Nanometrology in Biomedicine
-In-vitro diagnostics approach

EURACHEM Workshop on Quality Assurance of Measurements from Field to Laboratory

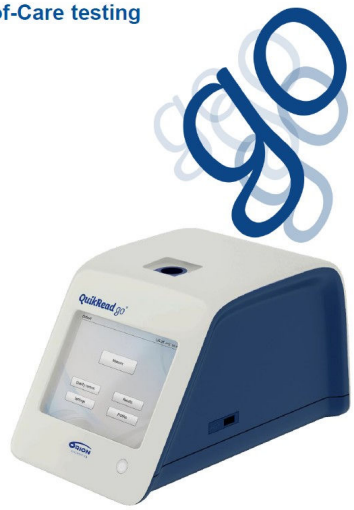
MIKES, Espoo Finland, 20-21 May 2013

Orion Diagnostica Oy
Company Presentation

Orion Diagnostica

An in-vitro diagnostic company focusing on Point-of-Care testing

- Well established over 35 years' experience
- Headquarters located in Espoo, Finland
- Compliance with ISO 9001 and ISO 13485 Quality Standards as well as FDA requirements
- Products marketed globally, main markets:
  - Nordic countries
  - China
  - Eastern, Southern and Central Europe
  - USA
  - Japan
- Net sales MEUR 54.0 (+ 9%) International sales were over 80 % of net sales
- Personel in 2012 c. 300 + 50 outside Finland
- Part of Orion Group, the largest health-care company in Finland.



Orion Diagnostica — Business Areas

Point-of-Care tests

- Tools for differential diagnosis between bacterial and viral infection
- Urinary tract infections

Laboratory tests

- Clinical microbiology
- Clinical chemistry

Dental tests

- Caries risk
- Oral hygiene status

Hygiene tests

- Surface hygiene in food processing and hospital environments
- Monitoring microbiological load in industrial fluids

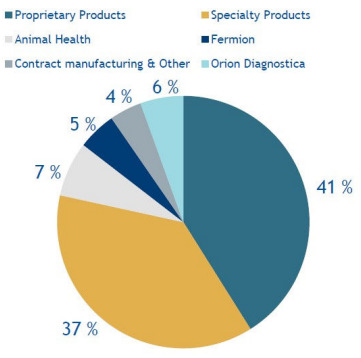
New: Molecular testing

ORION

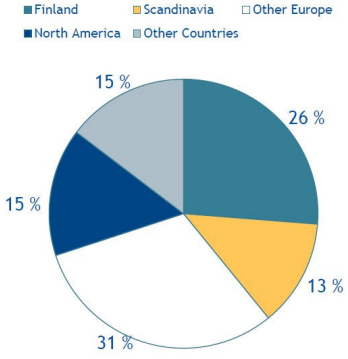
Building well-being

Breakdown of Net Sales of Orion Group in 2012 EUR 980 Million

By business divisions



By market area



Units share common clientele consisting of health care professionals. Focus on services to physicians, pharmacies, hospitals, clinics and other healthcare professionals as well as to their customers

ORION DIAGNOSTICA

Income Statement of Orion Group 2008–2012

Formation of profits, EUR million	2008	2009	2010	2011	2012	Change %
Net sales	710.7	771.5	849.9	917.9	**980.4**	+6.8%
Cost of goods sold	-243.4	-265.2	-283.2	-305.1	**-350.0**	+14.7%
Gross profit	467.4	506.3	566.8	612.8	**630.4**	+2.9%
Other operating income and expenses	3.1	6.0	1.2	3.0	**6.3**	+108.8%
Sales and marketing expenses	-143.9	-160.0	-188.9	-204.8	**-205.7**	+0.4%
R&D expenses	-90.0	-95.2	-85.5	-87.5	**-104.8**	+19.8%
Administrative expenses	-51.5	-50.2	-39.3	-40.6	**-45.3**	+11.5%
Operating profit	185.0	207.0	254.2	282.9	**280.9**	-0.7%
Profit before taxes	184.2	203.7	252.6	282.0	**279.3**	-0.9%
Profit for the period	136.3	151.4	184.7	209.5	**208.9**	-0.3%

In Vitro Diagnostics - IVD

In vitro diagnostic (IVD) tests

In vitro diagnostic (IVD) tests are medical devices intended to perform diagnoses from assays in a test tube, or more generally in a controlled environment outside a living organism. - *In vitro* means *in glass* in Latin.

