Nanobiomedizin:

Liegt die große Zukunft in den kleinen Dingen ?

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(University Rostock, Germany)

Phabos, 11.06.2017

"The trouble is that all the important discoveries have already been made"

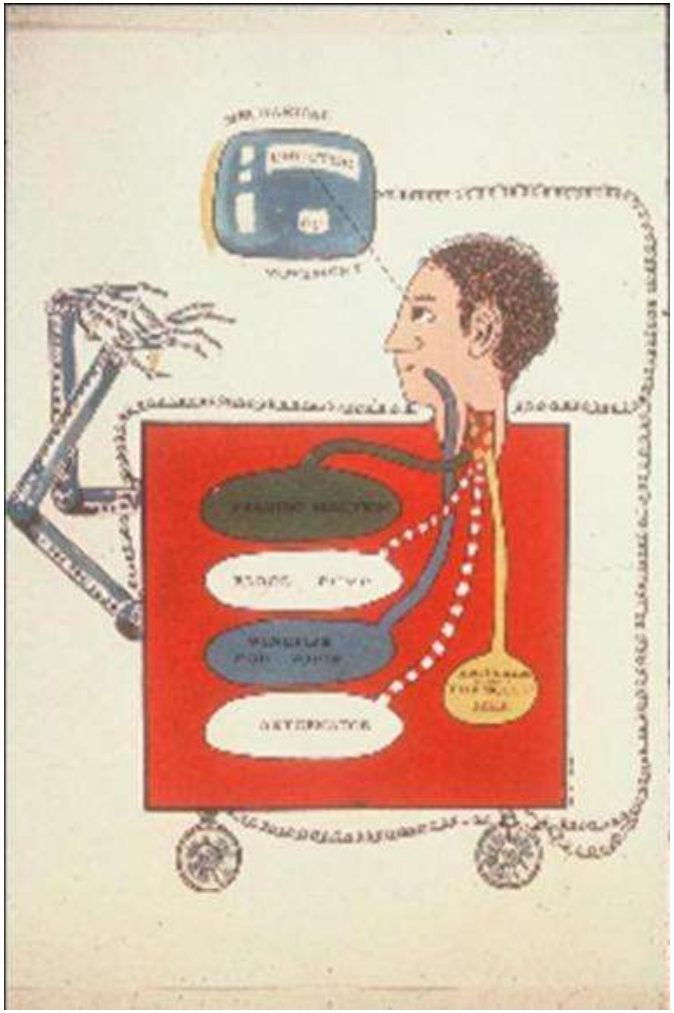
COLLIER'S

MORT WALKER

“Angebote an die Medizin”

von

Biotechnologen, Molekularbiologen, Physikern, Chemikern, Ingenieuren, Informatikern …. Bionikern



Früher : “Angebote von Handwerkern”

Wooden Toe Prosthesis of a 3000 - year old mummy

“The Next Big Thing is Really Small”

Bestseller von J. Uldrich, R. Small, USA

Nano: *nános* „Zwerg“ Faktor: 10^{-9}

Länge: Strukturen und Teilchen im Größenbereich vom Einzelatom bis zu einer Strukturgröße von 100 nm

Wichtigste Besonderheiten:

1. Oberflächeneigenschaften spielen gegenüber den Volumeneigenschaften der Materialien eine immer größere Rolle

2. Quantenphysikalische Effekte müssen berücksichtigt werden müssen.

Einsatzfelder:

Nano-Technologie ?

Nano-Biotechnologie ?

Nano-Biomedizin ?

Nano-Medizin ? => Liposomen als Wirkstoffcontainer

Nano-Kosmetik ? - “ -

Nano-Nahrung ? => “Nano-Milch” ! ????

sowie Umwelttechnik, Energie, Beton, Auto, Maschinen, …Militärtechnik

(Nano-Colors, H_2-Storage, Material propoerties) … (…?….)

Nano ➔ Quantenphysik =====➔ “Quantenmedizin” ? => 25 Mrd. € für Zuwendung

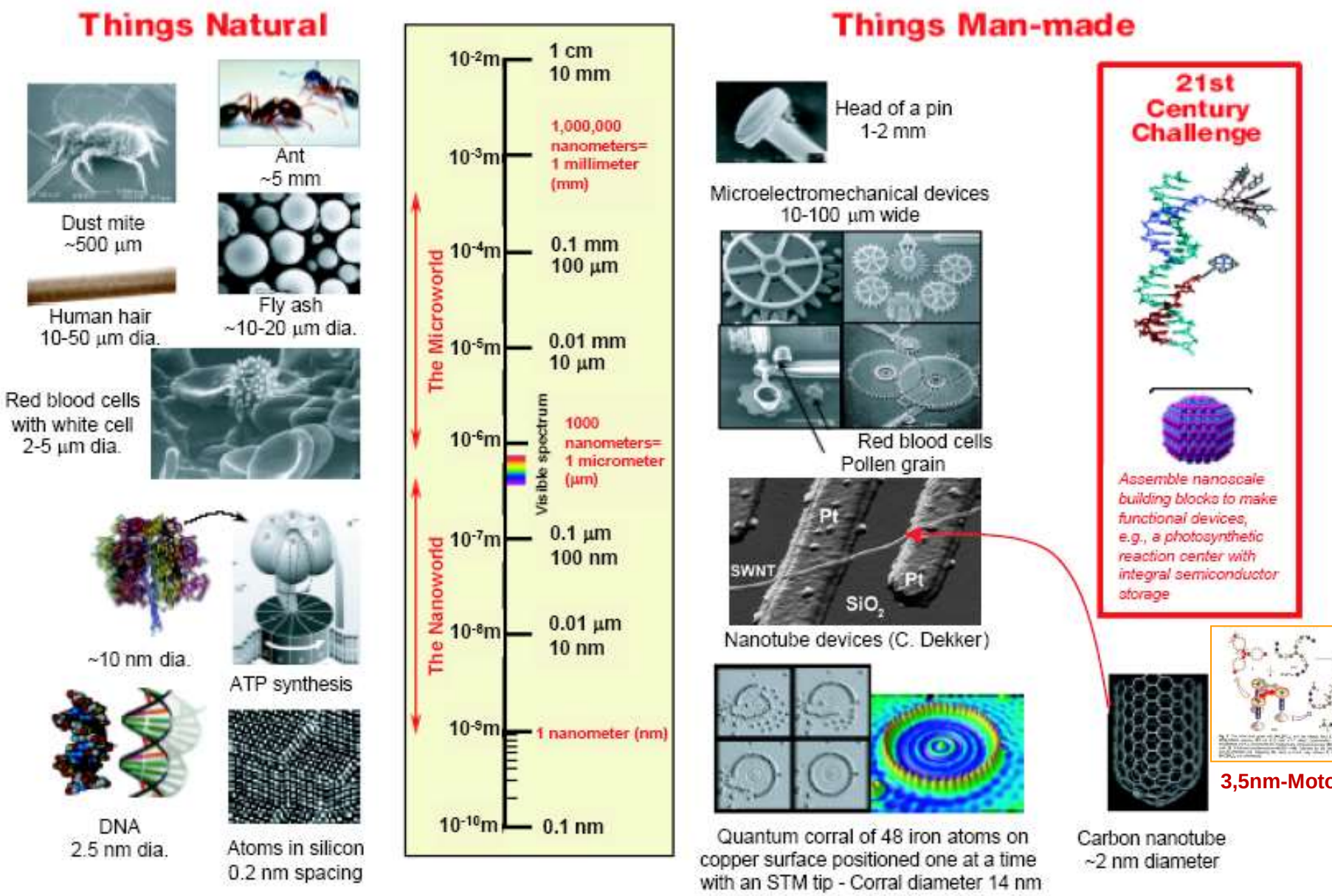


FIGURE 1.1 The size of nanoscale objects and phenomena compared with the size of small everyday objects. Courtesy of Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy.

Adapted from: NRC Report: Small Wonders, Endless Frontiers: Review of the National Nanotechnology Initiative (National Research Council, July 2002)

WS:	Nanotechnology fields involved	=>	Topics in Rostock, NYC, Tokyo, Krem
1969	Tunnelling through single molecules, monolayers	=>	Field emission microscopy (Giaever)
1974	Characterization of cells, particles, liposomes, BLM	=>	Automated single cell electrophoresis
1984	- " -	=>	Confocal laser scanning microscopy
1993	Stretch-activated ion channels	=>	Single channel manipulation
1995	Magnetic particles for isotope placement	=>	*Int. Conferences with Univ. Vanouver*
1997	Adsorbents for specific immunoadsorption	=>	Lab-system for pre-clinical investigatio
2002	Cell monolayer impedance (drug testing)	=>	Modified ECIS (Giaever)

Univ. Rostock 65-92 / 95-96, Cornell Univ. NY 93-95, Jikei Univ. Tokyo 96, JMS Tokyo 96-02, FH Krems 02-12

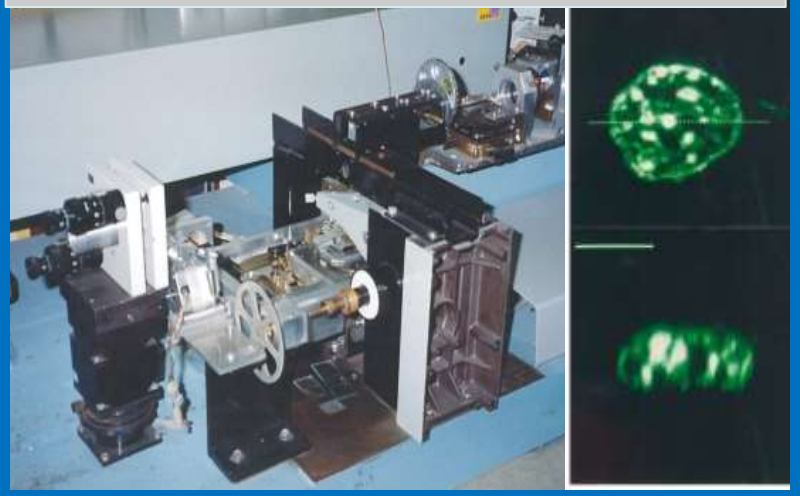
Electron Microscopy

1969-74

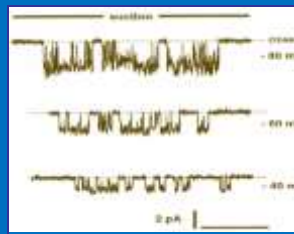
Single Particle Electrophoresis

1975-92

CONFOCAL LASER SCANNING MICROSCOPE



1983-1992



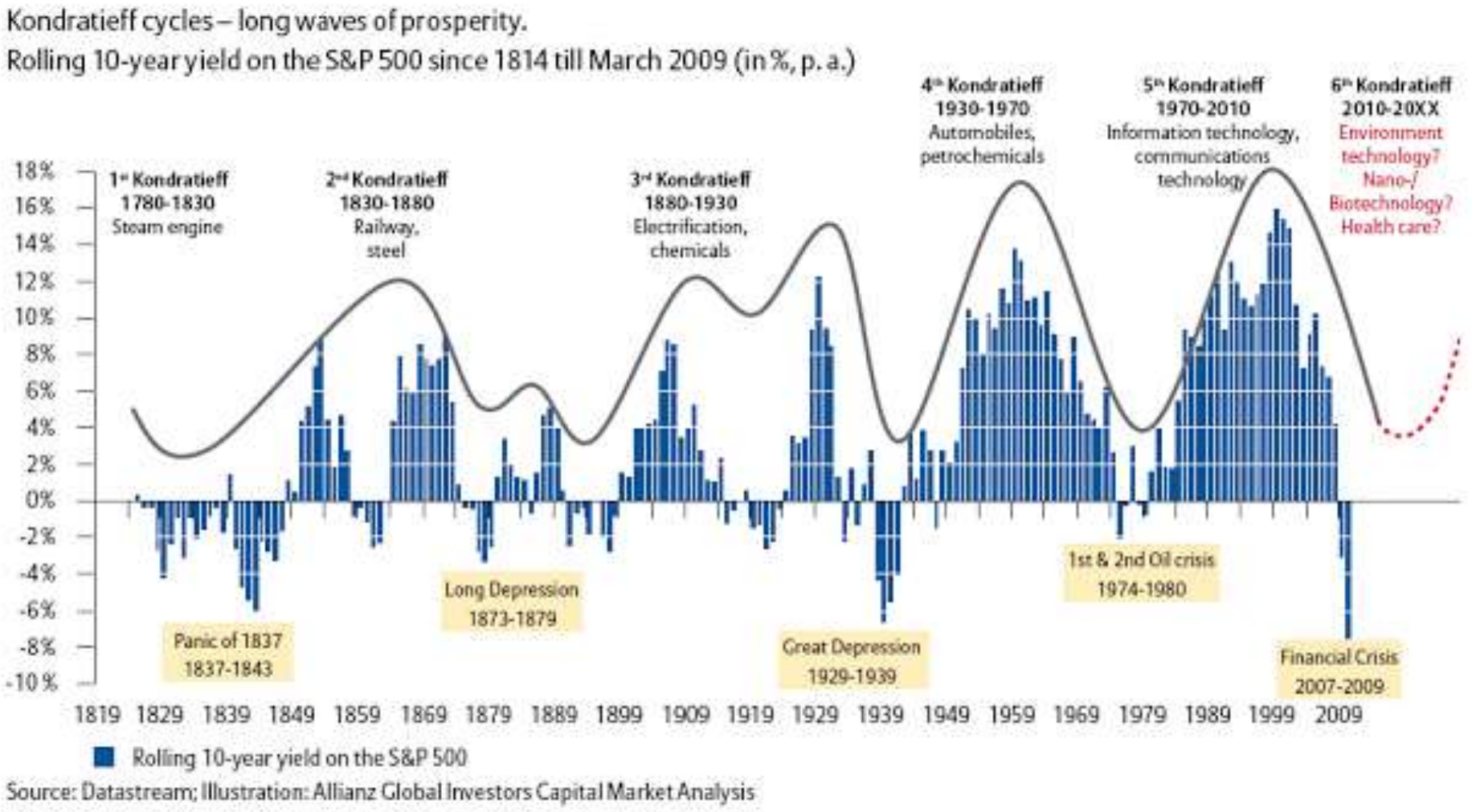
1993-95

1995-1997 -2002

Nikolai Kondratjew:
entwickelte 1920 die Theorie zur zyklischen Wirtschaftsentwicklung

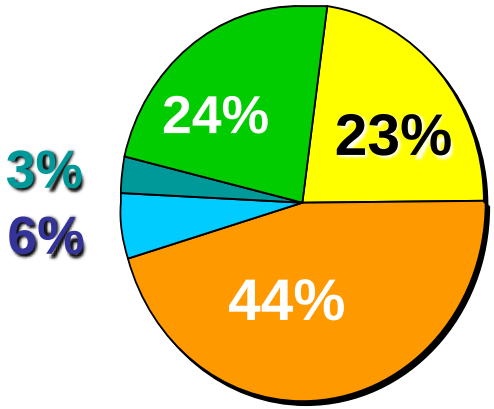
Ausgangspunkt für die *Langen Wellen* sind Paradigmenwechsel und die damit verbundenen innovationsinduzierten Investitionen.

Kondratjew war nach Oktoberrevolution für mehr Marktwirtschaft, hingerichtet 1938



Nanotechnology and Global Markets

2010: US-$ 220 Mrd



- Ultrathin layers
- Nanoparticles
- Ultra-surface modifications
- Lateral Nanostructures
- Analysis of Nanostructures

Areas

Elektronics, Optics, Sensors,…

Applications:
Chemical , Environmental, Energy, Military, Space Research

and

Med. Device-Technologies

Germany: >100.000 jobs

Re: Technical Insight Analysis Report Frost & Sullivan

Nanotechnology Global Market

e.g. Nanoparticles in life sciences:	2014	**29,6**	Mrd. US dollars
	2016	**45,1**	- “ -
forecast	2019	**78,8**	- “ -

76 %	**Drug delivery**
11 %	**in vitro diagnostics**
6 %	**lab on chip**

400 companies identified as active in nanomedicine worldwide

(50% in USA, 35% in EU)

Others Fields (2017):	Radiotherapy	7 Mrd.	US Dollars
	Dialysis	17 Mrd.	-”-
	Medical Laser	6 Mrd.	-”-
	Microscopy	5 Mrd.	-”-

bccresearch.com (2016)

Nanotechnology Publication

Range	Country	Articles	Share (%)
1	China	38146	32.27
2	USA	20437	17.29
3	India	8678	7.34
4	South Korea	7572	6.41
5	Germany	7192	6.08
6	Japan	6745	5.71
7	Iran	5026	4.25
8	France	4836	4.09
9	UK	3978	3.36
10	Spain	3642	3.08

Nanotech papers as a percentage of all papers

1	Singapore	16 %
2	China	15 %
3	South Korea	13 %
…	EU, USA	5 %

BCCReport 2017

Ein 1. Gedankenexperiment zur “Nano-Diagnostik”:

Die Lokalisierung und Vermessung eines Nanoteilchens ist gleichbedeutend mit folgender Herausforderung:

A. Ein Beobachter vom Flugzeug will auf der Erde eine Zeitung mit 1000mal kleineren Buchstaben lesen

=> Tumorzelle

B. Ein Beobachter vom Mond will die Struktur unser Sitzanordnung, unsere Größenverteilung herausfinden und ob wir z.B. im Takt Walzer tanzen oder ungeordnet herumhüpfen

=> Rezeptorenverteilung

=> Zell- und Membranfunktionen

Ein 2. Gedankenexperiment zur “Nano-Therapie”:

Die Manipulation (z.B. Targeting) eines Nanoteilchens ist gleichbedeutend mit folgender Herausforderung:

A. Der Beobachter vom Flugzeug will nun in dieser Zeitung einige Buchstaben austauschen

=> Gentherapie

B. Der Beobachter vom Mond will nun erreichen, dass ausgewählte Personen zunächst etwas schneller tanzen und sich dann gezielt neu plazieren und ihre Geldbörse erleichtern

=> Gezielte Wirkstofffreisetzung

=> Wirkstofftestung an Ionenkanälen

Nanobiotechnology: Statements

Two of 21st century **most promising technologies**

Revolutionary opportunities in the fight against cancer, cardiac and regenerative disorders and infection

Boundaries between physics, chemistry, material science and biology **disappear**

=> **a new way of thinking**:

Feynman (1959):

The nature does not know that we divide this universe into parts: physics, chemistry, biology, geology, astronomy, psychology,…

(in our small minds!)

General Task of (Nano)Medicine

Earlier detection of disease **….** on the single cell and molecular level

=> to stop cancer before it starts

Faster diagnosis and lower costs **…** mass production of lab-on-chip tools

Personalized treatment strategies **….** based on proteomics and cell culture studies

=> specific delivery of new bioactive substances

Follow-up control

Combination of treatment strategies …. on individual cells and cell substructures

=> autonomous and non-automomous nanosystems

= > Interdisciplinary cooperation

LIFE expectancy ! ?

Roman Empire	**~**	**30 Years**
1900	**~**	**46 Years**
2000	**~**	**76 Years**
2015	**~**	**80 Years**

Dürer´s Mother

Age 66 !

A better offer !

Lucas Cranach, d.Ä., 1545

5244-1

Nanobiotechnology in Medical Applications:

What is „new" in the Nanoworld?

=> New Technologies for Designing & Characterization

=> New Physical Effects

Nanotechnology:

=> Basis of Applications in Biomedicine

Nanomedicine:

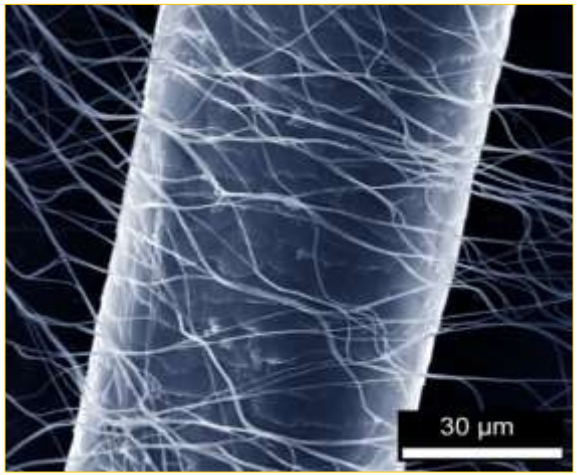
=> Clinical Applications and open Questions

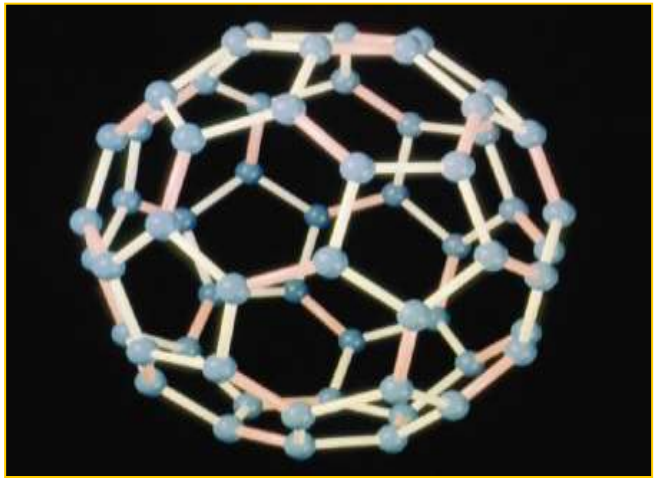
Quo vadis ?

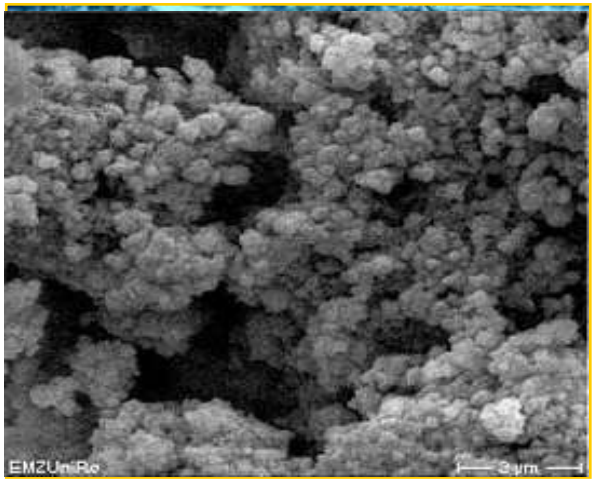
=> Economy, Risk and Public Discussions

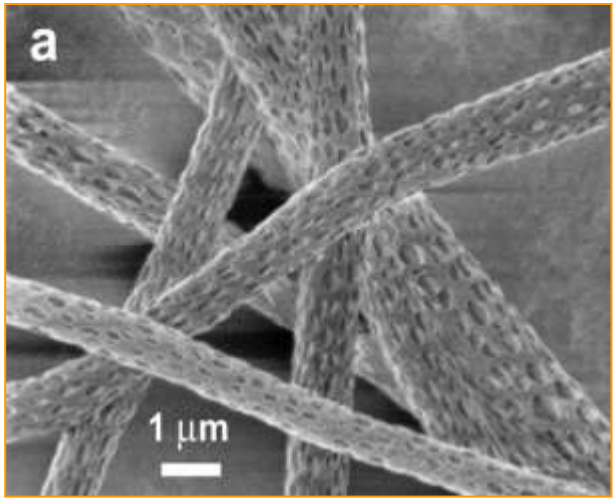
Variations of Surface Topology:

chemical composition, nano- and micro-topology, electrical charge distribution

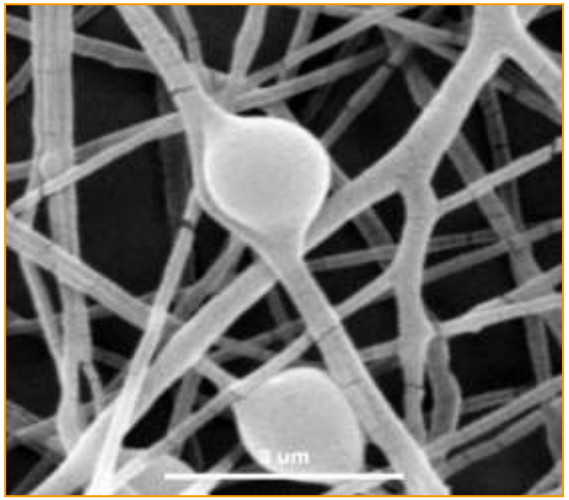




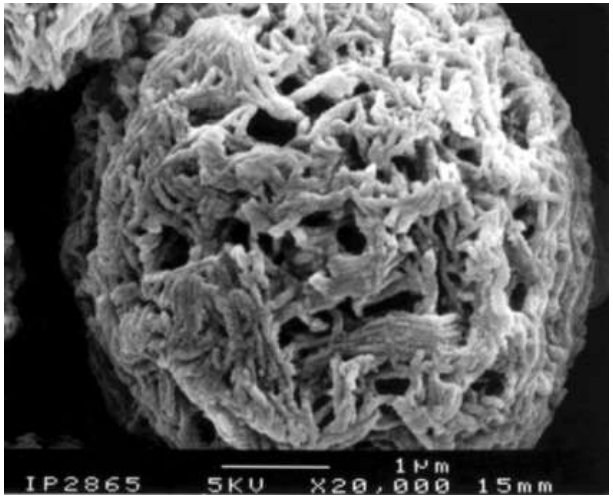




Phase separation during Electro-spinning



Inclusion of Latex Particles

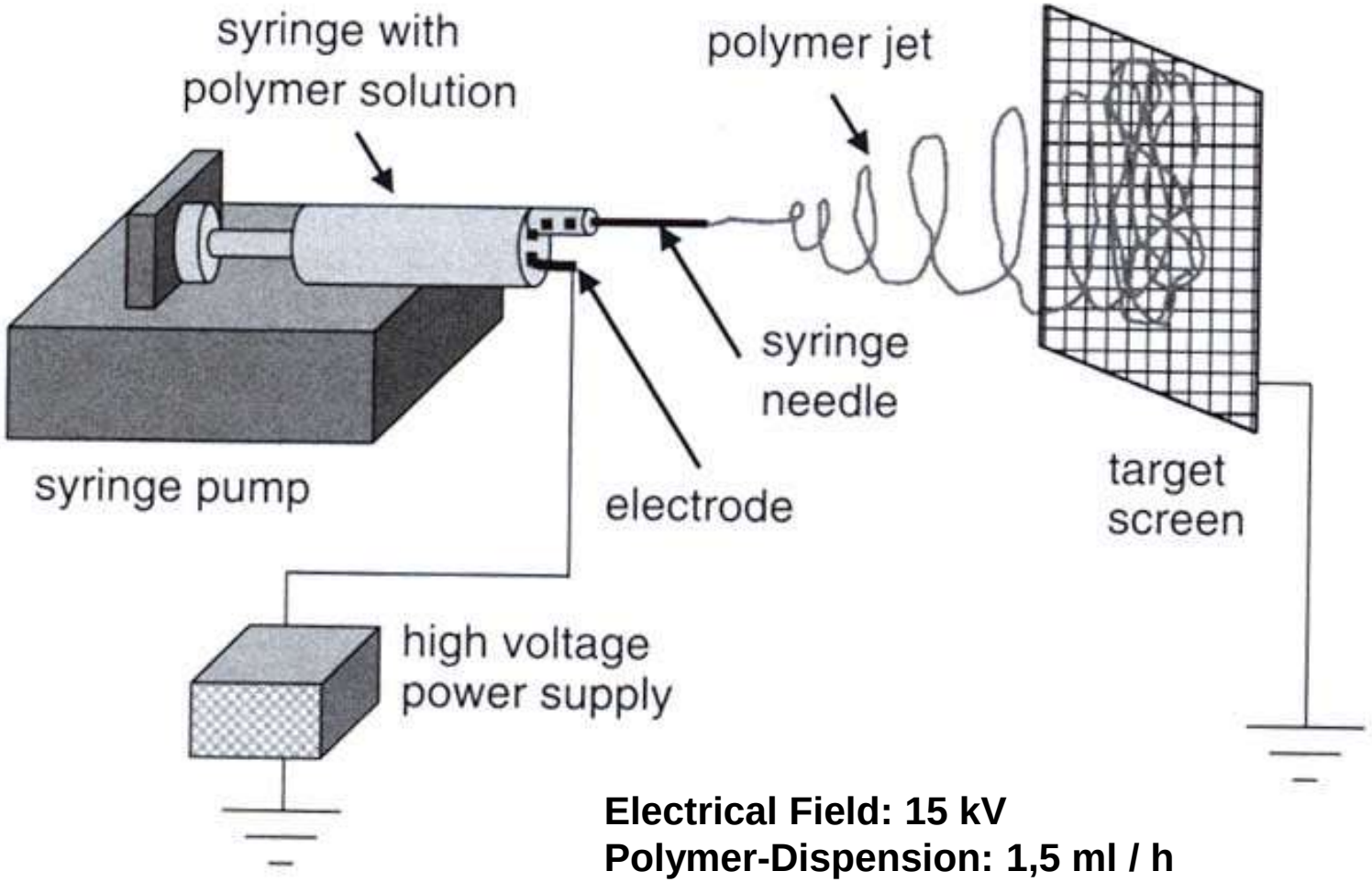


Adsorber Particles

Re: Bognitzky A et al., Polym Eng Sci, 41: 982 (2001)

How to manufacture Nanofibers?

Electrospinning – Technology

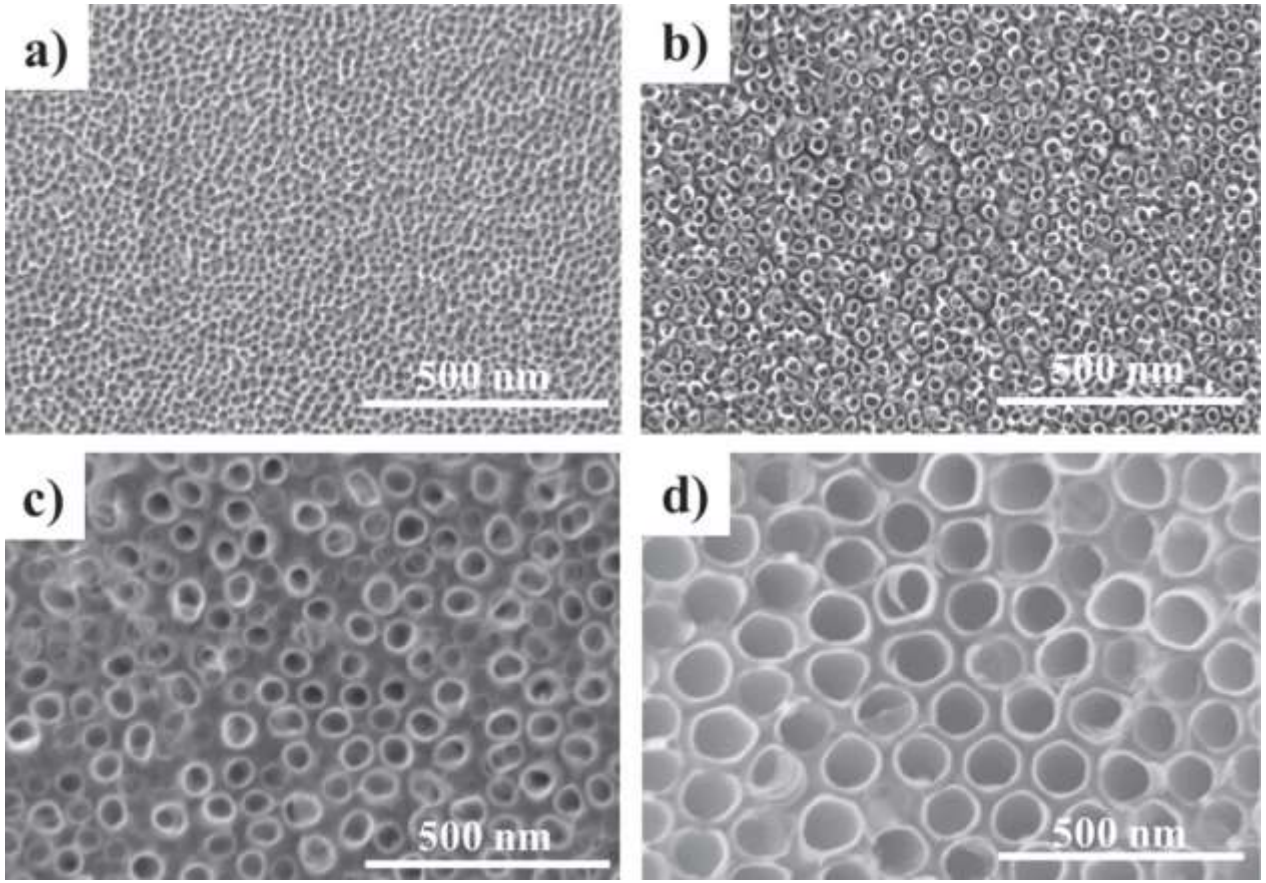


Electrical Field: 15 kV
Polymer-Dispension: 1,5 ml / h

Other techniques: vapor or electrochemical deposition ! -

A Badami et al., Biomaterials, 27:596-606 (2006)

TiO_2 Nanotubes

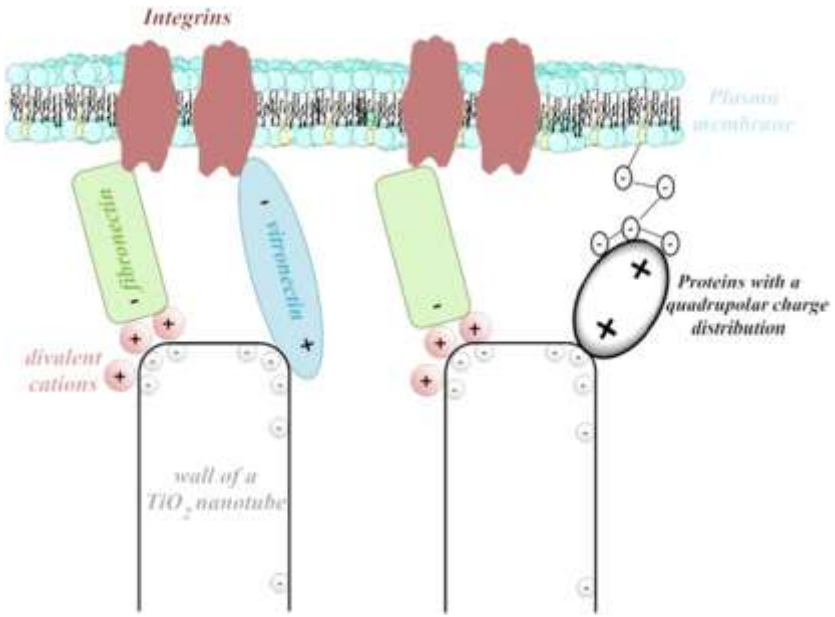


Top-view SEM images of different diameter TiO2 nanostructures :

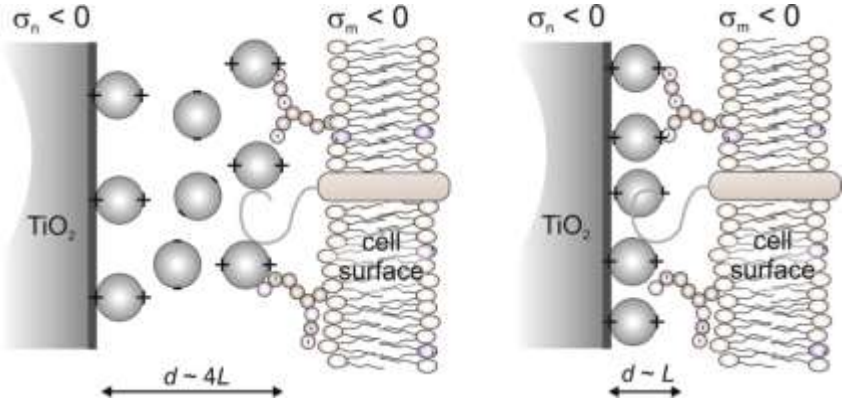
15 nm nanopores (a), 15 nm nanotubes (b), 50 nm nanotubes (c) and 100 nm nanotubes (d).

