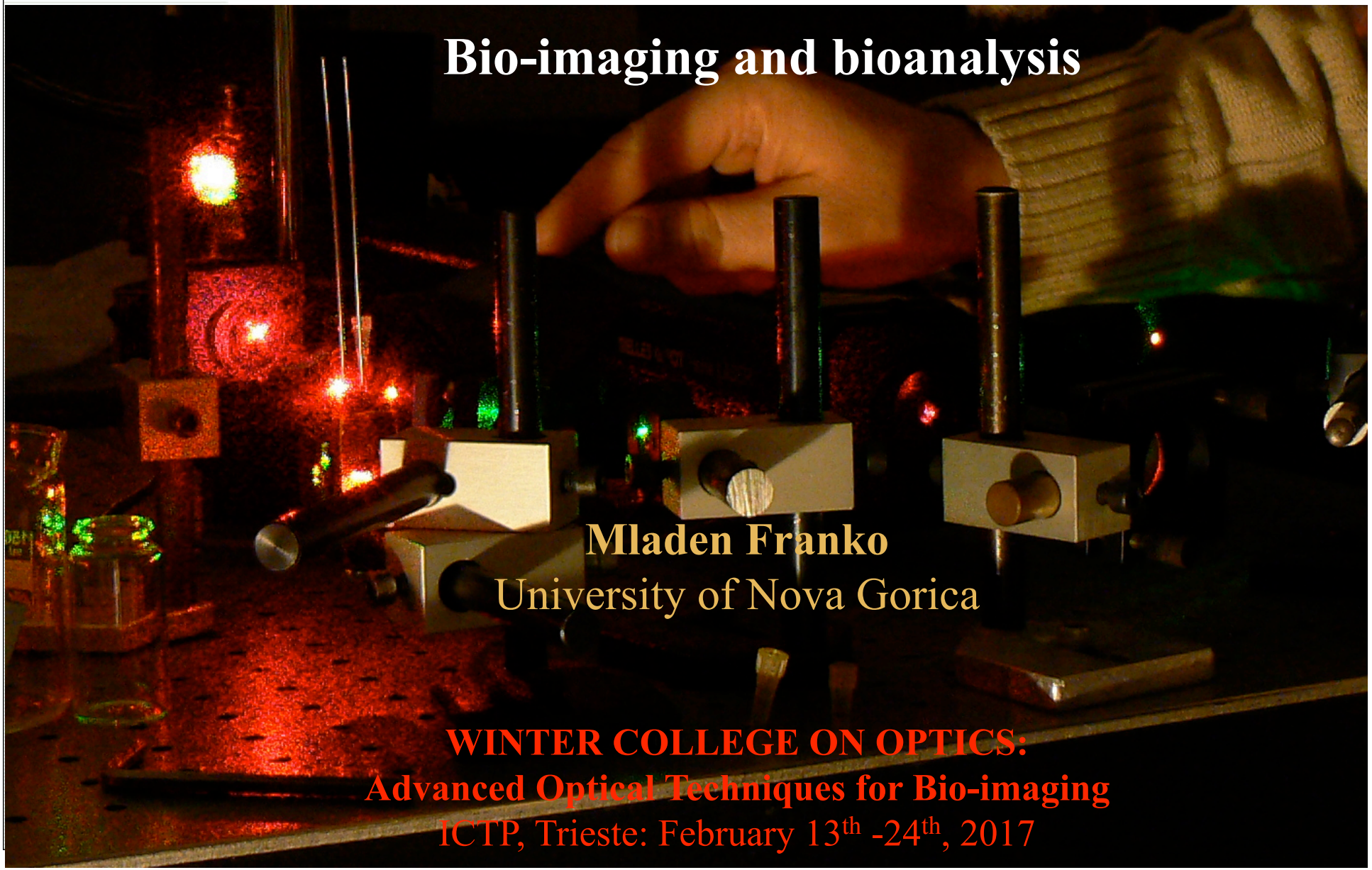
Applications of Thermal Lens Spectrometry and Microscopy

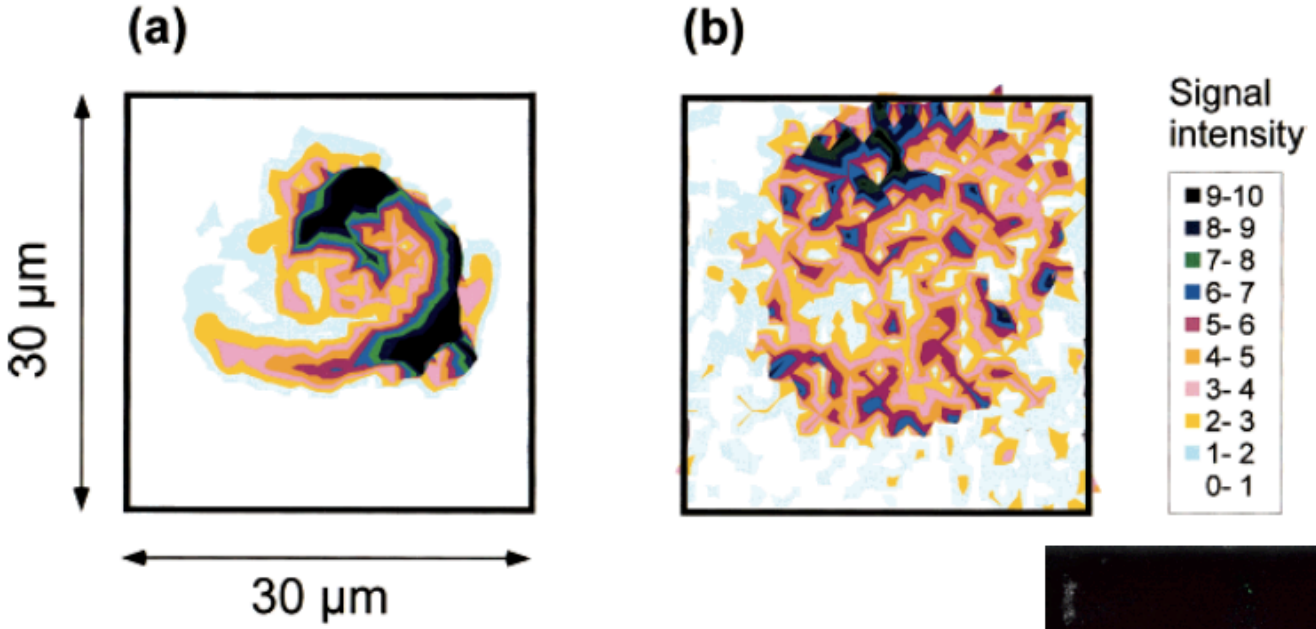


Bio-imaging and bioanalysis

Mladen Franko
University of Nova Gorica

WINTER COLLEGE ON OPTICS:
Advanced Optical Techniques for Bio-imaging
ICTP, Trieste: February 13th -24th, 2017

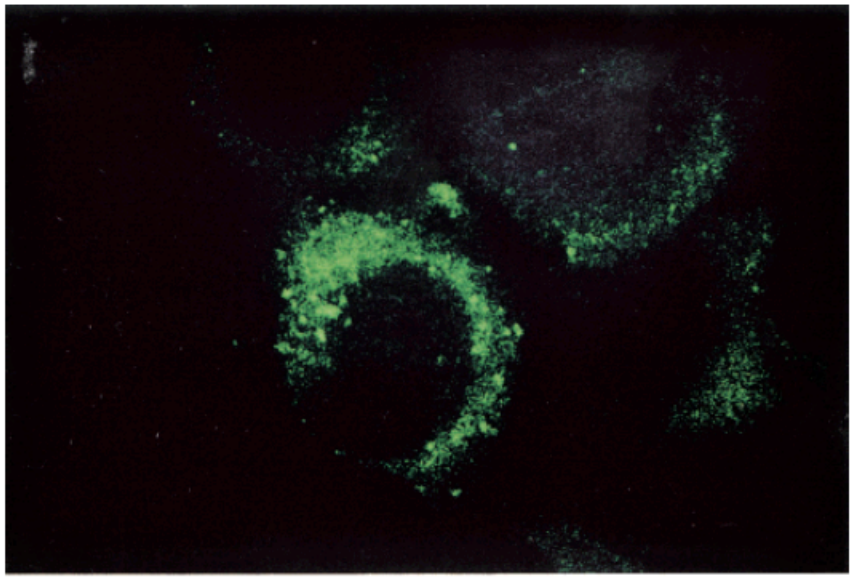
Single-Cell Analysis in a Microchip by a Scanning TLS Microscope



- Cytochrome c distribution during apoptosis

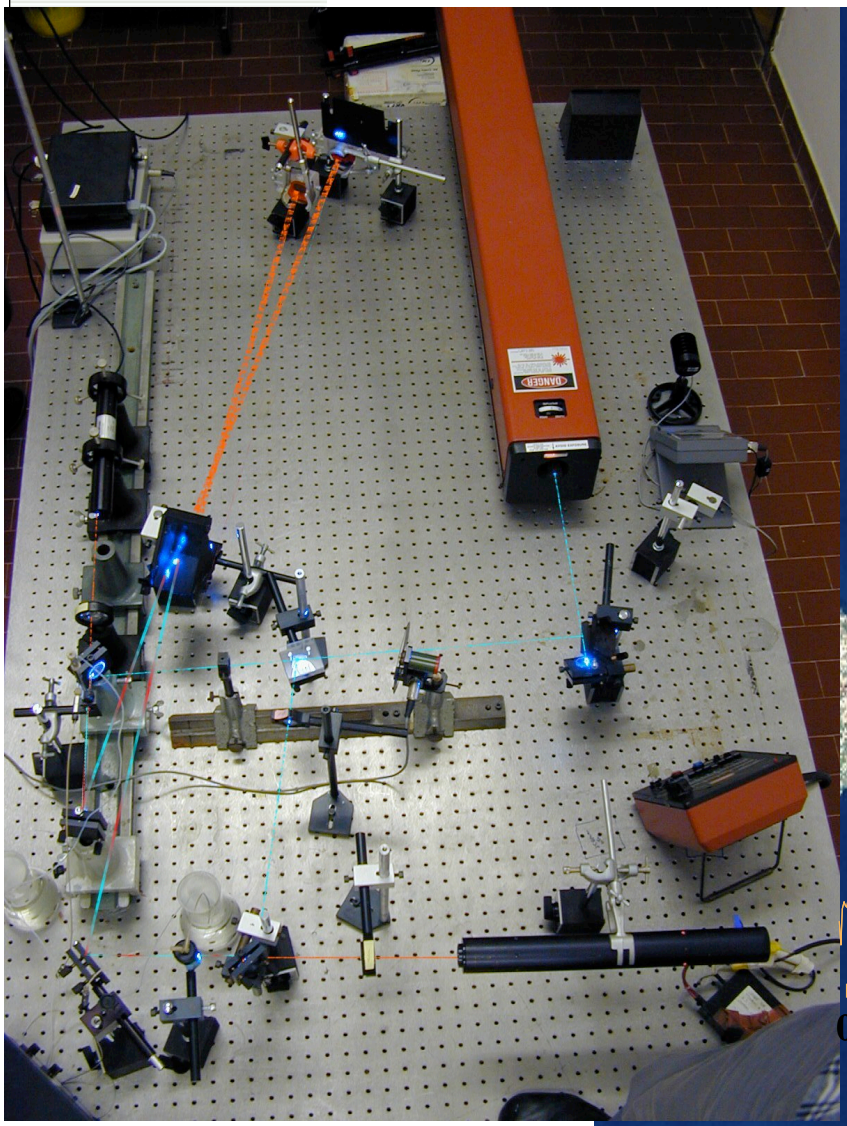
E. Tamaki et. al:

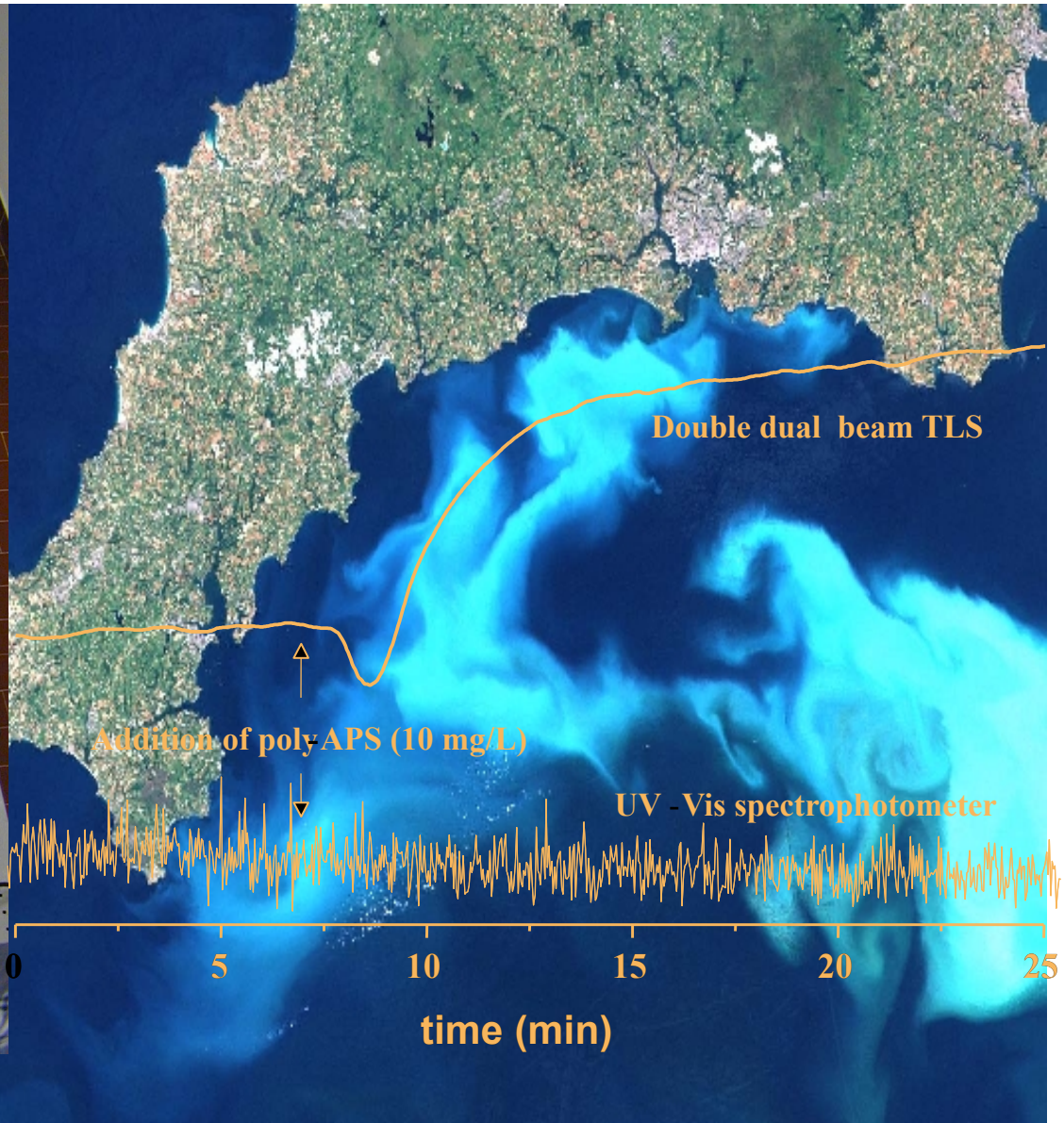
Anal. Chem. 2002, **74,** 1560-1564.



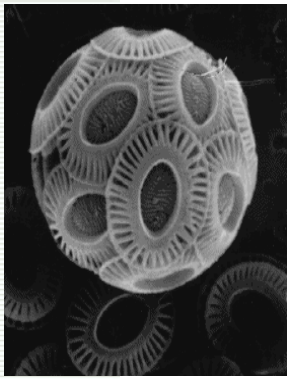
Advantages of TLS: extremely high sensitivity, small sample capability

(100 – 1000 times lower LOD than SF)





