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Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Concept of (Optical) Biosensors

Content

- Analysis of biomarkers, harmful compounds.
- Motivation and evolvement of implementations, from laboratory desktop devices to implanted sensors.
- Definition and types of biosensors
- Sensor schemes in close contact with human body.
- Performance characteristics - limit of detection, sensitivity.

Established Bioanalytical Technologies

Motivation

Common practice: Analysis of collected samples (e.g. blood, urine) in central laboratories which is time consuming, require trained personnel and is costly (ELISA, mass spectrometry, HPLC…), centralized laboratories.

Tecan, Infinite® F50

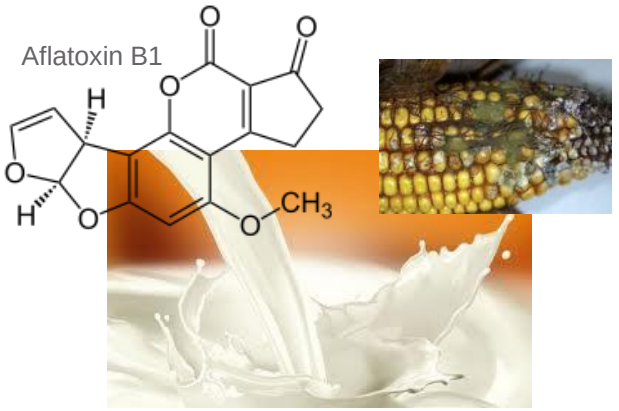
Strip tests

Quantitative, accurate, laborious

Simpler, qualitative

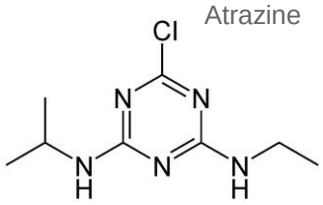
Application Areas

Food control (toxins, bacterial pathogens…)



Medical Diagnostics (biomarkers for cancer, cardiac, inflammation…)

Environmental Monitoring (pollutants in water and soil…)



Homeland Security, **Forensics**….

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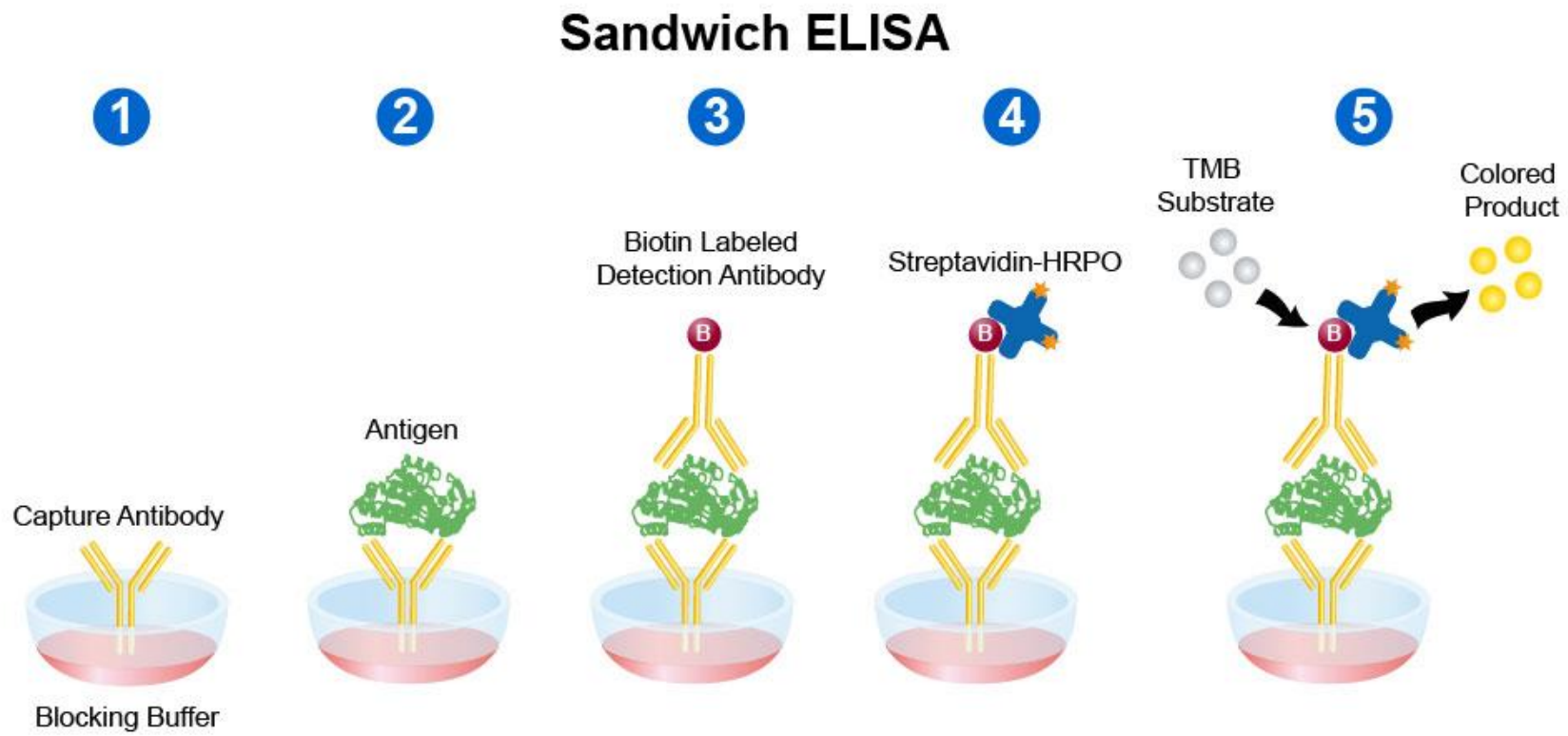
Enzyme-linker Immunosorbent Assays



https://www.leinco.com/sandwich-elisa-protocol/

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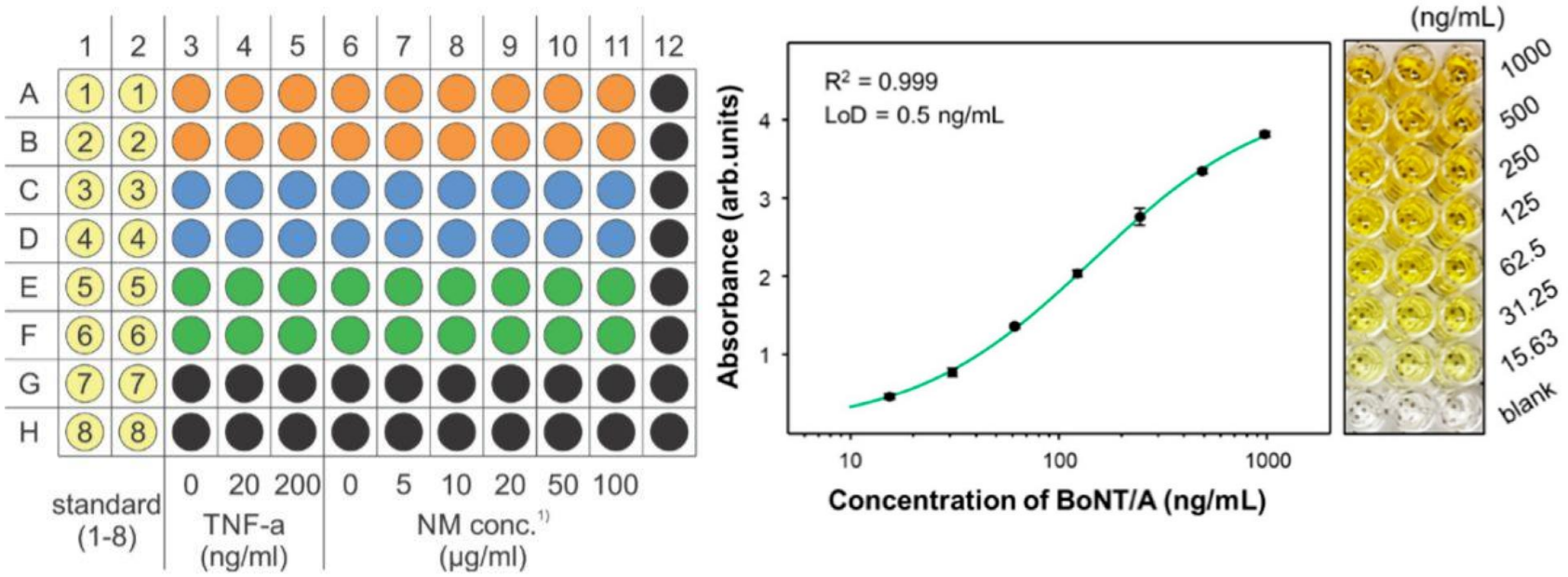


https://www.nanopartikel.info/files/methodik/VIGO/I_ELISA_A549.pdf

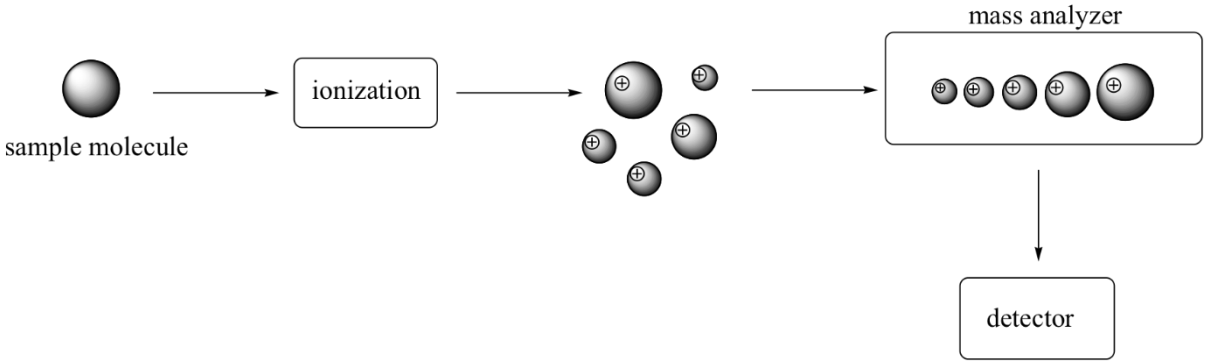
https://doi.org/10.3390/s19194081

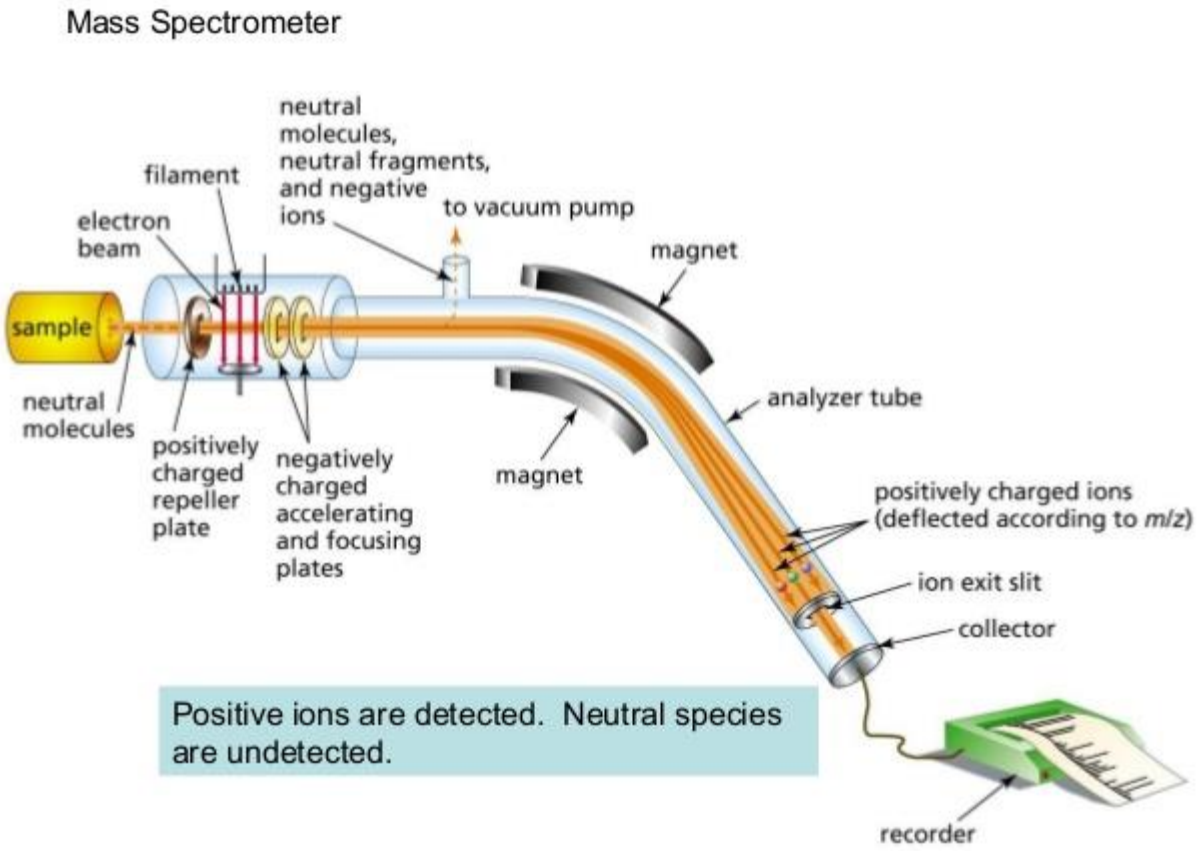
ELISA replaced radio-immunoassays in 1970ties, still routinely used and other enzymatic reactions exploited for faster and more sensitive readout.