In vitro diagnostics are tests that can detect diseases, conditions, or infections. Some tests are used in laboratory or other health professional settings and other tests are for consumers to use at home

According to the directive 98/79/EC '**in vitro diagnostic medical device**' means any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment, or system, whether used alone or in combination, intended by the manufacturer to be used in vitro for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information: — concerning a physiological or pathological state, or — concerning a congenital abnormality, or — to determine the safety and compatibility with potential recipients, or — to monitor therapeutic measures.

Nanotechnology @ IVD

Impact of Nanotechnology in Healthcare ?

Nanotechnology is an emerging scientific field creating materials, devices and systems at the molecular level. By being able to work at the ultra-small scale, given a nano is one billionth of a metre, nanotechnology is being used to deliver innovations in sectors including health.

What is NanoMedicine ?

NanoMedicine is the application of nanotechnology to achieve breakthroughs in healthcare. It exploits the improved and often novel physical, chemical and biological properties of materials at the nano scale, and offers the potential to enable early detection, prevention, improved diagnosis and imaging, treatment and follow-up of diseases. NanoMedicine embraces a wide field including in vivo and in vitro diagnostics to therapy including targeted delivery and regenerative medicine; it interfaces nanomaterials (surfaces, particles, etc) or analytical instruments with "living" human material (cells, tissue, body fluids).

In-vitro diagnostics and cost-efficient health care

In-vitro diagnostics are vital for the drive for cost effective healthcare, point of care monitoring and personalized medicine. The diagnosis and management of medical conditions is becoming increasingly reliant upon the detection and measurement of biochemical markers, or targets.

The general approach is to use specific recognition molecules, called probes, to capture targets; however, there are several issues with current diagnostic devices, including measurements of the interface, repeatability and reproducibility of assays and diagnostic performance.

Where and why nanometrology is needed in IVD ?

Nanometrology is needed for the characterisation of Biomolecular Interfaces for Diagnostic Devices

There are **3 main issues** with current diagnostic devices:

- **Measurements of the interface**
- **Repeatability and Reproducibility of Assays**
- **Diagnostic performance**

Examples of participating nanometrology-related projects which have direct linkage to IVD field.

EMRP
European Metrology Research Programme
Programme of EURAMET
The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

JRP HLT04 (BioSurf)

Metrology for the characterisation of Biomolecular Interfaces for Diagnostic Devices

The research within this EURAMET joint research project receives funding from the European Community's Seventh Framework Programme, ERA-NET Plus, under Grant Agreement No.217257.

BioSurf will develop measurement methods for the research, development and quality control of biomolecular interfaces in diagnostic devices, and establish accurate, traceable and comparable methods to determine the amount of biomolecular probe immobilised at both planar and nanoparticle interfaces. It will also investigate novel approaches for the measurement of biointerfacial structures that can be correlated with activity and binding efficiency and methods to measure and predict the activity of immobilised probes. With the goal of supporting high throughput and multiplexed diagnostic methods BioSurf will also assess the capabilities of emerging techniques capable of detecting many targets simultaneously.

Work Packages

WP1: Development of standard platforms, reference surfaces and measurement methods

Develop and produce materials that will be used as reference materials within the JRP and for an inter-laboratory comparison. Establish accurate, traceable and comparable methods to determine the amount of biomolecular probe immobilised at planar and nanoparticle interfaces.

(In WP1 a silane-based protocol for functionalization of silicon oxides and a protocol for the thiol-gold system have been developed. XPS measurements of epoxy-silane modified glass silicon surfaces and PEG-thiol modified gold surfaces are complete.)