Nanotechnology 26 (2015) M. Kulkarni et al

The structure of TiO2 and adsorbed protein influences the implant's success



Schematic of the adhesion of fibronectins, vitronectins and proteins with a quadrupolar internal charge distribution at sharp edges of vertically aligned TiO2 nanotubes.



Attraction between the negatively charged TiO2 surface and negatively charged cell membrane surface mediated by macro-ions at a larger distance (left) and the equilibrium distance corresponding to the minimal free energy of the system (right).

Nanotechnology 26 (2015) M. Kulkarni et al

Properties influencing
the biocompatibility, implant´s success and circulation time of particles in blood

Surface electrical charge density and distribution

“Crystal” structure (relation to integrin structure)

Size of pores, tubes, fibers and particles

Surface modification: **proteins (biofilm) as mediators between material and cell membrane**

functional groups

growth factors

nanoparticle decoration

⇒ **Influences the stem cell differentiation and cell growing**

⇒ **Effect of size on cytotoxicity is still not fully understood**

⇒ **Smaller-diameter nanotubes showed**

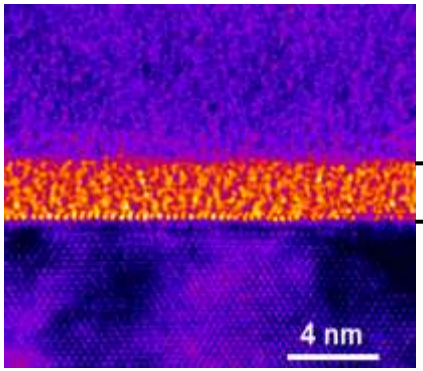
- **increased antibacterial effect against E.coli.**
- **enhanced human cell adhesion**

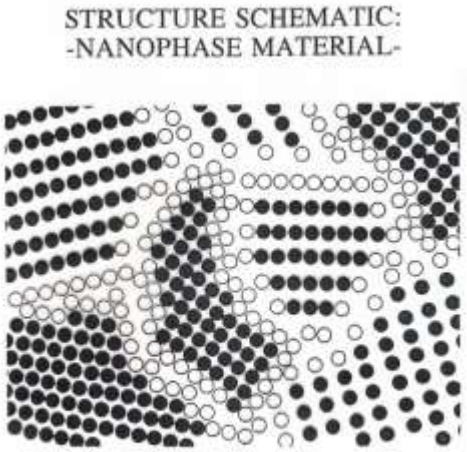
“Nano-sized structures”

=> Quantum effects !

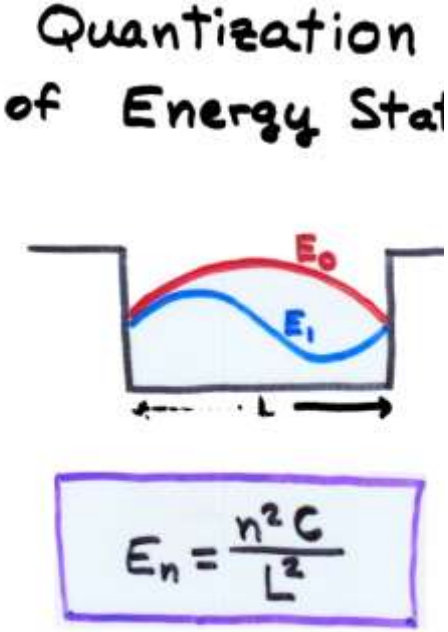
Micro => Nano-Technology ?

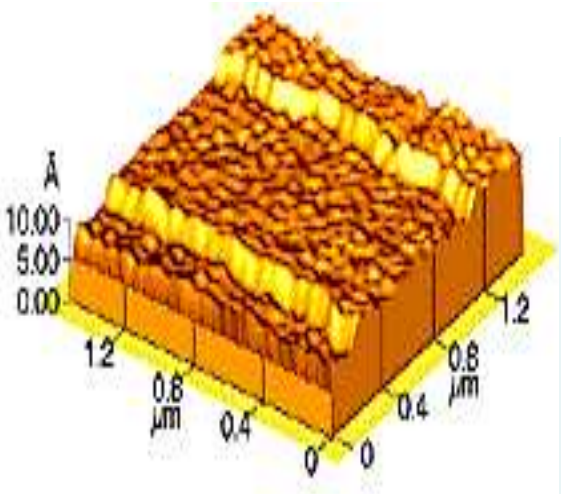
Is the ability to construct structures with atomic / molecule control
Transition from classic to quantum-mechanical physics





Schematic of an equiaxed nanocrystalline metal showing atoms associated with individual grains (filled circles) and those constituting the grain boundaries (open circles). [H. Gleiter, Prog. Mater. Sci. 89, 223 (1989)]



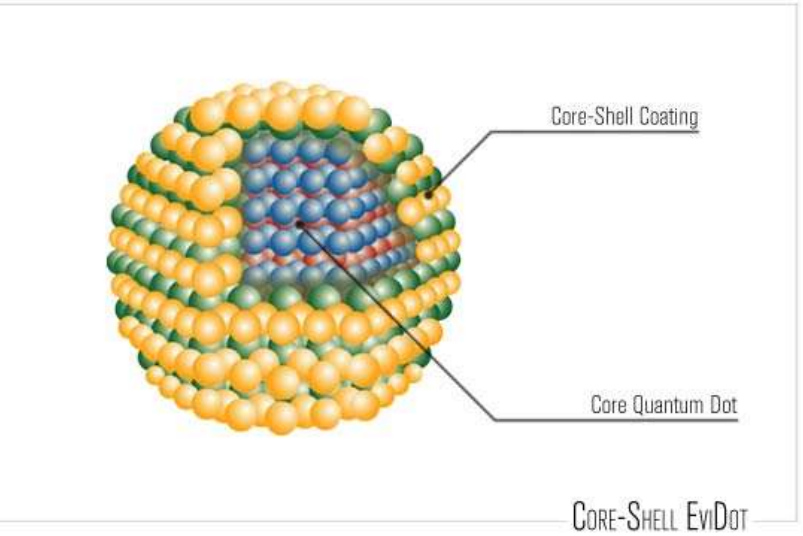


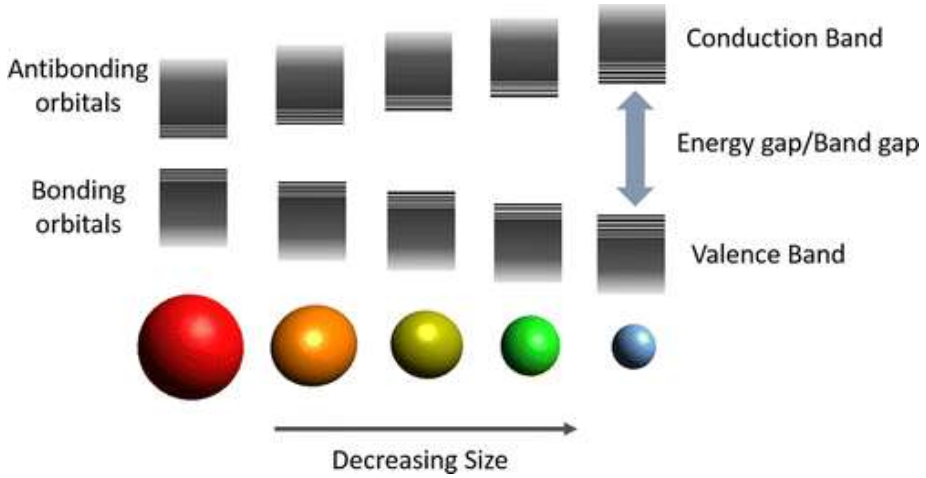
=> Defined quantum states:
new sensor technology

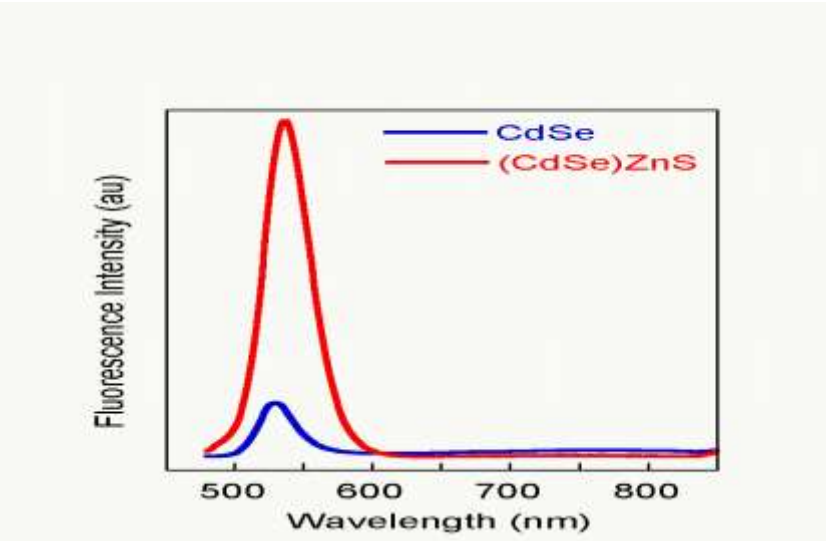
=> New construction technologies (IT)

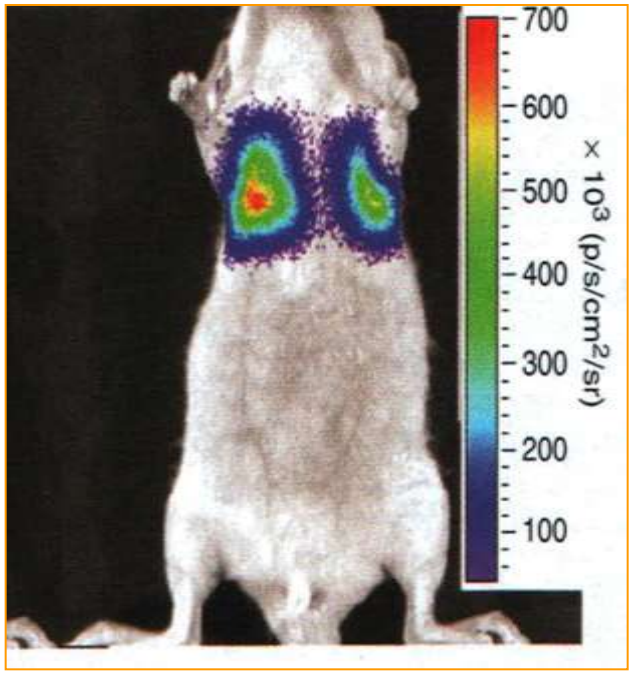
=> Tunneling & Atomic Force Microscopy:
surface inspection in atomic level
single molecule manipulation & depositing

„Quantum Dots“









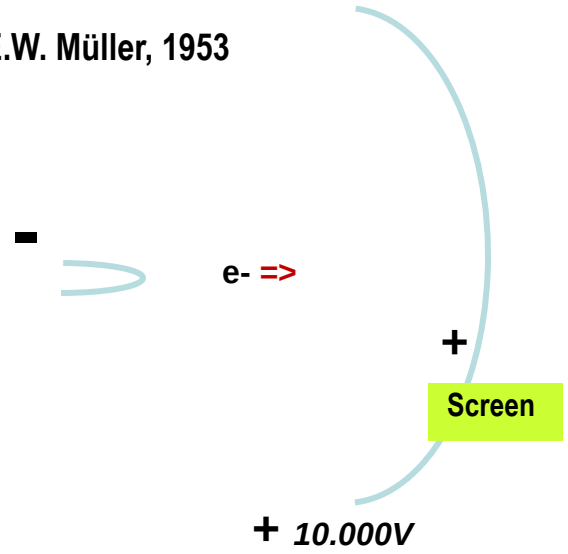
Vision !?

Tissue investigation during operation !

R. Shull, Nat. Inst. Standards

Quantum Mechanics:
Field Emission Microscopy

E.W. Müller, 1953



Quantum Mechanics:
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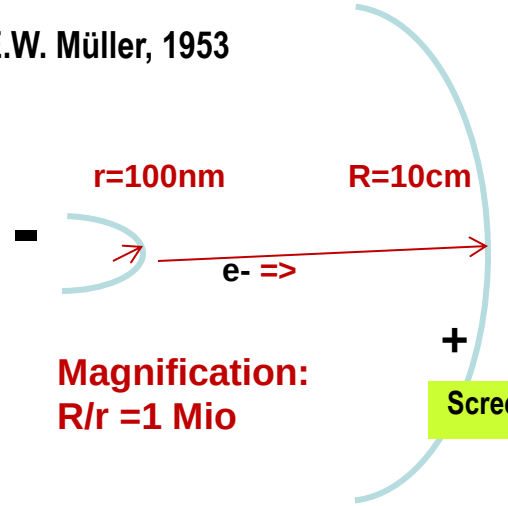
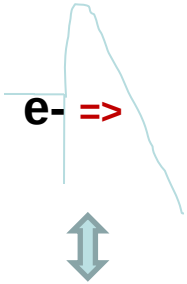


Magnification:
R/r =1 Mio

Field emission tip surface with a single molecule

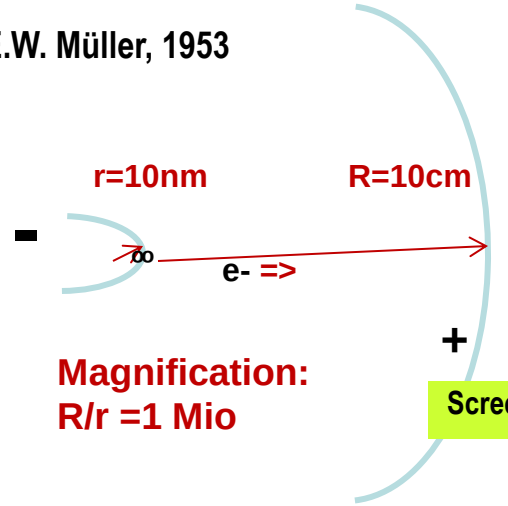


Potential wall thickness .
5-10 nm

Ivar Giaever, 1968 /1973 Nobel Award

Quantum Mechanics:
Field Emission Microscopy and Scanning Tunnelling Microscopy

E.W. Müller, 1953



Magnification:
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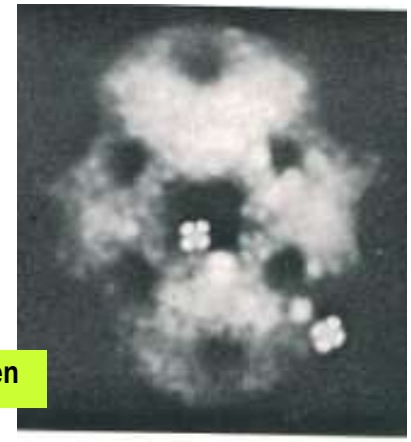
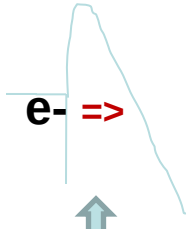


ıl-Bild von CuPc-Molekülen auf der W

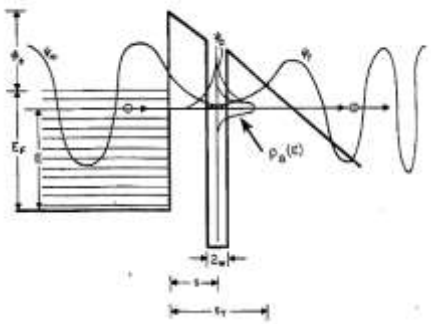
Field emission tip surface with a single molecule

Gerd Binnig, Nobel Award 1986

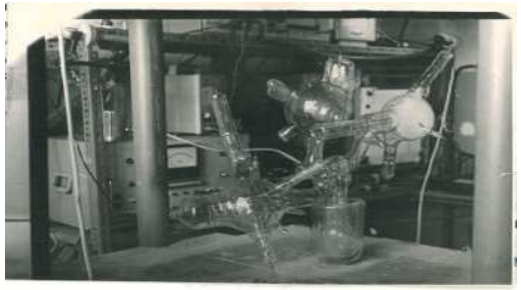
Field emission tip near the object surface: Scanning tunnelling microscope



Potential wall thickness . 5-10 nm



Resonance tunnelling through a single molecule (Schütt, 1973)



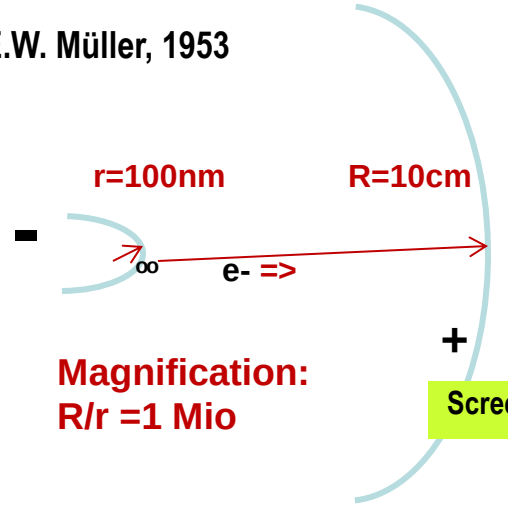
=> Atomic force microscope
=> Magnetic force microscope

=> Ab-Ag-force microscope (1986)

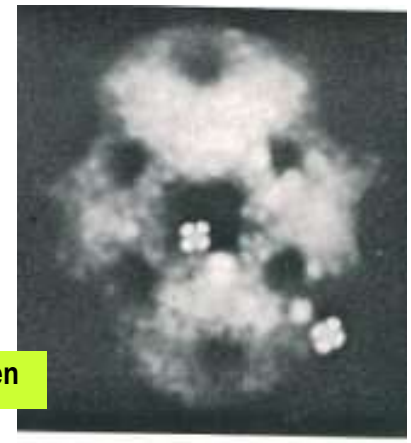
=> .....Resonance tunnelling ?

Quantum Mechanics:
Field Emission Microscopy and Scanning Tunnelling Microscopy

E.W. Müller, 1953



Magnification:
R/r =1 Mio



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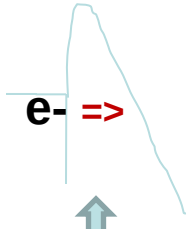
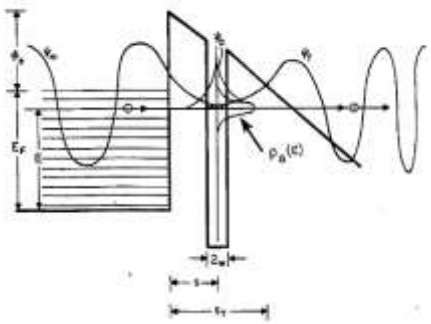
Gerd Binnig, 1986



Potential wall thickness .
5-10 nm



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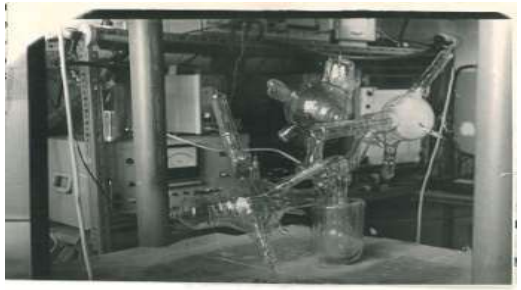
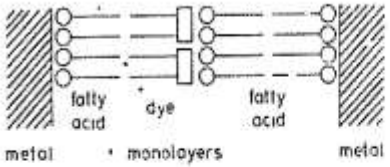


RESONANCE TUNNELLING IN MOLECULAR LAYER SYSTEMS



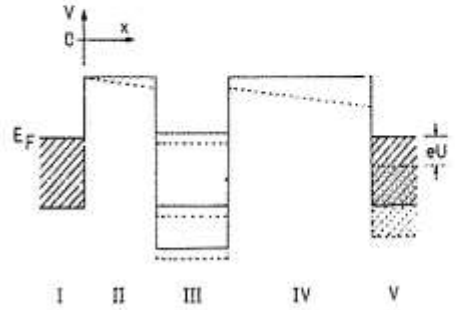
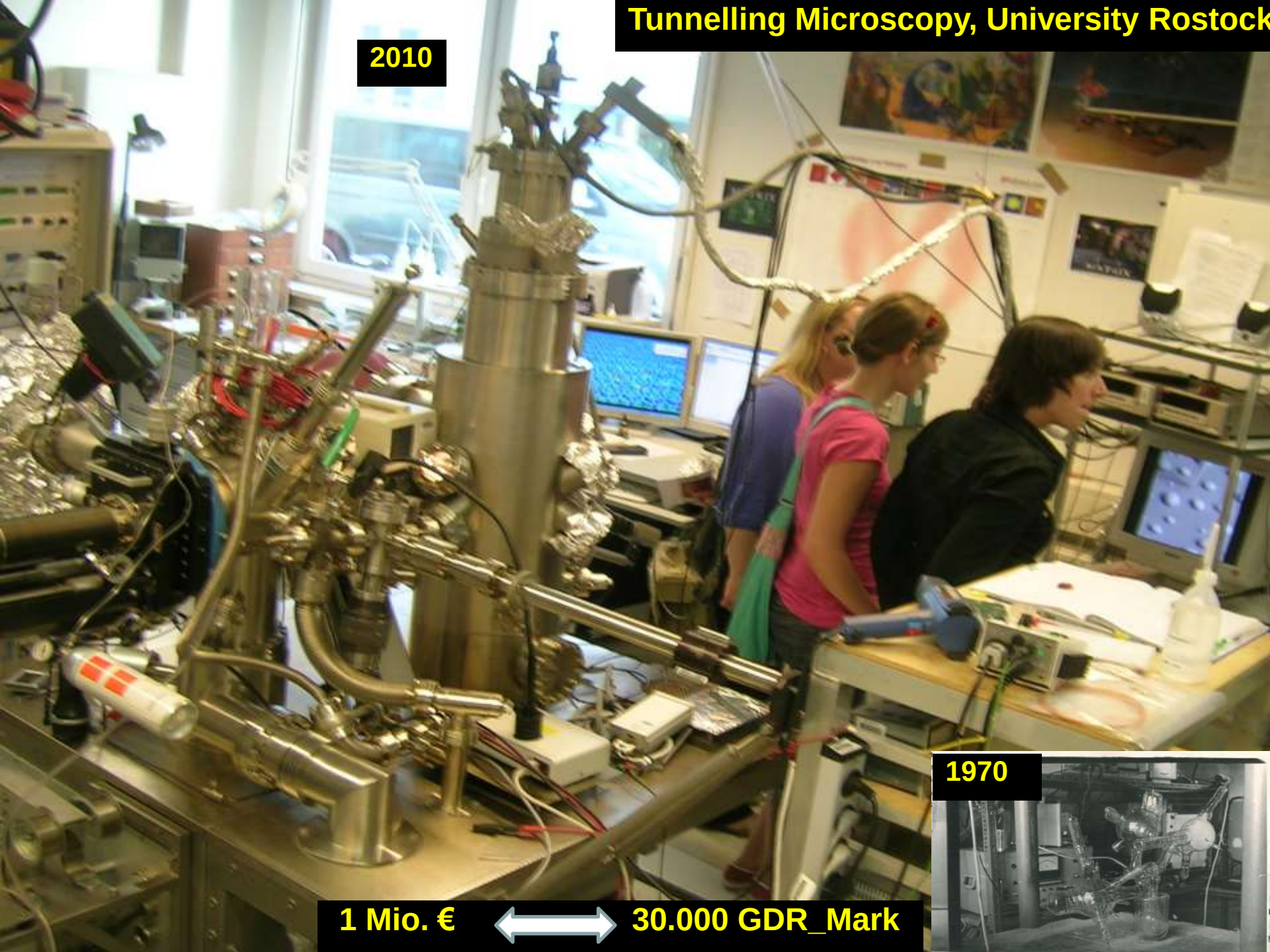


Fig. 1. Proposed layer system and associated potential energy.

proposed 1973

Tunnelling Microscopy, University Rostock

2010

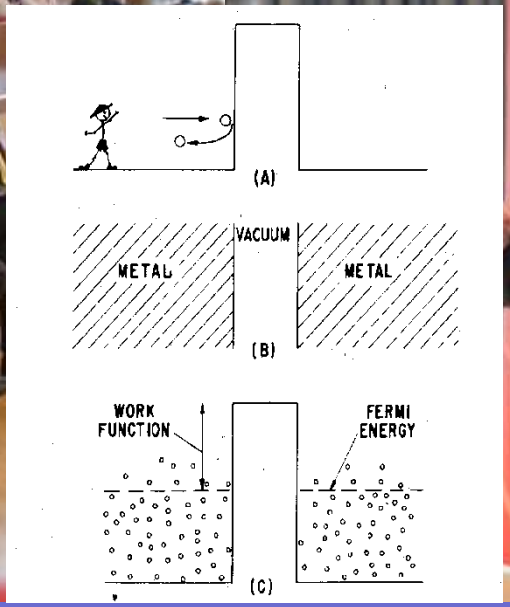


1970

1 Mio. € ⟺ 30.000 GDR_Mark

„Small“ => Quantum Mechanics:

“Electron Tunnelling”



Prof. Ivar Giaever, USA (Nobel Prize in 1973, since 1998 “Entrepreneur”
Students of the Institute of Biotechnology in Krems, 2009 / 2011 / 2013 / 2015

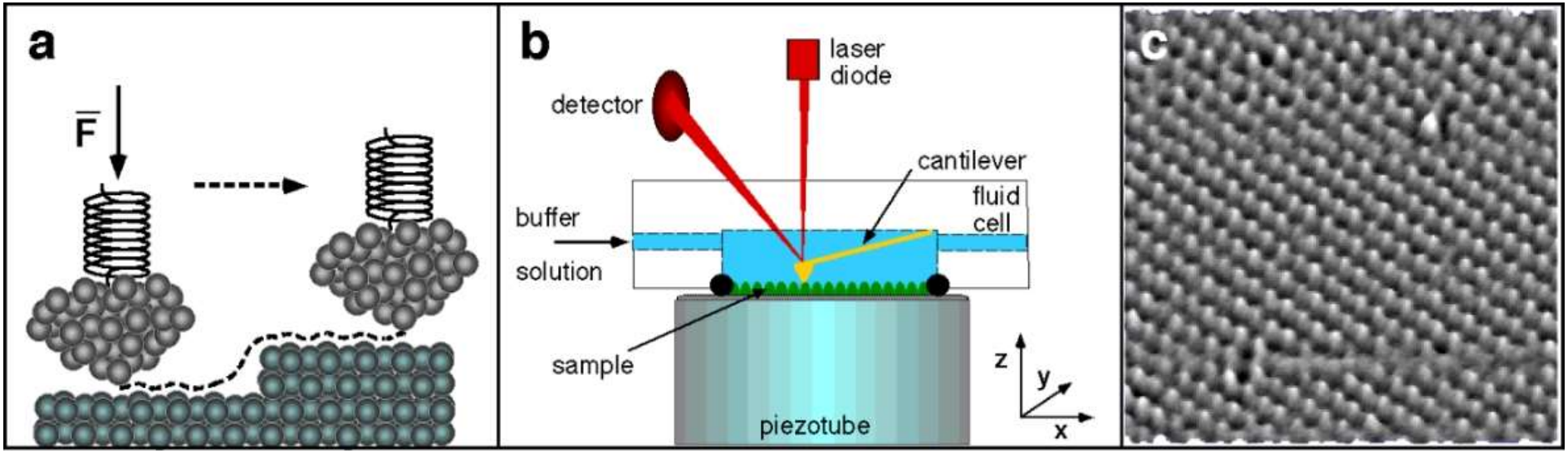
Nobel Laureate gives a lectures for Krems biotech students

(March 25. 2009)

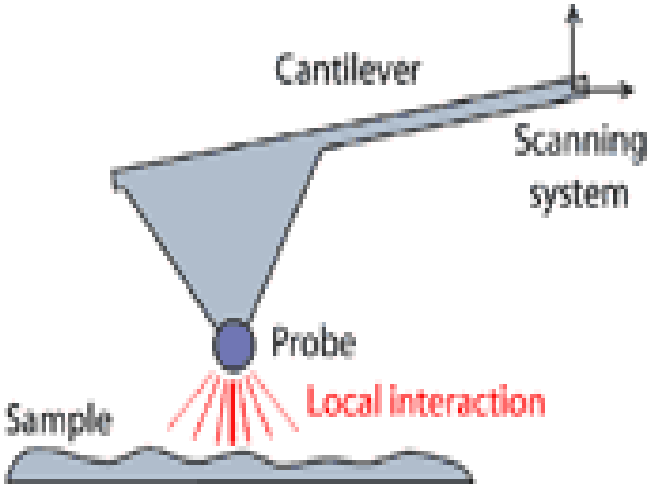
Prof. Ivar Giaever, USA
Nobel prize in 1973

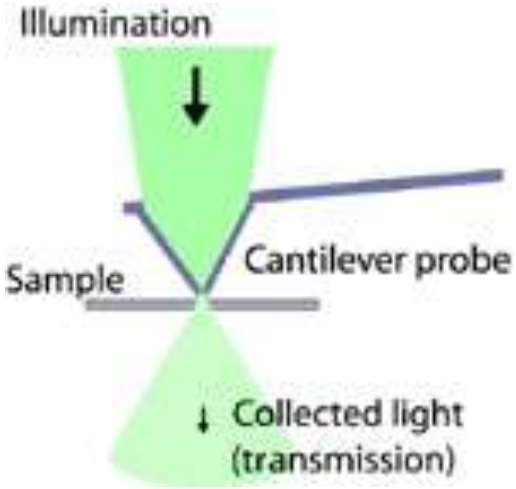
(Research corporation in the field of wound healing since 2005)

Atomic Force Microscopy for Surface Inspection

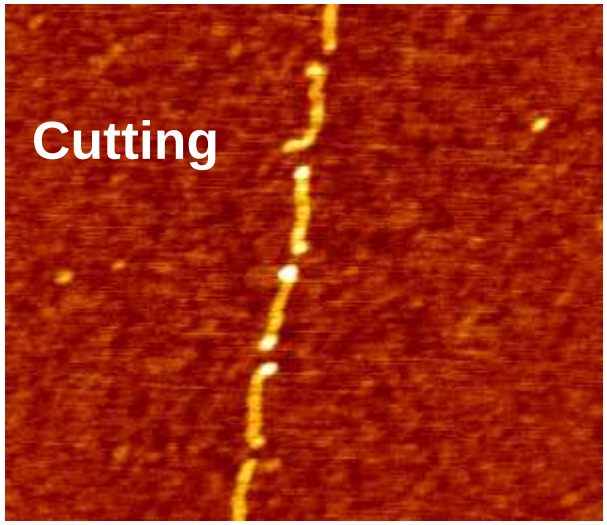


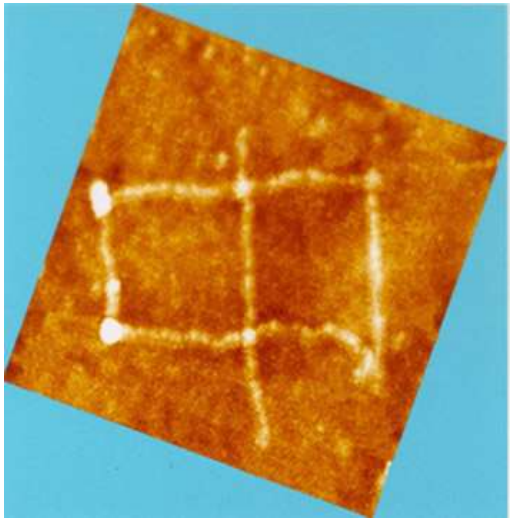
Plus "probes": ….molecule, antibody, magnet,……. light





Nano-manipulation of single DNA





Deposition of protein segments at the predesigned place

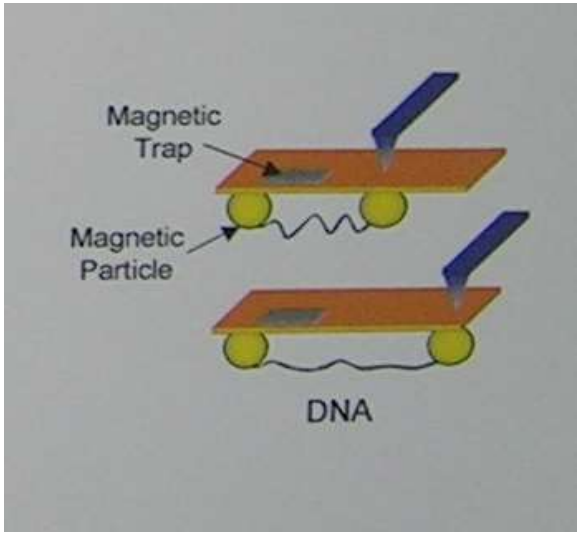
Stefani Maerz:

2003-2008 FH Krems-Biotech-Student

PTS and PhD at Shanghai JiaoTong University

Friction of a DNA molecule

Scanning Atomic Force Microscope with electro-magnetic Tip



Chad Mirkin,

Northwestern Univ. Chicago

”Nano World”: What is new for biomedicine?