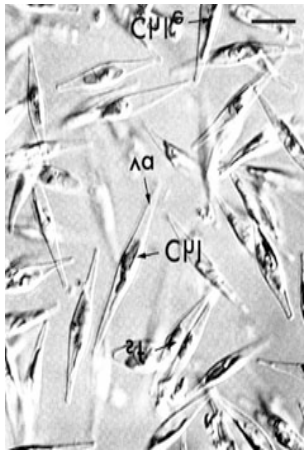
Investigated species of phytoplankton



Emiliania huxleyi

Skeletonema costatum

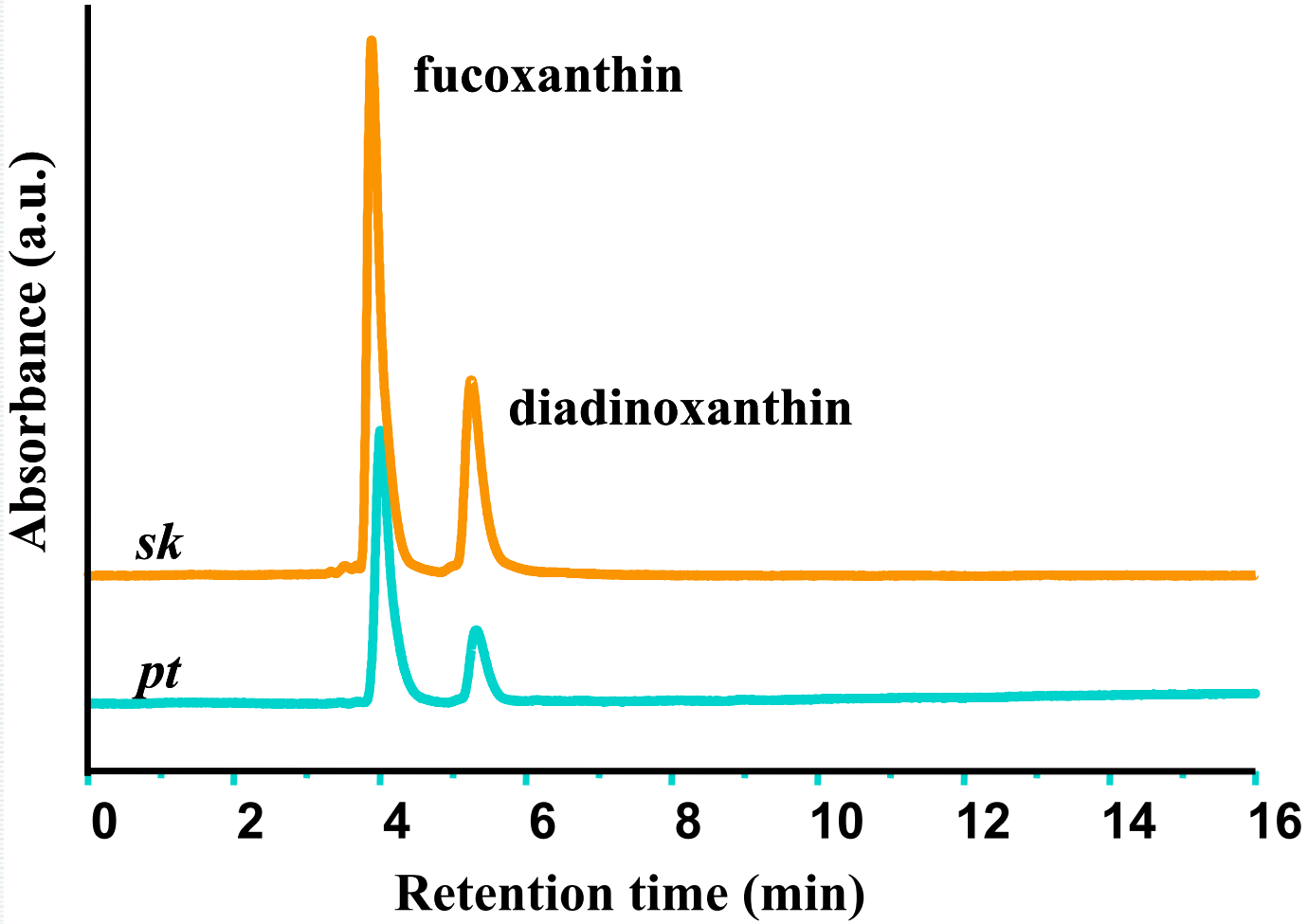
Chroomonas salina



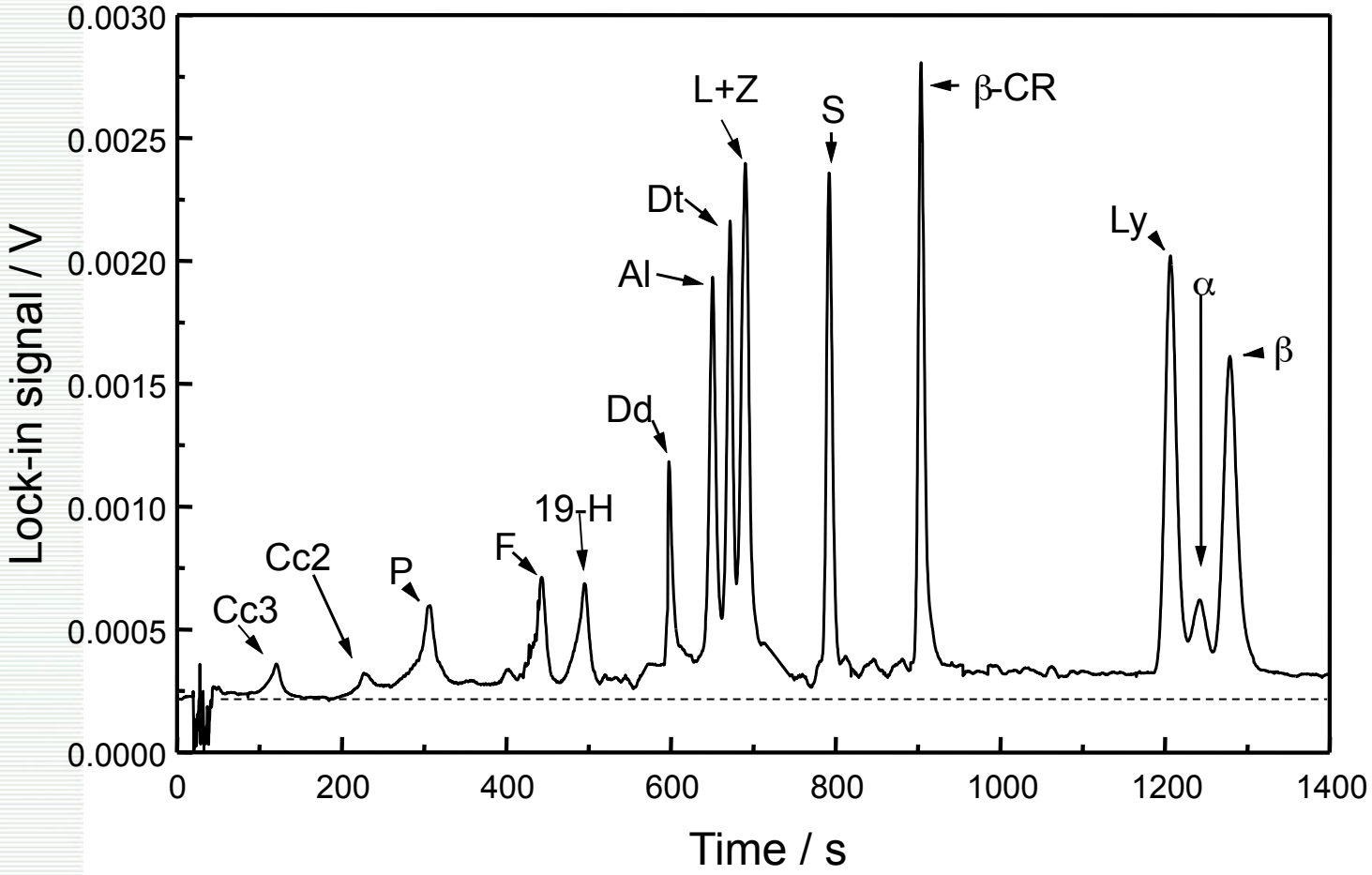
Phaeodactylum tricornutum

Amphidinium carterae

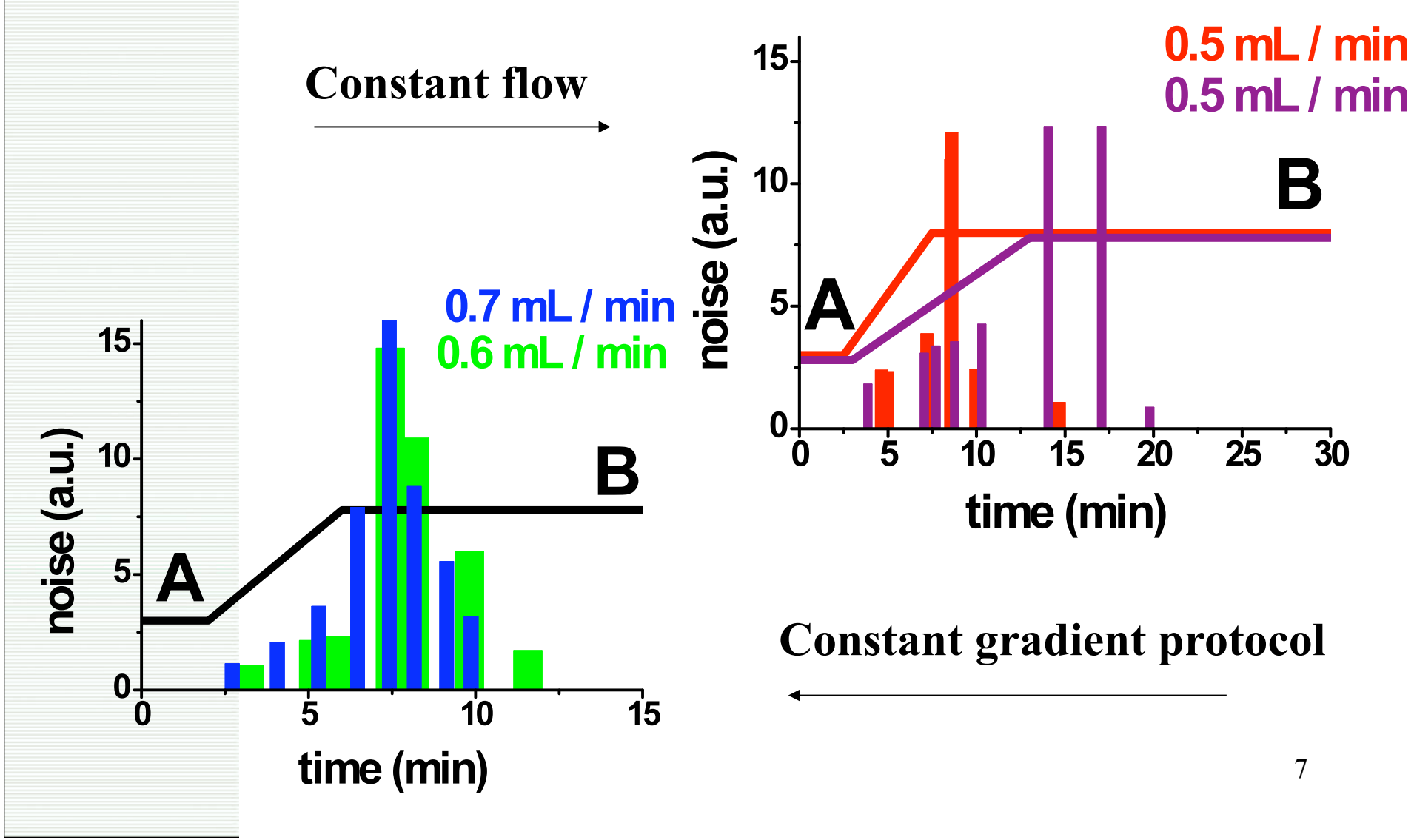
Carotenoids in diatoms



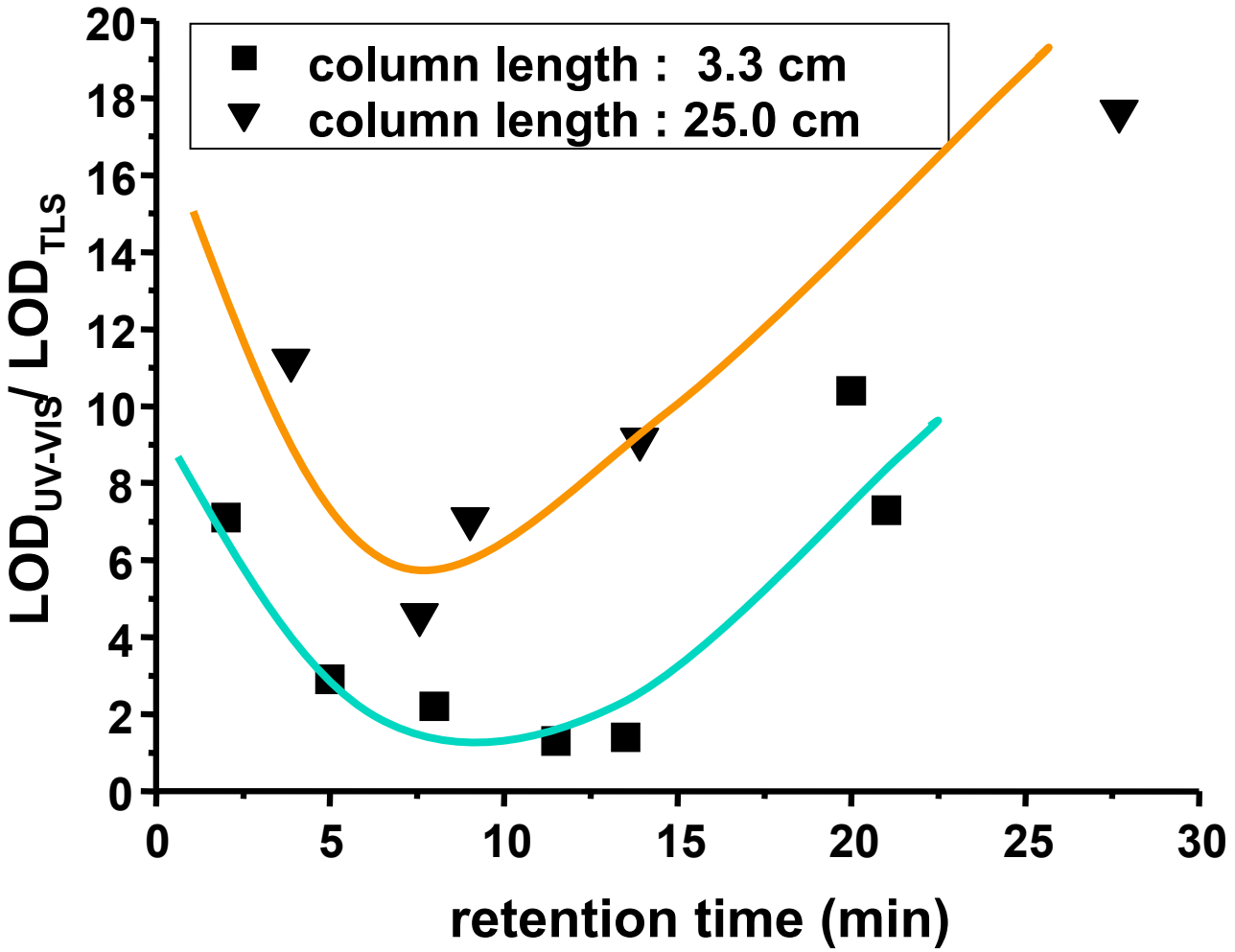
HPLC-TLS chromatogram of a mixed standard of carotenoids and chlorophylls



Signal noise in gradient HPLC-TLS



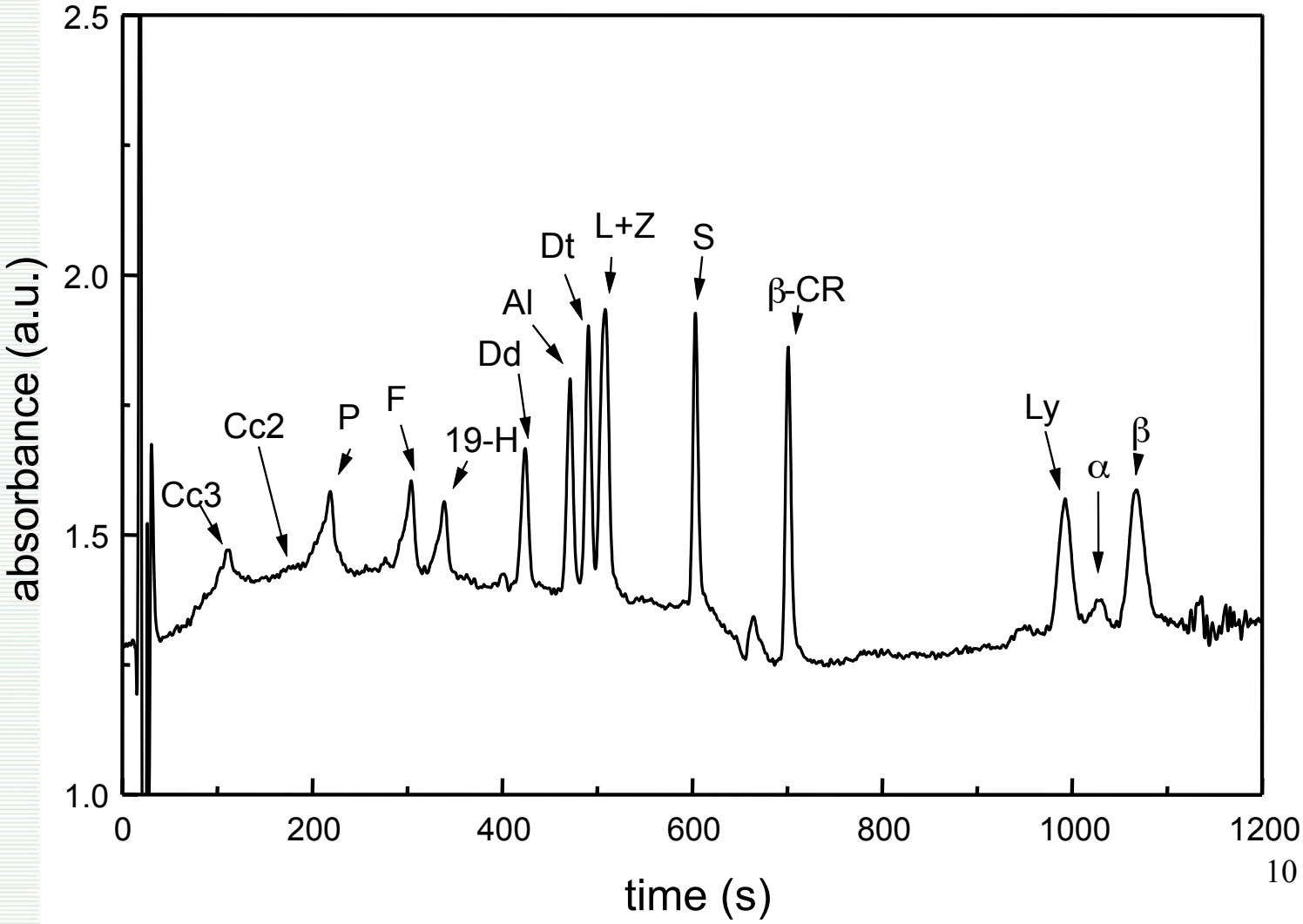
$LOD_{UV\text{-}Vis}$ / LOD_{TLS} (gradient HPLC)



