TL-Spectrometry and Applications in Biomedical Research and Diagnostics

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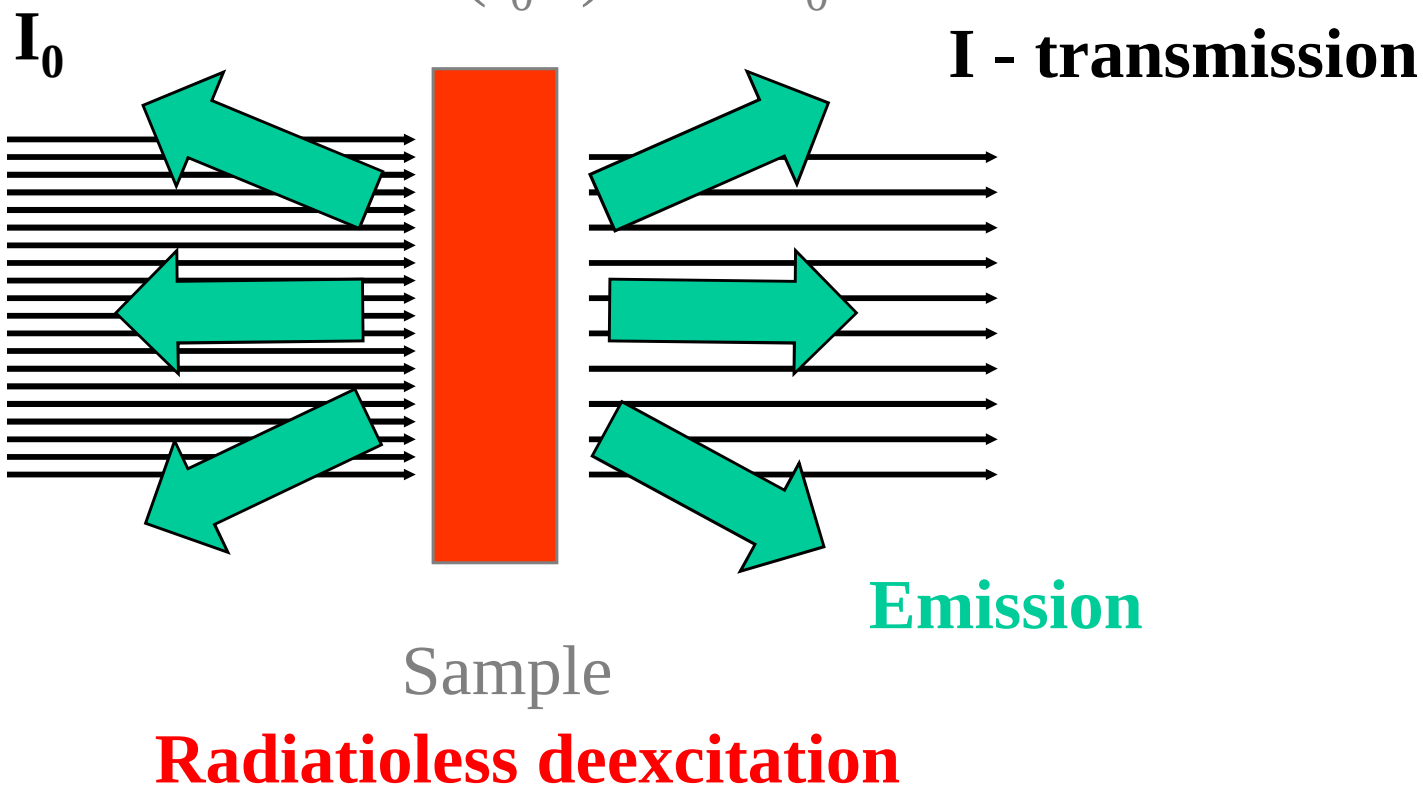
INTERNATIONAL SCHOOL OF QUANTUM ELECTRONICS -57th Course
ERICE - SICILY: October 19th -26th, 2016

Transmission techniques - how to improve sensitivity?