	Biomedical Application:
Quantum mechanical effects:	
colours	**bio- and cell-analytics**
magnetism	**nanomedicine**
=> Defined pore size	
membranes	**detoxification**
=> Enlarged surface area:	
adsorption	**immunoadsorption**
Molecular level:	
self organization	**particles, liposomes for drug delivery**
Nano-technologies:	
electro-spinning	**design of biomaterials**
chip-technology	**single particle / cell manipulation**
“nano-manipulation”:	**single molecule handling**

Extracorporal Blood Purification:

Blood Letting

Dialysis
(filters, water-solved toxins)

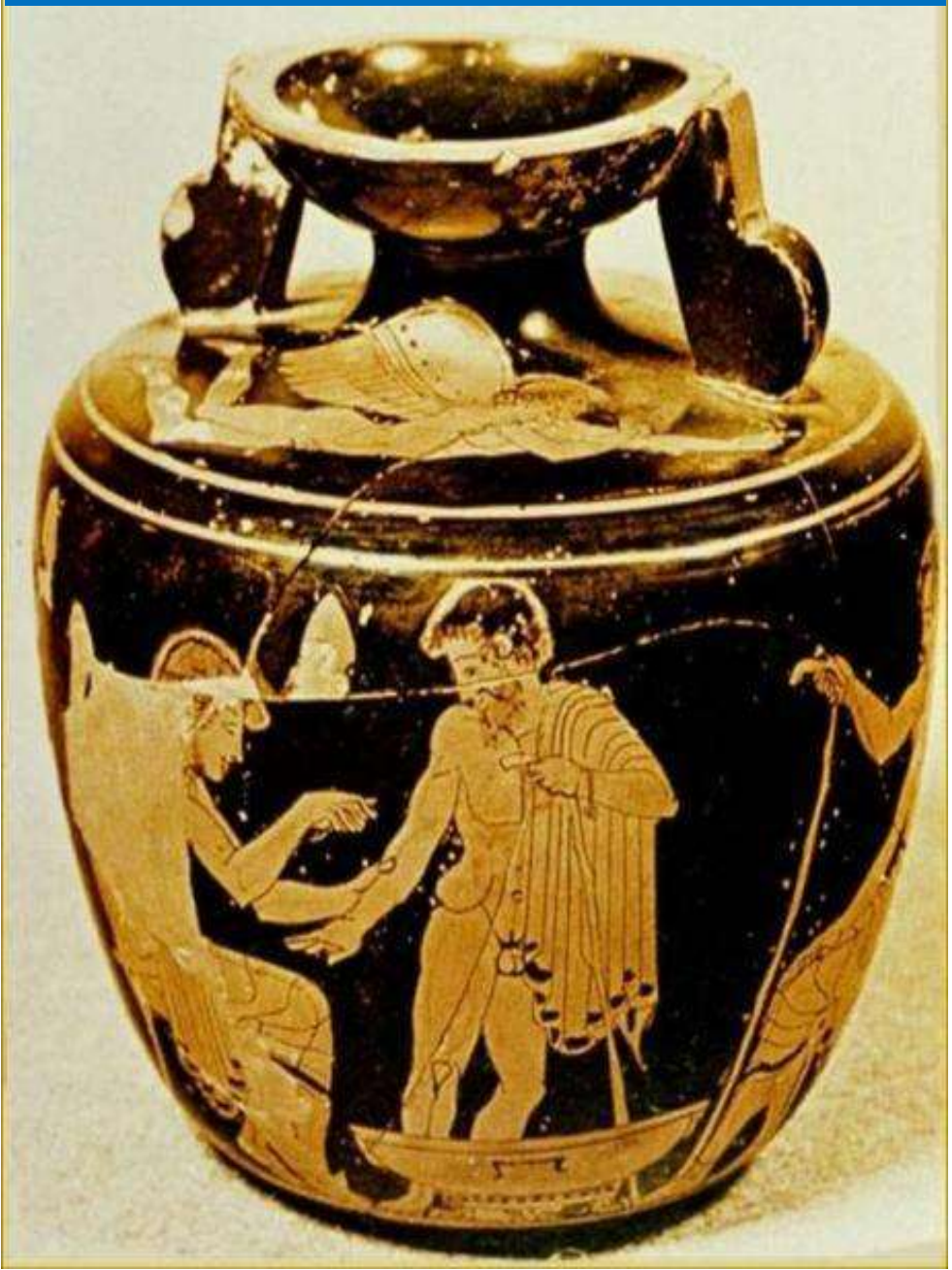
Plasma Exchange
(substitution of substances)

and

Apheresis
(adsorbents removing of certain mol. weight range)

Immunoadsorption
(specific adsorbents)

Bloodletting

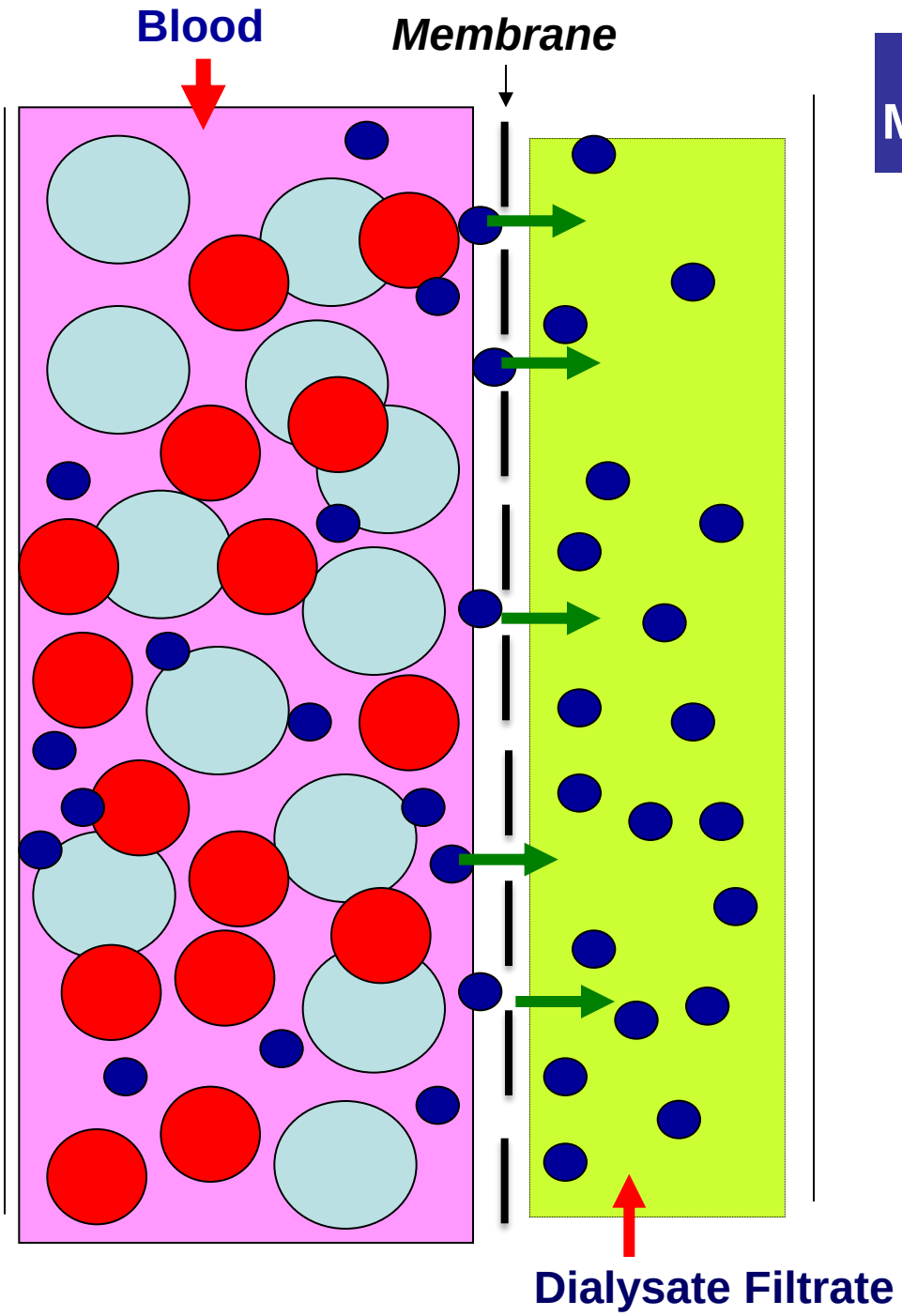


Early Dialysis !

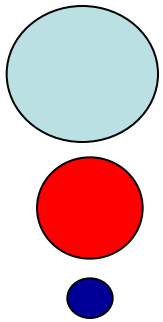
The Vartomans Enema (1516, British Museum)

Pioneers in Blood Purification

Selection by Membrane Separation



Different Molecules



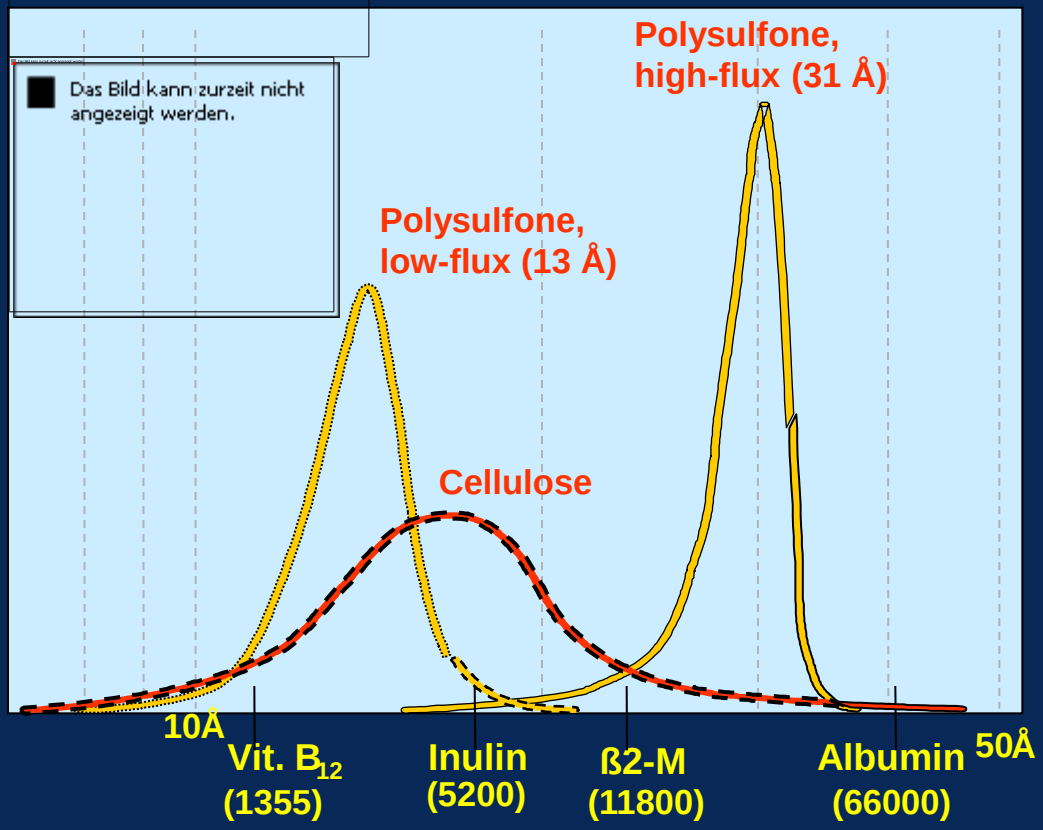
High-Flux Membranes: Pore size & Distribution determine SC & Q_F

0.000,000,001,3 m (low-flux)

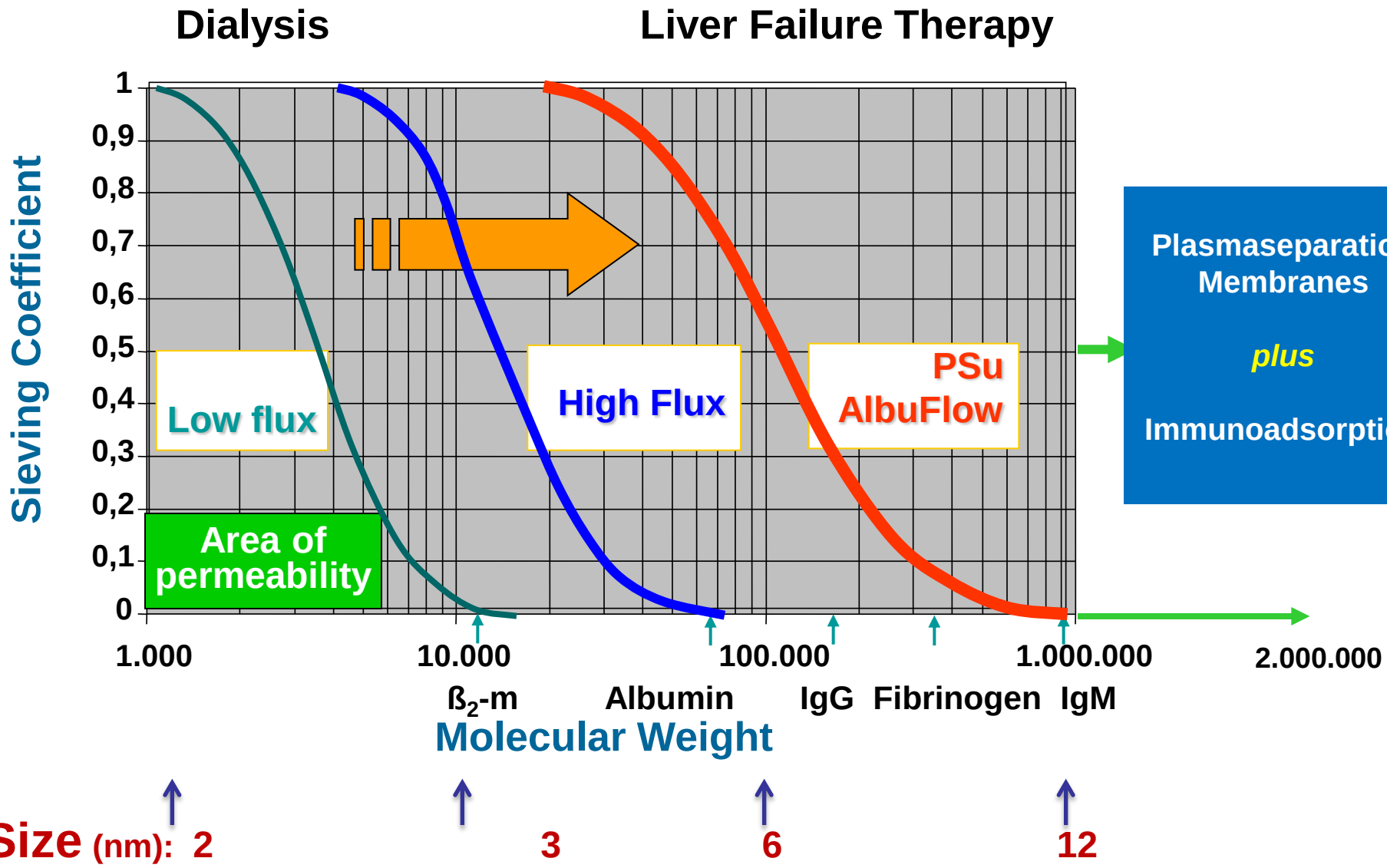
0.000,000,003,1 m (high flux)

Synthetic Membranes

Cellulosic Membranes



Characteristics of Different Membranes: Pore Size Effect



Global revenue for acute dialysis treatment

- Estimated revenue for dialysis products:

  € 9 Bill (incl. acute treatment!)

- Estimated revenue for the total dialysis therapy:

  € 42 Bill (incl. acute treatment)

Extracorporal Blood Purification:

Blood Letting

Dialysis
(filters, water-solved toxins)

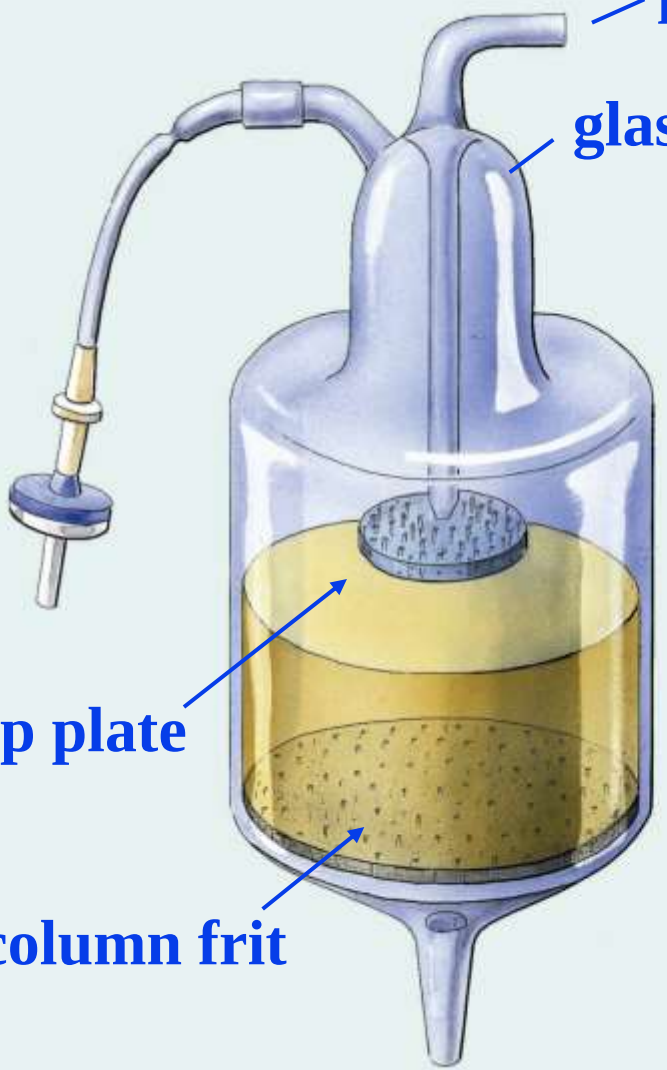
Plasma Exchange
(substitution of substances)

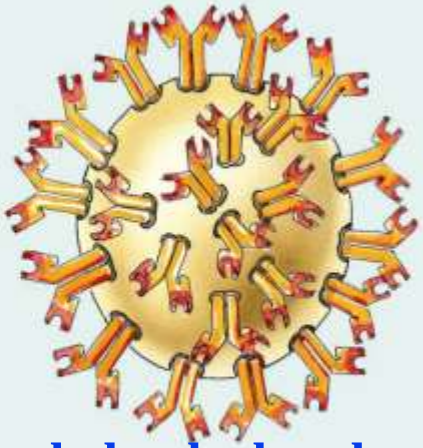
and

Apheresis
(adsorbents removing of certain mol. weight range)

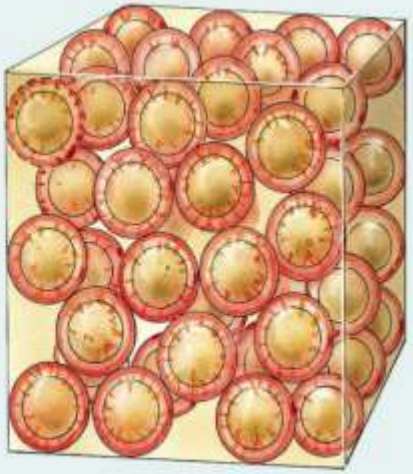
Immunoadsorption
(specific adsorbents)

Therasorb™-Ig pro / flex Adsorber





Fc-coupled polyclonal antibodies against human Ig



sepharose-matrix

Clinical relevant targets to be removed …

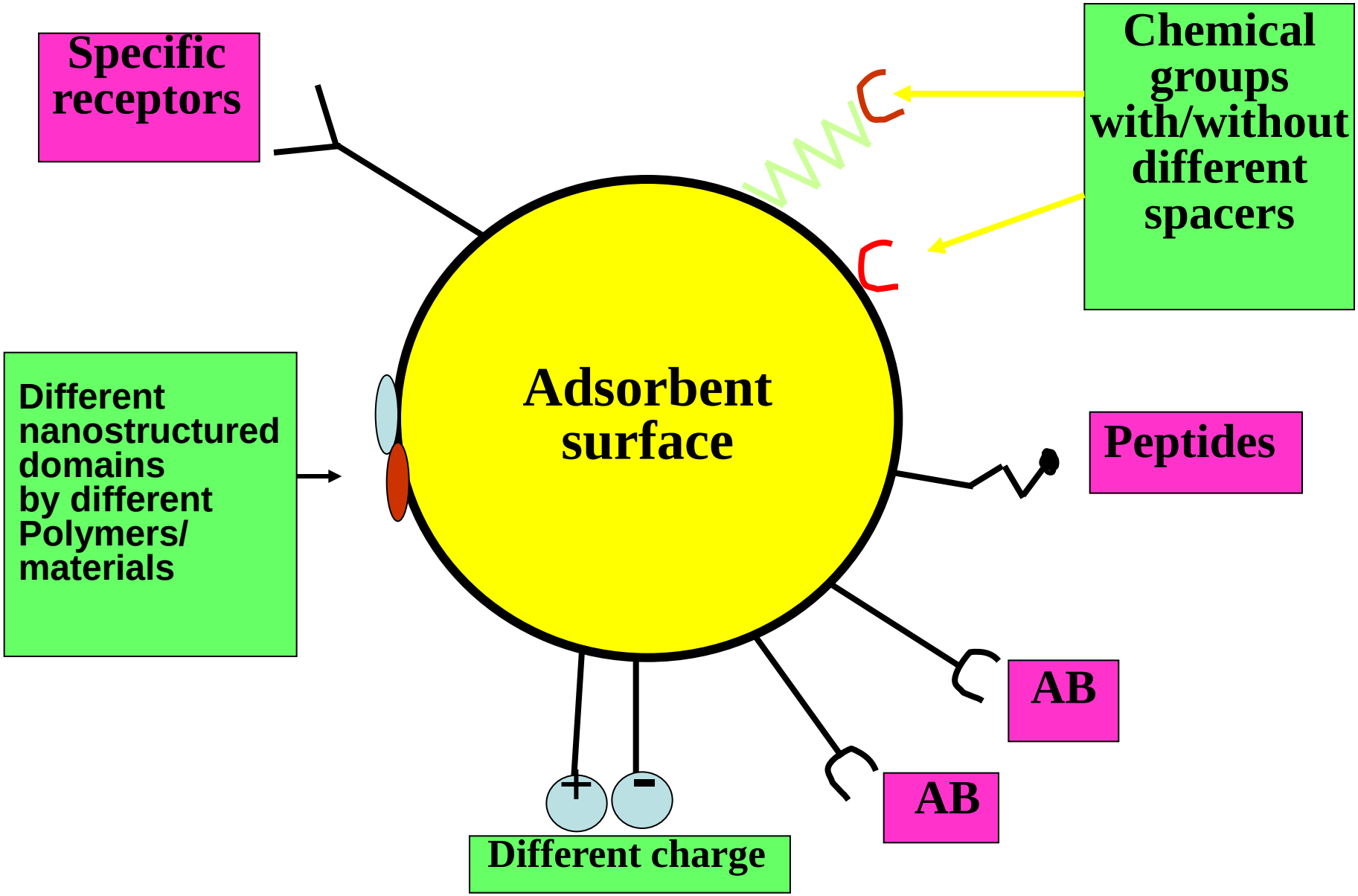
W. Ramlow, Rostock

- Anti-acetylcholine receptor autoantibodies
- Autoantibodies against neuronal surface antigens
- Coagulation factor inhibitors
- Desmoglein autoantibodies
- Anti-GBM autoantibodies
- Ds-DNA-Abs / immune complexes
- Cardiac structure autoantibodies
- HLA antibodies
- Blood group antibodies (anti-A, anti-B)
- Immunoglobulin E
- Activated granulocytes, monocytes, platelets

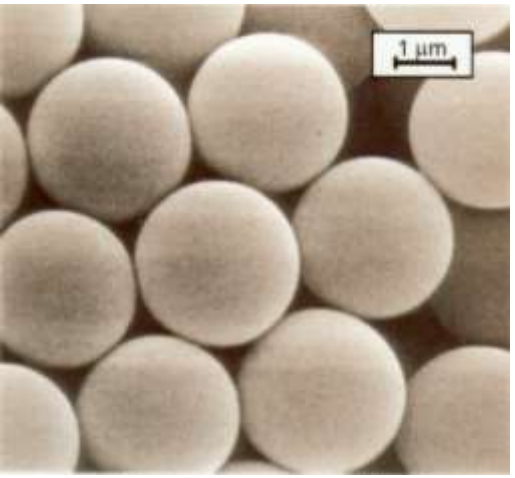
Long-term follow-up of a Pemphigus patient under immunadsorption with Protein A*

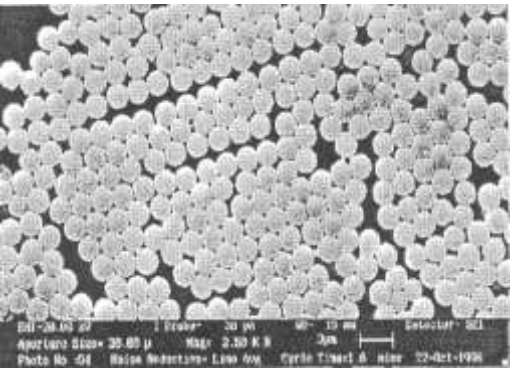
*** Source: Eming et al, Dermatology 2006;212:177–187**

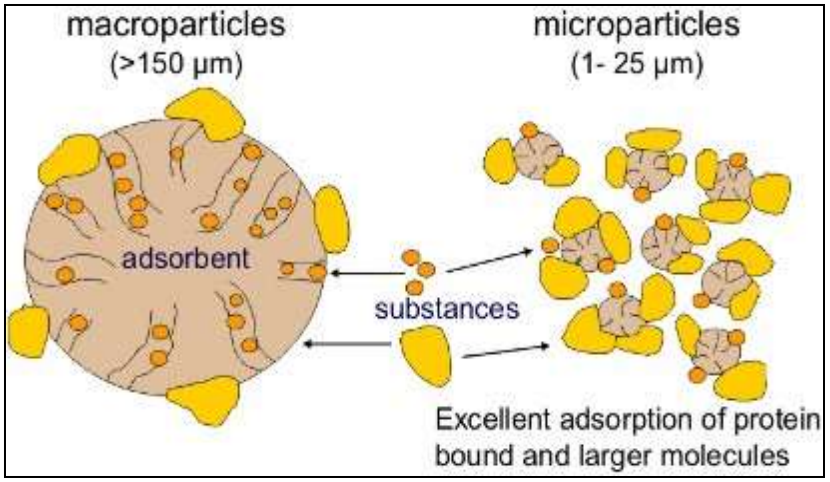
Surface modification for adsorbents for specific /selective adsorption

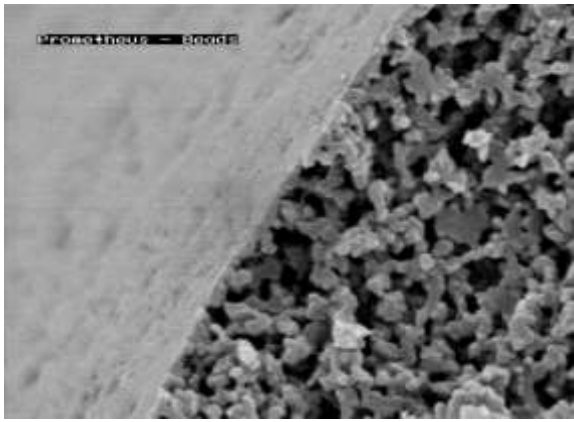


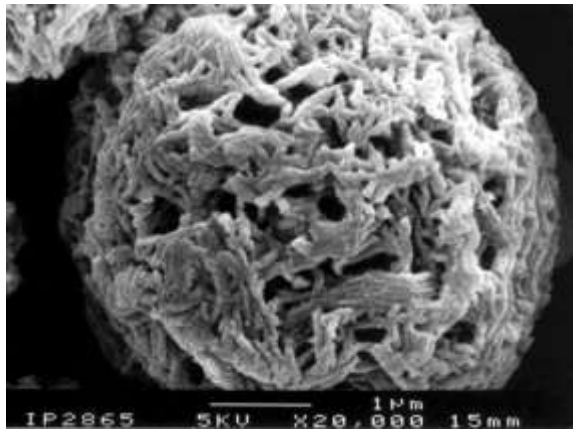
Uniform porous micro- and nano-particles











Nano-particles:

- higher surface-to-volume ratio

- higher adsorption capacity

- no limitation by diffusion

Particle Ref.: MicroCaps/Rostock, D.Falkenhagen,H.Loth/Potsdam,Krems, Fresenius/Bad Homburg

Nanoparticles and Dimensions - Comparison of Size, Area and Volume

Single particle

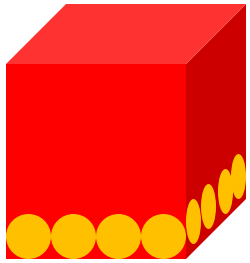
Filling the same compartment !

Size	Area	Volumen
d : 16 nm	A : 803 nm²	V : 2.144 nm³
d : 12 nm	A : 452 nm²	V : 904 nm³
d : 4 nm	A : 50 nm²	V : 35 nm³

With decreasing radius volume decreases faster than area!