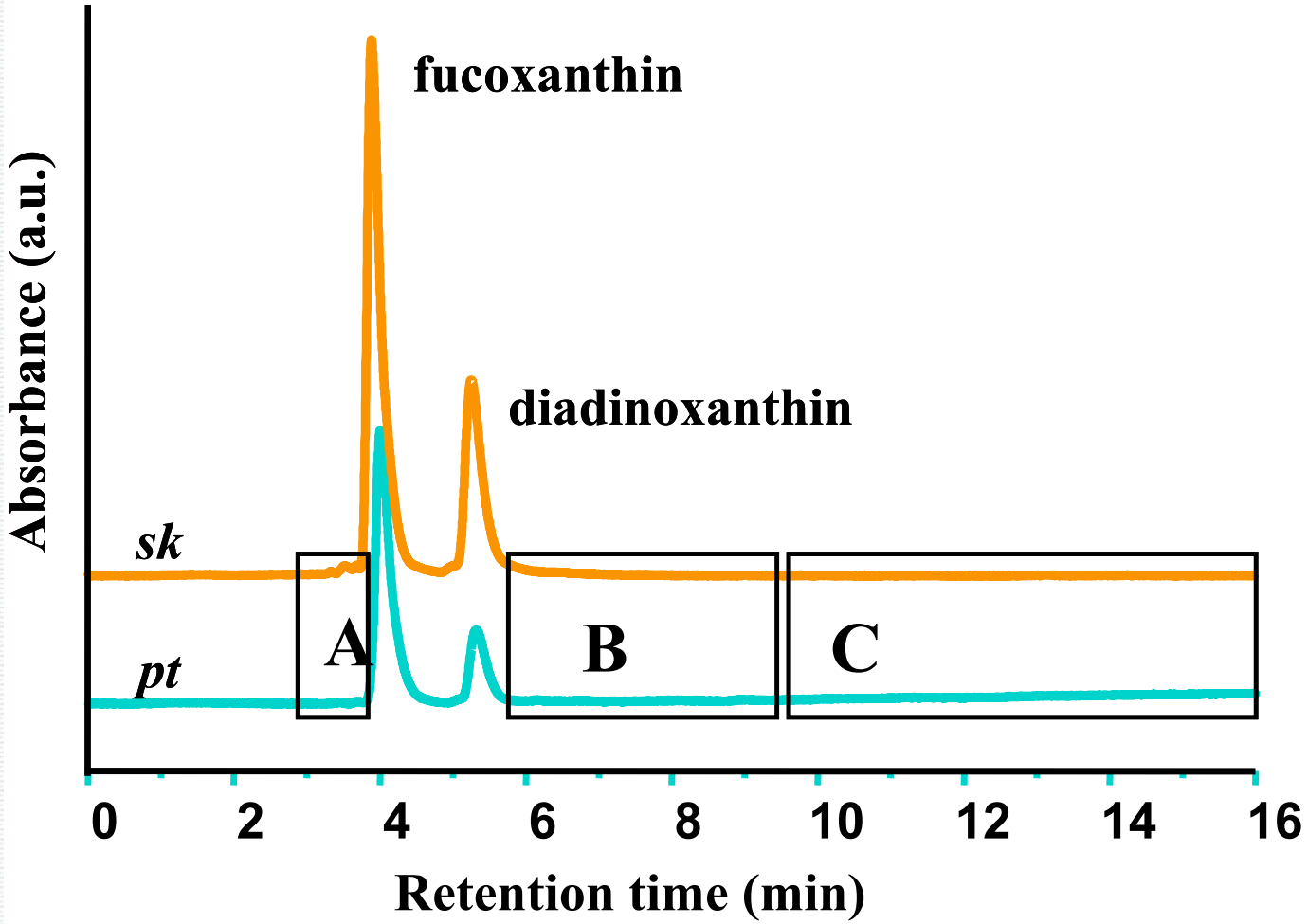
LODs for carotenoids and chlorophylls in gradient and isocratic HPLC-TLS

	gradient	isocratic
• β-cryptoxanthyn	0.1 $\mu g L^{-1}$	0.07 $\mu g L^{-1}$
• lycopen	0.2 $\mu g L^{-1}$	0.12 $\mu g L^{-1}$
• peridinin	0.5 $\mu g L^{-1}$	-
• chlorophill	> 1 $\mu g L^{-1}$	

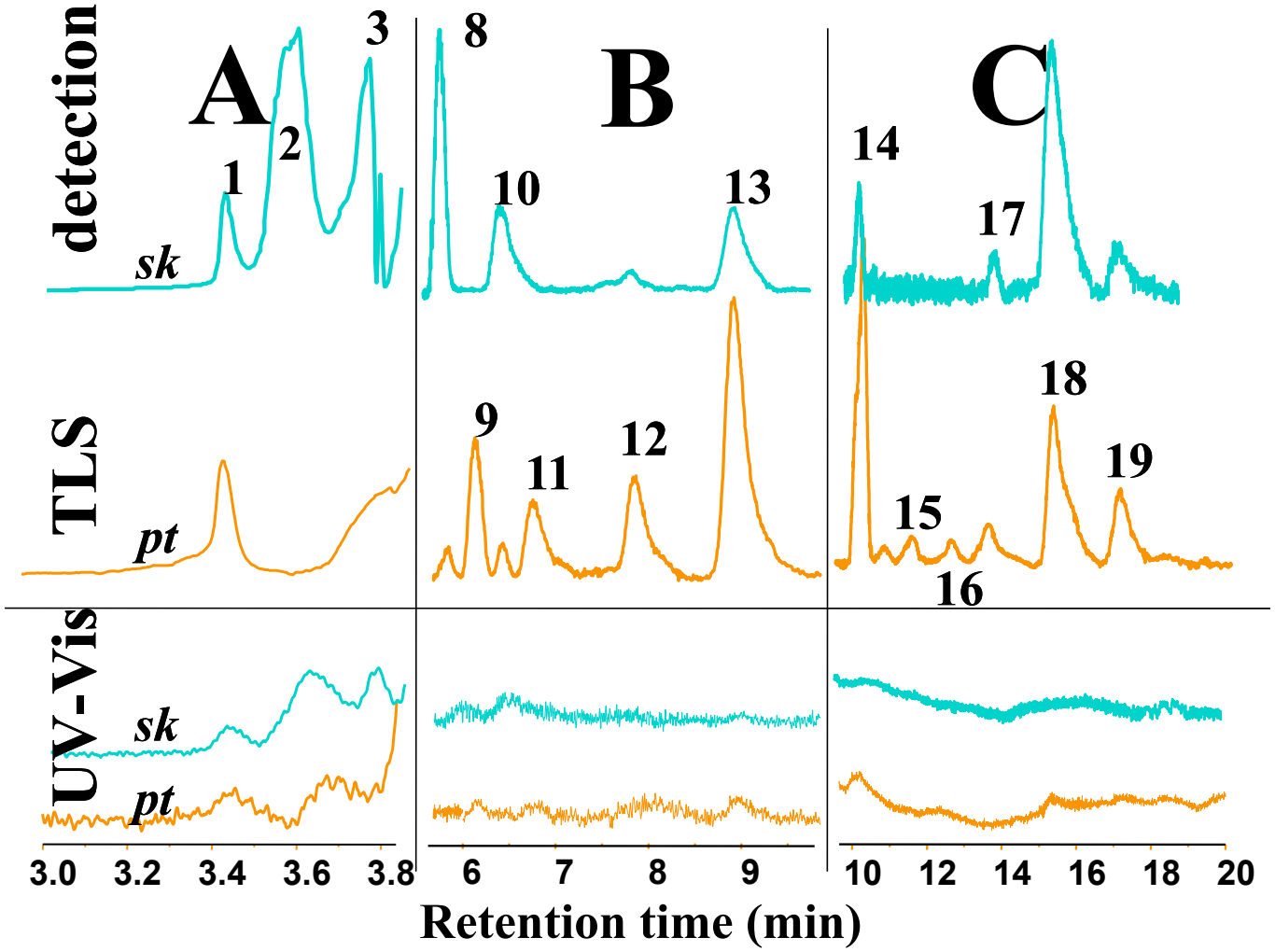
HPLC/UV-Vis chromatogram of a mixed standard of carotenoids and chlorophylls



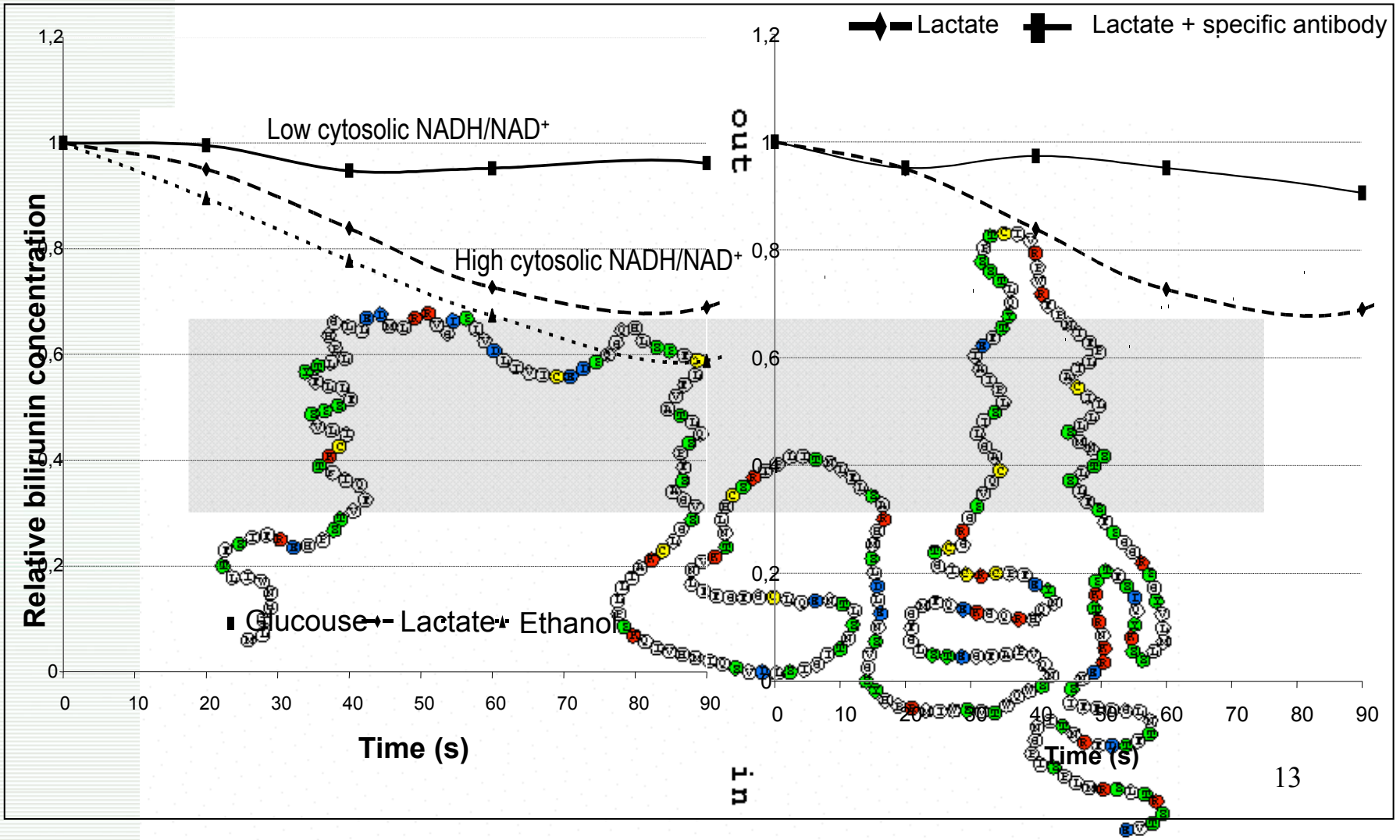
Carotenoids in diatoms



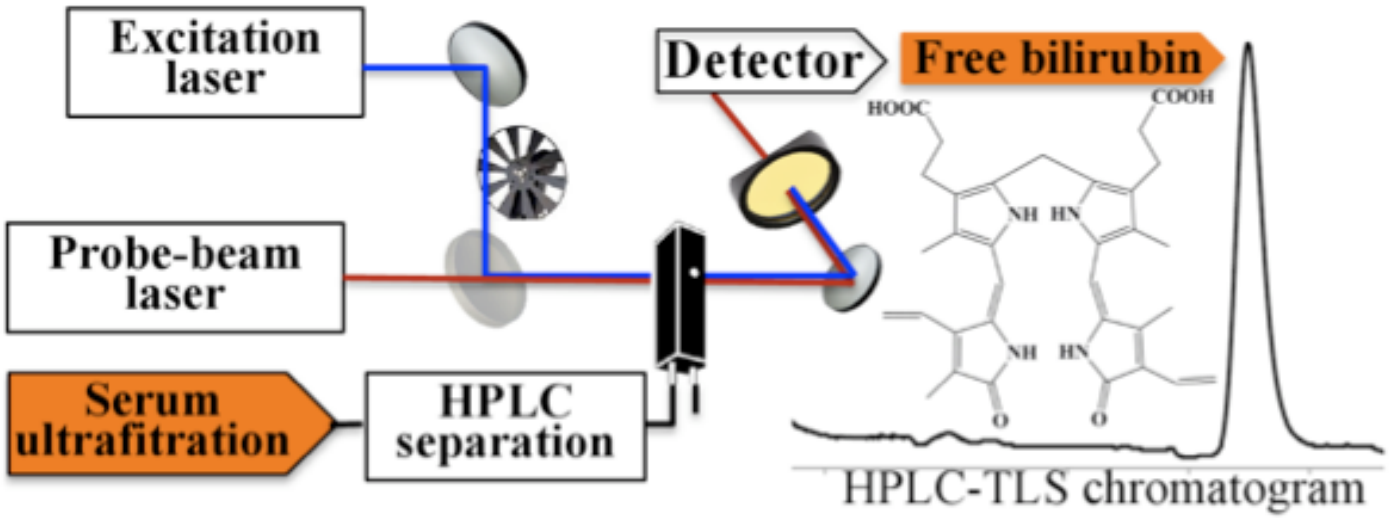
Detection of minor and trace carotenoids in diatoms



The role of BTL in the transport of antioxidants across the cellular wall



Improvement of selectivity by separation techniques (HPLC, IC)



LOD: 90 pM

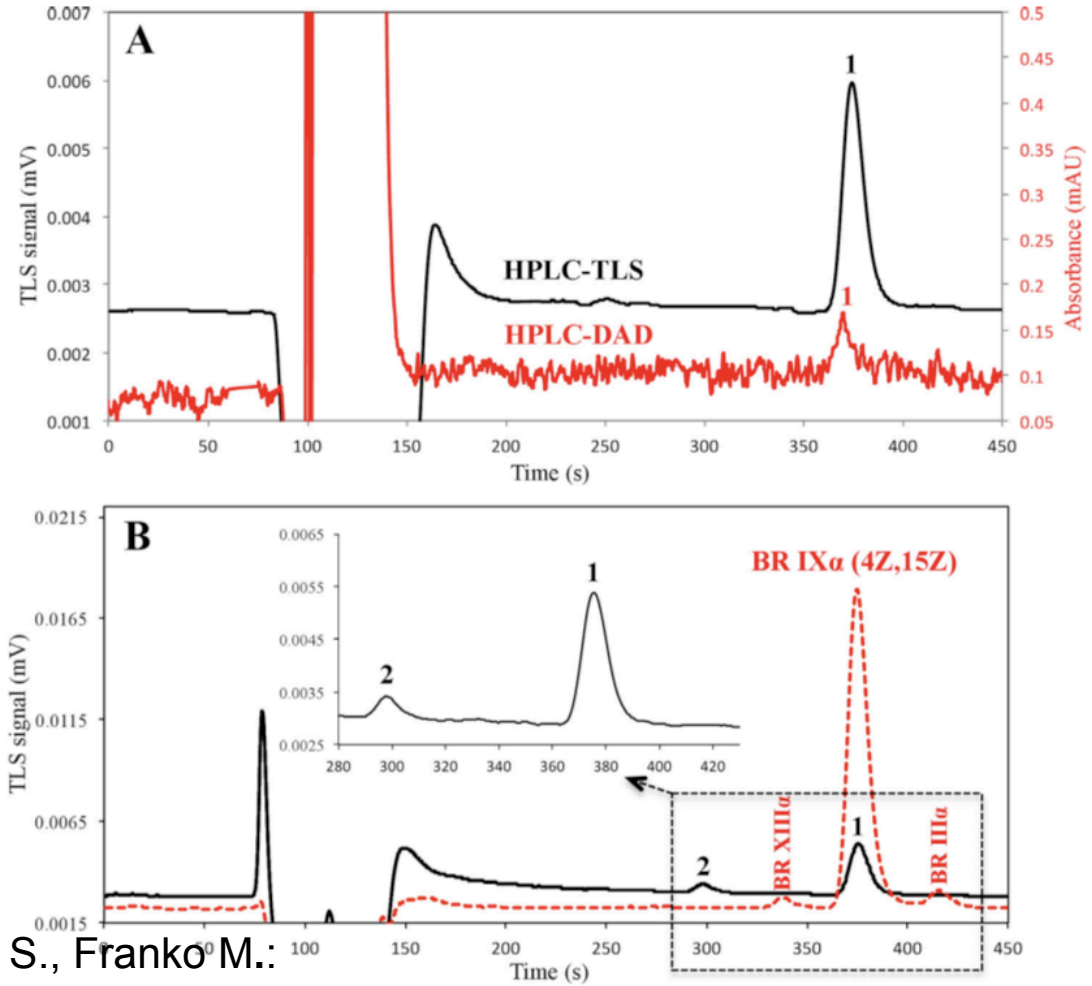
LOQ: 250 pM

Martelanc M., Žiberna L., Passamonti S., Franko M.:
Anal. Chim. Acta **809**, 2014, 174–182.