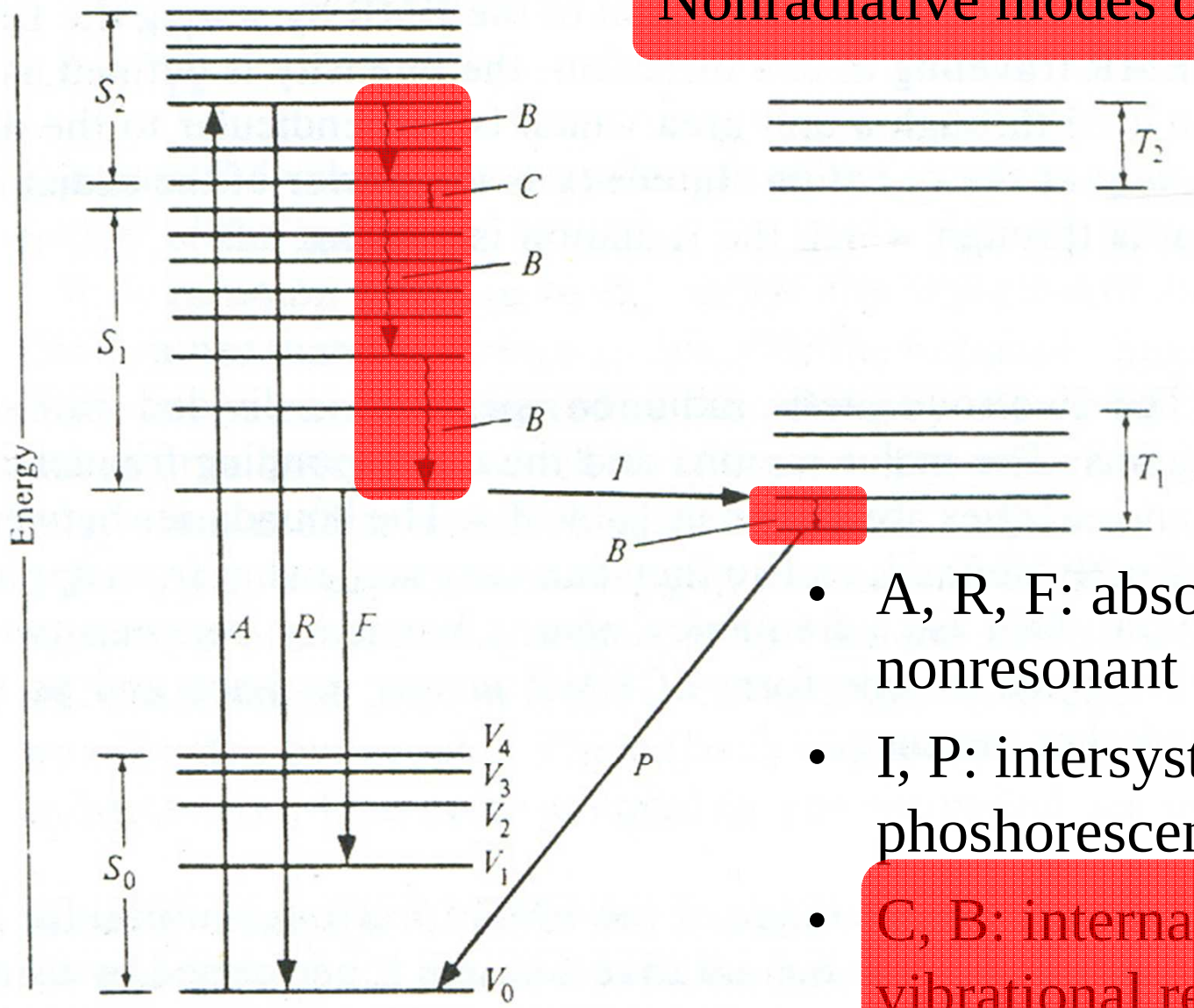
$A=-\log T=-\log(I/I_0)$

$A=\varepsilon LC \cong (I_0-I)/2.303I_0$



Molecular energy diagram and related excitation/deexcitation processes

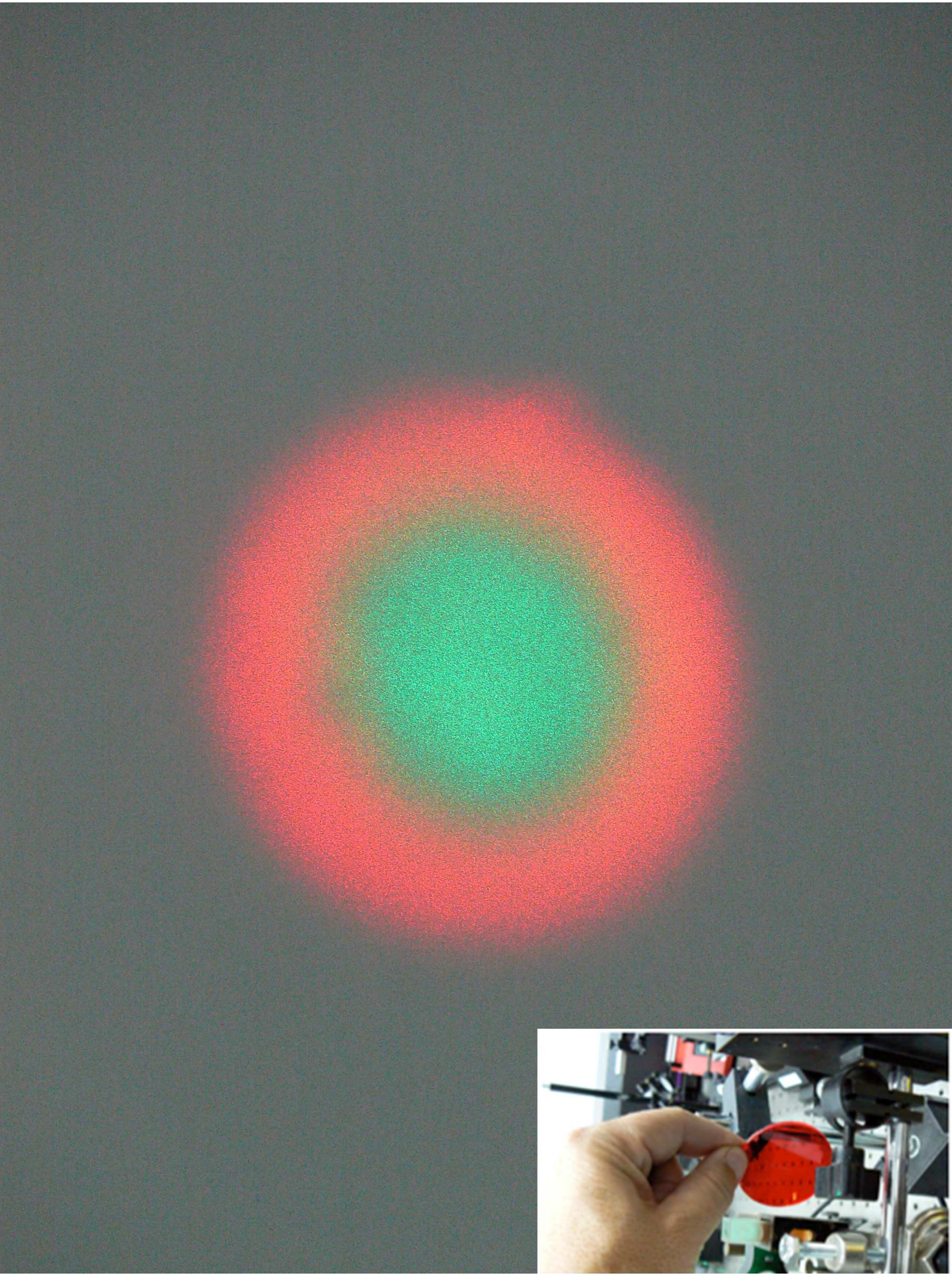
Nonradiative modes of relaxation (C, B)

