



Laboratory Techniques and Medical Device Approvals

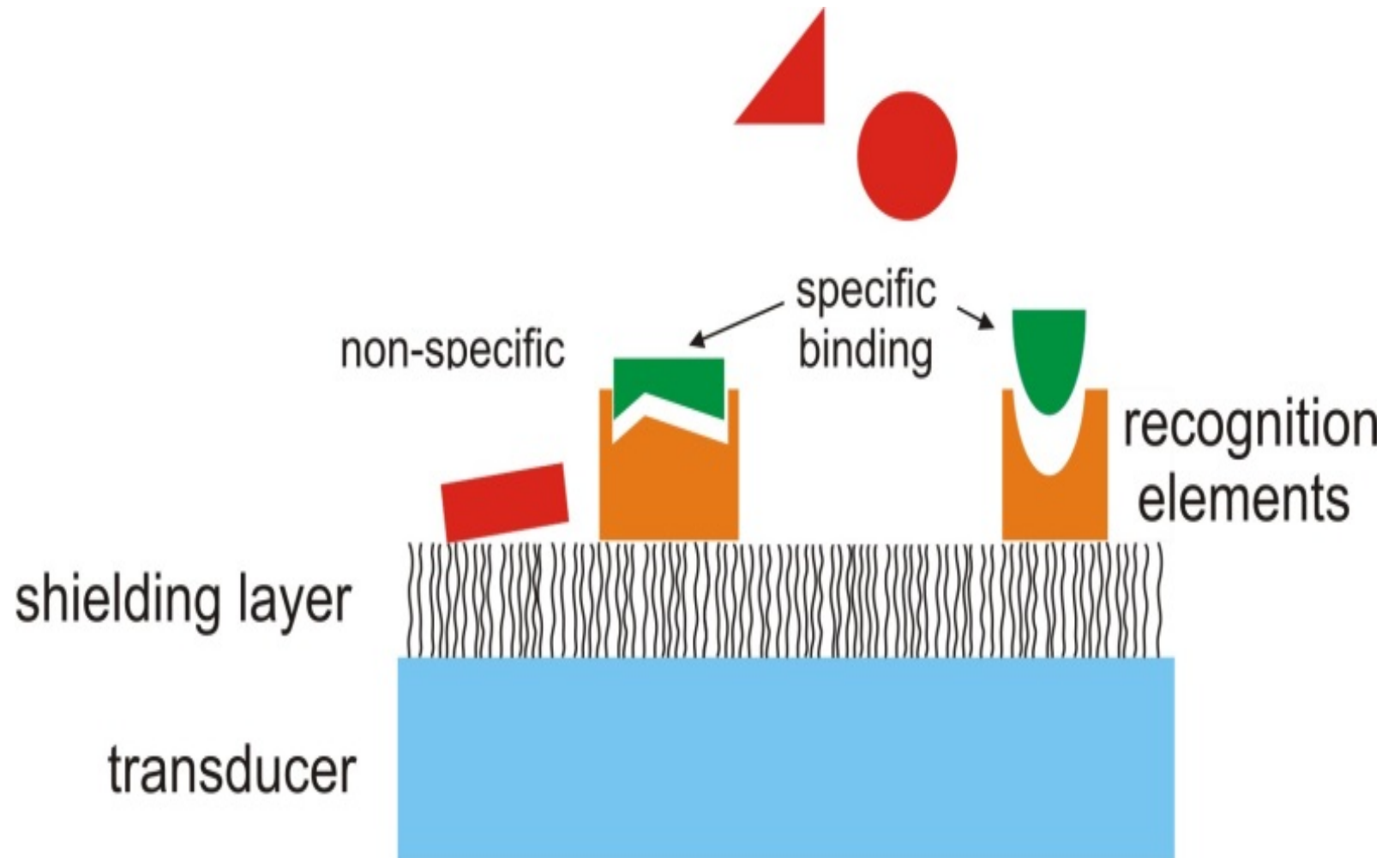
Part Gauglitz: Novel Bioanalytical Approaches
Part III: Optical Biosensors and POCT



Optical Methods in Biosensing



Biosensor principle





Optical Detection

- Amplitude
 - Frequency (spectra)
 - Phase
 - State of polarisation
 - Time dependence
- 
- Absorption/Transmission
 - Emission (Fluorescence, Phosphorescence)
 - Scattering (Rayleigh, Raman)
 - Reflection
 - Refraction



Classification I: interaction principle

- Absorption/Transmission

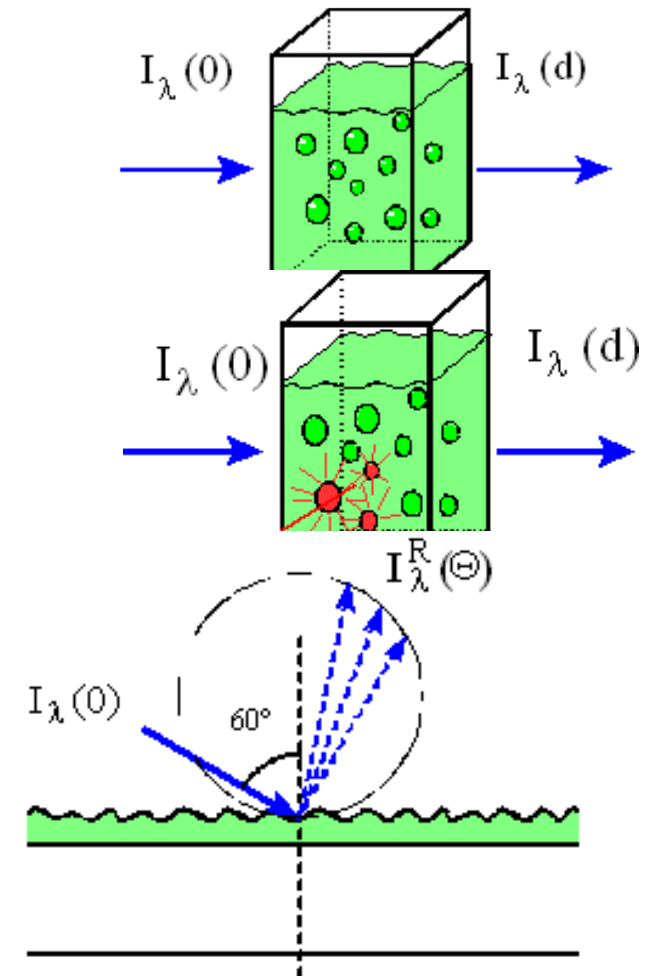
$$I_{\lambda}(d) = I_{\lambda}(0) \cdot \exp[d \cdot \varepsilon_{\lambda} \cdot c]$$

- Fluorescence

$$I_{\alpha}^F(t) = I_{\lambda}(x) \cdot \varepsilon_{\lambda} \cdot c(t) \cdot f_{\alpha} \cdot \eta \cdot \Gamma$$

- Reflection, diffuse (remission)

Lambert cosine law

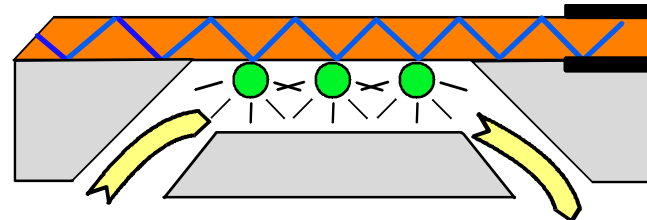




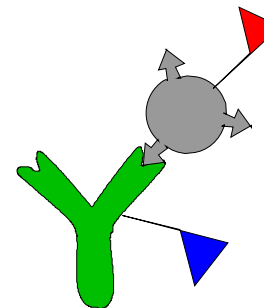
- Detection of labelled systems

- *Fluorescence intensity/lifetime*
- *Fluorescence anisotropy*
- *Fluorescence correlation*

- *TIRF*



- *FRET*





Classification of optical methods

➤ direct optical
detection
(label free)



heterogeneous
phase assay

➤ methods using
luminescence



homogeneous
phase assay

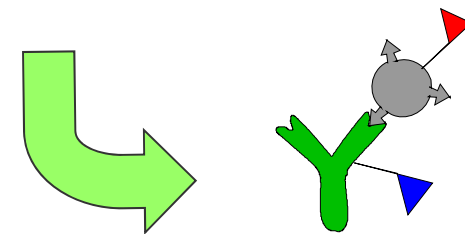


heterogeneous
phase assay



Optical detection of biomolecular interaction in homogeneous phase

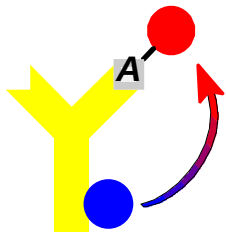
- *Fluorescence quenching assay*
 - analyte derivative labelled with fluorophore
 - quenching of fluorescence by binding of ligand to receptor
- *Fluorescence polarisation assay*
 - analyte derivative labelled with fluorophore
 - reduction of rotational diffusion
 - increase of fluorescence anisotropy
- *Fluorescence resonance energy transfer assay*
 - both analyte derivative and receptor labelled
 - energy transfer in the receptor-ligand complex
 - donor fluorescence must overlap acceptor absorption



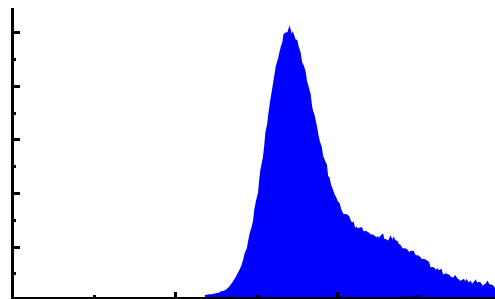


(Fluorescence) Förster Resonance Energy Transfer

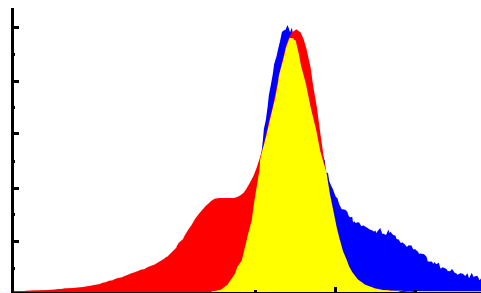
● *Use of intermolecular distance*



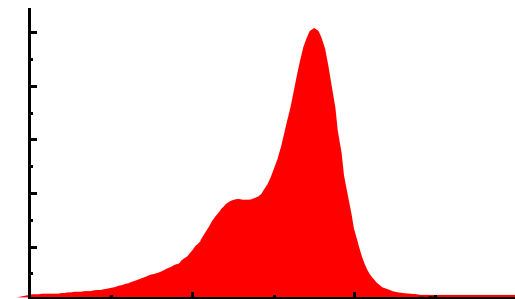
Rate constant of transfer: $k_{RET} = \frac{1}{\tau_D} \cdot \left(\frac{R_0}{R} \right)^6$



Fluorescence
(Donor)



Spectral overlap

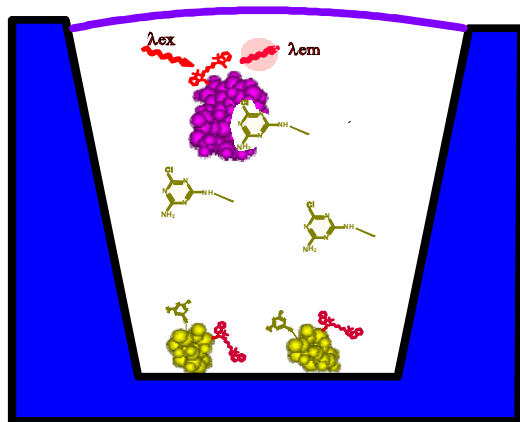


Absorption
(Acceptor)

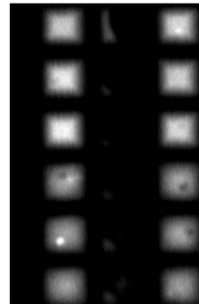
► *Quenching of the fluorescence depends strongly on the intermolecular distance*



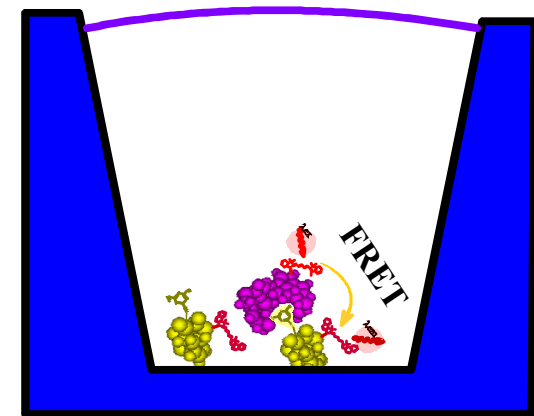
Phase separation assay



high fluorescence



low fluorescence



with analyte

- analytes bind to the paratope of antibodies in homogeneous phase
- no binding at the heterogeneous phase

fluorescence signal

without analyte

- antibodies bind to analyte derivatives immobilized at the surface

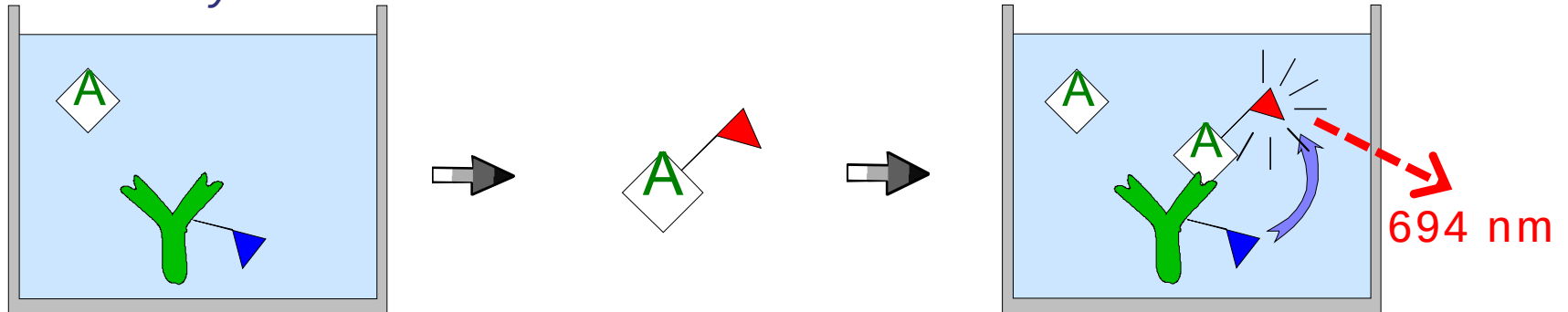
quenched fluorescence signal

Michael Seidel



Homogeneous test format (competitive test)

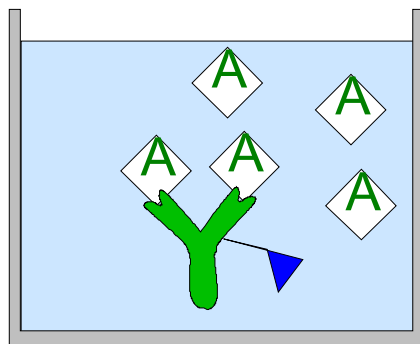
low analyte-concentration



Pre-incubation of
antibody-donor-
conjugate
with analyte

addition of
analyte-acceptor-
conjugate

647 nm



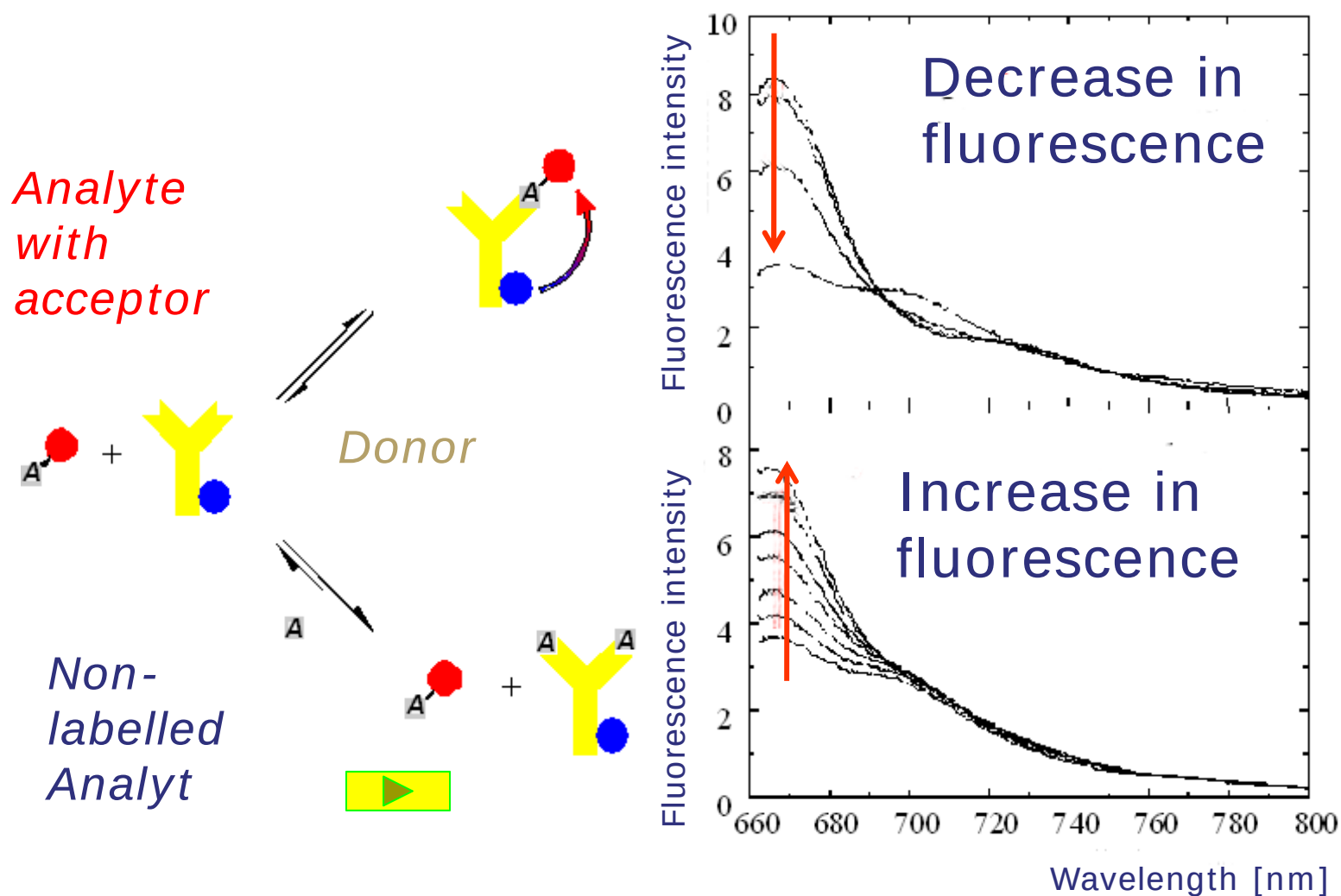
high analyte-concentration

674 nm

647 nm

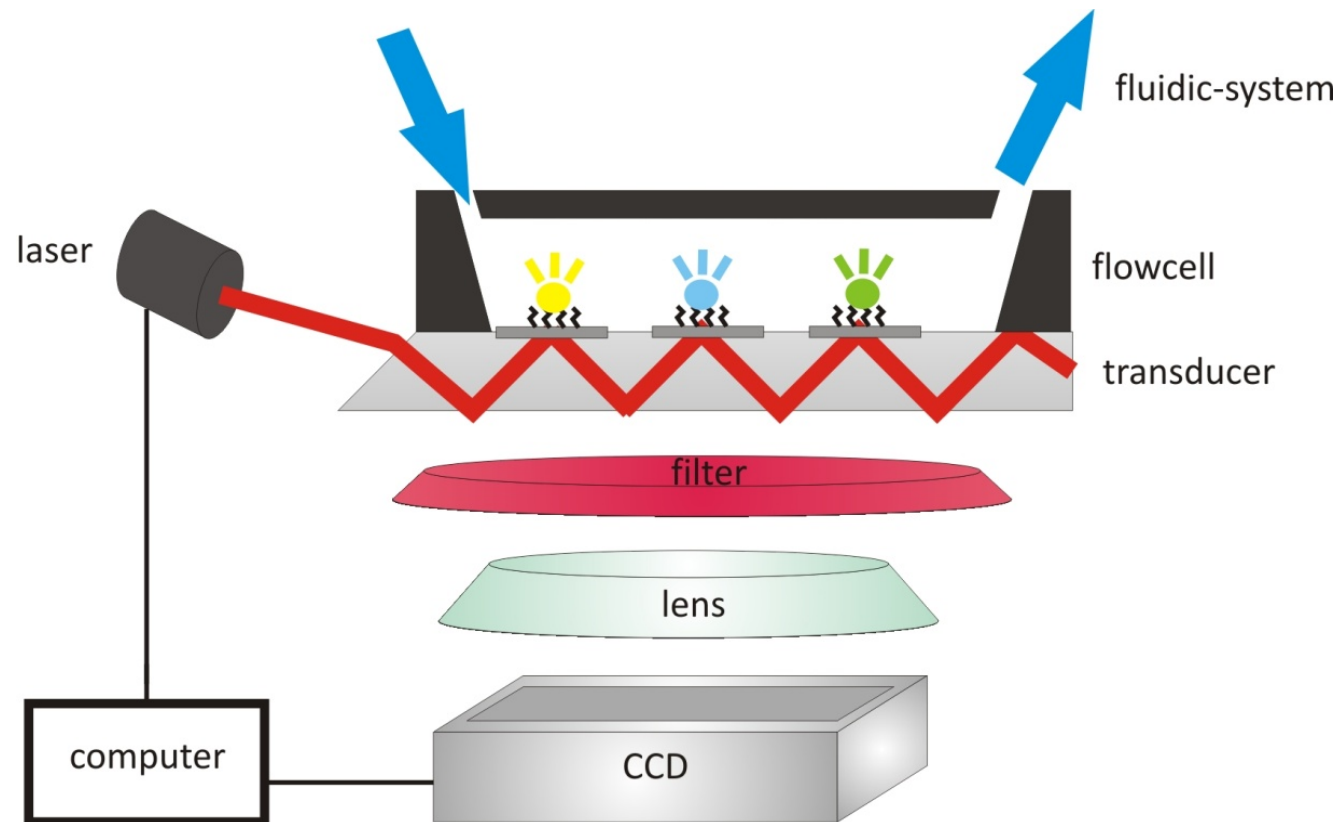


Donor-acceptor interaction





TIRF: total internal reflection fluorescence





Biosensor:

- *Interaction between analyte and recognition element*
- *measured by*
- *Labelling at least one partner*
- *Without label*



- **Only assays at heterogeneous phase**
- **Certain requirements for the interaction surface**
 - Non-specific binding
 - Regeneration necessary
 - Stability of the surface
 - Optimal specific binding
 - High loading
- **No problems with label**
 - Less costs
 - Less problems with bioactivity
 - Fast characterisation of the assay
 - No problems with photobleaching



Label-free principles

- Quartz micro balances
- Cantilevers
- Electrochemical methods
- Optical principles
 - Optical fibers, End fiber coupling
 - Grating couplers
 - Photonic crystals
 - Bragg gratings
 - Back scattering
 - Raman, SERS
 - Resonant mirror
 - Surface plasmon resonance
 - Mach-Zehnder Interferometer
 - Young Interferometer
 - Ring resonators
 - Ellipsometry
 - Reflectometric interference spectroscopy