Mass Spectrometry

Airport security – after Lockerbie bomb attack in 1988, mass spectrometry become used for screening of explosives.

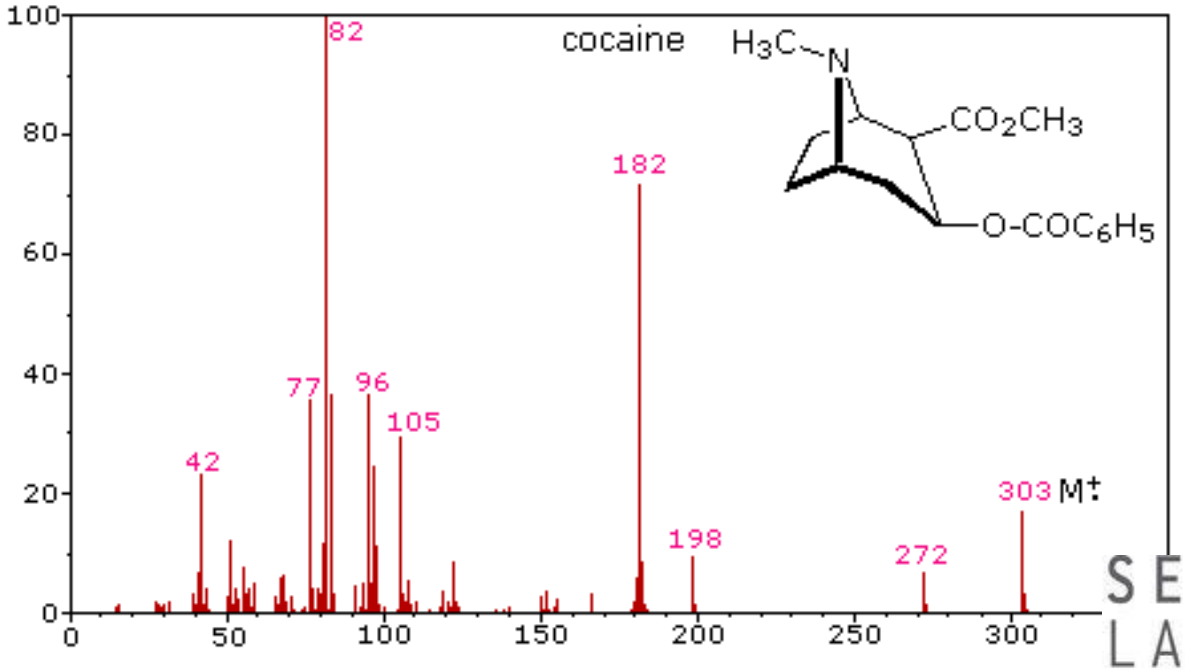
Mass Spectrometry





https://chem.libretexts.org/Courses/SUNY_Oneonta/Chem_221%3A_Organic_Chemistry_I_(Bennett)/1%3ALecture_Textbook/04%3A_Structure_Determination_I-_UV-Vis_and_Infrared_Spectroscopy_Mass_Spectrometry/4.03%3A_Mass_Spectrometry

Mass Spectrometry

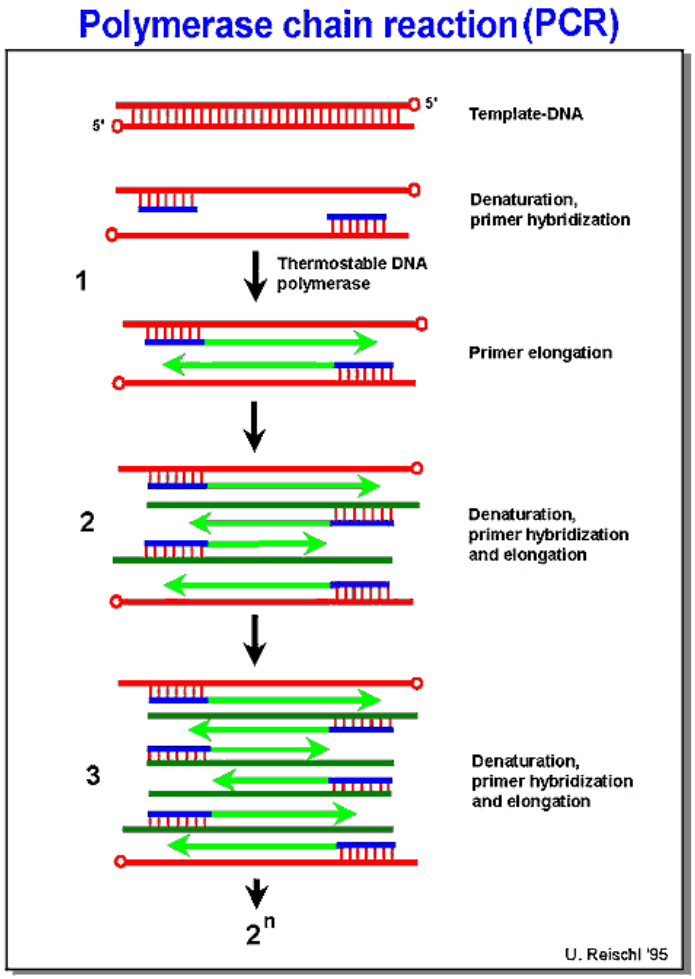


SEIBERSDORF LABORATORIES

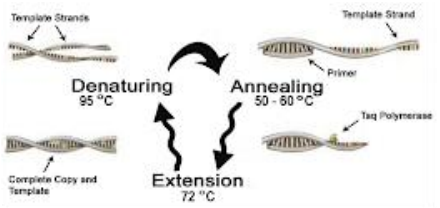
World-Anti-Doping-Agency (WADA).

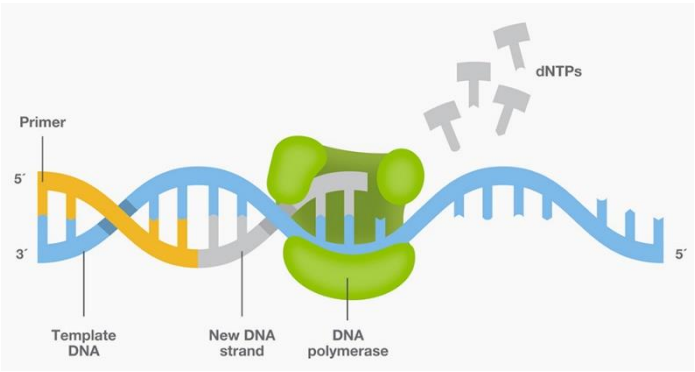
Used also in e.g. doping control and analysis of banned narcotics and stimulants. Legally assumed as a direct method that can be used at court.

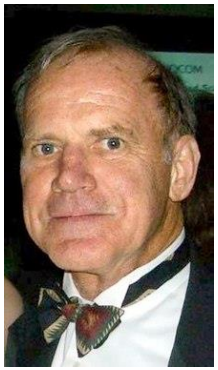
Polymerase Chain Reaction - PCR



Thermocycling

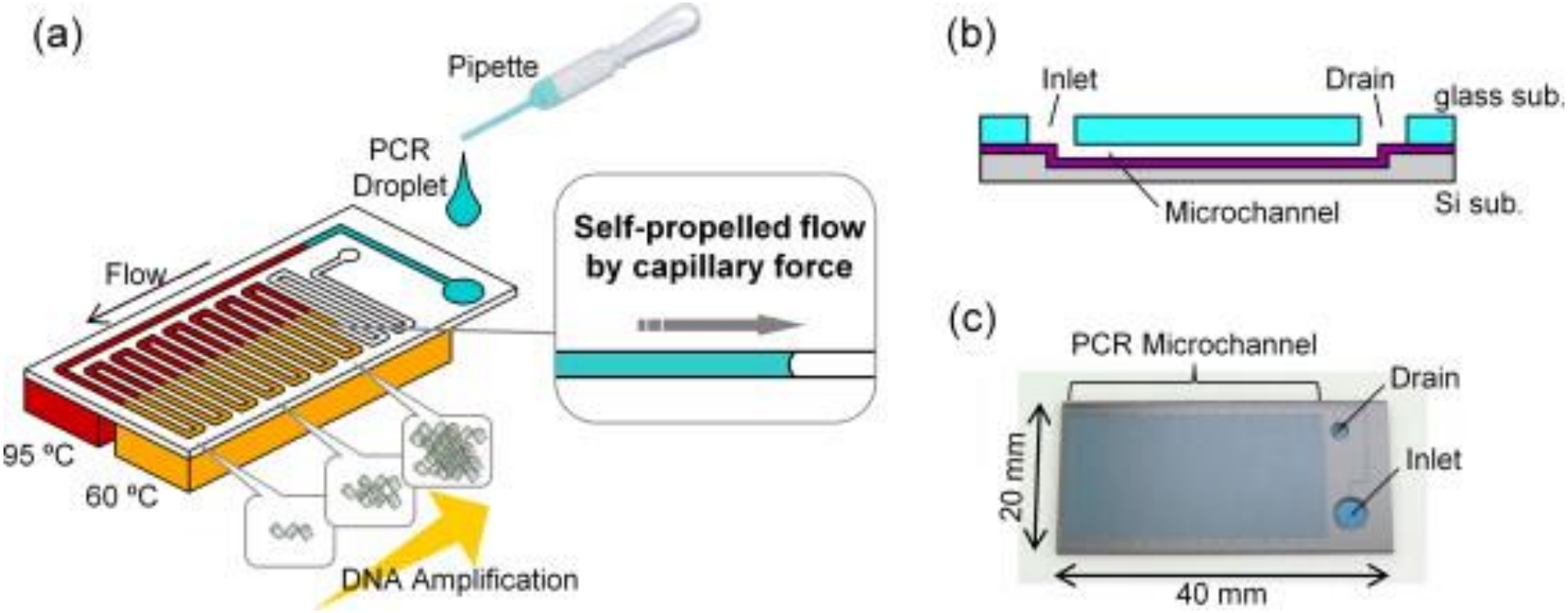






Karry Mullis was awarded the Nobel Prize in Chemistry in 1993 for inventing PCR

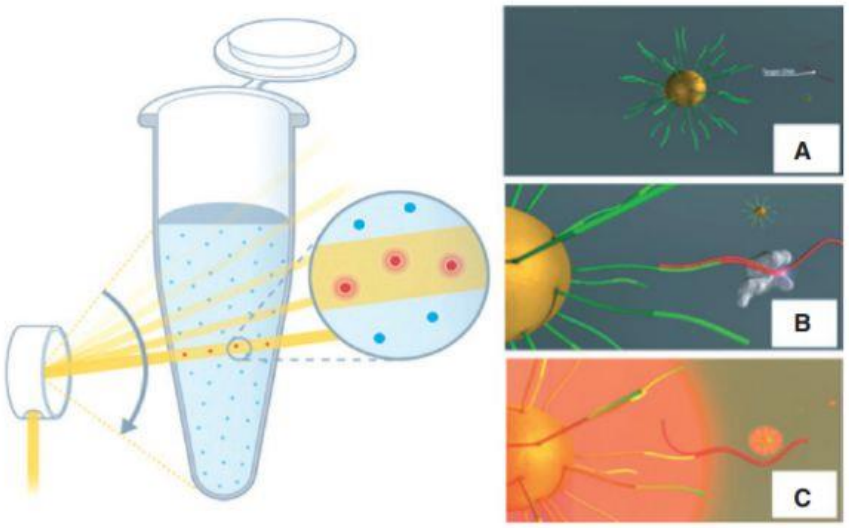
Polymerase Chain Reaction

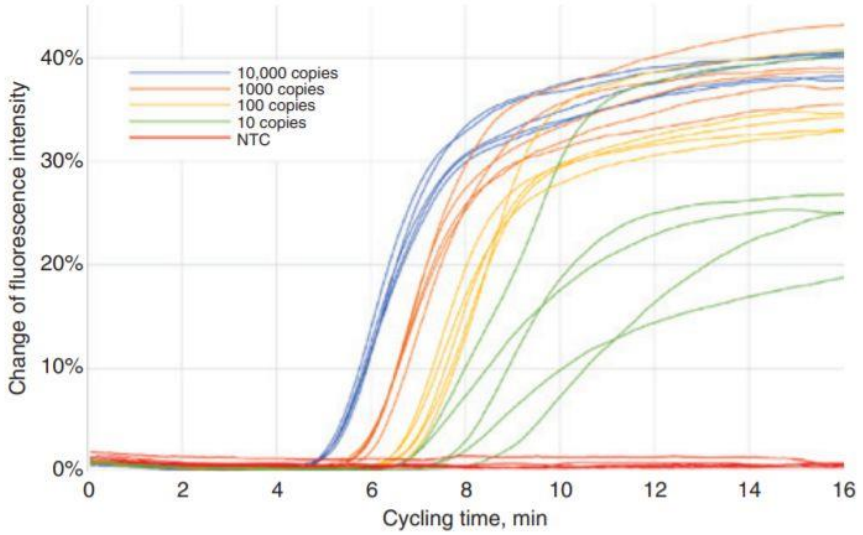


https://doi.org/10.1016/j.snb.2014.09.004

Thermocycling is typically rather slow limiting the speed of PCR.
Possible solution provides microfluidics dealing with small volumes.