Radius dependence:

r^3 vs r^2



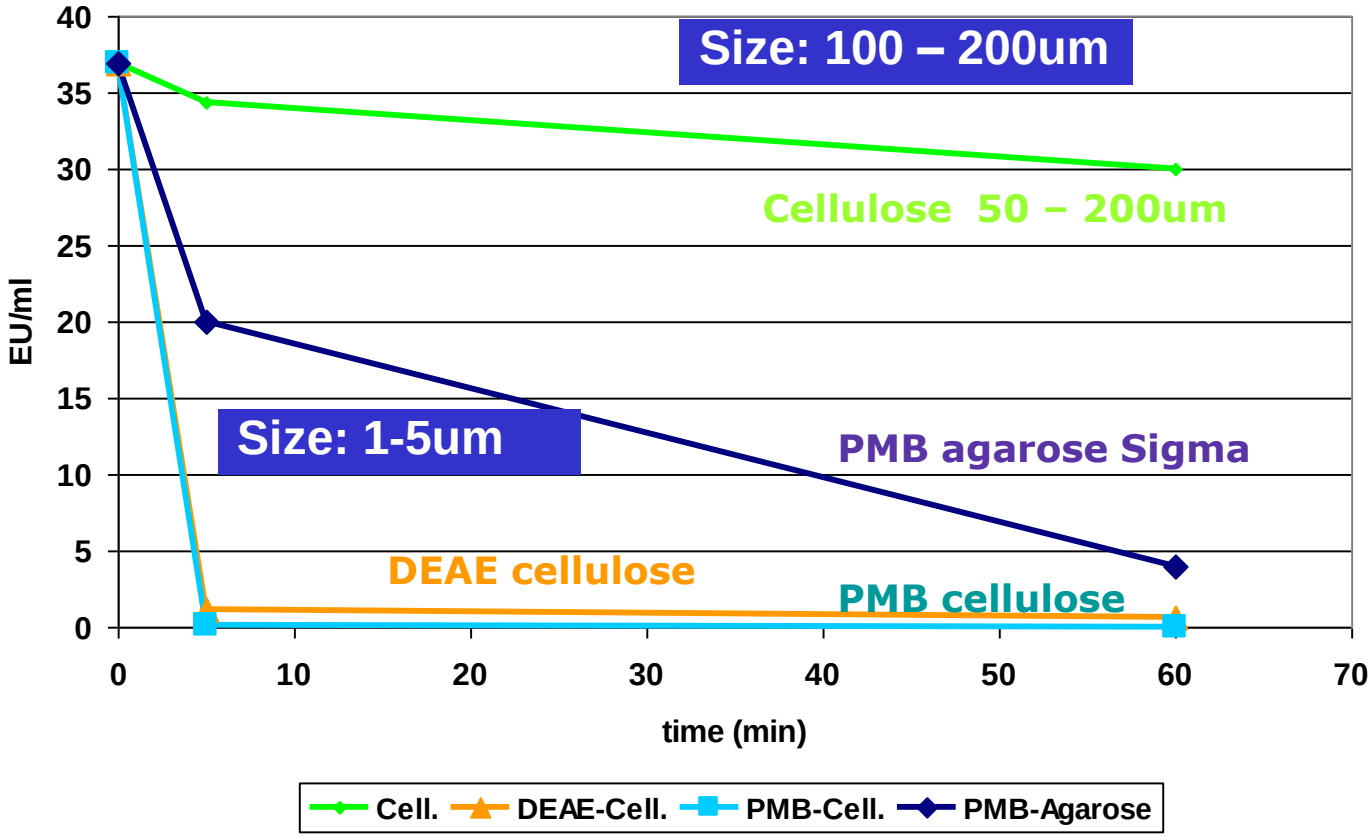
a = b = c = 16 nm

1 particle *A : 803 nm²* V : 2.144 nm³

4 times

64 particles *A : 3.200 nm²* V : 2.240 nm³

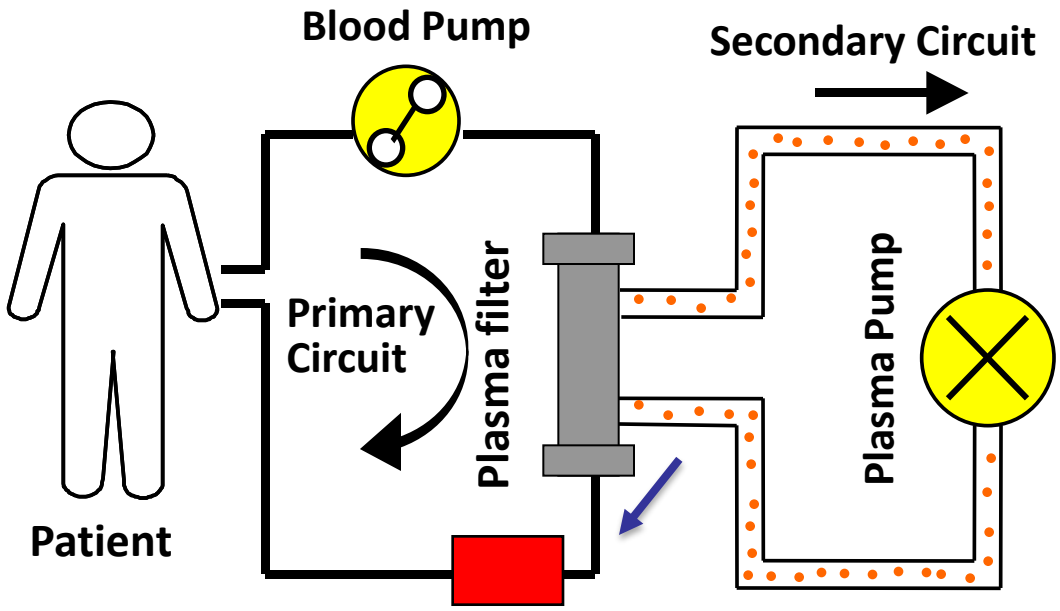
Adsorption of Endotoxins: *in vitro* Results



D. Falkenhagen, Krems

Microspheres based Detoxification System MDS

with a magnetic particle security system for membrane defects



Microparticle-Plasma Suspension
plus:
Fluorescent magnetic beads

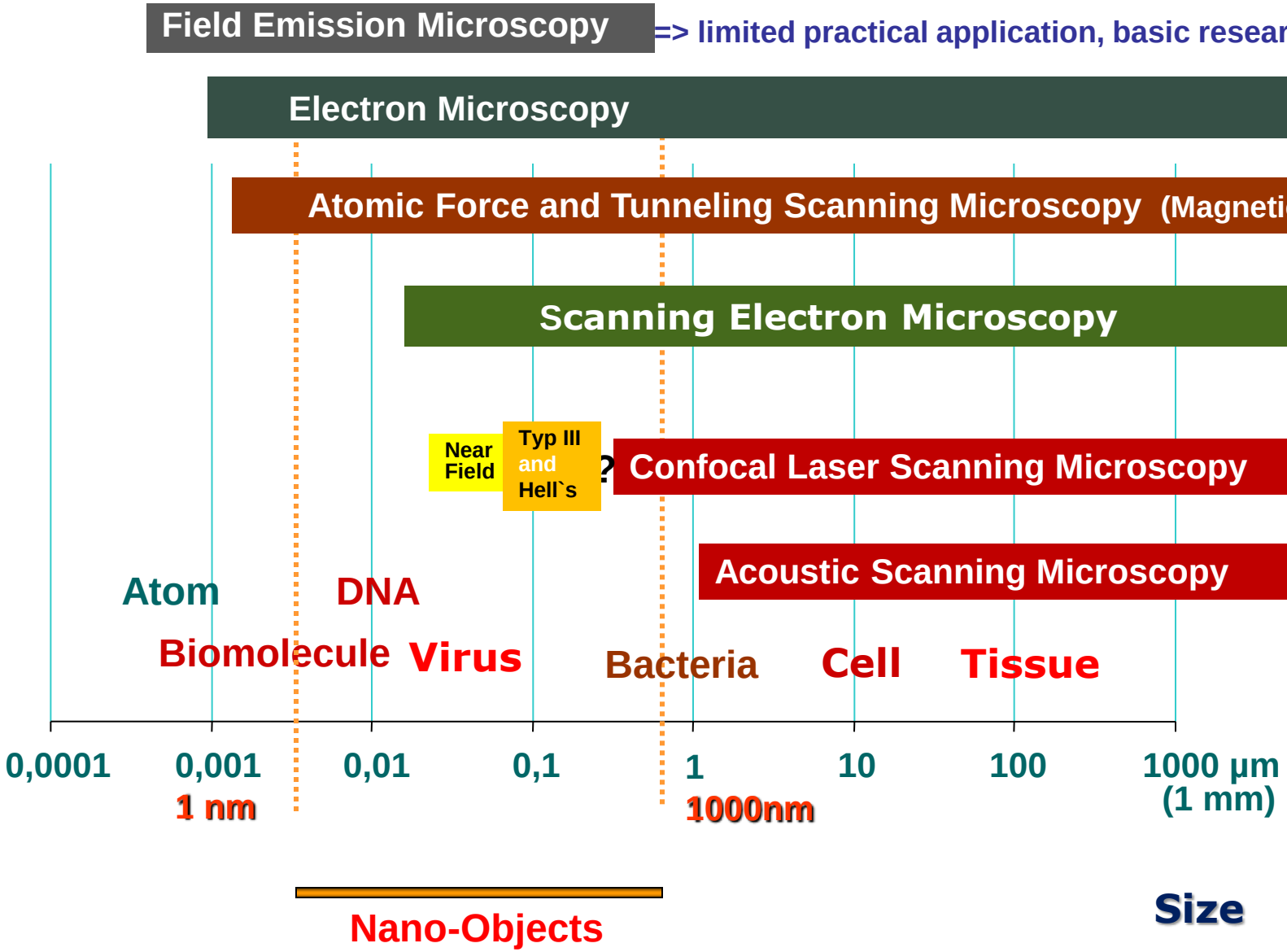
Security system:
Fluorescence detector for magnetic microparticles retended by magnetic field

D. Falkenhagen, Danube Univ. Krems

Characterization
of
Nano- and Microstructures
and Particles

for biomedical use?

Nanoscale Observation – Physical Instruments



Characterization of Particles:

size and size distribution

surface charge

magnetic susceptibility

…

…

in vivo:

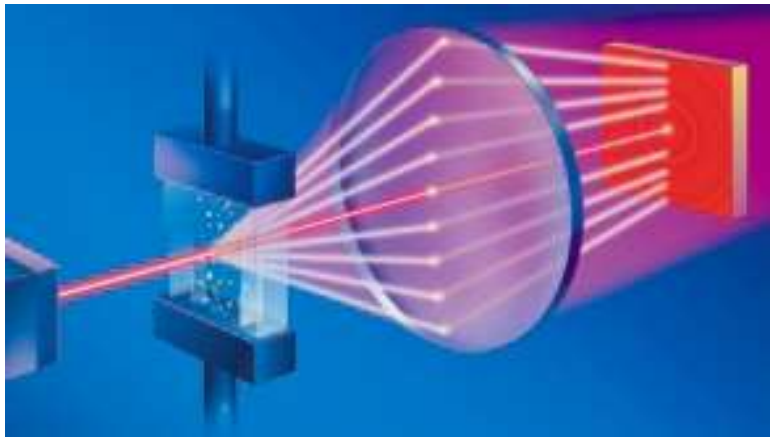
biocompatibility and circulation time

localization

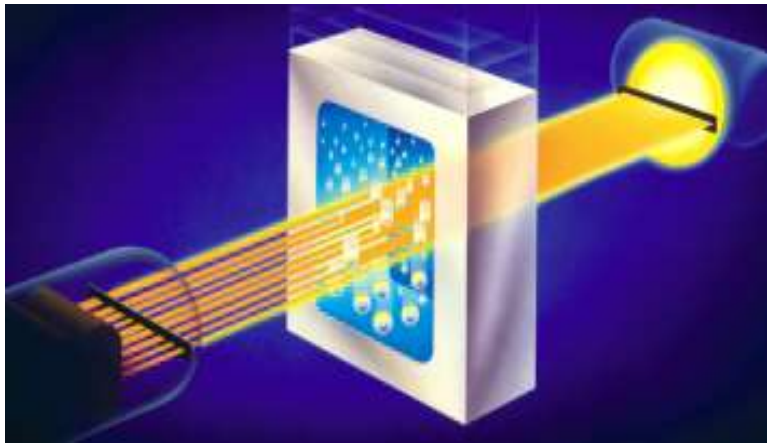
…

Size of Beads: Selected Methods

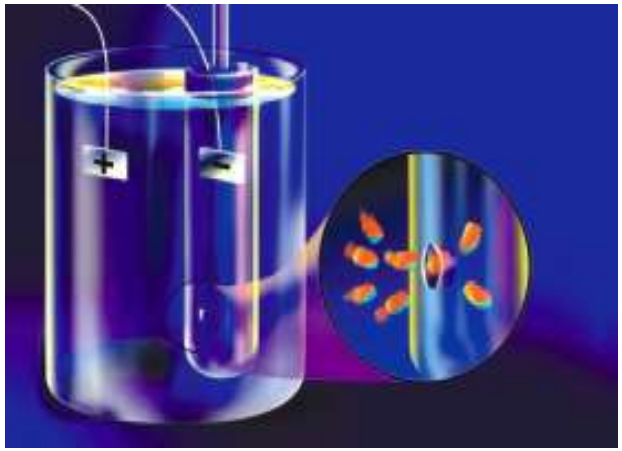
Light Scattering



Slit Scan Tech for Sedimentation Profil



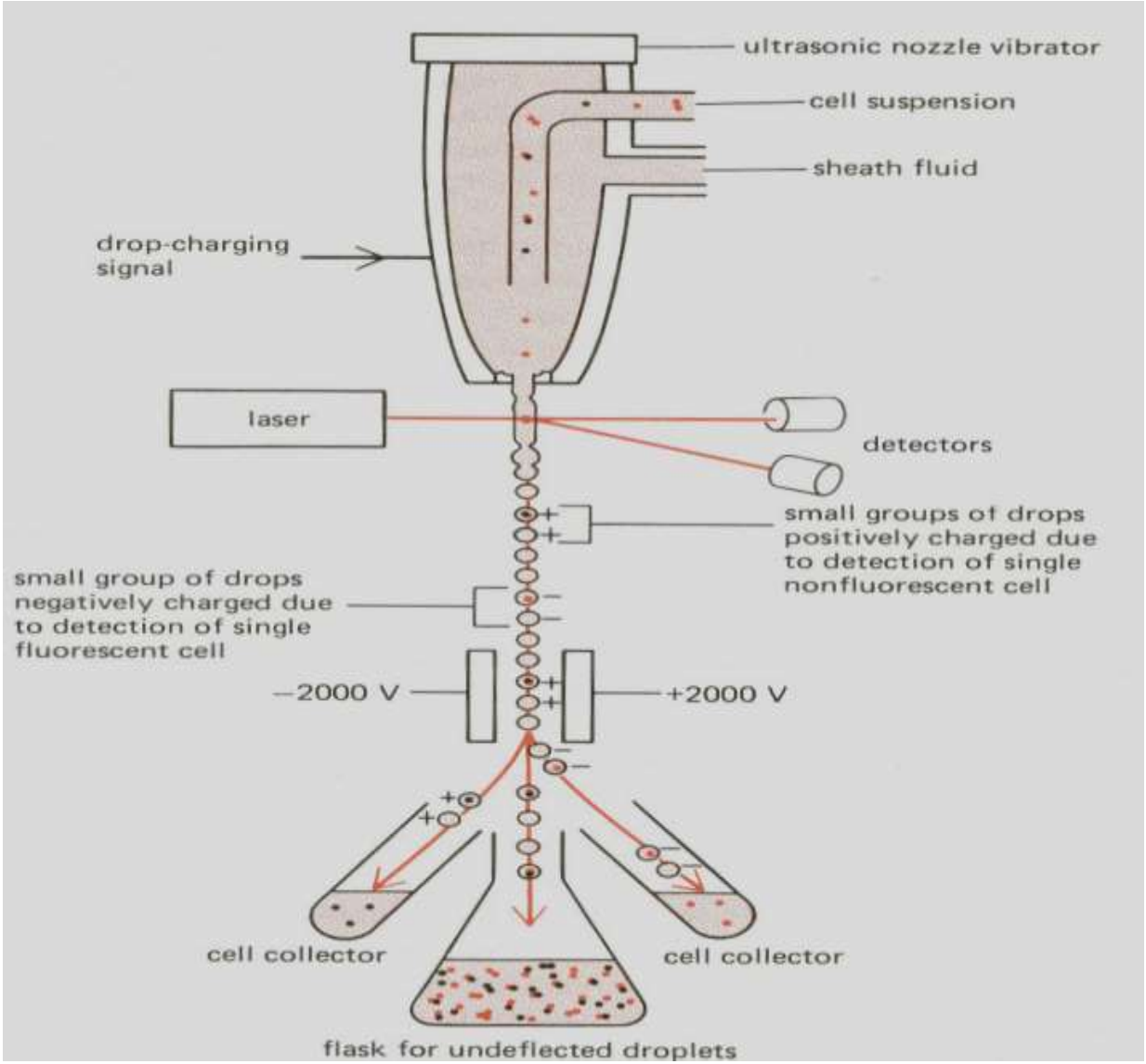
Impedance – Coulter Principle



Others:

- **Electron Microscopy**
- **Fluctuation Analysis by Laser Doppler (Dynamic Light Scattering)**
- **Flow Cytometry and Sorting (FACS)**
- **…**

FACS - Fluorescence activated cell sorting:



Steps:

Hydrodynamic focusing of cell suspension
(cells marked with fluorescent labeled antibodies)

Drops ultrasonically produced with single cells

Identification by Laser (scattered, transmitted or fluorescent light detection)

Charging of drops

Electric depletion of drops

Sorting of drops with cells according to

In combination with fluorescence labeled antibodies the most successful analytic method for cells and particles in suspension.

Characterization of Particles:

size and size distribution

surface charge

magnetic susceptibility

…

…

in vivo:

biocompatibility and circulation time

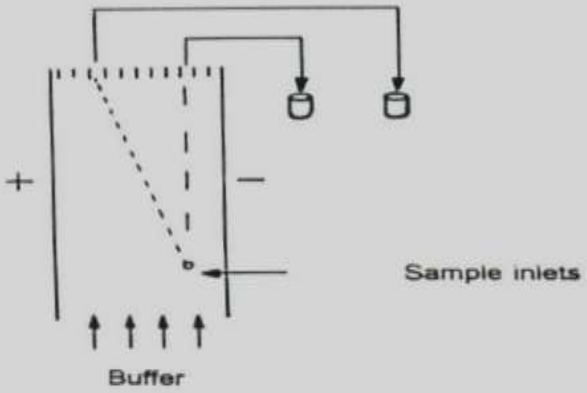
localization

…

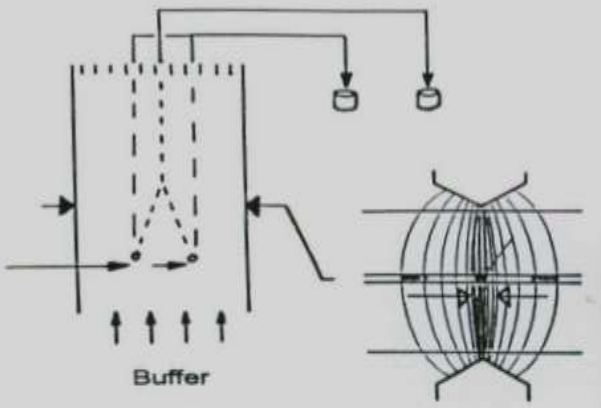
Analytical & Preparative Electrophoresis & Magnetophoresis

1975: Univ. Rostock and Zeiss Jena, 1986: with Collotec, NY (NASA)

Free Flow Electrophoresis

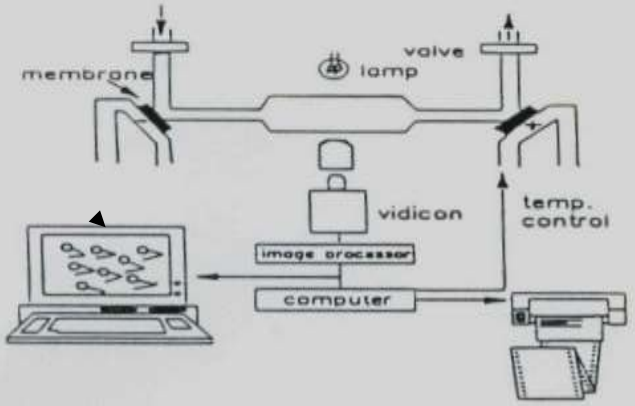


Free Flow Magnetophoresis



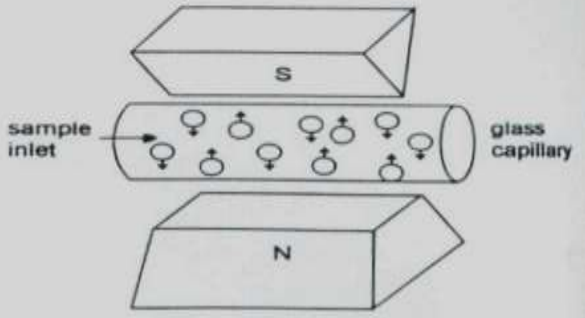
Single Particle Electrophoresis

Rostock /Zeiss Jena : PARMOQUANT



Single Particle Magnetophoresis

Rostock, Kiel, Moskau: MAGNETOQUANT



Single particle analytics: **charge density:** **&** **receptor density:**

Tumor cells, drug testing

(Magnetic Beads with Ab)

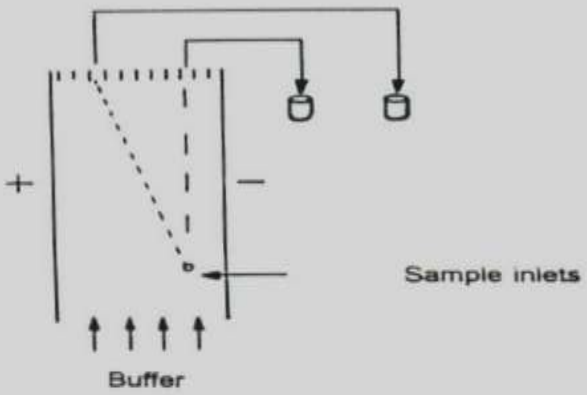
Analytical & Preparative Electrophoresis & Magnetophoresis

1975: Univ. Rostock and Zeiss Jena, 1986: with Collotec, NY (NASA)

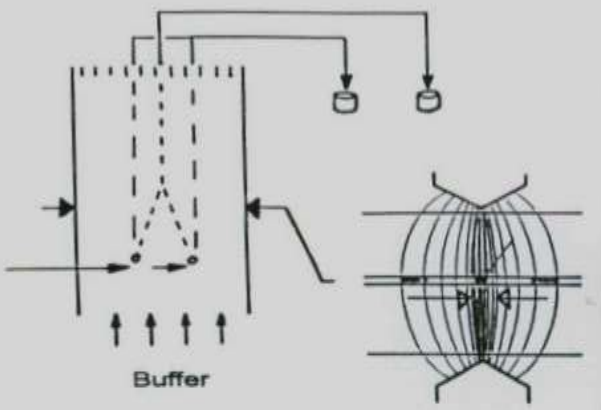
Zero-gravity cell separation
(since Sojus-Apollo-Project 1972)
=> no sedimentation
=> no convection

VIDEOPHOR
Rostock` System (NASA)
1987-1992

Free Flow Electrophoresis

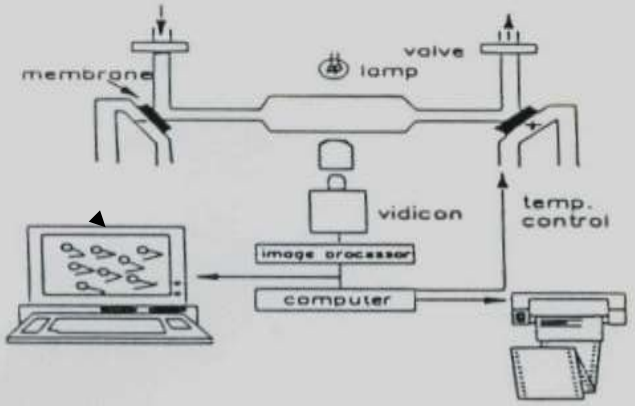


Free Flow Magnetophoresis



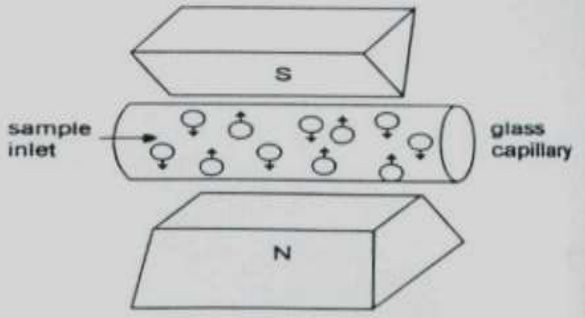
Single Particle Electrophoresis

Rostock /Zeiss Jena : PARMOQUANT



Single Particle Magnetophoresis

Rostock, Kiel, Moskau: MAGNETOQUANT

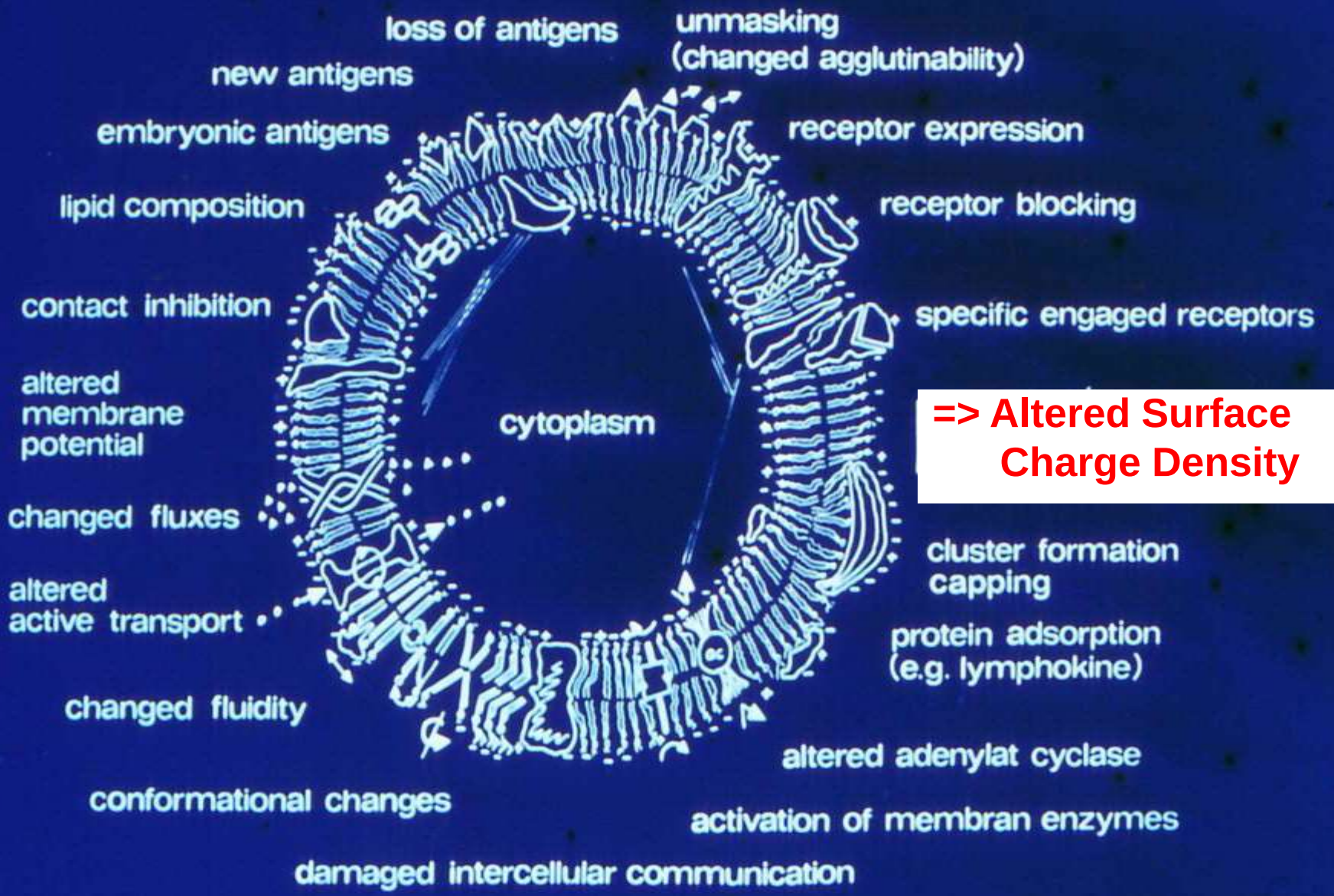


Single particle analytics: **charge density:** **&** **receptor density:**

Tumor cells, drug testing

(Magnetic Beads with Ab)

Potential Applications of Cell Electrophoresis



Parmoquant Single Cell Electrophoresis: Biomedical Applications Rostock 1976-1992

Table 1: Objects of investigation

Cells (untreated):	Biomedical Fields:
Erythrocytes:	Anemia, Stress, Incorporation of Parasites, Viruses, Storage
Thrombocytes:	Bernard-Soulier Syndrome, van Willebrand Disease, Hyperlipidemia, Acute myeloid leukemia
Lymphoid Cells:	(Percentage of B-, T- and other Subpopulations), Cancer, Transplantation, Immunopathies, Dialysis, Irradiation
Leukaemic Cells:	Differentiation, Bacteria, Algae, Parasites: Differentiation, Pathogenicity, Virulence
Liposomes:	Prenatal Lung Maturity (Amniotic Fluids)
Cells influenced by:	
Irradiation:	Sensitors, Protectors, Cancer Treatment
Parmaca:	Neuroleptics, Narcotics, Endotoxin, Antibiotics, Chemotherapeutics, Immunomodulators (e.g. TNF), Aggregating, Substances
Antibodies:	Blood Group Ab, Anti-Lymphocyte Sera, Monoclonal Ab.
Lectins:	Receptor Identification
Mitogenes:	Stimulation
Toxic Substances:	Toxicology
Biomaterial:	Selective Adherence, Lymphokine Production
Artificial Particles:	
Liposomes	Pathological Changes in Liquor
Latex	(Meningitis), Sera (Cystic Fibrosis, LDL/HDL-Relation),

Characterization of Particles:

size and size distribution

surface charge

magnetic susceptibility

…

…

in vivo:

biocompatibility and circulation time

localization

…

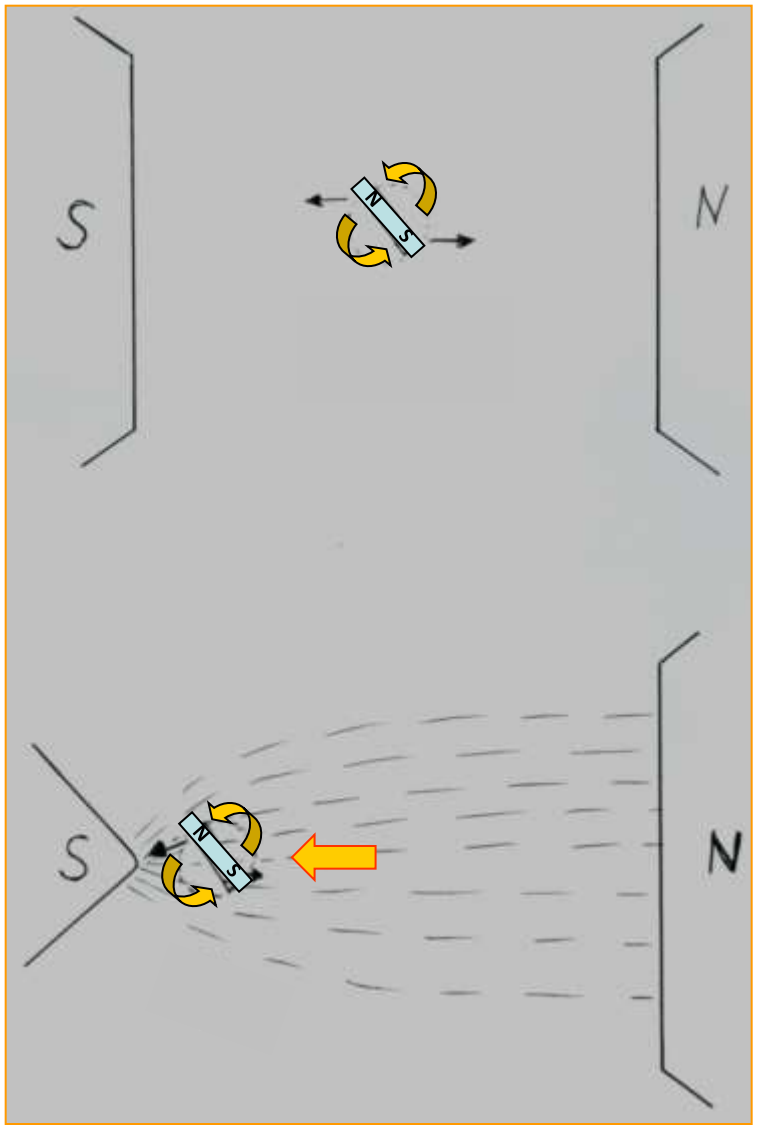
Physics: Magnetic particles (dipoles) in a magnetic field

Homogeneous Field

- Twist and friction
- Heat formation

Inhomogeneous Field

- Turns and moves



Characterization of Particles:

size and size distribution

surface charge

magnetic susceptibility

…

…

in vivo:

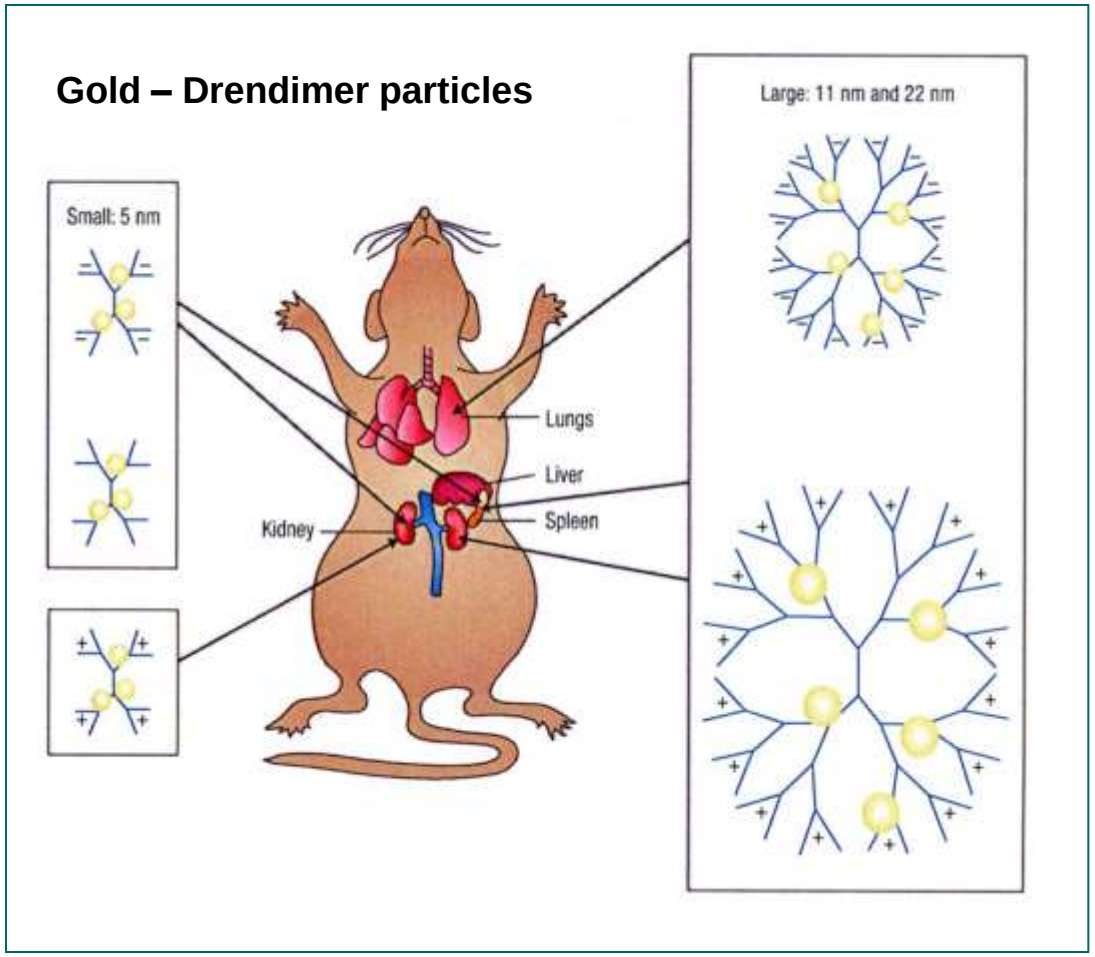
biocompatibility and circulation time

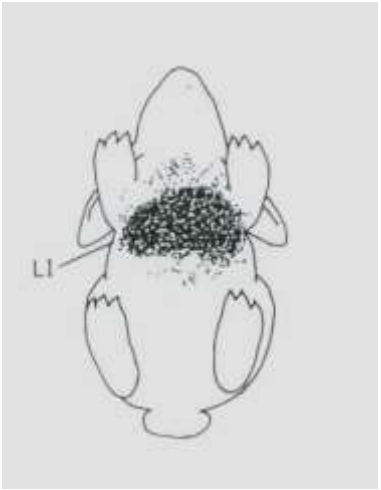
localization

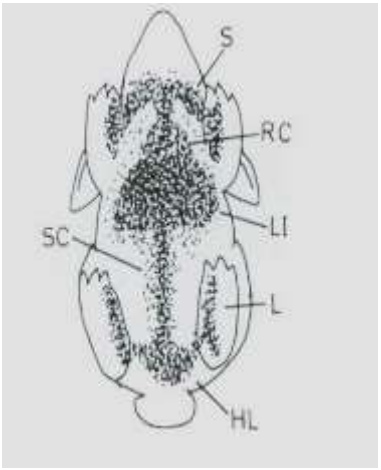
…

Biodistribution of modified Nanospheres
(size: 5 - 50nm)

Surface modification allows organ-specific targeting



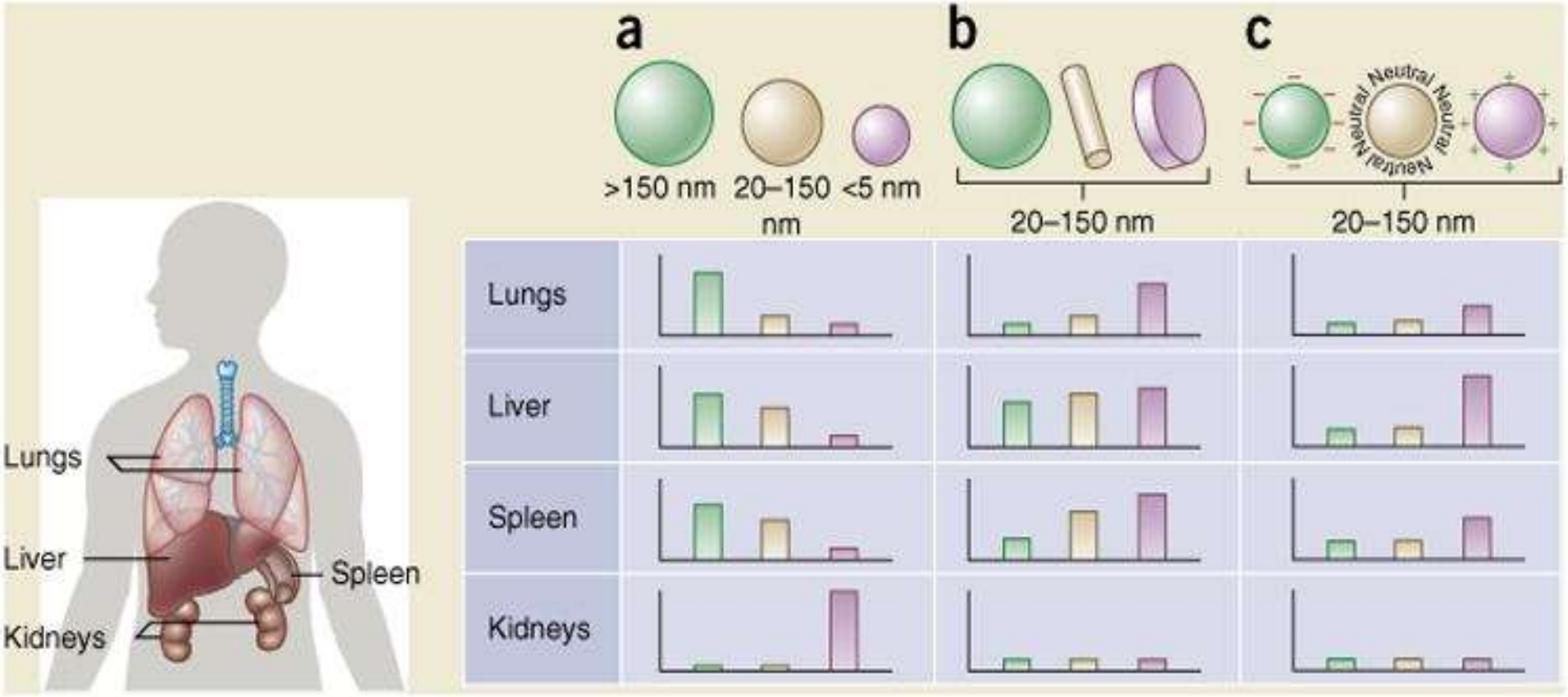




R Minchin, Nature Nanotech, 3:12-13 (2008)

R. Müller, Dept. Pharamcology, FU Berlin

Chemical Structure, Porosity and Surface Charge



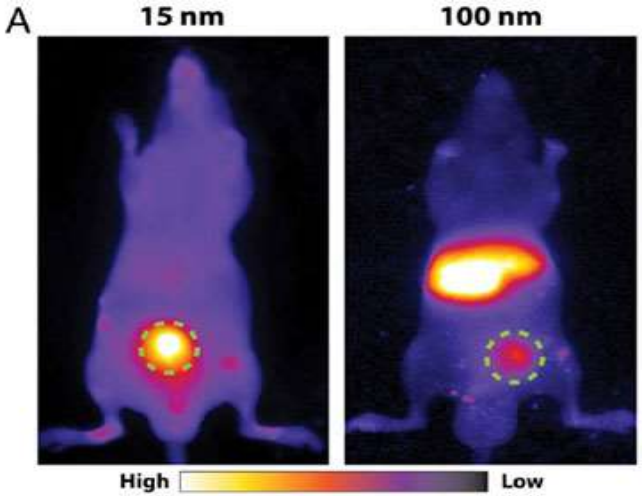
Strategies for nanoparticle biomimicry for prolonged circulation.

- **PEGylation provides a hydrating layer that hinders formation of a protein corona.**

- **CD47 peptides are attached to the surface of nanoparticles, after which macrophages identify the nanoparticle as ‘self’, whereby the nanoparticle avoids phagocytosis**

- **Coating of nanoparticles with cell membranes extracted from autologous leukocytes and red blood cells**

=> „Personalize nanoparticles to target tumours“



A. Whole-animal fluorescent images (mice, prostate tumours, Au-nanoparticles

B. Magnetic resonance images

Nanoparticles can be used to treat cancer, but a "one size fits all" method is far from ideal, say researchers from the universities of Toronto and Calgary in Canada and the University Health Network in Toronto. Instead, the size, shape and composition of the particles need to be tailored to the biological and physical properties of a tumour, which can vary from patient to patient and the stage at which a tumour.
"We are introducing here the idea of personalized nanomedicine," explains team leader W. Chan, Toronto

Characterization of Particles:

size and size distribution

surface charge

magnetic susceptibility

…

…

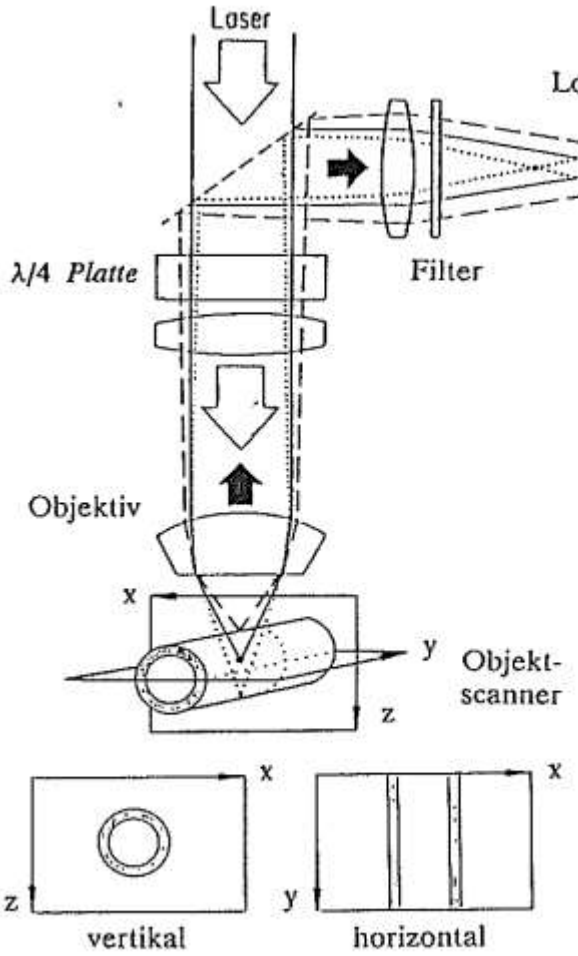
in vivo:

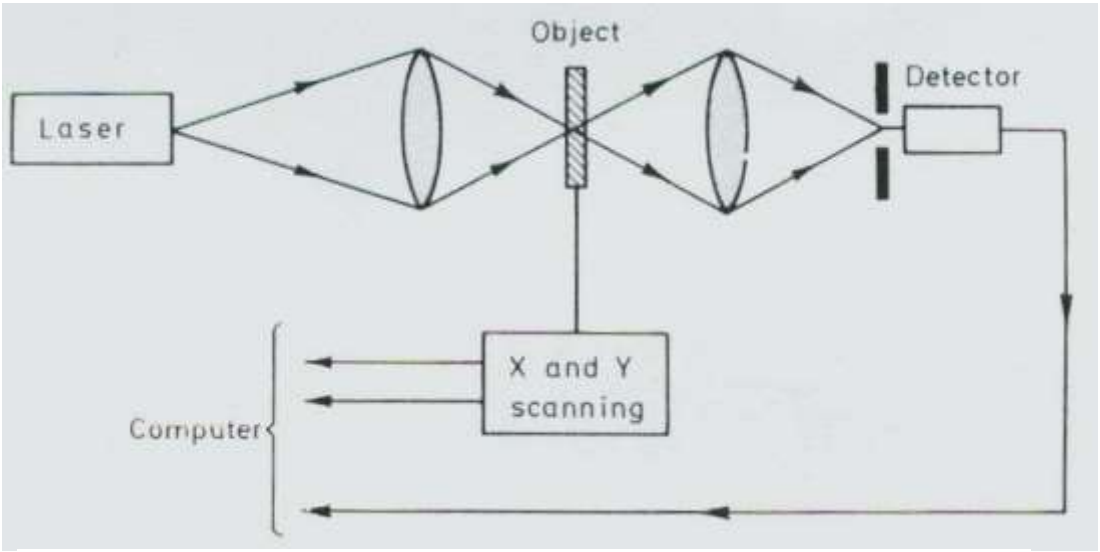
biocompatibility and circulation time

localization (in vitro and in vivo)

…

Confocal Laser Scanning Microscopy Typ:
=> Optical Sectioning of Cell Nucleus, Tissue, Eye Lens

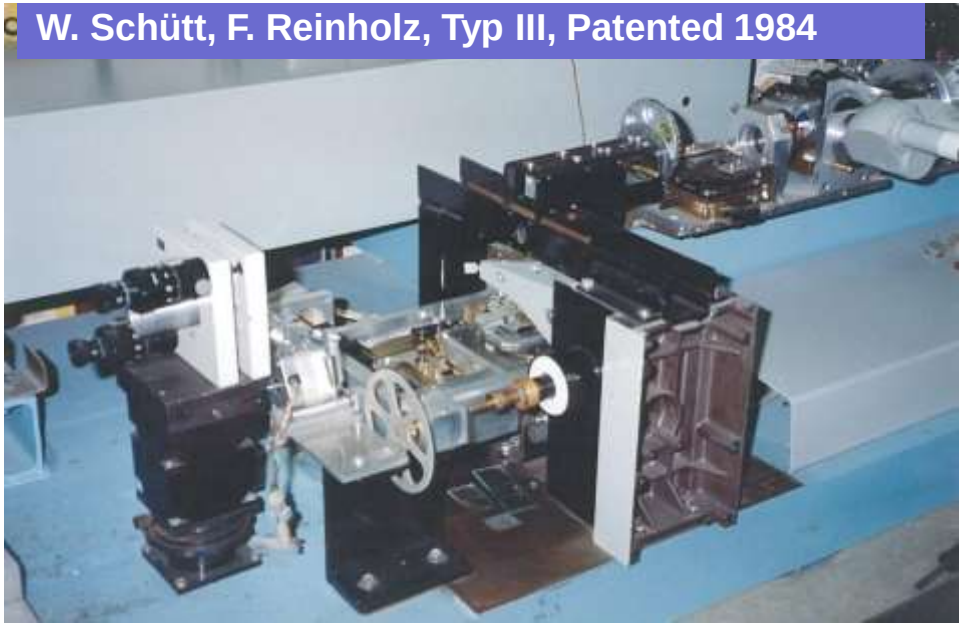


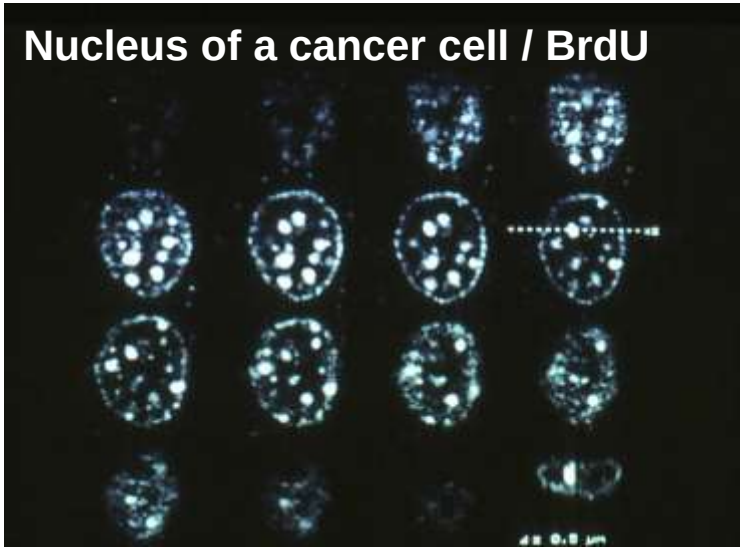


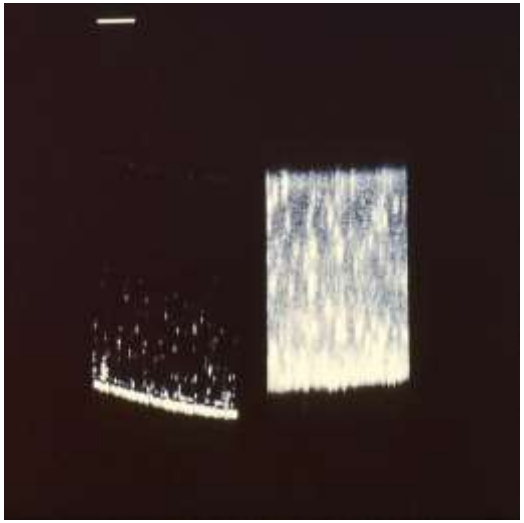
Theory: Tony Wilson, Oxford University

Prize: 0,2 – 1 Mio. €
Global Market: around 6 Mrd. €

First Confocal Laser Microscope Scans in Rostock, 1984

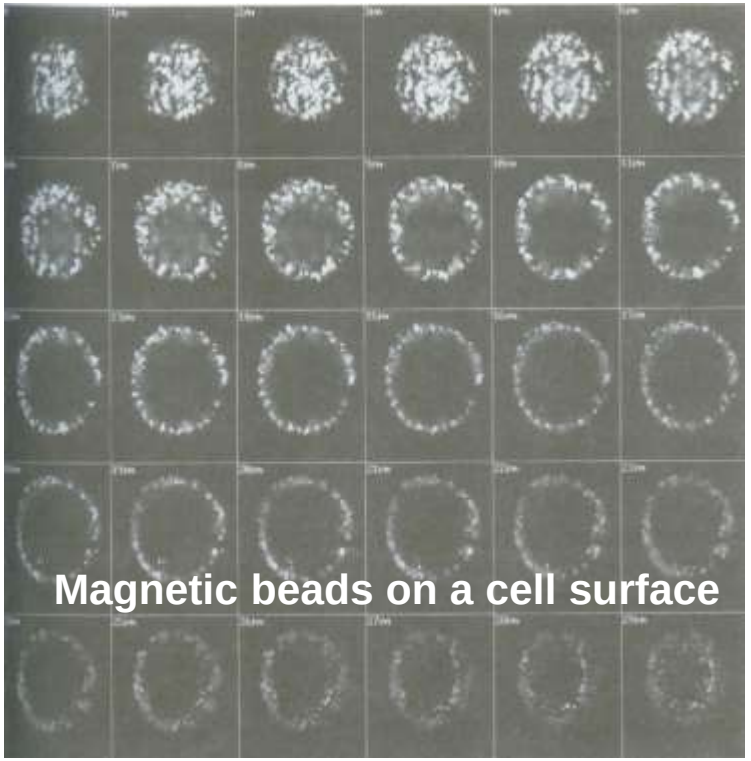






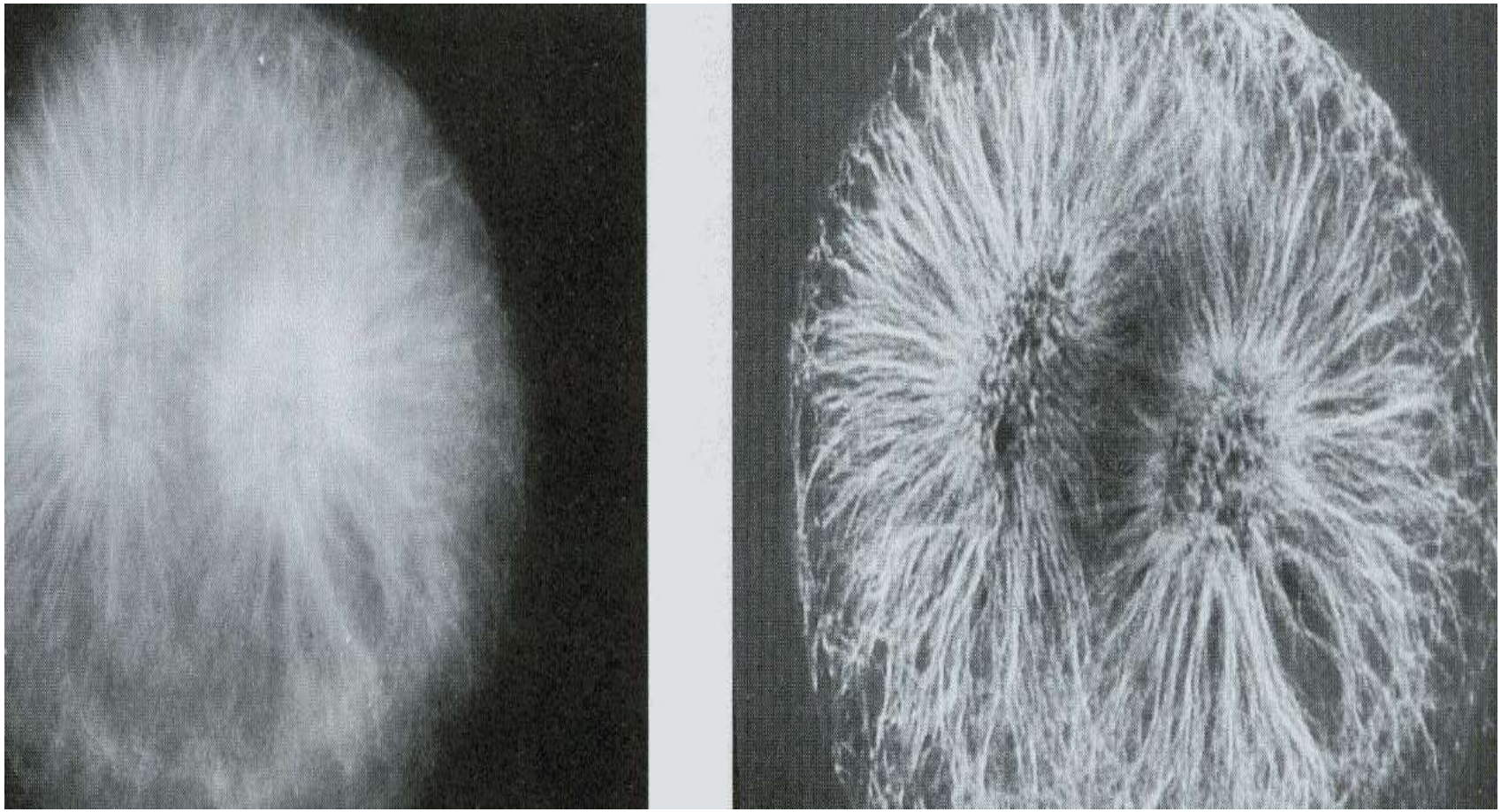
Vertical Sectioning:

Dialysis membrane with charge sensitive fluorescent dyes



Stine Kraeft, Rostock Univ. and Harvard Univ.

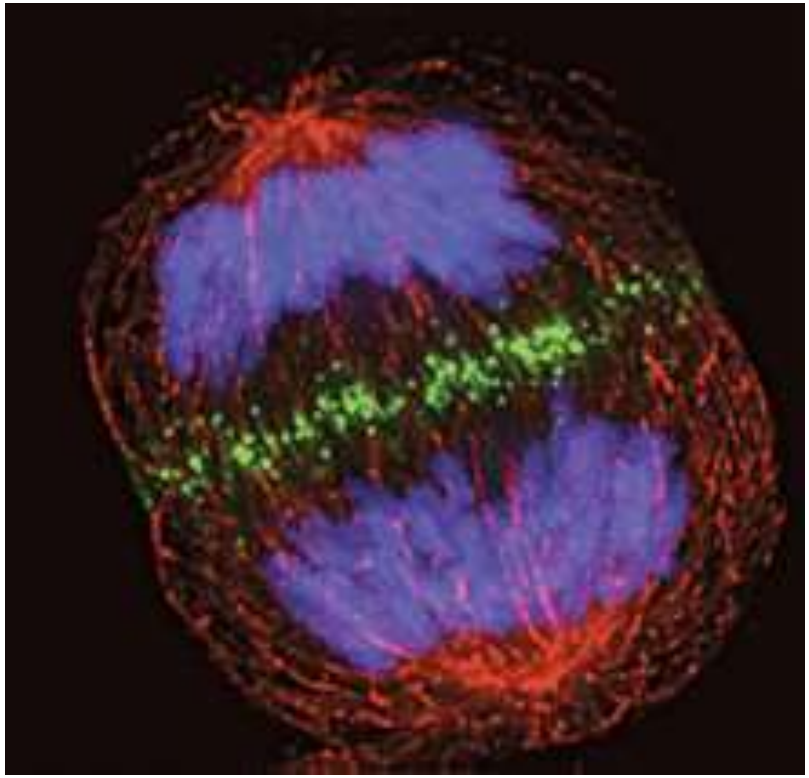
Conventional light microscopy and Confocal laser scanning microscopy

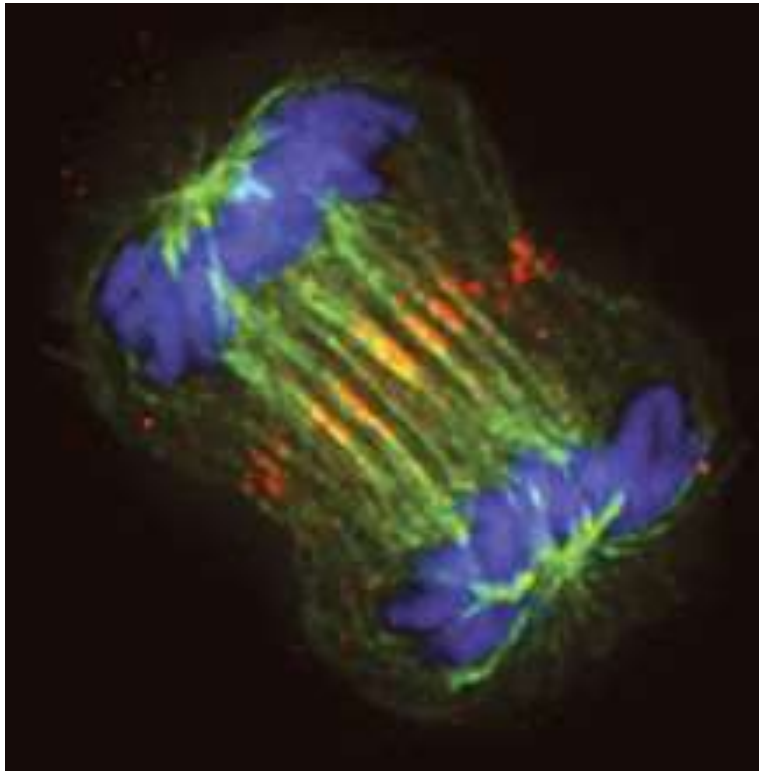


Scattered light from "out-of-focus" plane reduced

Source b.7.

Laser Scanning Fluorescence Microscopy



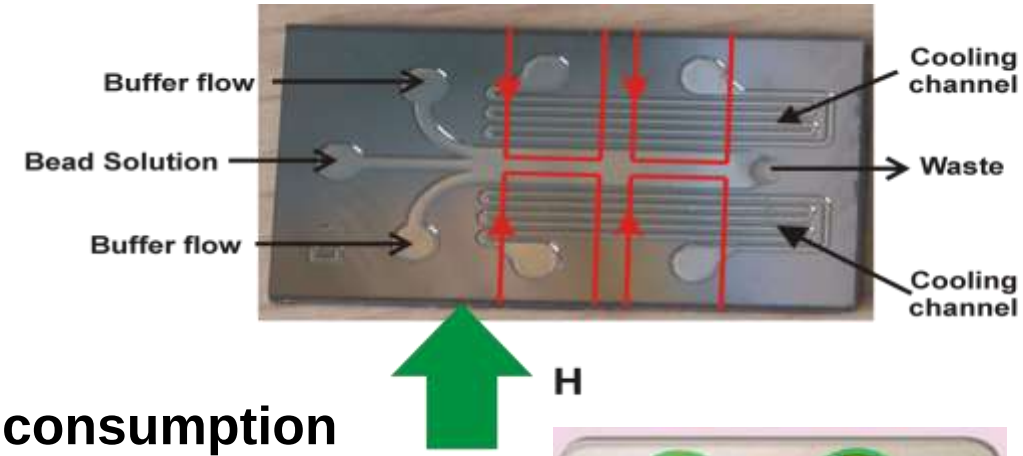


Cell division

Source c.2.

Lab-on-a-ship Technologies

Lab-on-a-chip



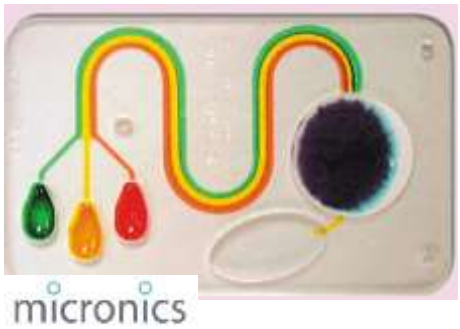
→ **reduced** sample and reagent **consumption**

→ **superior performance**
(speed, efficiency, control)

→ **combination**
of sampling, pre-treatment,
separation and detection
onto one device

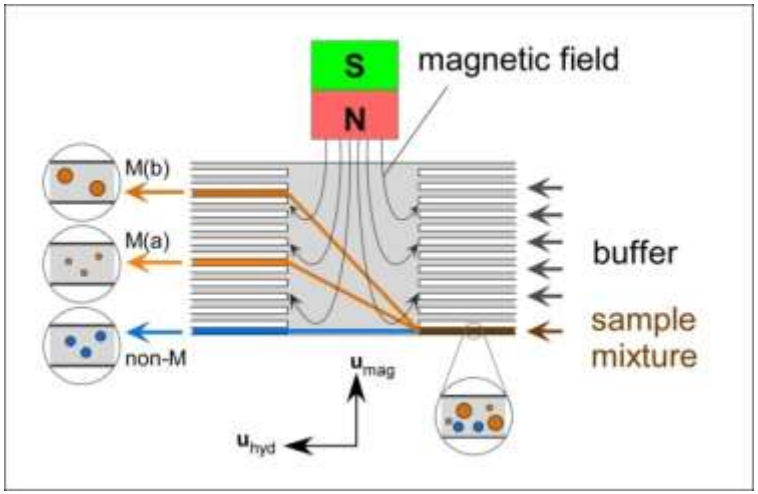
→ **facile integration**
of sensors and microelectric components

→ **portability** (point of care / in the field detection)

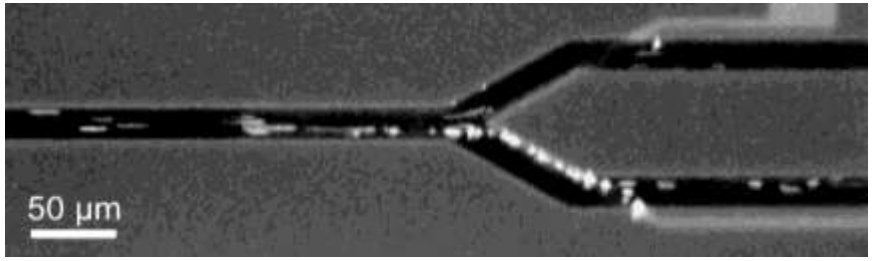


Micronics, U.S.A.