Combination
electrochemical and
optical



Optical monitoring

■ Direct optical detection

■ Reflectometry

- *RfS*
- *ellipsometry*

$f(d)$

$f(d,n)$

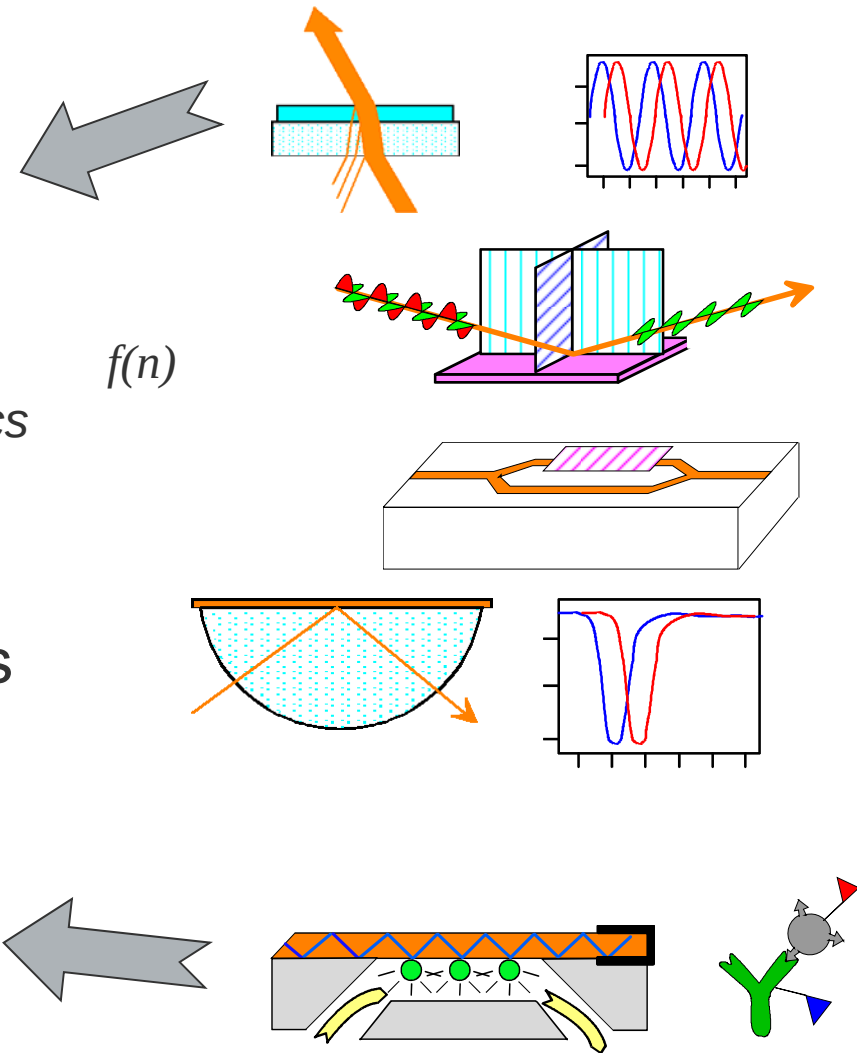
■ Evanescent field techniques

- *Mach-Zehnder integrated optics*
- *Surface plasmon resonance*
- *Grating couplers*
- *Resonant mirror*

$f(n)$

■ Detection of labelled systems

- *Fluorescence intensity/lifetime*
- *Fluorescence anisotropy*
- *Fluorescence correlation*
- *TIRF*
- *FRET*





Classification of direct optical

- Refractometric
 - Evanescent field techniques
 - Measuring **refractive index**
 - Methods
 - SPR
- Reflectometric
 - Measuring optical **thickness**
 - Methods
 - Reflectometric Interference Spectroscopy

$f(n)$

Optical thickness
 $= n \cdot d$

Both dependent on
temperature

n adds effect by volume
dependent polarisation

$f(d)$

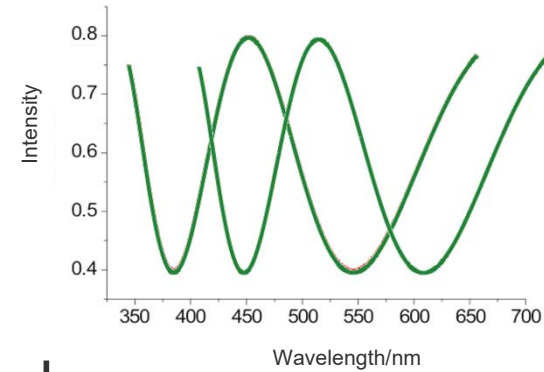
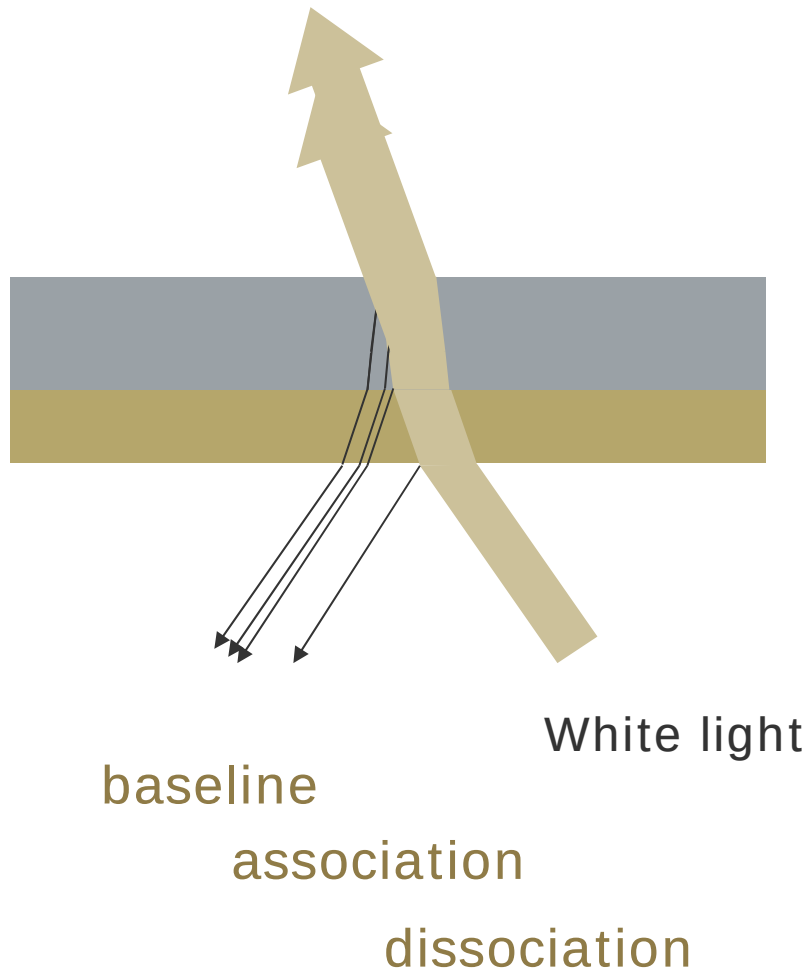
Negligible temperature influence



RlfS: assoziation and dissoziation

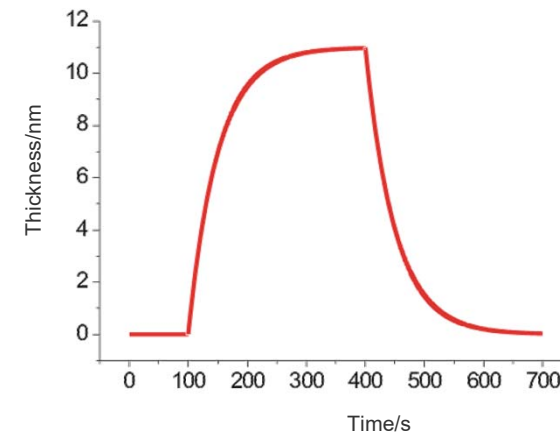
Transducer + Biolayer (biopolymer/recognition sites)

Interference spectrum



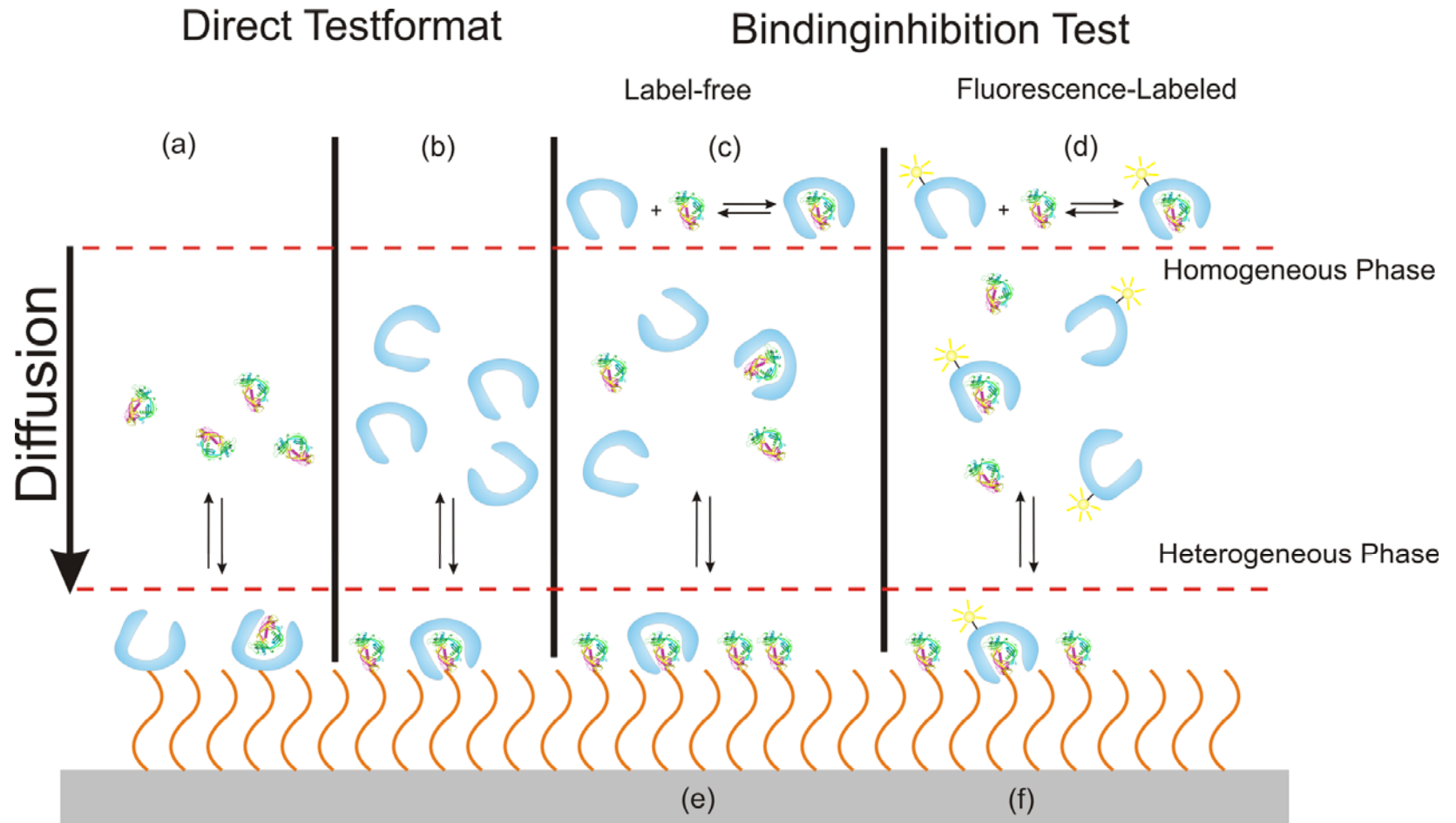
$\Delta n d$

Binding curve





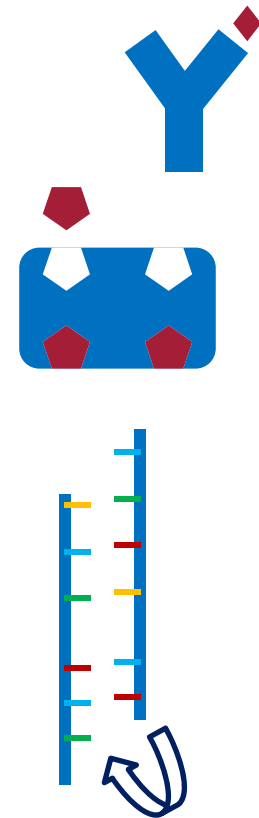
Binding at heterogeneous phase





Recognition elements

- Polymers
- Functionalised polymers
- Molecular imprinted polymers
- **Biolayers as recognition structures**
 - Antigen/antibody
 - DNA/DNA
 - Peptide/protein
 - Protein/protein
 - Small ligands/protein
- Scaffolds
- Aptameres
- Membrane like structures
- Membranes





- **Certain requirements for the interaction surface / material**
 - Non-specific binding
 - Optimal specific binding
 - High loading
 - Regeneration necessary
 - Stability of the surface

- **Surface specific interaction**
 - Assay conditions
 - Boundary conditions of molecular interaction
 - Quality of monitoring
 - Quantification of interaction



Applications

- Central labs at hospitals
- POCT



- Most in central labs
 - Only at certain locations
 - Not always available (limited beginning Friday afternoon)
- Requirements
 - Fast results (turn around time)
 - reliable
 - Real-time diagnostics
 - costs
 - Hospital wards
 - Ambulance vehicle
 - Medical practices
 - Epidemic studies
- Relevant in
 - Human health control and care
 - Urgent need for animal diagnostics

POCT

point of care diagnostics



Lecture on POCT: Clinical and organizational aspects of POCT

CARE-MAN. 3rd annual meeting, Oct 20 – 22, 2008. Graz, Austria

Und Fms-Regionaltag „Entwicklungen und Perspektiven
der Patientennahen Diagnostik

Tübingen, 7. Juni 2010



Peter B. Lupp
Institute of Clinical Chemistry and Pathobiochemistry, TU München, Germany



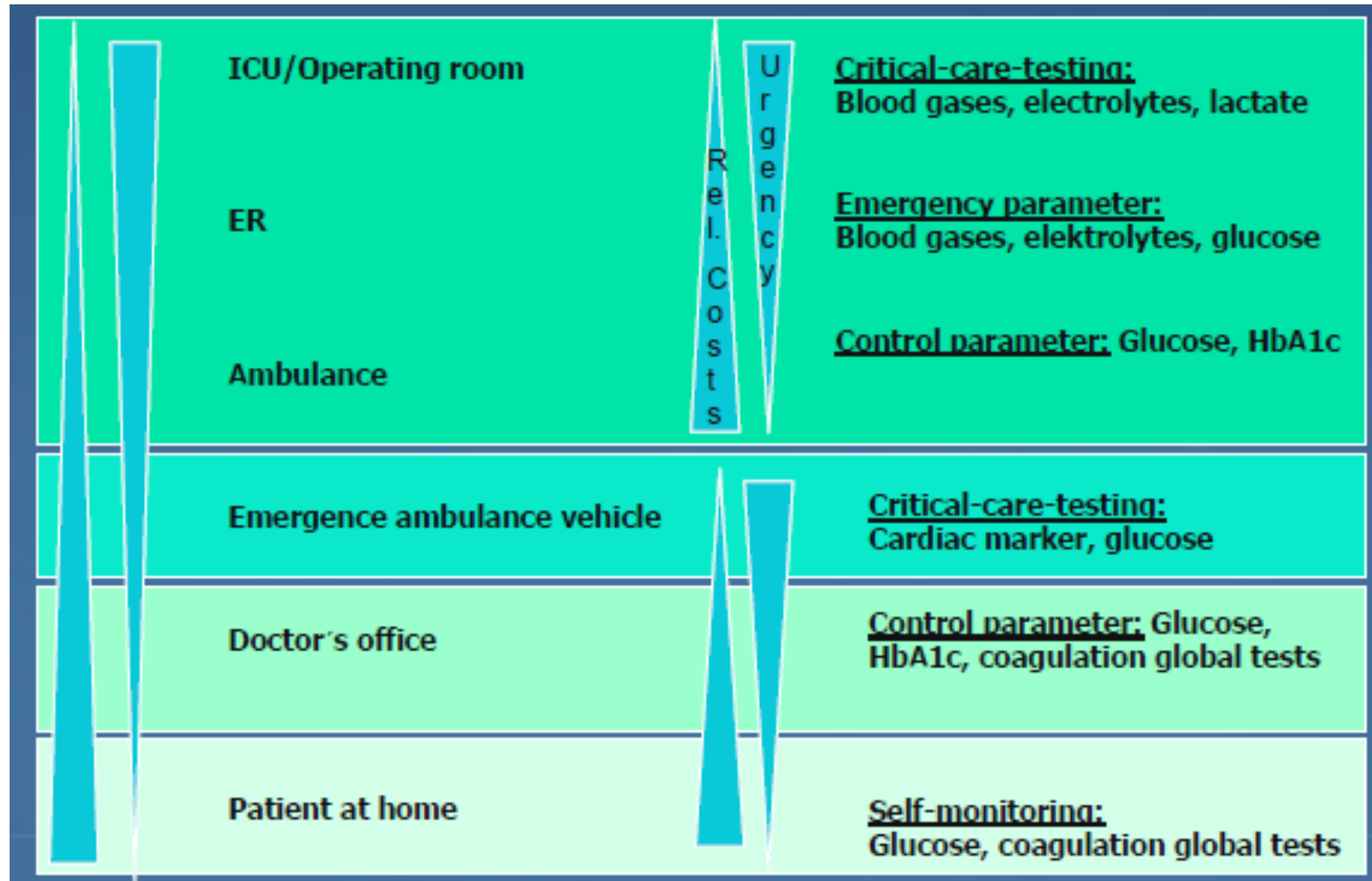
Characteristics of POCT

1. (Quantitative) laboratory analyses near the patient (bed-side testing)
2. Being performed outside a central (core) or decentralized lab
3. No sample pretreatment, whole blood being used as sample material
4. No pipetting steps
5. Use of ready-to-use reagents (cassettes or unit-use devices)
6. Purpose-made measuring devices for single (not serial) measurements
7. Operation by non-technicians (care-givers, physicians...)
8. Rapid availability of results
9. This allows immediate therapeutic responses by the attending physician

Definition is important for the QC regulations



Fields of application





POCT-devices - State-of-the-art



POCT parameters

<u>Blood gases, acid/base balance</u>	pH, pCO ₂ , pO ₂ (+ CO-oxymetry for Hb fractions)
Electrolytes	Na ⁺ , K ⁺ , Cl ⁻ , ion. Ca ⁺⁺ , ion. Mg ⁺⁺
Metabolites	(HDL)-Cholesterol, triglycerides, creatinine, urea, uric acid, bilirubin, lactate, ammonium
Enzymes	Amylase, Alkaline phosphatase, CK, AST, ALT, γ-GT
Haemostaseology	ACT, aPTT, TPT (INR), D-Dimer, platelet function tests,
Haematology	Hb, hematocrit, erythrocytes, leukocytes, thrombocytes
<u>Cardiac markers</u>	TroponinT, troponin I, myoglobin, CK-MB, BNP/NT-pro-BNP
<u>Diabetes mellitus</u>	Glucose, HbA _{1c} , microalbumin, min. invasive contin. glucose-monitoring
<u>Infection/sepsis</u> ←	CRP, PCT, LBP, IL-6, IL-8
In-vitro allergy diagnosis	Allergen specific IgE
Therapeutic and drugs-of-abuse	Therapeutic drugs, alcohol, amphetamines, barbiturates, benzodiazepines, cannabinoides, cocaine, methadon, opiates
Infectiology	HIV, inf. mononucleosis, <i>Chlamydia</i> , <i>Trichomonas</i> , <i>Plasmodium</i> ...
Fertility	HCG, LH and FSH in urine, sperm count
Urine diagnostics	Strips (pH, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, ...)
Feces diagnostics	Blood in stool, albumin in stool
Non-invasive measurements	Transcutaneous pCO ₂ - and pO ₂ -analyses, neonatal bilirubin
Patient-self monitoring	Blood sugar, TPT (INR)



Types of POCT devices

- **Types of POCT devices I – Unit-use devices**

simple, single determination steps

- These dedicated systems apply ready-to-use reagents for single determinations. Sample matrix is unprocessed whole blood samples.
- One characteristic for these devices is that the sensors are integrated in the
- test strips and are to be inserted in the reader instrument. Reagents and specimen react on the strip surface.

- **Types of POCT devices II - Benchtop instruments**

needs power supply, experience to handle, ...

- These are more complex analyzers being placed primarily in the functional areas of an ICU or in the ER. These instruments use various analytical principles



Types of POCT devices I

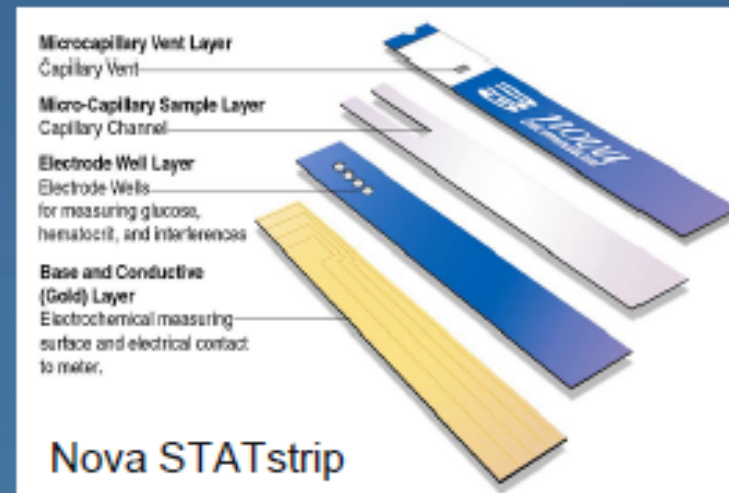
e.g. pregnancy test

Unit-use devices

These dedicated systems apply ready-to-use reagents for single determinations. Sample matrix is unprocessed whole blood samples.

One characteristic for these devices is that the sensors are integrated in the test strips and are to be inserted in the reader instrument. Reagents and specimen react on the strip surface.

