Dr. Krausa
Dr. Pinkwart

Battery technology
Fuel cells
Sensors
Electrochemical catalysis

Environmental Engineering
R. Schweppe
Dr. Woydasky

Environmental friendly production processes
Disposal procedures
Recycling economy
Environment simulation

Polymer Engineering
Dr. Henning
Dr. Bräuning
Dr. Diemert

Products
Tool technology
Processing
Materials

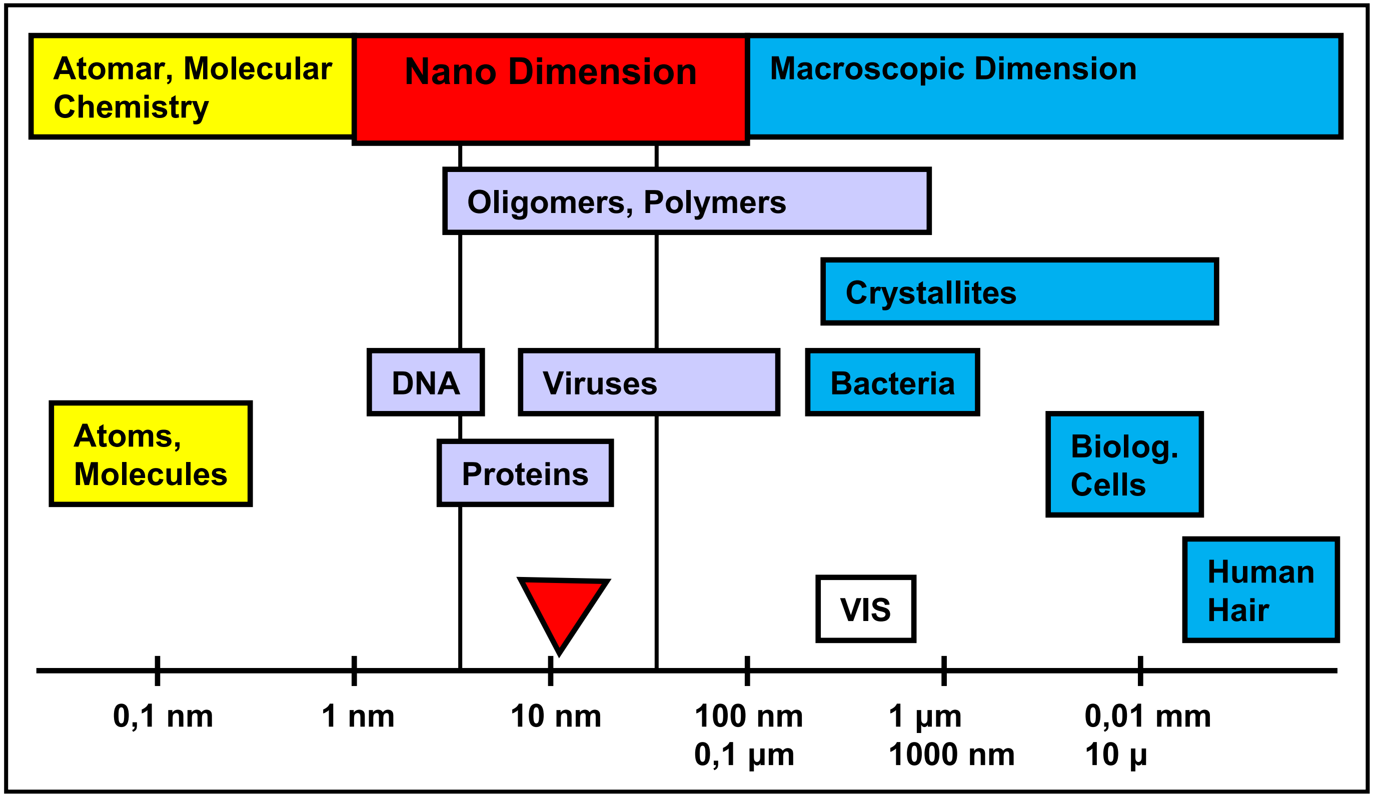


Figure 6.1

Size classification of well known objects. Interesting Nano-Region: 5 – 50 nm.

Medical Applications

General Medical Research

1. Drug Discovery
2. Analysis of Cell Signal Pathways

Therapy

1. Drug Delivery (Targeting, Controlled Release)
2. Gene Delivery
3. Personalized Medicine
4. Regenerative Medicine (Bioactive Surfaces)
5. Tissue Engineering
6. Antimicrobial Surfaces
7. Cancer Therapy

Diagnostics In-vitro, In-vivo

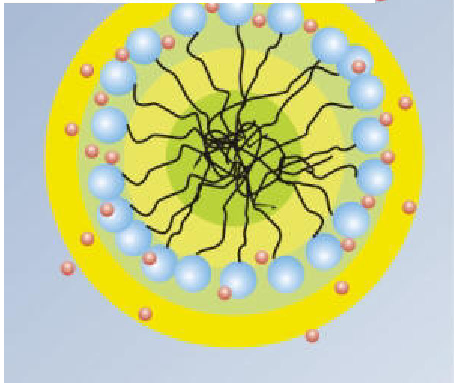
1. Biosensors
2. Biochips (DNA, Proteins, Cells)
3. Medical Imaging (Ultrasonic, NMR, X-Ray)

„Thera-Nostics“

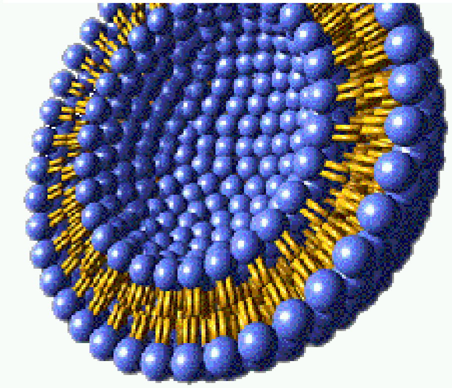
Organic and Inorganic Nano-Particles as Drugs / Carriers

Sources: Uni Dortmund, Lenz

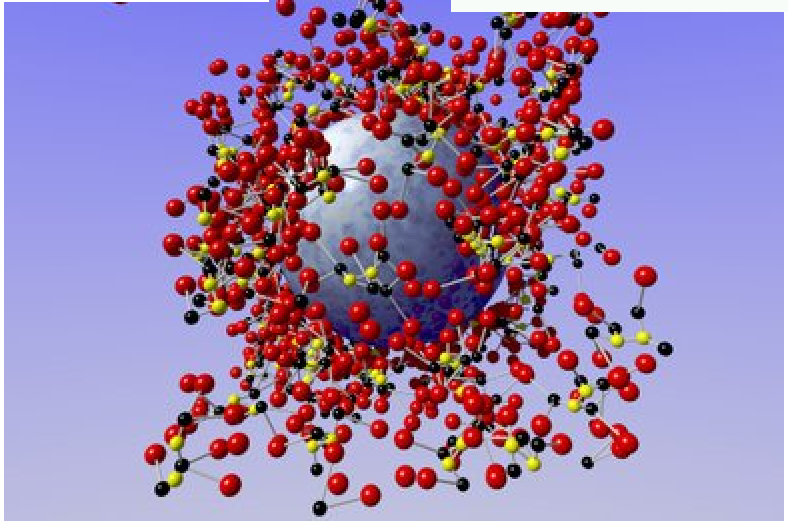
Micells (Vesicles)



Liposomes (Vesicles), Phospho-Lipids



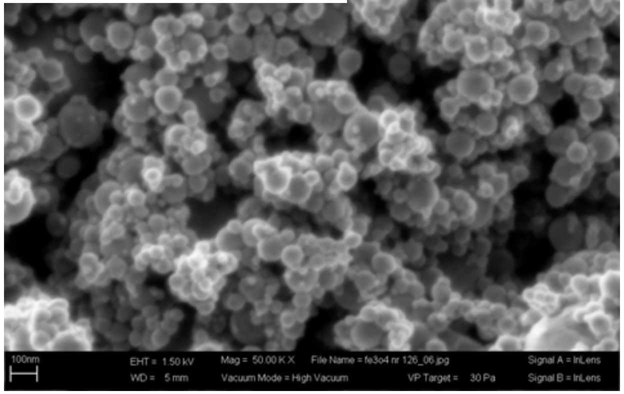
Dendrimers



Polymer-Protein-Conjugates

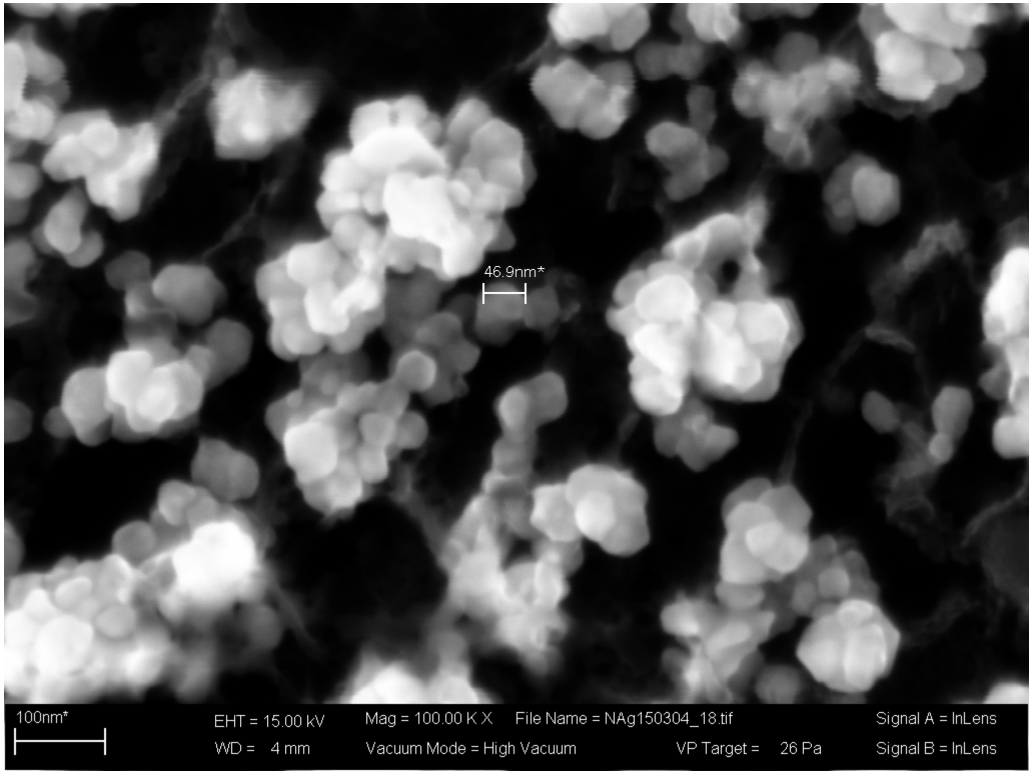
Complex Compounds
Gd-DTPA (Chelate)
Gadolinium-dietylene-triamino-pentaacetate
Gd^{3+}, 7 unpaired f-e^{-},
Paramagnetism
Contrast Agent MRI:
Toxicity -> Nephrogenic Systemic Fibrosis