WP2: Investigation into the sensitivity of emerging techniques to biomolecular structure

Use innovative approaches to determine the orientation and structure of biomolecules at an interface, and develop useful measurement approaches for the research, development and quality control of biomolecular interfaces in diagnostic devices.

(In WP2 the design of the UHV compatible liquid cells has been finalised)

WP3: Measuring the attachment of targets to reference surfaces

Develop novel approaches for the measurement of biointerfacial structure that can be correlated with activity and binding efficiency. Develop methods to measure and predict the activity of immobilised probes, by measuring the activity of diagnostic surfaces, quantifying and modeling the interaction between probes and targets. Develop validated numerical tools, based on Poisson-Boltzmann and Molecular Dynamics models, for the analysis of the binding mechanism between target and probe molecules

(In WP3, a software plan of the physical model to be implemented in the numerical code for the biomolecular simulation software, focusing on PB and MD models has been created.)

WP4: Novel Microscopic and mass spectrometry methods for identifying and measuring target binding

Assess and evaluate the capabilities of new, emerging techniques and approaches to biomolecular sensing which enable multiplexed and label-free identification and quantification of bound targets.

(In WP4 biotin-avidin has been selected as the probe-target pair for future experiments.)

WP5: Creating Impact

BioSurf will provide guides, standards and protocols for the quantitative analysis of biomolecular interfaces relevant to the needs of diagnostic device manufacturers.

This initiates a new area of activity for European National Measurement Institutes (NMIs) and addresses the needs of the rapidly growing and competitive in-vitro diagnostic device industry within the context of the European Commission in-vitro diagnostic device directive (IVD Directive 98/79/EC). BioSurf will provide input to standards and protocols on the preparation and measurement of biointerfaces through the work carried out, proposing VAMAS interlaboratory studies, attending and contributing to relevant new work items within ISO TC201, TC212 and TC229.

JRP start date and duration:	01 May 2012: 3 years
JRP-Coordinator: Alice Harling, NPL Tel: +44 208 943 7025 E-mail: alice.harling@npl.co.uk JRP website address: http://projects.npl.co.uk/HLT04-BioSurf/	
JRP-Partners: JRP-Partner 1: BAM, Germany JRP-Partner 2: INRIM, Italy JRP-Partner 3: PTB, Germany JRP-Partner 4: SP, Sweden	
REG1-Researcher: (associated Home Organisation):	Björn Agnarsson, Sweden Chalmers, Sweden
REG2-Researcher: (associated Home Organisation):	Wolfgang Malzer, Germany TUB, Germany
REG3-Researcher: (associated Home Organisation):	Veronique Blanchard Charite, Germany

The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

SUCCESS STORIES

IntelliTip: Ultra-sensitive, stable and easy to use AFM bio sensor tips

PARTNERS
Project coordinator: JKU, Austria
Project partners: SCL, Austria
FMFIUK, Slovakia
IBGA SAS, Slovakia
UTA, Finland
OD, Finland

PROJECT DESCRIPTION

Atomic Force Microscopy (AFM) has developed to a key tool in Nanomedicine offering methodologies to understand molecular and cellular biology at the single molecule level. Advanced Bio-AFM techniques are based on using monomolecular biological sensors tips for Molecular Recognition Force Spectroscopy (MRFS) and Topography and RECognition Imaging (TREC).

This project was targeting the stability, sensitivity, accuracy, and usability of AFM biosensor tips. Instead of complicated and risky multi step coupling protocols biosensor tips were developed which are either ready to use or can be functionalized in one easy coupling step.