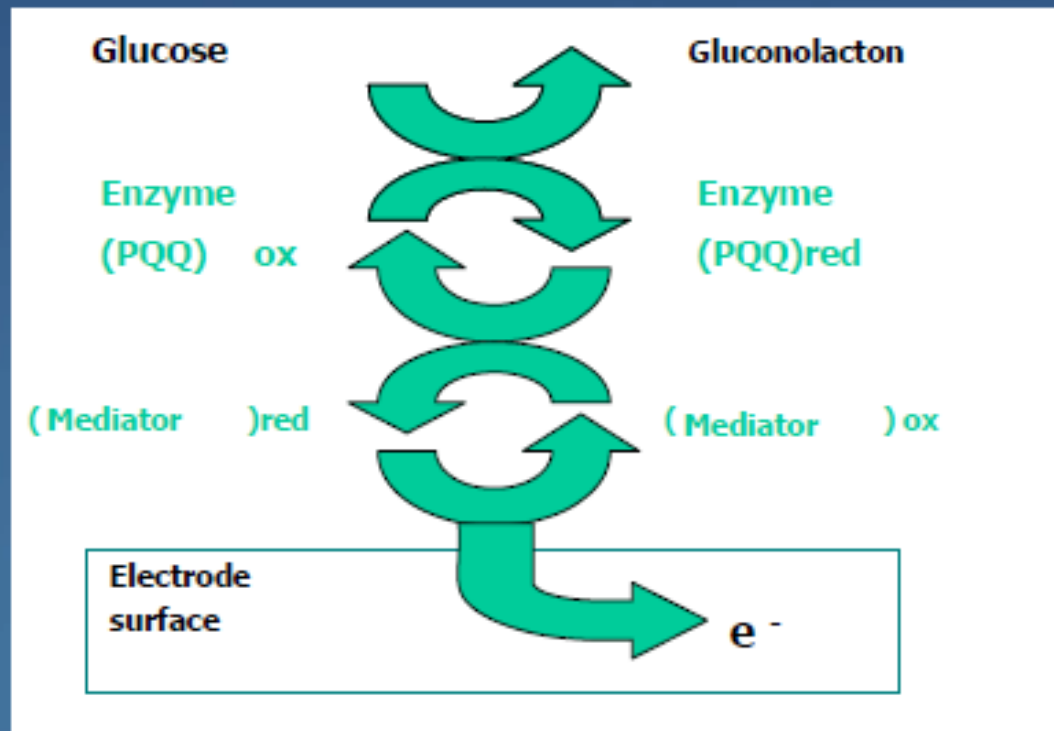
It is also characteristic for these systems that calibration is replaced by an electronic or physical standard which is measured whenever the device is turned on.





Glucose test

enzyme based, e.g. for color scale



Beta-D-glucose is oxidized by the pyrrochinolinchinon (PQQ)-dependent glucose-dye-oxidoreductase to form gluconolactone and PQQH. Amperometric detection .



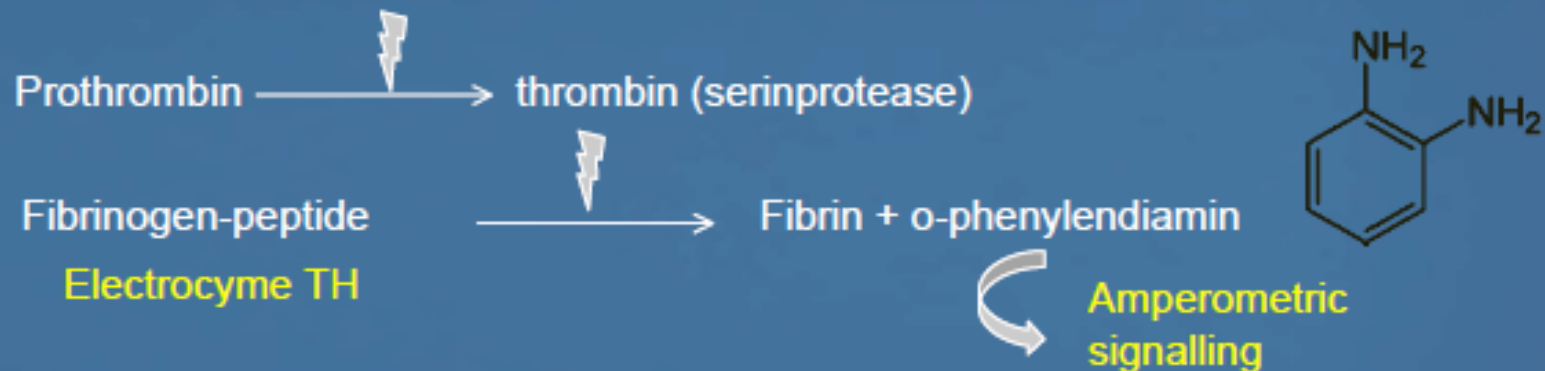
Test principle of Coagu-Check

Test principle of the Coagu-Chek CX TPT measurement

When a blood sample is applied to the test strip, thromboplastin activates the coagulation cascade which leads to the formation of thrombin. Thrombin cleaves its substrate creating an electrochemically active peptide, which generates an electrical signal.

The signal is converted to an INR value and displayed by the CoaguChek XS System.

Recombinant human thromboplastin





Glucometers for a hospital POCT network



Roche Accu-Chek Inform

Care Eco Solo II

Hemocue 201 DM

Nova StatStrip

Abbott Precision PCx



Unit-use multiparameter hospital POCT device



The i-STAT® handheld system performs a comprehensive panel of critical tests. Utilizing self-contained test **cartridges**, the i-STAT 1 system delivers lab-accurate testing for blood gases, electrolytes, substrates, coagulation, hematology, glucose, and cardiac markers.





Roche Diagnostics: cobas h 232

Fluorescence intensity detection

<http://www.keller-medical.de/media/archive1/produkte/cobas-h-232-broschuere.pdf>

<http://pubs.rsc.org/en/content/articlehtml/2011/lc/c0lc00204f>

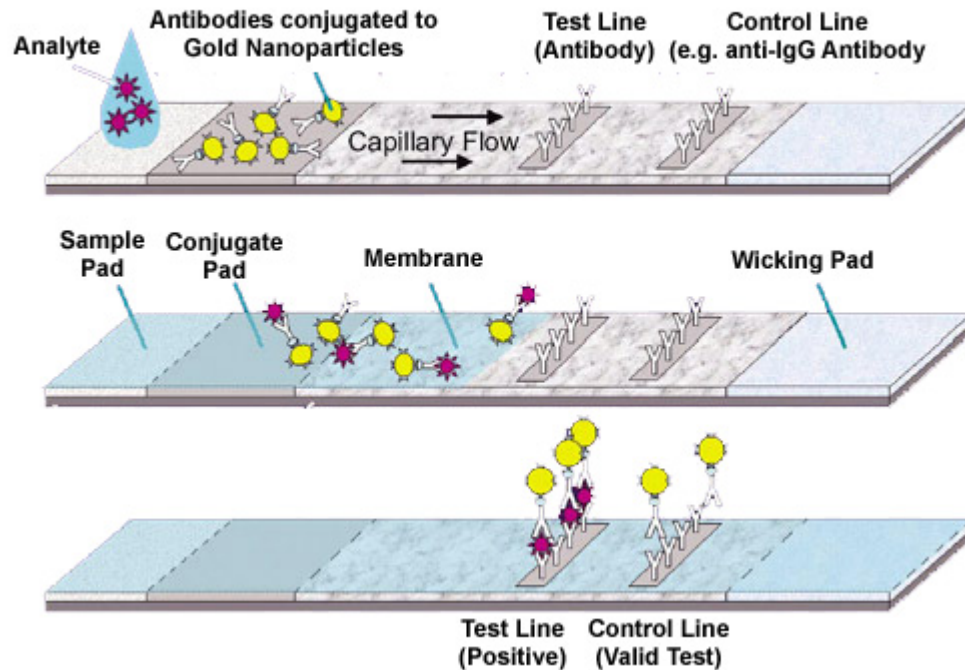
<http://www.appn.net.au/Data/Sites/1/appn/02implementation/technicalresources/troponint/roche-cobas-h232-troponint-method-and-sample-collection.pdf>





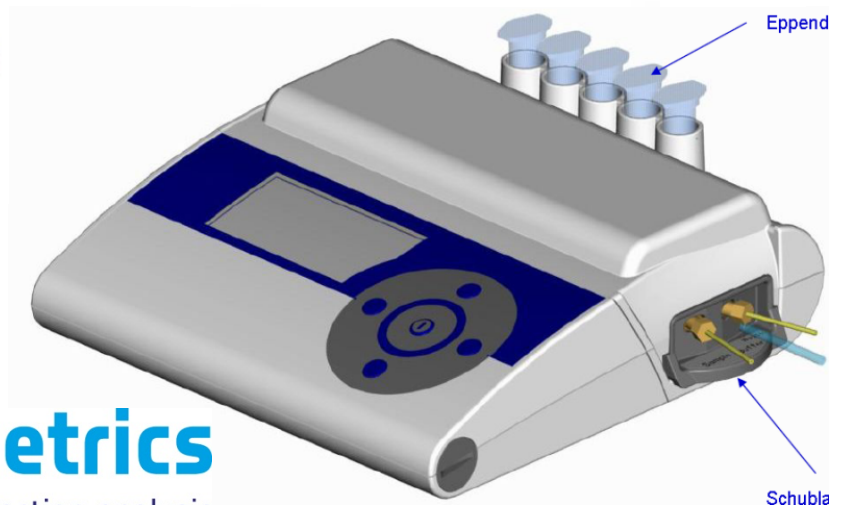
Strips, lateral flow, read-out

Lateral Flow Assay Architecture



<http://www.cytodiagnostics.com/lateral-flow-immunoassays.php>

<http://www.safetyfirstaid.co.uk/Product/SFA/Diagnostics/Roche-Diagnostic-Strips.aspx>



biometrics
biomolecular interaction analysis



Blood gas analyzers

used during operation,
oxygen content, CO₂ or CO level, ...
even control anestheticum





disk used
spinning: distribution of reagent, analyte
certain stations on disk, several tasks at a
time

Further analyzers



Clinical chemistry analyzers



Haematology analyzers



Immunoassay analyzers

1. no non-specific binding process
2. working in complex matrix (blood), difficult, usually blood injection analyses ...





Types of POCT devices

Types of POCT devices III – Hemostasis analyzers

use viscosity

POCT-suitable methods with a high grade of complexity. In this case the control of a specialist (clinical chemist) is essential for operation

- Combined analysis of plasmatic coagulation, thrombocyte function and fibrinolysis (Viscoelastic coagulation tests):
 - Rotation thrombelastometry (ROTEM) (Pentapharm, Germany)
 - Thrombelastography (TEG; Haemoscope, USA)
 - Sonoclot (Sienco, USA)

- Analysis of the platelet function (in vitro bleeding time and optical aggregometry):
 - PFA-100 (Siemens, Germany)
 - Whole blood aggregation (Multiplate, Dynabyte, Germany)
 - Verify now (Accumetrics, USA)



Platelet function: P2Y12 Assay

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[PATIENTS](#)
[INVESTORS & MEDIA](#)
[DISTRIBUTORS](#)
[SUPPORT](#)

Our Technology

- VerifyNow Instrument
- VerifyNow Aspirin
- VerifyNow P2Y12
- VerifyNow IIb/IIIa

VerifyNow P2Y12 Assay

Thienopyridines such as Plavix® (clopidogrel) block the P2Y12 ADP binding site on the platelet. The VerifyNow® P2Y12 Assay uses ADP as agonists to induce platelet activation and ascertain the level of platelet function impaired by these medication. It also incorporates a second channel containing thrombin receptor agonists to approximate an absolute baseline function so that there is no need to withdraw patients from their medication. Studies show that up to 30% of patients taking Plavix have low levels of platelet inhibition (response)^{1,2} and do not receive the expected antiplatelet effect which may increase the risk of ischemic events or unnecessarily delayed from surgery. The quantitative assay provides a simple measurement to determine the patient's level of response to their therapy.

Additional Documentation

[Instructions for Use \(PDF\)](#),
 [VerifyNow System \(PDF\)](#),
 [Waiting for Clopidogrel \(PDF\)](#),
 [Interventional Cardiology Brochure \(PDF\)](#)

INTENDED USE

The VerifyNow P2Y12 Assay is a whole blood assay used in the laboratory or point of care setting to measure the level of platelet P2Y12 receptor blockade.

References:

1. Malinin A, et al. Monitoring Platelet Inhibition after Clopidogrel with the VerifyNow P2Y12 Analyzer: The Verify Thrombosis Risk Assessment (Veritas Study). Thromb Res 2007;119(3):277-284.
2. Serebruany VL, et al. Variability in Platelet Responsiveness to Clopidogrel among 544 Individuals. J Am Coll Cardiol 2005;45:246-51

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Multiplate detection principle



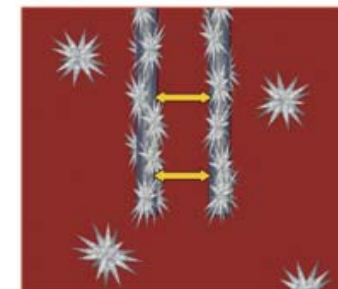
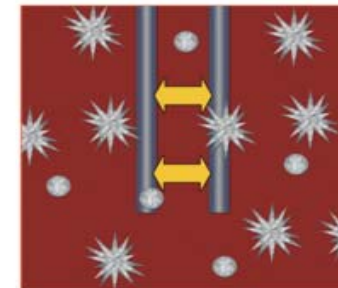
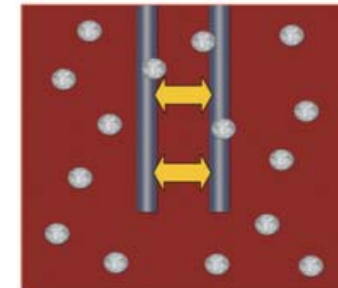
Multiplate® detection principle:

Impedance aggregometry was developed by Cardinal and Flower and has been used since the 1980's for the assessment of platelet function in whole blood.

Impedance aggregometry is based on the principle that blood platelets are non-thrombogenic in their resting state, but expose receptors on their surface when they get activated which allow them to attach on vascular injuries and artificial surfaces.

When platelets stick on the Multiplate® sensor wires, they enhance the electrical resistance between them, which is continuously recorded. In order to enhance the resistance on the sensor wires a tight attachment of the platelets is required.

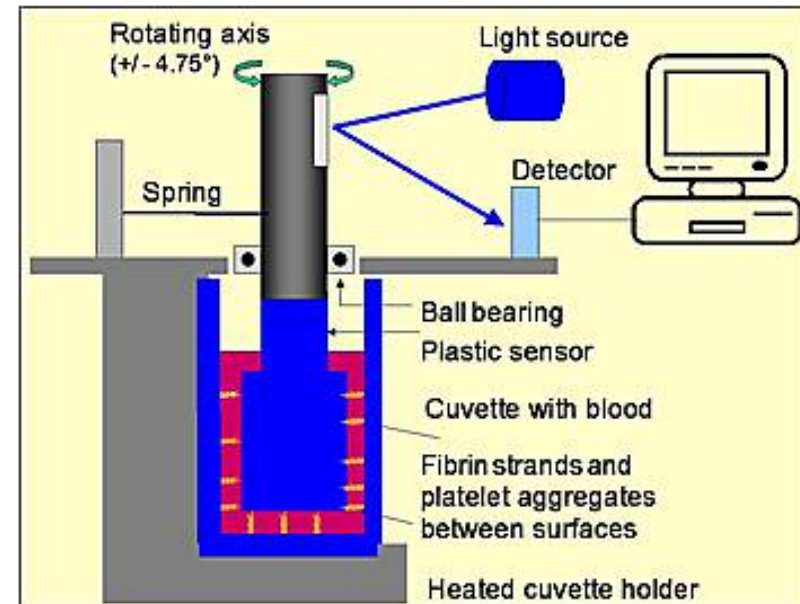
The fact that aggregation in Multiplate® takes place on surfaces is a major difference compared to methods such as Born aggregometry and single platelet counting. In Born aggregometry and single platelet counting methods, platelets aggregate with each other in the liquid phase. This presumably happens only in severely ill patients (e.g. ...), so usually only take place on surface.





Thromboelastometry

ROTEM® stands for rotation thromboelastometry and is an enhancement of classical thromboelastography, a powerful technique for the assessment of blood coagulation disorders.

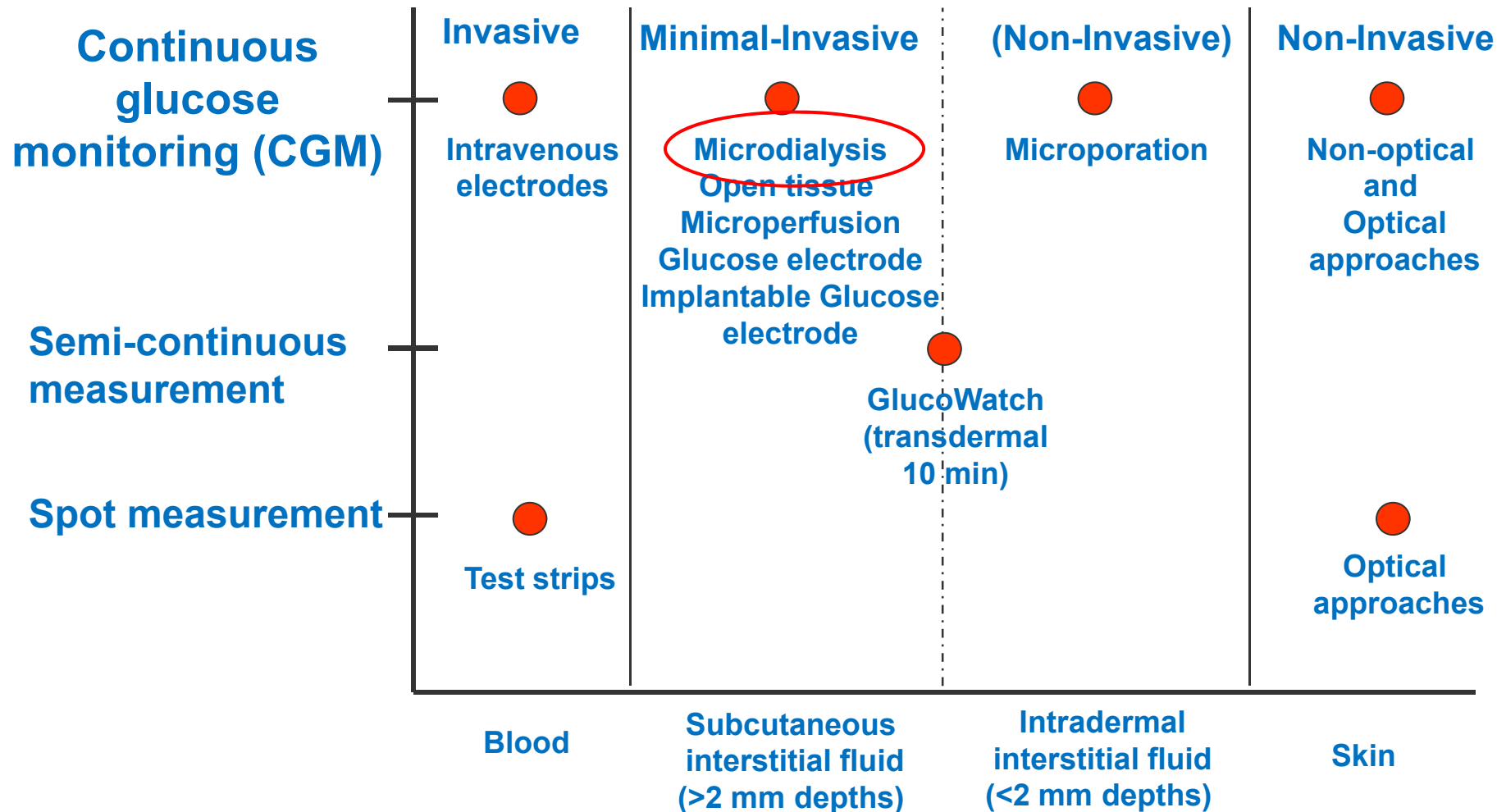


- Blood is added into disposable cuvette in heated cuvette holder
- Disposable pin (sensor) is fixed on tip of rotating axis
- Axis is guided by high precision ball bearing system
- Axis rotates back and forth
- Axis is connected with spring for measurement of elasticity.
- Exact position of the axis is detected by reflection of light on small mirror on axis.
- Obtained data are then analysed on computer
- The loss of the elasticity when the sample clots leads to a change in the rotation of the axis.



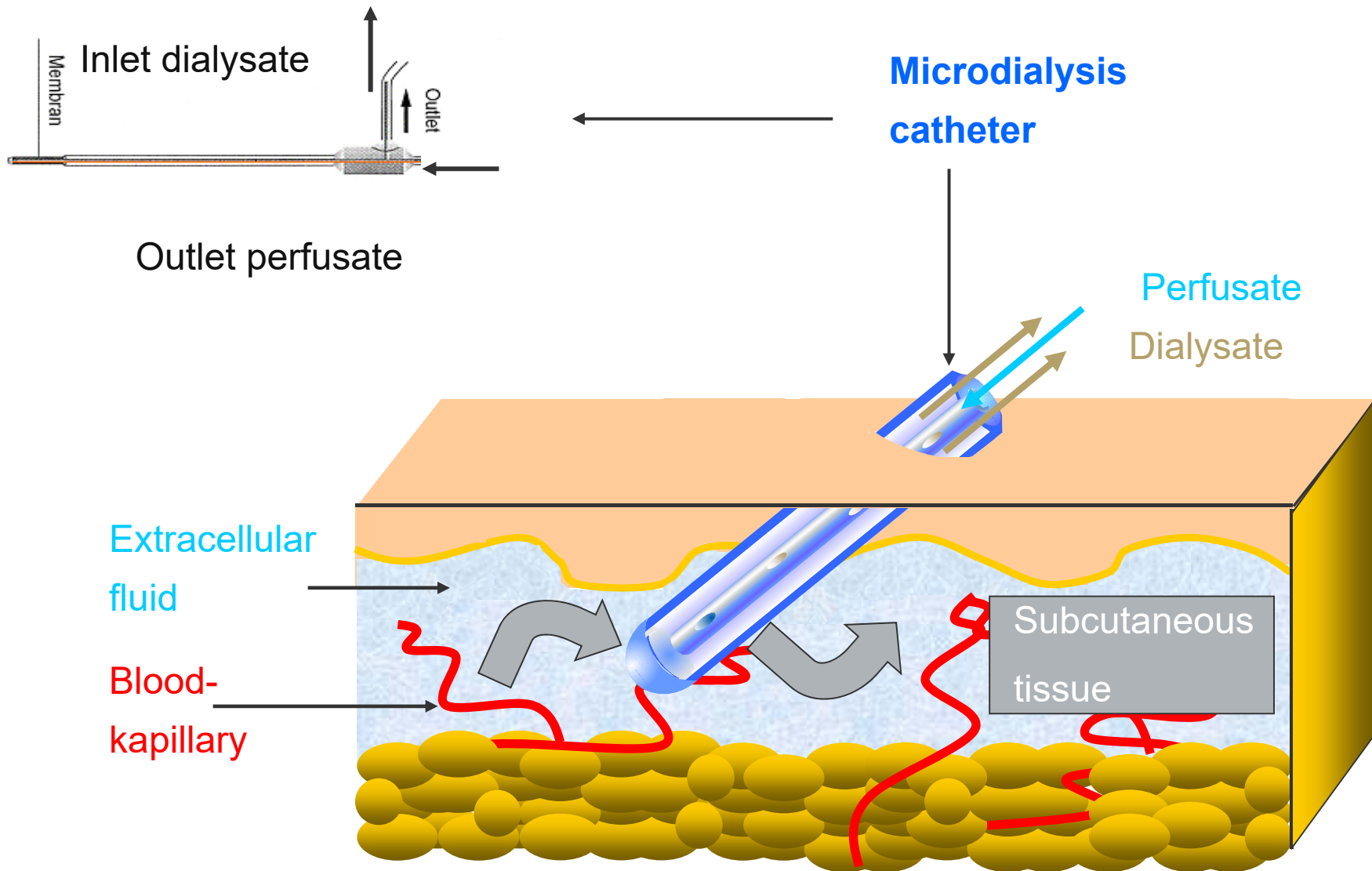
Types of POCT devices

- Types of POCT devices I – continuous monitoring systems





Microdialysis





MicroEye



MicroEye™



Provides a continuous stream of blood biomarkers without the need for blood withdrawal“

- Enables continuous, online and automated monitoring of substances in circulating blood without withdrawing blood from the patient
- Can provide real-time information
- Inserted using common peripheral venous catheters
- Easy to use and reduces risk of contact with blood borne pathogens
- Produces a 'plasma-like' sample without the need for blood centrifugation
- Samples can be collected for off-line analysis if required.