Laser Polymerase Chain Reaction





https://doi.org/10.1515/labmed-2017-0093

https://www.gna-bio.com/solutions/

Plasmonic nanoparticles may serve as miniature heat sources to rapidly modulate temperature in extremely low sample volumes.

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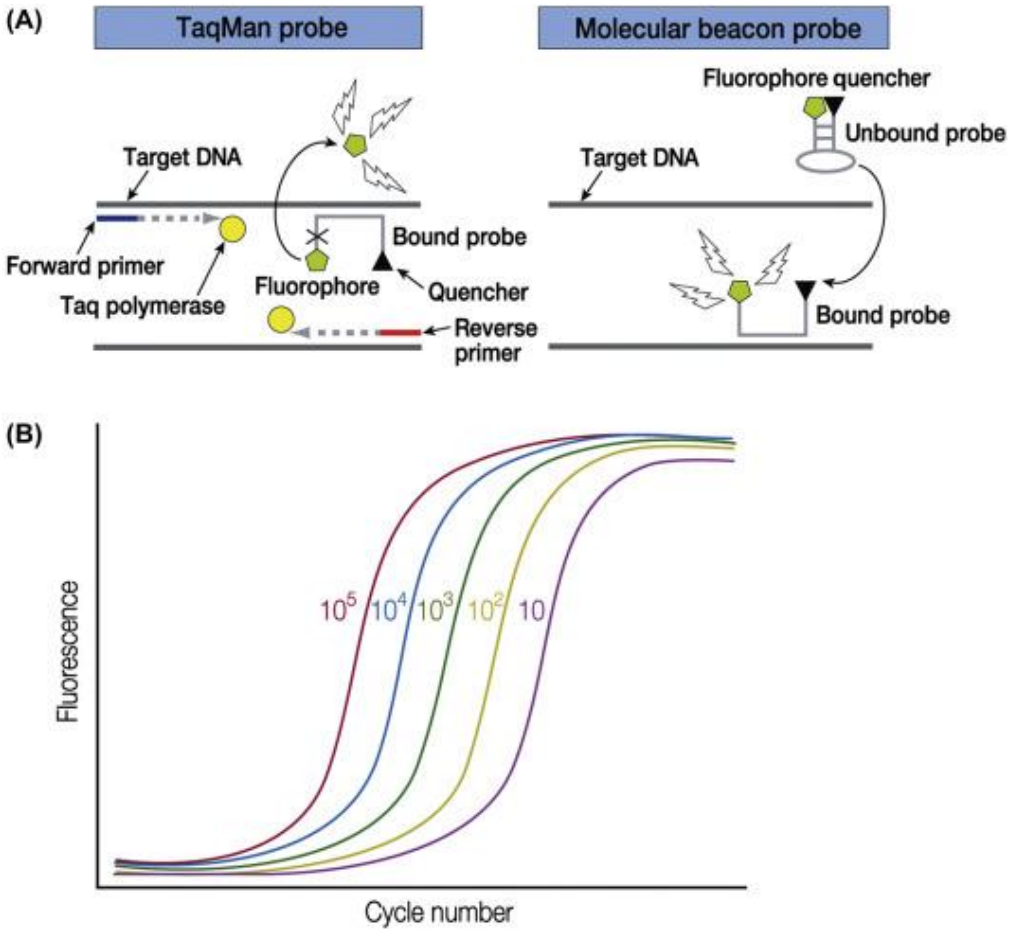
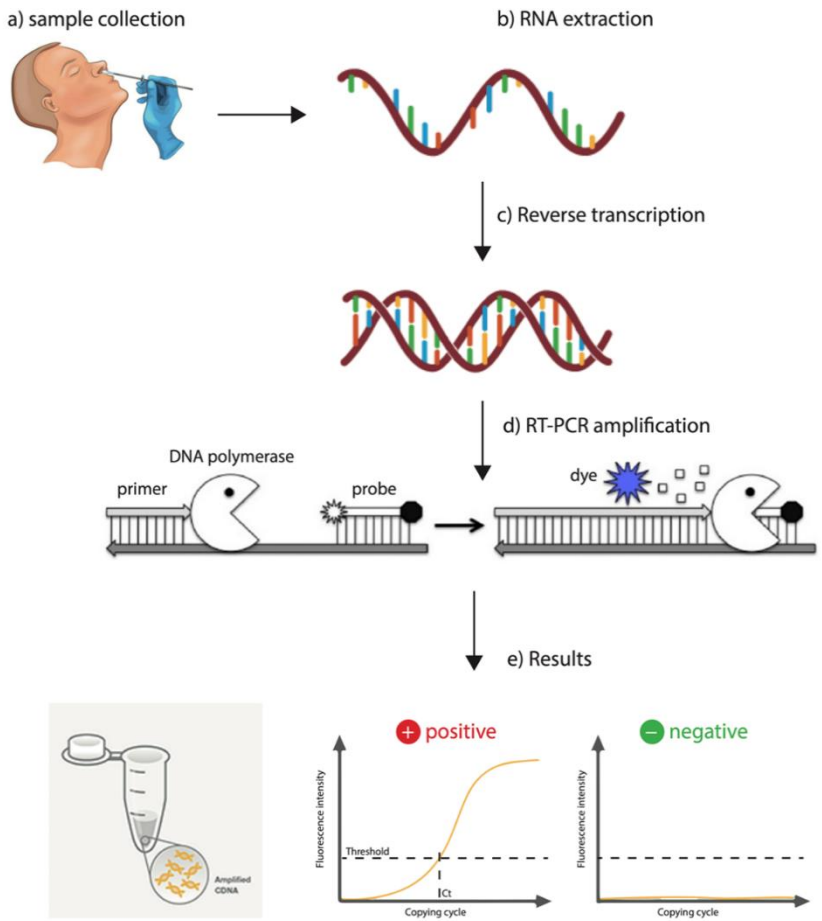
Real Time - Polymerase Chain Reaction



https://doi.org/10.1016/B978-0-12-800838-6.00004-7

- Fluorescence is typically employed for the readout of the presence of the amplified oligonucleotide chains.
- In RT-PCT the FRET or quenching of fluorophores allows for reporting on the presence of selected amplified strands.
- Many other versions – digital PCR, RCA, LAMP…

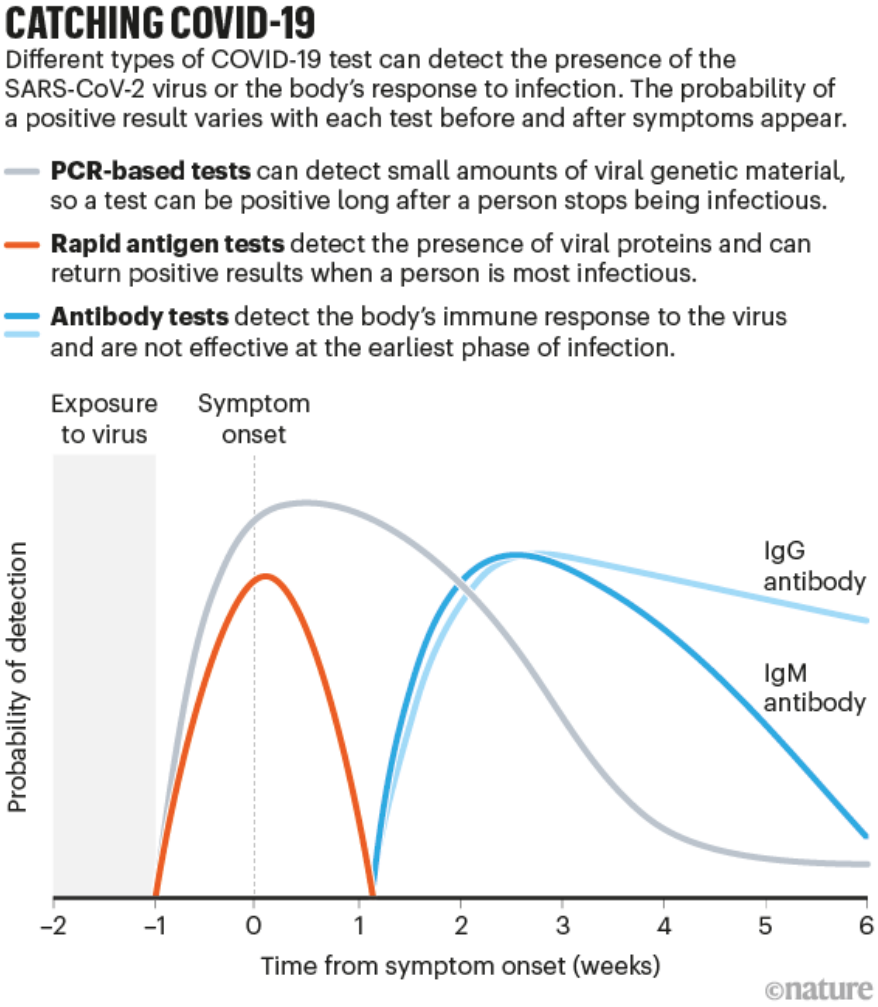
PCR – Covid 19



https://www.globalbiotechinsights.com/articles/20247/the-worldwide-test-for-covid-19

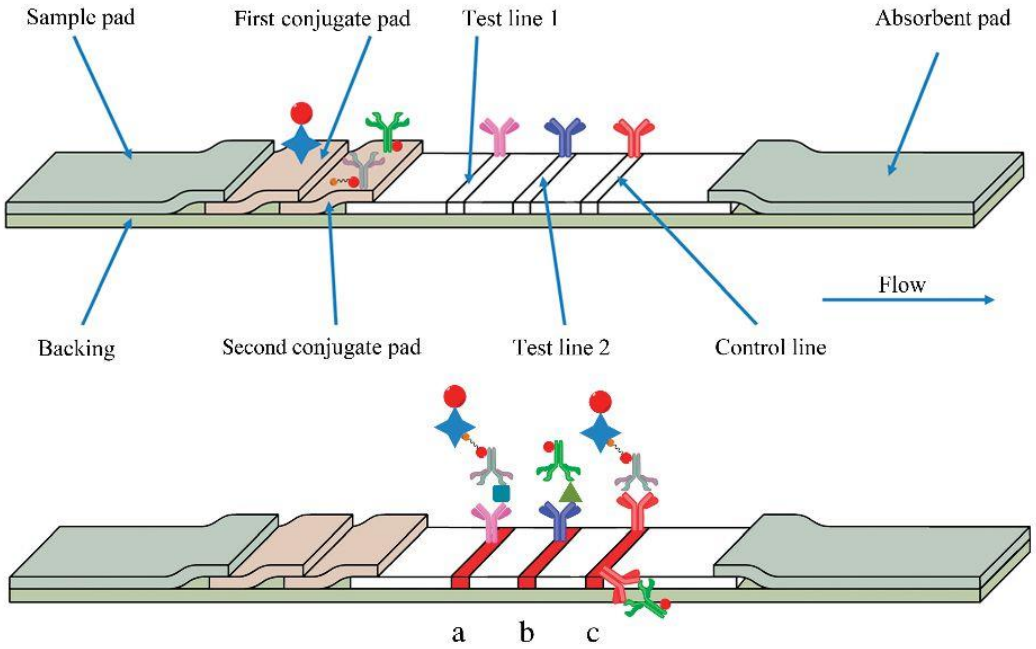
- Reverse Transcription Polymerase Chain Reaction (RT-PCR)
- Whole process takes hours
- Not quatitative, gives either positive or negative result.
- In principle PCT is sensitive technique that can detect several copies of target species in a sample