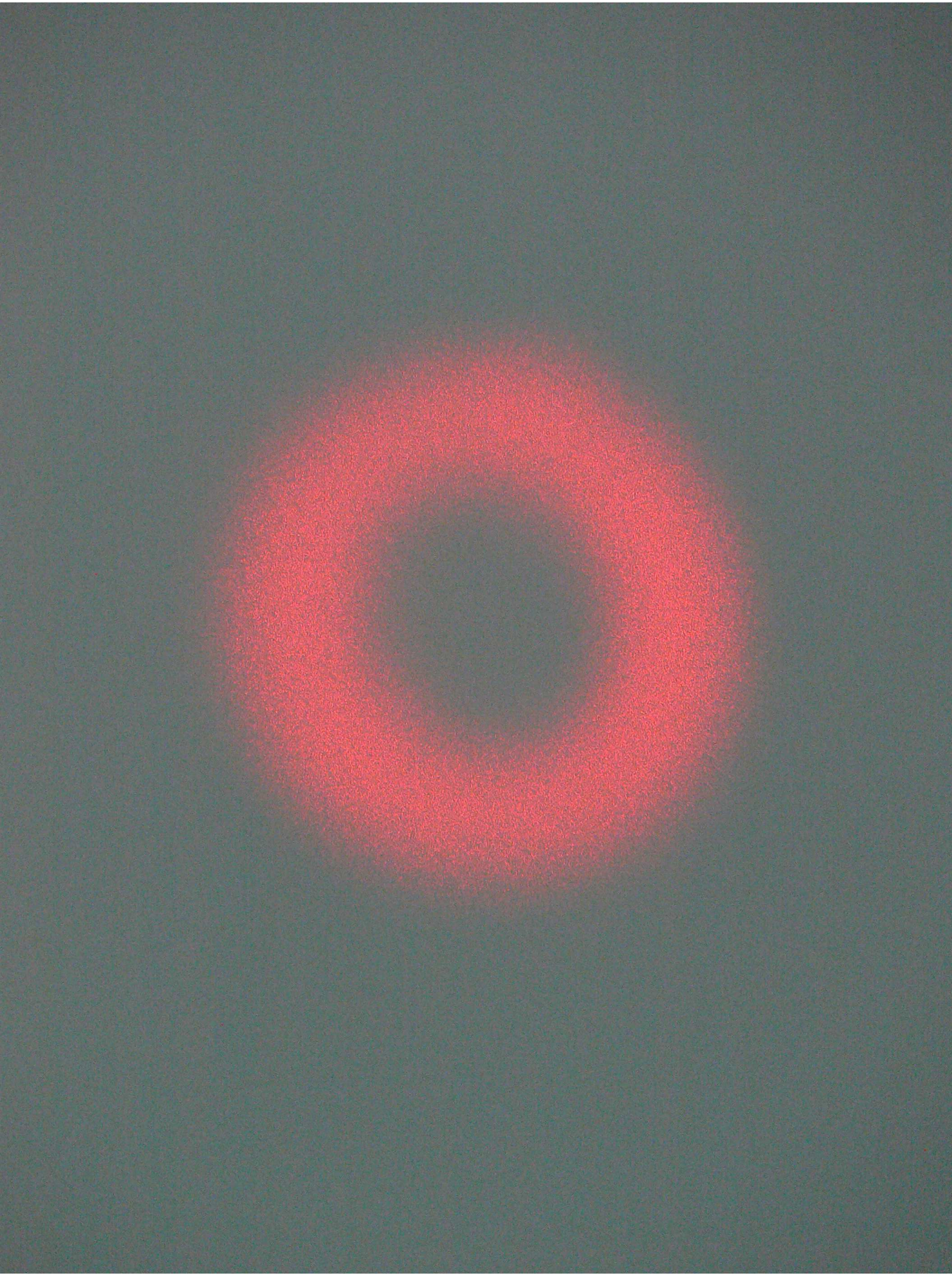


- A, R, F: absorbance, resonant and nonresonant fluorescence
- I, P: intersystem crossing, phoshorescence
- C, B: internal conversion, vibrational relaxation

Basics of thermal lens effect

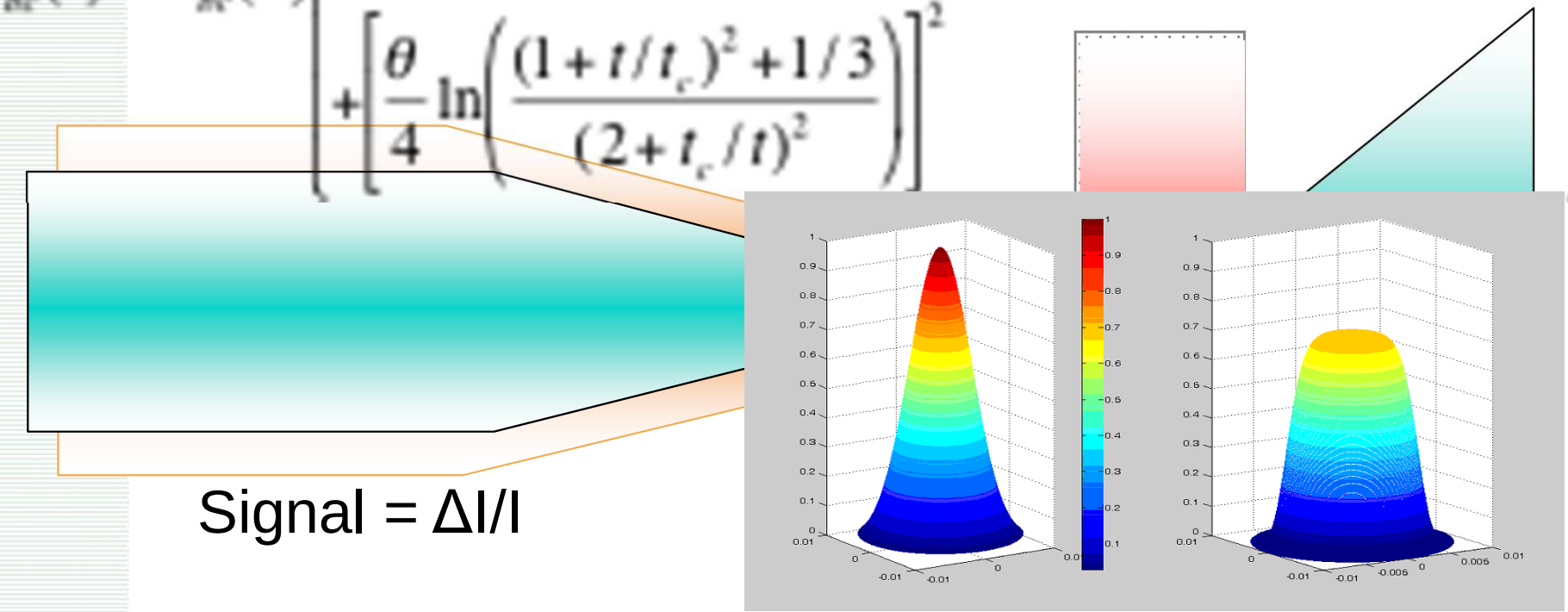
- During non-radiative relaxation of excited species temperature in the sample increases (10^{-4} - 10^{-3} K)
- a temperature gradient is generated with maximum temperature at the axis of the excitation beam
- the resulting refractive index gradient acts as a lens (mostly: $dn/dT < 0$, diverging lens)
- laser beam is defocused (single beam or pump/probe configuration)
- beam radius and its intensity at the beam axis changes
- relative change in the beam intensity is proportional to the absorbance of the sample and to the power of the excitation beam.





TLS – how to make good use of something not desired in laser technology?

$$I_{bc}(t) = I_{bc}(0)\left\{1 - \theta \tan^{-1}\left(\frac{1}{(1+t_c/t)\sqrt{3}}\right) + \left[\frac{\theta}{2}\tan^{-1}\left(\frac{1}{(1+t_c/t)\sqrt{3}}\right)\right]^2 + \left[\frac{\theta}{4}\ln\left(\frac{(1+t/t_c)^2+1/3}{(2+t_c/t)^2}\right)\right]^2\right\}$$



$$t_c = \frac{a^2 \rho C_p}{4k} \qquad \theta = \frac{2.303AP(-dn/dT)}{\lambda k}$$

Nonsteady thermal diffusion

$$\frac{\partial T(r,t)}{\partial t} = D\nabla^2 T(r,t) - v_x \frac{\partial T(r,t)}{\partial x} + \frac{1}{\rho C_p} Q(r,t)$$

- T(r,t)….. temperature
- D……thermal diffusivity
- ρ……. density
- c_p…….heat capacity
- Q(r,t)……source term (“heat”)
- v_x……..velocity of the medium in x direction

By solving nonsteady the thermal diffusion equation, changes in refractive index and related TLS signal can be calculated for different beam geometries and excitation regimes (pulsed, cw)

Pulsed and cw excitation with a Gaussian beam

- Pulsed:

$$Q(r,t) = \frac{2\alpha E_0}{\pi a^2 t_0} \exp\left[-2(x^2 + y^2)/a^2\right]$$

- cw:

$$Q(r,t) = \frac{2\alpha P_{av}}{\pi a^2} \left\{\exp\left[-2(x^2 + y^2)/a^2\right]\right\} \times (1 + \cos \omega t)$$

E_0 ….pulse energy a…..pump laser beam radius

t_0 …..pulse width P_{av}…cw laser average power

α……absorbance (cm^{-1}) ω…..modulation frequency

Thermal lens signal

$$s(t) = \frac{w_2^2(t) - w_2^2(0)}{w_2^2(0)}$$

- $w_2(0)$….radius of an unperturbed probe beam at the detector site
- $w_2(t)$…..time dependent radius of a probe beam perturbed by the thermal lens
- w_0…….radius of the probe beam at its waist

$$w_2^2(t) = w_0^2\left[\left(1 - \frac{z_2}{f(t)}\right)^2 + \frac{1}{z_0^2}\left(z_1 + z_2 - \frac{z_1 z_2}{f(t)}\right)^2\right]$$