Available CGM system : Guardian RT/Medtronic MiniMed



- Self application by the patient possible
- On-line graphics of results
- Alarm signaling for extremes
- Measurements every 5 min
- Sensor electrode with telecommunication
- Two recalibrations per day
- Gestation time **72 h**



Available CGM system : GlucoDay/Menarini Diagnostics



- Application by the physician
- On-line graphics of results
- Alarm signaling for extremes
- Measurements every 3 min
- Calibrations every 4 h
- Gestation time **48 h**

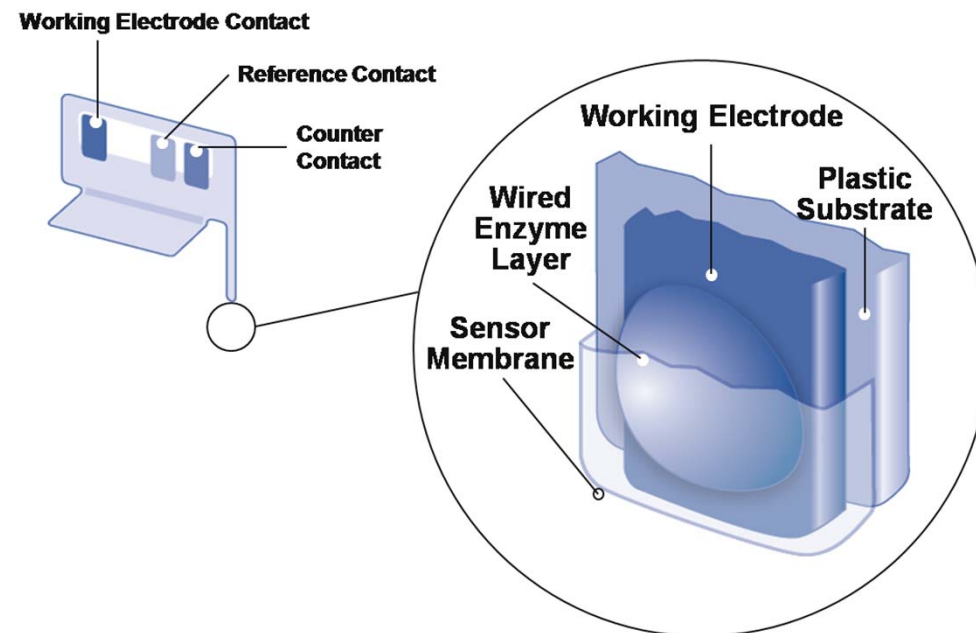




Available CGM system : Abbott Navigator™



Glucose sensor with “Wired Enzyme” technology





Other CGM systems



STS/DexCom

- Application by patient or physician
- Wireless data transfer to reader
- Gestation time up to 7 days
- Alarm signaling for extremes
- Trend messages 1, 3 or



Gluco-Watch Cygnus/J&J



PENDRA Pendragon Medical



Types of POCT devices V

- **Molecular biology-based POCT-devices for the detection of infectious agents**

The Xpert® GBS advantage: **Simplicity**

Detection of group B streptococcus





Clinical perspectives for POCT

“Time issues” and
“Evidence-based medicine”



Intensive care needs

What the intensive care clinician needs are rapid analyses of vital parameters

Blood gases

Contemplative differential diagnosis discussions on the basis of useless analytes are obsolete.

Coagulation

Troponin I/T

„Vital parameter“: analytes with a diagnostic efficiency $> 95\%$ in a ROC plot (the prevalence of the patients being diagnosed is $> 15\%$)

Hemoglobin

Electrolytes



POCT applications

- **Hospital POCT for rapid assessment of life-threatening crisis**

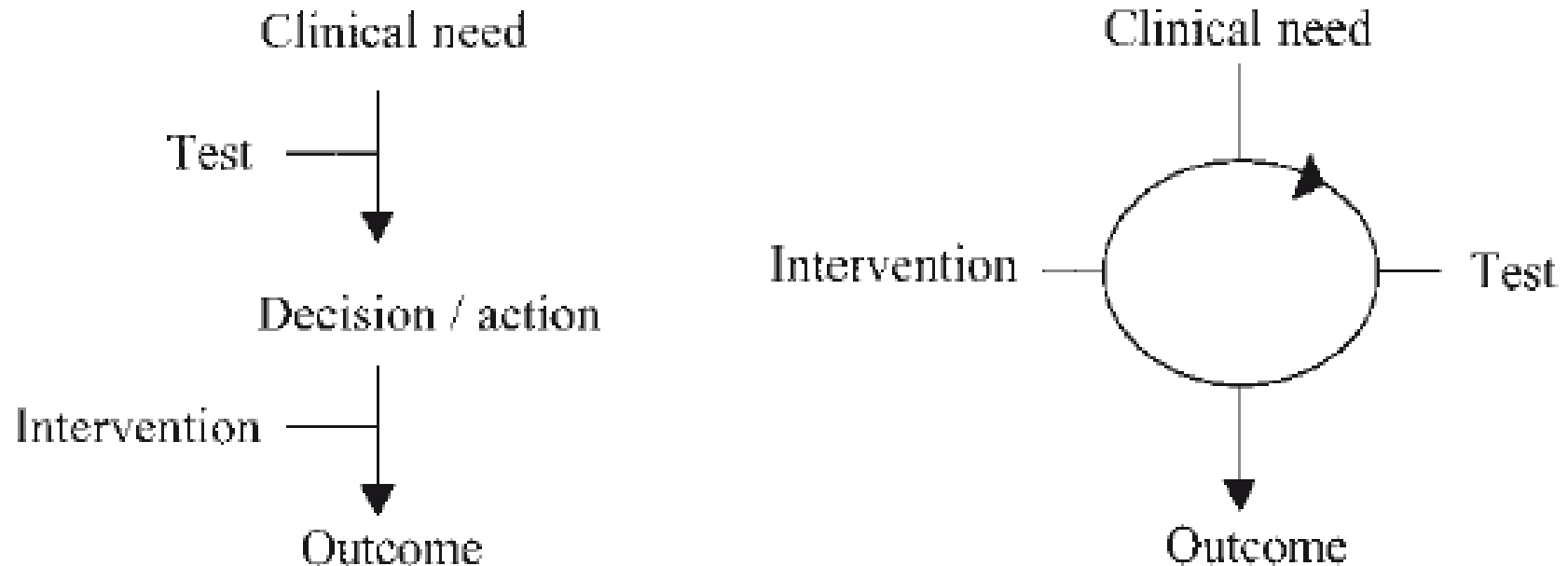
- Hypoglycaemia and hyperglycaemia
- Paracetamol poisoning
- Head injury
- Drug and alcohol intoxication
- Acute chest pain
- Calcium and electrolyte disturbances
- Impaired gas exchange

- **Home care POCT supporting compliance with therapy**

- Diabetes mellitus
- Gestational diabetes
- Coagulation status
- Optimisation of anticonvulsant therapy
- Antiresorption therapy for osteoporosis
- Cholesterol-lowering therapy



The two major scenarios for POCT



The two major scenarios for POCT

- (a) where the speed from clinical need to action is imperative and
- (b) where the continuous cycle of test → decision → intervention produces the improved outcome.



Rapid analysis

- What the ICU clinician needs are rapid analyses of vital parameters
- Contemplative differential diagnosis discussion on the basis of useless analytes are obsolete.
- „Vital parameter“: analytes with a diagnostic efficiency $> 95\%$ in a ROC plot (the prevalence of the patients being diagnosed is $> 15\%$)



Golden Hour

Blood gases

Coagulation

Troponin I/T

Hemoglobin

Electrolytes



for rapid assessment of life-threatening crisis

- In the described scenario, the core lab with optimized response times and additional services competes with POCT techniques.

- Paula Santrach:

It's all about process and value!

Quality = Outcomes + Safety + Service



Turn-around-time



Clinical Outcomes of Point-of-Care Testing in the Interventional Radiology and Invasive Cardiology Setting

JAMES H. NICHOLS,^{1*} THOMAS S. KICKLER,¹ KAREN L. DYER,¹ SANDRA K. HUMBERTSON,¹
PEG C. COOPER,² WILLIAM L. MAUGHAN,³ and DENISE G. OECHSLE²

Conclusions: Although POCT has the potential to provide beneficial patient outcomes, merely moving testing from a central laboratory to the medical unit does not guarantee improved outcomes. Systematic changes in patient management may be required.



The Disposition Impacted by Serial POC Markers in Acute Coronary Syndromes (DISPO-ACS) Trial

A randomized trial, evaluating 2,000 patients in 4 US ED settings

Conclusion: The effect of POCT on length of stay in the ED varies between settings. At one site, POCT decreased time to admission, whereas at another, POCT increased time to discharge. Potential effects of POCT on patients throughput should be considered in the full context of ED operations.

Editor's Capsule Summary: At some institutions, troponin bedside testing may decrease length of stay, but other patient flow considerations may mitigate this effect at many others.

Richard J. Ryan et al. *Annals of Emergency Medicine* 2009;53:321-8



2007

Available online at www.sciencedirect.com



ScienceDirect

Clinica Chimica Acta 379 (2007) 14–28



www.elsevier.com/locate/clinchim

Executive summary. The National Academy of Clinical Biochemistry
Laboratory Medicine Practice Guideline: Evidence-based practice
for point-of-care testing

James H. Nichols ^{a,*}, Robert H. Christenson ^b, William Clarke ^c, Ann Gronowski ^d,
Catherine A. Hammett-Stabler ^e, Ellis Jacobs ^f, Steve Kazmierczak ^g, Kent Lewandrowski ^h,
Christopher Price ⁱ, David B. Sacks ^j, Robert L. Sautter ^k, Gregg Shipp ^l, Lori Sokoll ^c,
Ian D. Watson ^m, William Winter ⁿ, Marcia L. Zucker ^o

Background: POCT has the potential to provide faster test results and therapeutic intervention with improved patient outcomes. However, when over-utilized or used inappropriately POCT results can be misleading and increase healthcare costs.

Results: The „Laboratory Medicine Practice Guidelines“ systematically review the scientific literature relating POCT to clinical outcomes and offer recommendations to improve the clinical utility of POCT.



Need of POCT: Parameters

Coagulation tests:	These POC tests are evidence-based methods which are able to improve the clinical outcome
Blood gases and glucose	Both POCT applications are clearly evidence-based in terms of an improved outcome
Cardiac marker	No evidence for an improved patient outcome but with improved analytical devices an evidence will be likely in the future
Renal function parameter	No evidence for an improved patient outcome
Bilirubin in neonates	No evidence for an improved patient outcome when using POC techniques
...	

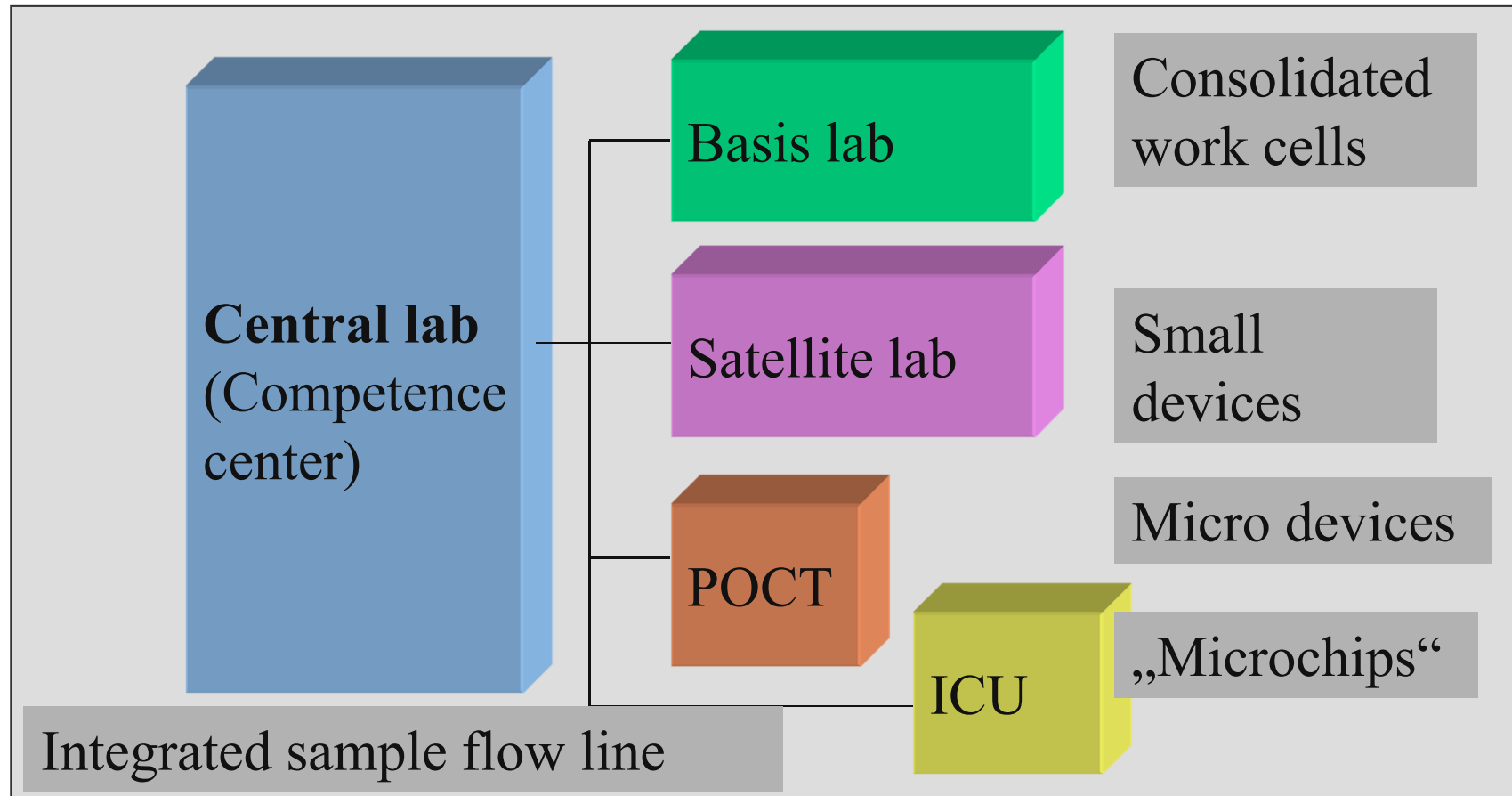


Trends for the next years – Application fields

- Home care POCT:
New applications possible (if novel markers are available)
- Doctor's office POCT:
Still increasing market (POCT for infectious agents)
- Hospital POCT:
Still slightly increasing market (new sepsis markers, arrays?)



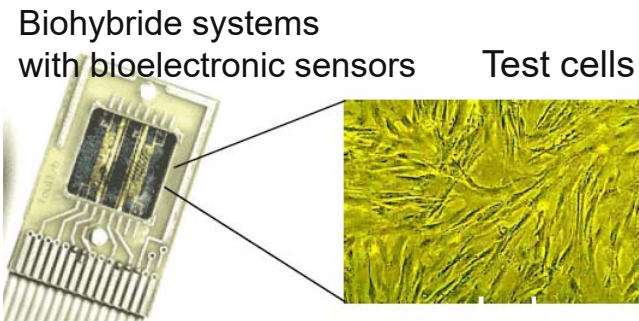
Trends for the next years – Parallelization



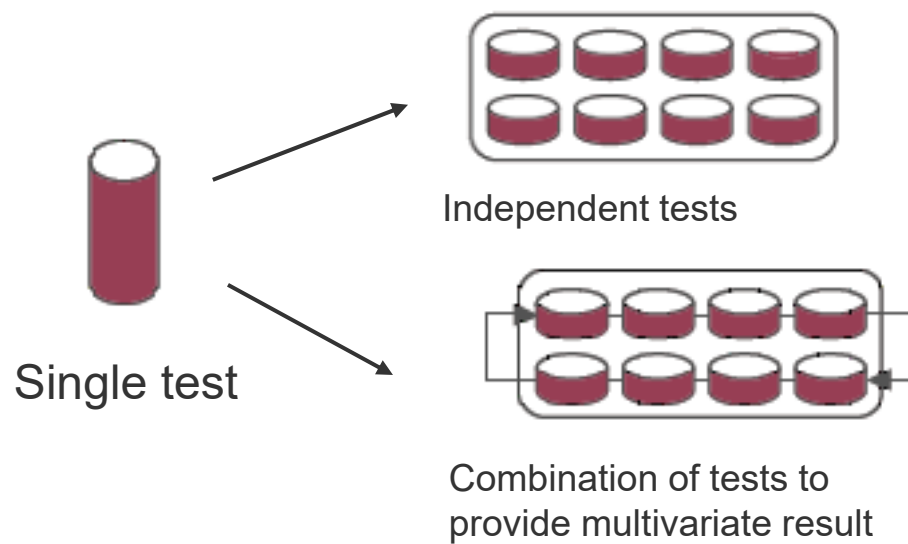
G. Hoffmann, Bayern innovativ, Forum MedTech Pharma, 4. Juli 2007



Trends for the next years



Miniaturization

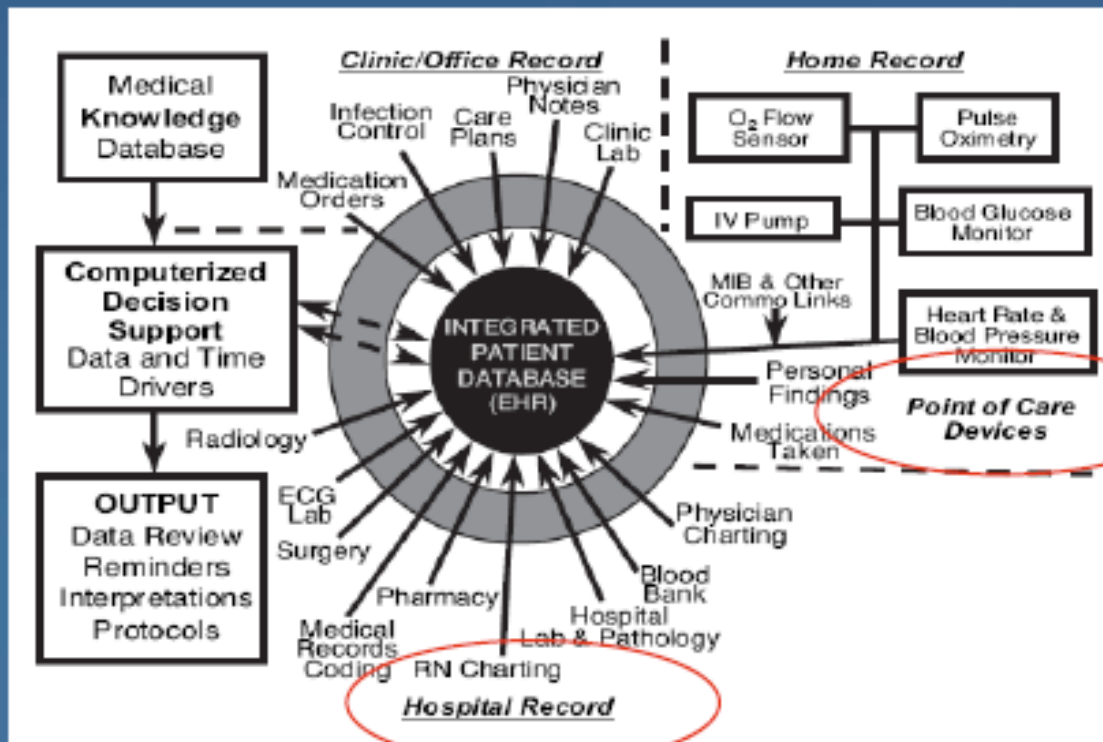


Multiplexing



Trends for the next years – Networking

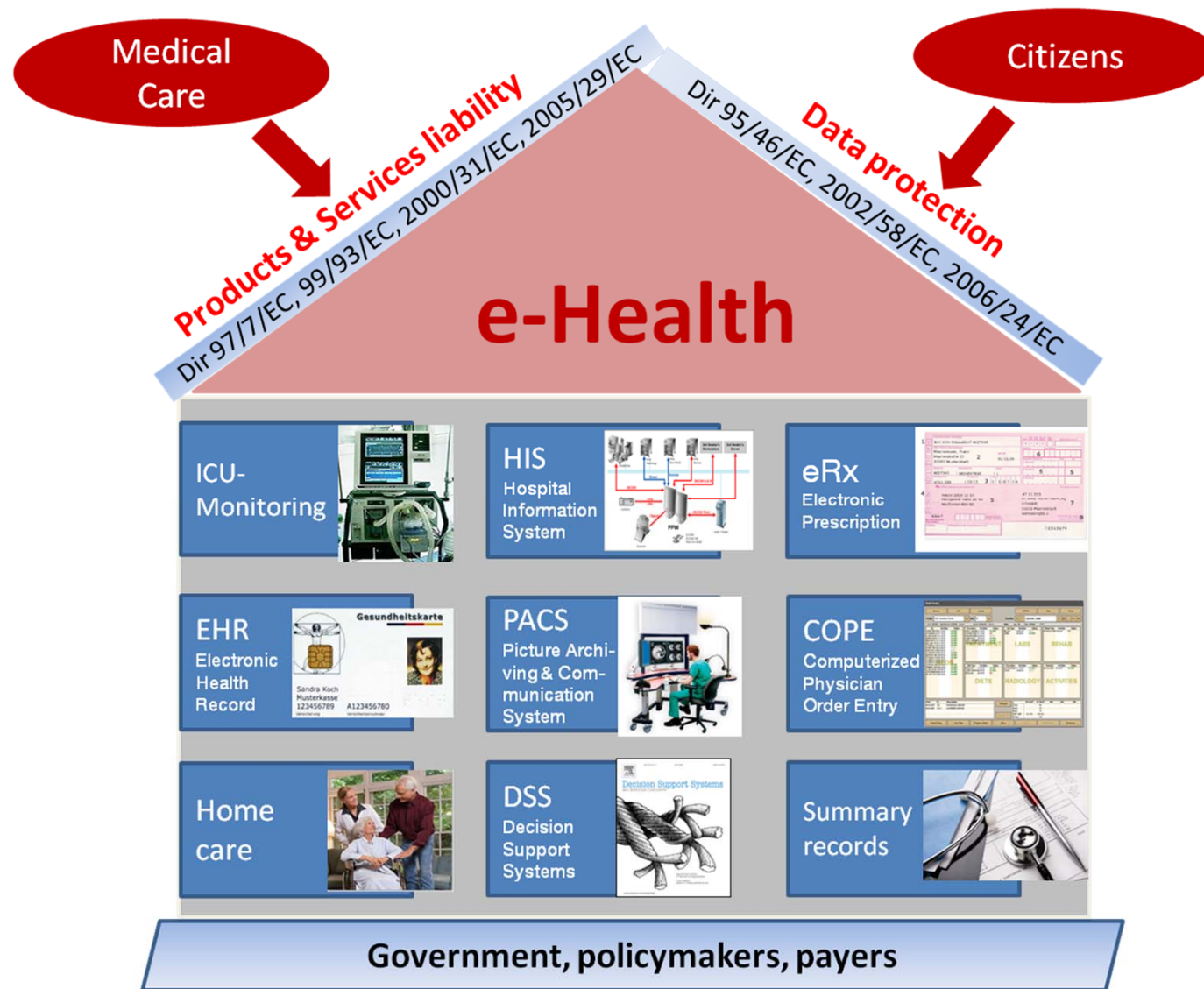
Integrating POCT into the electronic health record (EHR)
The conceptual block diagram depicts the complexity of modern patient management: inpatient, outpatient and self-monitoring



C. P. Price & L. Kricka
Clinical Chemistry 53,
No. 9, 2007 1669



E-Health

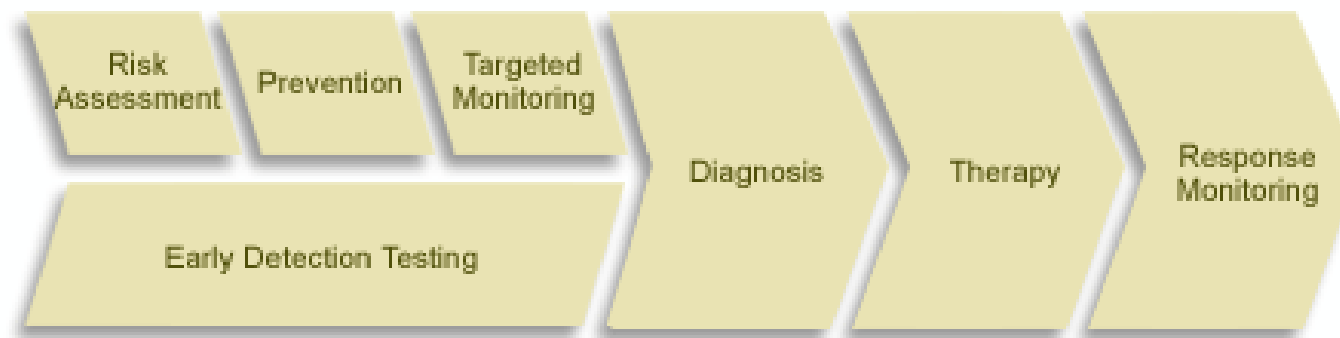




Trends for the next years – Personalized medicine

- For a series of chronic diseases (e.g. diabetes) the following scheme of the personalized medicine paradigm promises a better outcome.
- Novel analytical devices in clinical chemistry (central lab + POCT), together with enhanced collaborations with other medical disciplines (genetics etc.) are needed to reach the goal.