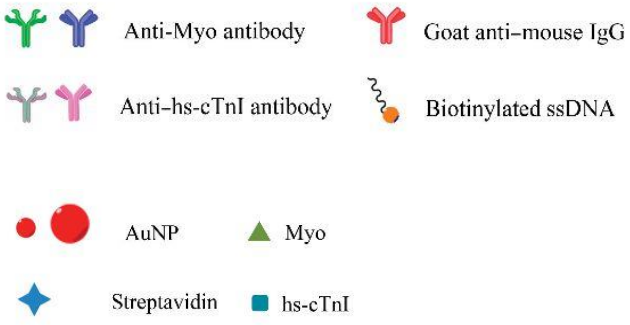
Tracking Covid Infections



https://www.nature.com/articles/d41586-020-02661-2

Strip Immunoassays – Concept of Lateral Flow Assay

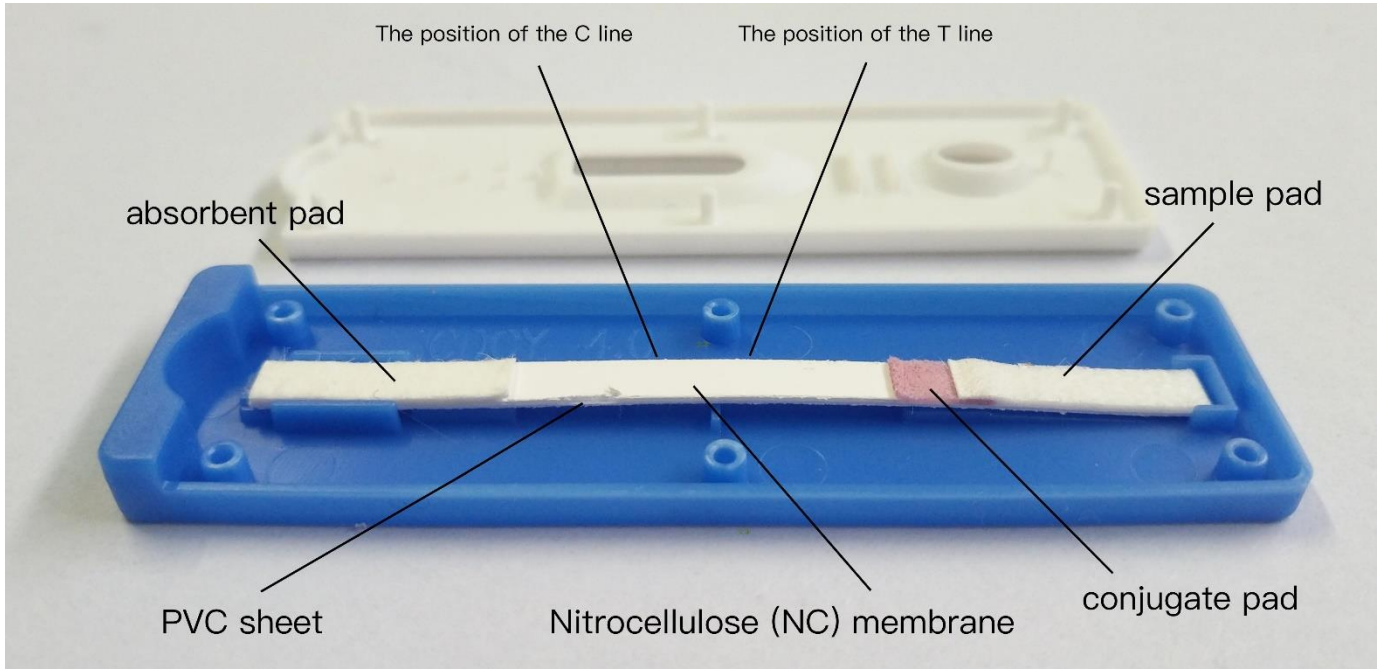




10.1373/clinchem.2011.171694

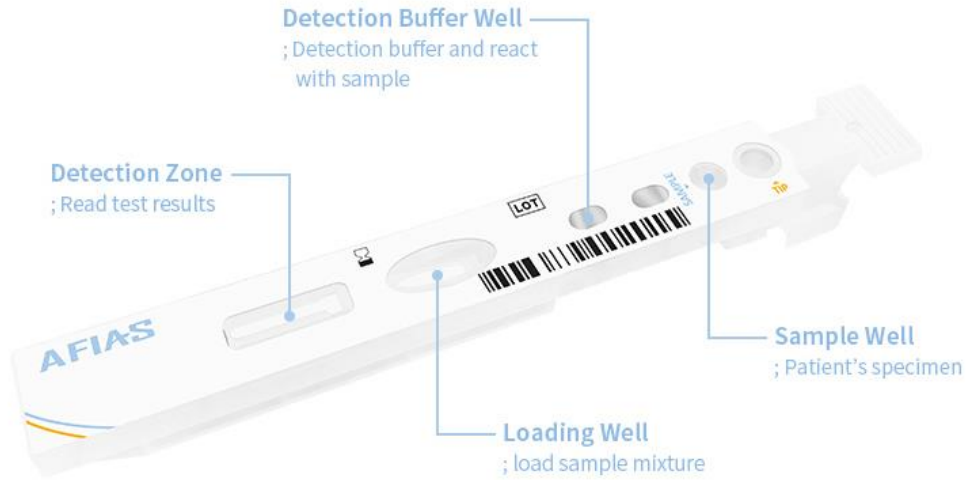
In order to visualize the specific and conteol stripes, originally enzymatic reactions were used. Nowadays increasingly using plasmonic nanoparticle labels offering bright colors.

Lateral Flow Assay - Implementation



https://doi.org/10.3389/fmicb.2018.00953

Rapid Protein Tests



http://www.boditech.co.kr/

Recently, more advanced cartridges are derived from the strips, allowing for quantiative (concentration) and rapid (15 min) detection in rather automatized manner.

Rapid Protein Covid Tests

Category	Item	Platform					
		ichroma™	iCHROMA II	AFIAS-1	AFIAS-6	iCHROMA-50	iCHROMA M2
Cardiac	Tn-I	●	●				
	Tn-I Plus		●	●	●		
	CK-MB	●	●	●	●		
	D-Dimer	●	●	●	●		
	NT-proBNP		●	●	●		
	Myoglobin	●	●	●	●		
	hsCRP	●	●				
	ST2		*	*	*		
Cancer	PSA	●	●	●	●		
	AFP	●	●	●	●		
	CEA	●	●	●	●		
	iFOB Neo	●	●			●	
Diabetes	HbA1c	●	●	●	●		
	Microalbumin	●	●	●	●		
	Cystatin C	●	●				
Hormone	TSH	●	●	●	●		
	TSH Plus		●	●	●		
	T3	●	●	●	●		
	T4	●	●	●	●		
	FSH	●	●	●	●		
	FSH Plus			*	*		
	Progesterone	●	●				
	β-hCG	●	●	●	●		
	β-hCG Plus		●	●	●		
	LH	●	●	●	●		
	PRL	●	●	●	●		
	Testosterone	●	●	●	●		
	Cortisol	●	●	●	●		
	AMH			●	●		

Detection with strip-like catridges is not done only colorimetrically, but also via fluorescence gaining sensitivity and making possible analysis of e.g. cardiac markers present at pM concentrations.

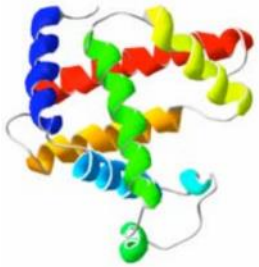
Biosensors

Biosensor

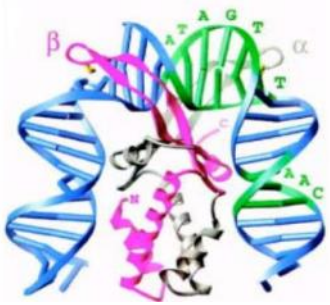
… is self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC 1996).

DNA/DNA

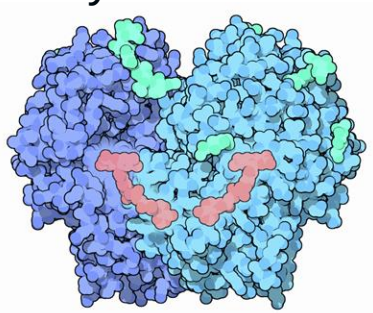
Protein/Protein

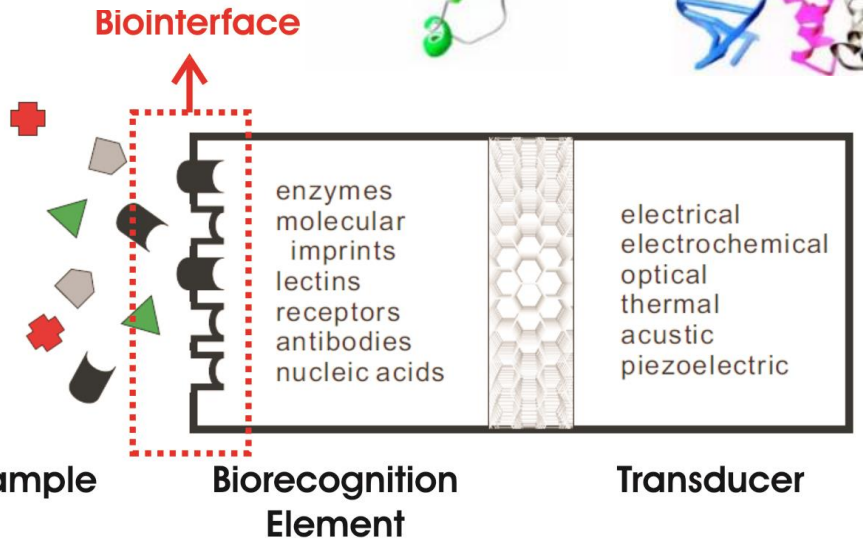


Protein/DNA



Enzyme





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Physico-Chemical Transducers

Transducer converts molecular binding events to measurable (physical) signal. Those can be based on various physical quantities:

Mass (quartz crystal microbalance…)

Conductivity (amperometry, voltametry…)

Heat release or absorption (calorimetry)

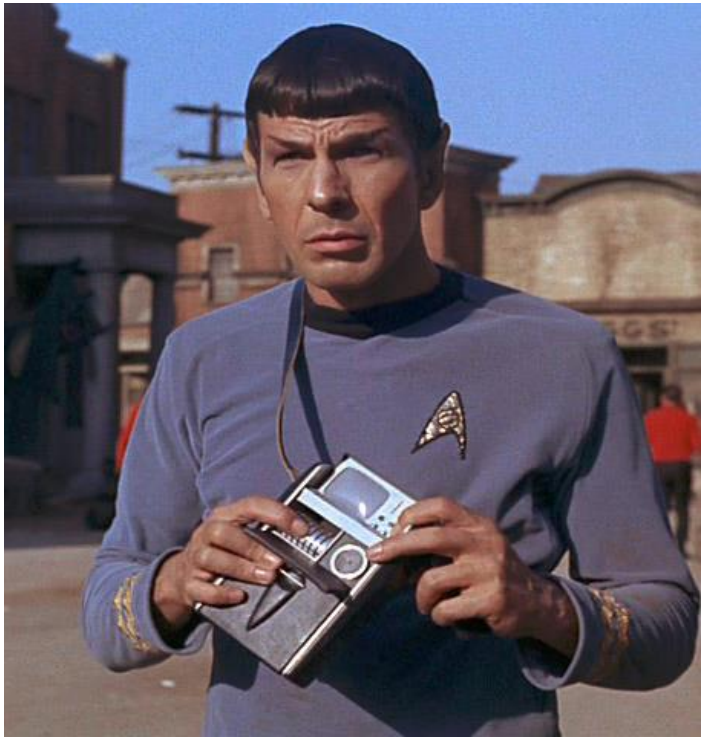
Refractive index (surface plasmon resonance)

Absorption (colorimetric detection)

Non-linear optical interaction with matter (fluorescence, SERS)

…

Envisioned quite some time ago…



Vision of a device that can “analyze everything at once…”.

Tricorder were used for sensor scanning, data analysis, and recording data

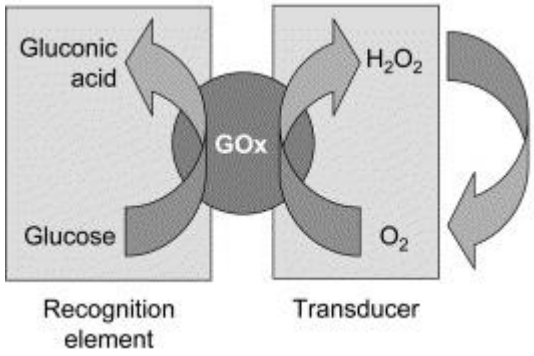
http://www.rounds.com/blog/star-trek-predicted/

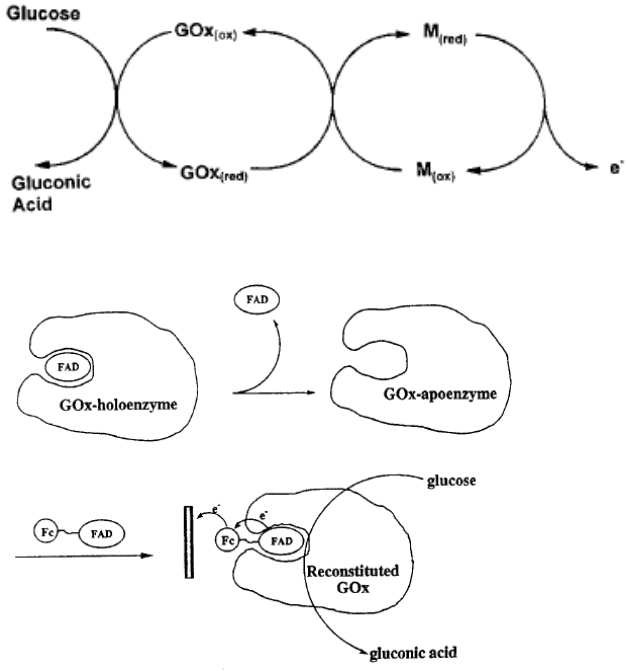
Historical Examples

1962	Invention of a biosensor: an amperometric enzyme electrode for glucose (Clark).
1975	Commercial glucose biosensor (Yellow Springs Instruments)
1980	First fiber optic pH sensor for in vivo blood gases (Peterson)
1983	First surface plasmon resonance (SPR) immunosensor (Liedberg, Nylander, and Lundstrom)
1990	Commercial SPR based biosensor by Pharmacia BIACore
….	

Electro-Chemical Biosensor

Amperometric detection of glucose by using glucose oxidase (GOx) is prominent example of electrochemical biosensor.