Inorganic-NP´s



Fraunhofer ICT

Example: Nano-Silver with Antimicrobial Functionality



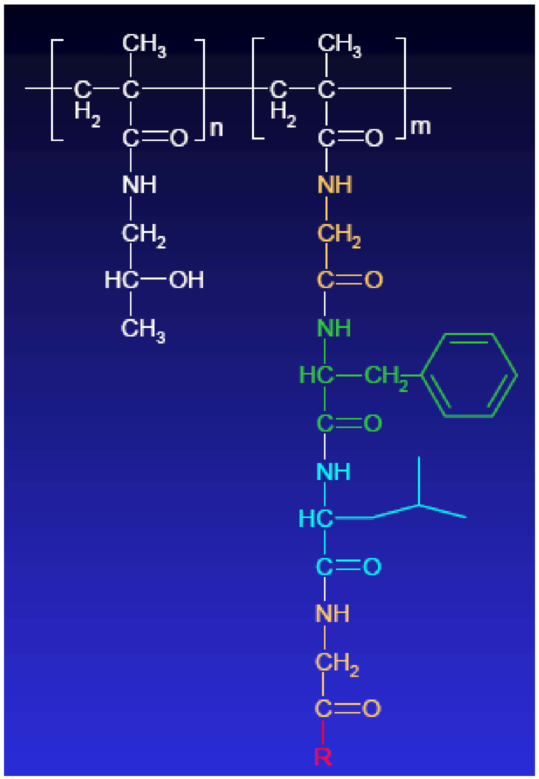
File: NAg150304_18.Tif

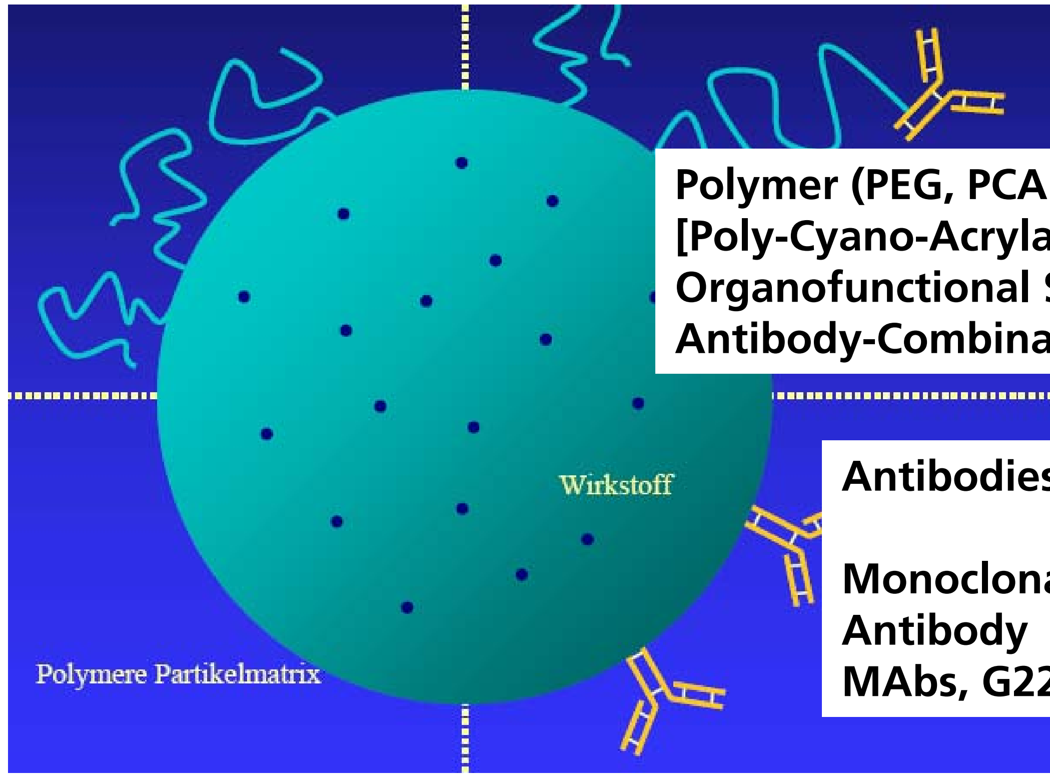
Figure 9.1 SEM-Record of Nano-Ag-Particles. A stable suspension was applied to an Al-plate and investigated after drying. Particle size approx. 50 nm.

Suggested Combination of Principles for Drug-Functionalization

Polymer-Protein-Conjugates
Hydrolyzed-Polymaleic-Anhydride (HPMA)-
Copolymer-Gly-Phe (Phenylalanine)-Leu-Gly-R

Source: Uni Frankfurt





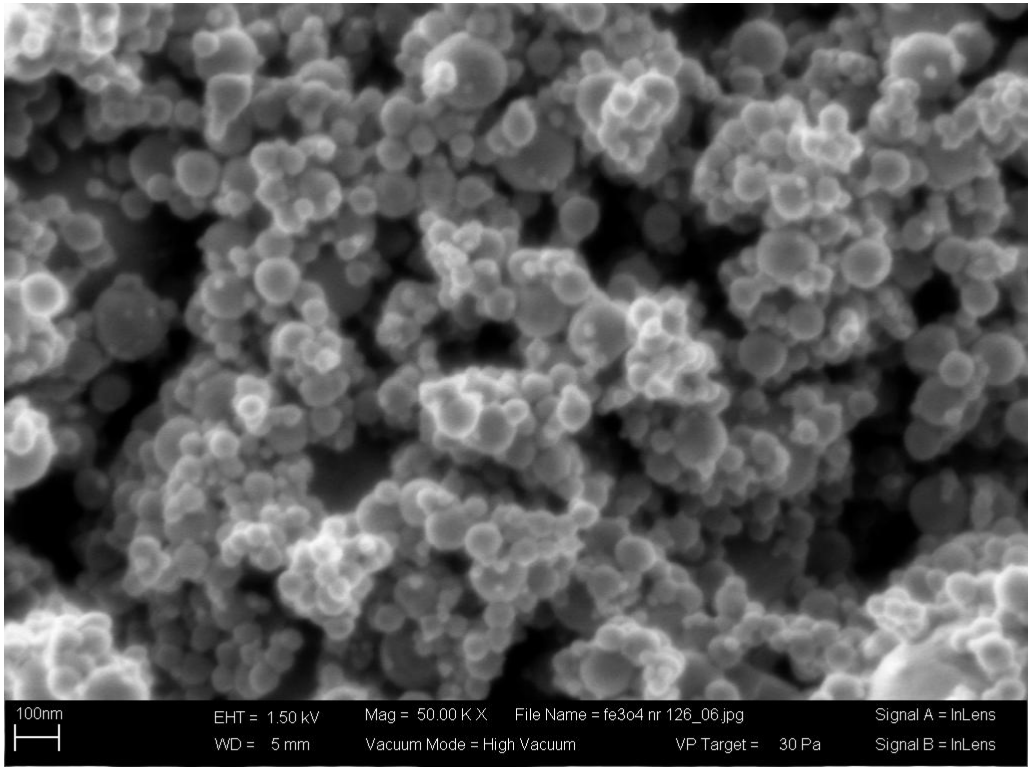
Polymer (PEG, PCA [Poly-Cyano-Acrylate], Organofunctional Silanes)-Antibody-Combination

Antibodies

Monoclonal Antibody MAbs, G22

R: Doxorubicin -> Apoptosis, Programmed Cell Death (PCD), Galactosamin

Example: Ferrimagnetic Nano-Magnetite



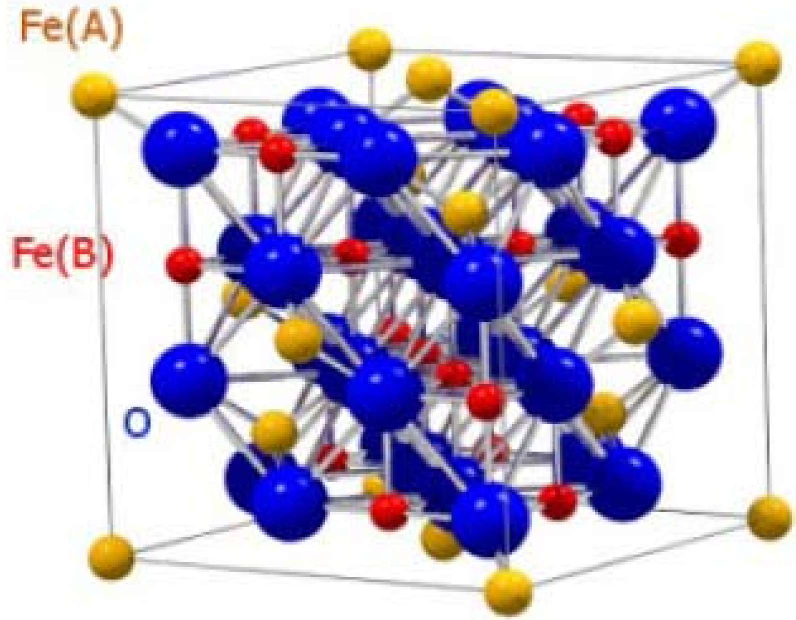
File: fe3o4 nr 126_06.jpg

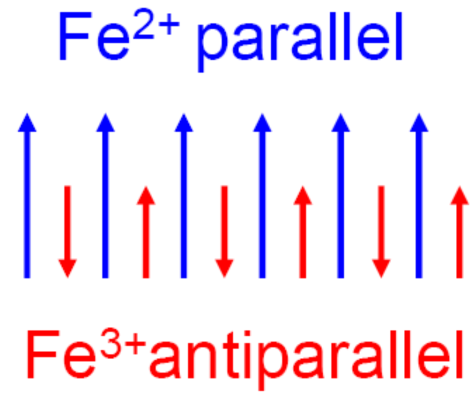
Figure 11.1 SEM-Record of Nano-Magnetite-Particles (Fe_3O_4). A stable suspension was applied to an Al-plate and investigated after drying. Particle size approx. 50 nm.

Crystal Structure of Magnetite Fe_3O_4

+III

+II/+III





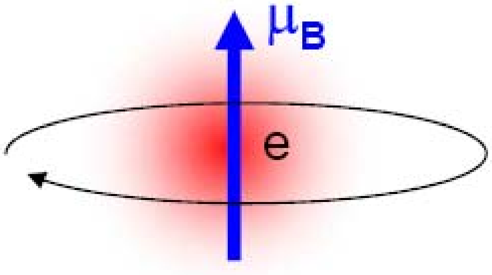
<u>Figure 12.1:</u> Crystal structure of Magnetite. The iron cations are located at two different positions (A and B) of the crystal lattice. The A-position is occupied only by Fe^{3+}. The B-position is occupied by Fe^{3+} as well as Fe^{2+}. The magnetic moments of the Fe^{3+} cations in positions A and B are antiparallel and therefore compensate. **Resulting is the magnetic moment of Fe^{2+}, which leads to ferrimagnetism.**

Example: Ferrimagnetic Nano-Magnetite

Atomic number	Element	Electronic structure of 3d					Moment (μ_B)
21	Sc	↑					1
22	Ti	↑	↑				2
23	V	↑	↑	↑			3
24	Cr	↑	↑	↑	↑	↑	5
25	Mn	↑	↑	↑	↑	↑	5
26	Fe	↑↓	↑	↑	↑	↑	4
27	Co	↑↓	↑↓	↑	↑	↑	3
28	Ni	↑↓	↑↓	↑↓	↑	↑	2
29	Cu	↑↓	↑↓	↑↓	↑↓	↑↓	0