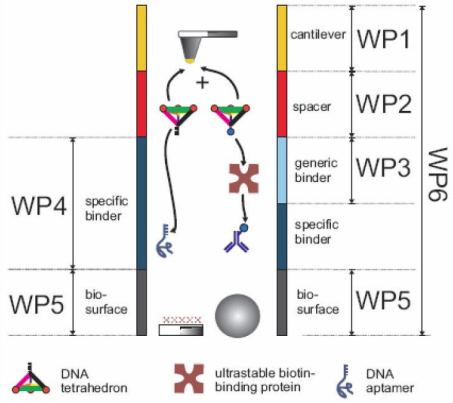
Aptamers

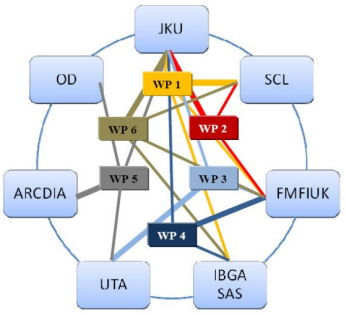
Ready to use biosensor tips have to overcome the protein stability problem. This will be accomplished by substitution of tip bound proteins with DNA aptamers – a new class of biopolymers with affinities comparable with antibodies. Aptamers can be developed and synthesized in vitro with high reproducibility and purity, and show an incredibly high stability (for decades instead of days) compared to classical proteins. In addition, aptamers can easily be generated with two different bio-reactive sites, allowing recognition of two kinds of receptors at the same time or screening for surface embedded receptors as part of a ternary complex.

Chimeric avidins

Extraordinary stable biotin-binding proteins (chimeric avidins) were produced by genetically engineering and bound onto the pre-functionalized AFM tips. Furthermore, these molecular building blocks were utilized to exactly adjust one binding site on the apex of the tips for increasing the success rates for the preparation of strictly single molecular tips. Developments of ultra-force-sensitive cantilevers were further applied for increasing the performance of the bio-sensors.

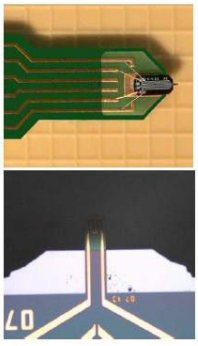
MNT-ERA.net/IntelliTip
(2010-2012)





Institute of Medical Technology

Multifunctional Cantilevers



- Integration of sensor
- Integration of actuator
- Integration of other sensing capabilities

Active cantilevers designed for fluid operation

Rangelow, IW, Microelectronic Engineering 2006

Fantner G.E. et al, ASME-DSMC in press 2009

SCL SENSOR.TECH. 2010-09-23 **Intellitip Meeting - Orion Diagnostica** E.J.Fantner

Partner 1 Key Competence

MRFS (Molecular Recognition Force Spectroscopy):
Single Molecule Interaction Force Measurements

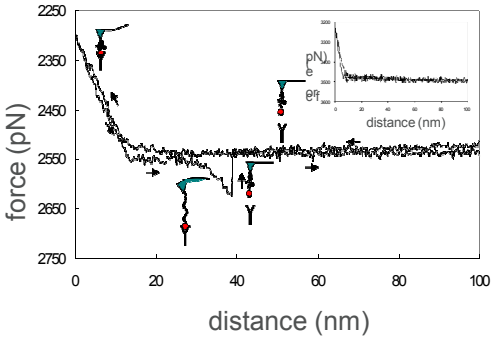
TREC (Topography and RECognition imaging):
Simulateous scanning of the surface topography while mapping specific interaction sites (on membranes, cells, …)

Single Molecule Biosensors
for MRFS and TREC.

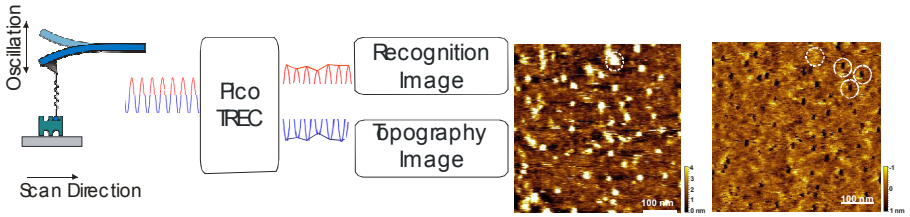
JOHANNES KEPLER UNIVERSITY LINZ | JKU

Molecular Recognition Force Spectroscopy

- Approaching
- Approaching and molecular recognition
- Approaching and molecular recognition & max. bending
- Withdrawing
- Withdrawing with stretching of the linker
- Unbinding event