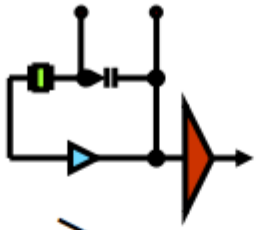
Original Clark concept – detection of depletion of O_2

Challenges to compensate to other effects (e.g. O_2 fluctuations). Amperometric detection of $H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$ and its replacement by mediators to eliminate effect of other electroactive species.

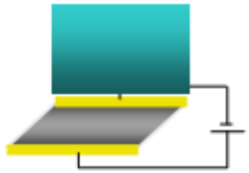
Wang, Glucose Biosensors: 40 years of advances and challenges, Electroanalysis (2001), 13, No.12

Quartz Crystal Microbalance

QCM – technique based on piezoelectric effect, coupled mechanical stress with charge



$$\Delta m = -C\frac{1}{n}\Delta f$$



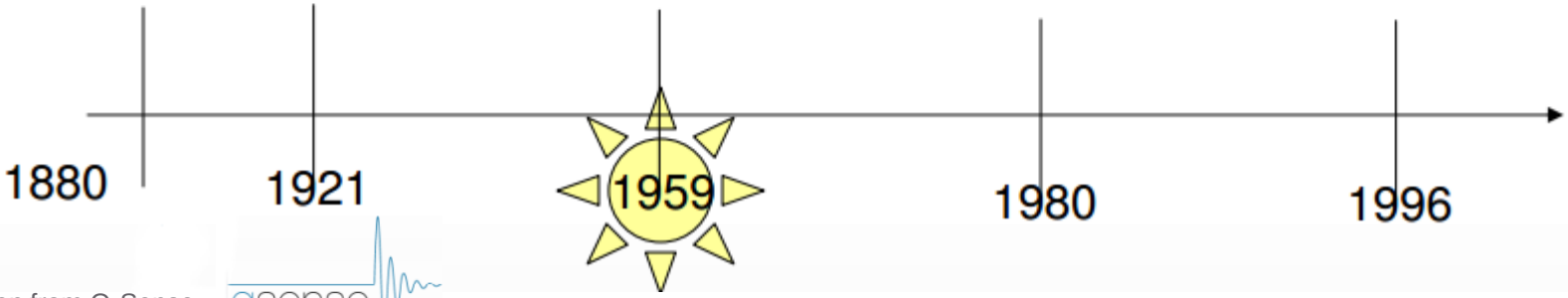
Curie brothers discover piezoelectricity

First quartz crystal controlled oscillator

Sauerbrey relates frequency and mass

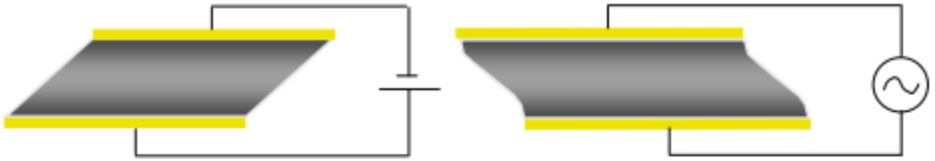
First QCM operating in liquid

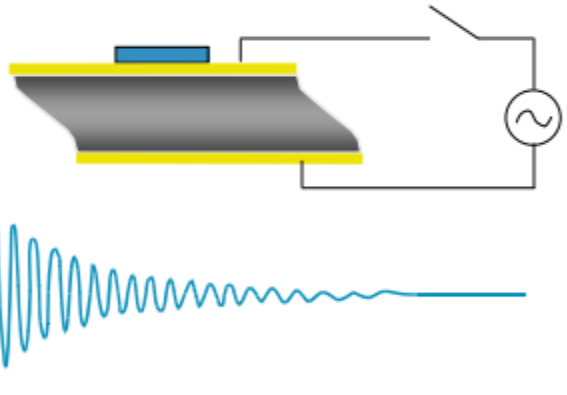
QCM-D Patent filed

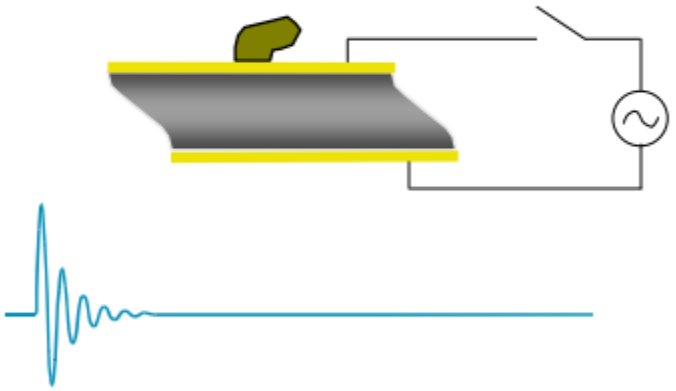


Taken from Q-Sense

Quartz Crystal Microbalance





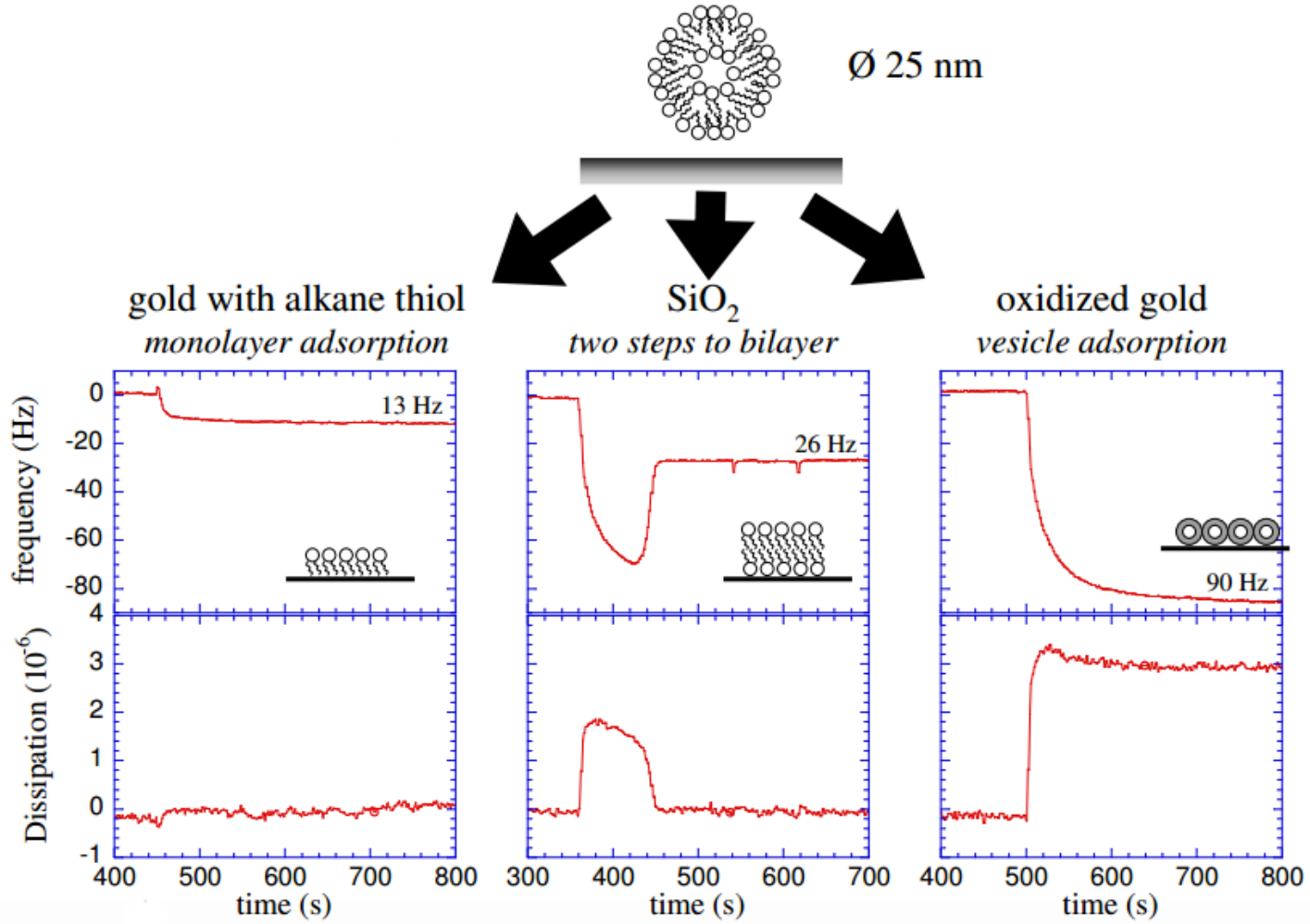


Δf is related to the mass of the attached film (Sauerbrey relation)

ΔD is related to the viscoelasticity

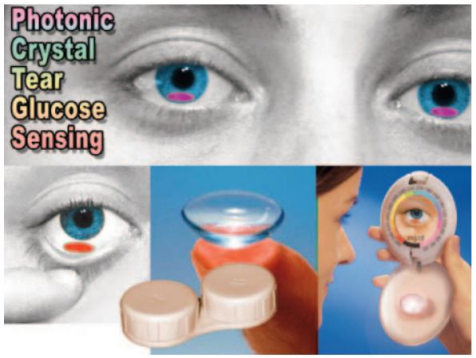
Taken from Q-Sense

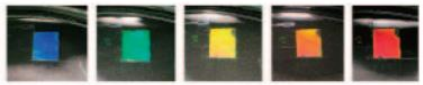
Quartz Crystal Microbalance

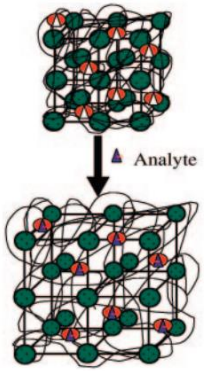


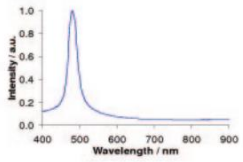
C.A. Keller and B. Kasemo, Biophysical J. 75 (1998) 1397.

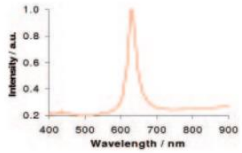
Contact Lens - Tear Fluid Analysis



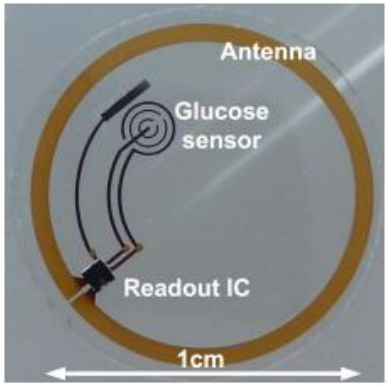


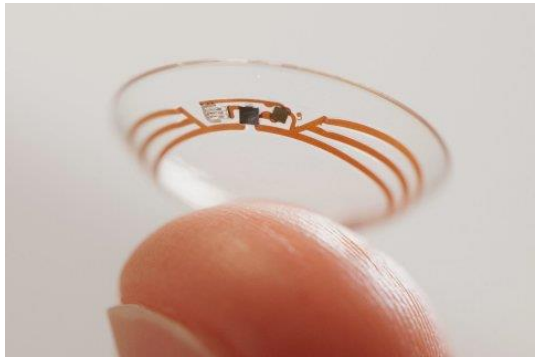






Photonic Crystal Glucose-Sensing Material for Noninvasive Monitoring of Glucose in Tear Fluid," V. Alexeev, S. Das, D.N. Finegold and S.A. Asher, Clinical Chemistry, 50, 2353 - 2360 (2004)

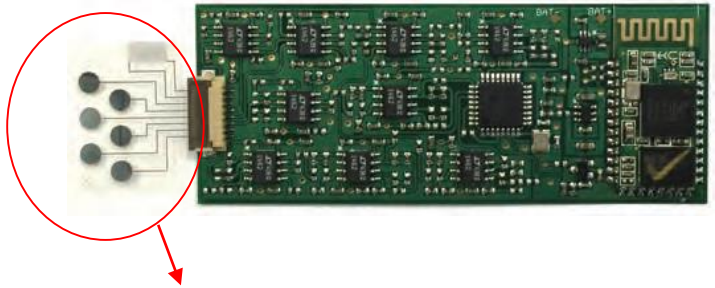




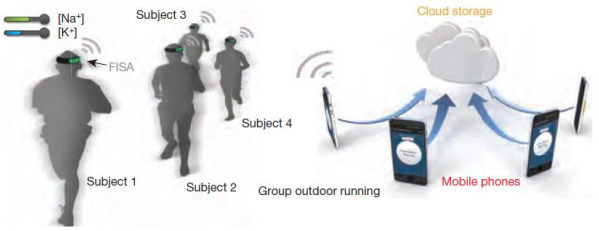
Liao Y-T, Yao H, Lingley A, Parviz B, Otis BP. A 3-uW CMOS glucose sensor for wireless contact-lens tear glucose monitoring. IEEE JSSC 2012;47:335Y44

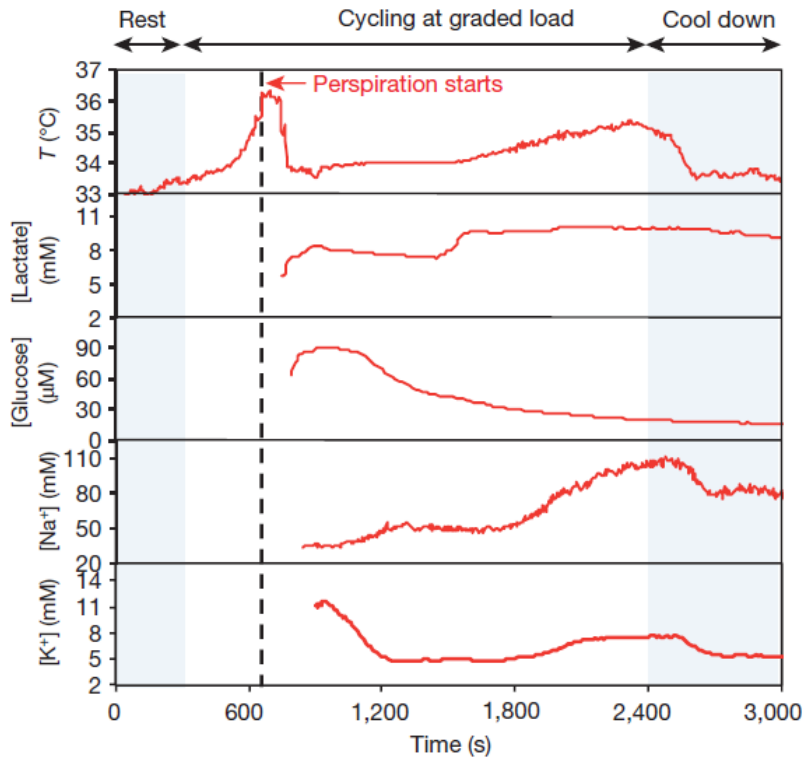
http://noviosense.com/

Wearable Sensors – Sweat Analysis



Electrochemical analysis of sweat at molecular level by arrays of sensors in close contact with skin.