Free bilirubin in blood serum samples

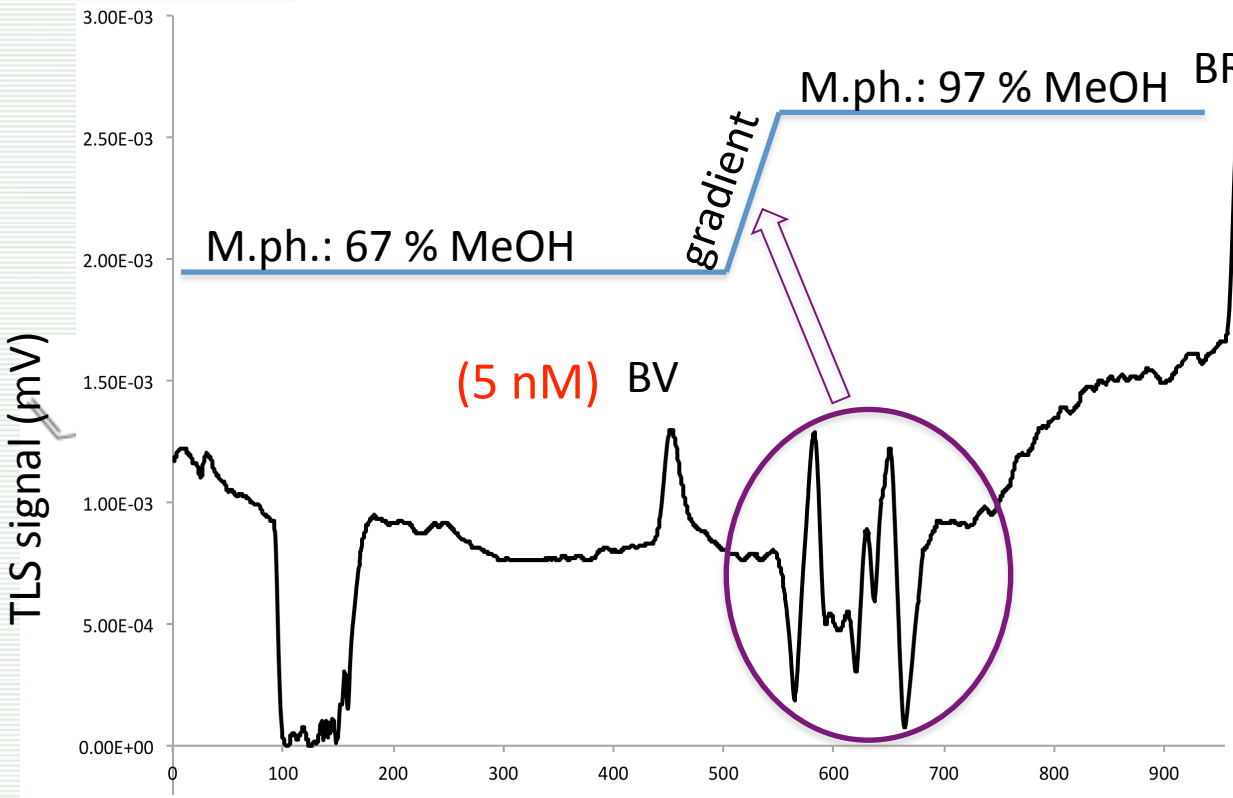
Serum samples	Male (aged 25)	Male (aged 30)	Male (aged 32)	FBSHI	FCSHI
Serum free bilirubin (nM)	13.1 ± 1.1	9.1 ± 0.6	8.5 ± 0.7	13.6 ± 1.2	17.3 ± 1.3

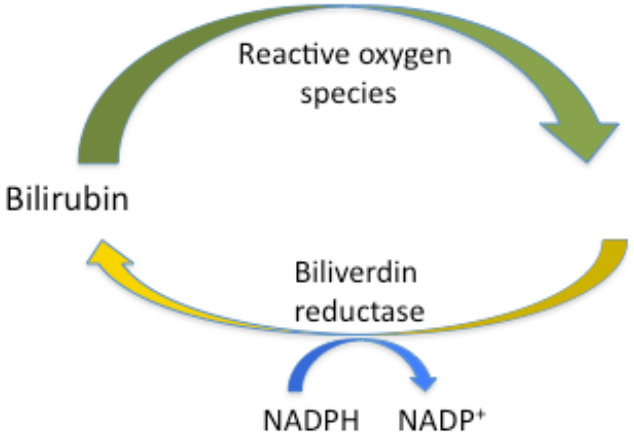
LOD: 90 pM
LOQ: 250 pM

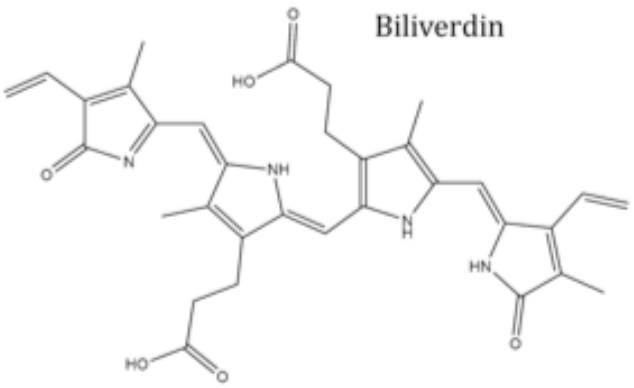


Martelanc M., Žiberna L., Passamonti S., Franko M.:
Anal. Chim. Acta **809**, 2014, 174–182.

Simultaneous determination of bilirubin and biliverdin







Contents lists available at ScienceDirect

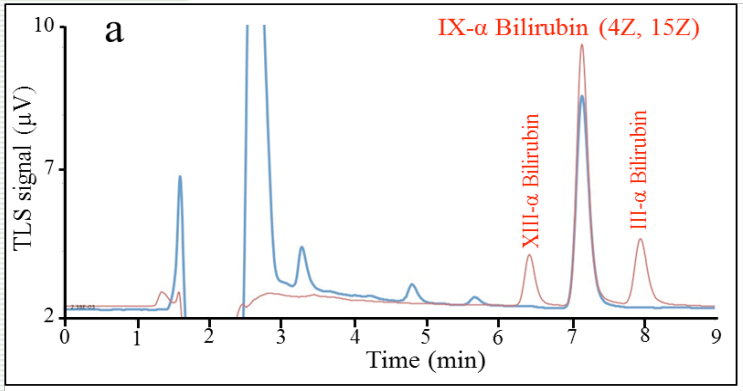
Talanta 154(2016)92–98

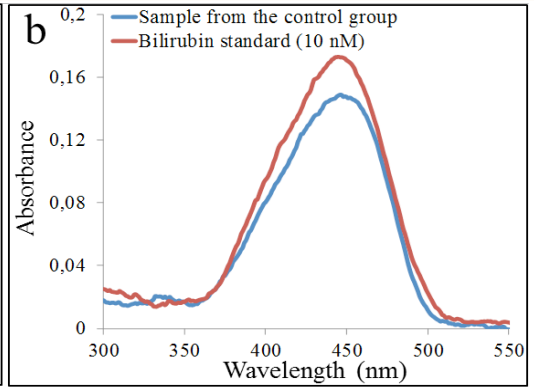
journal homepage: www.elsevier.com/locate/talanta

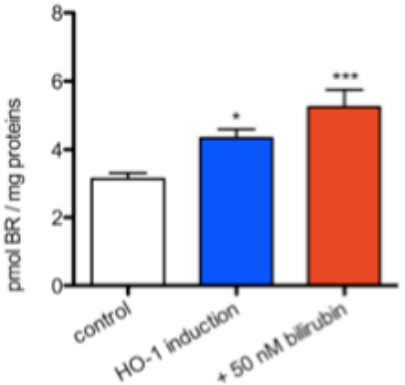
Application of high-performance liquid chromatography combined with ultra-sensitive thermal lens spectrometric detection for simultaneous biliverdin and bilirubin assessment at trace levels in human serum

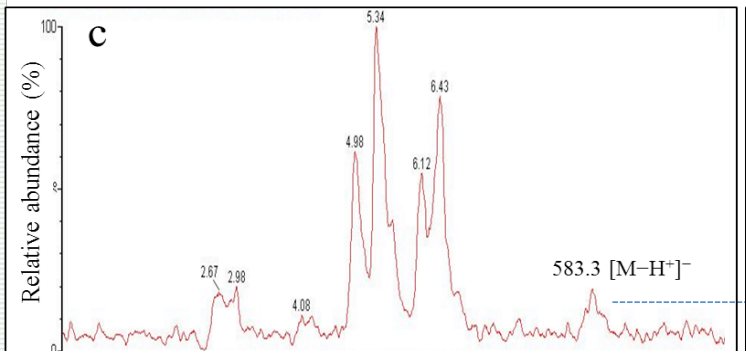
Mitja Martelanc [a], Lovro Žiberna [b], Sabina Passamonti [b], Mladen Franko [a,*]

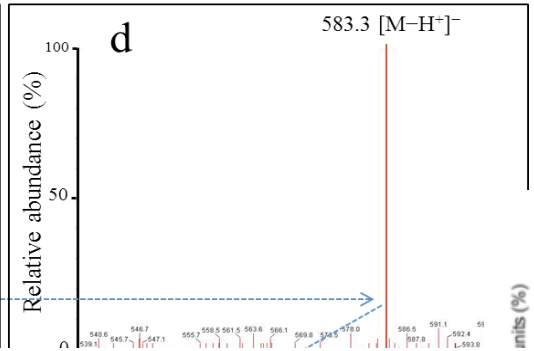
First detection and modulation of bilirubin in vascular endothelial cels

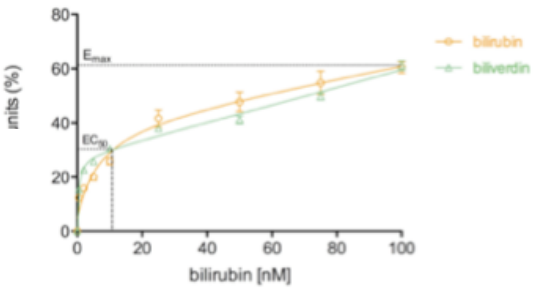












SCIENTIFIC REPORTS

OPEN

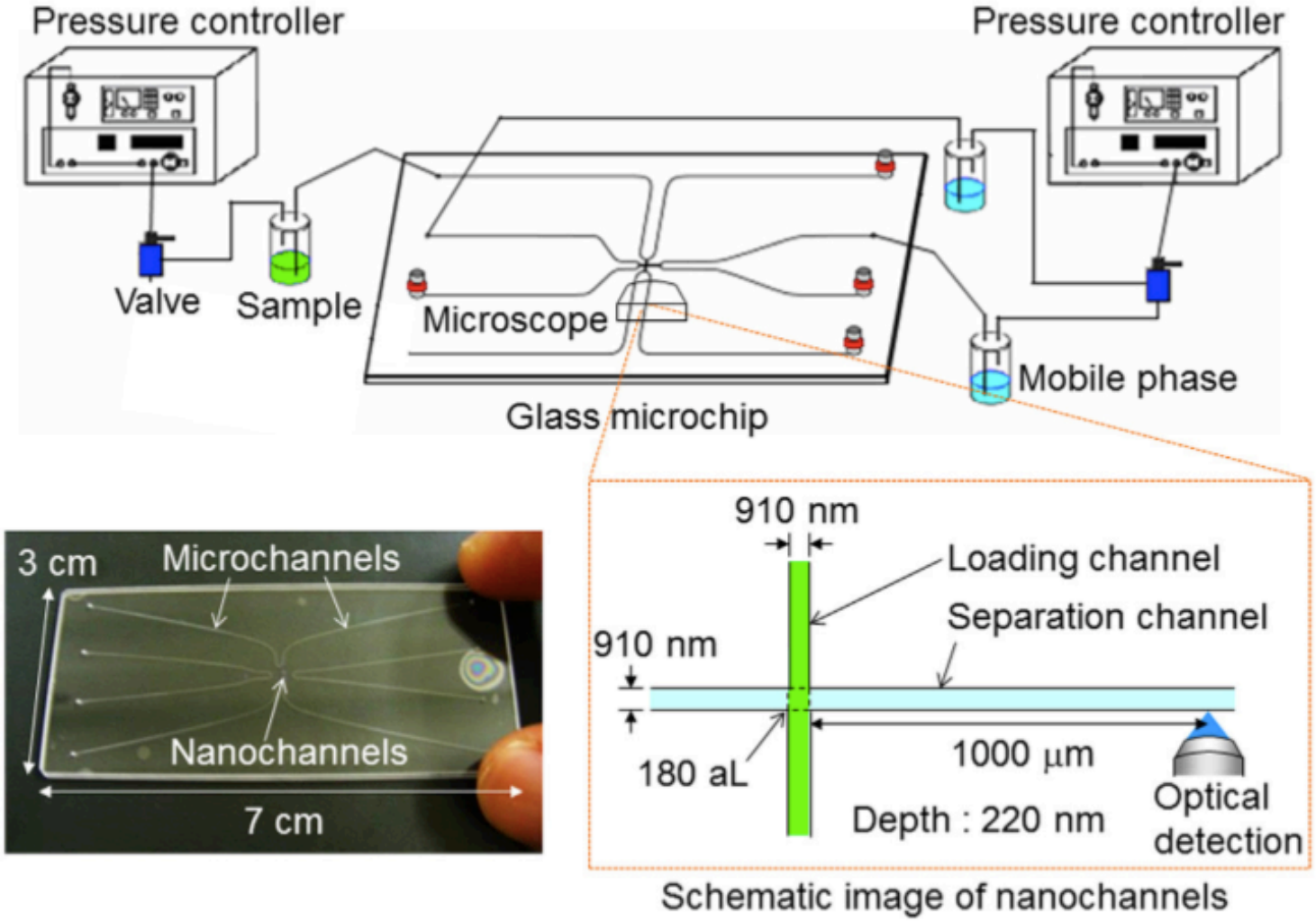
Bilirubin is an Endogenous Antioxidant in Human Vascular Endothelial Cells

Received: 29 December 2015
Accepted: 14 June 2016

Lovro Ziberna[1,2], Mitja Martelanc[3], Mladen Franko[3] & Sabina Passamonti[1]

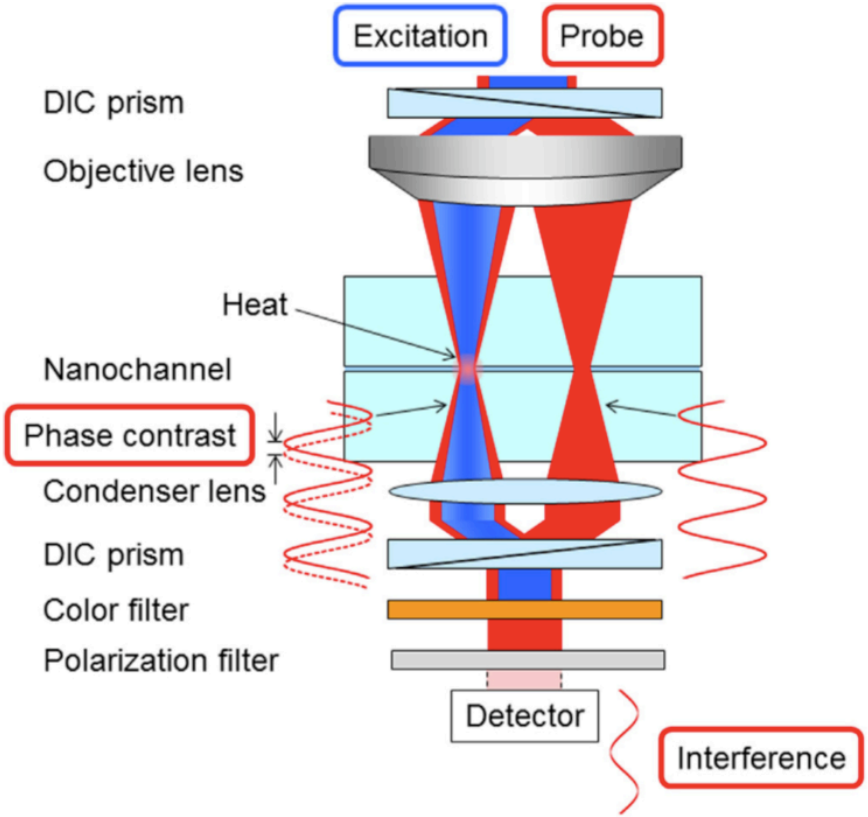
Scientific Reports 6:29240
DOI: 10.1038/srep29240