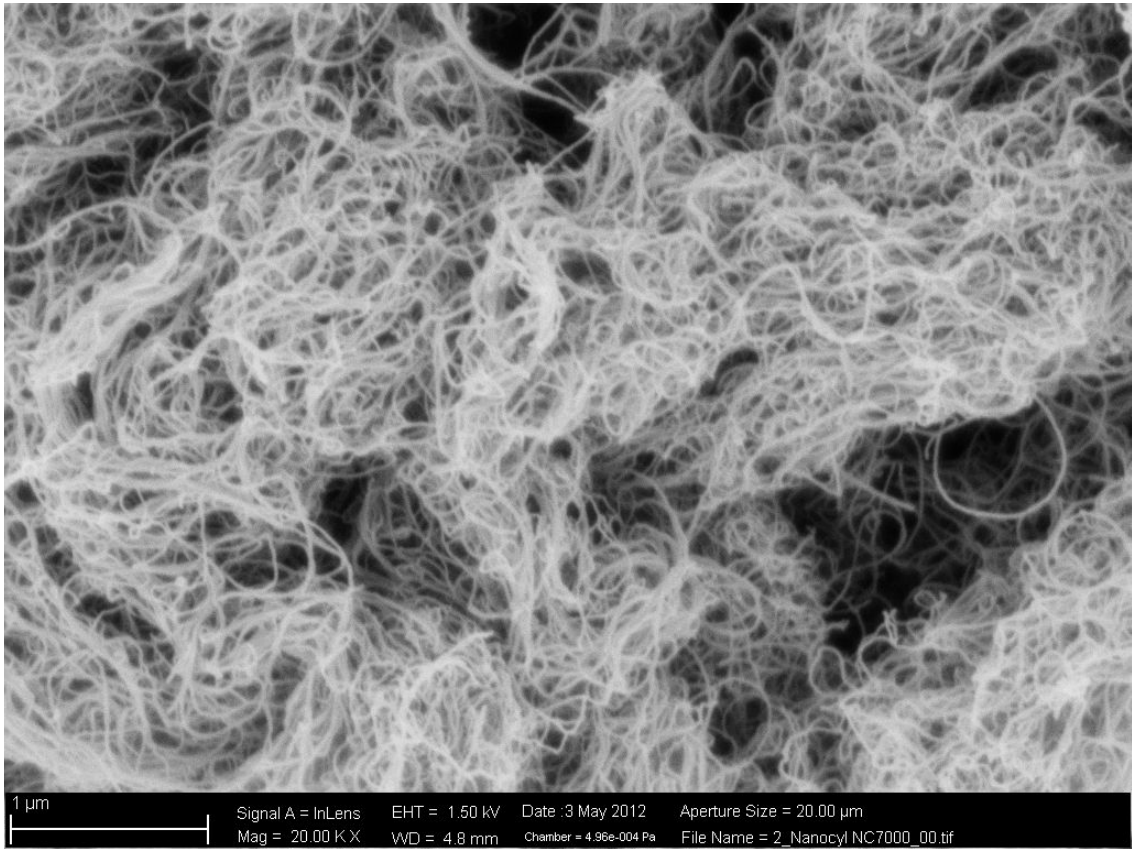
↑ = electronic spin orientation

$$\mu_B = \frac{e\hbar}{2m_e c}$$



Example: Degradable Carbo Nano-Tubes for Controlled Drug Delivery

Source: Wikipedia



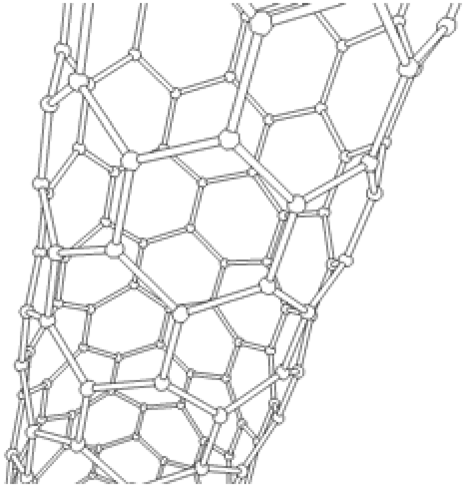
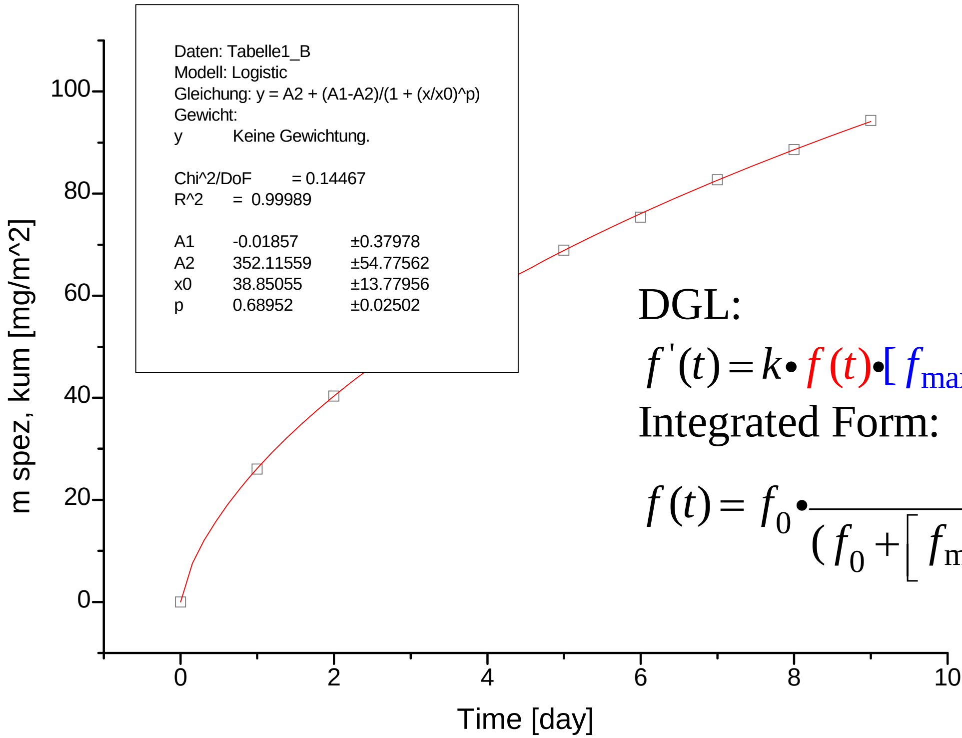


Figure 14.1 SEM-Record of Carbon Nano-Tubes for Drug Delivery.

Fraunhofer ICT

Mathematical Modeling of Cumulative Drug-Realease



DGL:

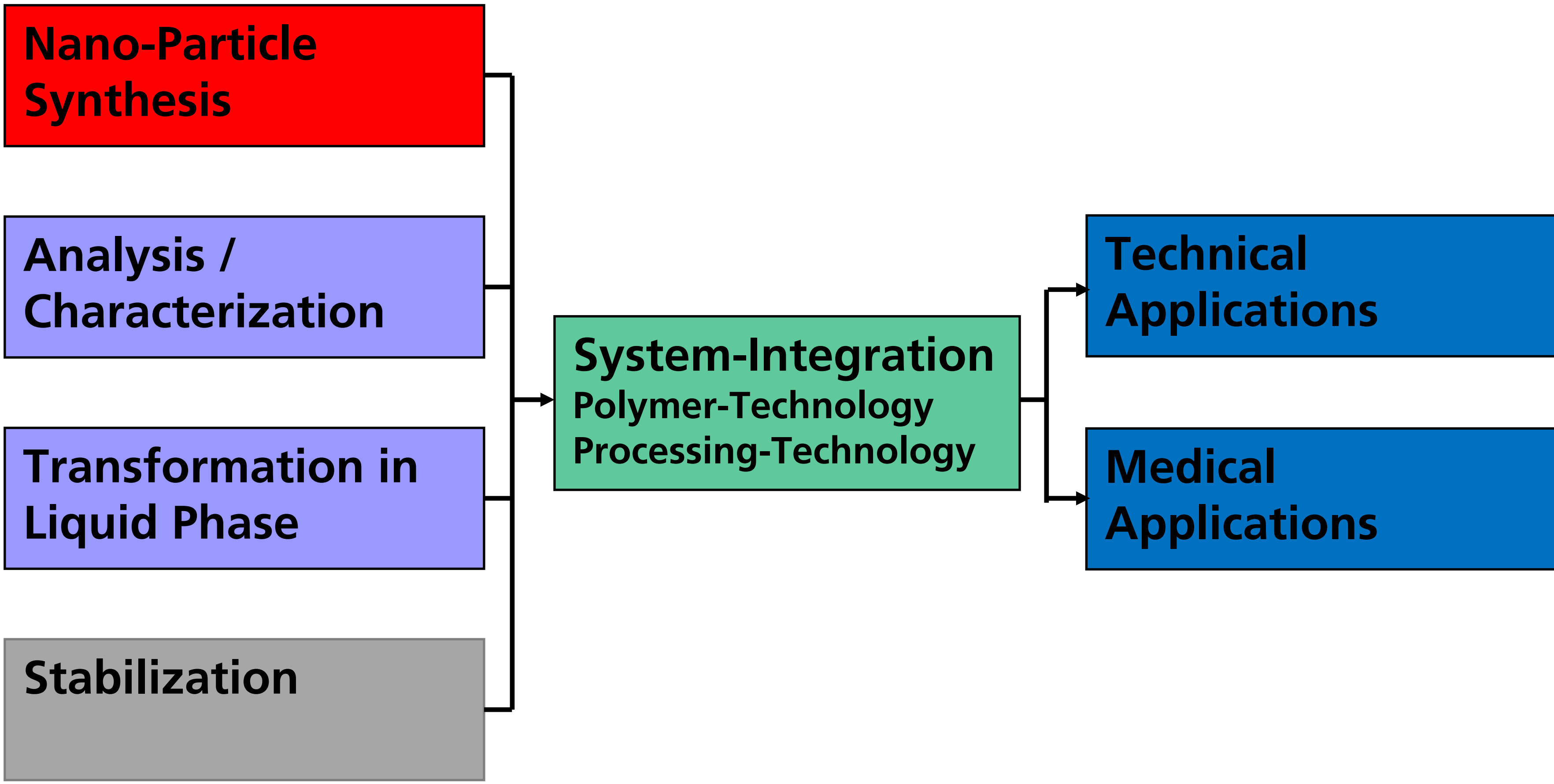
$$f'(t) = k \cdot f(t) \cdot [f_{max} - f(t)]$$

Integrated Form:

$$f(t) = f_0 \cdot \frac{f_{max}}{(f_0 + [f_{max} - f_0] \cdot e^{-f_{max} \cdot k \cdot t})}$$

<u>Figure 15.1</u> Example for Mathematical Modeling of Drug-Release under Consideration of a Superimposed Solution and Diffusion Process using an Exponential-Function representing „Logistic Growth“.