Nature 529(7587):509-514 · January 2016
DOI: 10.1038/nature16521

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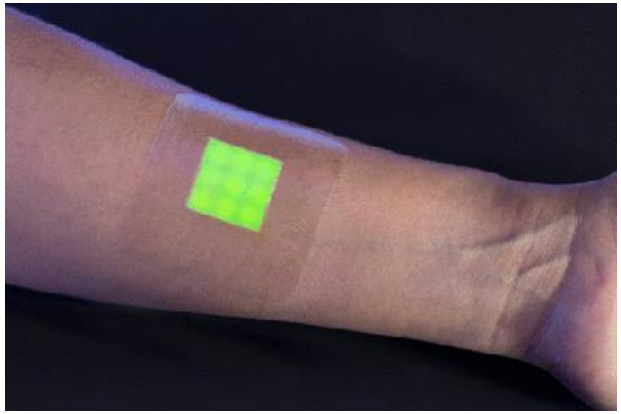
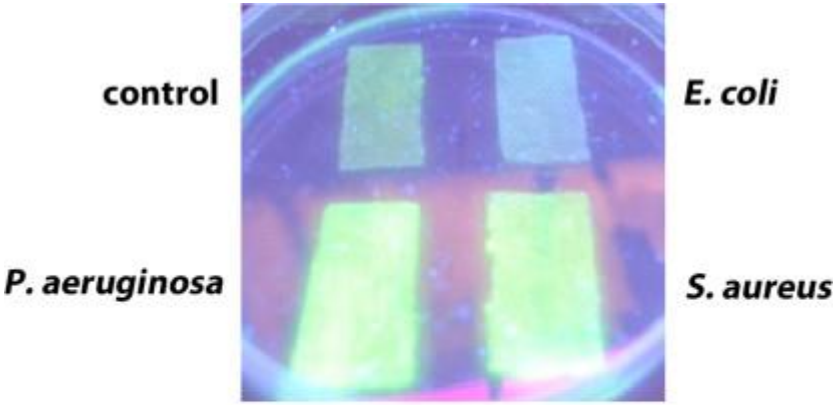
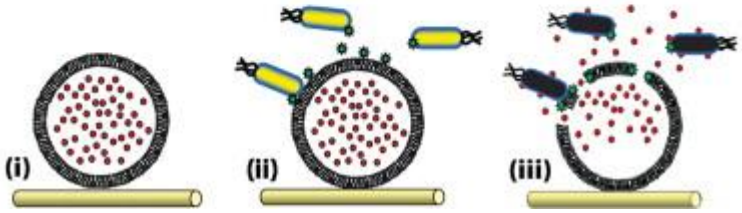
Smart Wound Dressing



Biosensors embedded in wound dressings to monitor bacterial infections. Possible incorporation of triggered release of a drug.





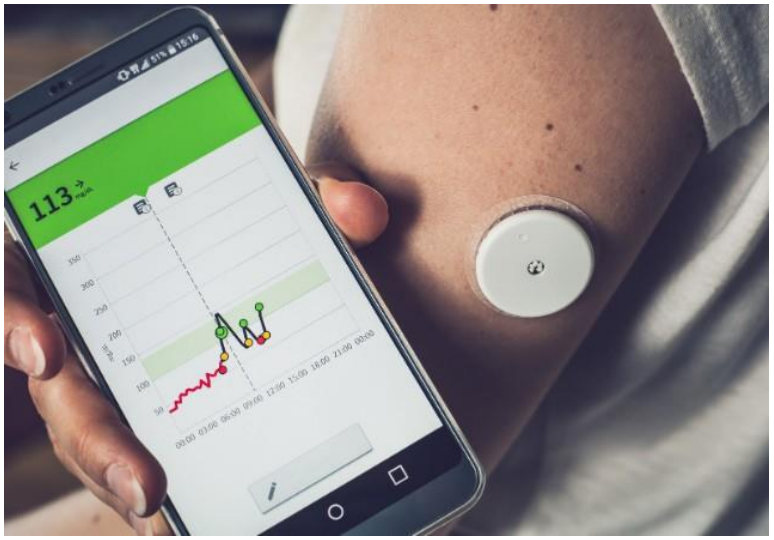
Dye self quenched

Dye bright

Fluorescent dye loaded to lipid vesicle, toxic bacteria destroy the lipid bilayer wall and leaches the dye reporter.

Toby Jenkins laboratory - 10.1021/acsami.5b07372

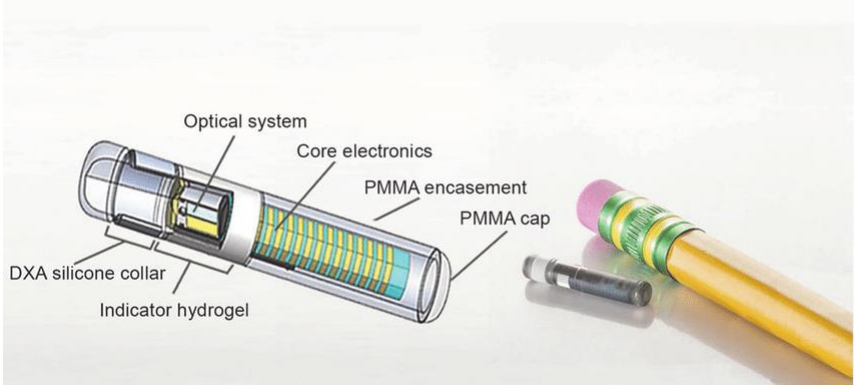
Implanted Glucose Sensing

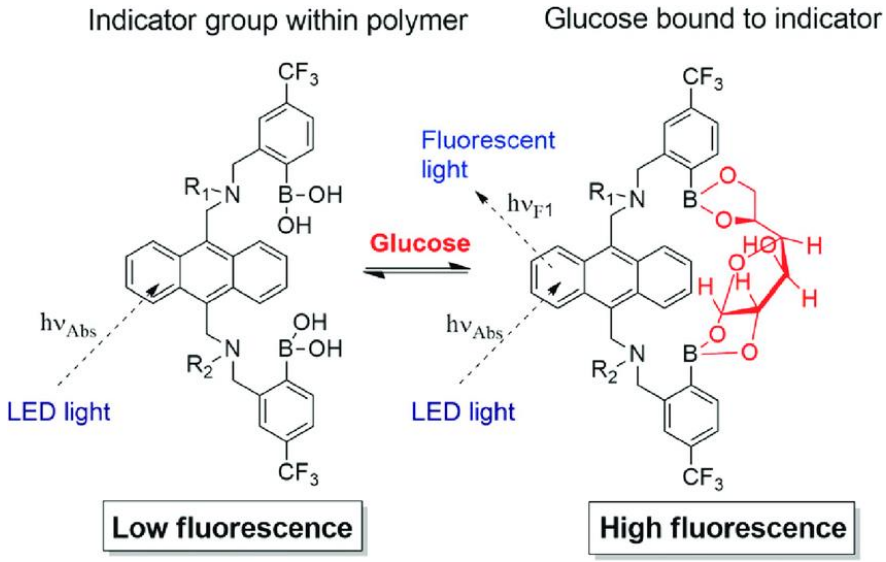


Eversense provides continuous blood glucose monitoring for up to 90 days via an under-the-skin sensor, a removable and rechargeable smart transmitter, and a convenient app for real-time diabetes monitoring and management.

https://www.eversensediabetes.com/sensor

Implanted Glucose Sensing

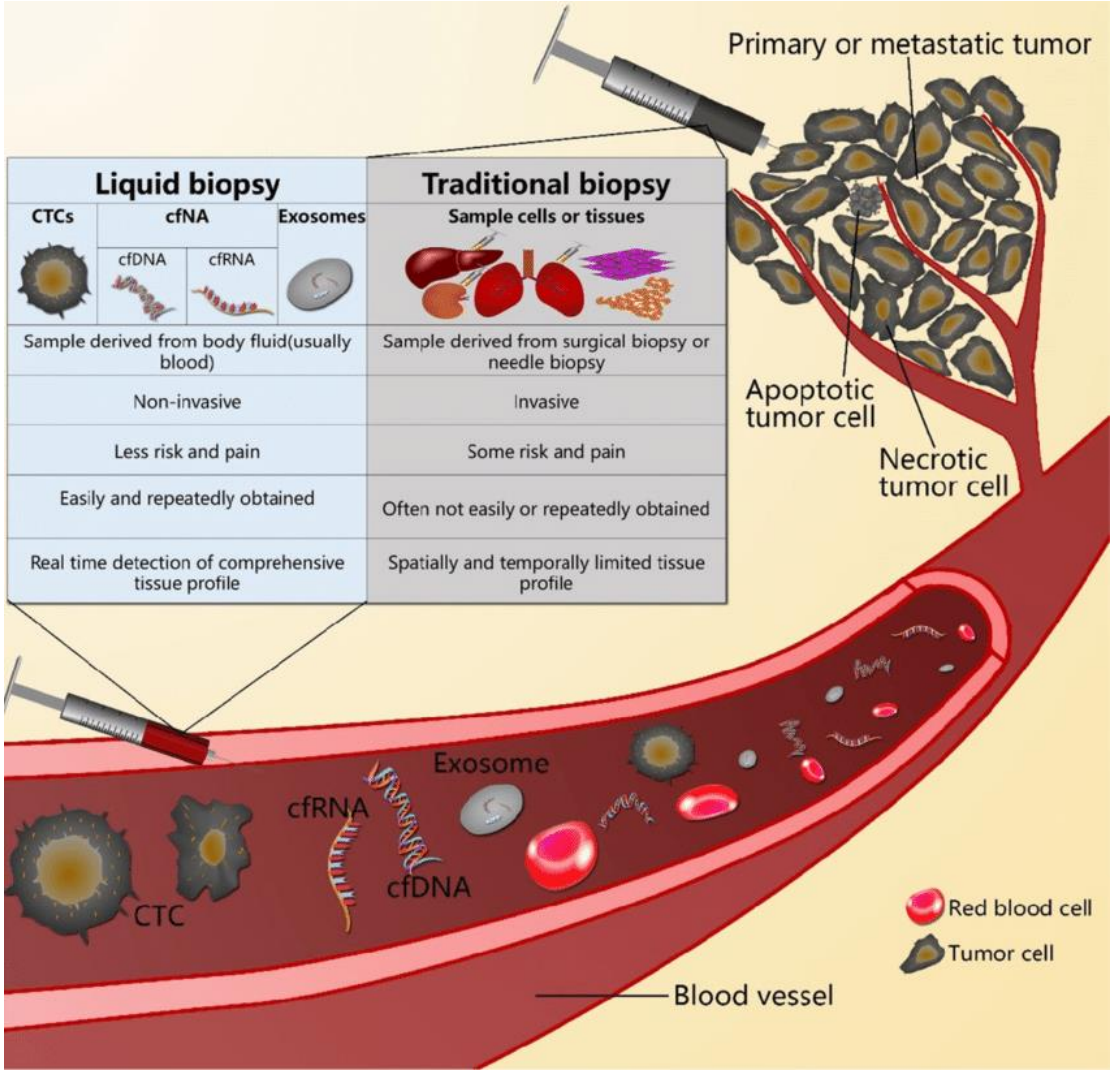




It uses a patented **fluorescent** glucose-indicating polymer technology to measure glucose in the **interstitial fluid** (a thin layer of fluid that surrounds the body's .