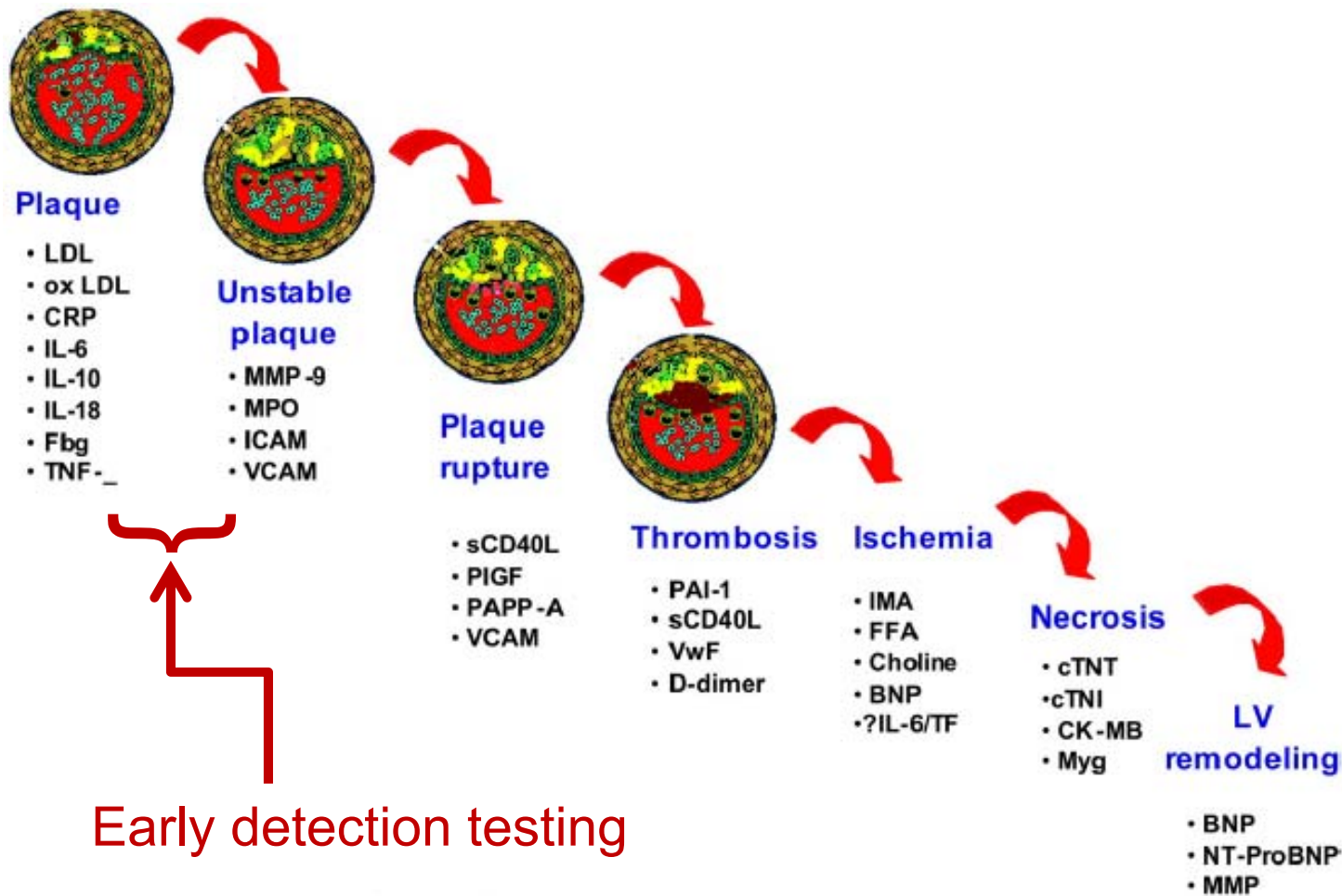
www.personalizedmedicinecoalition.org



The "4P" Healthcare Approach: Predictive, Personalized, Preventive and Participatory



Cardiac markers for different steps of the ischemia cascade



Early detection testing



New technologies and analytical devices



Worldwide research activities are focussed on novel POCT devices

There are trends to use novel:

- ❖ Biological recognition elements (scaffolds, aptamers, lipocalins ...)
- ❖ Optical signal generation techniques (TIRF, SPR, piezo-optical ...)
- ❖ Magnetoresistance

- ❖ Arrays for infectious agents, sepsis, tumors, autoantibodies ...



At present there are tremendous activities for new products:

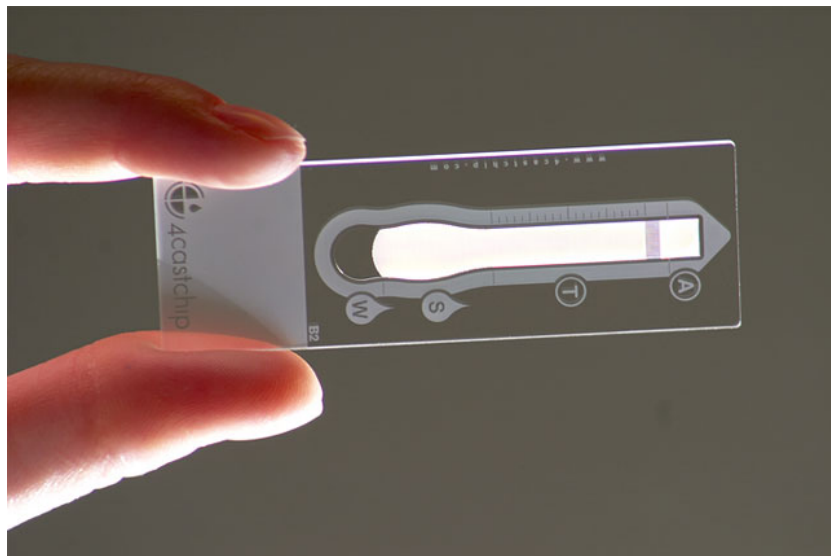
- ❖ Ortho-Clinical Diagnostics acquisition of Amic (**4Castchip** & Reader),
- ❖ Roche's collaboration with Response Biomedical (RAMP 200),
- ❖ Inverness's acquisition of Biosite products (Triage cardiac reader)
- ❖ Radiometer with the new cardiac system **AQT90 FLEX**
- ❖ Nanoentek (Korea) with the new heArt2 system
- ❖ Philips with the novel **Magnatech** POCT immunoanalyzer
- ❖ **Vivacta** with a piezoelectric detection immunoassay
- ❖ Bio-Rad **In2It**, Sysmex **Smart 546** ...





4Castchip & Reader

Amic, recently acquired by Johnson & Johnson, introduced the Forecastchip technology, a generic microcapillary driven chip featuring covalent surface chemistry thought to replace conventional lateral flow membranes. The assay itself is a simple sandwich immunoassay with fluorescence detection.



Relevant parameters:

cTnI

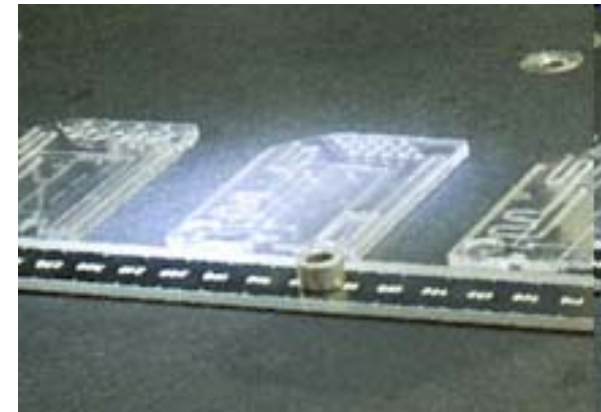
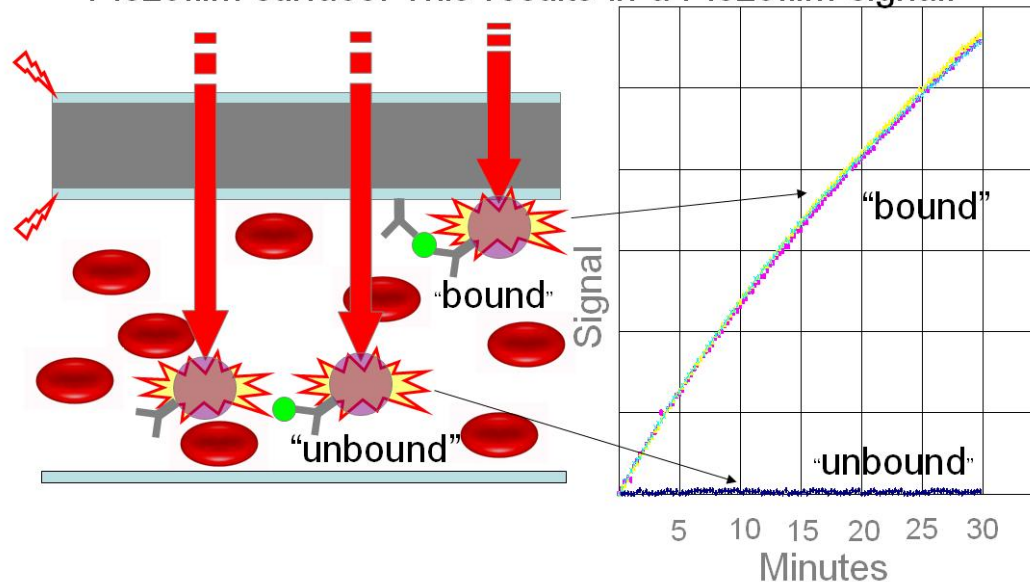
CRP

TSH

NT-proBNP

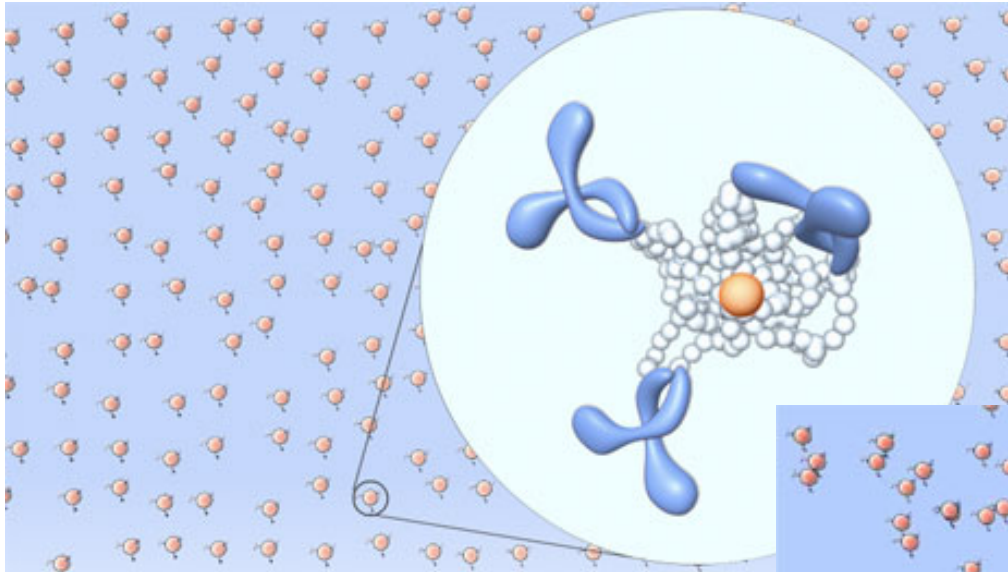
Piezofilm (polyvinylidene fluoride as pyroelectric film) detection: A new, rapid, ultra-sensitive and non separation assay designed for POC immunoassays

Flashing a light source (LED) through the transparent Piezofilm, causes a thermal perturbation where label is bound to the Piezofilm surface. This results in a Piezofilm signal.



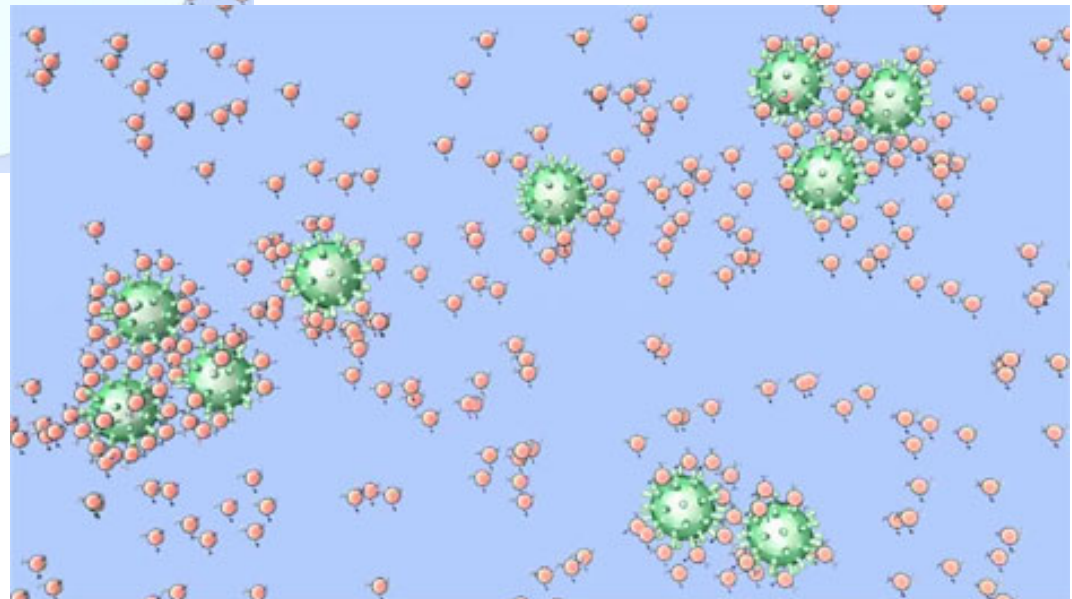


T2 Biosystems' Magnetic Resonance Detection



T2 Biosystems' nanoparticles are composed of an iron oxide metal core covered with dextran polymer. These are in turn functionalized with a detection moiety, such as an antibody recognizing a virus.

Nanoparticles are coated to bind to the target analyte, forming clusters. The presence of clusters changes the T2 of the solution.



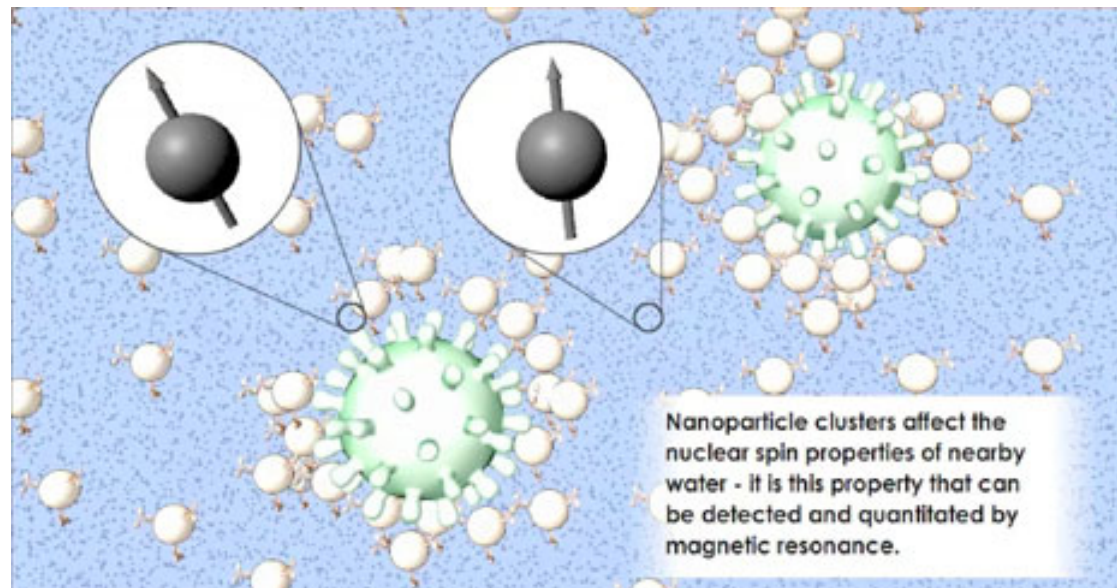


T2 Biosystems' Magnetic Resonance Detection

When the molecular or cellular target is in the presence of T2 Biosystems nanoparticle assay, a change in a characteristic signal is produced that can be measured by magnetic resonance detectors.

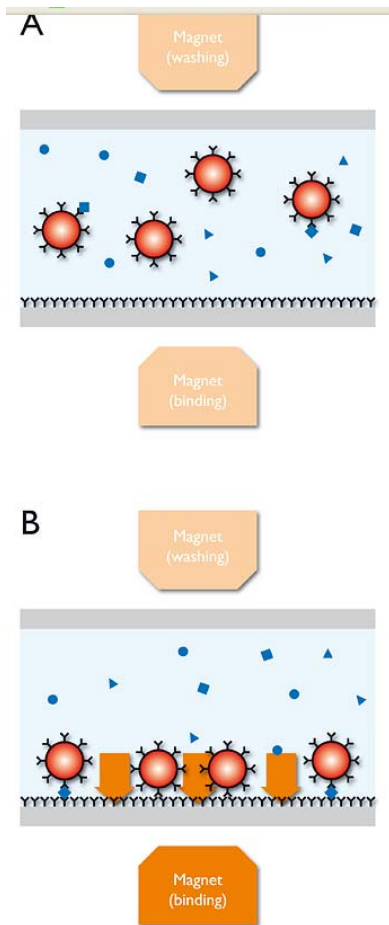
These detectors, which are based on clinically-proven MRI technology, consist of miniaturized magnets, detection coils, and spectrometers and are specifically tailored to provide a rapid output of the nanoparticle assay result.

While conventional MR instruments require large components and have limited range of application, T2 Biosystems' highly miniaturized technology analyzes microliter sample volumes and therefore require much smaller magnets and coils, and less complex electronics, ultimately allowing the technology to be small and portable



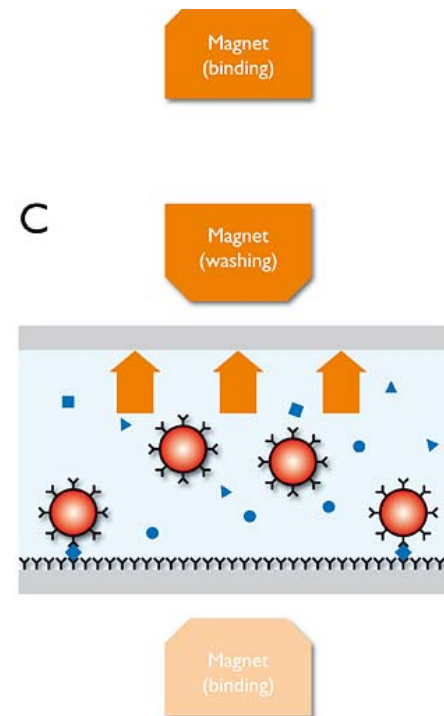


Philips Magnotech



Coated with appropriate ligand molecules, the magnetic nanoparticles bind to the target protein molecules in the sample blood or saliva.

The target molecules end up locked between the active surface and attached nanoparticles.

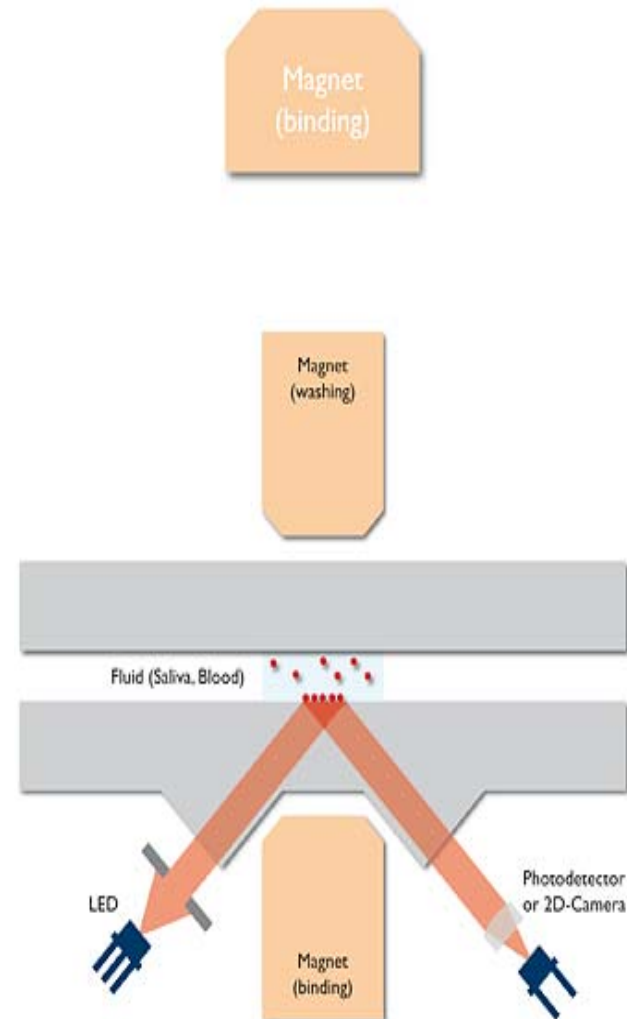


A 2nd magnetic field is then generated to pull unbound magnetic nanoparticles away from the active surface. A fast and accurately controlled separation between bound and unbound magnetic nanoparticles is thereby achieved.