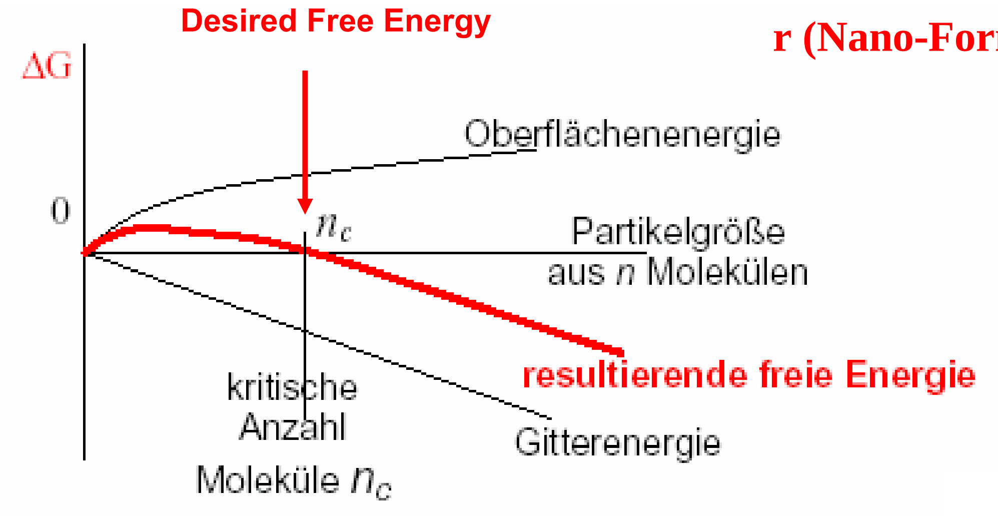
Main Steps from Nano-Particle-Production to Applications



2 Methods and General Tools, Nano-Production, Stabilization

Source: Penth

Thermodynamic Effect

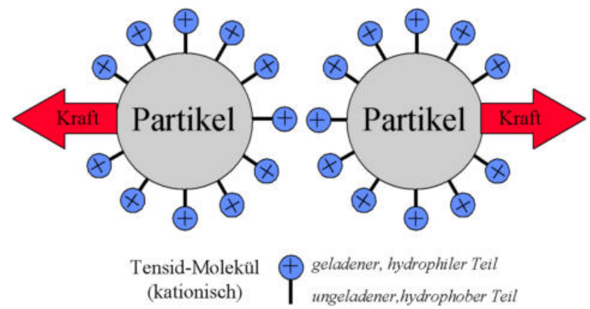


Kinetic Aspect

r (Nano-Formation) >> r (Growth, Agglomeration)

Necessity for Stabilization ->

Surface Treatment



$$\nabla^2 U(\eta,\theta) = \frac{(\cosh\eta - \cos\theta)^2}{B^2} \bullet \frac{\partial^2 U}{\partial \eta^2} + \frac{(\cosh\eta - \cos\theta)^2}{B^2} \bullet \frac{\partial^2 U}{\partial \theta^2} - \frac{\sinh\eta \bullet (\cos\eta - \cos\theta)}{B^2} \bullet \frac{\partial U}{\partial \eta} +$$

$$\left(\frac{(\cosh\eta - \cos\theta)^2}{\tan\theta} - (\cosh\eta - \cos\theta) \bullet \sin\theta \right) \bullet \frac{1}{B^2} \bullet \frac{\partial U}{\partial \theta} = \sinh(U(\eta,\theta)) \quad (1)$$

U: Reduced Electrostatic Potential, B: Constant in Bispheric System of Coordinates

Equation 1

Calculation of surface potential U according to Poisson-Boltzmann-Equation (1) in order to make a suitable selection of chemical additives enabling stabilization and preventing from reagglomeration.

Source: TU-Darmstadt

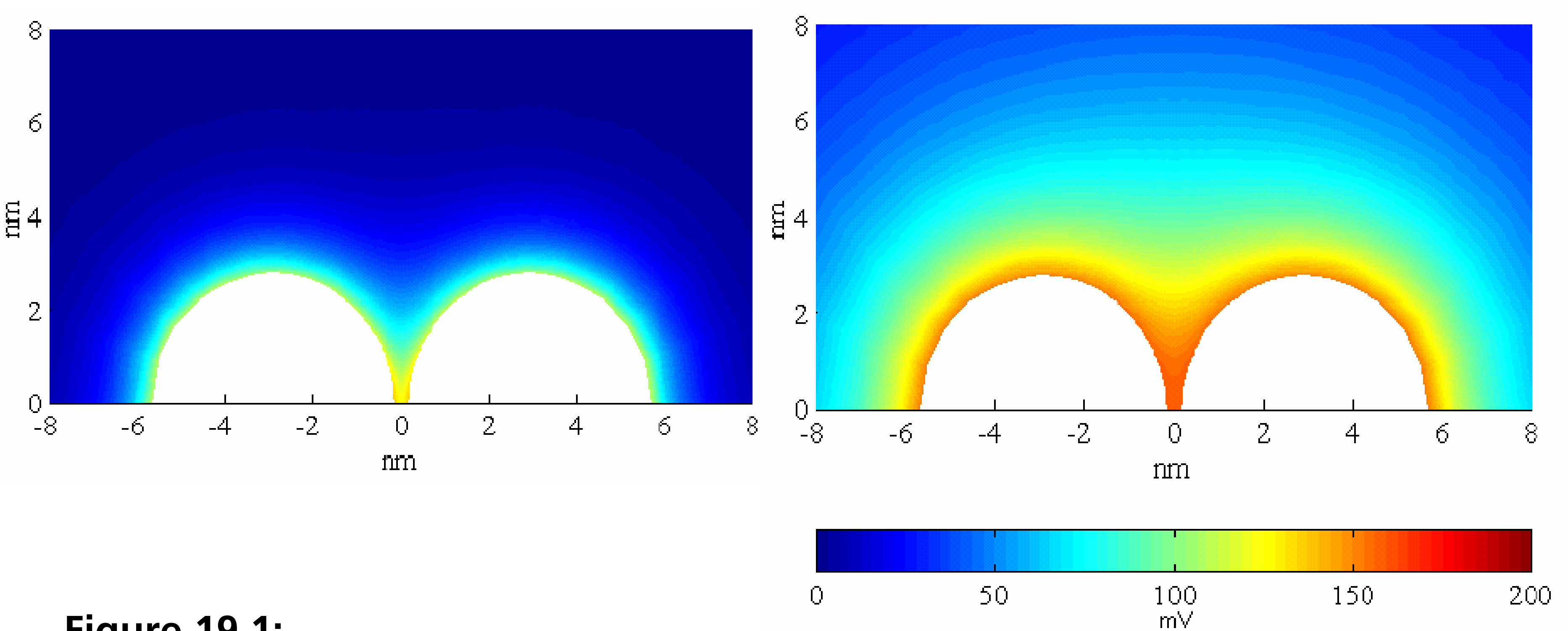
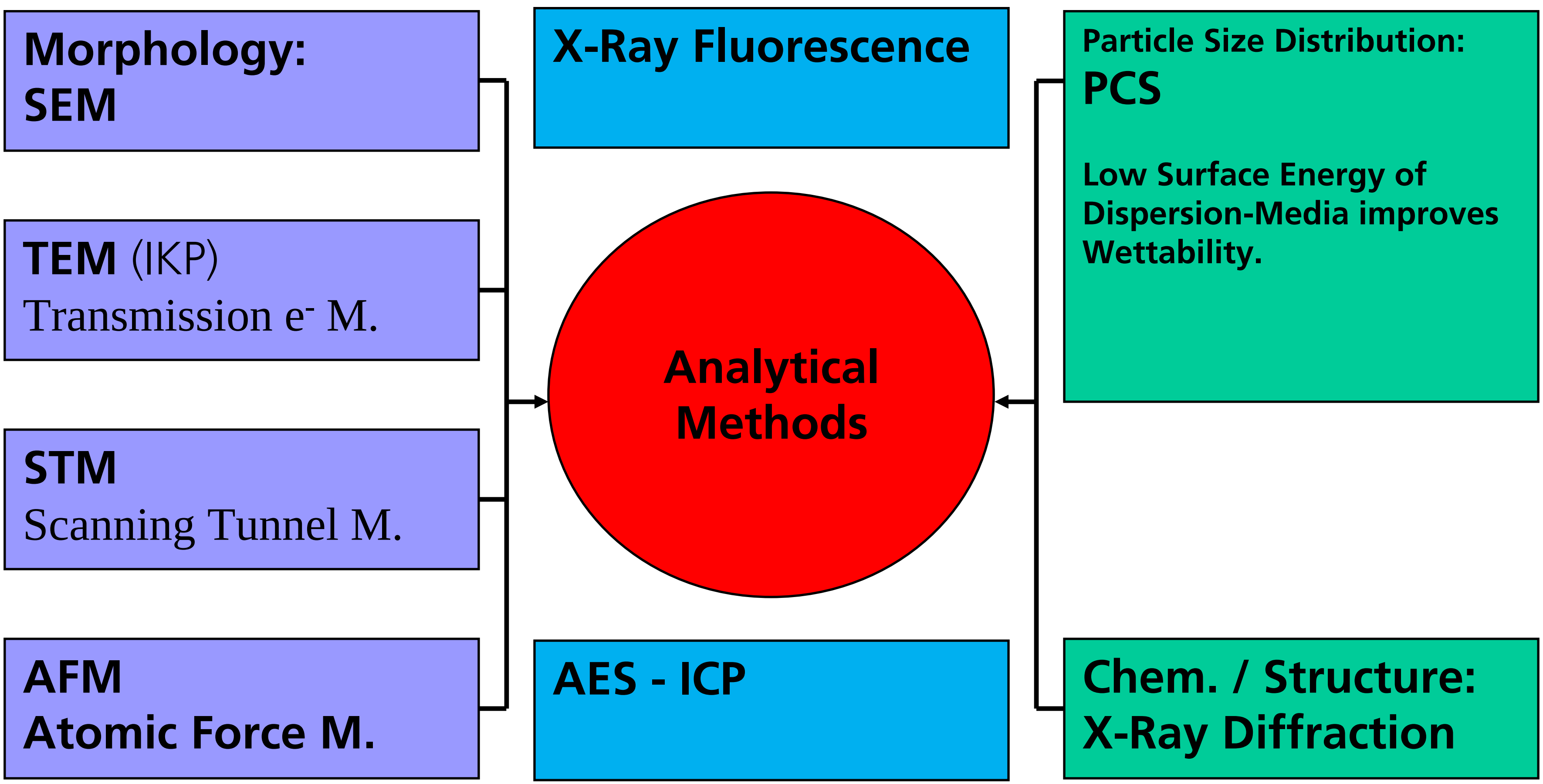


Figure 19.1:
Theory of Nano-Particle-Stabilization:
FE-Calculation of Electrostatic-potential-gradient between two Al_2O_3-Particles (5 nm) as a Function of pH and Electrolyte-concentration (left pH2, 0.1 M NaCl, right pH4, 0.001 M NaCl)

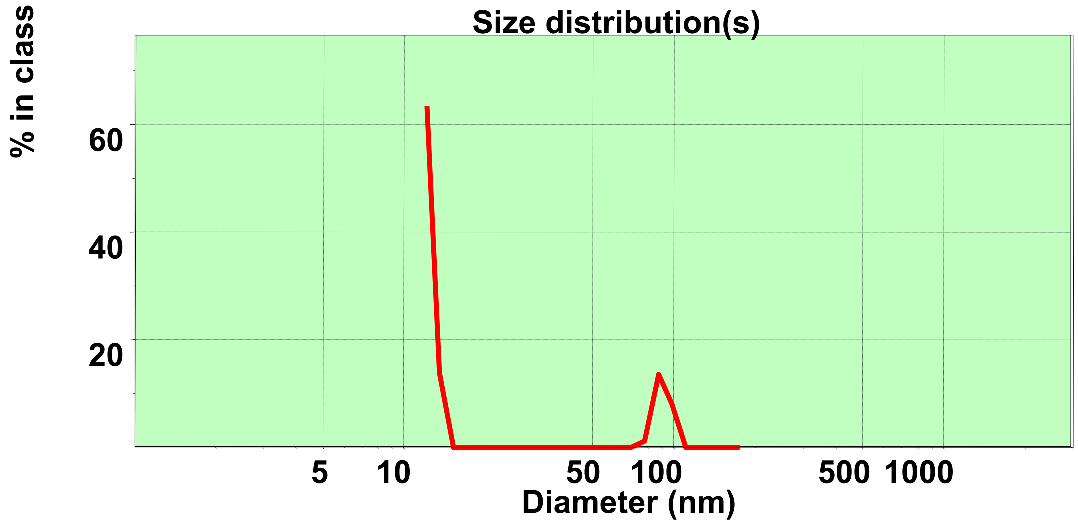
Nano-Analysis, Characterization



AAg00103

Zetasizer 3000
Data taken on 22/07/04 at 17:07:06
Temperature 25.0   Viscosity 0.890 cP   Angle 90.0 deg
RI medium 1.33   RI particle 1.92 + Abs. 1.00

Cumulant Z Ave   46.3 nm   Polydispersity 0.599



Size (nm)	% Intensity	Size (nm)	% Intensity	Size (nm)	% Intensity
12.1	63.3	30.8	0.0	78.2	1.2
13.6	13.8	34.6	0.0	87.8	13.6
15.3	0.0	38.9	0.0	98.7	8.1
17.2	0.0	43.7	0.0	110.9	0.0
19.3	0.0	49.0	0.0	124.6	0.0
21.7	0.0	55.1	0.0	140.0	0.0
24.4	0.0	61.9	0.0	157.3	0.0
27.4	0.0	69.6	0.0	176.8	0.0

Peak :   Mean 12.4 width 0.5

Figure 21.1
Particle Size Distribution
Results of **PCS-Analysis**
of Nano-Ag, 1 Vol.-%
Suspension in H_2O
(CAg00103).