https://www.eversensediabetes.com/sensor

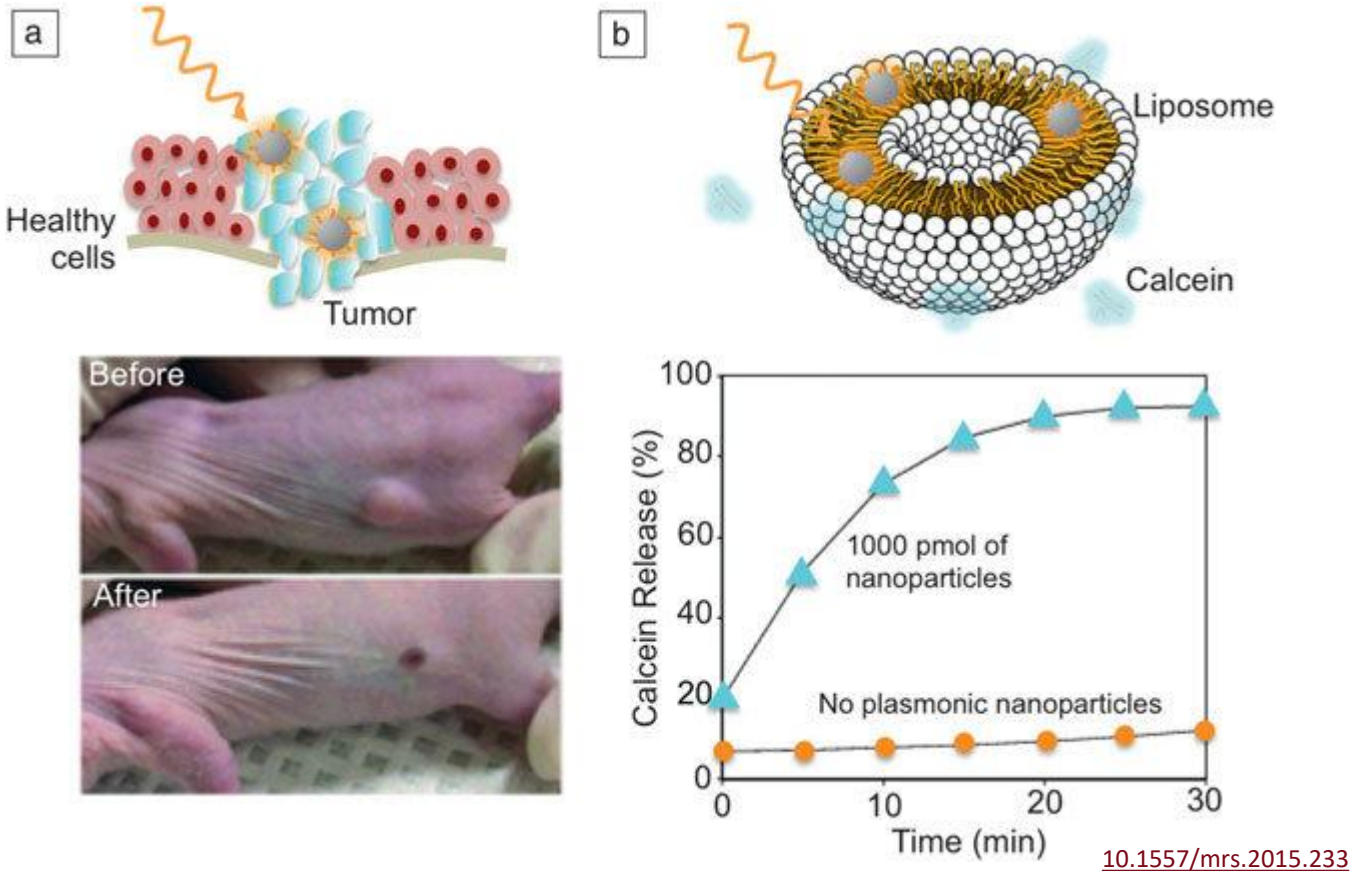
Liquid Biopsy



Liquid biopsy			Traditional biopsy
CTCs	cfNA (cfDNA, cfRNA)	Exosomes	Sample cells or tissues
Sample derived from body fluid(usually blood)			Sample derived from surgical biopsy or needle biopsy
Non-invasive			Invasive
Less risk and pain			Some risk and pain
Easily and repeatedly obtained			Often not easily or repeatedly obtained
Real time detection of comprehensive tissue profile			Spatially and temporally limited tissue profile

•10.7150/jca.24591

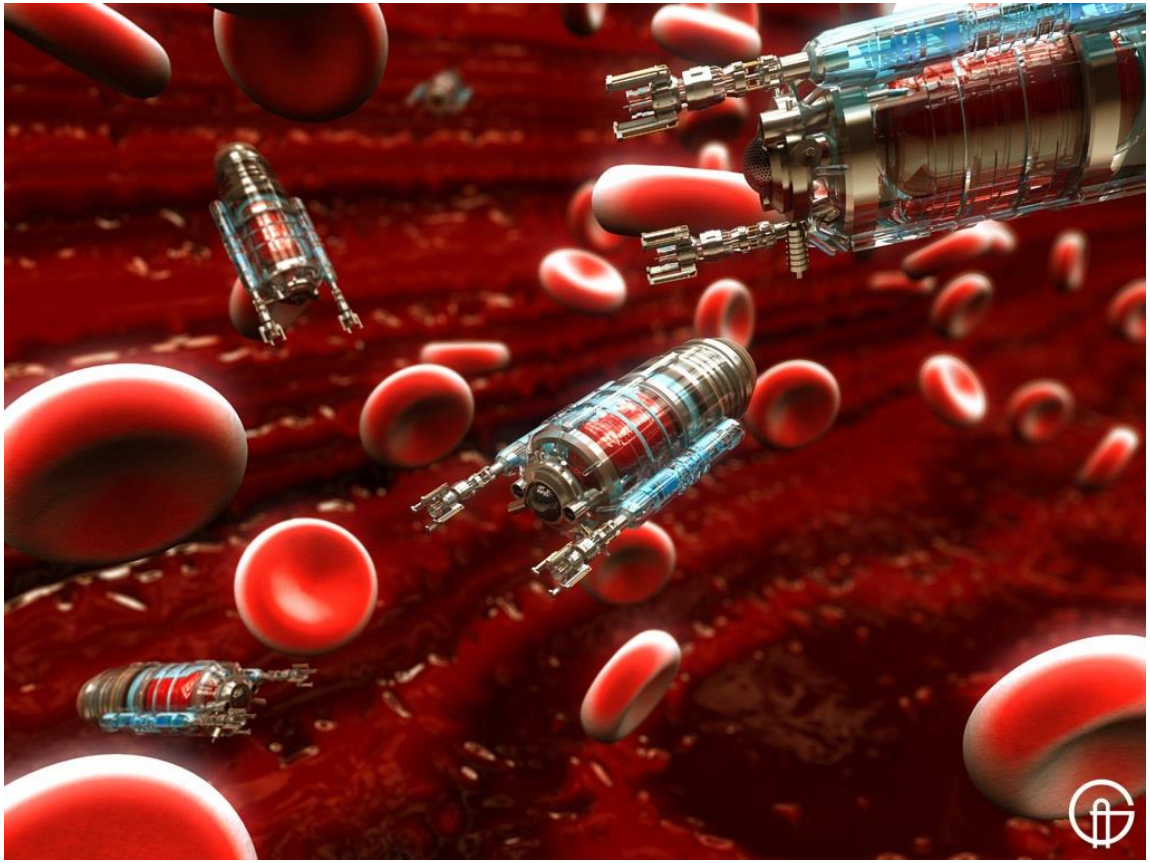
Thera(g)nostic



10.1557/mrs.2015.233

Thera(g)nostics is a patient management strategy in precision medicine. Aims ay molecular targeting and kills cancer cells whilst sparing healthy tissue.

Swallowed Surgeon

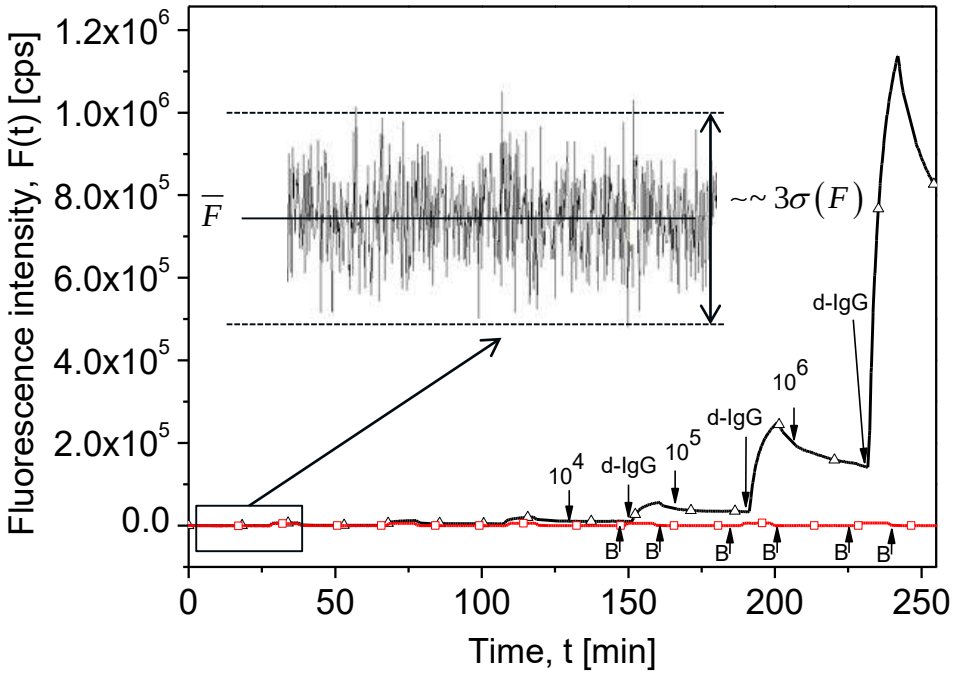


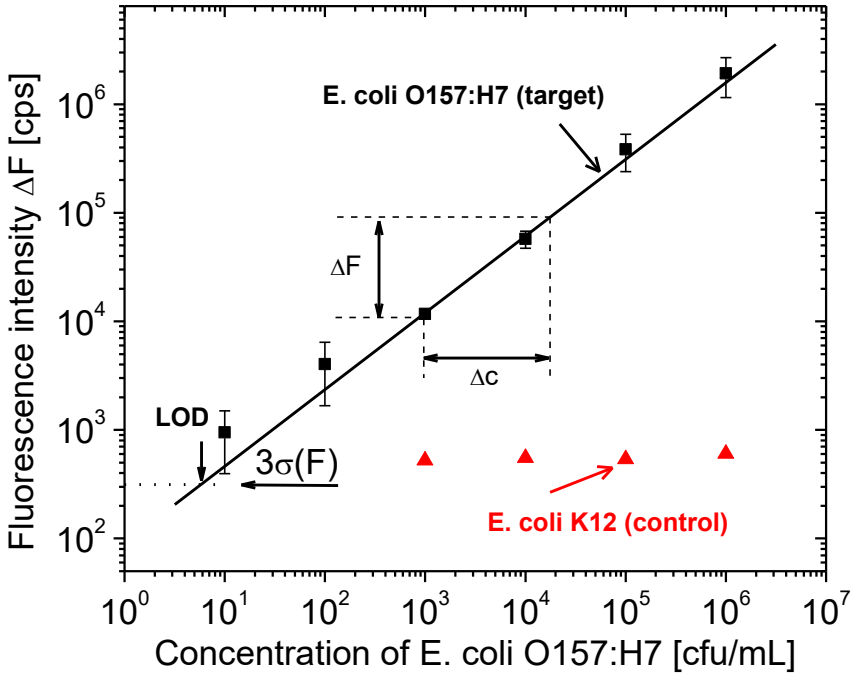
1966 movie, *Fantastic Voyage* explored shrinking a medical team to microscopic size in order to save a renowned scientist's life. The Argonauts travel through the bloodstream into the brain where the crew uses a laser gun to blast away a blood clot.

http://internetmedicine.com/2016/11/06/52871/

Biosensors Characteristics

Calibration Curve





C.J. Huang et al , Biosensors and Bioelectronics (2010), 26, 4, 1425-1431.

Sensitivity S=ΔF/ Δc

Sensor signal noise described by stand. deviation $\sigma(F)=\sqrt{\frac{1}{N-1}\sum_i\left(F_i-\overline{F}\right)^2}$

Limit of detection (LOD) determined from sensor noise as LOD=3σ(F)/S

Limit of quantification (LOQ) determined from sensor noise as LOD=10σ(F)/S

Performance Characteristics

Detection range:	Concentrations of analyte that can be determined.
Sensitivity:	The value of the sensor response per analyte concentration.
Limit of detection	Minimum concentration of analyte that can be detected
Specificity / selectivity:	Interference of the presence of other compounds must be minimized for obtaining the correct result.
Matrix effect	Detection in real samples (e.g. blood serum) is rather more difficult than in model ones (e.g. buffer)
Analysis time:	The necessary time to carry out the analysis
Reusability:	Sensor chips are used only once or can be regenerated for multiple detections.