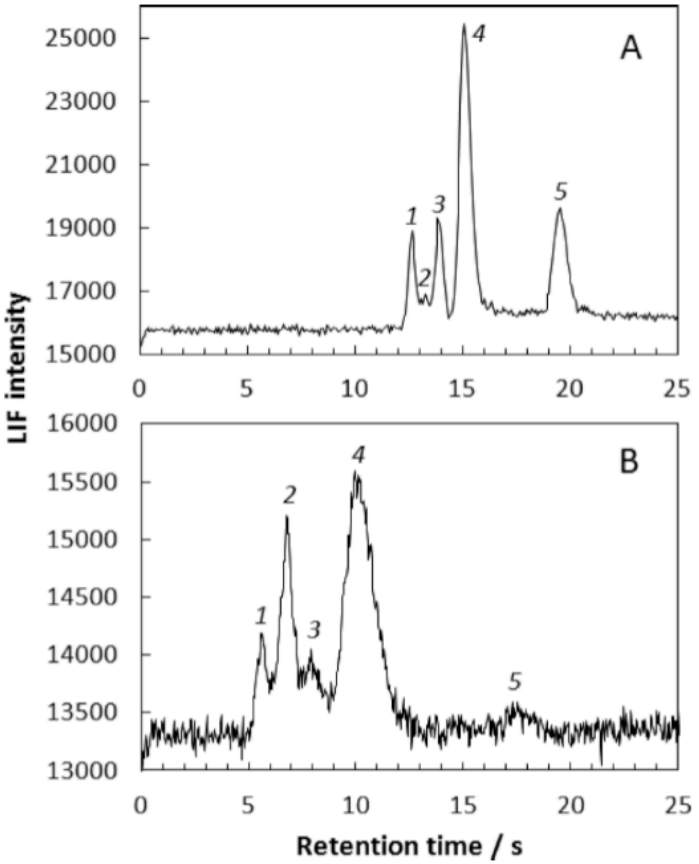
HPLC in extended nano-space



From: H. Shimizu et al. Journal of Chromatography A, xxx (2016) xxx–xxx

Differential interference contrast thermal lens microscope



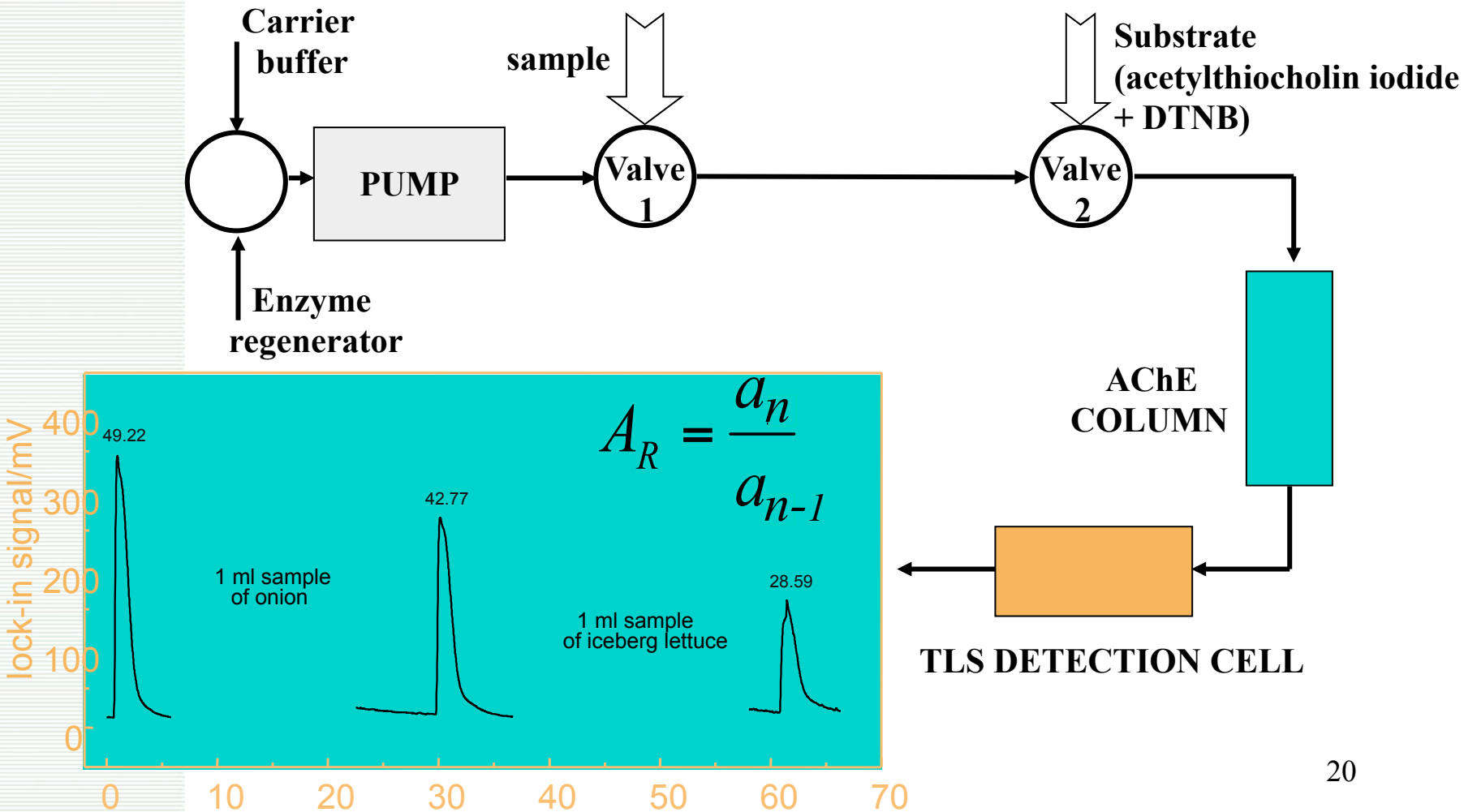


LOD: 370 molecules in a 350-nm-deep microchannel few μM i.e. 10-100 ppb

Separation of NBD-F derivatized amino acids: 1- serine, 2 - NBD-OH, 3 – alanine, 4 – proline, 5 – valine

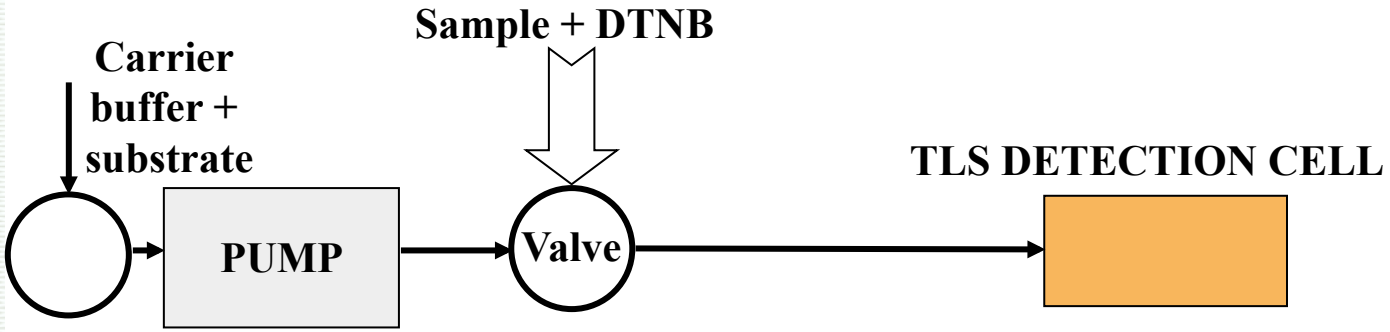
From: A. Smirnova et al. Anal. Sci. 31(2015)1201

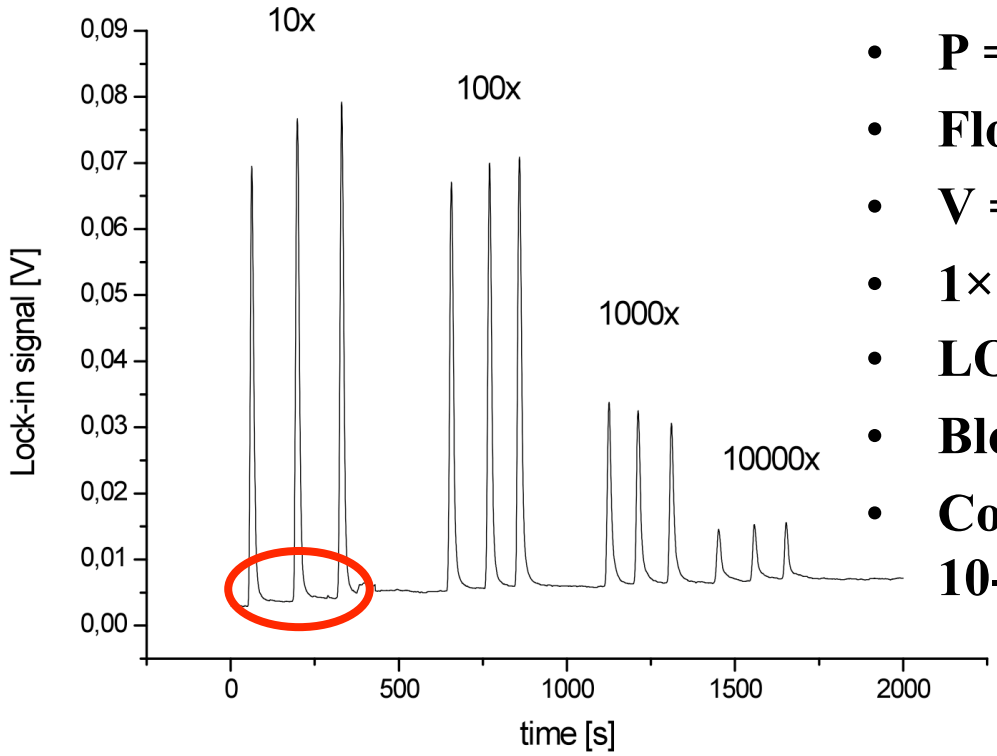
Bioanalytical FIA system



L. POGAČNIK, M. FRANKO, Biosens. Bioelectron.. **2003**, 18, 1-9

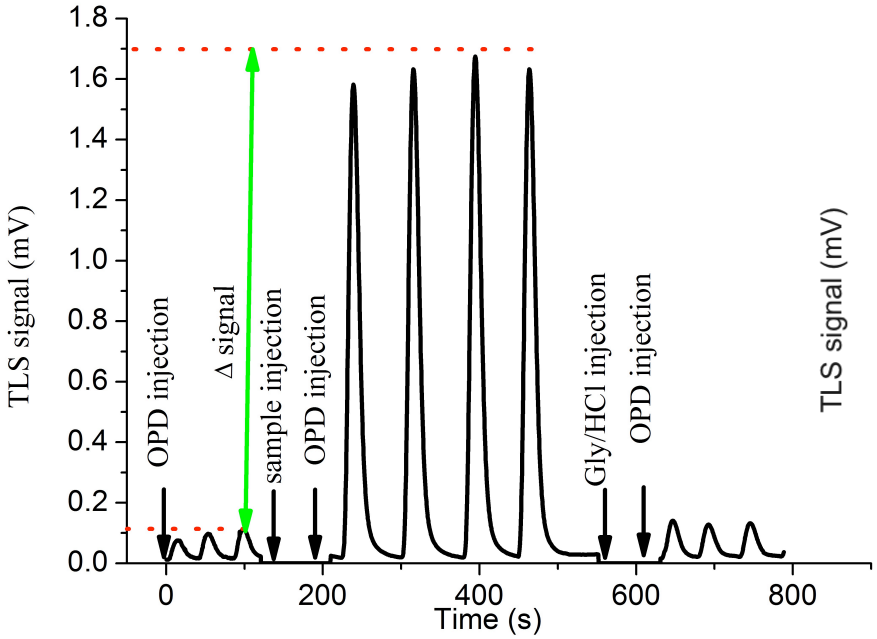
FIA-TLS for determination of AChE activity in human blood

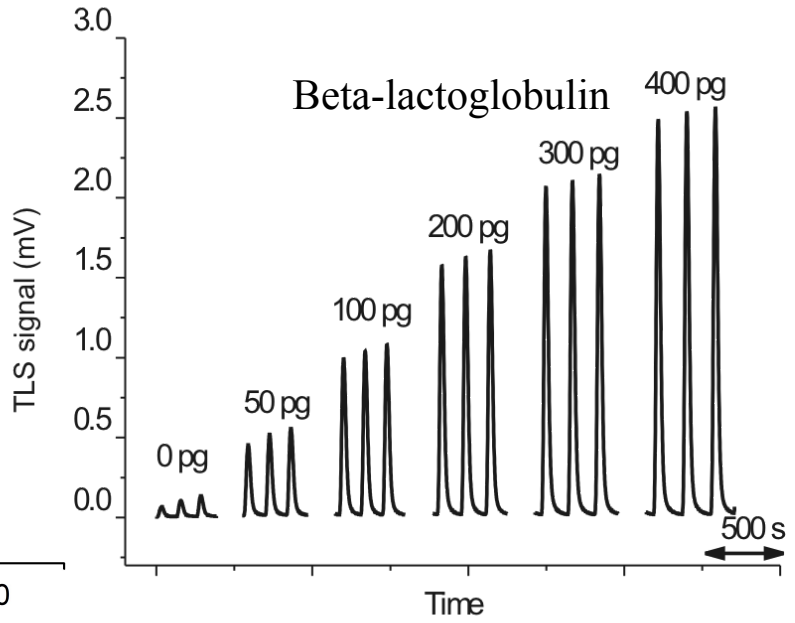




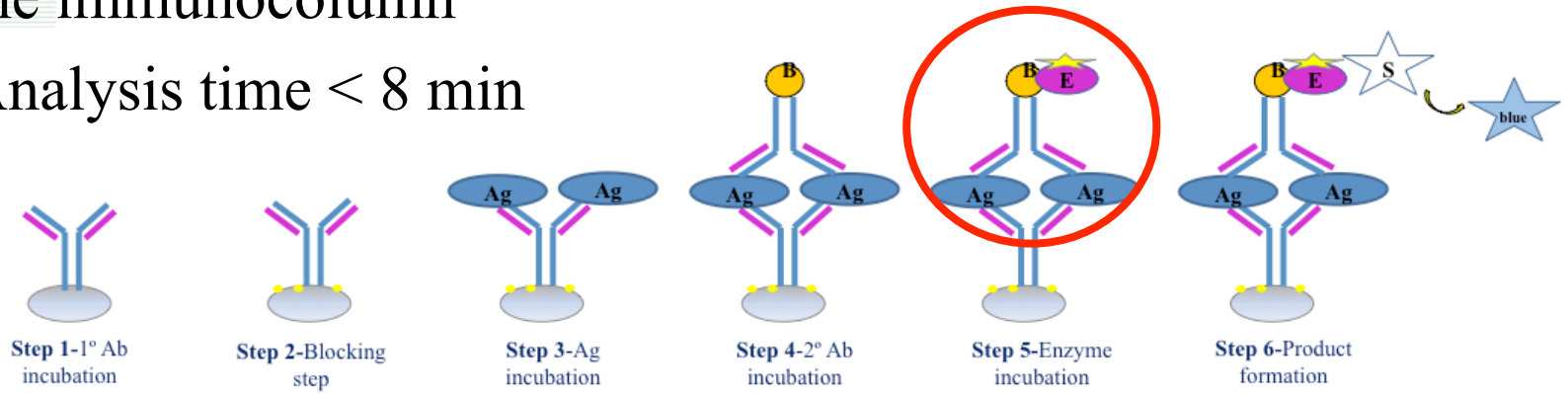
- P = 30 mW
- Flow rate = 1 mL/min
- V = 20 µL
- 1× = 146 U/mL
- LOD = 9.4 mU/mL
- Blood levels: 2-8 U/mL
- Commercial assay time 10-30 min (ABCAM)

FIA-ELISA-TLS detection of food allergens

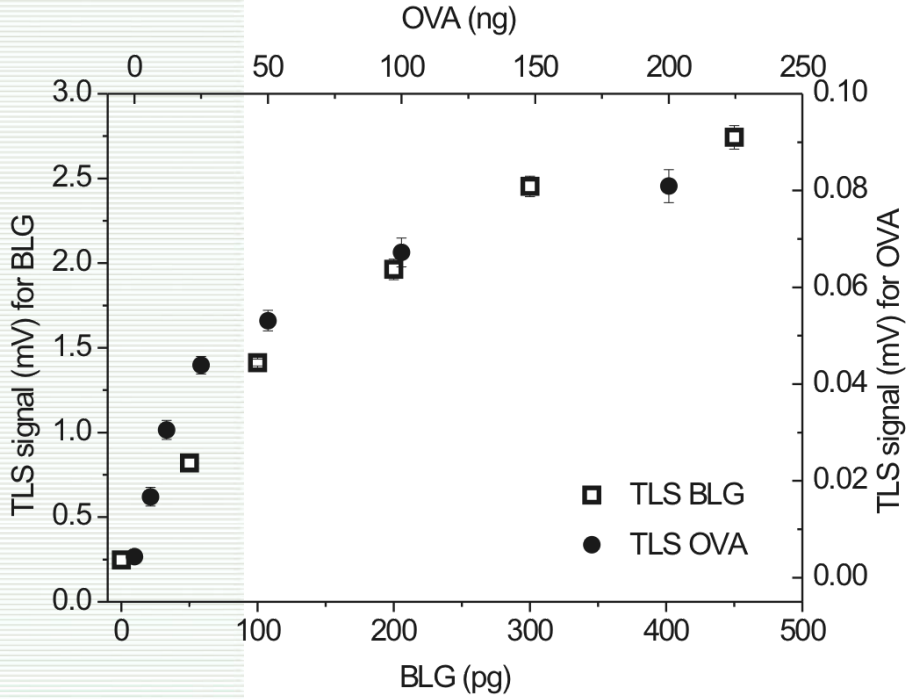


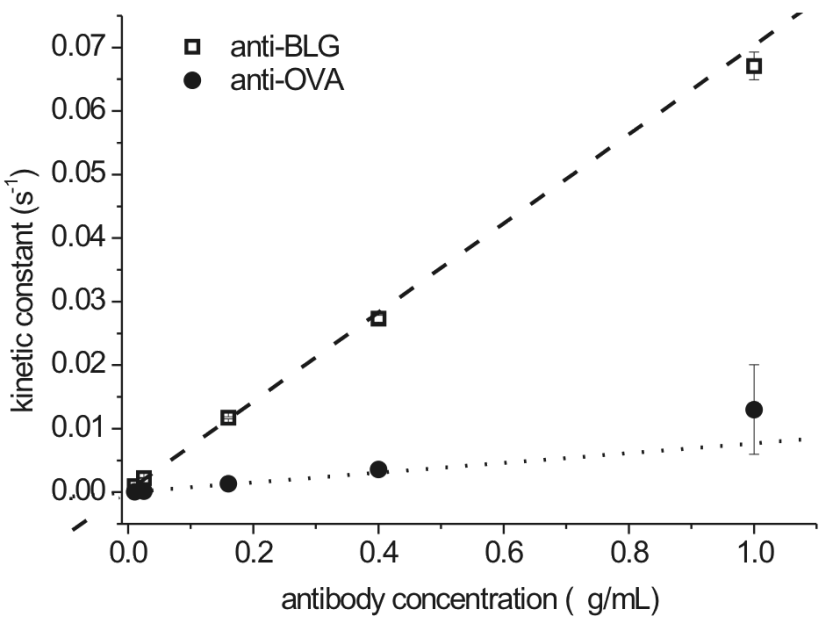


- TLS signal proportional to the amount of allergen retained on the immunocolumn
- Analysis time < 8 min



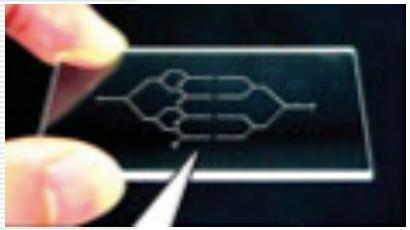
Determination of BLG and OVA by FIA-ELISA-TLS





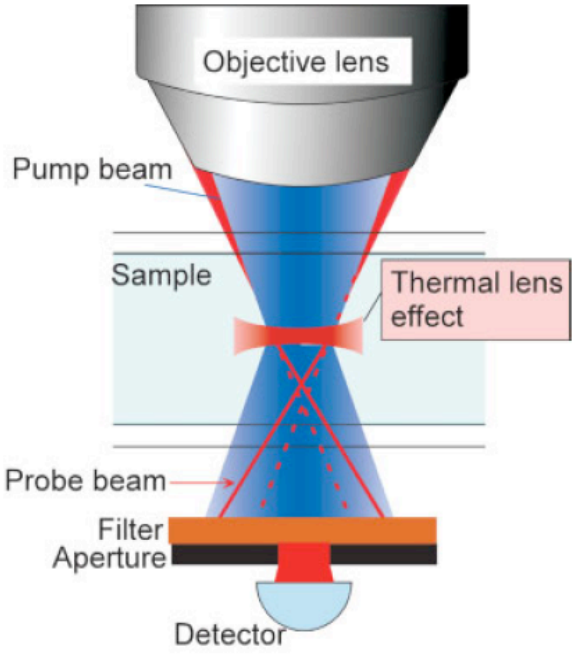
LOD for beta-lactoglobulin (BLG) =2.3 pg/100 µL (190 pg by ELISA – Bethyl)
LOD for ovalbumin (OVA) =1 ng/100 µL (1 µg by ELISA – Abcam)