Temperature-resolved X-Ray Diffraction

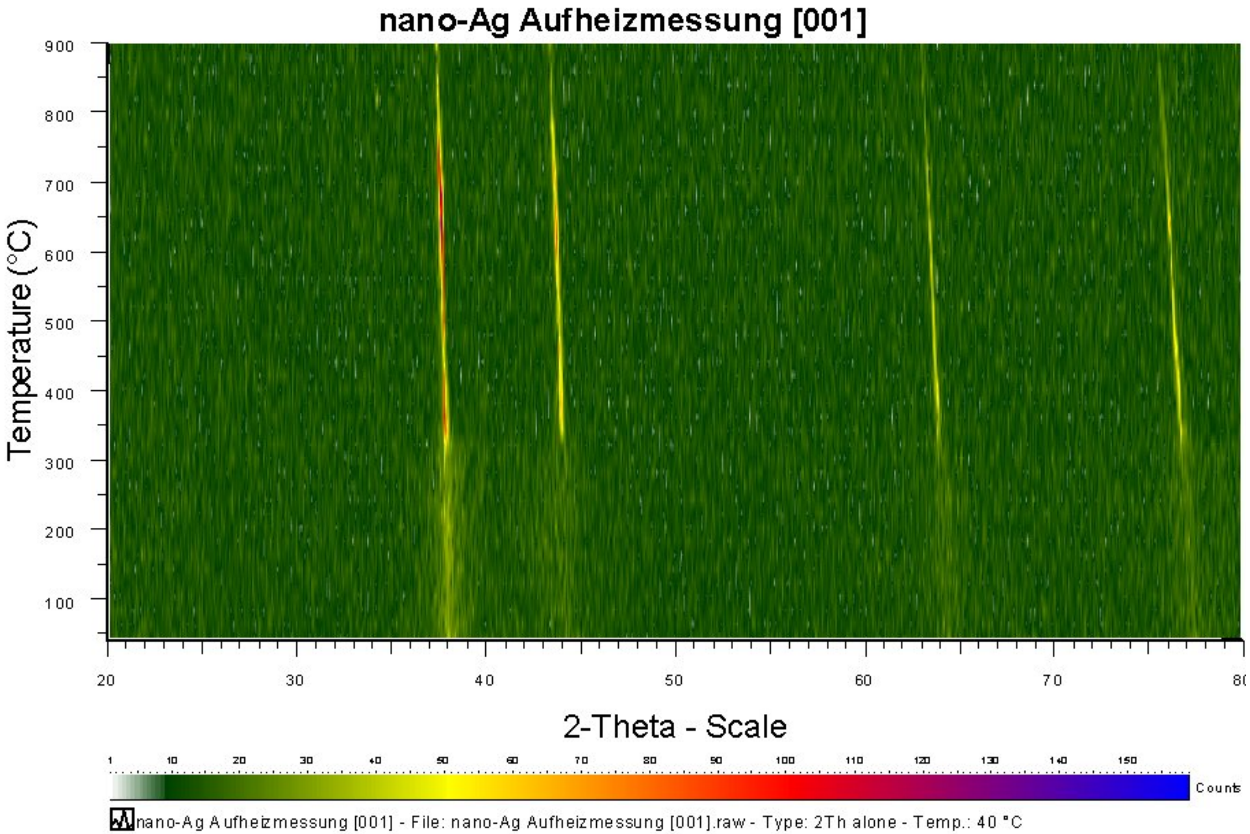


Figure 22.1 Temperature-resolved **X-Ray Diffraction** Diagrams of a Nano-Ag sample in air in a temperature-range from 40 – 900 ^{0}C, χ = 10 K/min (Fp Ag: 961.9 ^{0}C) [Nano-Ag Aufheizmessung_02.jpg]. Peaks represent Ag-reflexes in different phases.

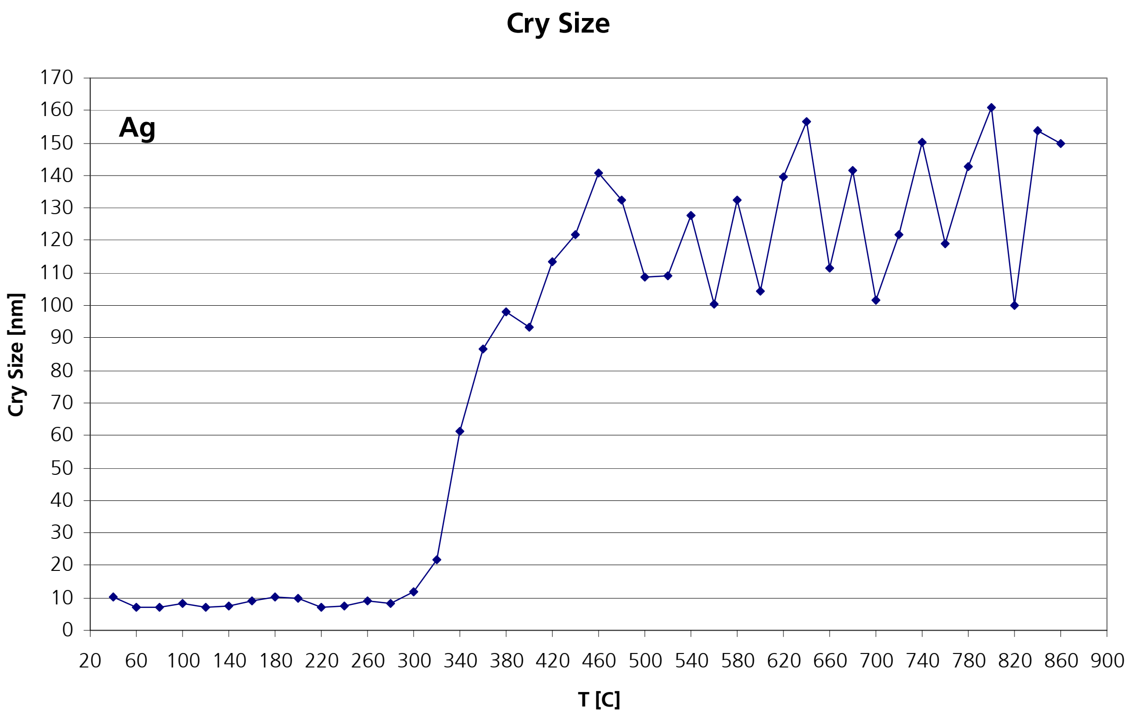
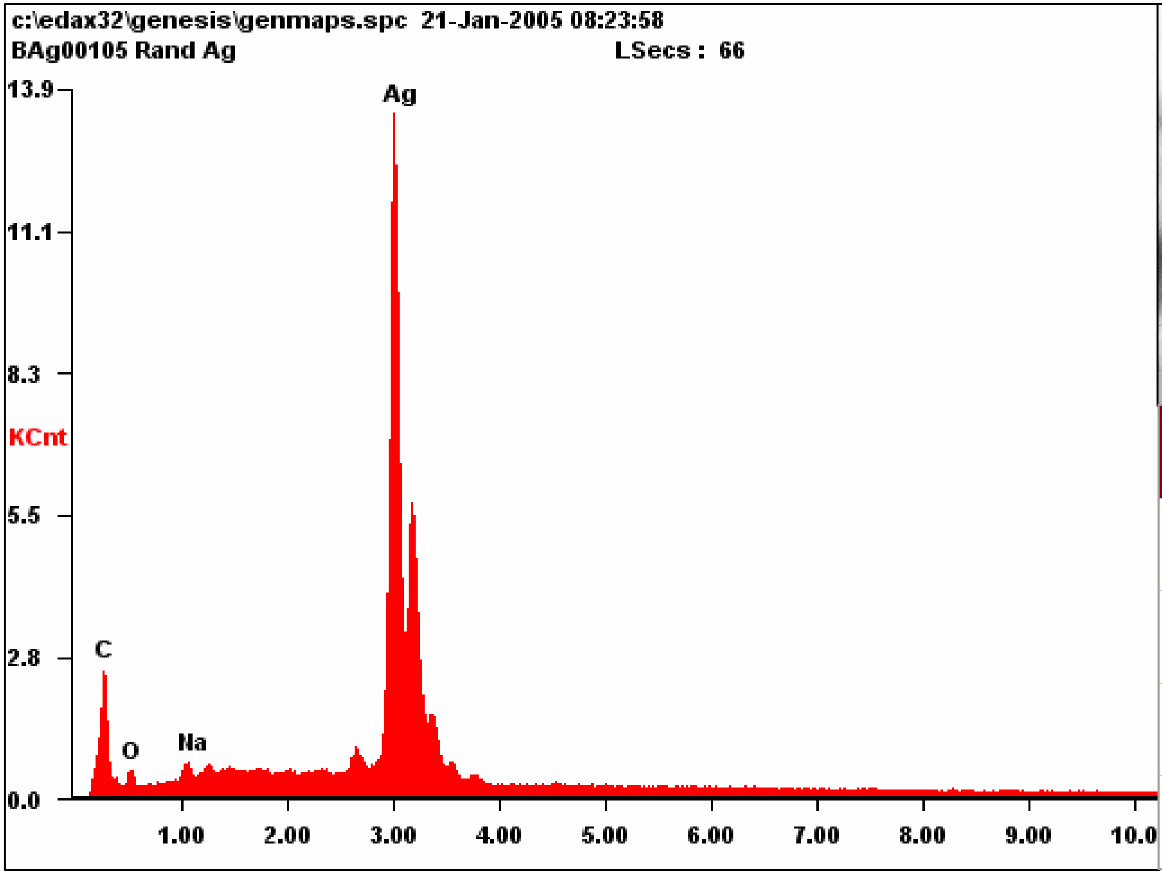
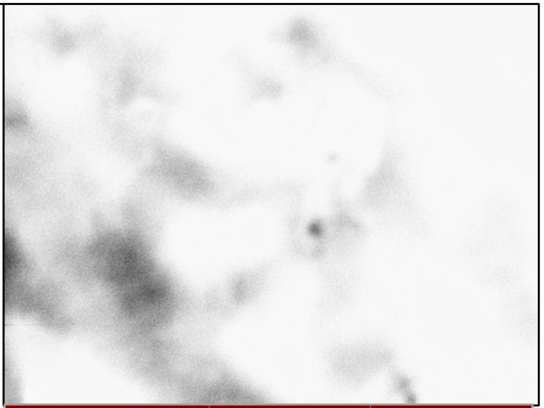


Figure 23.1 By means of **temperature-resolved** X-Ray Diffraction evaluated primary crystallite dimension of Nano-Ag as a function of temperature. Grain growth starting from 300 ^{0}C.