TREC



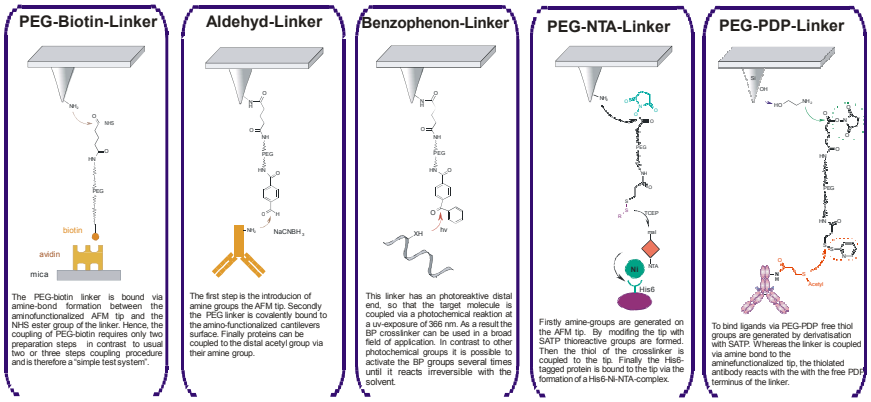
Upper Amplitude

- Specific molecular interaction
- → **Recognition Image**