TLM detection in microfluidic systems



Microchip

TLM



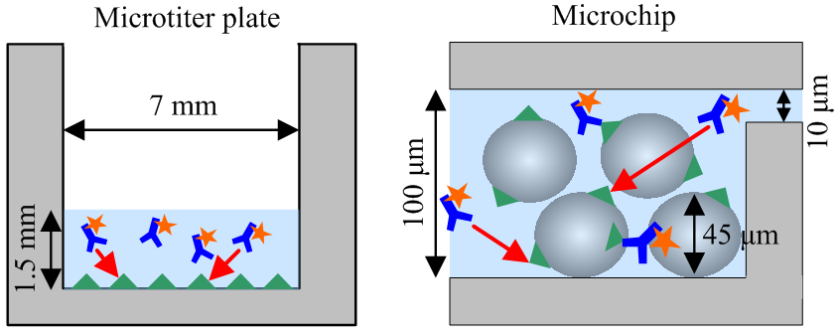
Microchannel-based immunoassay

assay time of s-IgA
24 h → 20 min

channel size

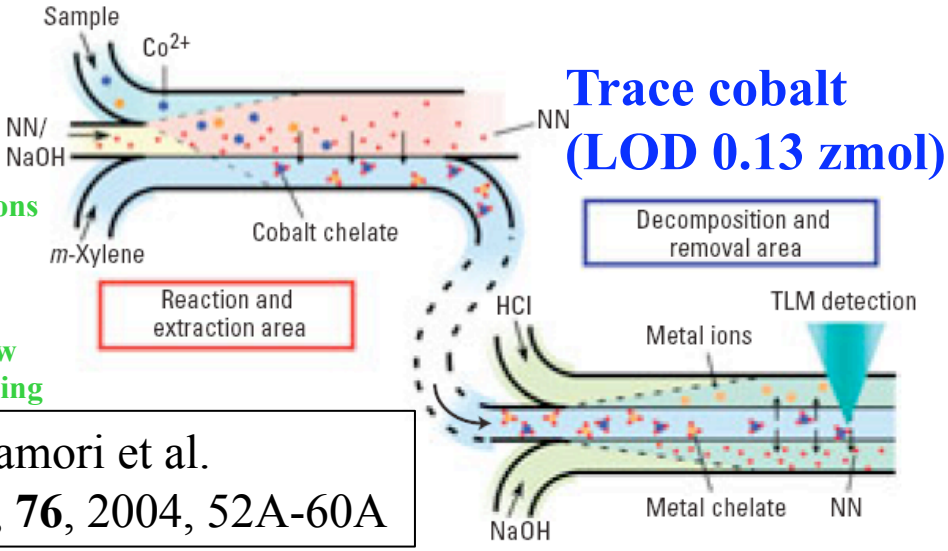
$$t_m = L_m^2 / D_m$$

$$\sigma_m = S_m / V_m \propto 1 / L_m$$



MUOs
Microunit operations

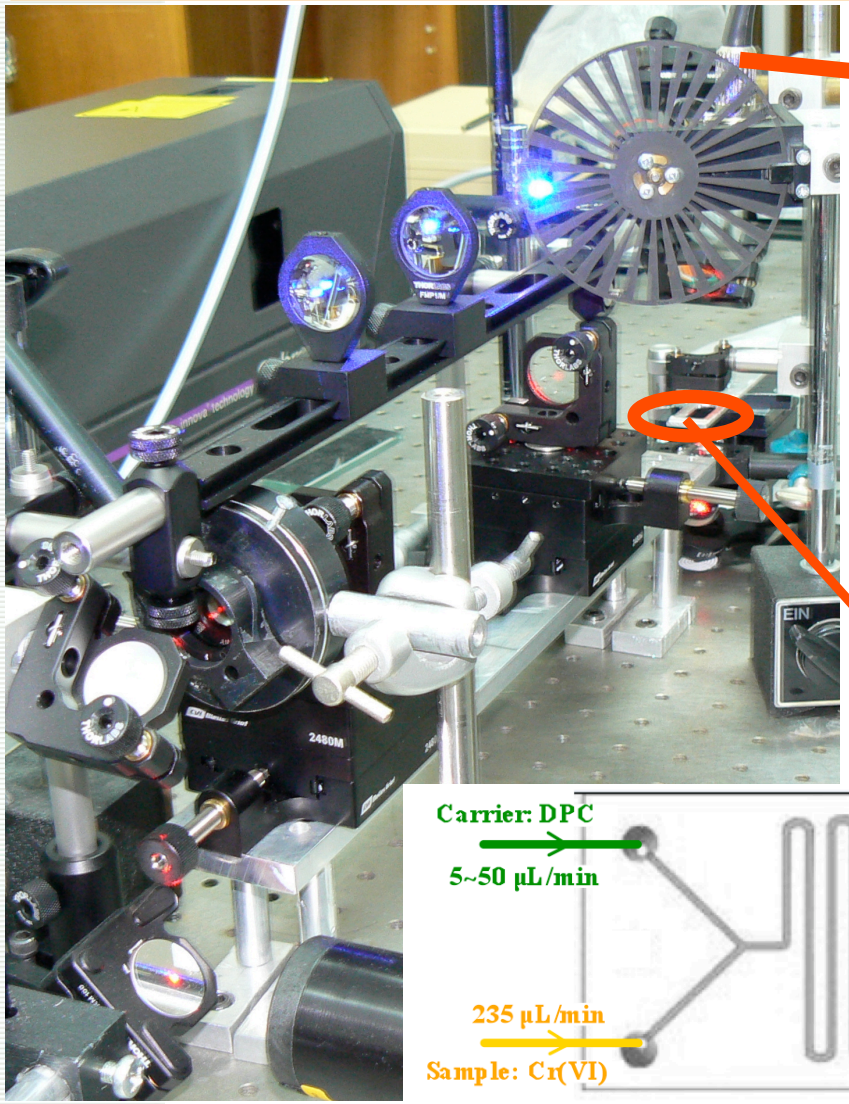
CFCP
Continuous flow chemical processing

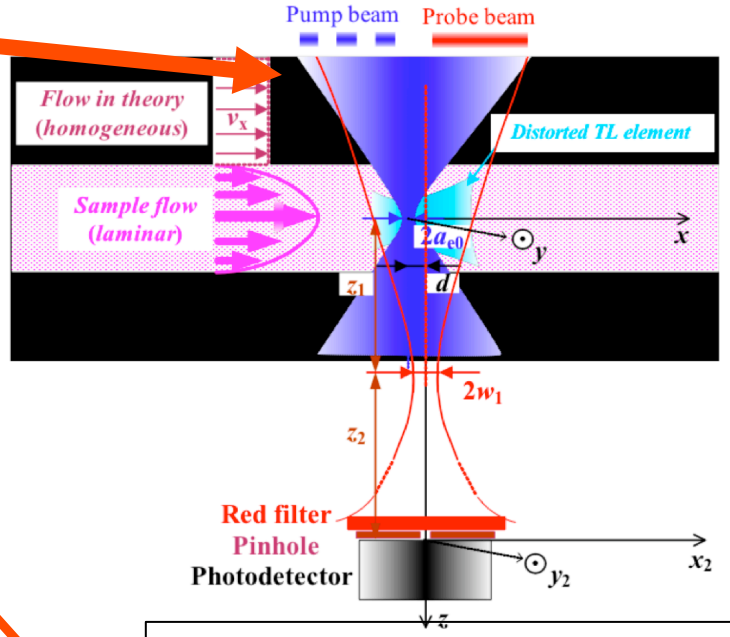


Trace cobalt (LOD 0.13 zmol)

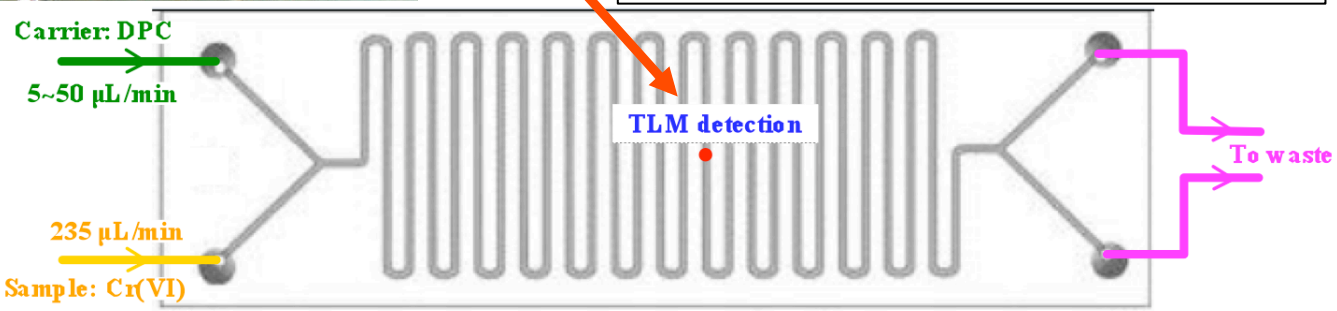
From: T. Kitamori et al.
Anal. Chem., **76**, 2004, 52A-60A

Microfluidic-FIA and TLM

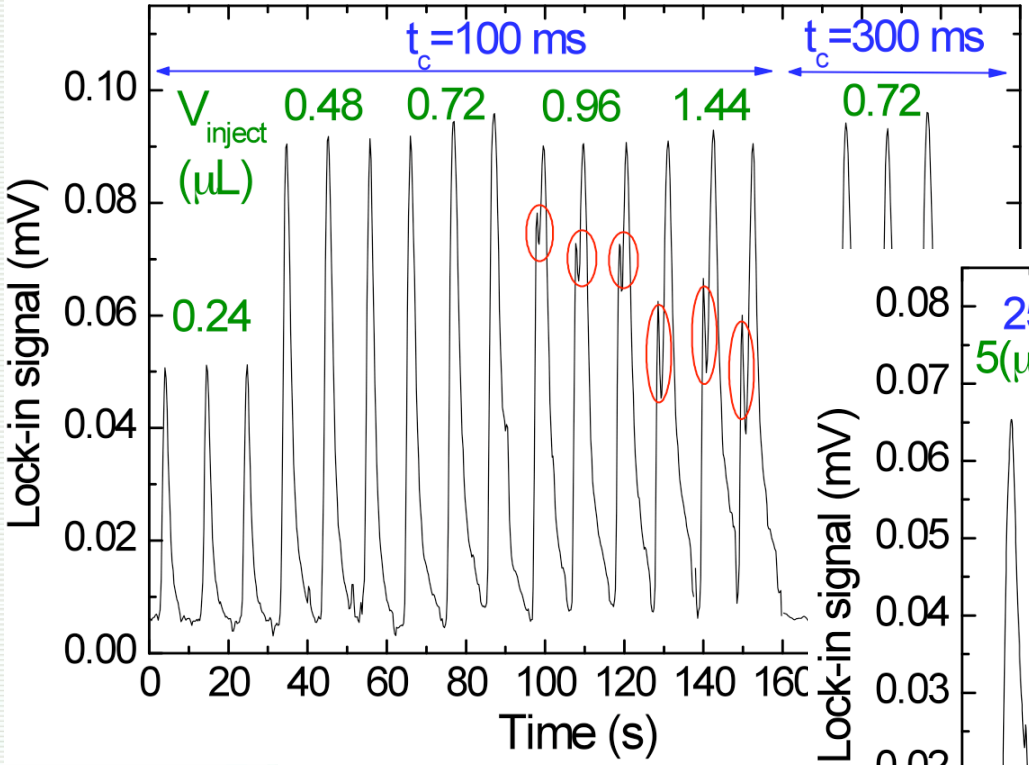


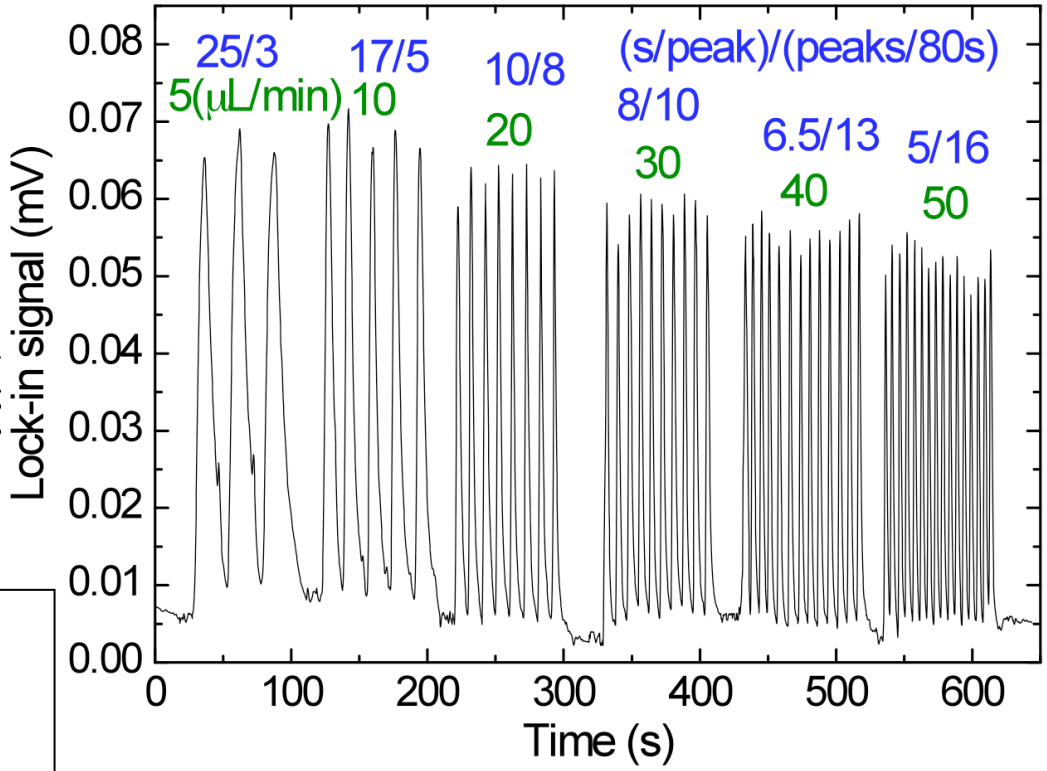


Liu M., Franko M. *Crit. Rev. Anal. Chem.* **44**, 2014, 328-353.



Optimization of carrier flow and sample volume for µFIA-TLM detection of Cr(VI)

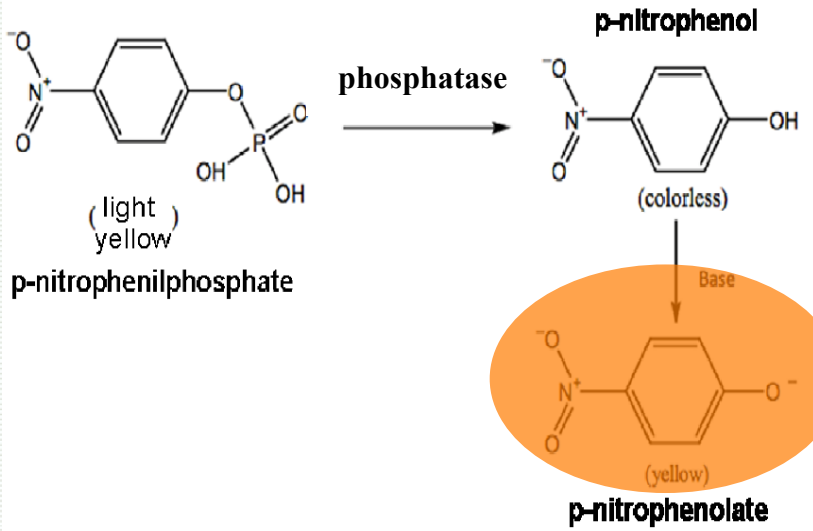




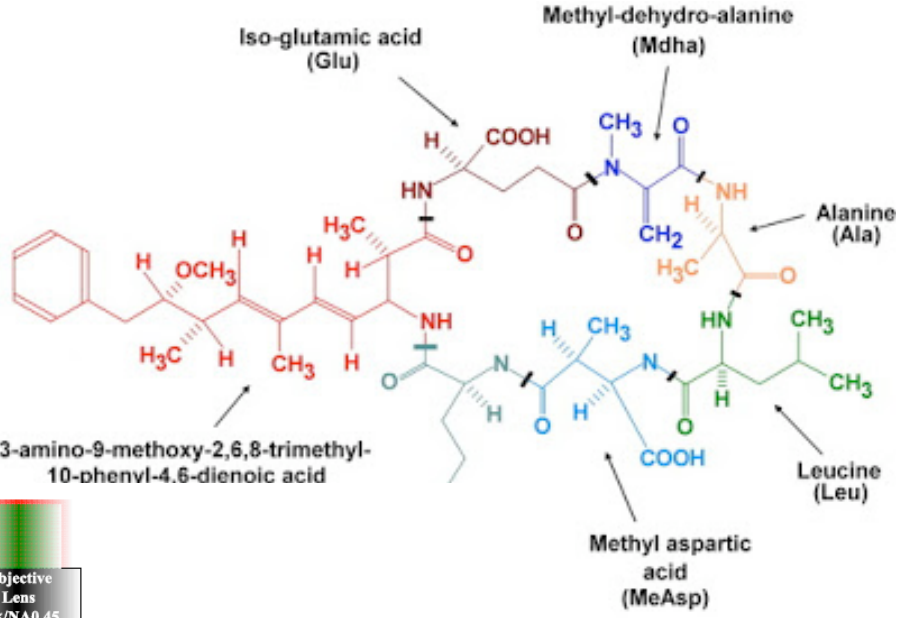
At P=60 mW, and V_{inject}=0.72 µL:
LOD = 3.5 ng/mL, corresponding to 9×10^{-6} AU for Cr(VI)-DPC