Energy Dispersive X-Ray Spectroscopy (EDX)





Element	*Wt%*	*At%*
C K	41.08	78.57
O K	06.71	09.64
NaK	00.86	00.85
AgL	51.35	10.94

Figure 24.1 Quantitative Ag-Analysis in Sample BAg00105 with X-Ray Fluorescence

<u>Figure 25.1</u> Mini-Twin-screw-extruder for sample-production in lager scale (500 g / h)

Nano-Strukturing / Coating of Polymer-Granulate to Achieve System Functionality

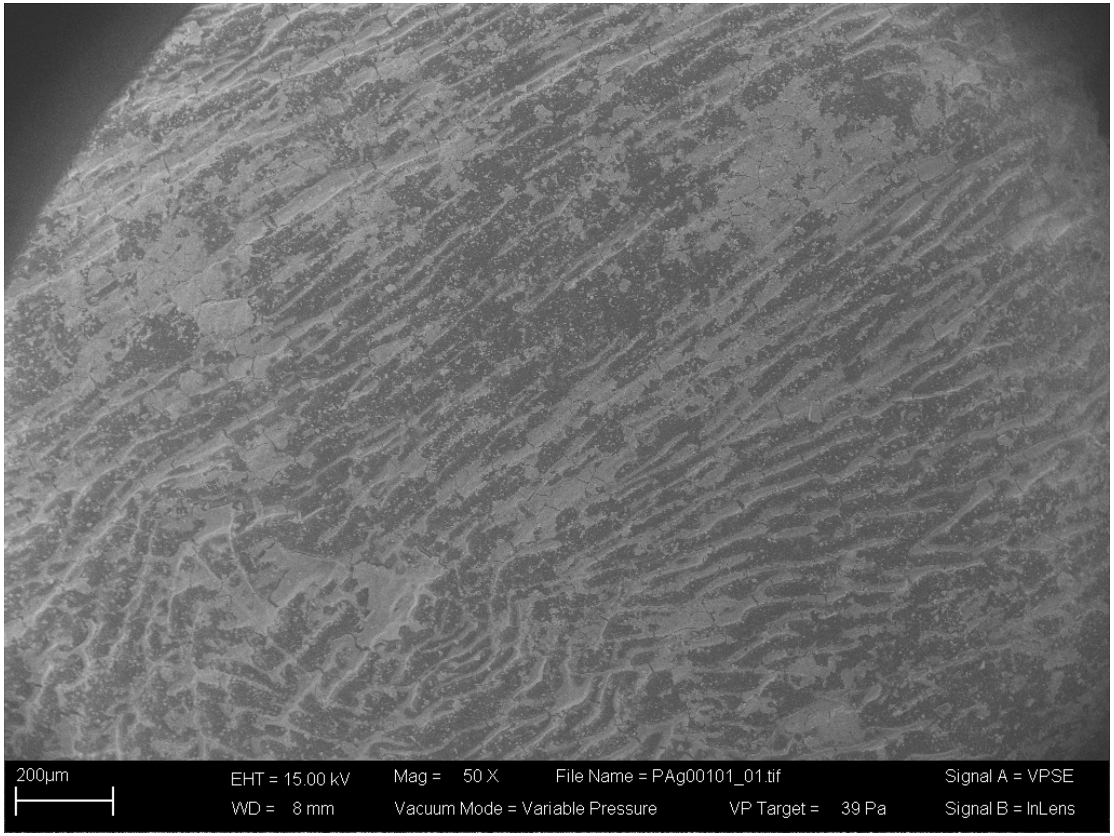
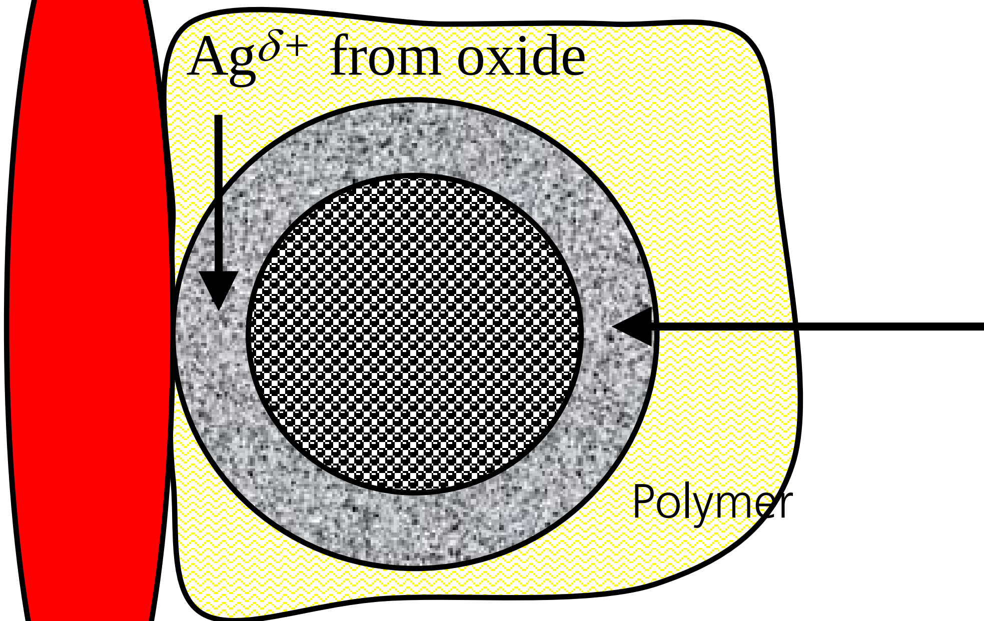


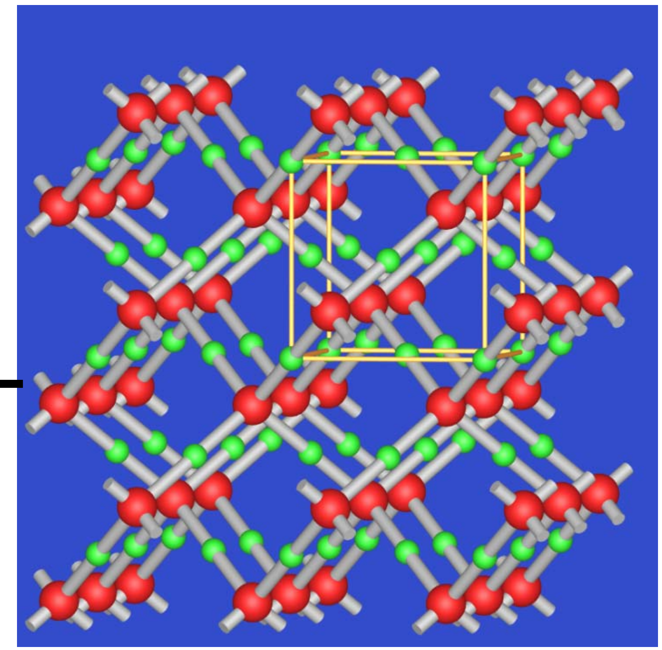
Figure 26.1 SEM-Data of Nano-Ag-Particles on PP-Granulate (PAg00101).

3 Results / Discussion, Selected Applications, Nano-Silver-Technology

Source: Uni Freiburg

Approx. 25-30 mass-% as porous oxide-phases: **Ag^{+I}_2O**, $Ag^{+II}O$, $Ag^{+2.66}_3O_4$, $Ag^{+III}_2O_3$





Micro organism, reduction factor 100 - 1000.

Crystal structure Ag_2O:
Cubic closest packed **Ag^+**,
O^{2-} occupy ¼ of tetrahedral gaps.

Ag, $Ag^{\delta+}$, Ag^{+} as Biocide Substances – Chemical Mechanisms

Electrophile -> Electrophile Mechanism

Oxidant, Redox-Mechanism

Complex-, Chelate Forming Mechanism

Ionic Exchange Reactions

Superimposed Processes via Split Ionic Charges

Biocides, Sub-group Micro-Biocides – Working Mechanism

Source/Autorization: Wortundbildverlag, 08.02.06

Golgi-Apparatus
Last modification of proteins produced by Endoplasmic Reticulum

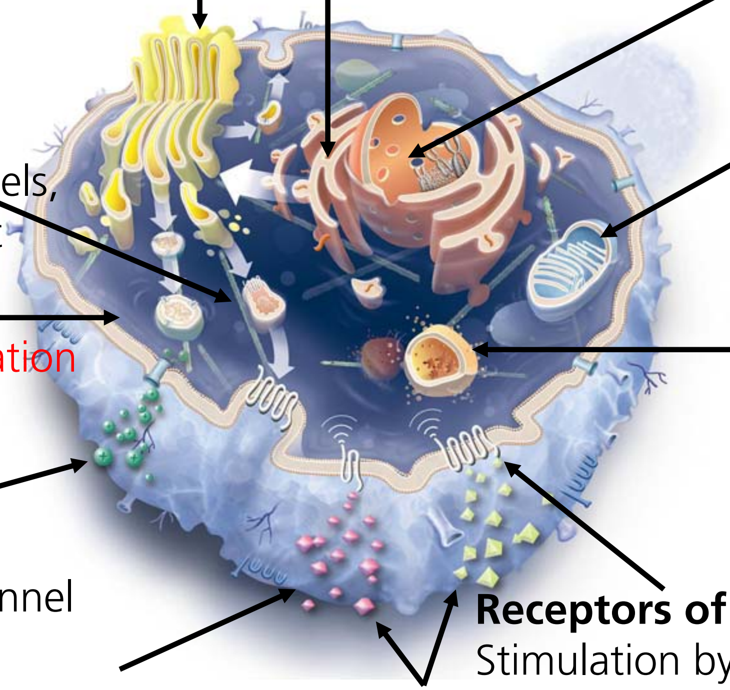
Endoplasmic Reticulum
Protein production guided by nucleus in order to control metabolism

Nucleus
Location of genetic informations and control center

Microtubuli
Internal transport channels, mechanic reinforcement

Mitochondrium
Energy delivery by glucose degradation

Peroxisom
Waste disposal, degradation of internal pollutants (RCOOH, ROH)



Lysosom
Waste disposal, acid degradation of foreign substances

Ion Channel
Two-directional Na^+ channel
50 – 99 pm

Receptors of Cell Membrane
Stimulation by external hormons, signal generation and transmission in to the cell, protein production, action

External Hormons

Theoretical Aspects – Safety Concept

Intra- and intermolecular Bounding

Physisorption

Chemisorption

Mech. Anchoring

Van-der-Waals

Weak Elektrostatic Interakt.

Partial-Charge, Dipole-I.,
(H-)Bridges: 20 x VdW

Particle-Size Dependency

Kovalent

Metallic

Potential-Theory

Ionic

Transition-State-Theory

Molecule-Orbital-Theory

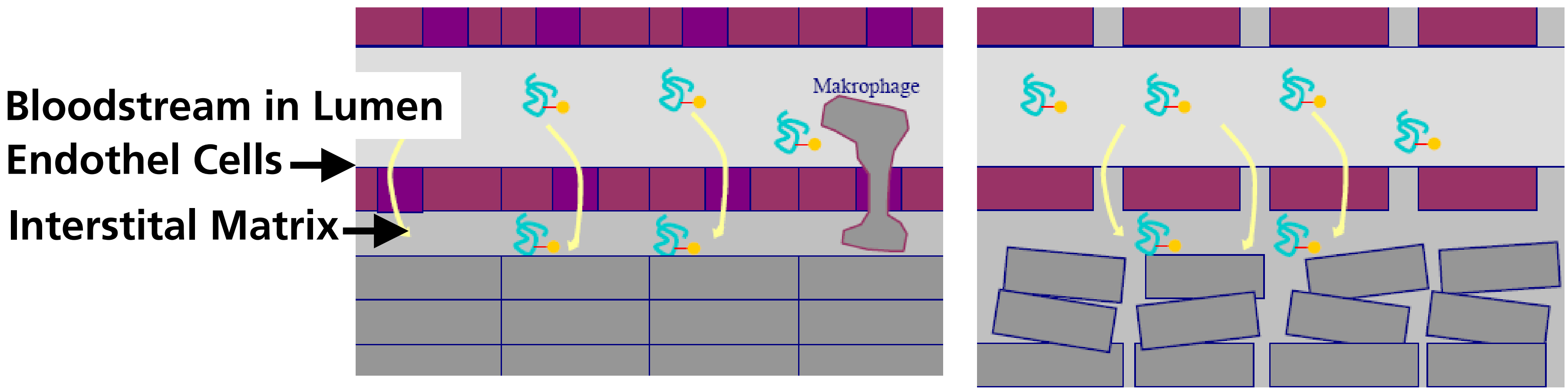
Surface-Thermodynamics

Crystal-Field-Theory

Transportation, Targeting and Release of Nano-Particles

Source: Uni Frankfurt

Intravascular Transportation
Passive Targeting -> Accumulation in Reticular Connective Tissue (Liver, Spleen)