Simplifications for usual far field experimental configuration

- $z_2 >> z_1$, $z_2 >> z_0 = \pi w_0^2/\lambda$
- $f(t) >> z_1$, $f(t) >> z_0$
- @ $t=0$, $f(0)=\infty$
  - λ …..probe beam wavelength
  - z_0…..confocal distance

$$s(t) = -\frac{2z_1}{f(t)}$$

Refractive index change and focal distance of thermal lens

$$n(x,y,t) = n_0 + \left(\frac{\partial n}{\partial T}\right)_{T_A} \times T(x,y,t)$$

- n_0….. unperturbed refractive index at ambient temperature T_A

collinear:

$$\frac{1}{f} = -\frac{\partial n}{\partial T}\,\ell\left(\frac{\partial^2 T}{\partial r^2}\right)$$

transversal:

$$\frac{1}{f} = -\frac{\partial n}{\partial T}\int_{-\infty}^{\infty}\left(\frac{\partial^2 T}{\partial x^2}\right)dy$$

- f…..thermal lens focal length
- ℓ….interaction length

TLS signal for collinear configuration

- Pulsed: ($t_0 \rightarrow 0$)

$$s(t) = -\frac{4AE_0 z_1 (\partial n / \partial T)}{\pi k a^2 t_c} \frac{1}{(1 + 2t / t_c)^2}$$

- cw:

$$A = \alpha \ell$$

$$s(t) = -\frac{2APz_1 (\partial n / \partial T)}{\pi k a^2} \frac{1}{(1 + t_c / 2t)}$$

  – t_c….time constant = $a^2 \rho c_p / 4k = 4a^2 D$
  – k….thermal conductivity of the sample

TLS signal for transversal configuration

- Pulsed: ($t_0 \rightarrow 0$)

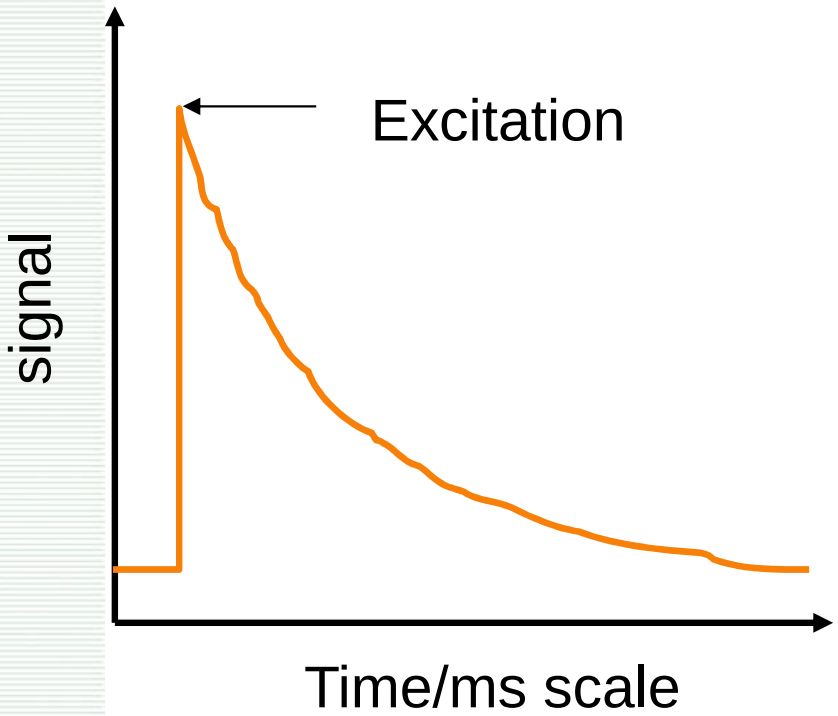
$$s(t) = -\frac{2\alpha E_0 z_1 (\partial n / \partial T)}{\sqrt{2\pi} k a t_c} \frac{1}{(1 + 2t / t_c)^{3/2}}$$

- cw:

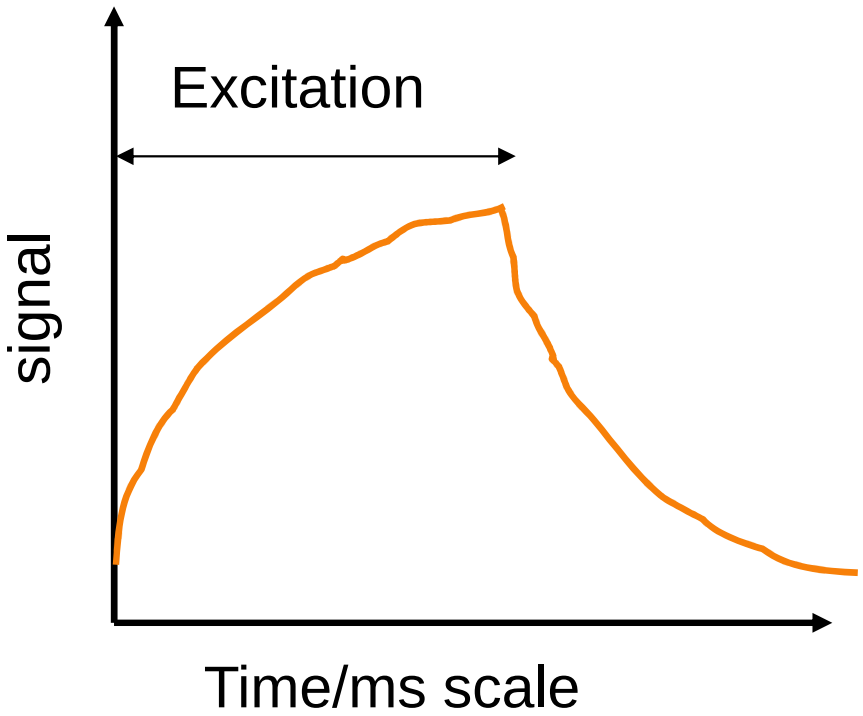
$$s(t) = -\frac{2\alpha P z_1 (\partial n / \partial T)}{\sqrt{2\pi} k a} \frac{1}{(1 + t_c / 2t)^{1/2}}$$

TLS signal form

Pulsed



CW



TLS signal in a single beam experiment

- P/a^2 changes with increasing z_1
- the signal maximum is found at $z_1=z_0$ (parabolic model) $z_0 = \pi a_0^2/\lambda$

$$s(t) = -\frac{AP(\partial n/\partial T)}{\lambda k}\frac{1}{(1+t_c/2t)}$$

- or at $z_1=z_0\sqrt{3}$ (aberrant model)

$$s(t) = -\frac{AP(\partial n/\partial T)}{\lambda k}\tan^{-1}\left[\frac{1}{(1+t_c/t)\sqrt{3}}\right]$$

E - Enhancement factor in TLS

$$\frac{\Delta I}{I} = \frac{2.303P\left(-dn/dT\right)A}{\lambda k} arctg\left(1/\sqrt{3}\right) = 2.303EA$$

Solvent	$-dn/dT$ (10^4 K^{-1})	k ($W\,m^{-1}K^{-1}$)	E (10^{-3} W^{-1})
H_2O	0.91	0.607	0.12
CCl_4	5.9	0.103	4.74
acetone	5.42	0.190	2.36