Magnetic Micro-Channel System for Continuous Cell Separation:

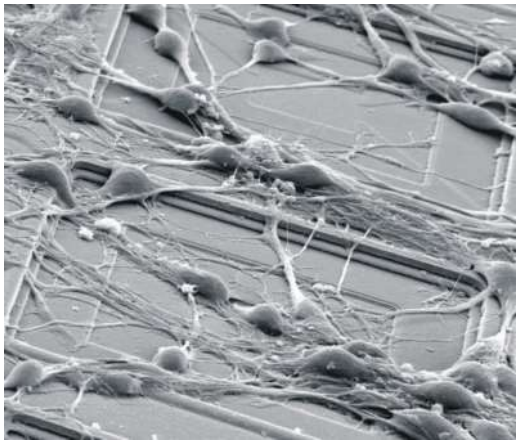


N. Pekas, Iowa State Univ.



G. Hansen, Technical Univ. Copenhagen

"Neuro-electronics": Testing of Communications (Cancer, Bioactive Substances)



Integration
of sensors and electric components
(microelectronics know how !)

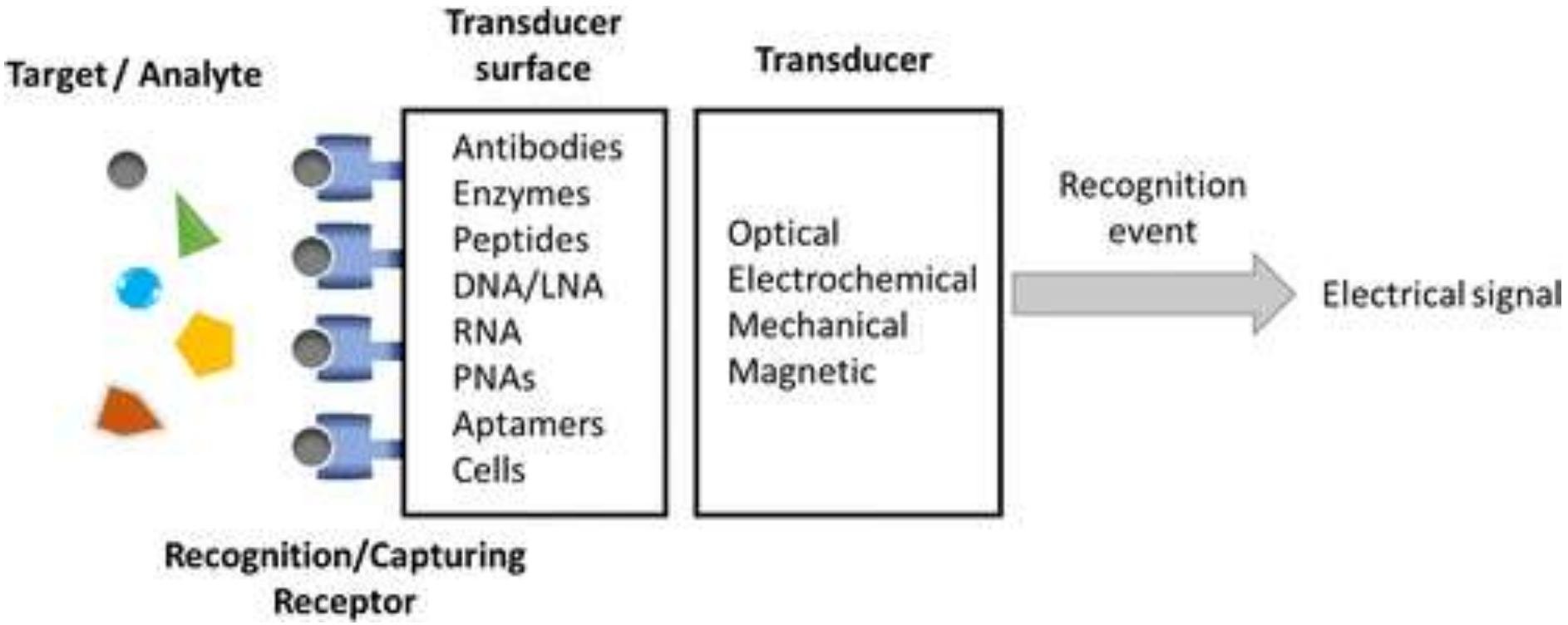
Detection of cell-cell-communication:
Toxicology
Cancer

D. Weiss, Univ. Rostock

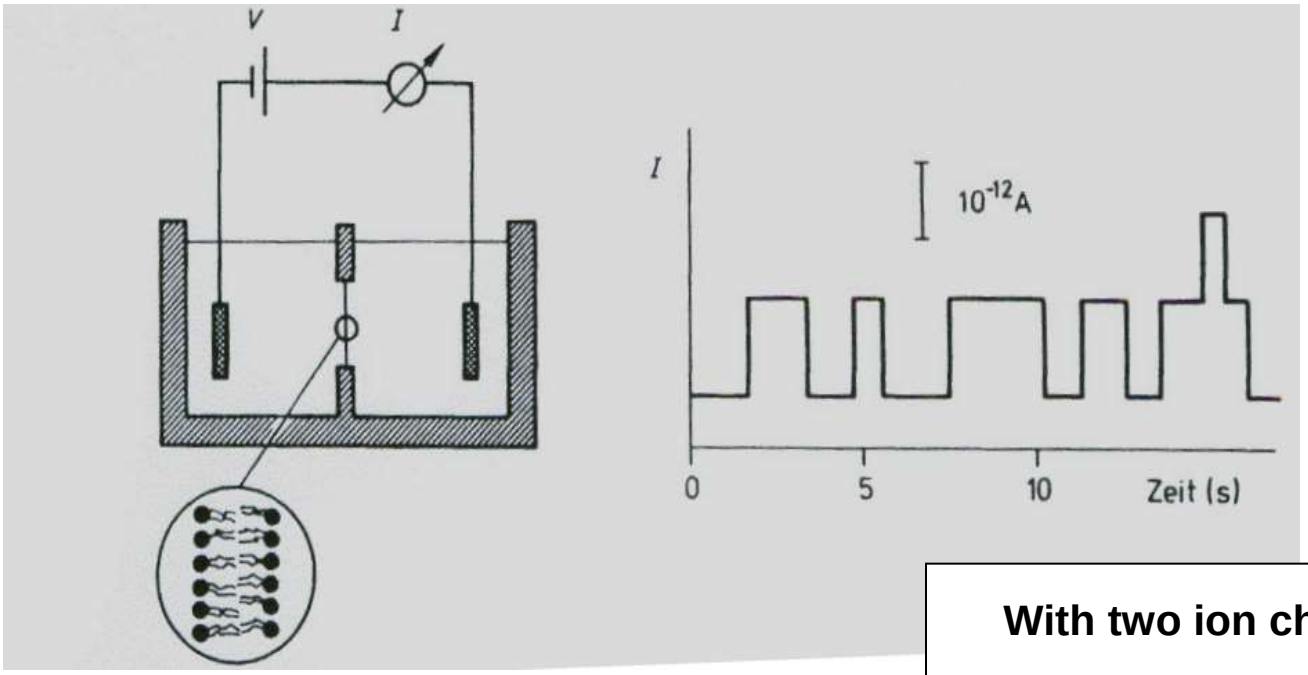
Nanosensors

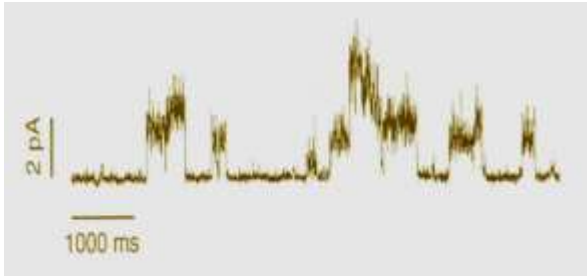
An example for cooperation between

Medicine, Immunology, Molecular Biology, Chemistry, Physics, IT



Model Membrane: "Black-Lipid-Layer" with Ion Channel





With two ion channels

Thickness: 10 nm => Electron microscope

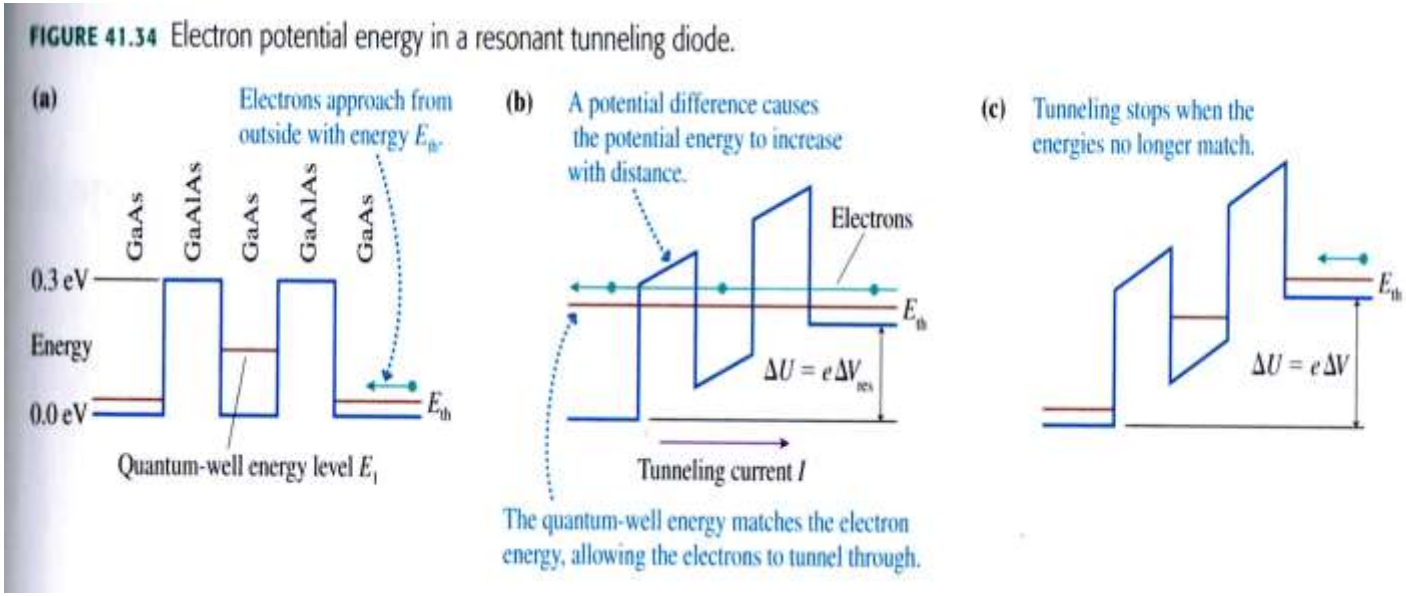
Electrical resistance: $1 - 10^4$ Ohm cm^2 (natural membrane material)

10^8 Ohm cm^2 (artificial protein-free material)

Electrical capacity: 1µ Fcm^{-2} (for all cell types !)

Incorporation of transport systems => electronic detection of switch-on an -off

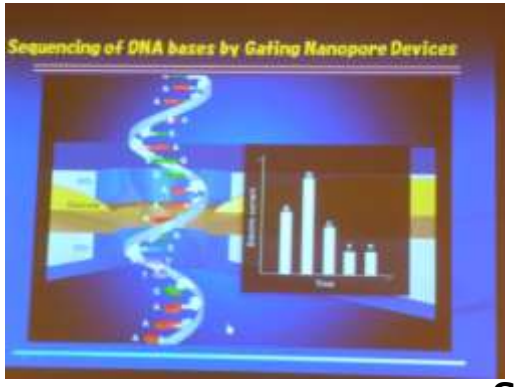
Resonance Tunneling through double barriers

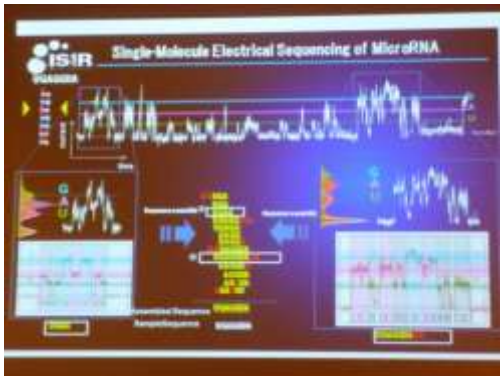


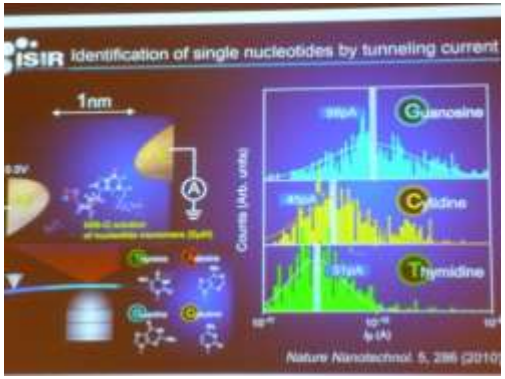
DNA – Sequencing via tunneling effect

P1040027.MOV

N. Kawai, Tokyo
AsiaSense Conference, Seoul 2011







Slides taken from Kawai´s conference presentation, Schütt

High-throughput Cell Separation for Delivery of Highly Enriched Cell Subpopulations for Gene Expression Microarray Analysis

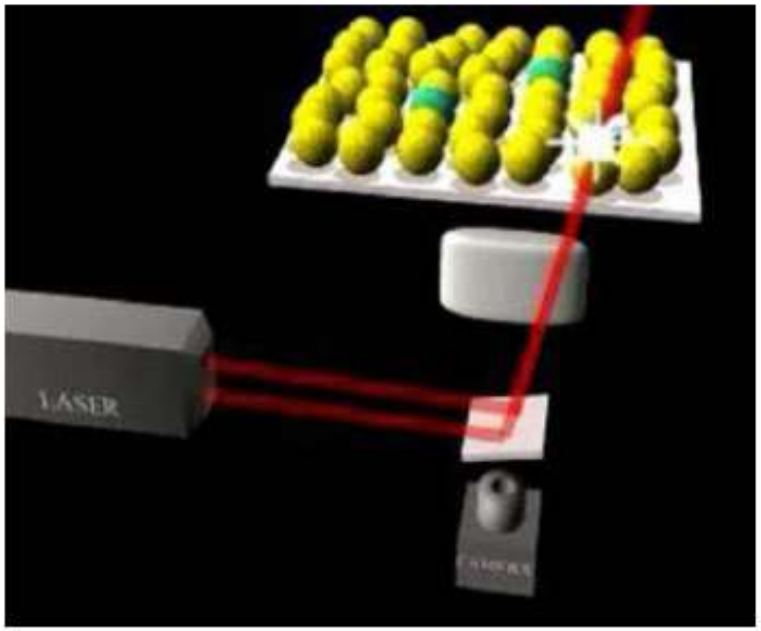


Figure 1: A new cell separation technology (LEAP™, Oncosis, Inc.) first analyzes cells by high-speed, bright-field and/or fluorescence imaging. Then living cells not-of-interest are eliminated from the cell suspension or tissue. The laser can also be used to opto-inject cells at very high speeds for introduction of molecules into living cells without the need for conventional transfection techniques.

LEAP™ (Laser-Enabled Analysis and Processing) has throughputs greater than 100,000 events/sec, high cell purity, yield and viability. It can process several cells or a billion cells with an expanded cell range including fragile cells. Another advantage is that it can analyze and purify biohazardous cells without generating aerosols .

Fluorescence collection optics of LEAP instrument

Shooting at cells inside 384-well plates to eliminate undesired cells and capture desired cells for subsequent gene expression microarray analysis

Source: Oncosis, Inc.

R&D Profil of the Inst. of Biotechnology, IMC FH Krems:

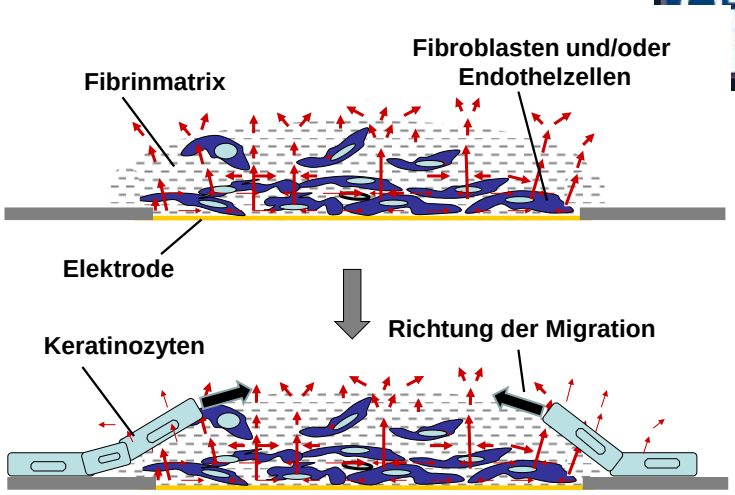
New cell-based technologies for identification and testing of bioactive substances (20 Mio.€)

e.g.:

Wound healing assay

Metastasis model

Intestinal models for Colitis



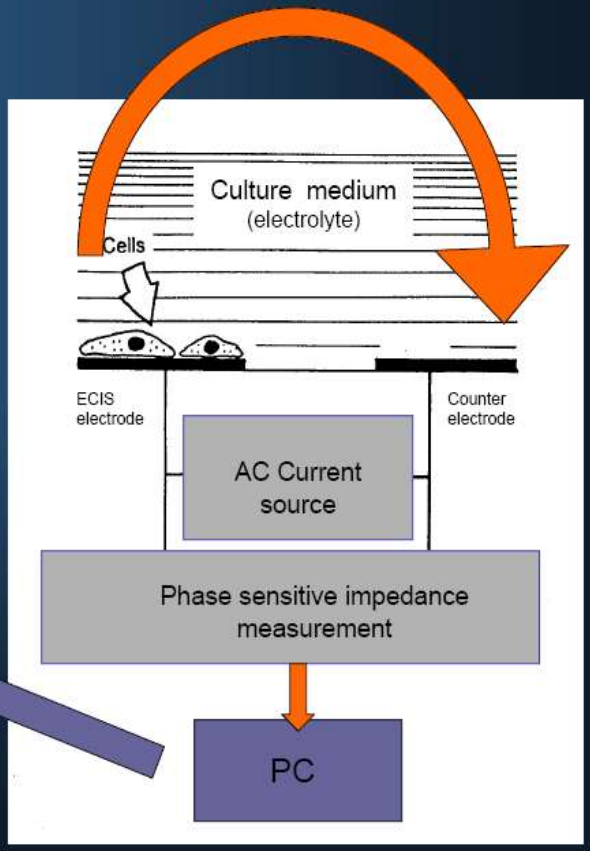
Applied Biophysics, Inc. Troy
Founded by Ivar Gieaver, 2004

ECIS™

Electric Cell-substrate Impedance Sensing

A cell morphology biosensor

The measurement is non-invasive



Ion channels ?
Pores ?
Cell growth ?
Cell interaction ?
Cell migration ?

=> Influence of bioactive substances

See: FH Krems Master-Lab-Course !

Magnetic Particles

Rostock (1996), Cleveland (1998), Rostock (2000), Tallahassee (2002), Lyon (2004)

6th International Conference on the

Scientific and Clinical Applications of Magnetic Carriers

May 17 – 20, 2006

Krems, Austria (Vienna Region)

THE CLEVELAND CLINIC FOUNDATION

Krems an der Donau, a one hour drive from Vienna Airport, is the eastern gateway to the Wachau
one of Europe's loveliest river landscapes with a famous wine culture. Its history which extends back
thousand years is evident everywhere - in the streets and squares, in the old monasteries and chu
the hundreds of original cellars of the winemakers.

The historical center of Krems is one of the most beautiful in Europe. In 1975 Krems was singled ou
City for Historical Preservation" and in 2000, it was added to the UNESCO World Heritage List.

You are cordially invited to our next and 6th Conference by:

Urs Häfeli
University of British Columbia
Vancouver, Canada

Wolfgang Schütt
IMC University of Applied Sciences
Krems, Austria

Maciej Zborowski
The Cleveland Clinic Foundation
Cleveland, U.S.A.

Mirka and Ivo Safarik
Academy of Science
Ceske Budejovice, Czech Republic

Topics:

- Biocompatible Magnetic Particles
- Molecular Biology and Genetics
- Cell Biology and Tissue Engineering
- Specific Drug Delivery
- Hyperthermia
- Blood Purification
- Imaging Technologies
- Quantum Dot Sensors
- Lab-on-Chip-Systems
- Nanobiotechnology

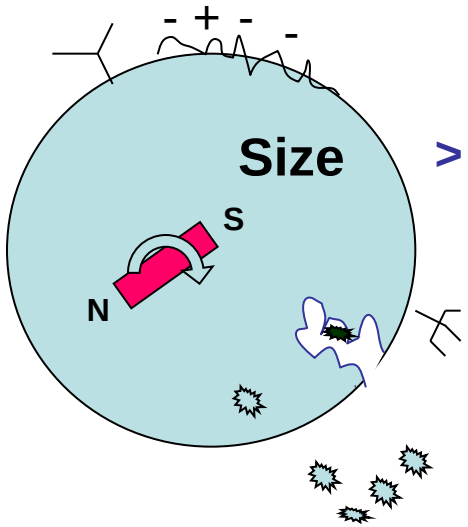
350 participants from 43 countries in Krems

Biomedical applications of magnetic particles

Targeting

Imaging

Biodistribution



> 100nm – 10µm

Tracking

Adsorption

Heating

Release

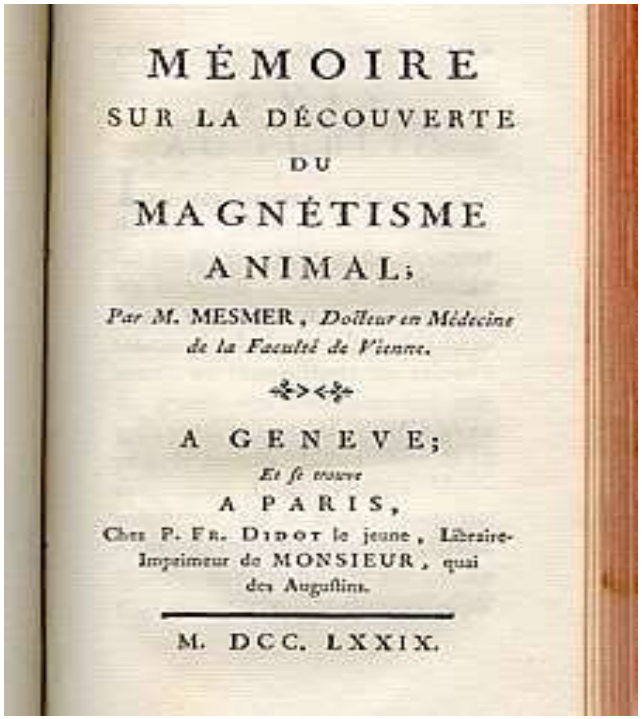
5-100nm

Nanobiotechnology

Imaging Transporter Nanoprobes Sensors Lab-on-a-Chip

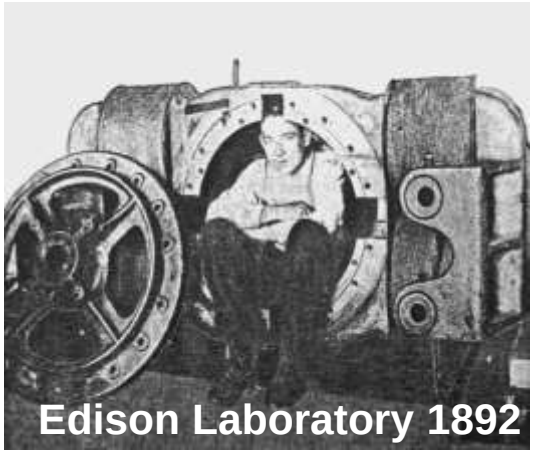
"Magnetism"

Mesmerized in Vienna



MÉMOIRE
SUR LA DÉCOUVERTE
DU
MAGNÉTISME
ANIMAL;
Par M. MESMER, *Docteur en Médecine de la Faculté de Vienne.*
A GENEVE;
Et se trouve
A PARIS,
Chez P. Fr. Didot le jeune, Libraire-Imprimeur de MONSIEUR, quai des Augustins.
M. DCC. LXXIX.

Mesmer
1734 – 1815



Edison Laboratory 1892

???? 2007

What kind of magnetic particles are useful ?

e.g.

Magnetic Bacteria

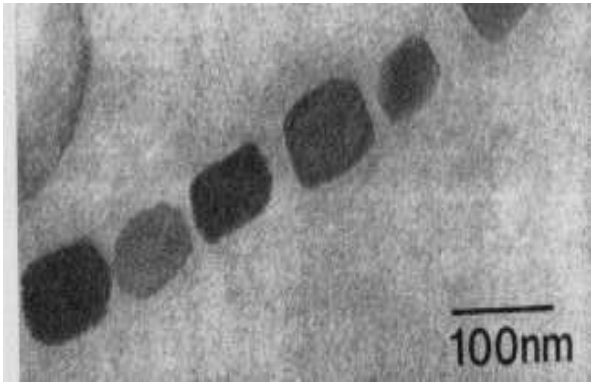
Polymers

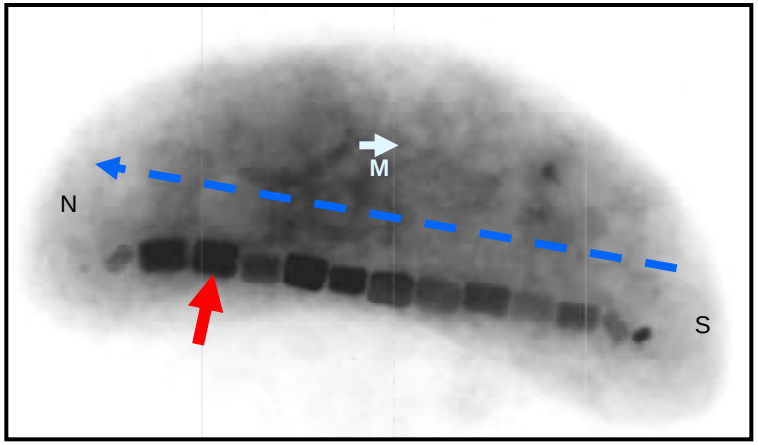
Cells labelled

Erythrocytes loaded with magnetic particles

Magneto-Liposomes

Magnetotactic Bacteria





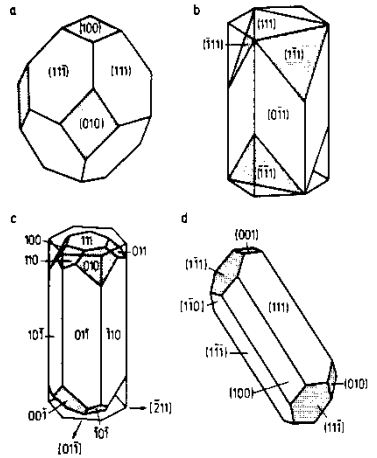
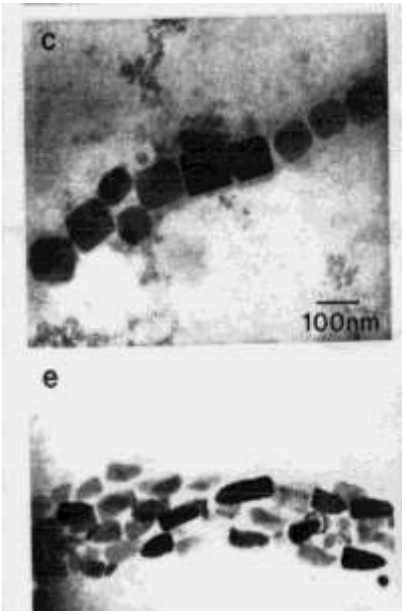
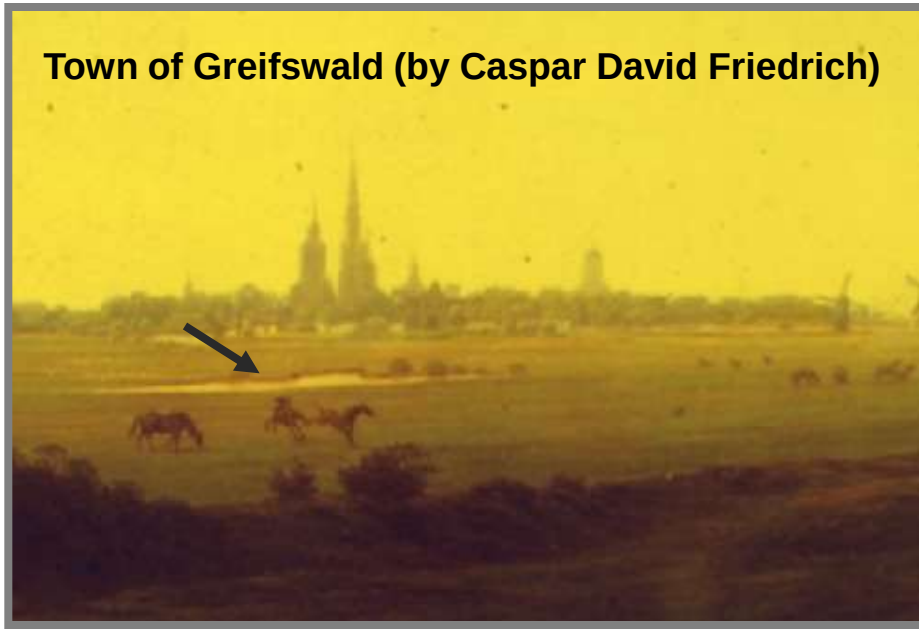


Fig. 5. Idealized crystal morphologies of magnetite in magnetotactic bacteria derived from HRTEM studies: (a) cube-octahedron; (b) and (c) variations of hexagonal prisms; and (d) elongated cubo-octahedron. (Reproduced from Mann and Frankel, 1989, with permission of VCH.)