In the final phase, the number of bound nanoparticles is measured using an optical technique based on **frustrated total internal reflection**.

During proof-of-concept tests, the biosensor's magnetic field actuation was shown to speed up assays by a factor of more than 100 when compared to simply letting the nanoparticles diffuse to the sensor's active surface. In the cTnI assay, concentrations below 1 pM were detected in blood plasma in under 5 min.





Giant magnetoresistance (GMR)

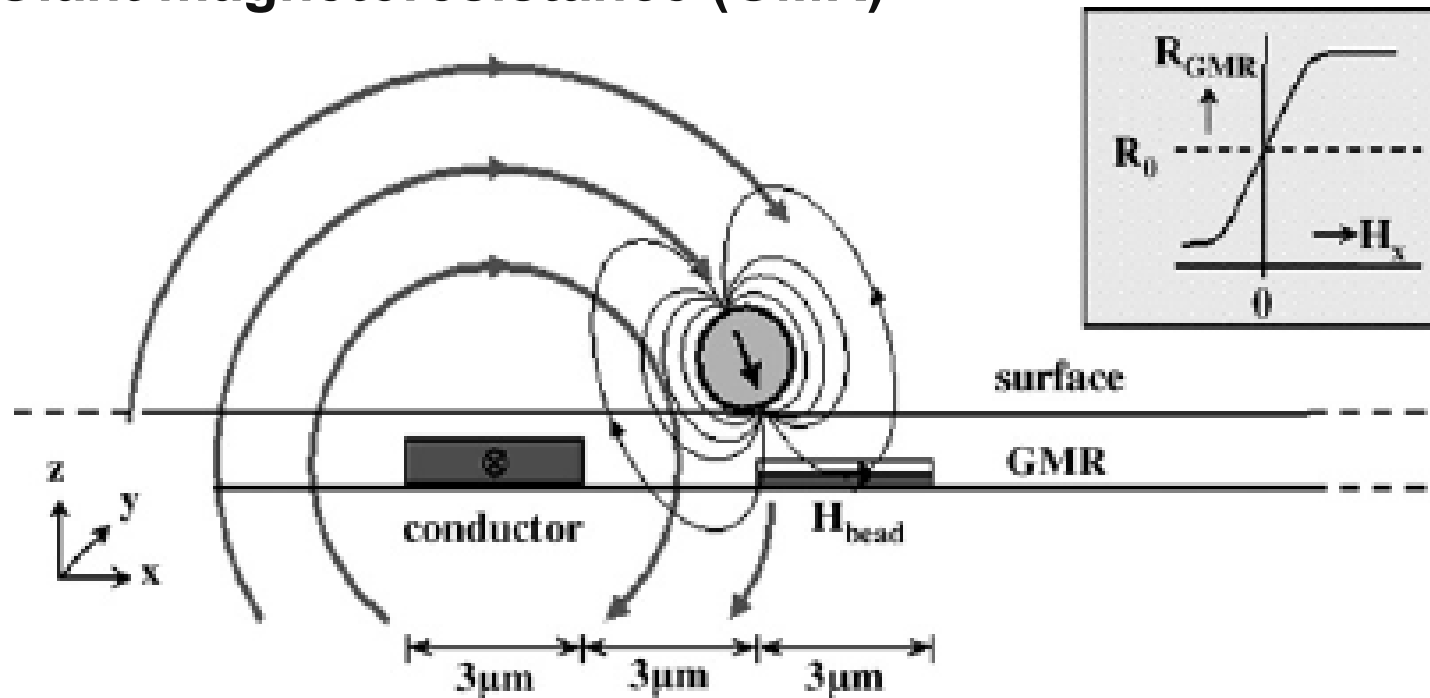


Fig. 1. A sketch of a sensor cross-section with a magnetic label on the chip surface. An excitation current that flows through the integrated conductors produces the excitation field. The stray field from a label that is magnetized by the excitation field results in a resistance variation of the GMR sensor.

An integrated and sensitive detection platform for magneto-resistive biosensors. B.M. de Boer, J.A.H.M. Kahlman, T.P.G.H. Jansen, H. Duric, J. Veen. *Biosensors and Bioelectronics* 22 (2007) 2366–2370



Radiometer AQT 90 FLEX

Immunoassay analyzer

The AQT90 FLEX analyzer combines high measuring accuracy vital to acute care testing with the ease of use you need in your everyday work – whether in the lab or at the point of care.



Parameters	Units	Measuring ranges
Troponin I	µg/L	0.010-50
CKMB	µg/L	2-500
Myoglobin	µg/L	20-900
NT-proBNP	ng/L	12 - 35,000
CRP	mg/L	5-500
BhCG	IU/L	2-5,000
D-dimer*	µg/L	80-100,000

Sysmex Smart 546

CRP analyzer

Smart 546 is designed for highly sensitive CRP measurement





Afinion AS100 Analyzer



The Afinion AS100 Analyzer from **Axis-Shield** is designed to enable on-the-spot testing, regardless of the sample type.

Key features

Multiple analytes on one instrument

Very easy to use

Short assay times

No mess - no maintenance

All-in-one reagent cartridge

Long shelf life of reagents

Convenient kit size: 15 tests

Integrated failsafe mechanism





Bio-Rad In2It - HbA1c-Analyzer

This system provides results while the patient waits. The in2it assay requires only 10µL of capillary or venous blood and uses boronate affinity chromatography for results that are free from Hb variant interference and traceable to the worldwide DCCT reference.



Nanogen DXpress™ Reader

The DXpress Reader is a multi-functional portable tabletop instrument used to read results of quantitative *in vitro* immunodiagnostic assays. The reader works in conjunction with Nanogen's Vvent product line to measure and display quantitative results. This easy-to-use instrument is ideal for use at the point-of-care and in central laboratory settings.





epoc SmartCard Technology

- Easy to use
- Room temperature storage
- Ready for use at any time
- Integrated calibration and quality checks
- Results in just 30 seconds
- Barcoded for error-free panel identification & lot number, and inventory records



epoc
BLOOD ANALYSIS

*A cost-effective point of care solution
for the entire healthcare enterprise.*





Nanoentek heArt²



“Membrane-free” immunoassay technology with fluorescence detection:
FRENDO™ = Fluorescence Response Enhanced Nanodrop Diagnosis

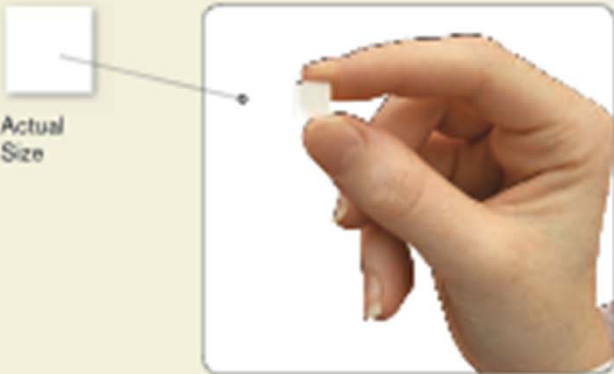
The FRENDO is a portable fluorometer that read and interpret information from a diagnostic device and report the result in electronic format. The FRENDO™ data management software provides access to all diagnostic information at one central location. It is real ubiquitous environment instrument.



Biochip Array Technology (BAT) by Randox Ltd.



BAT is an immunoassay testing platform for the simultaneous multianalyte testing of a panel of related tests. After addition of a single patient sample to the biochip, analytes present in the sample bind to the specific ligands. The degree of binding is determined using a chemiluminescent light source and quantified using a CCD camera and imaging system. Randox offer three biochip analysers, the Evidence, Evidence Investigator and the Evidence MultiStat for POCT.



Biochip Array Technology

- A single 9x9mm biochip acts as the reaction vessel
- Up to 23 discrete test regions (DTRs) on each biochip
- Immobilised ligands specific to different markers on each DTR
- Two DTRs reserved for quality control
- Chemiluminescent immunoassays
- Charge coupled device camera detects the light signal generated by each DTR
- Advanced image-processing software compares each signal against a calibration curve



Speed

- Multiple results available within 20 minutes
- Dual sample capacity – up to 6 samples per hour

Ease of use

- 3-step process - add sample to cartridge, insert cartridge, read results
- Colour Touch Screen with Windows® based software

3-step process



Add sample to cartridge



Insert cartridge



Read results



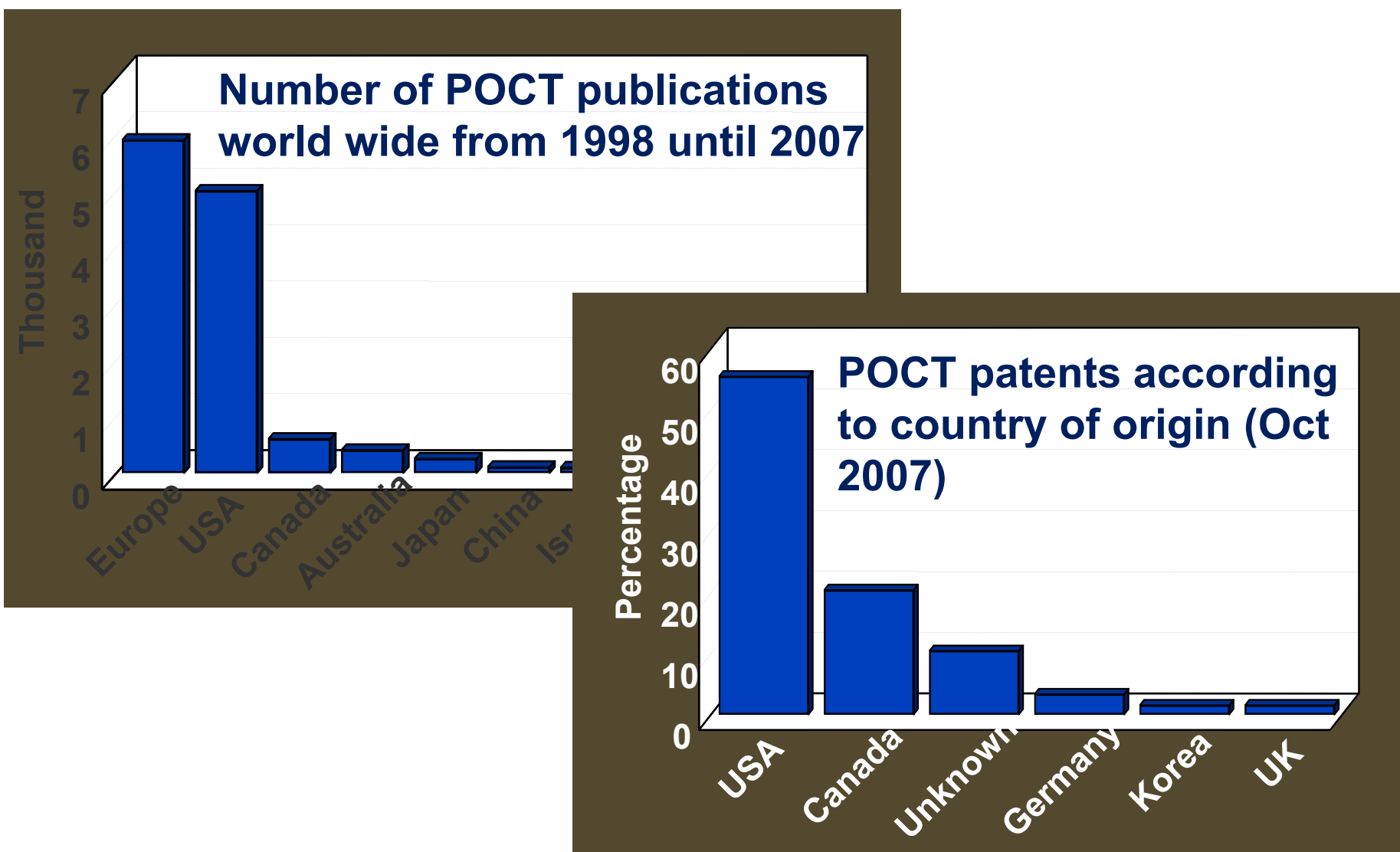
- Full connectivity to central laboratory without additional software

Flexibility

- Suitable for use in a wide range of near patient applications

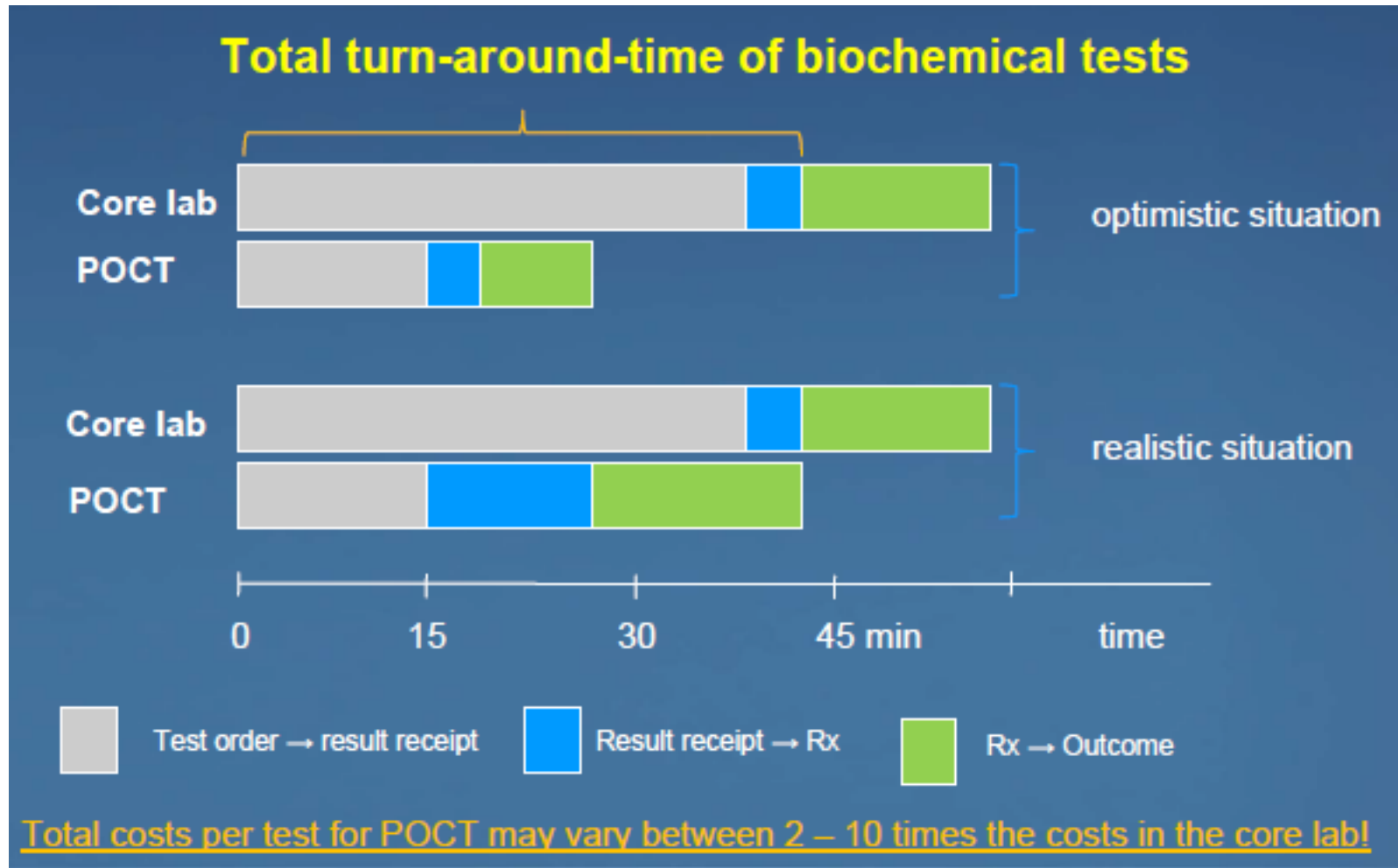


Publications - patents





Costs / turn-around-time





Benchtop instruments

These are more complex analyzers being placed primarily in the functional areas of an ICU or in the ER.

These instruments use various analytical principles:

- Spectrophotometric clinical-chemistry analyzers with several channels (e.g. Abaxis Piccolo)
- Clinical-chemistry test strip/cassette devices (Roche Reflotron)
- Hematological multi-channel analyzers (Sysmex pocH-100i)
- Blood gas analyzers with/without oxymetry and biosensoric electrolyte/substrate determinations (IL, Nova, Radiometer, Roche, Siemens)
- Immunological multi-channel devices (Mitsubishi Chemical Pathfast)



Luppa
Schlebusch



POCT – Patientennahe Labordiagnostik

 Springer



Application fields

The question **“What is clinically useful?”** must be seen for POCT applications not only under aspects of evidence-based diagnostics, but also under legislative and economical aspects



POCT: selected rules and standards for quality assessment of the clinical laboratory

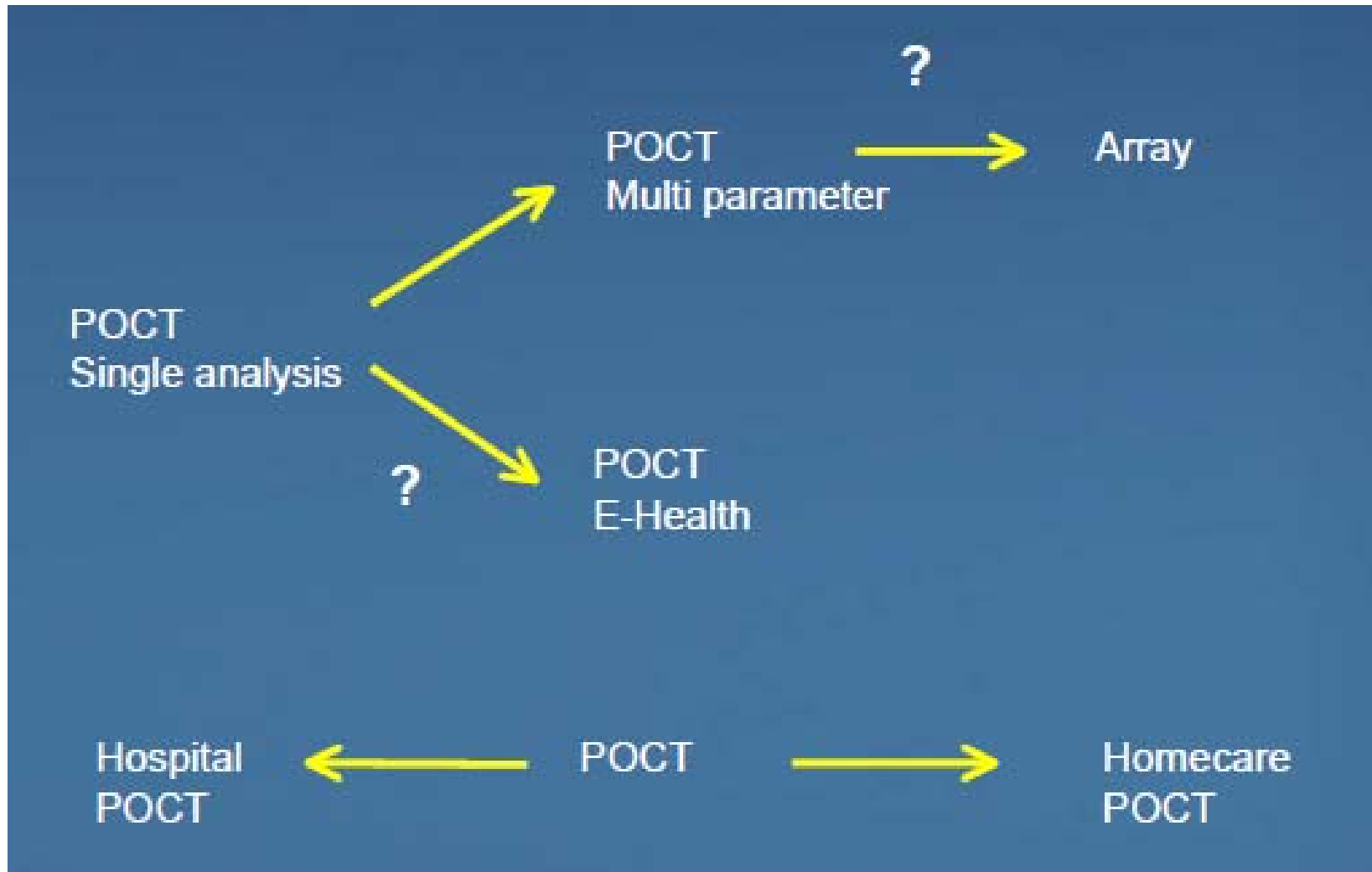
- ISO15189: Quality management in the med. laboratory (2003)
- ISO 22870: Point-of-care testing (POCT) – Requirements for quality and competence (2006)
- NCCLS* AST2-A: Point-of-care in-vitro diagnostic testing (1999)
- NCCLS* EP 18-A: Quality management for unit-use testing (2002)
- NCCLS* POCT1-A: Point-of-care connectivity (2004)



* NCCLS renamed as CLSI

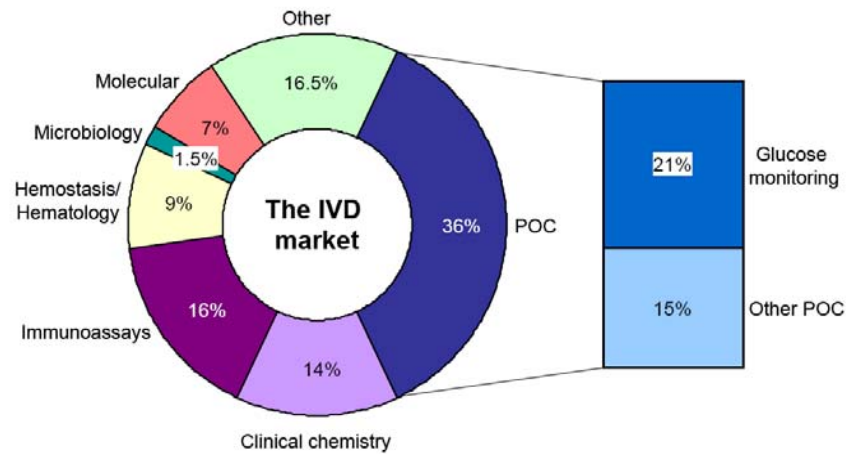


Future of POCT



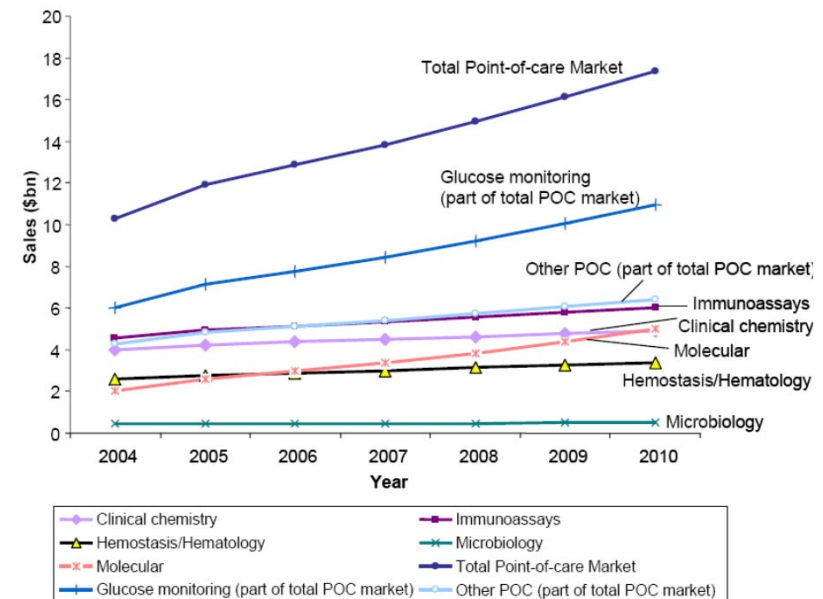


Situation on market



Source: Merrill Lynch; Roche; Digene; Author's research & analysis

Business Insights Ltd



Source: Merrill Lynch; Roche Diagnostics 2005; Digene; Author's Research & Analysis

Business Insights Ltd

→ But: there are much more applications!