$E = (-dn/dT)/(1.91\ \lambda k)$ is calculated for $\lambda = 632.8$ nm

Thermo-optical properties of solvents for TLS measurements

Solvent	Thermal conductivity, k $mWcm^{-1}K^{-1}$	$10^4(dn/dT)$ K^{-1}	$-\frac{10^4(dn/dT)}{k}$ $cm\ mW^{-1}$
CO_2 (SC)	0.7	-100	143
CCl_4	1.03	-5.9	5.73
Benzene	1.24	-6.4	5.16
C_8MImTf_2N	n.d.	n.d.	4.55
cyclohexane	1.24	-5.4	4.35
$BMImBF_4$	1.78	-7.54	4.24
n-heptane	1.26	-5.0	3.97
$BMImTf_2N$	1.06	-4.0	3.78
dioxane	1.39	-4.6	3.31
$EMImTf_2N$	n.d.	n.d.	2.37
methanol	2.20	-4.7	2.14
water	6.11	-0.8	0.13

Calc. values (except CO2) taken from Chieu D. Tran and T. A. Van Fleet, Anal. Chem. 60, (1988) 2478

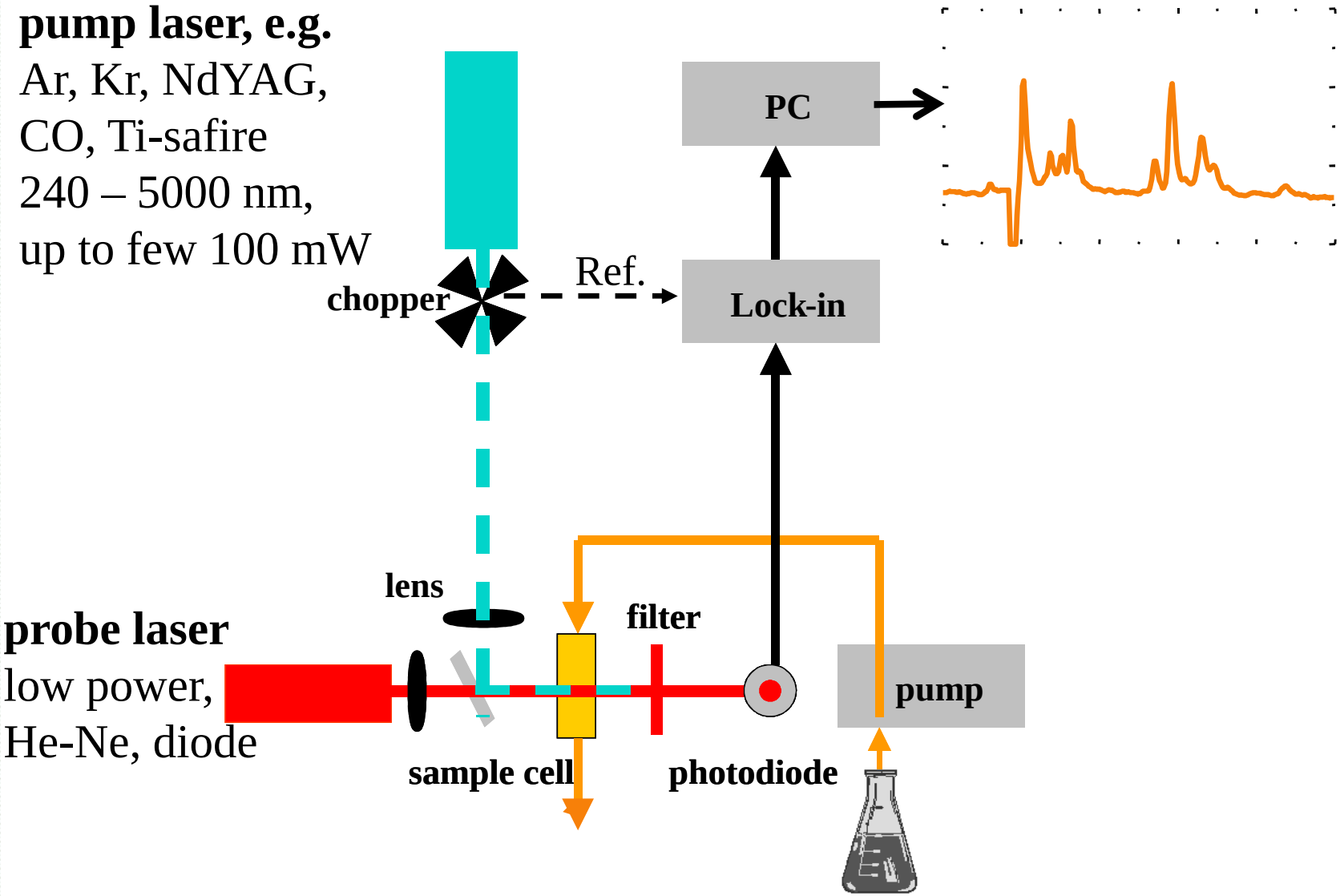
TLS - advantages

- High sensitivity
  - signal proportional to excitation laser power
  - absorbances as low as 10^{-7} can be measured
- Enables On-line detection
  - fast response of TLS signal (on μs to ms time scale)
- Capability of measuring small samples
  - sub-pL volumes can be probed
  - detection in microfluidic systems