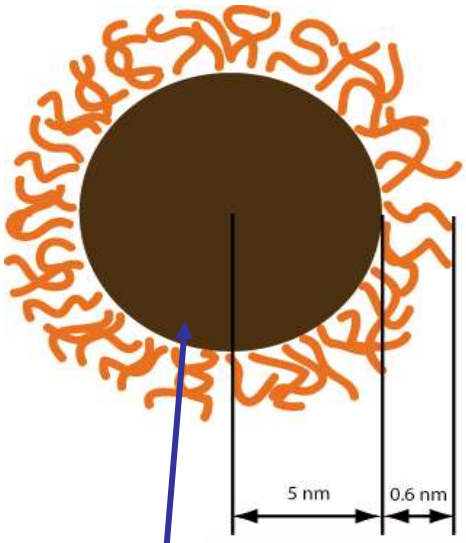


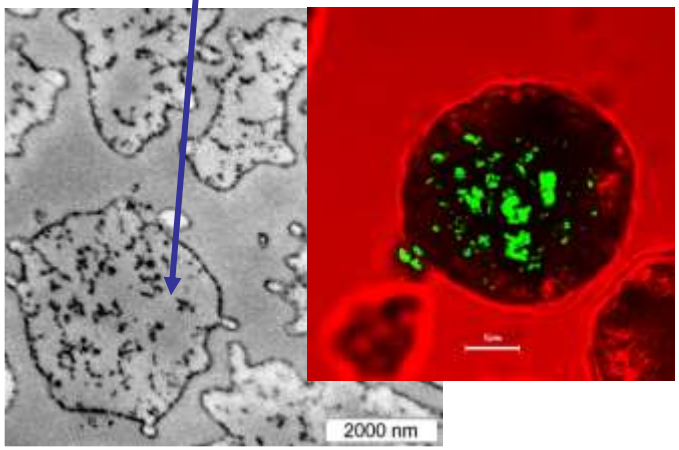


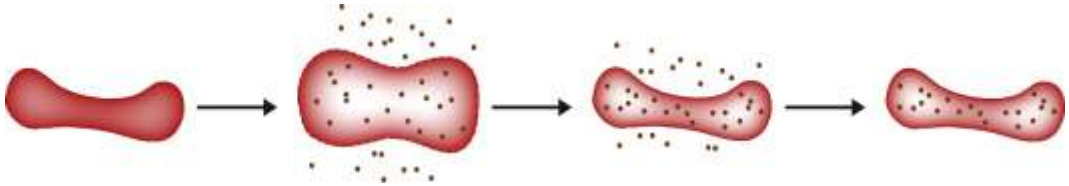
Natural Habitat of *M. gryphiswaldense*

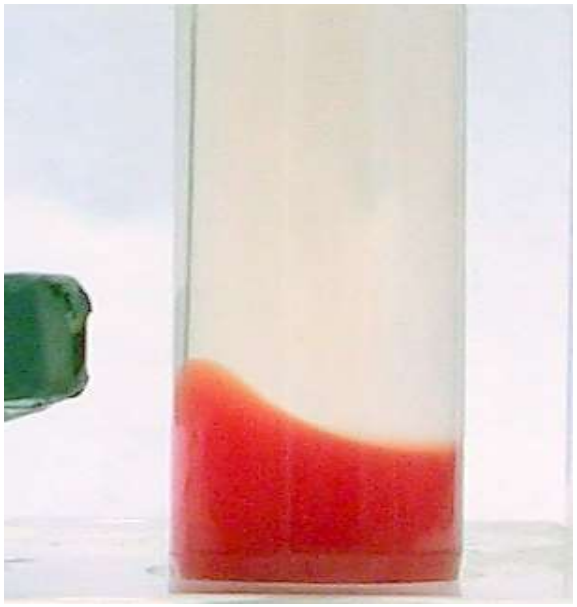
Magnetite loaded RBC

Loading with superparamagnetic iron nanoparticles by osmotic swelling, resealing, washing





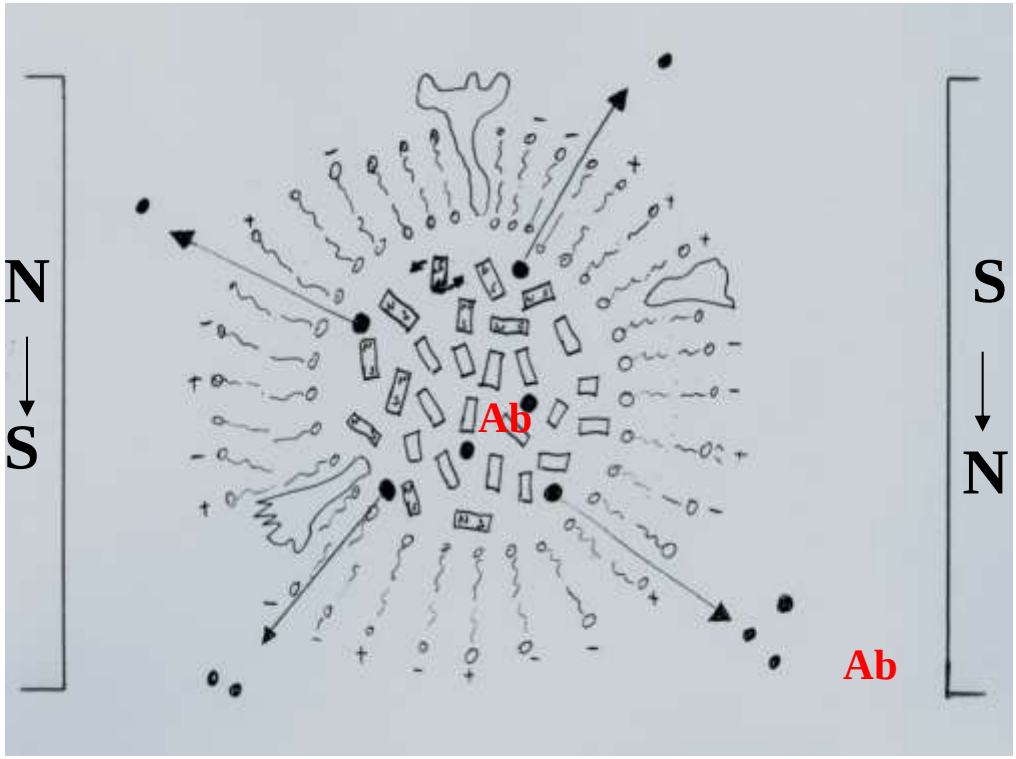




=> attractable by a permanent magnet
=> long circulation time

M. Brähler, Charité-Universitätsmedizin Berlin

Biocompatible magneto-liposomes



External alternating magnetic field:

=> twist / orientation of crystaloids

=> friction and heating (41 C !)

=> enhanced membrane permeability

=> Release of drugs, markers,…

Proposed at Rostock sailors pub "Kogge":

P. Todd, NASA,
De Cupers, Univ. Gent,
A. Kusnetzov, Acad. Sci. Moskau

International Cell Biophysics Conference, Rostock 1984

Biomedical Applications of Magnetic Particles based upon:

Movement

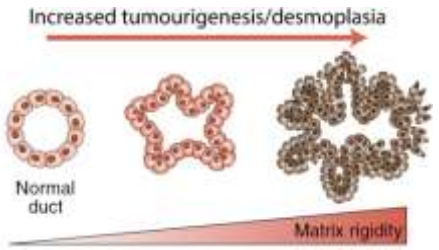
Retention

Heating

Localization

Magnetic Single Cell Viscosity Measurement

Tumour progression => matrix rigidity => circulation
(poor size)



=> settlement of tumour cells
=> migration through tissue
=> vesicle release
=> ion channel activity

Task of NanoMedicine:

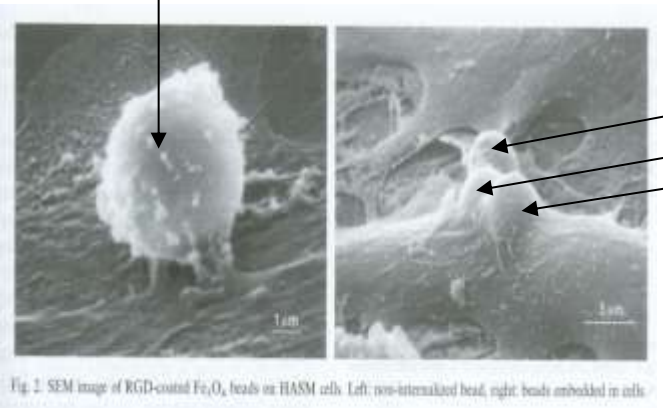
=> To find the right particle size for specific targeting and drug delivery

(A. Chronopoulos, in: Converting Science Physical Oncology, Feb, 2017)

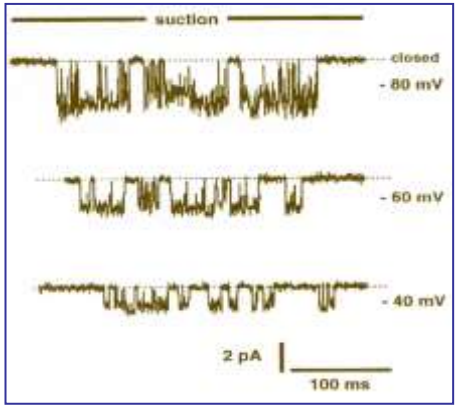
External inhomogeneous magnetic field

- **Displacement of the magnetic bead … viscosity ?**
- **Opening of stretch- activated ion-channels**

External beads

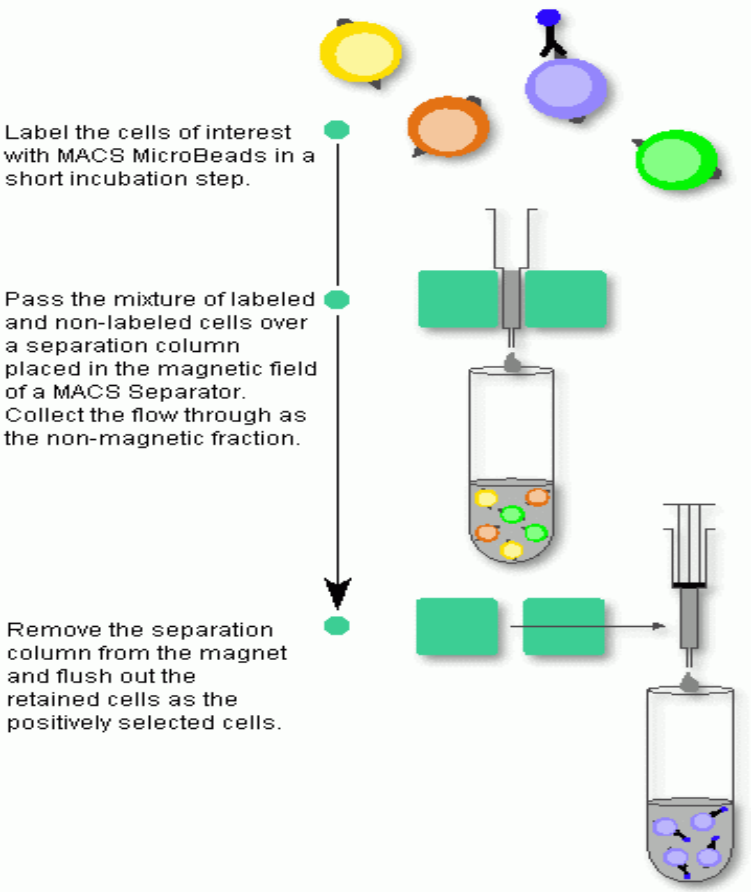


Internalized beads



W. Schütt, 1993-95, Cornell Univ., New York

Miltenyi Magnetic Cell Sorting System MACS

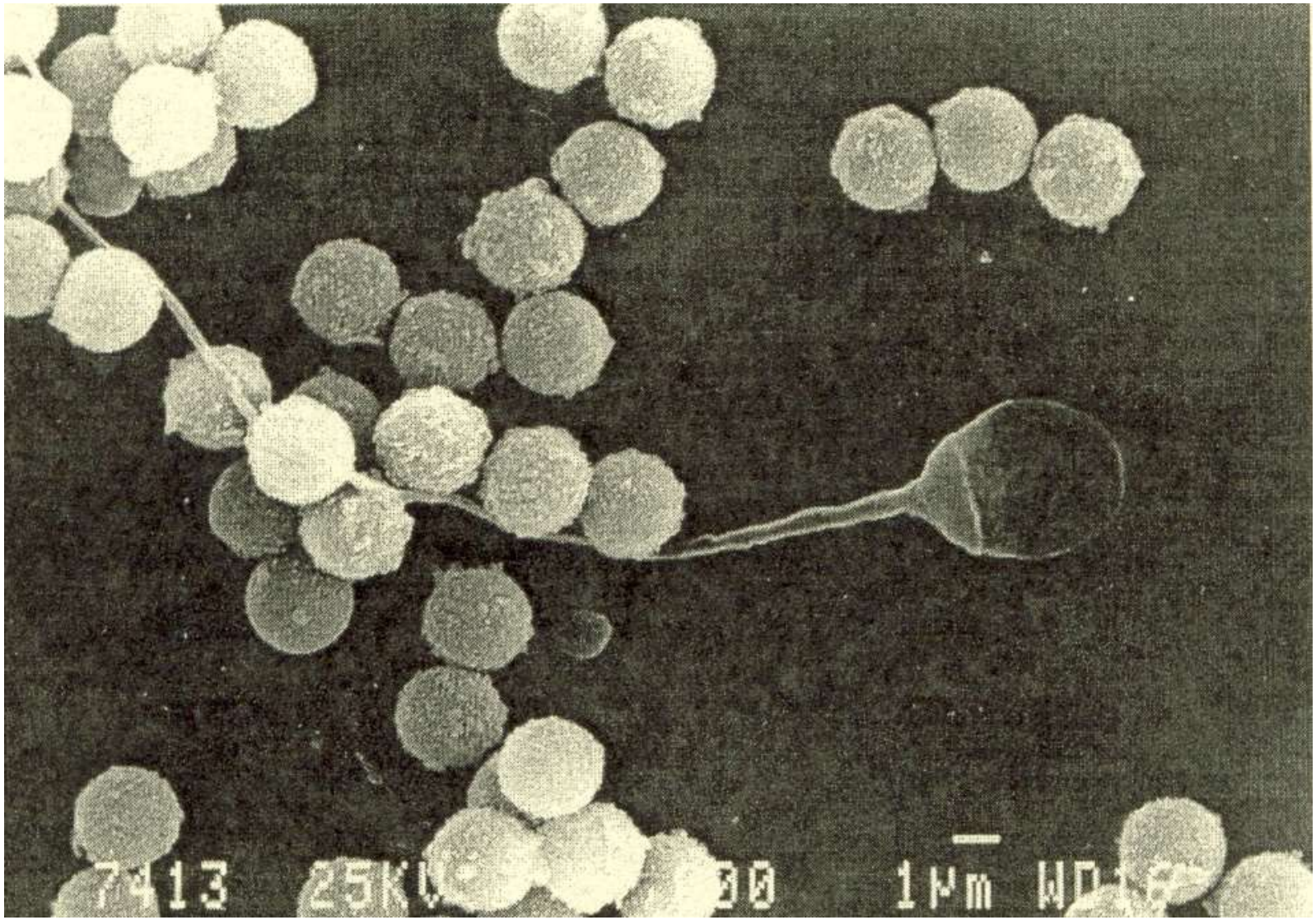


=> e.g. Bone marrow cells for clinical use

St. Miltenyi, Bergisch-Gladbach / Rostock

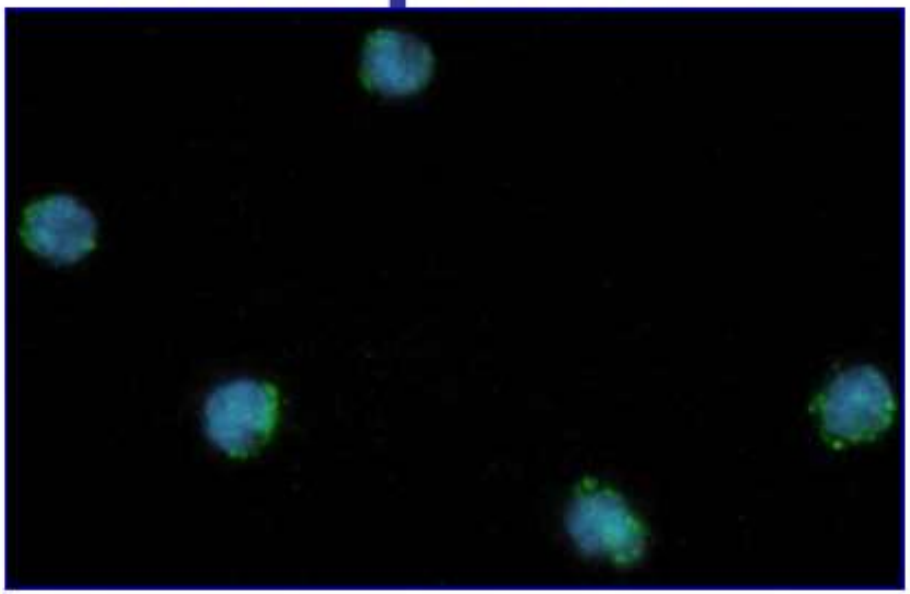
a.o.

Magnetic particles coated with antibodies:
Identification and selection of a single sperm cell

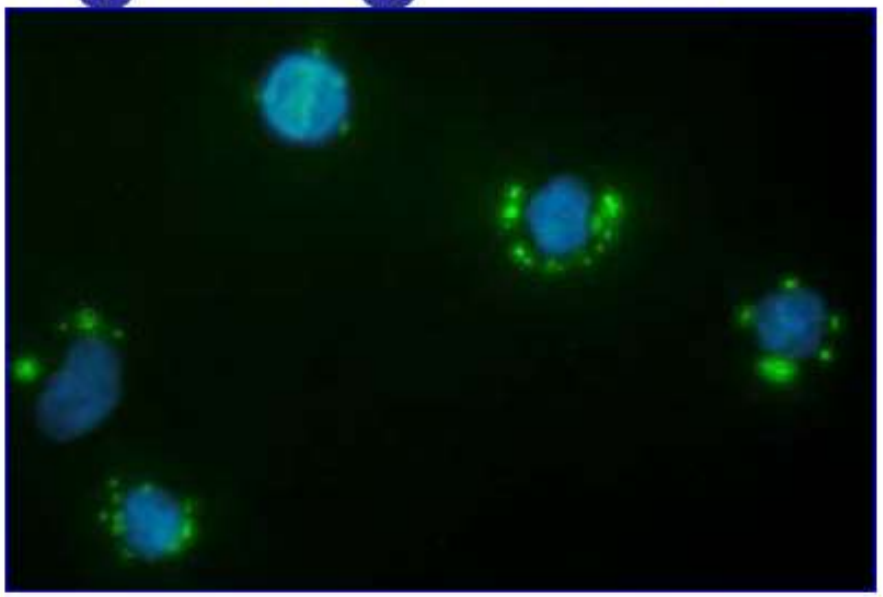


Is it feasible to find rare cancer cells in astronauts?

Nanoparticle Targeting Data



Conventional antibody labeling



Nanoparticle labeling

Targeting strategies already developed can detect one rare cell in a million other cells (similar to the expected frequency of cancer cells in astronauts exposed to space radiation)

For review see: Leary, 1994; Leary, 2000; Leary, 2005.

Note: Nuclei of cells are counter-stained blue with a DNA dye

Single cell detection sensitivity :

1 cell per	**1 000**	**with flow cytometry**
1 cell per	**200 000**	**immunochemistry**
1 cell per	**10 000 000**	**using PCR**

1 cell per 14 000 000 using nanoparticles

In vivo - Clinical Applications

of

Large and Small Particles

Particle size: > 200-300 nm

higher container capacity

directed movement and retention in streaming media possible

e.g. blood, digestive and interstitial fluid, air

Particle size: < 50 nm

increasing Brownian movement (magnetic guidance is not possible !!!)

longer circulation time

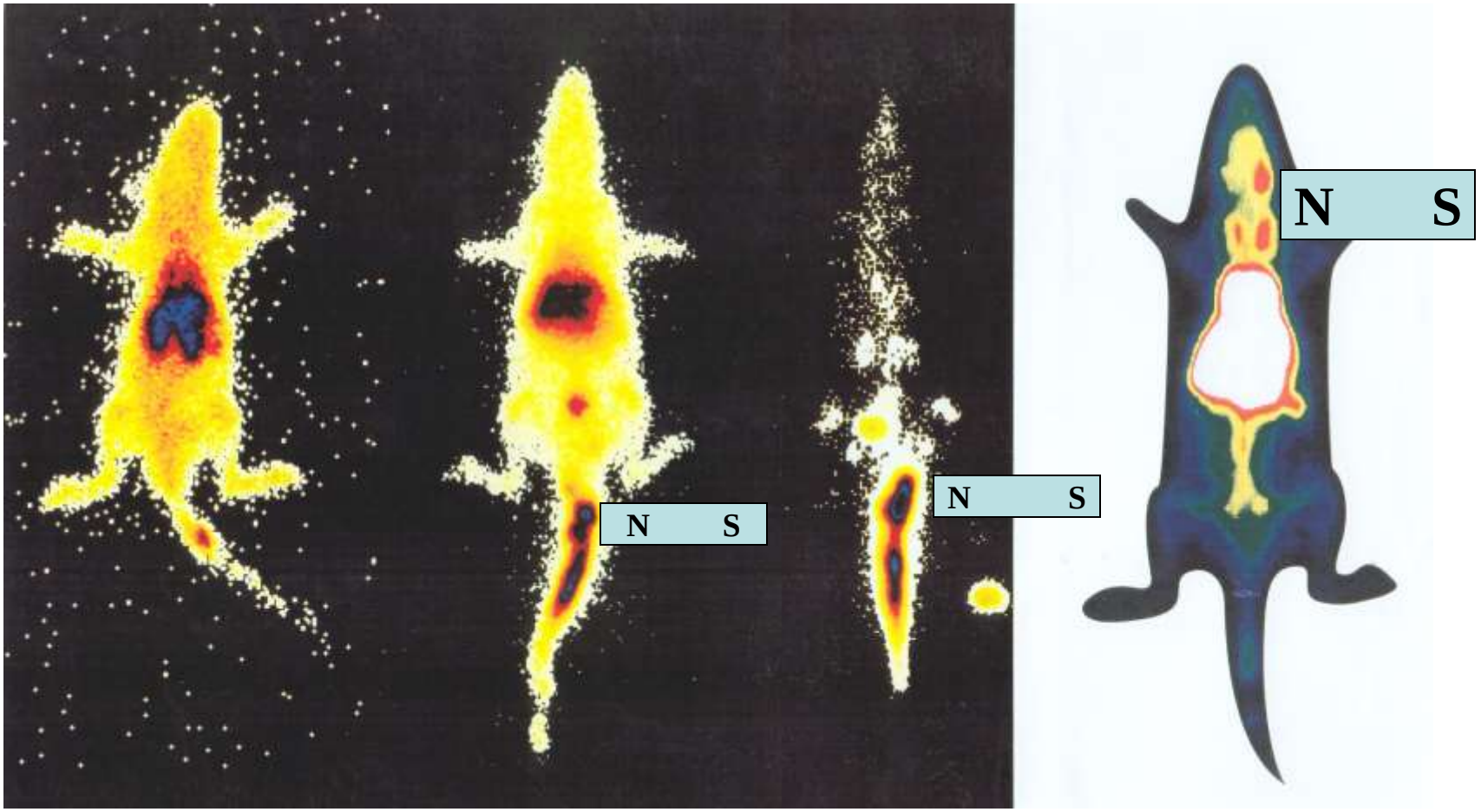
surface modification (electric charge, markers to influence the biodistribution)

crossing blood – brain – barrier

=> Toxic effects, degradation of materials?

Retention of Magnetic Particle:

Effect of size and surface modification !



200nm *600nm* *600nm - PEG*

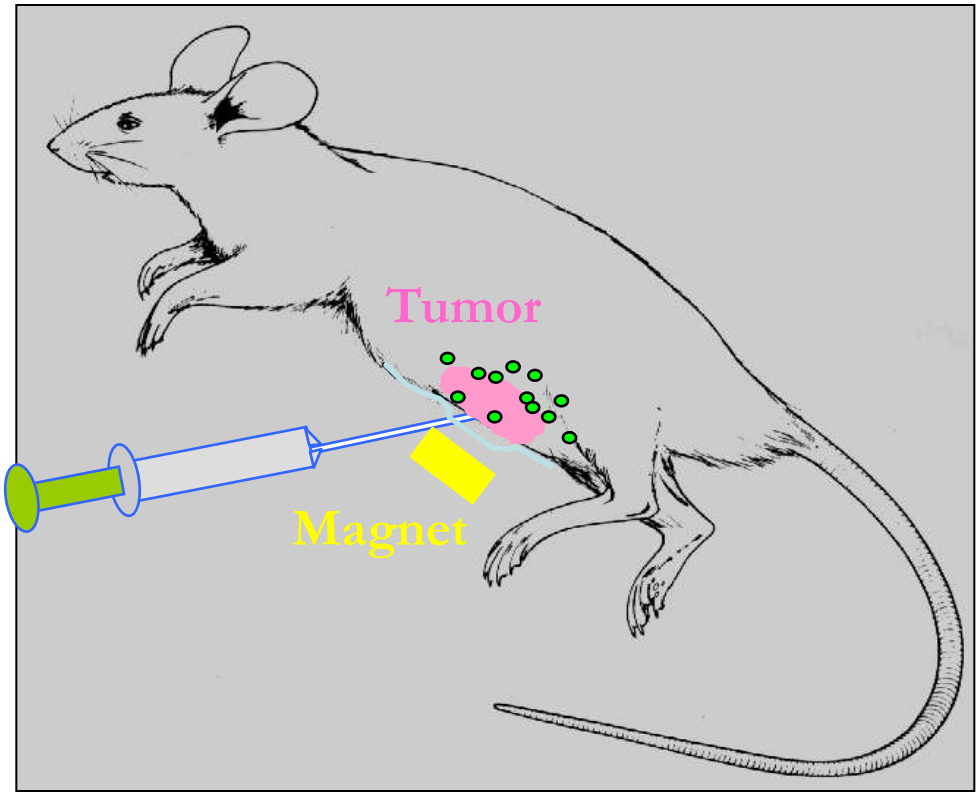
W.Schütt (1995 - 96)

How to overcome the low retention ?

How to target the magnetic carriers ?

Injection of magnetic beads into the tumor or the blood line, magnetic retention and heating

=> Necrosis and or Drug release.

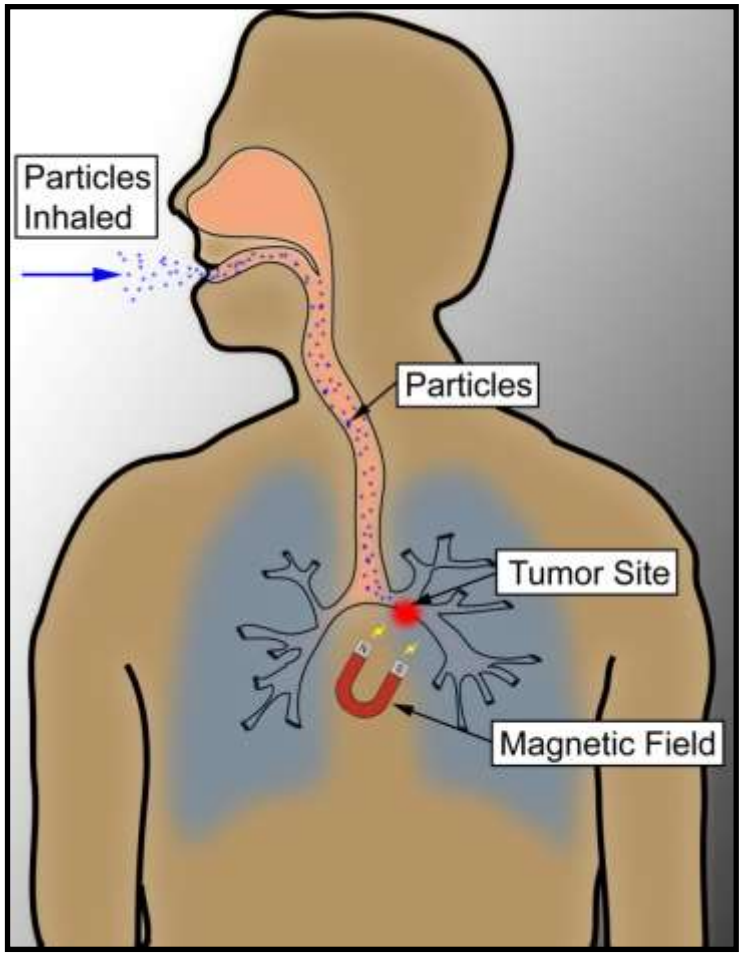


=> Problem: spread-out of suspension !

B. Kossisnek, PhD Alumni FH Krems (PTS in Vancouver) and Royal Inst. London

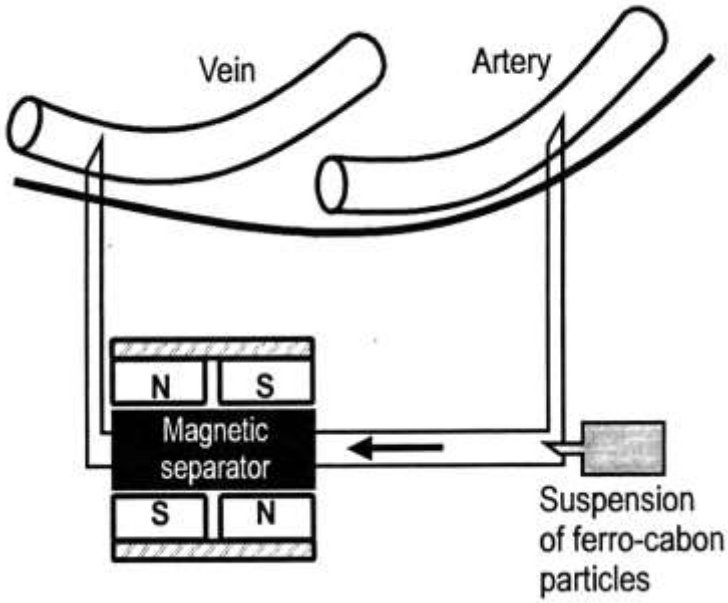
Magnetic targeting of aerosols to cancer sites in the conducting airways

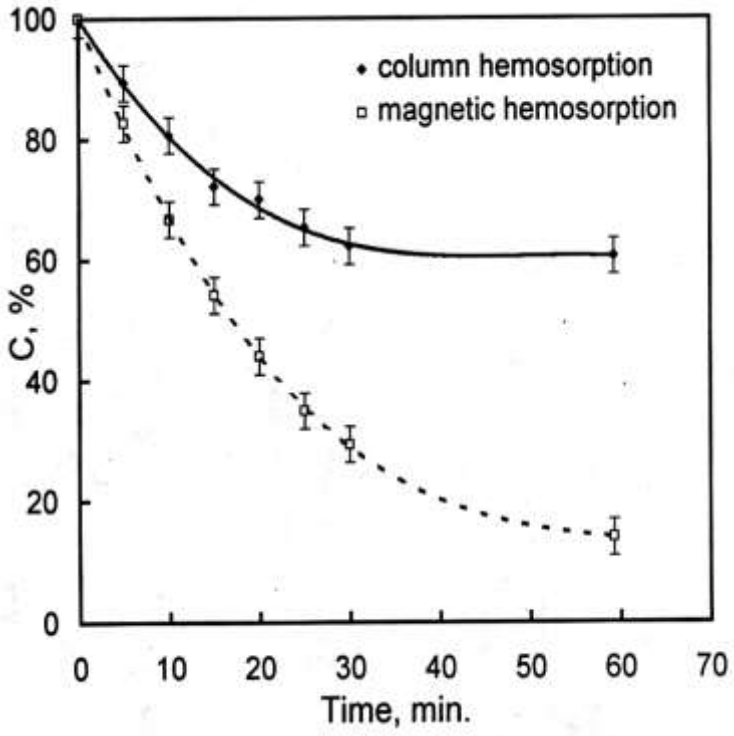
Inhalation of magnetic particles loaded with radioactive isotopes ?



J. Ally, University of Alberta

“Proposed” Magnetic Hemo- or Cell-sorption !





Extracorporal magnetic removal of

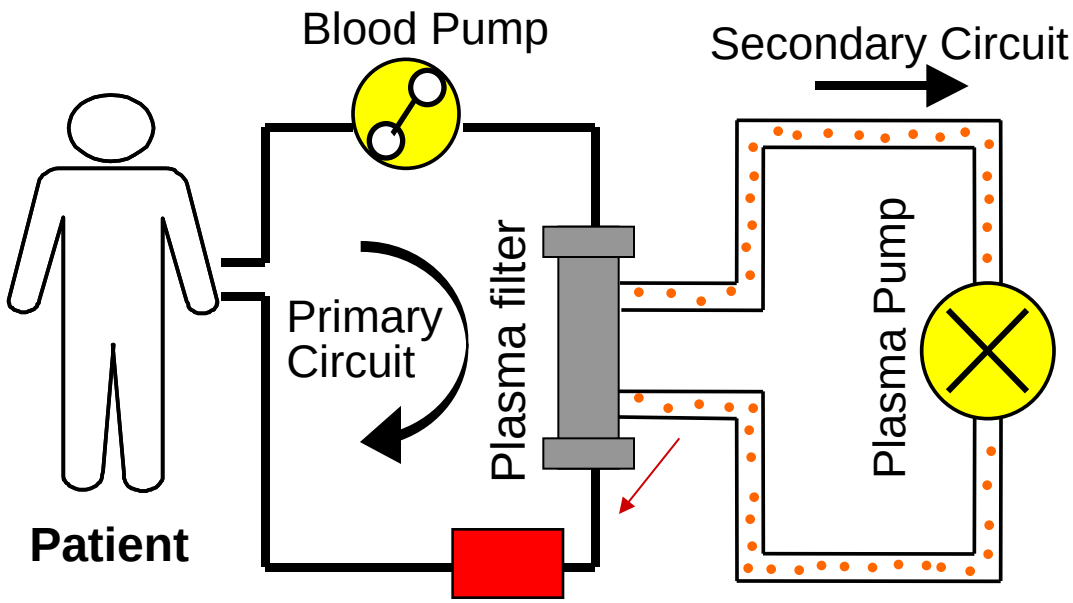
⇒ Toxins

⇒ Circulating tumor cells

Creatinine extraction from blood

A. Kusnetzov, 1989, Russ.Akad.Sci. Moscow

Microspheres based Detoxification System MDS
with a magnetic particle security system



Microparticle-Plasma Suspension

plus:

Fluorescence detector for magnetic microparticles retended by magnetic field

Fluorescent magnetic beads

Security System:

for the detection of membrane defect

D. Falkenhagen, Krems

Particle size: > 200-300 nm

higher container capacity

directed movement and retention in streaming media possible

e.g. blood, digestive and interstitial fluid, air

Particle size: < 50 nm

increasing Brownian movement (magnetic guidance is not possible !!!)

longer circulation time

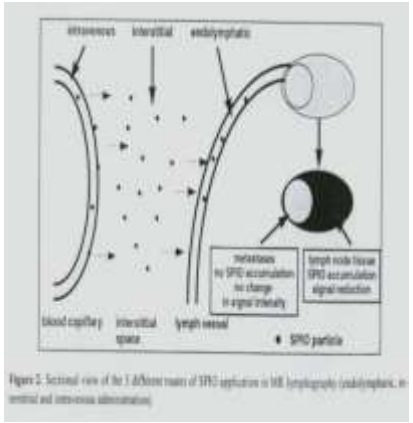
surface modification (electric charge, markers to influence the biodistribution)

crossing blood – brain – barrier

=> Toxic effects, degradation of materials?