Dr. Alexander Jung



Lateral Flow systems for POCT

Tumor Markers: GlysBy/Italy



Cardiac Markers: Spectral Diagnostics/Canada





Market trends

- Most current POCT systems are based on lateral flow devices
- Electronic readers are employed for quantification of lateral flow immunoassays
- Most readers have desktop size
- Fluorescence labels increase sensitivity in lateral flow immunoassays
- First real wet chemistry devices are launched now
- Asian manufacturers focus on optimized interface device/patient
- User-friendly and safe sampling is an important issue @ POCT
- Rapid blood sampling methods increase application sites for POCT
- Ultrastable enzymes and stabilizers increase shelf-life of POC systems
- POCT is also an issue in the wellness industry



POC Testing

- Directly test at the bedside, ICU, emergency room etc.
- Not limited to medical diagnostics
(wellness, food control, ...)
- Gives results quickly → faster than central lab (1-20 min)
- Measuring emergency parameters for fast decisions
- Workflow is minimal → user friendly device
- *Current major issues/problems of POCT:*
 - Single Parameter testing only – too unspecific
 - More expensive than lab testing (but value added!)
 - Lower analytical quality
(needs improvement of technology & education!)
 - Low throughput (not a flaw for single patient testing)



Some Applications



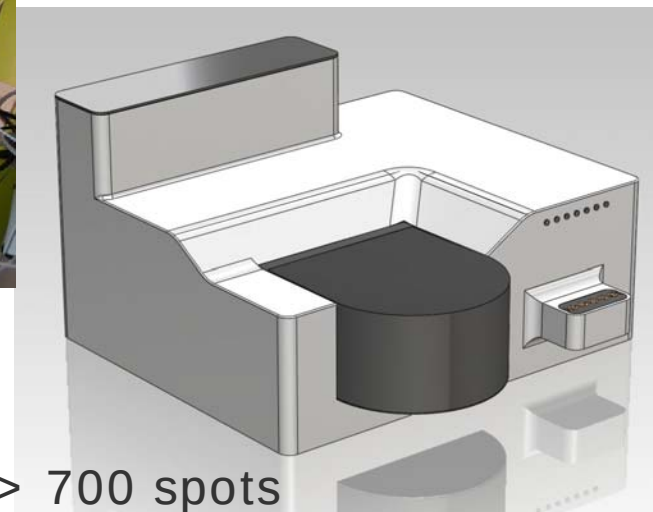
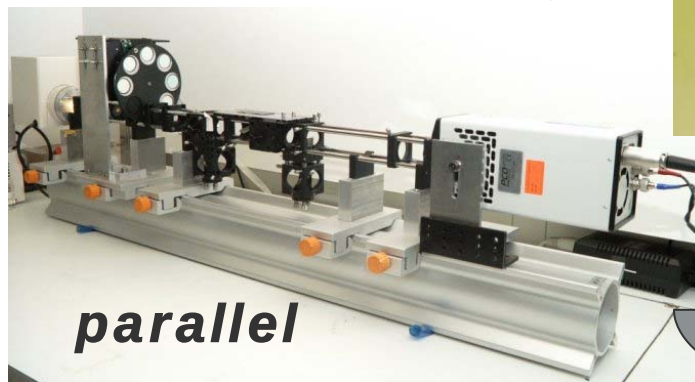
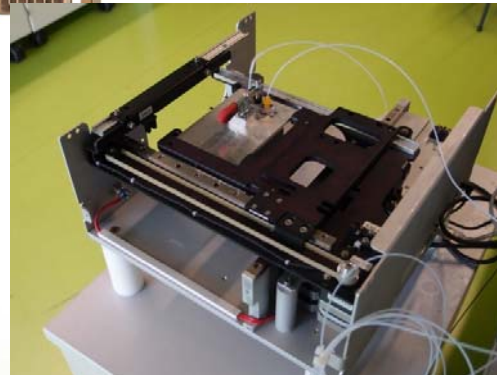
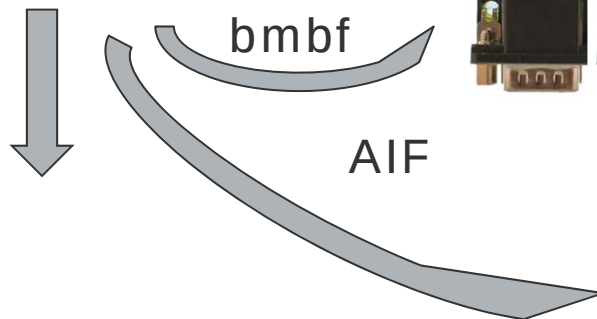
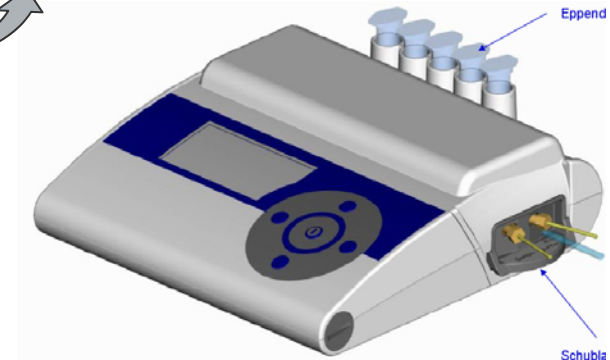
Device development



White light with
modified Spekol

biametrics
biomolecular interaction analysis

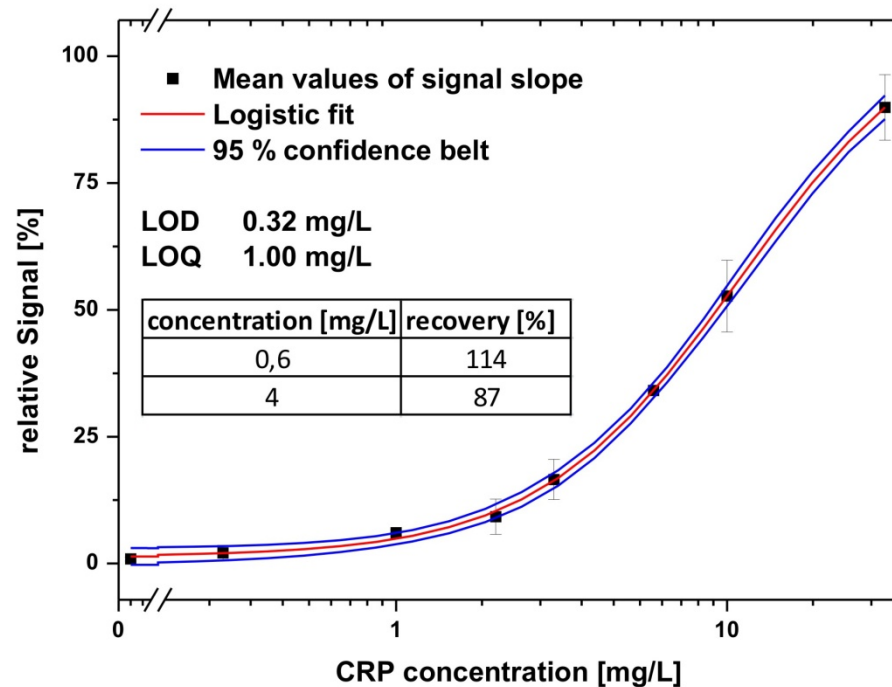
Single wavelength



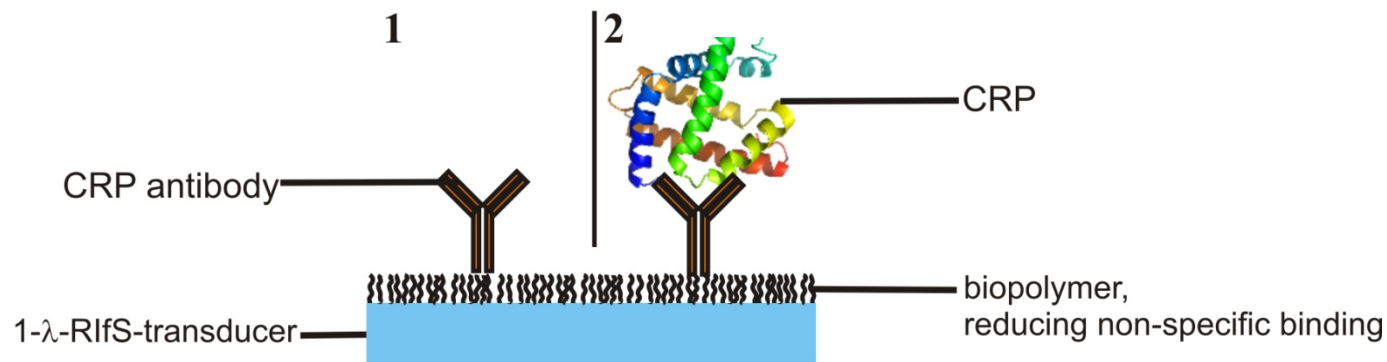


CRP: general parameter for inflammation

- C-reactive protein (CRP) is an **inflammation** marker in blood
- CRP increases in case of infection (bacterial, viral)
- Challenge: antibodies as recognition elements on the sensor surface problematic immob.



RfS



Melanie Ewald



CRP: working range optimised by scaffolds

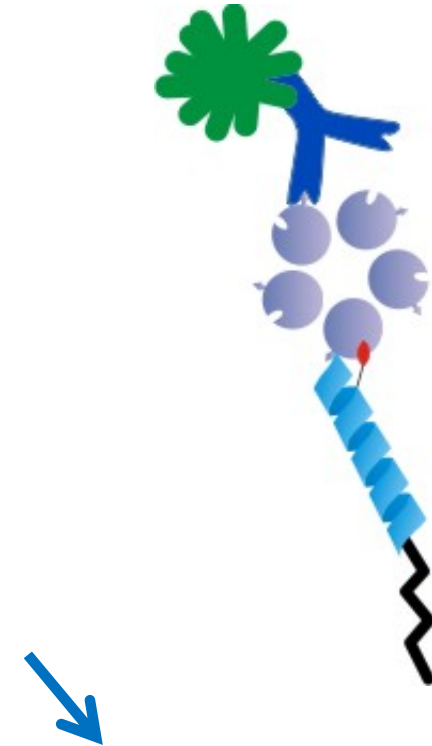
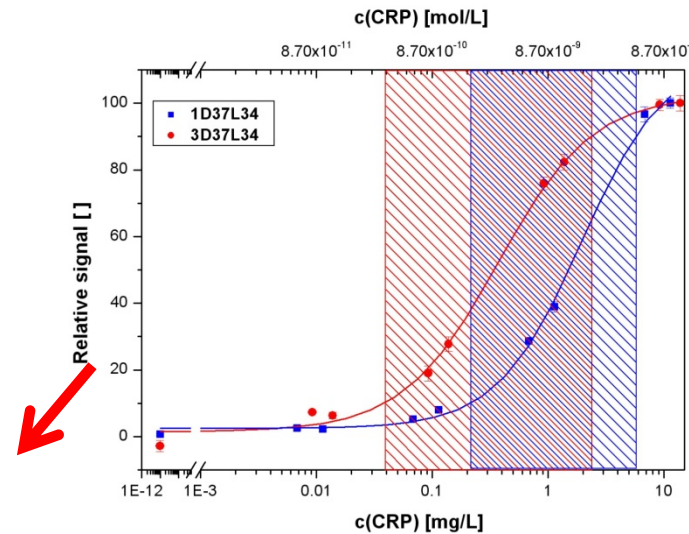
Detection

antibody carrying
a **fluorescence**
label

Analyte **CRP**

Capture element:
peptide scaffold
with independently
linked
ligand and **spacer**

Christiane Albrecht



TIRF

Sandwich

3-D37L34

0.04 – 2.3

$3 \cdot 10^{-10}$ - $2 \cdot 10^{-8}$

Binder

Working range [mg/L]

Working range [M]

1-D37L34

0.22 – 5.75

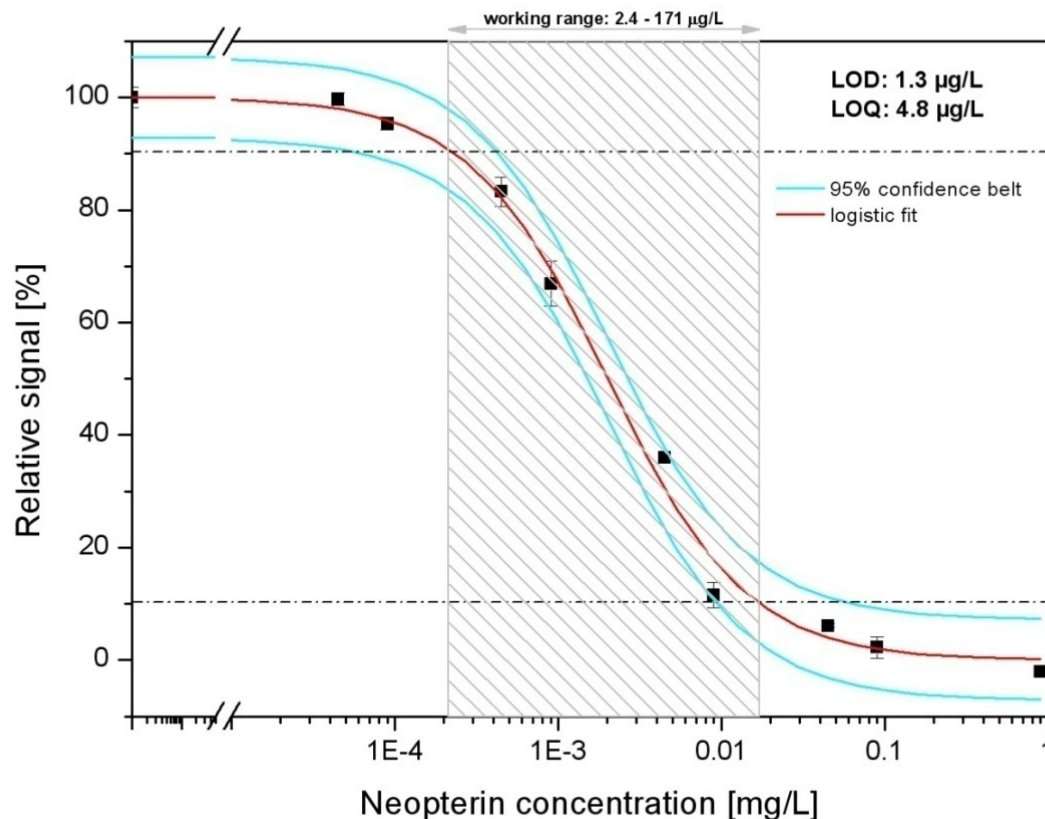
$2 \cdot 10^{-9}$ - $5 \cdot 10^{-8}$



Neopterin assay

TIRF platform, binding inhibition

Markus Ehni



Neopterin marker for

- HIV infection status
- Cardiovascular disease
- Organ transplantation
- Autoimmune disease
- Tumors
- T-lymphocytes-activity

Binding inhibition assay

- Surface: immobilised Neopterin- OV-conjugate from VRI (Franek)
- Buffer: PBS
- Samples in 10% pooled plasma
- LOD and LOQ sufficient, even in 10% plasma



Antiphospholipid Syndrome

Background

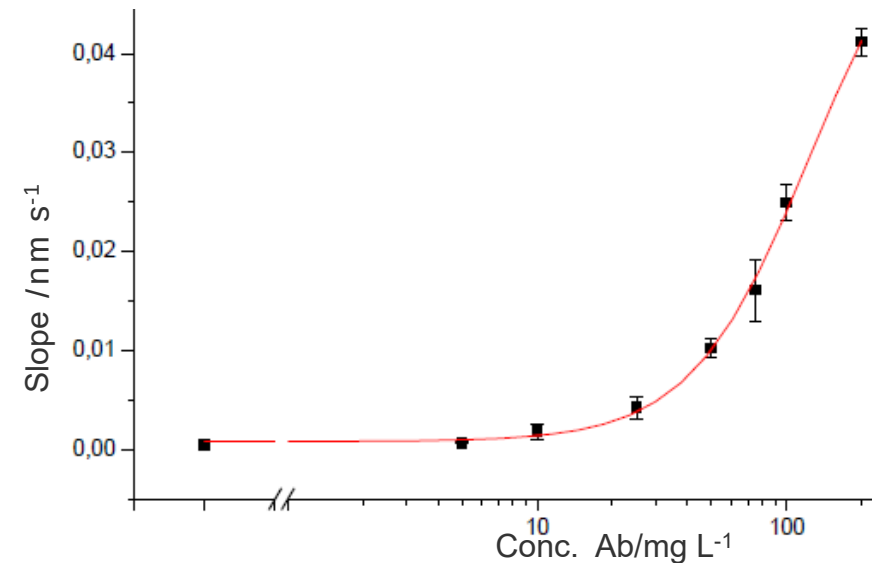
- Antiphospholipid syndrome is an auto-immune disease leading to dysfunctions in **blood coagulation**
- β_2 -glycoprotein 1 (β_2 GP-I) is the main auto-antigen
- Binding of β_2 GP-I to cell membrane is required for reaction with antibody

Assay

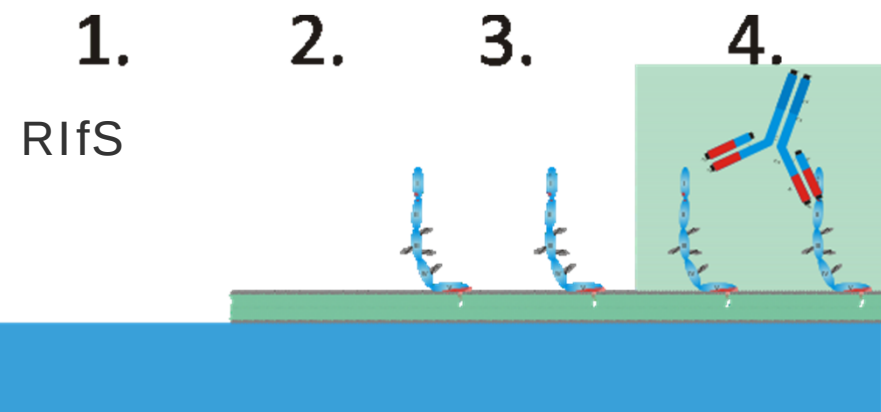
- Transducer (1.) coated with model membrane (2.)
- β_2 GP-I is bound to membrane leading to reactive conformation (3.)

- Biomimetic approach mimicing the disease on the transducer
- Label-free detection of antibody through RfS (4.)

Essential for emergency diagnostics during pregnancy



Urs Hilbig



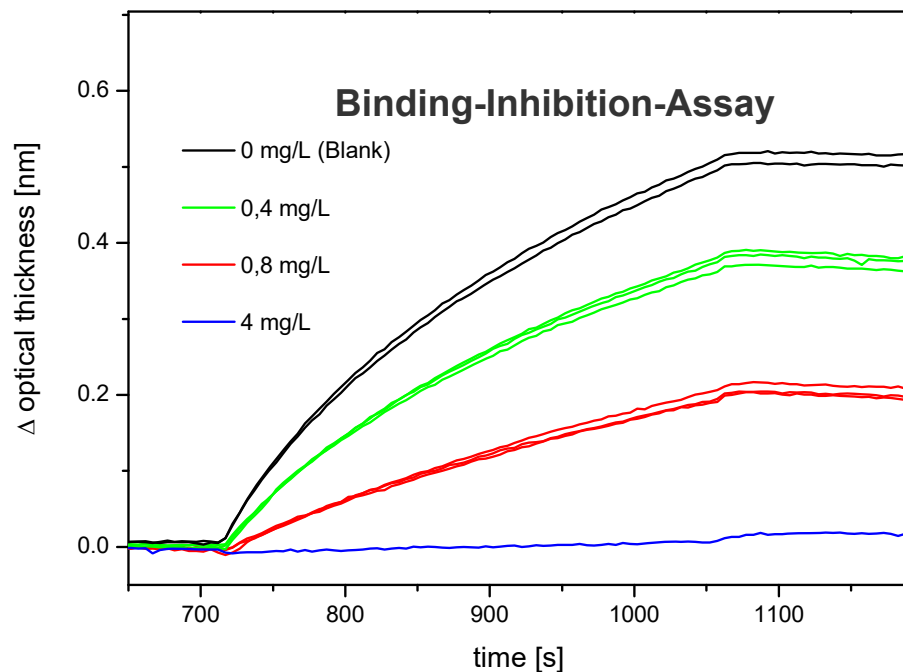


Cystatin-C

- Advantages compared to creatinine
- Cystatin C: Marker for renal failure
- small protein (13,6 kDa)



R. Janowski, M. Abrahamson, A. Grubb, M. Jaskolski,
Domain swapping in N-truncated
human cystatin C *Journal of Molecular Biology* **2004**, **341**,
151-160.