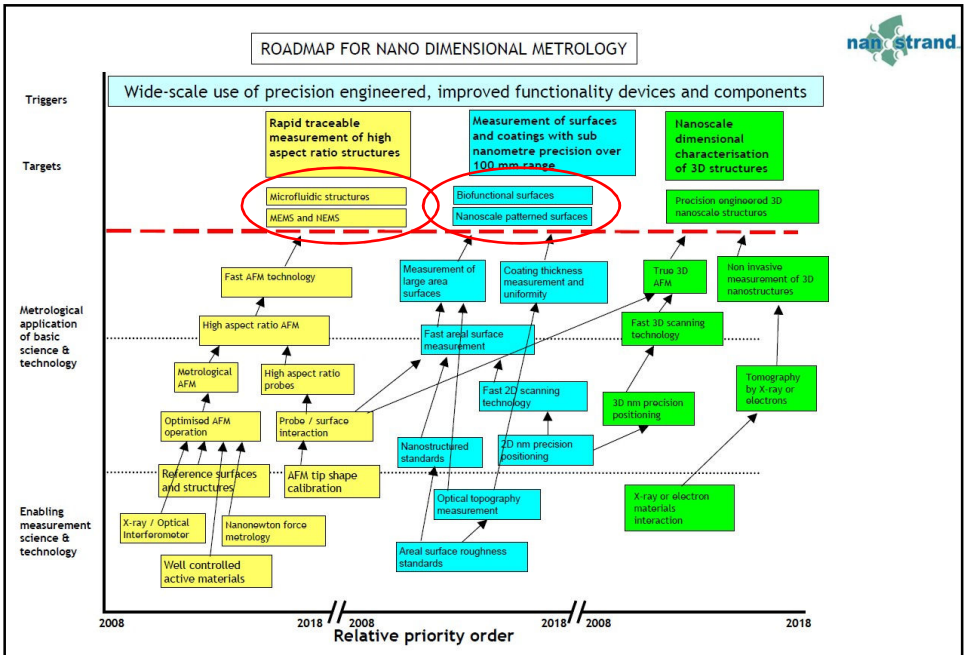
Lower Amplitude

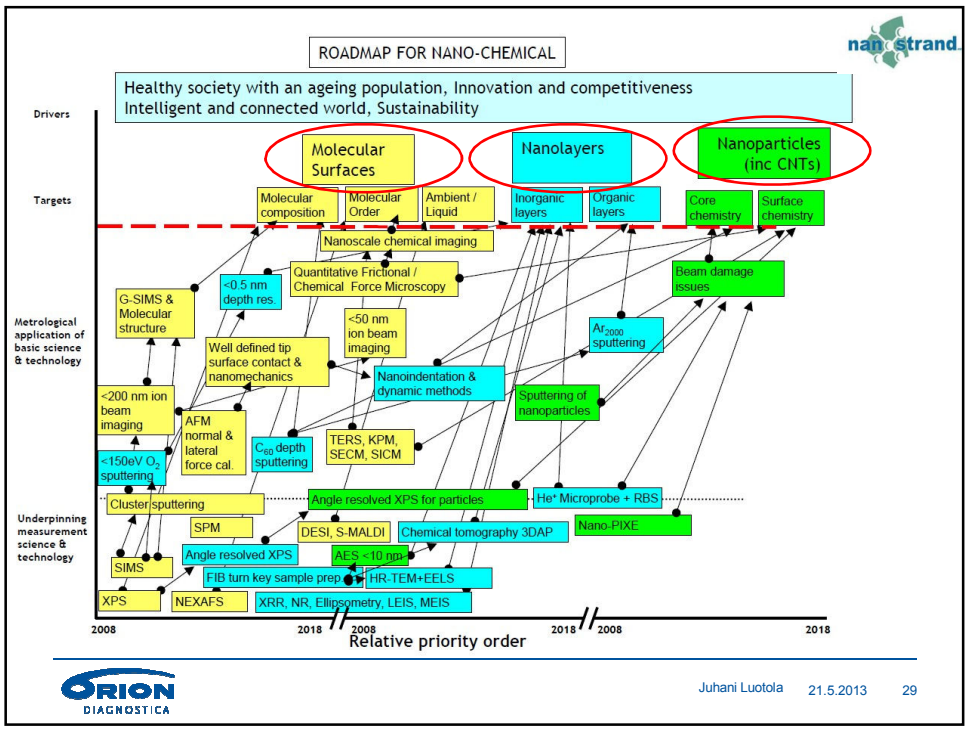
- Used for the Piezo Feedback loop
- → **Topography Image**