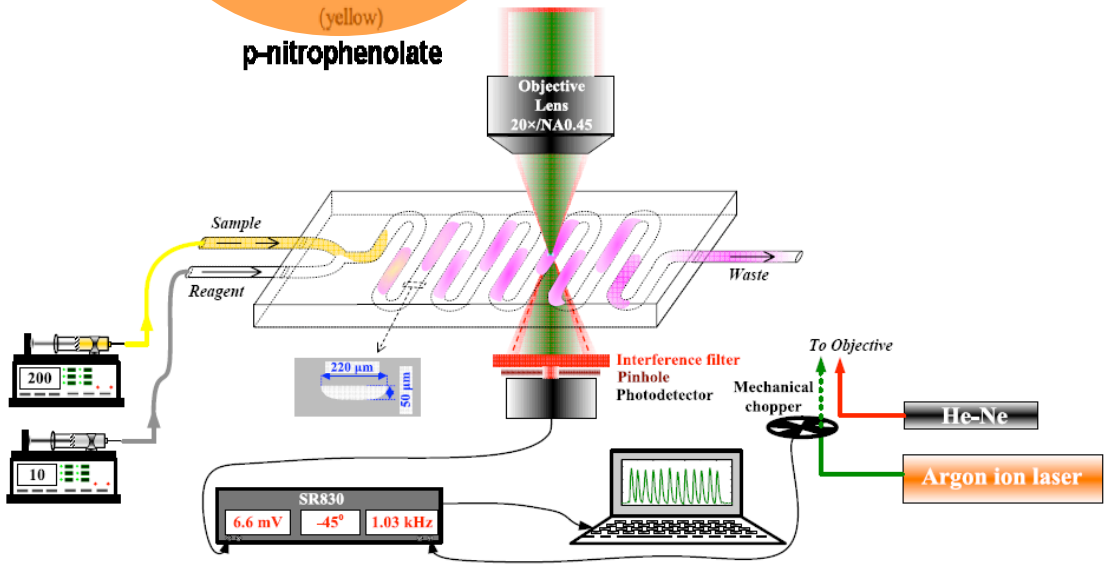
Determination of microcystin by PP2A inhibition assay

Colorimetric reaction catalyzed by PP

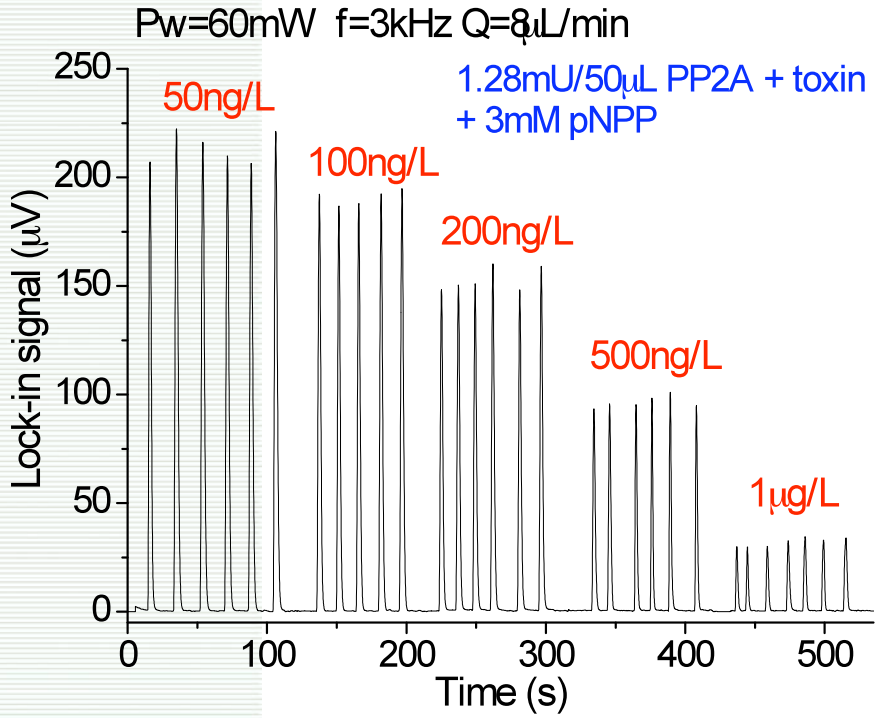


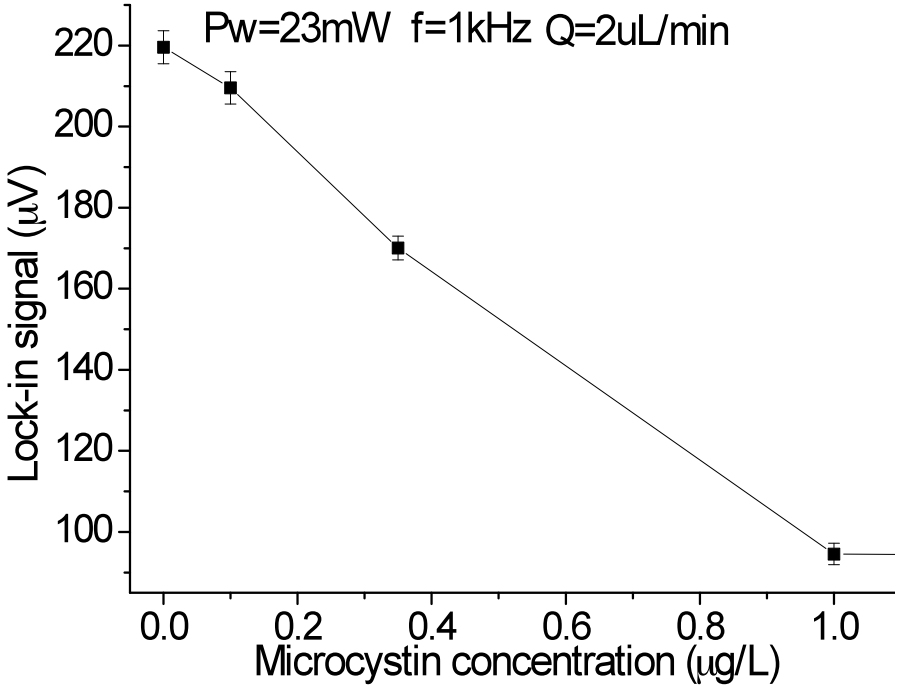
Reaction inhibited by cyanotoxin





TLM-PP2A inhibition assay





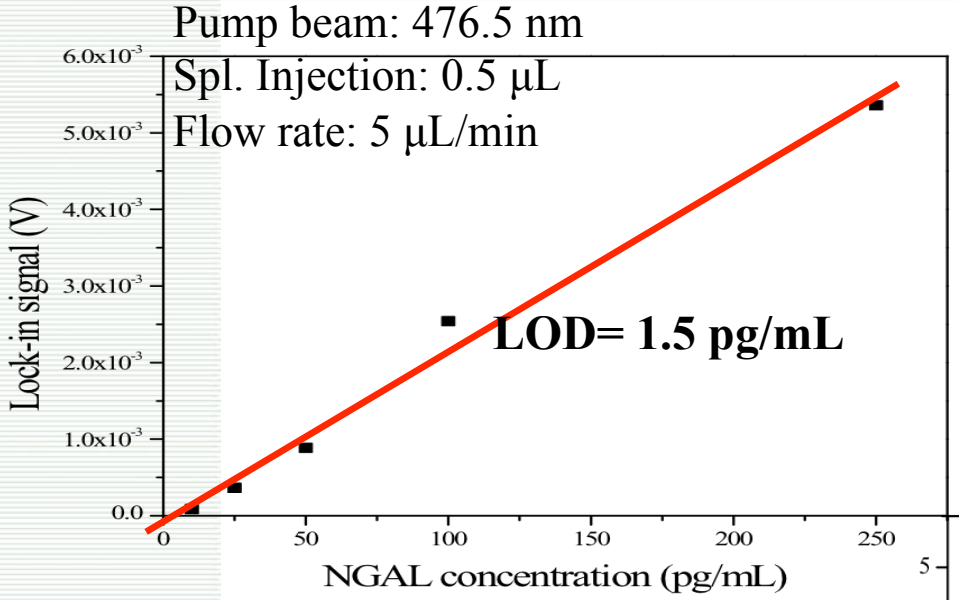
Enzyme consumption: 0.5 µL per injection

Detection limit: ~ 80ng/L

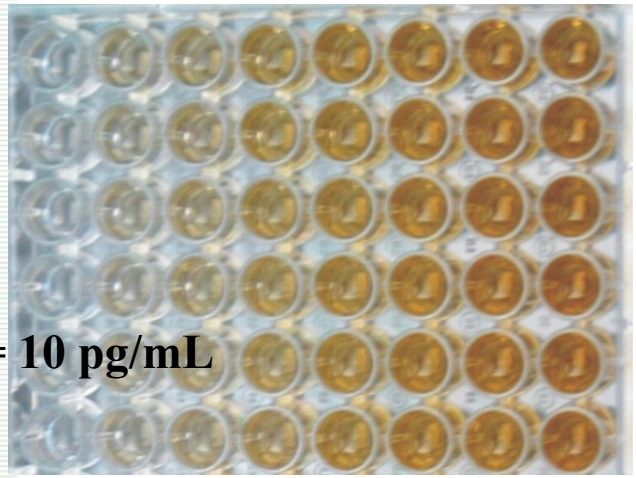
-12 times lower below the WHO limit for drinking water

-8 times faster than batch mode assay

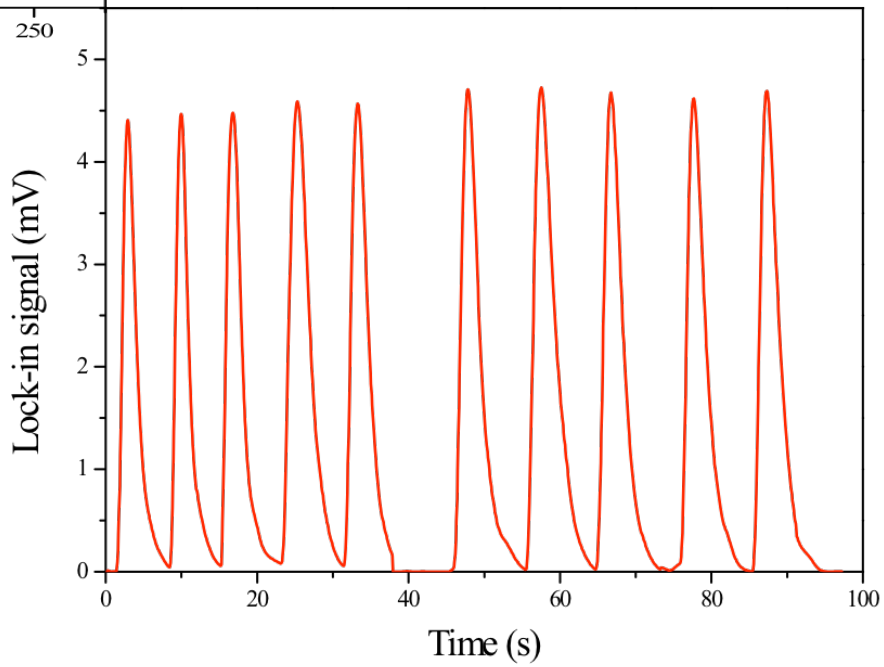
Determination of NGAL - a biomarker of acute kidney injury



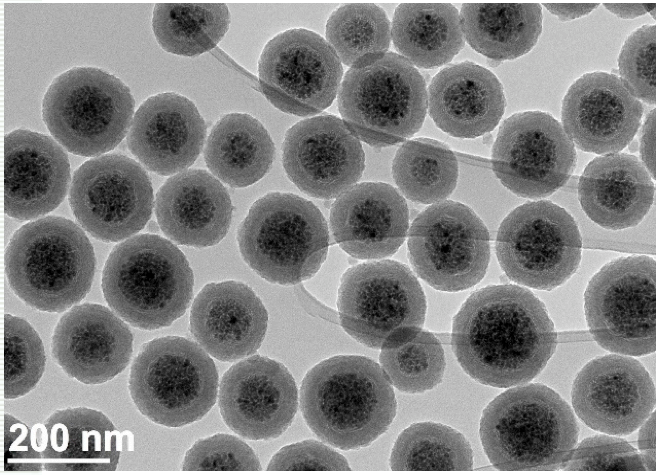
TLS signals for replicate injections of two aliquots of 500-times diluted blood plasma sample (217 pg/mL)

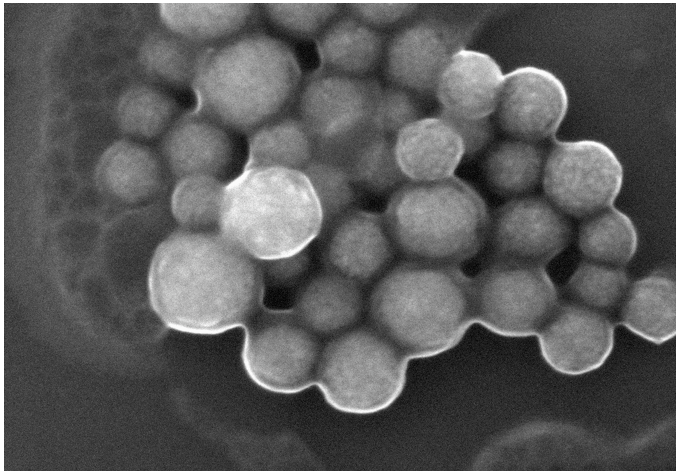


LOD= 10 pg/mL

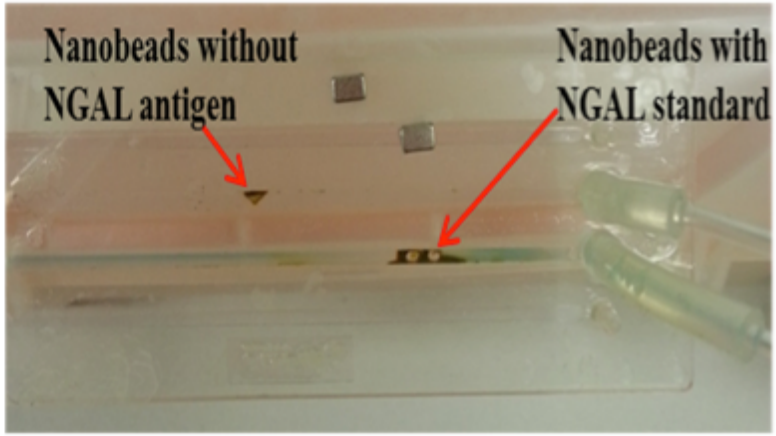


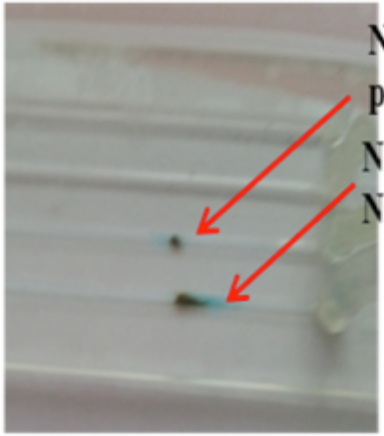
Immobilization of NGAL antibodies on magnetic nanobeads



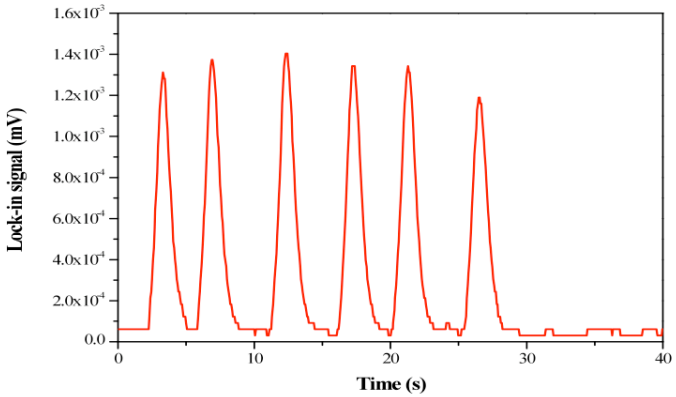


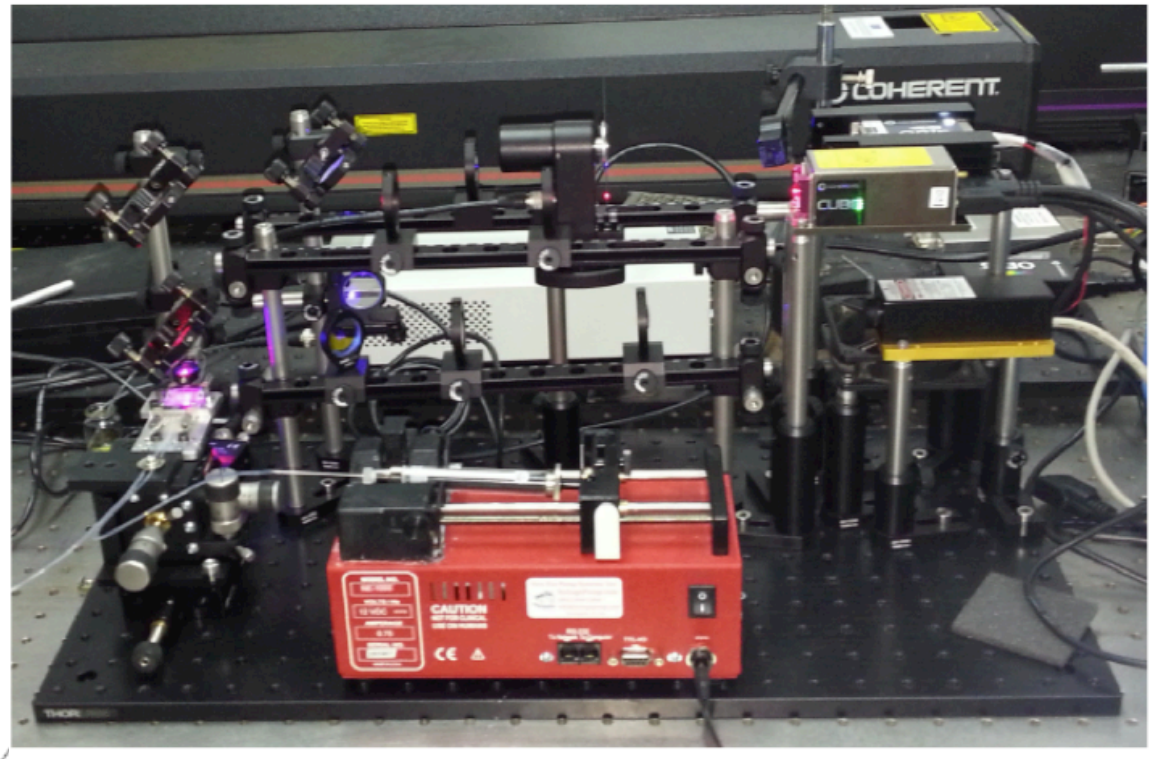
iNANOvative™|BIO amine nano beads by: NANOS SCI.