RfS

Oliver Bleher

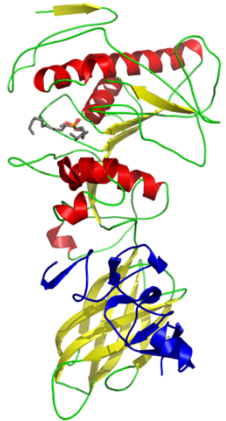
- constant serum-level of
0,53 – 0,95 mg/L
in healthy individuals



increase of serum
level indicates
kidney dysfunction



Detection of acute pancreatic failure

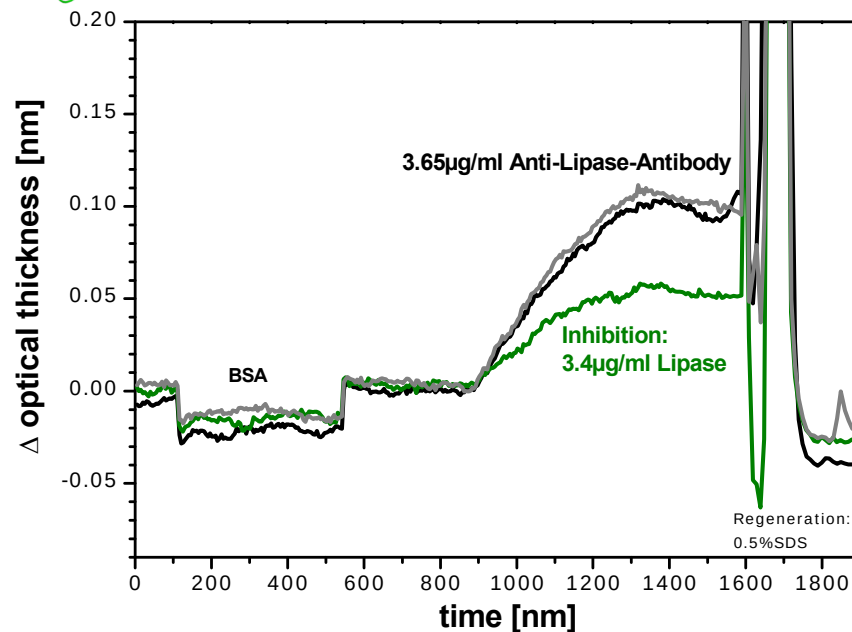


At failure of pancreas high conc. of enzyme lipase can be monitored in blood

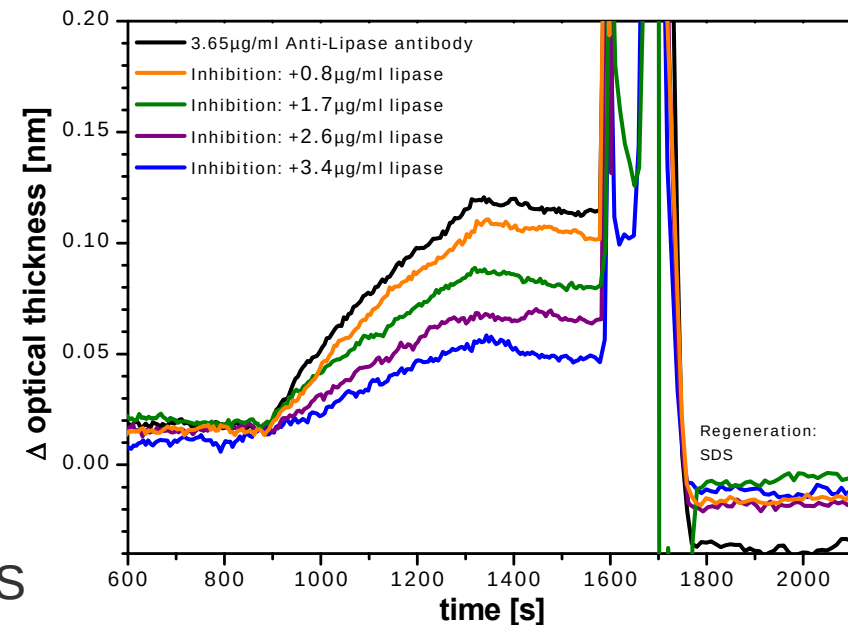
Binding-inhibition assay to measure lipase concentration:

- Immobilisation of pancreatic lipase
- Measurement of antibody
- Control: Inhibition with various lipase concentrations
- currently: measurements in buffer
- Regeneration of the surface possible

Katrin Krieg



RfS

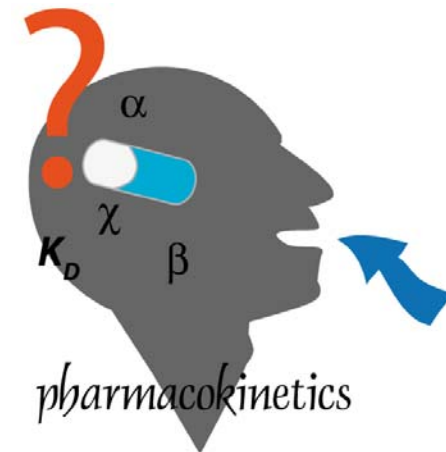




Necessity for TDM?

- **Inter- and intra-patient-variability** (up to 20fold)
related to pharmacokinetics and blood level
- **Control of blood level** influenced by some pharmaceuticals
- Faster/better **Response to therapy**
- Reduction of **spillover effects**
- **Safety** (avoidance of toxic concentrations)
- **Cost efficiency** of therapy

Katrin Krieg

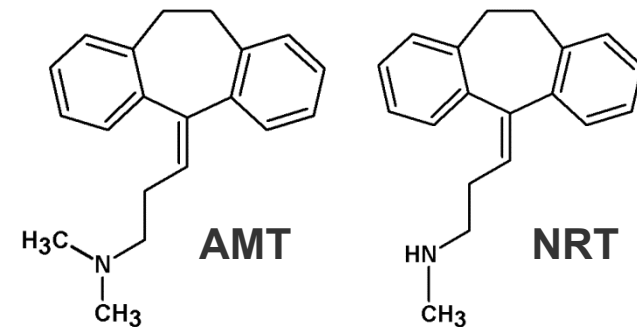




Application of amitriptylin

Amitriptylin (AMT)

- AMT was one of the first anti-depressiva (**1961**)
- belongs to the group of **tricyclic anti-depressiva (TCA)**
- Main metabolite: nortriptylin (NRT)
- small „therapeutic window“



Depressionen

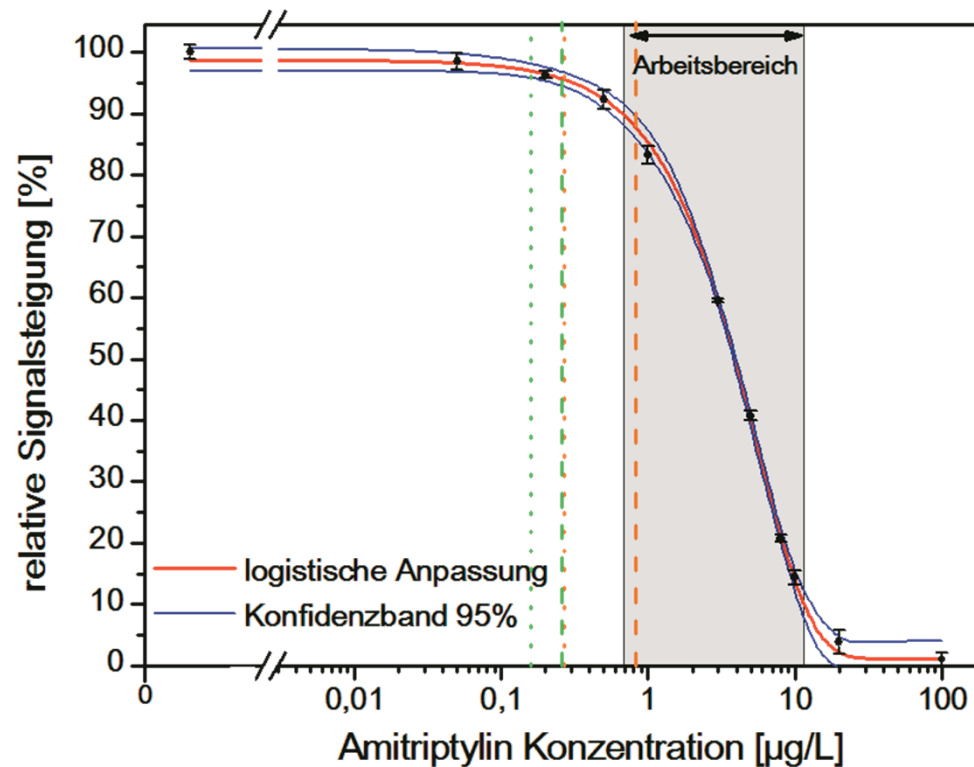
- **350 millions persons concerned** world-wide (WHO, 2012)
- **Third-leading cause** for occupational disability world-wide (WHO-Global Burden of Disease Study)
- depressive diseases are treated by **psychotherapy** and **anti-depressiva** according to type and degree of seriousness



Calibration of amitriptylin



- Very good match of calibration function to data ($R^2=0,999$; $\chi^2=1,101$)
- Low standard deviation (0,3-2,0%)
- Range fits well to expected range of calibration
- dilution 1:10 (1:100) possible



MDC . . . 0.16 µg/L
RDL - - - 0.26 µg/L
LOD . . . 0.27 µg/L
LOQ - - - 0.83 µg/L

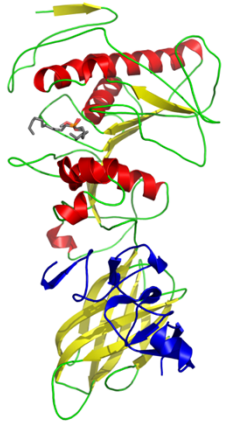
Arbeitsbereich:
 0.68 – 11.99 µg/L

$$y = A_2 + \frac{A_1 - A_2}{\left[1 + (2^{s-1} - 1) \cdot \left(\frac{x}{x_0} \right)^p \right]^s}$$

RfS Binding inhibition test



Detection of acute pancreatic failure

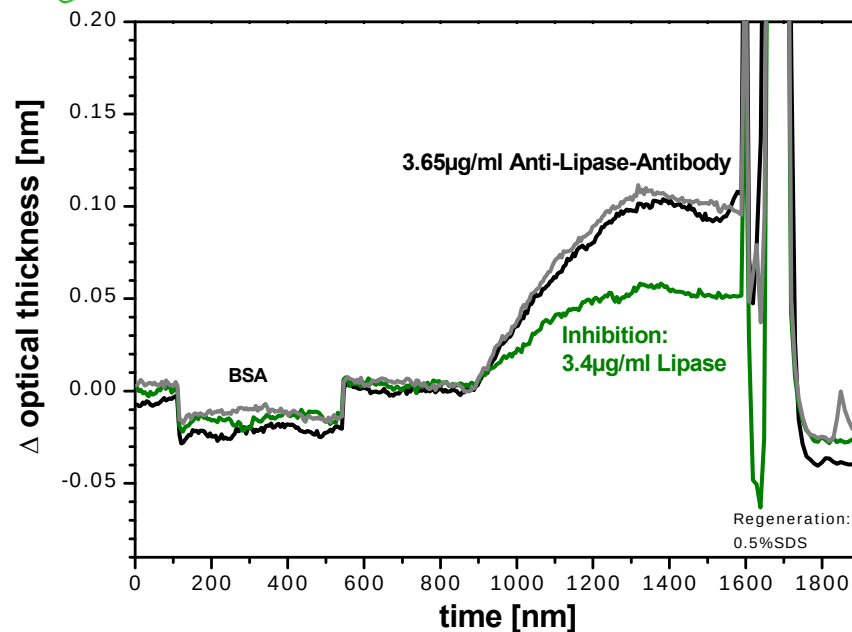


At failure of pancreas high conc. of enzyme lipase can be monitored in blood

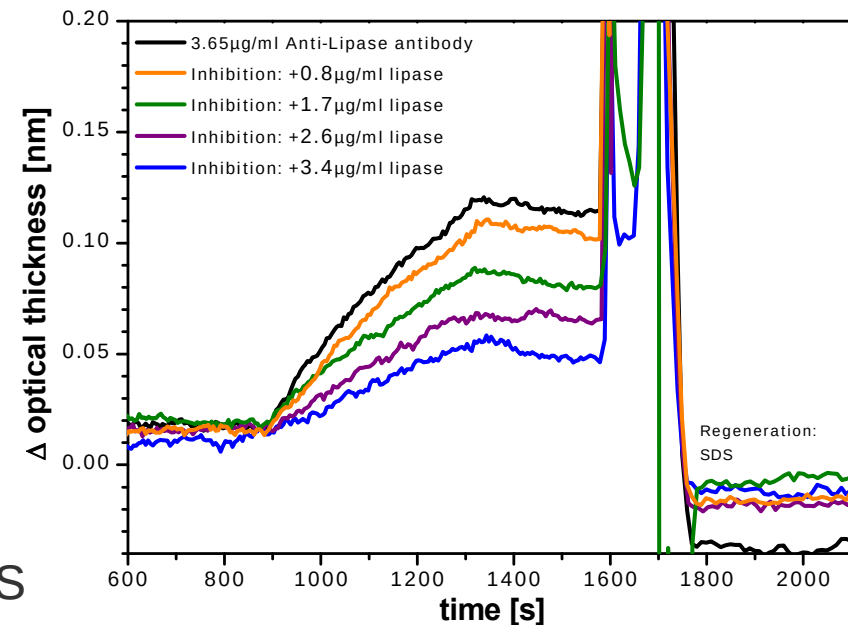
Binding-inhibition assay to measure lipase concentration:

- Immobilisation of pancreatic lipase
- Measurement of antibody
- Control: Inhibition with various lipase concentrations
- currently: measurements in buffer
- Regeneration of the surface possible

Katrin Krieg

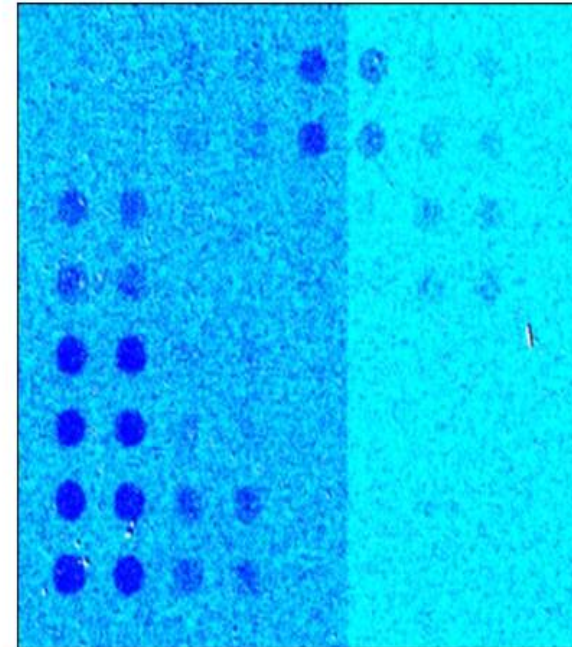
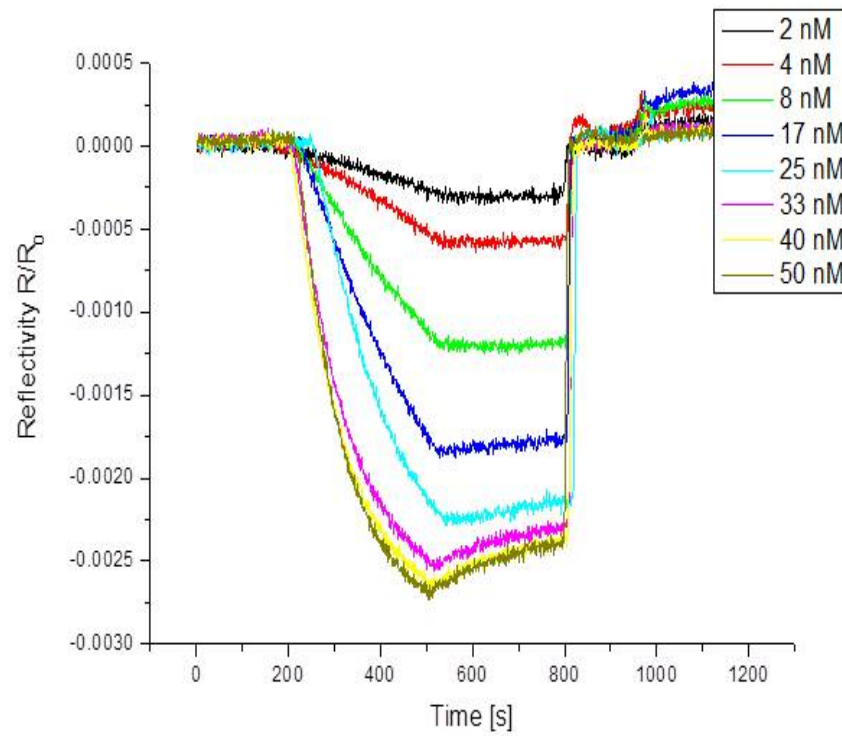


RfS





Parallelized RfS



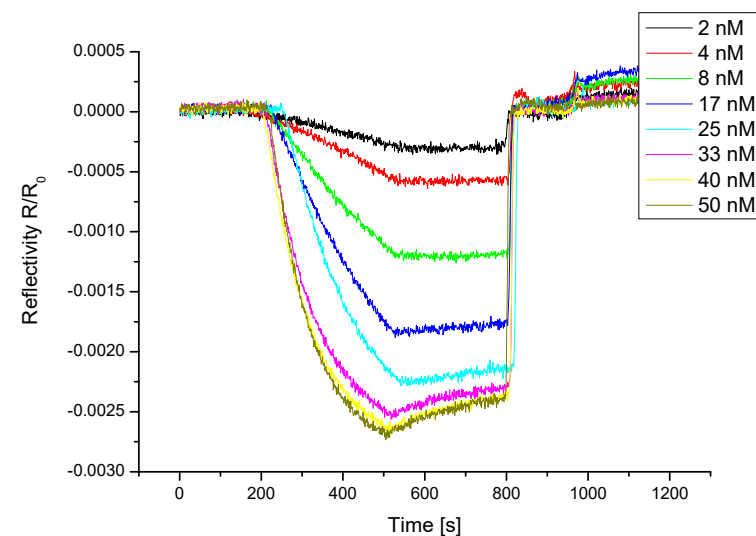
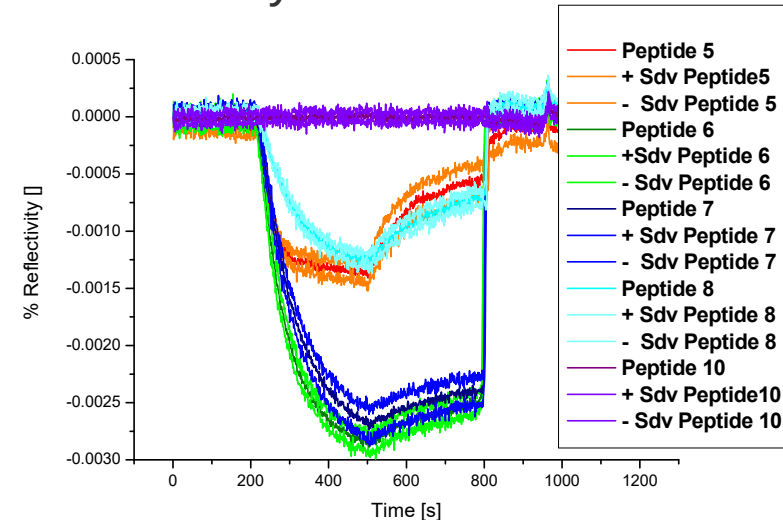


Parallelized Peptide-Antibody-Interaction

Epitopemapping for anti-Transglutaminase Antibody: **Coeliac disease**

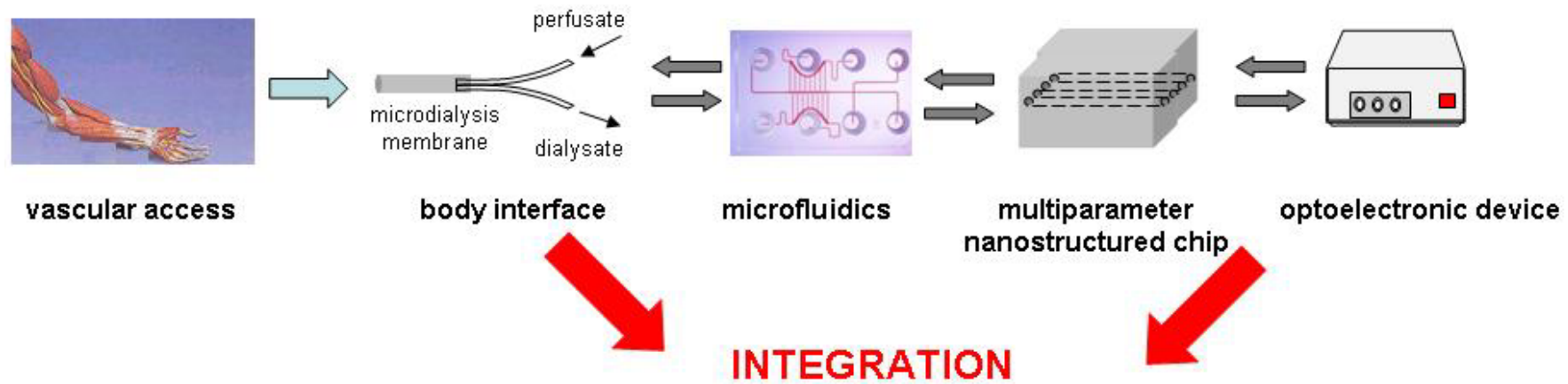
- Autoimmune disease
 - Different interaction behaviour between 5 sequence consecutive peptides and a monoclonal anti-Transglutaminase-Antibody
 - Measurements with 50 nM antibody
 - Measurements represent mean and sdv of four replica
 - Peptide “7” is best
-
- Binding signal for peptide “7” dependent on antibody concentration
 - Antibody concentration varies between 2 nM and 50 nM
 - Transition between mass controlled and kinetically controlled conditions

Barbara Schwarz





Nanodem



FP7-NMP-2010-LARGE-4

318372

NANODEM

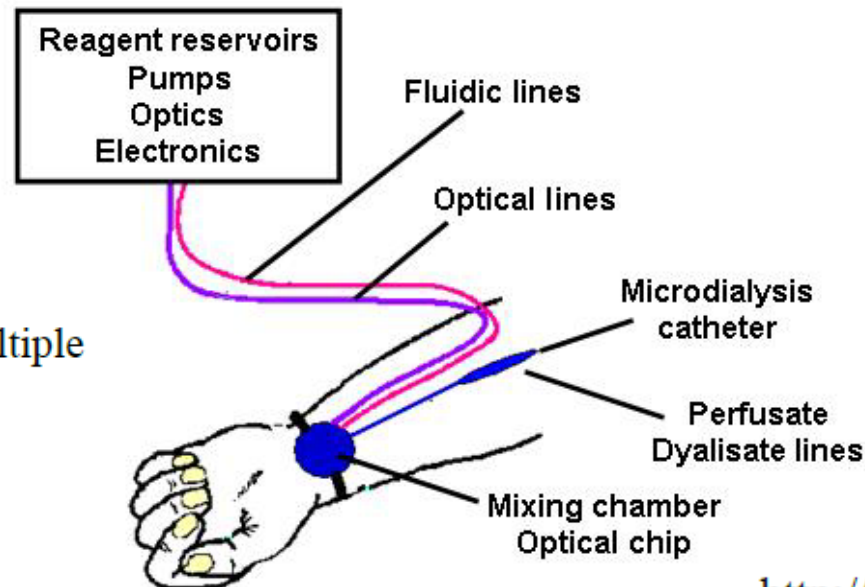
NANOphtonic DEvice for Multiple
therapeutic drug monitoring

Collaborative project

01/10/2012

48 months

Francesco Baldini, CNR



<http://nanodem.ifac.cnr.it/>



Content of lecture

- Optics
 - Electromagnetic radiation
 - Polarised radiation
 - ORD
 - Interference
 - Coherence
- Interaction EM radiation with matter
 - Jablonski
 - Orbitals
 - Band shift
- Sensors
 - Principle
 - Components
 - Transducer
 - Recognition
 - Surface chemistry, shielding
- Assays
 - Types
 - Kinetics
 - Homogeneous/heterogeneous



Content of lecture

- Biomolecular interaction analysis
 - Kinetics
 - Thermodynamics
- POCT
 - Characteristics
 - Requirements
 - Realisations
 - Strips
 - Laminar flow
 - Bench top
 - Using microdialysis
 - Trends
 - New instrumentation and principles



Laboratory Techniques and Medical Device Approvals

Gaiglitz Part III: Optical Biosensors and POCT

End