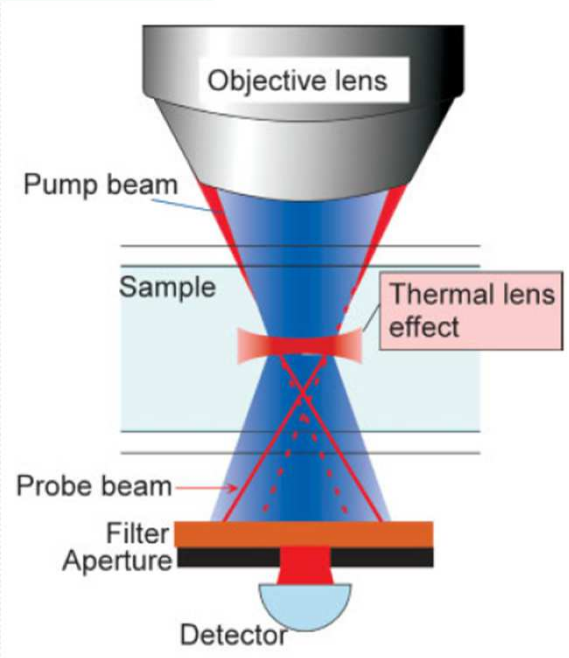
TLS – drawbacks and solutions

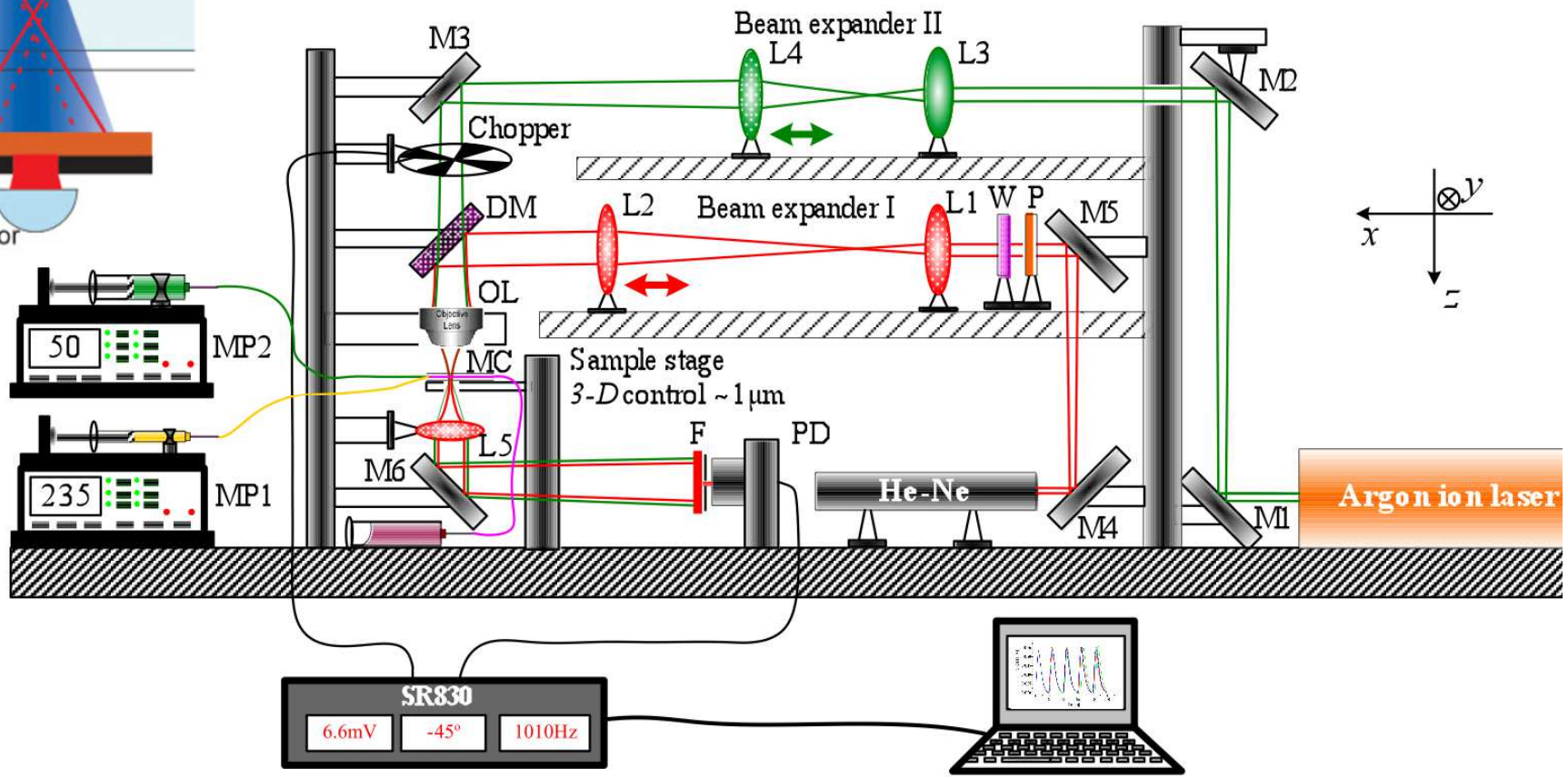
- Sensitivity still needs improvement
  - Higher laser power? (photo-labile compounds)
  - Modify solvents
- Limited availability of laser sources
  - Coloring reactions, indirect detection
- Poor selectivity
  - Single wavelength measurements
  - Coupling to separation techniques (HPLC, IC, CE)
- Photodegradation
  - Measure in flowing systems