Normal Tissue

Cancer Tissue

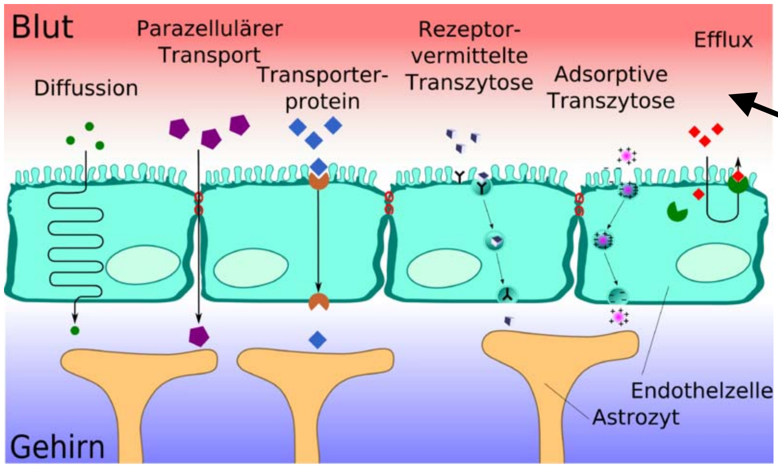
Extavascular, Interstitial Transportation
Active Targeting with Antibodies

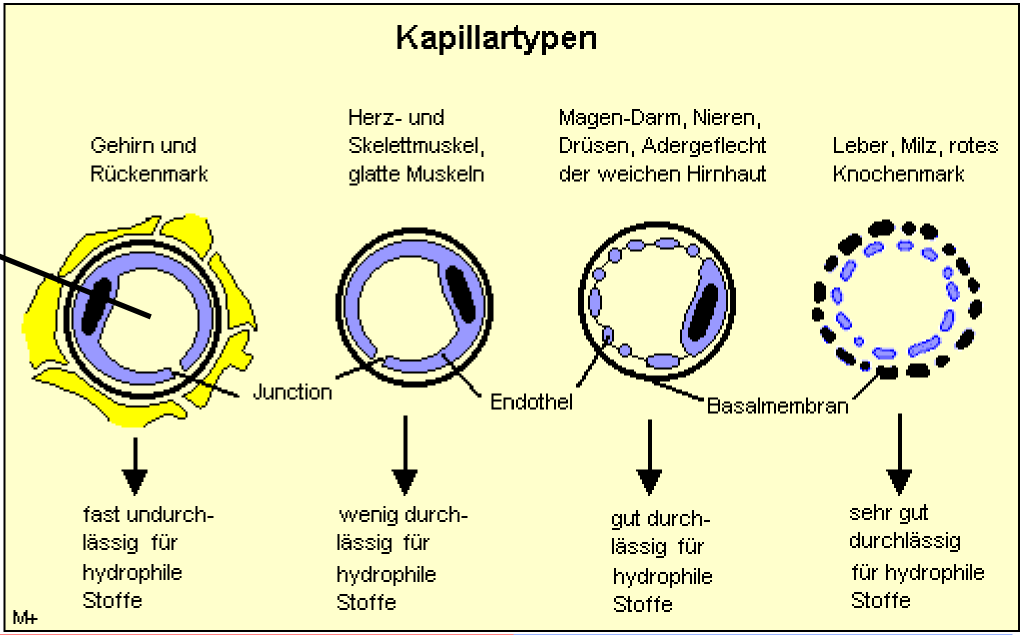
Capillary Penetration, Blood-Brain-Barrier (BBB)

Source: wikipedia, medizinfo

Penetration is not only a function of size but of surface charge (hydrophobia, hydrophilicity)

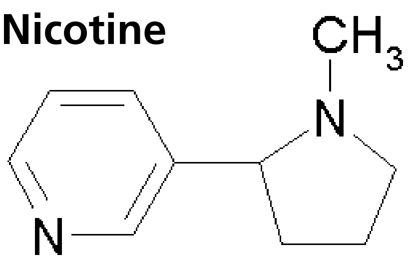
Blood-Brain-Barrier





Permeability of hydrophobic substances	Permeability of Hydrophilic substances

Nicotine



Test-Methods – Fungicide Activity

Direct-Feed-Test / Direct-Cell-Contakt-Test / Pharm. Eu. 1997

Sterile delivery.
Sample preparation: Inkubation with 10-100 colony forming units (malt-extract) in a closed test glass, breeding: 3-5 days.
Visual evaluation, comparison with reference.
Additional performance as **Row-Dilution-Test** to evaluate acting concentration.

(Konservation-)Stress-Test, similar to Direct-Feed-Test

No sterile delivery.
Sample preparation as described below.
Visual evaluation.
Additional performance as **Row-Dilution-Test** to evaluate acting concentration.

Quantitative Suspension-Test

If necessary, autoclave treatment of sample to prohibit external influence; disadvantage p,T.
0.1 g fungal solution in Mueller-Hinton-Bouillon + 9.9 g sample are to be united.
Germs will be re-isolated.
Rearing / breeding of re-isolated germs.
-Better rearing -> increase of germ number
-Normal rearing -> constant gern number
-No rearing -> killing of germs
Additional performance as **Row-Dilution-Test** to evaluate acting concentration.

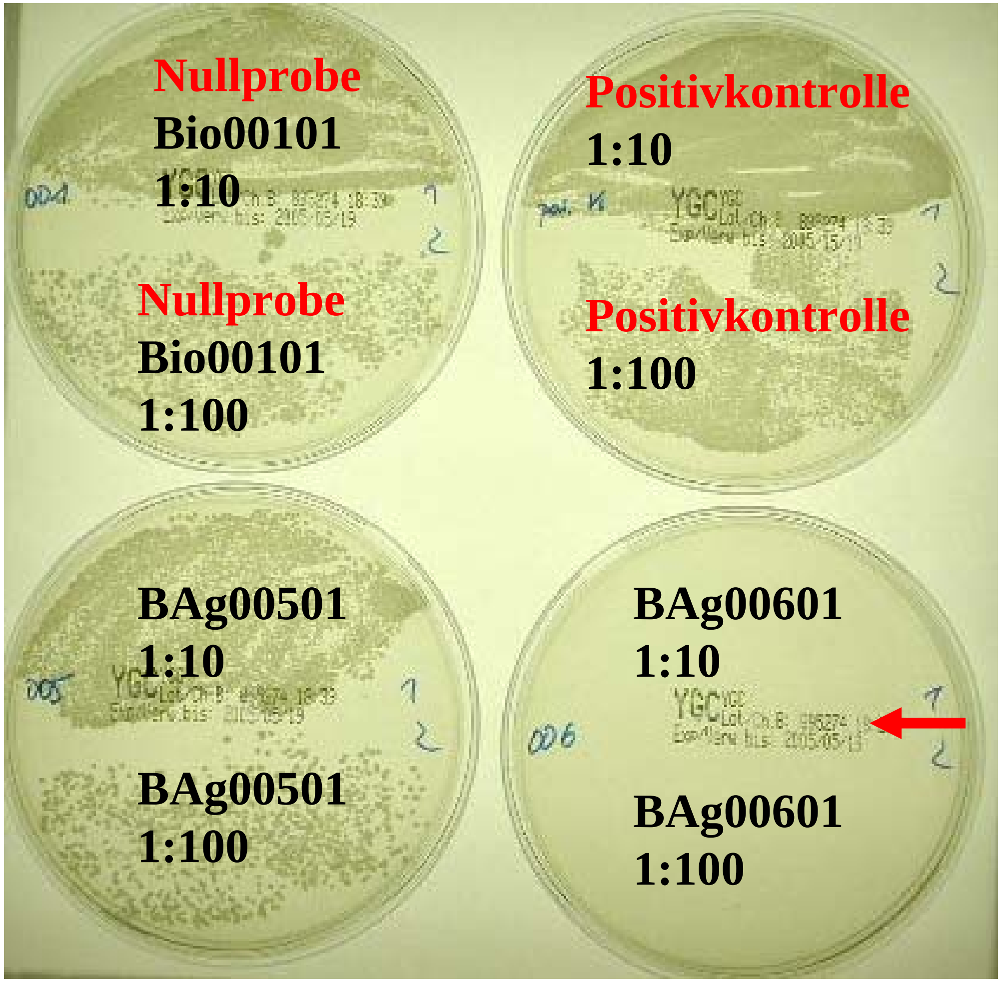
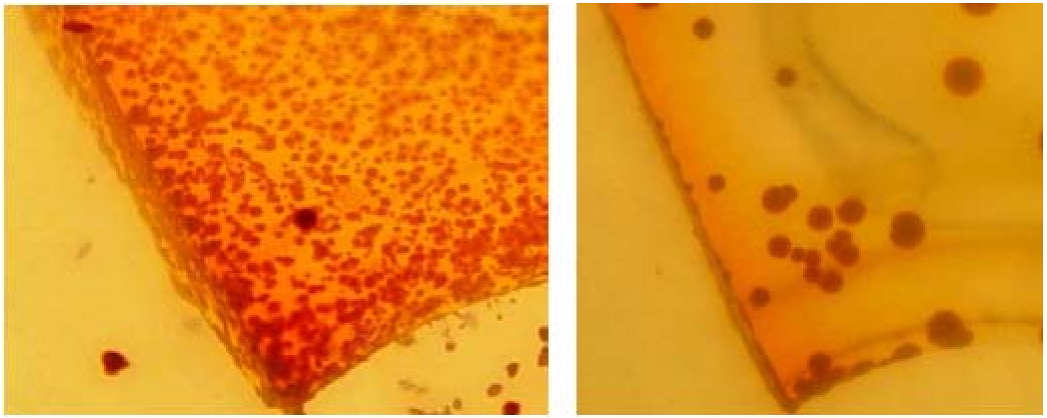


Figure 34.1 Result of breeded diluted crop-out of the samples (mP/mNL=const.)



<u>Figure 35.1</u> Example of a Nano-Ag-coated Silicon-Polymer (on the right) compared to a untreated sample (on the left) with colonies of **Staphylococcus Aureus**.

A reduction rate of factor 100 was detected based on the applied Nano-Ag-content (Test-setup: Spray-test).

Depending on Nano-Ag-content reduction rates of 99,999 % (5 log-levels) are possible.