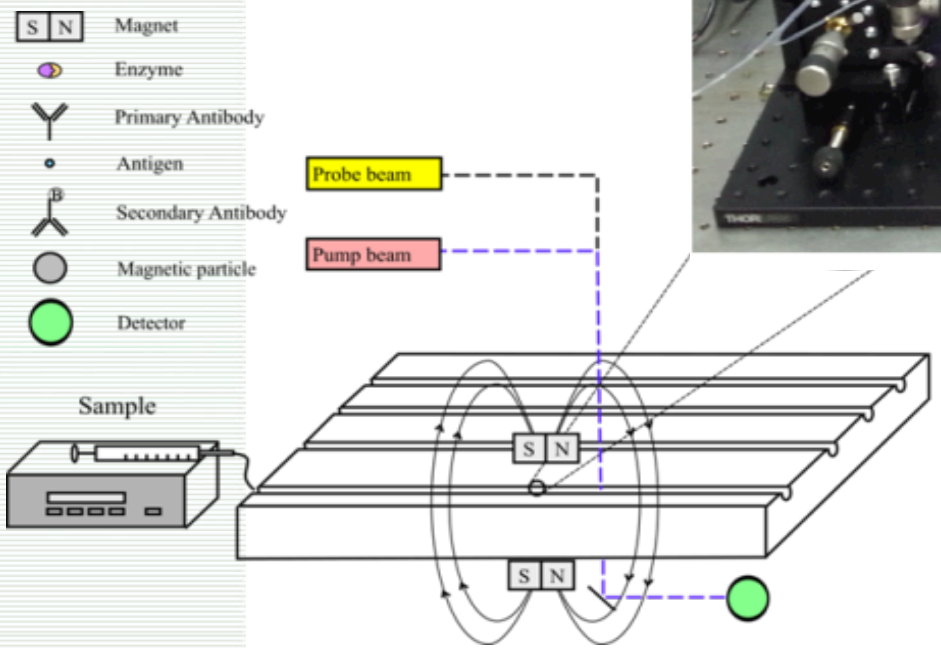




Activity of HRP on secondary NGAL antibodies retained by magnetic NPs

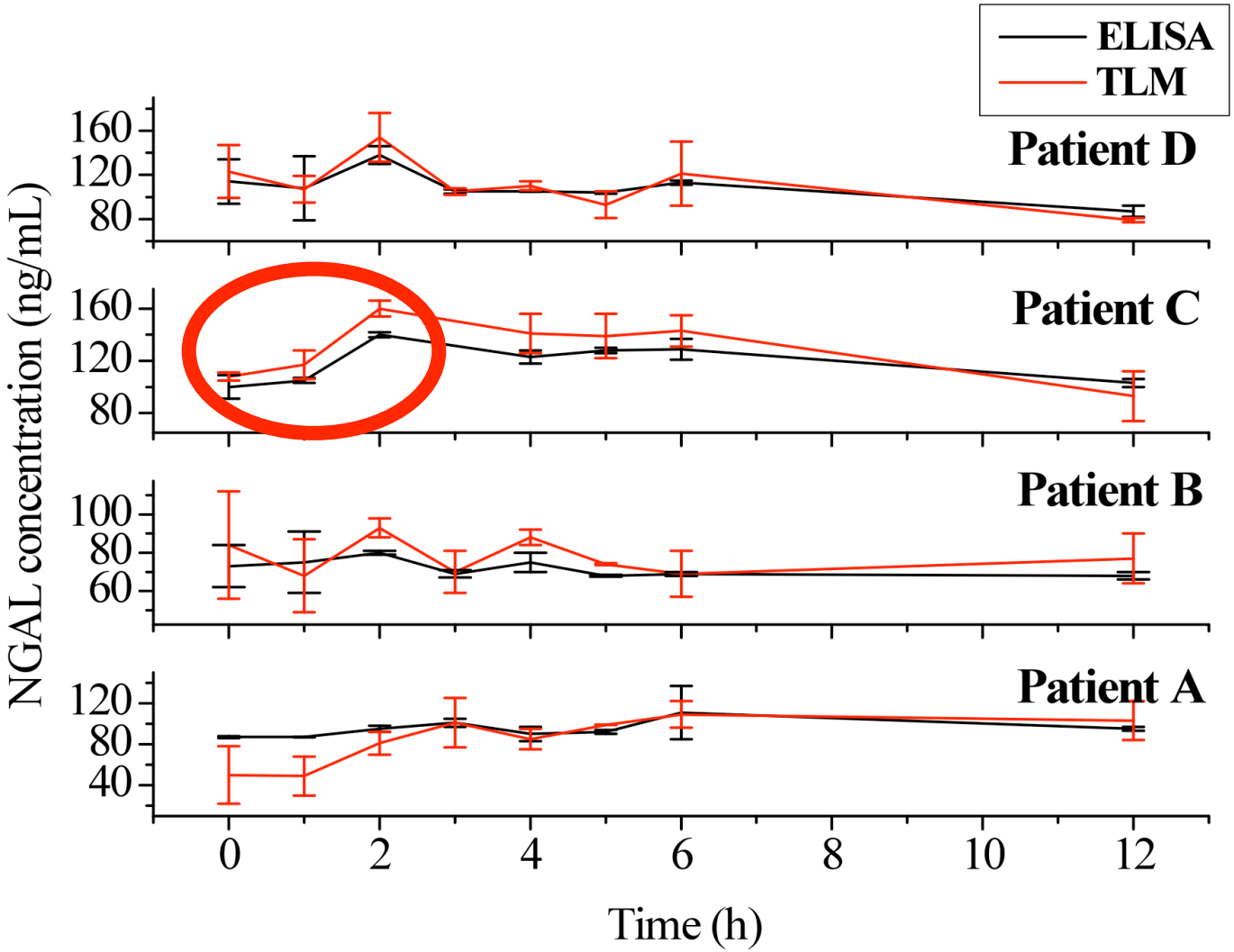






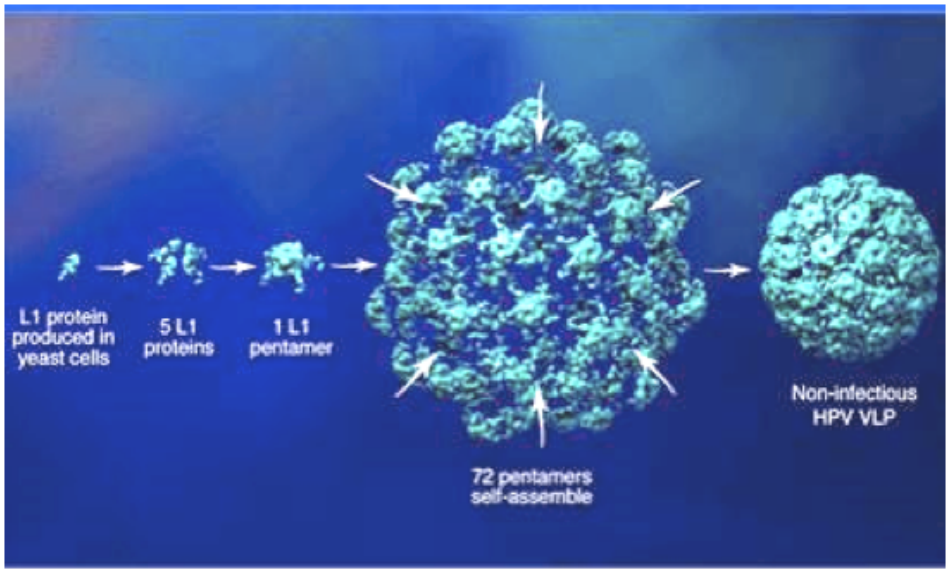
LOD = 5 pg/mL
Total analysis time 35 min
(for ELISA 4h)

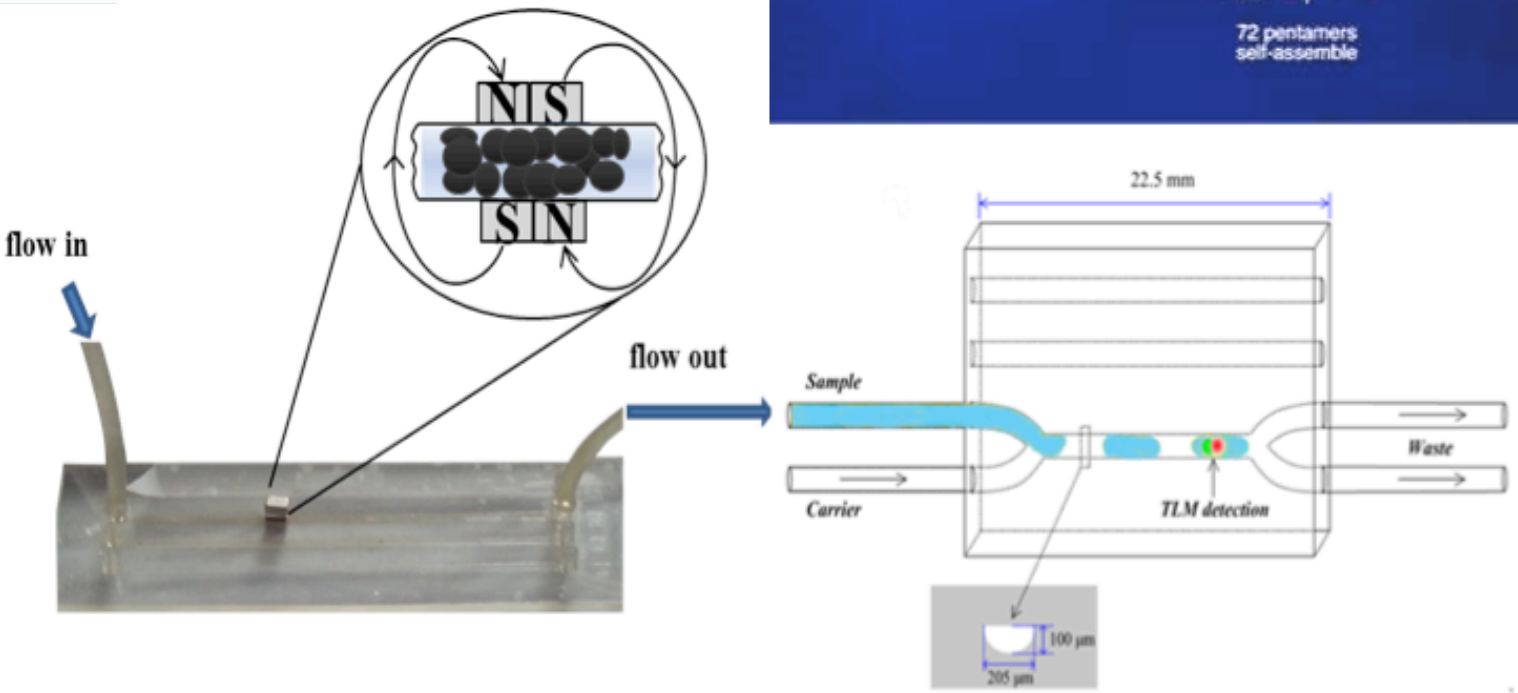
Detection of NGAL in blood plasma of patients after percutaneous coronary angiography (injection of contrast agents) by



Detection and utilization of virus-like proteins and pseudovirions

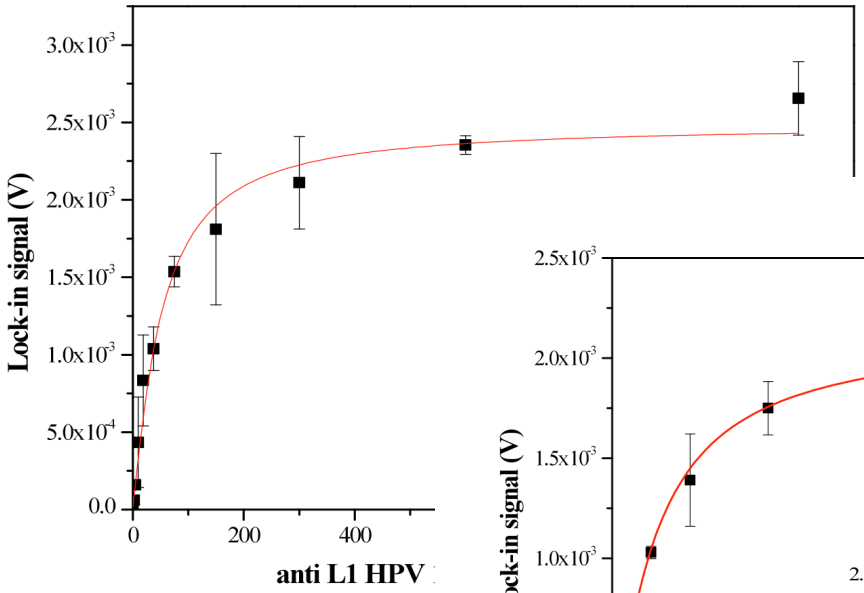
HPV is a cause of cervical cancer.
By using HPV antibodies one can detect HPV.
By using HPV-VLPs one can detect HPV antibodies and past or current infection



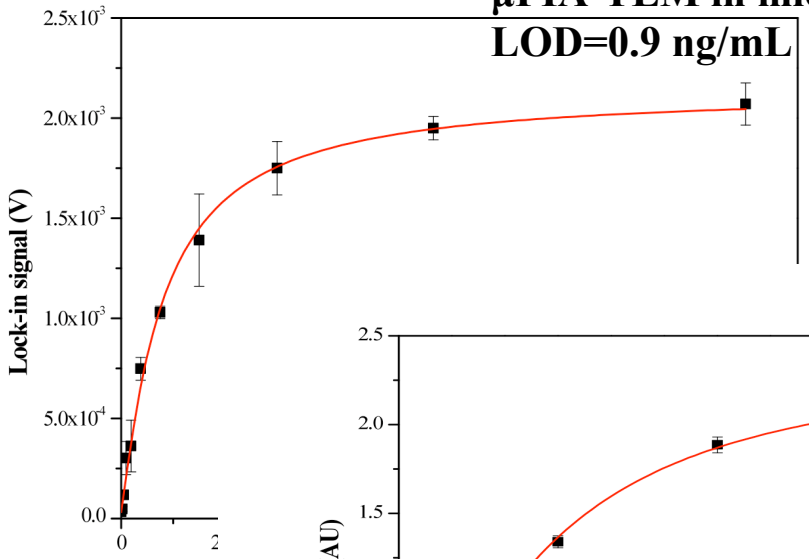


Calibration curves for HPV-16 antibodies

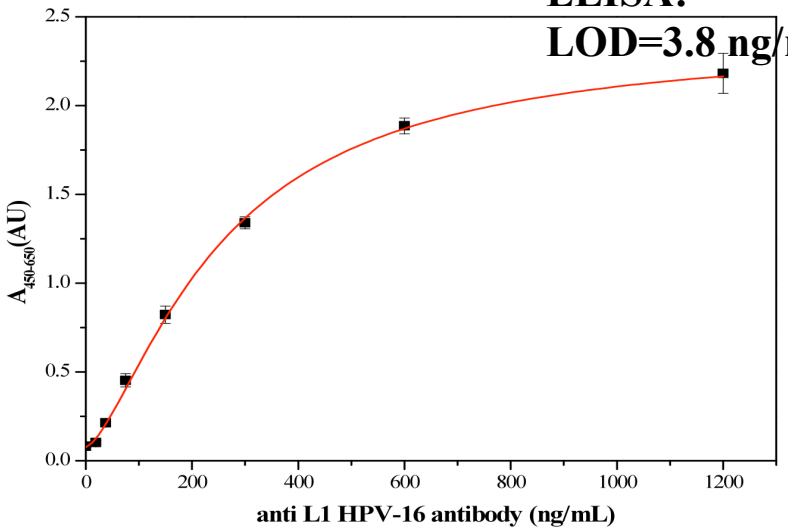
µFIA-TLM on nanobeads: LOD=0.65 ng/mL



µFIA-TLM in microtiter plate: LOD=0.9 ng/mL



ELISA: LOD=3.8 ng/mL



Concentrations of L1 HPV 16 antibodies in HPV diagnosed persons

Sample	*ELISA (ng/mL)*	*Microtiter µFIA-TLM (ng/mL)*	*Nanobeads µFIA-TLM (ng/mL)*
1	6.9 ± 0.1	7 ± 1	6.8 ± 0.9
2	8.2 ± 0.8	7.3 ± 0.2	8 ± 1
3	5.9 ± 0.5	5.2 ± 0.8	5.4 ± 0.2
4	below LOD	3.0 ± 0.4	3.8 ± 0.6
5	3.9 ± 0.3	4 ± 2	4 ± 1
6	8.4 ± 0.7	8.2 ± 0.6	8 ± 2
7	10 ± 2	9.5 ± 0.5	9 ± 2
8	6 ± 1	7.7 ± 0.4	/
9	10.7 ± 0.7	11.2 ± 0.7	/
10	9 ± 1	10.2 ± 0.8	/
11	9 ± 1	9.7 ± 0.2	/

Conclusions

- μFIA-TLM enables high throughput (over 10 samples/minute) analysis of sub μL samples with LODs in sub ppb concentration range
- Spatial and temporal resolution of μFIA-TLM enabled studies of diffusion processes in microfluidic systems, which are dominated by convective diffusion
- TLM is at the brink of becoming a routine portable “point of care” technique for medical diagnostics