Dual beam TLS spectrometer for detection in FIA, HPLC and bioassays

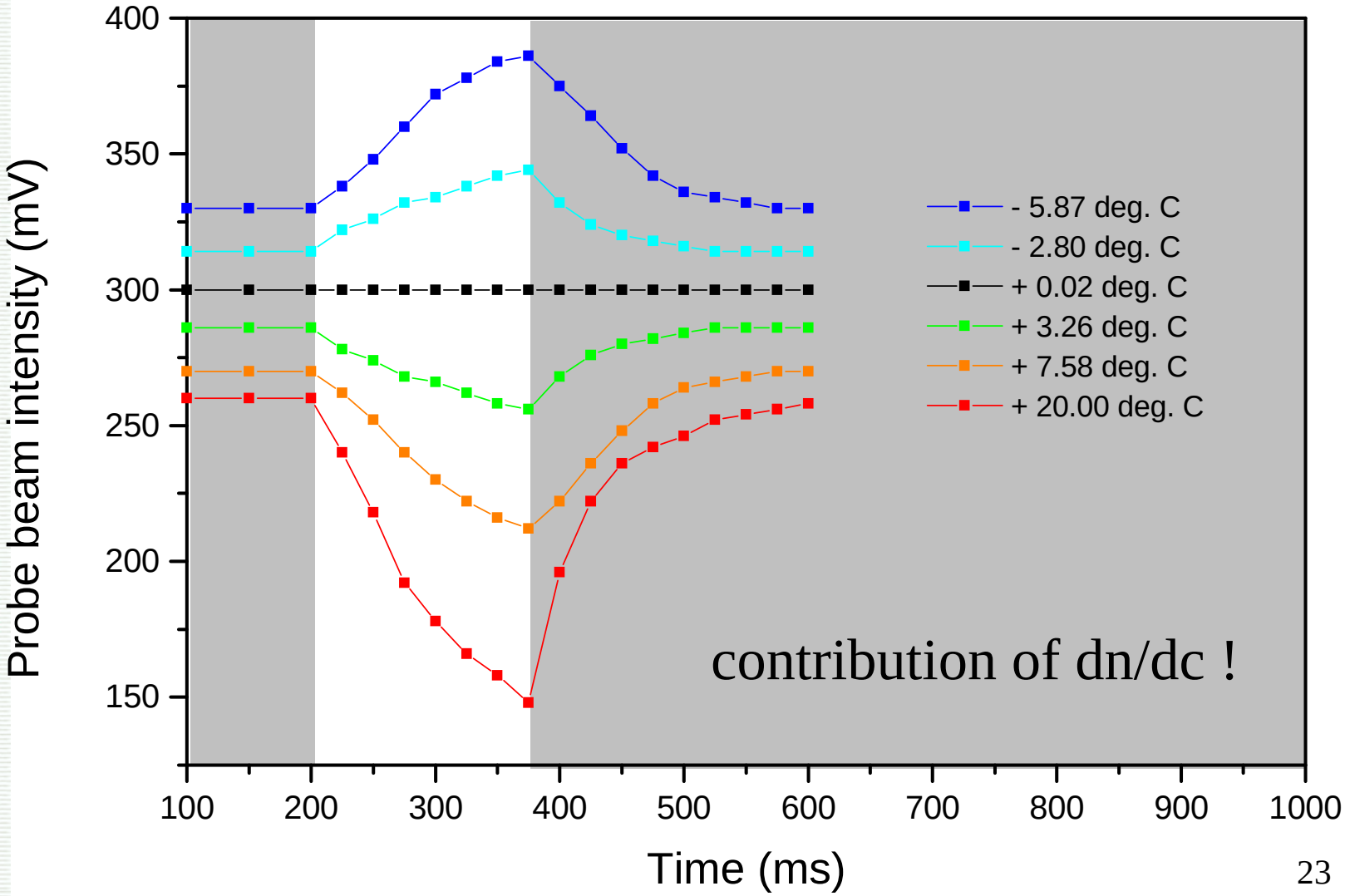


Adjustable beam size/position TLM

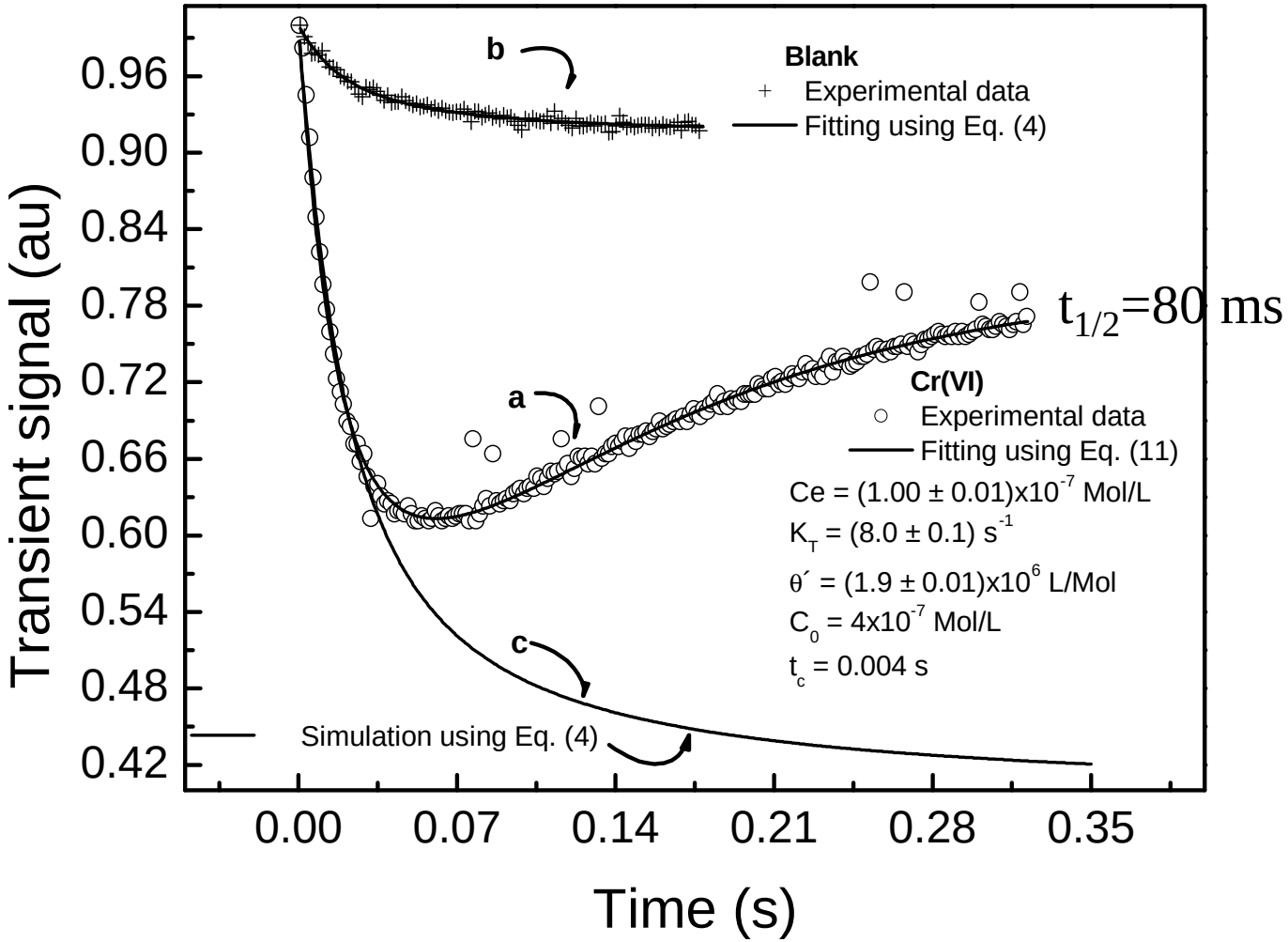




Temperature dependent TLS signal in water



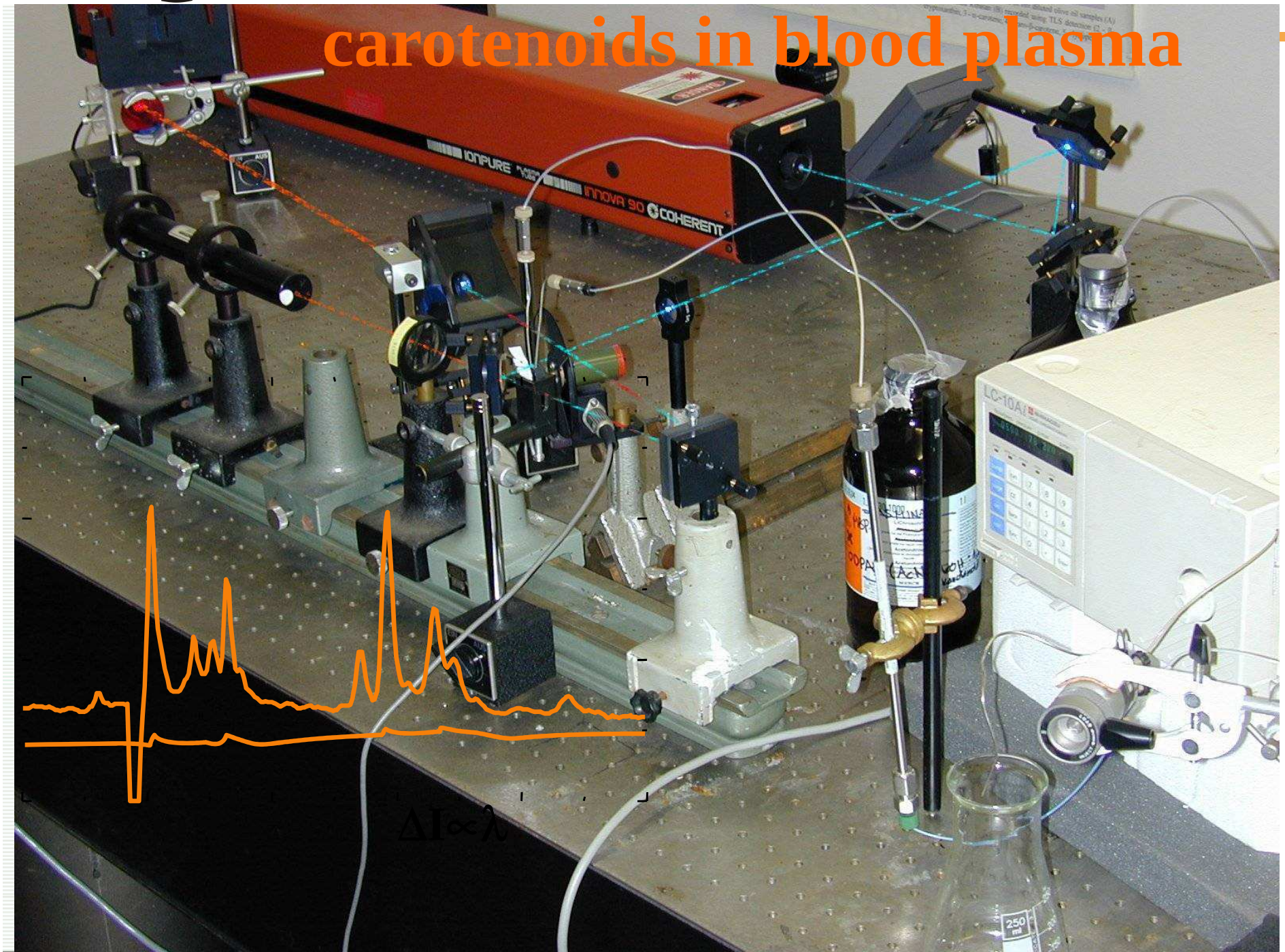
The effect of photosensitivity on TLS signal (case of Cr-DPC)