Mathematical Modeling of Microbial Growth

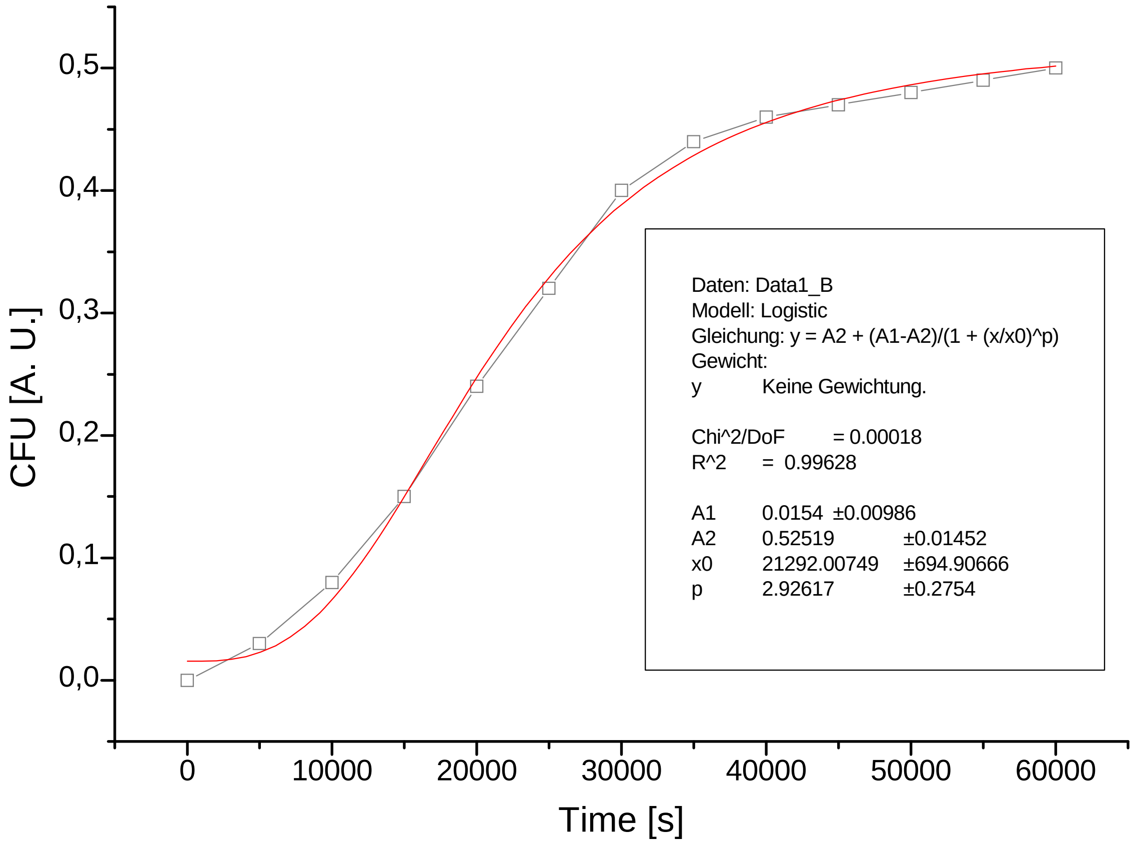


Figure 36.1 Example for Mathematical Modeling of Microbial Growth (Exp. Data) with an Exponential-Function representing „Logistic Growth".

Nano-Structuring / Coating of Fibres

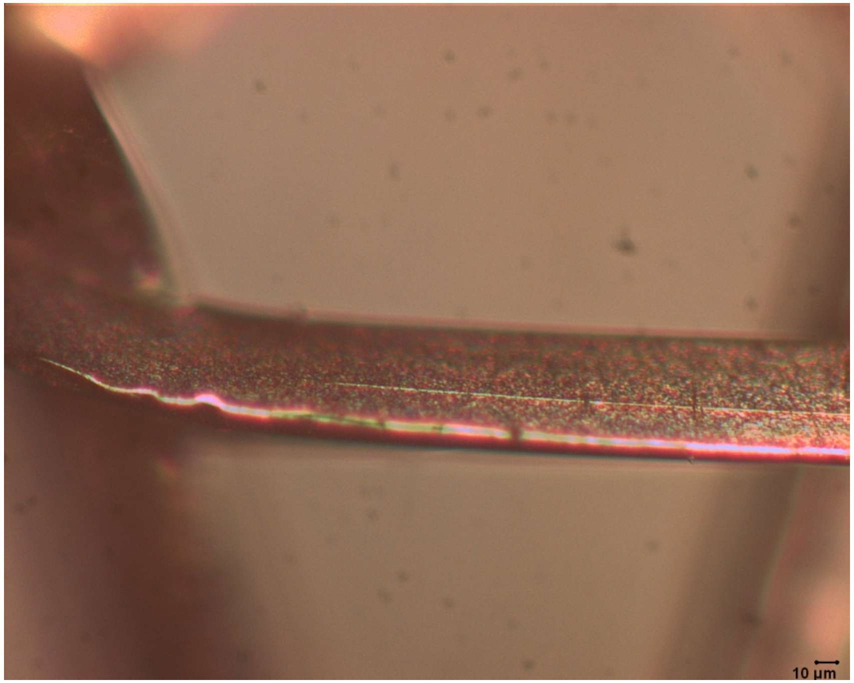


Figure 37.1
Microscopic image (incident-microscopy) of a metal-coated PA-fibre (File: TüllA.Jpg, 33%)

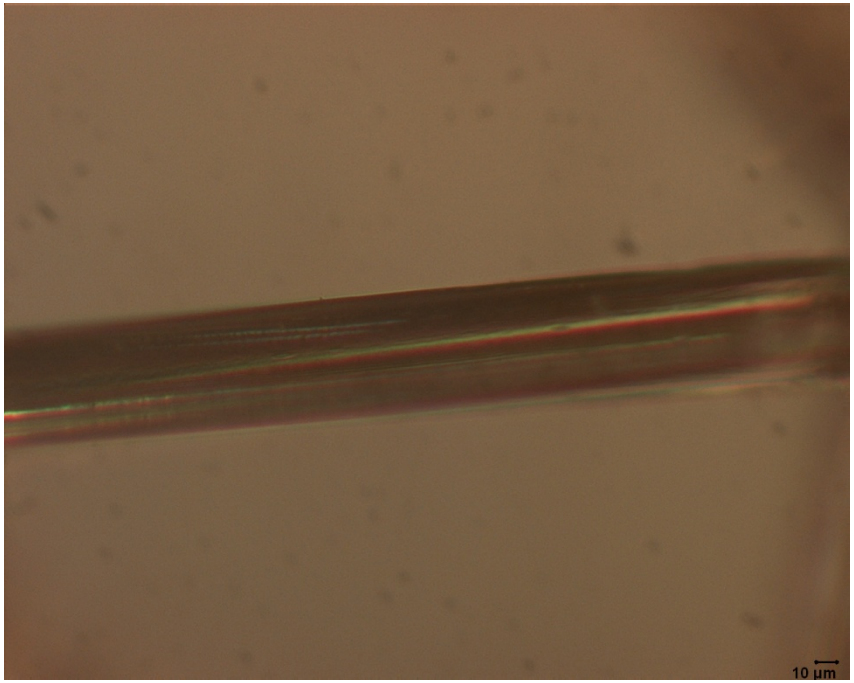


Figure 37.2
Microscopic image (incident-microscopy) of a **nano**-metal-coated PA-fibre (File: TüllRohA.Jpg, 33%)

Wound-Pad Application

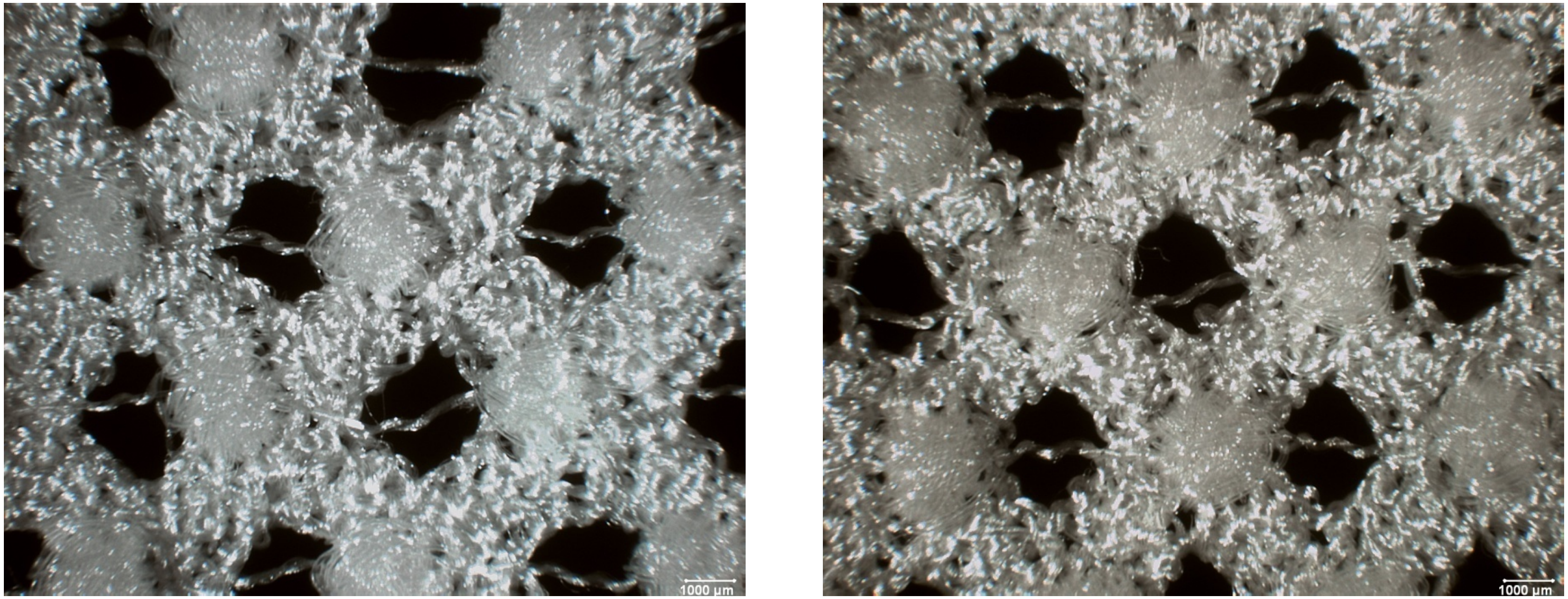


Figure 38.1 Macroscopic Image of a PE-Biopad NURPES-001 (Wound-Pad). Left sample uncoated, right sample Nano-Ag-coated, x < 100 ppm (GAg00001, GAg00101).

Goal: Medical Device Class 2b.

Fraunhofer ICT

Product Example: Antimicrobial Nano-Silver-Dispersion-Paint

Product Safety by Life Cycle Analysis

Production → **Transport** → **Application** → **Utilisation, Disposal**

Production
- Waste water
- Factory wasts

Transport
- Emission in case of accidents

Application
- Abrasion
- Aging
- Wash out

Utilisation, Disposal
- Recycling
- Therm. untilization
- Dumping

Product Example: Antimicrobial Nano-Silver-Dispersion-Paint

Figure 40.1 Al Wasl Hospital, Dubai, U.A.E.

Product Example: Antimicrobial Nano-Silver-Dispersion-Paint

Figure 41.1 Al Wasl Hospital (Building 2), Dubai, U.A.E.

Product Example: Antimicrobial Nano-Silver-Dispersion-Paint

Figure 42.1 Hospital in Moscow which was equipped with the developed Nano-Silver-Dispersion-Color as a protection against Staphylococcus Aureus (MRSA).

4 Abstract / Summary

Nanotechnology Special Branch, Product Area Energetic Systems at ICT has it´s key competences according to following items:

1. **Production of nearly all kinds of Nano-Particles (metals, transition-elements, oxides) on laboratory scale as well as in bulk quantities**
2. **Adaptation process of particle size distribution developed**
3. **Production of stabilized Nano-Suspensions -> Interface to application**
4. **Know-how of system integration especially in combination with polymer systems**
5. **Nano-Products already estabilshed with several years of field experience**
6. **Nano-Safety-Concept developed and analytically proofed**
7. **Full compatibility to all legislative injunctions / homologations**
8. **Until now the developments were recognized with 5 national resp. international awards**

Fraunhofer ICT

- **Further Nano-Products with additional antimicrobial effect, will be put to market soon**
- **Numerous promising systems are still under development**
- **Nanomedicine is identified as an outstanding field of application**
- **Recognizing the overall benefit, investments in this technology makes sense. Fraunhofer-ICT is looking for cooperations in order to perform in-vitro and in-vivo experiments**

Dziekuje za uwage!

Danke für Ihre Aufmerksamkeit!

Thanks for your attention!