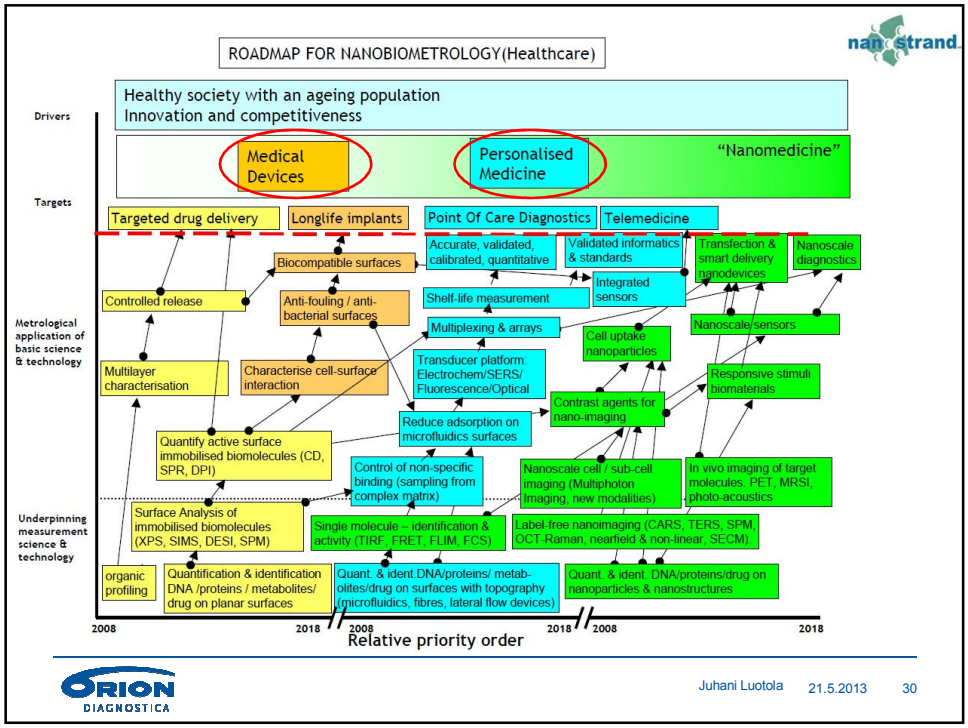
Single Molecule Biosensors



Metrology Roadmaps for Nanotechnologies




ROADMAP FOR NANO-CHEMICAL
nanostrand
Healthy society with an ageing population, Innovation and competitiveness
Intelligent and connected world, Sustainability
Drivers
Targets
Metrological application of basic science & technology
Underpinning measurement science & technology
Molecular Surfaces
Nanolayers
Nanoparticles (inc CNTs)
Molecular composition
Molecular Order
Ambient / Liquid
Inorganic layers
Organic layers
Core chemistry
Surface chemistry
Nanoscale chemical imaging
Quantitative Frictional / Chemical Force Microscopy
Beam damage issues
G-SIMS & Molecular structure
<0.5 nm depth res.
<50 nm ion beam imaging
Ar2000 sputtering
Well defined tip surface contact & nanomechanics
Nanoindentation & dynamic methods
<200 nm ion beam imaging
Sputtering of nanoparticles
AFM normal & lateral force cal.
C60 depth sputtering
TERS, KPM, SECM, SICM
<150eV O2 sputtering
Cluster sputtering
Angle resolved XPS for particles
He+ Microprobe + RBS
SPM
DESI, S-MALDI
Chemical tomography 3DAP
Nano-PIXE
SIMS
Angle resolved XPS
AES <10 nm
FIB turn key sample prep
HR-TEM+EELS
XPS
NEXAFS
XRR, NR, Ellipsometry, LEIS, MEIS
2008
2018
2008
2018
2008
2018
Relative priority order
ORION DIAGNOSTICA
Juhani Luotola 21.5.2013 29


ROADMAP FOR NANOBIOMETROLOGY(Healthcare)
nanostrand
Healthy society with an ageing population
Innovation and competitiveness
Drivers
Targets
Metrological application of basic science & technology
Underpinning measurement science & technology
Medical Devices
Personalised Medicine
"Nanomedicine"
Targeted drug delivery
Longlife implants
Point Of Care Diagnostics
Telemedicine
Accurate, validated, calibrated, quantitative
Validated informatics & standards
Transfection & smart delivery nanodevices
Nanoscale diagnostics
Biocompatible surfaces
Integrated sensors
Controlled release
Anti-fouling / anti-bacterial surfaces
Shelf-life measurement
Multiplexing & arrays
Nanoscale sensors
Cell uptake nanoparticles
Multilayer characterisation
Characterise cell-surface interaction
Transducer platform: Electrochem/SERS/ Fluorescence/Optical
Responsive stimuli biomaterials
Contrast agents for nano-imaging
Reduce adsorption on microfluidics surfaces
Quantify active surface immobilised biomolecules (CD, SPR, DPI)
Control of non-specific binding (sampling from complex matrix)
Nanoscale cell / sub-cell imaging (Multiphoton Imaging, new modalities)
In vivo imaging of target molecules. PET, MRSI, photo-acoustics
Surface Analysis of immobilised biomolecules (XPS, SIMS, DESI, SPM)
Single molecule – identification & activity (TIRF, FRET, FLIM, FCS)
Label-free nanoimaging (CARS, TERS, SPM, OCT-Raman, nearfield & non-linear, SECM)
organic profiling
Quantification & identification DNA /proteins / metabolites/ drug on planar surfaces
Quant. & ident.DNA/proteins/ metab-olites/drug on surfaces with topography (microfluidics, fibres, lateral flow devices)
Quant. & ident. DNA/proteins/drug on nanoparticles & nanostructures
2008
2018
2008
2018
2008
2018
Relative priority order
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Thank you for your attention !