Clinical Applications of *small* particles

Diagnostic: **Contrast enhancement of MRT**



Filling defect because of metatstasis

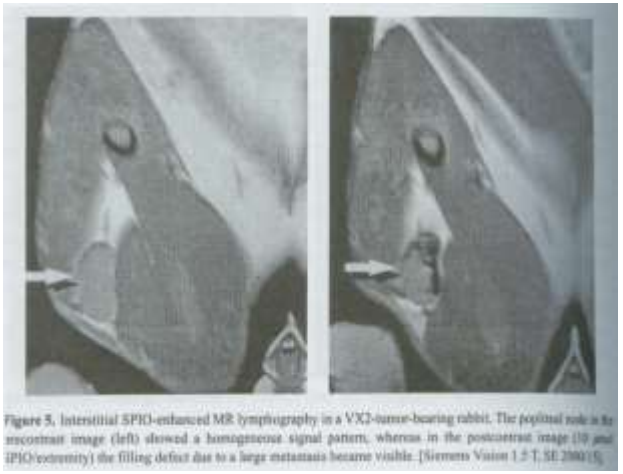


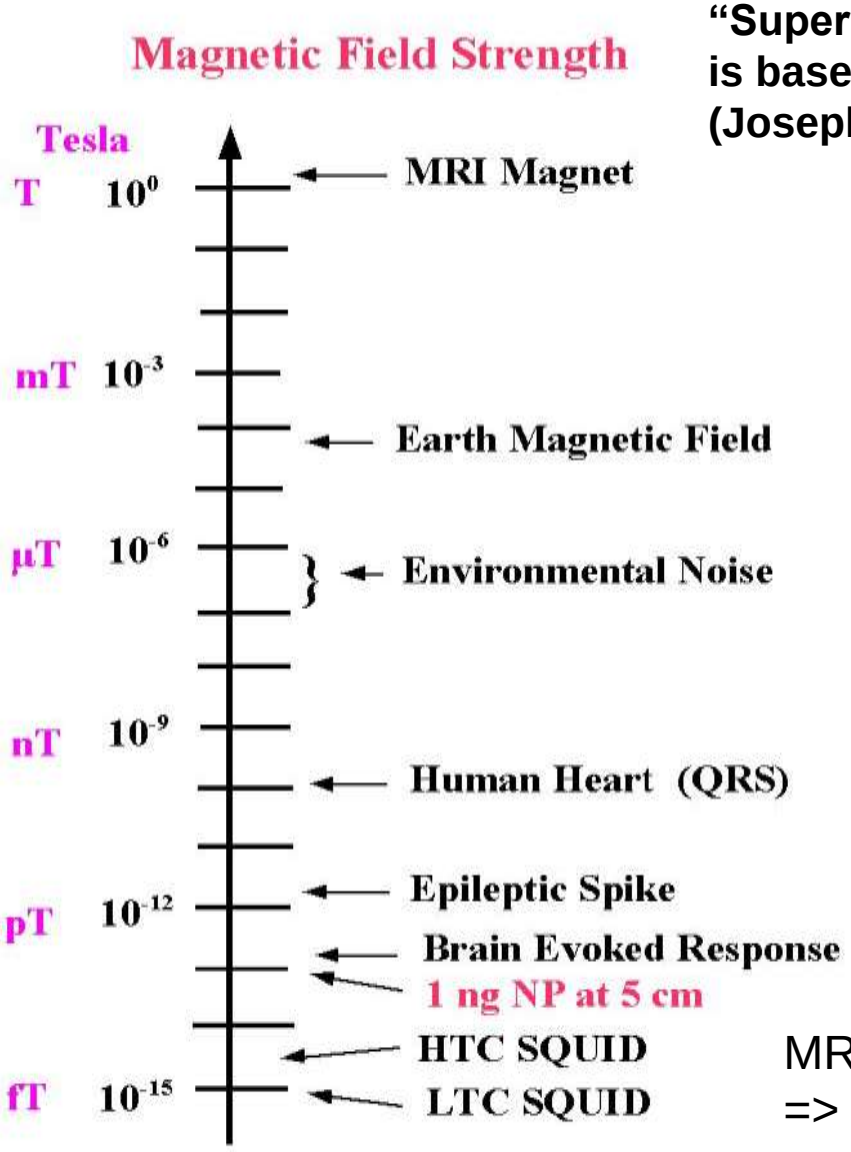
Figure 5. Interstitial SPIO-enhanced MR lymphography in a VX2-tumor-bearing rabbit. The popliteal node in the precontrast image (left) showed a homogeneous signal pattern, whereas in the postcontrast image (10 min after SPIO/extremity) the filling defect due to a large metastasis became visible. [Siemens Vision 1.5 T, SE 2000/15]

Localization of magnetic markers

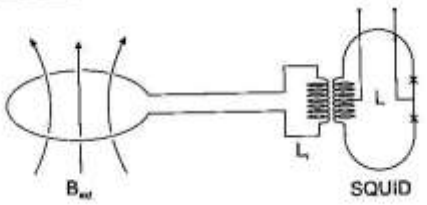
=> SQUID system is capable of detecting 10^5 cells

at a distance of 4 cm (~100-150 micron clusters.)

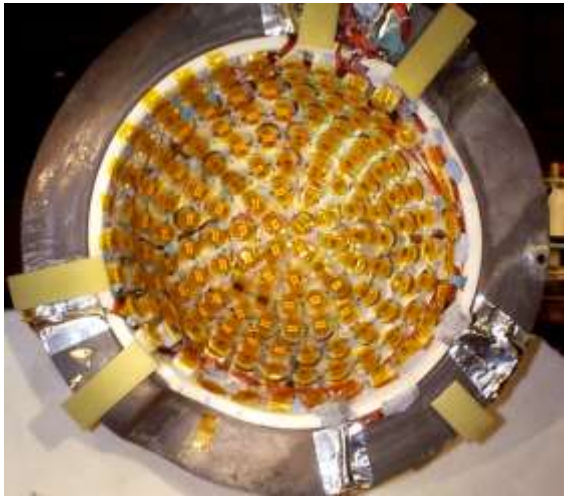
SQUID Array Scanner



"Superconducting Quantum Interference Device" is based on a quantum mechanical tunneling effect (Josephson & Giaver 1973).



superconducting SQUID array



MRT-source and SQUID-sensor field strength:
=> diameter of hair and the distance to the sun !

Ed Flynn, US Department of Defense, Los Alamos

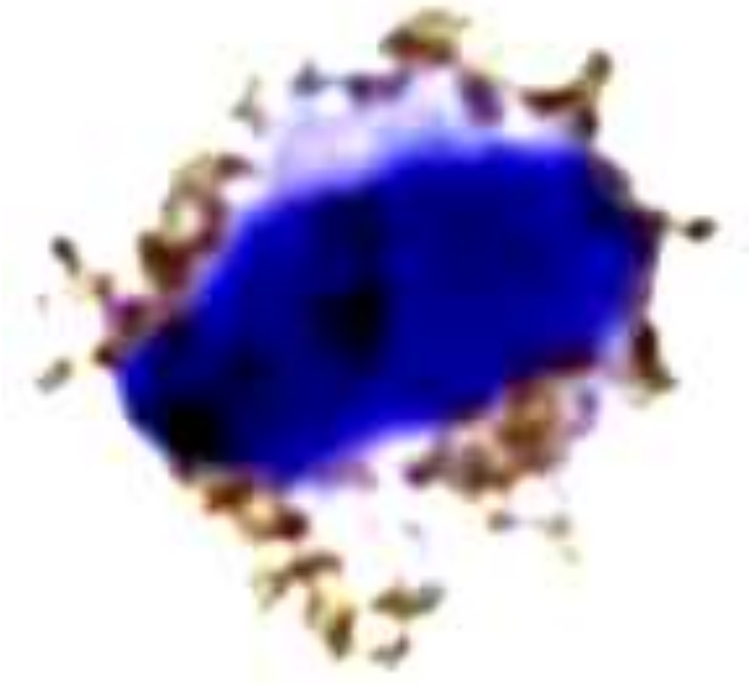
Live T-cell with 5 x 10^4 CD3 Antibodies conjugated to nanoparticles

Current SQUID system is capable of detecting:

10^5 of these cells at a distance of 4 cm

This corresponds to the amount of cells

in ~100-150 micron diameter clusters.



In comparison:

10^8 tumor cells visible on x-ray:

Ed Flynn, US Department of Defense, Los Alamos

The Future of NanoParticles in Medicine:

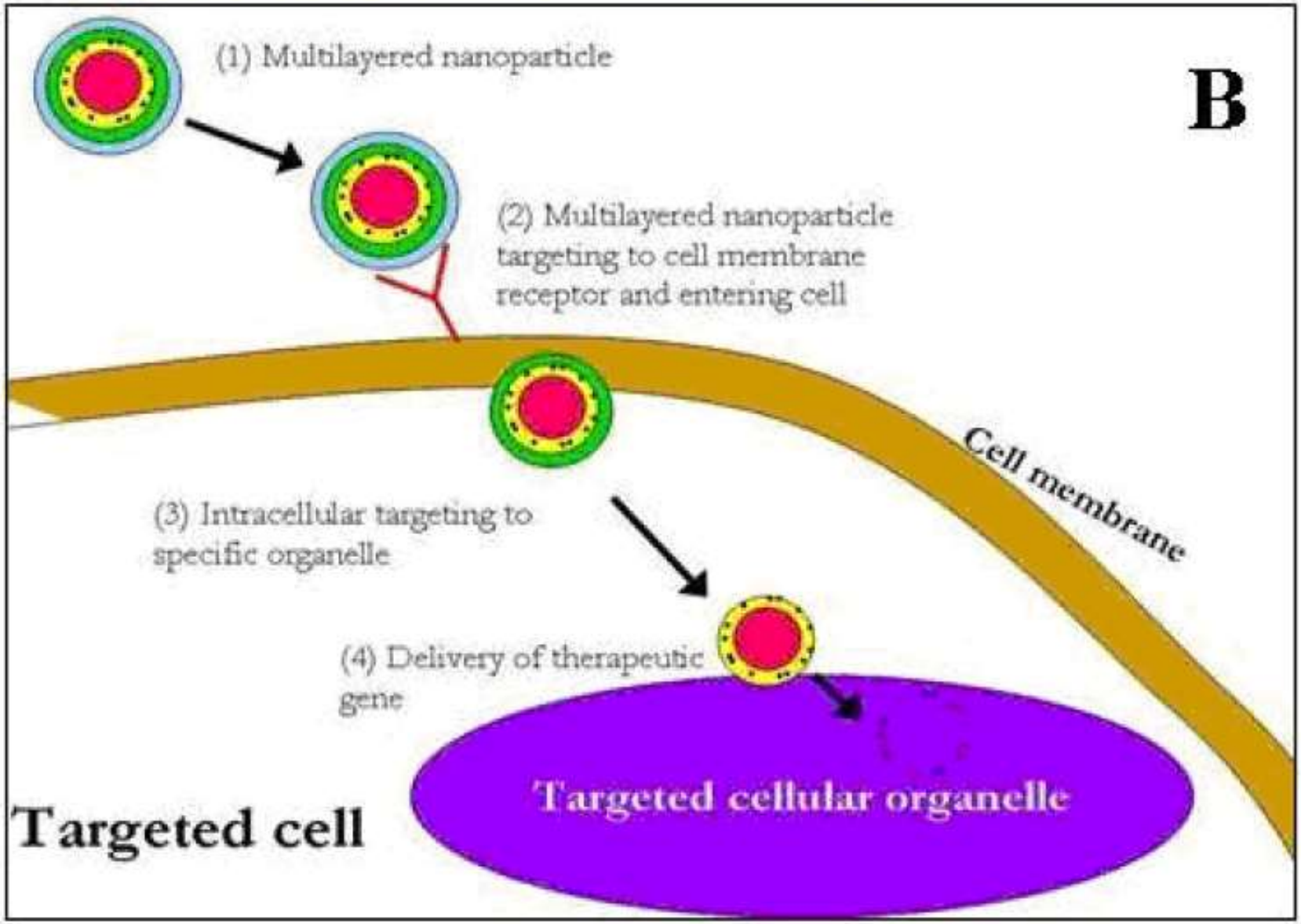
Therapy ?

⇒ **Specific Targeting and Retention ?**

⇒ **Stimulated Delivery of Drugs, Genes a.o. ?**

⇒ **Local Hyperthermia ?**

The Multi-Step Drug/Gene Delivery Process in Nanomedical Systems

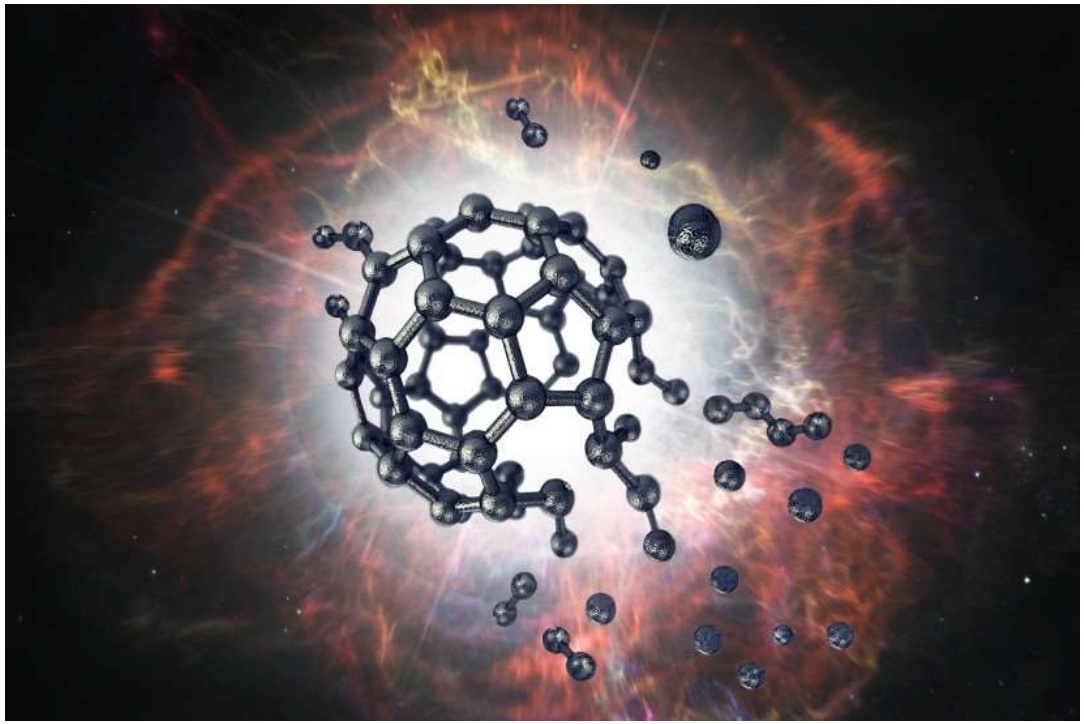


Leary, Pow (USA)

“Nanoscale explosives show potential in fight against cancer”

The creation of Buckybombs could one day be used for demolition of individual cancer cells

By Robert Perkins, March 2016



Artist's rendering of an exploding Buckyball (USC/Holly Wilder)

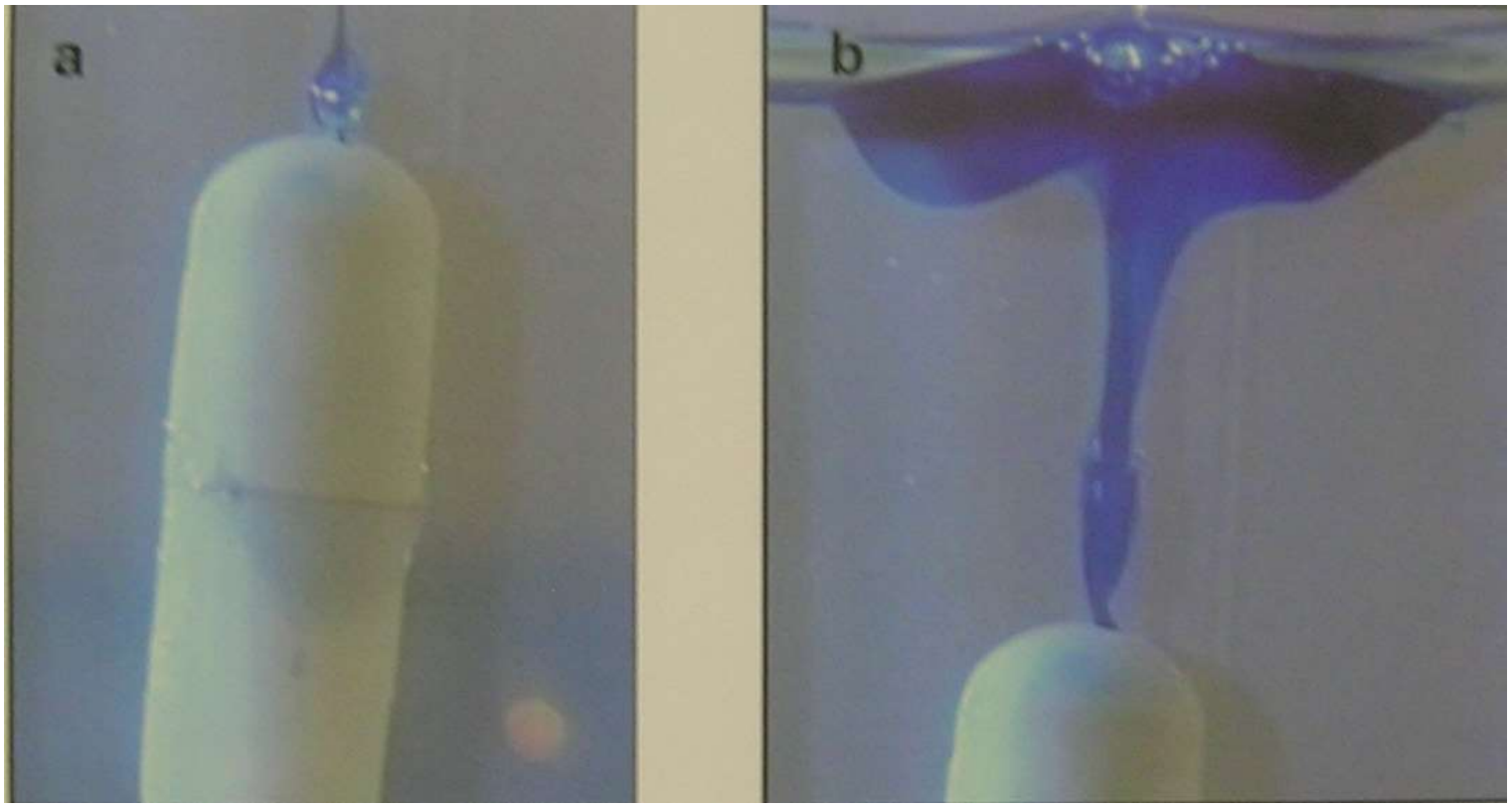
In 1996, a trio of scientists won the Nobel Prize for Chemistry for their discovery of Buckminsterfullerene — soccer-ball-shaped spheres of 60 joined carbon atoms that exhibit special physical properties.
Now 20 years later, scientists have figured out how to turn them into Buckybombs. These nanoscale explosives show potential for use in fighting cancer, with the hope that they could one day target and eliminate cancer at the cellular level — triggering tiny explosions that kill cancer cells without affecting surrounding tissue.

Futuristic view of nanobiomedicine: A clinical scenario (The "NanoDoctor" in Nature 2014) !

Patient N.N., 35 years with no medical complaints and normal physical exam

1. **Patient receives an out-patient *infusion* of biocompatible nanoparticles**
2. **Nanoparticles designed to circulate for a long time and to *capture molecules* in the blood**
3. ***Patient`s visit***
4. **Particles will be *harvested***
5. **Evaluation for the presence of *biomarkers* indicative of diseases processes**
6. **Particles containing an intrinsic *"barcode"***
7. **Molecular content will be extracted and *identified* using mass spectroscopy, PCR ....**
8. ***Diagnosis* is cancer !**
9. ***Tissue or single cell extraction* and investigation**
10. **Using modified nanoparticles for the *separation* of cancer cells**
11. **Using *lab-on-a-chip* techniques**
12. **Molecular profiling using silicon microarrays**
13. ***Using 3-dimensional tumor model for identification of patient-specific bioactive substances* (Inst. Biotechnology, FH Krems)**
14. **Nanotechnology based *treatment*: *target specific drug delivery, hyperthermia, irradiation***
15. ***Follow up studies* with *imaging* techniques using nanoparticles for localisation of tumor cells**

Defined Heating



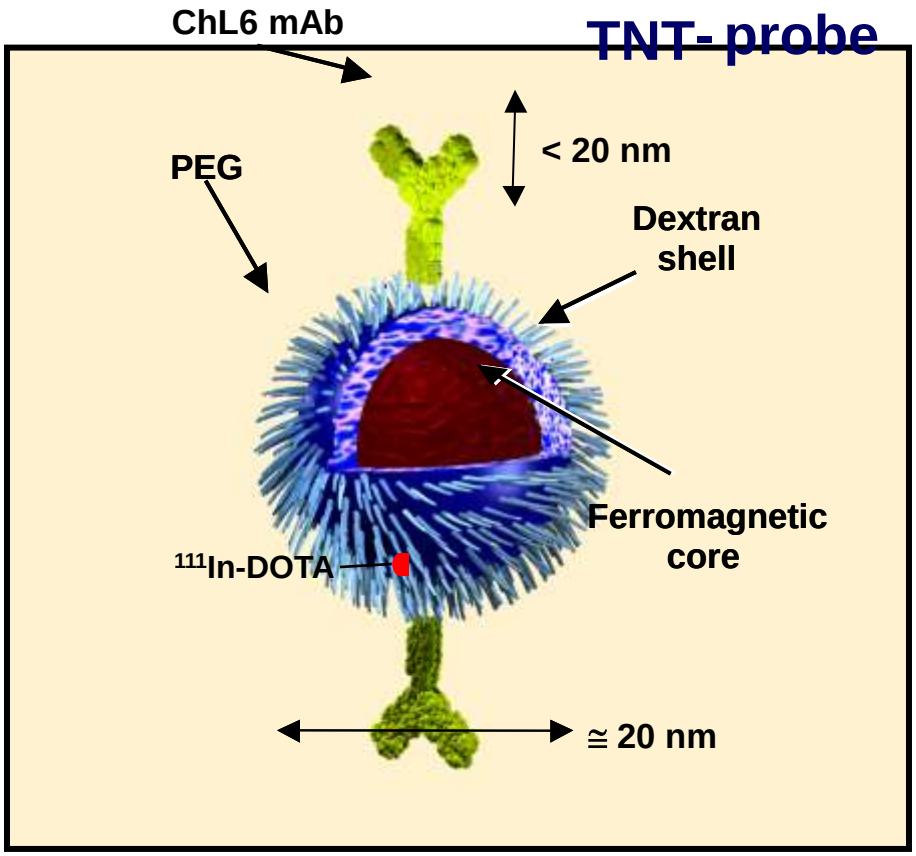
1.Localizating the capsule with external sensors in the target region of the GI-tract
2.Induction of drug release by an external magnetic field

C. Bräher, Jena

Design of nano-probes

Nano-probe Components:

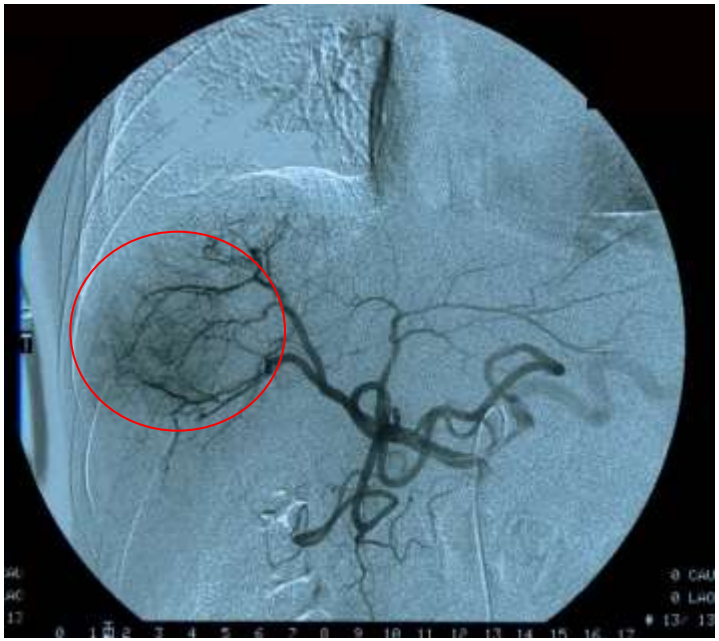
1. **Therapeutic heating:**
   Ferromagnetic core
2. **Selective uptake in tumor:**
   biologic targeting molecule – Chimeric L6 monoclonal antibody
3. **Tumor uptake control:**
   radioactive imaging label – ^{111}In DOTA
3. **Biocompatible:**
   polymer-dextran+PEG-COOH



S.J. DeNardo, University of California

Clinical Trial: Liver Tumor Treatment

- Application of 50-600 mg MTC containing up to 10% doxorubicin
- Clinical phase I/II study with 32 patients in US



Angiogram

MRI

Jeff Bulte, John Hopkins Univ., Baltimore

Therapeutic Superconducting Magnets

Niobe-Artis System for Stereotaxis

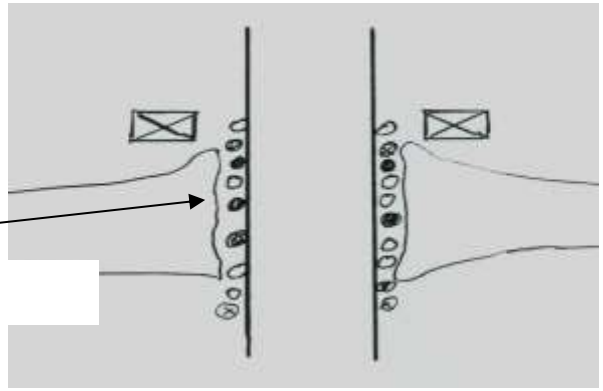
Combination of permanent magnets and a Siemens Axiom Artis dFC digital fluoroscopy system

FDA approved

Controlled Drug Release and Heating

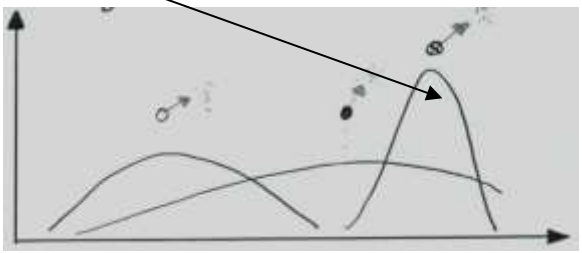
Catheter coated with different particles (800 nm) non-magnetic (two) and magnetic

=> loaded with different drugs



Application of external alternating magnetic field

=> Release of different drugs



and:

=> Stimulation of wound healing?

Schütt, Teller, Lothmann (Patent, 1988)

time

Local Magnetic Particle Hyperthermia (MPH)

Usual heating methods:

- heat bath
- microwave radiation
- implanted electrodes
- ultrasound
- **lasers (and nano particles !)**

Magnetic nanoparticle-mediated thermotherapy is:

- Selective
- Minimally invasive
- Controlled dose deposition and rate
- Integrative – provides imaging to diagnose, treat, and assess
- Enabling – repeated treatments in short time possible

$\Rightarrow$ **Heat** as therapeutic agent is analogous to **Radiation**

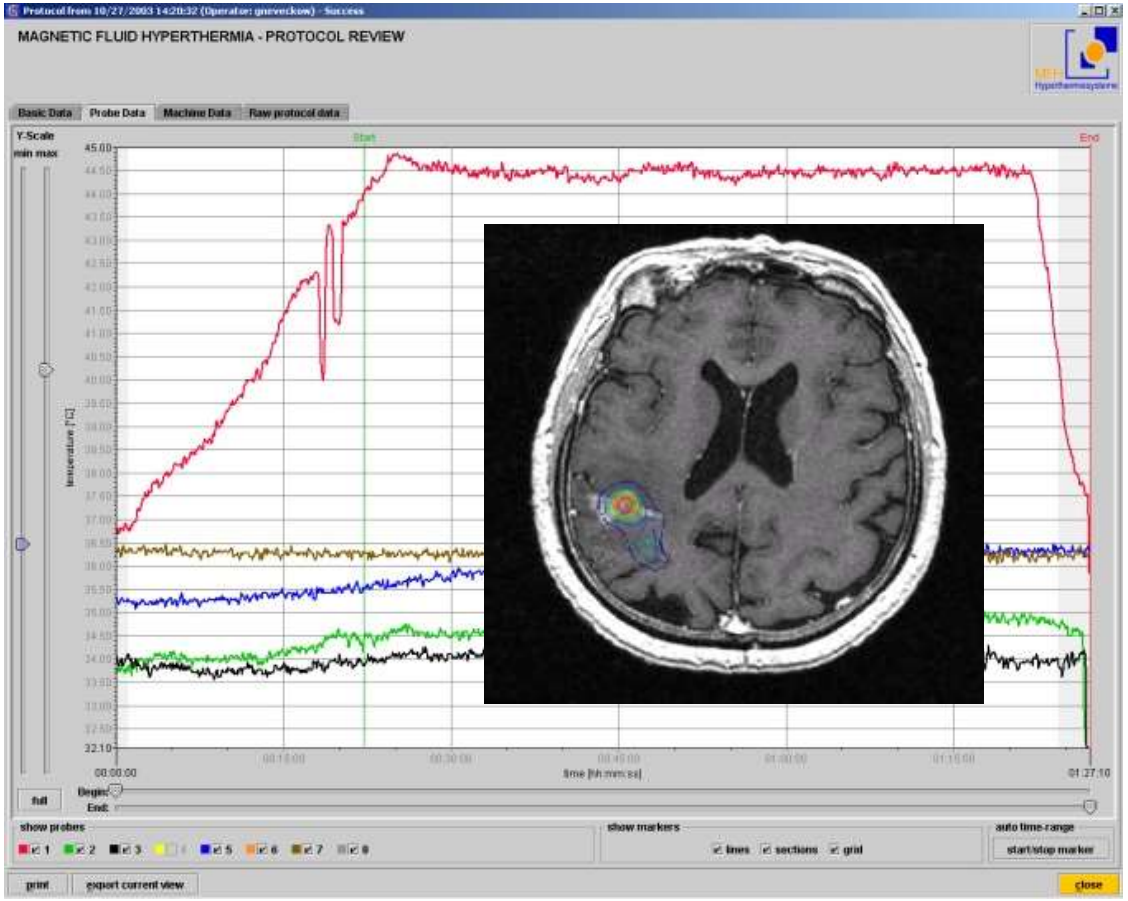
Problems: unrealistic expectations, physical limitations …and ….

but: 12 groups are active

A. Jordan, Charite` Berlin

Local Magnetic Particle Hyperthermia

Clinical Trials started in 2005



Selective and minimally invasive

Controlled dose deposition

Provides imaging for diagnosis, treatment and control

Repeated treatments in short time possible

A. Jordan, Charite, Berlin

Science Fiction or ?

“Nano-robots” — **Transfer of “computational” genes into the body in order to repair or detect damages and infections**

=> 0,5 – 3 µm max. size for capillary passage

“Cell repair machines” — **Entering of cell in order to rebuild of damaged molecular structures**

Freitas, R. (2005)

=> “Quantum- Food”

Expectations and Risk Assessement of Nanotechnology not in Line!

COMMENTARY

Scientists worry about some risks more than the public

DIETRAM A. SCHEUFELE[1]*, ELIZABETH A. CORLEY[2], SHARON DUNWOODY[3], TSUNG-JEN SHIH[3], ELLIOTT HILLBACK[3] AND DAVID H. GUSTON[4]

are in [1]the Department of Life Sciences Communication, University of Wisconsin–Madison, 440 Henry Mall, Madison, Wisconsin 53706, USA; [2]the School of Public Affairs, Arizona State University, 411 North Central Avenue, Phoenix, Arizona 85004, USA; [3]the School of Journalism & Mass Communication, University of Wisconsin–Madison, 821 University Avenue, Madison, Wisconsin 53706, USA; [4]the Department of Political Science, Arizona State University, PO Box 874401, Tempe, Arizona 85287, USA.
*e-mail: scheufele@wisc.edu

A comparison between two recent national surveys among nanoscientists and the general public in the US shows that, in general, nanoscientists are more optimistic than the public about the potential benefits of nanotechnology. However, for some issues related to the environmental and long-term health impacts of nanotechnology, nanoscientists were significantly more concerned than the public.

Re: Nature Nanotechnology, 2:732-734 (2007)

Thank you for your attention !

Int. Conf. on Scientific and Clinical Application of Magnetic Carriers

Rostock (1996), Cleveland (1998), Rostock (2000), Tallahassee (2002), Lyon (2040), Krems (2006), Vancouver (2008), Rostock (2010), Minneapolis (2012), Dresden (2014), Vancouver (2016), Copenhagen (2018)

Organized by:

U. Hafeli **W.Schütt**

University British Columbia and **IMC FH Krems**

“Building a ship not only means to organize the tools, but also to create aspiration for the sea in your team!”
....Exyperie

Thank you!

Rostock !