Optical Biosensors

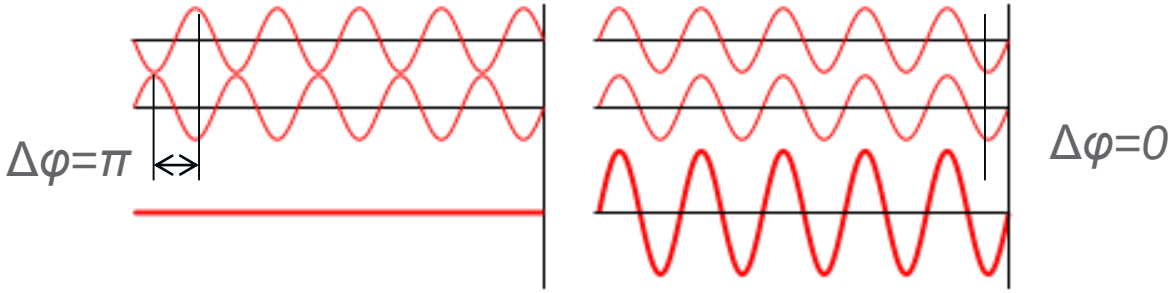
Refractive index (surface plasmon resonance, integrated optics waveguides, grating coupler, reflectometric interference spectroscopy)

Absorption (colorimetric detection, plasmonic nanoparticle aggregation assays)

Non-linear optical interaction with matter (fluorescence, Raman scattering, infrared absorption)

Interference

Optical phenomenon arising from (coherent) superimposing of amplitudes of two spatially overlapping waves. When changing a phase φ of one of the waves, intensity is varied.



Field amplitudes are subtracted – destructive interference

Field amplitudes are added – constructive interference

$\Delta\varphi = knd$ – a minute changes in refractive index shifts the phase and alters the intensity

Exploited in (arguably) most sensitive optical measurements: Frequency stabilized lasers for metrology, microscopy with phase contrast, narrow optical filters,…

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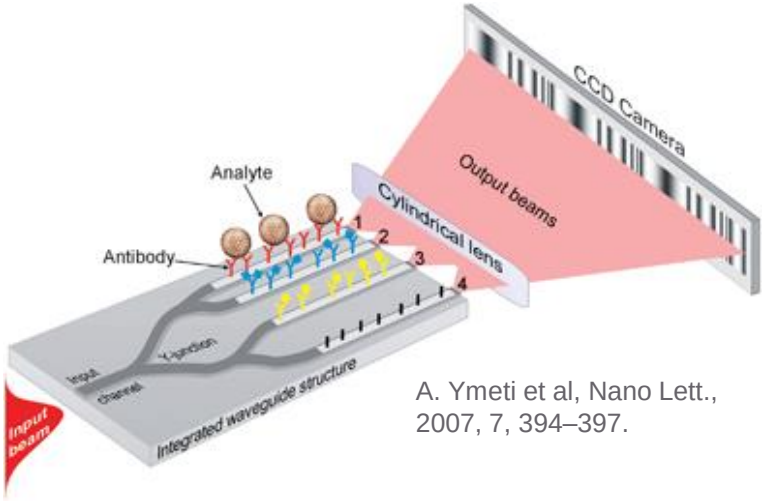
Dielectric Waveguides

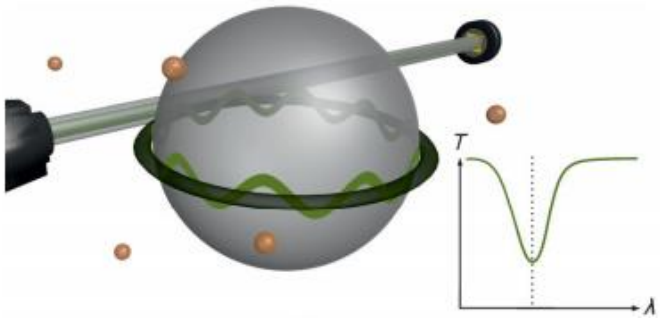
Various other optical biosensor platforms that are sensitive to binding-induced refractive changes have been developed. Further two examples that holds potential for highly compact devices based on integrated optics will be presented:

Waveguide interferometer



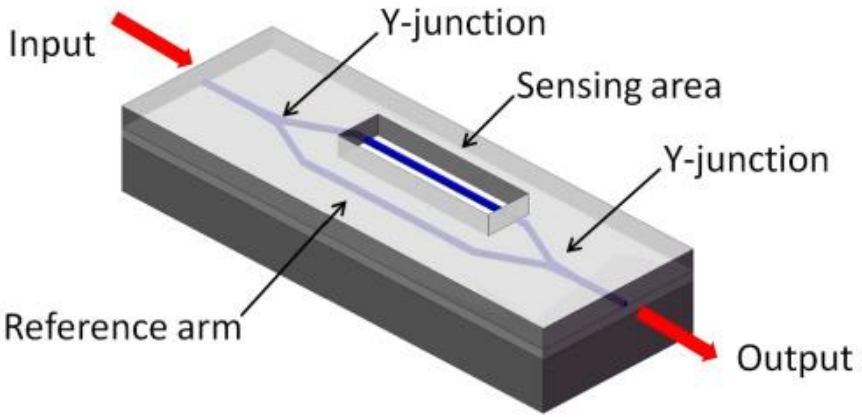
A. Ymeti et al, Nano Lett., 2007, 7, 394–397.

Ring resonators

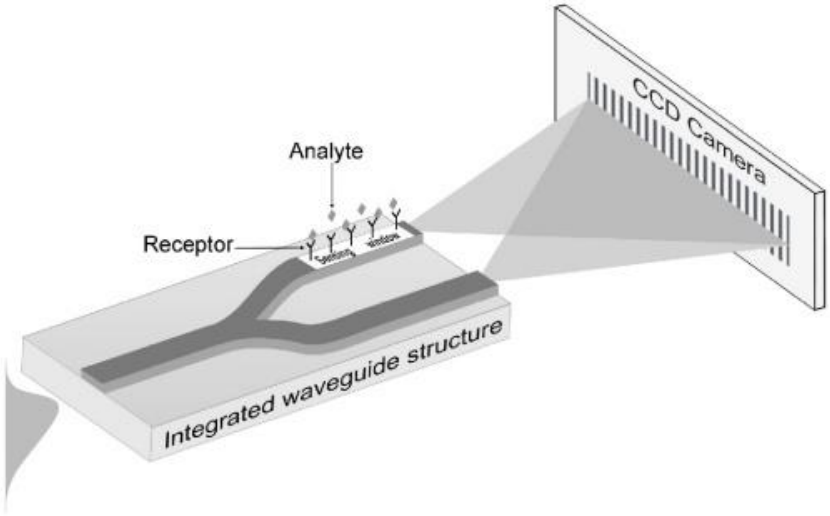


Martin Baaske and Frank Vollmer, ChemPhysChem 2012, 13, 427 – 436

Implementation of Interferometers



Optics Express, Vol. 20, Issue 7, pp. 7195-7205 (2012)



Optics Express, Vol. 20, Issue 19, pp. 20934-20950 (2012)

Mach Zehnder interferometer:

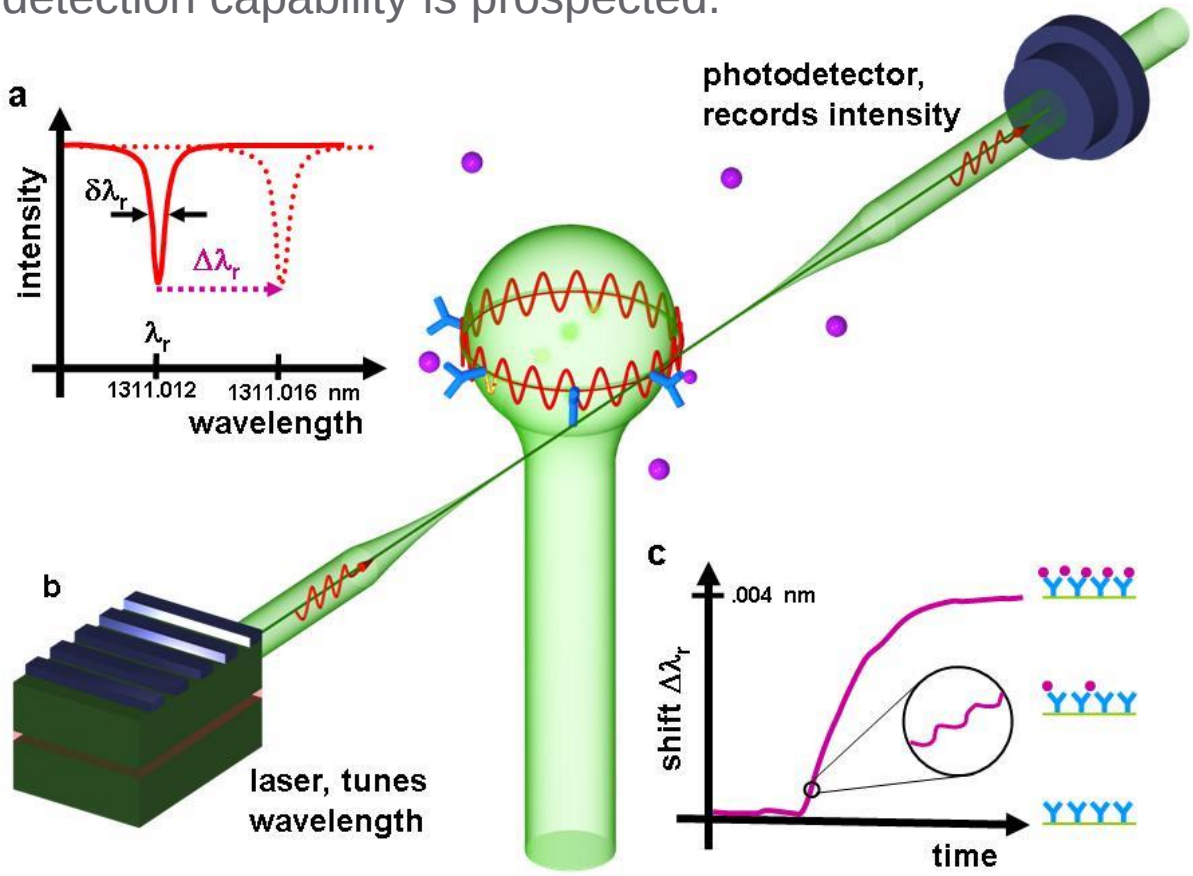
Detection of output intensity change induced by the capture of analyte on the sensing area

Young interferometer:

Detection of interference pattern shifts induced by the capture of analyte

Ring Resonators

Optical micro-resonators that exhibit a large Q-factor and small modal volume V (large Q/V) - highest sensitivity for label-free detection of molecules. Single-molecule detection capability is prospected.



Vollmer group: http://mpl.mpg.de/mpf/php/bfp/research/wgm_biosensing.php

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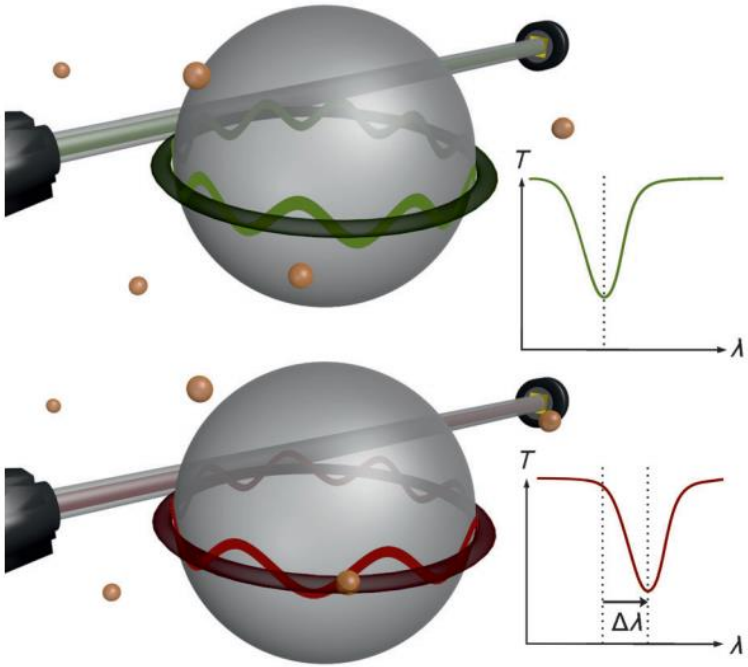
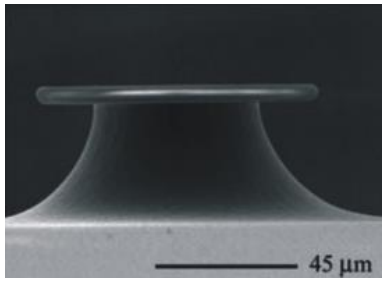
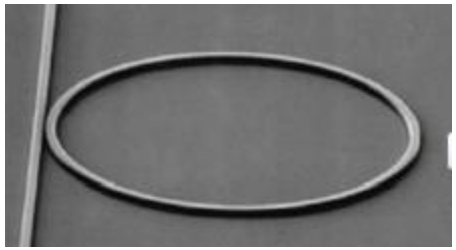
Implementations of Ring Resonators

Possible implementations include those based on silica microspheres coupled to tapered optical fibers (left) as well as integrated optical structures prepared by lithography (right).



Martin Baaske and Frank Vollmer,
ChemPhysChem 2012, 13, 427 – 436





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Volume 620, Issues 1–2, 14 July 2008, Pages 8–26