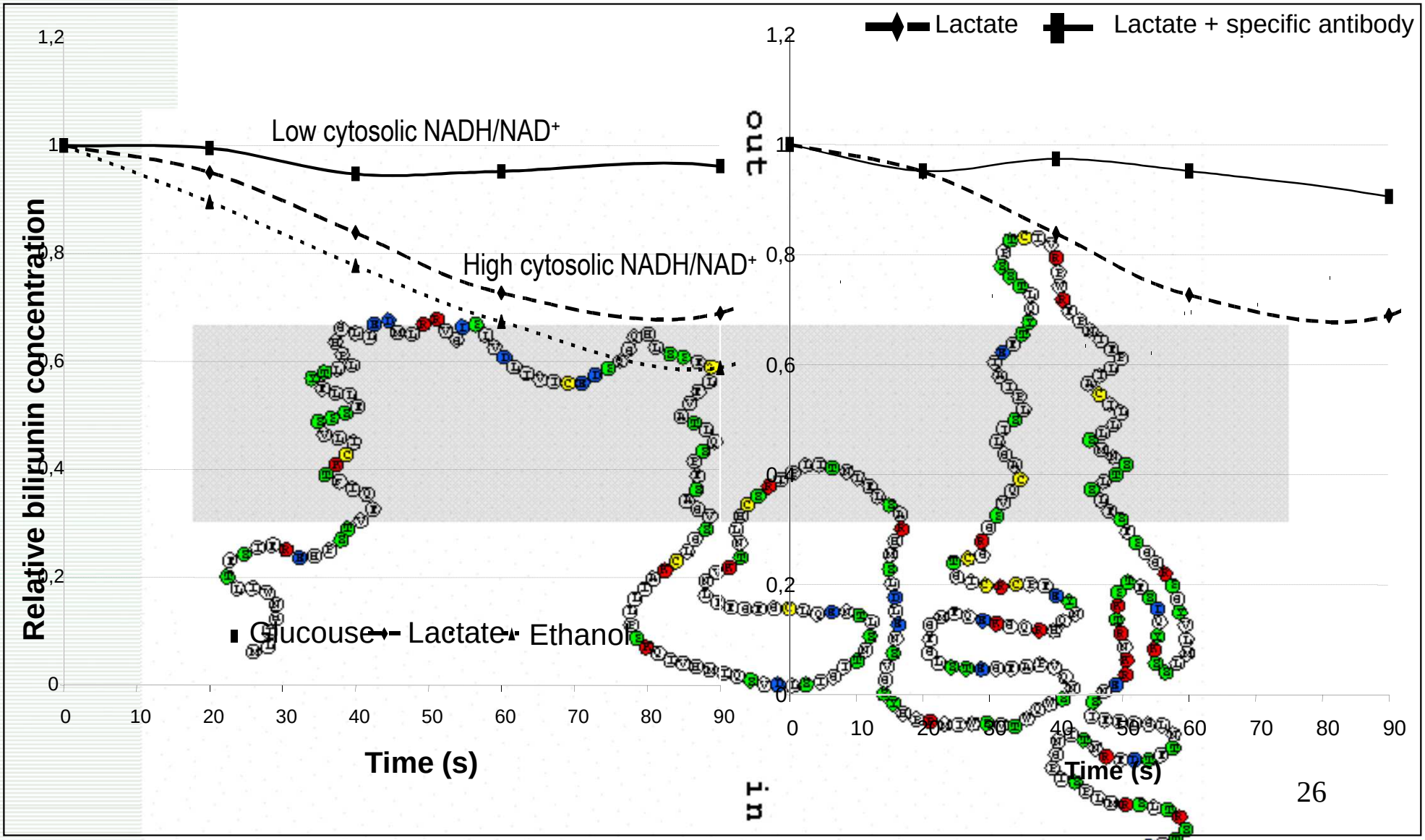
$$I_{bc}(t) = I_{bc}(0)\left[1 - \frac{\theta'\left[(C_0 - C_e)\exp(-(k_e + k_D)t) + C_e\right]}{2}\tan^{-1}\left(\frac{1}{(1 + t_c/t)\sqrt{3}}\right)\right]^2$$

Pedreira et al.: J. Appl. Phys., **100** (2006)
Chem. Phys. Lett. **396(**2004)221

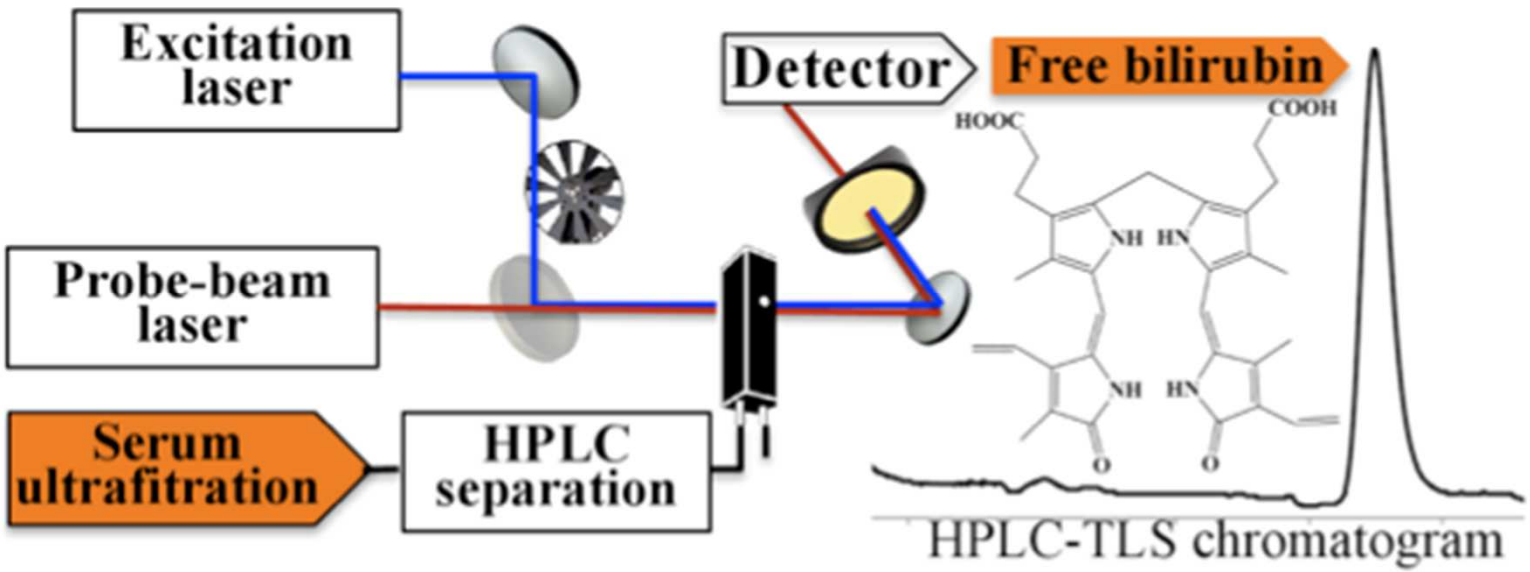
HPLC-TLS degtermination of carotenoids in blood plasma



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



Improvement of selectivity by separation techniques (HPLC, IC)



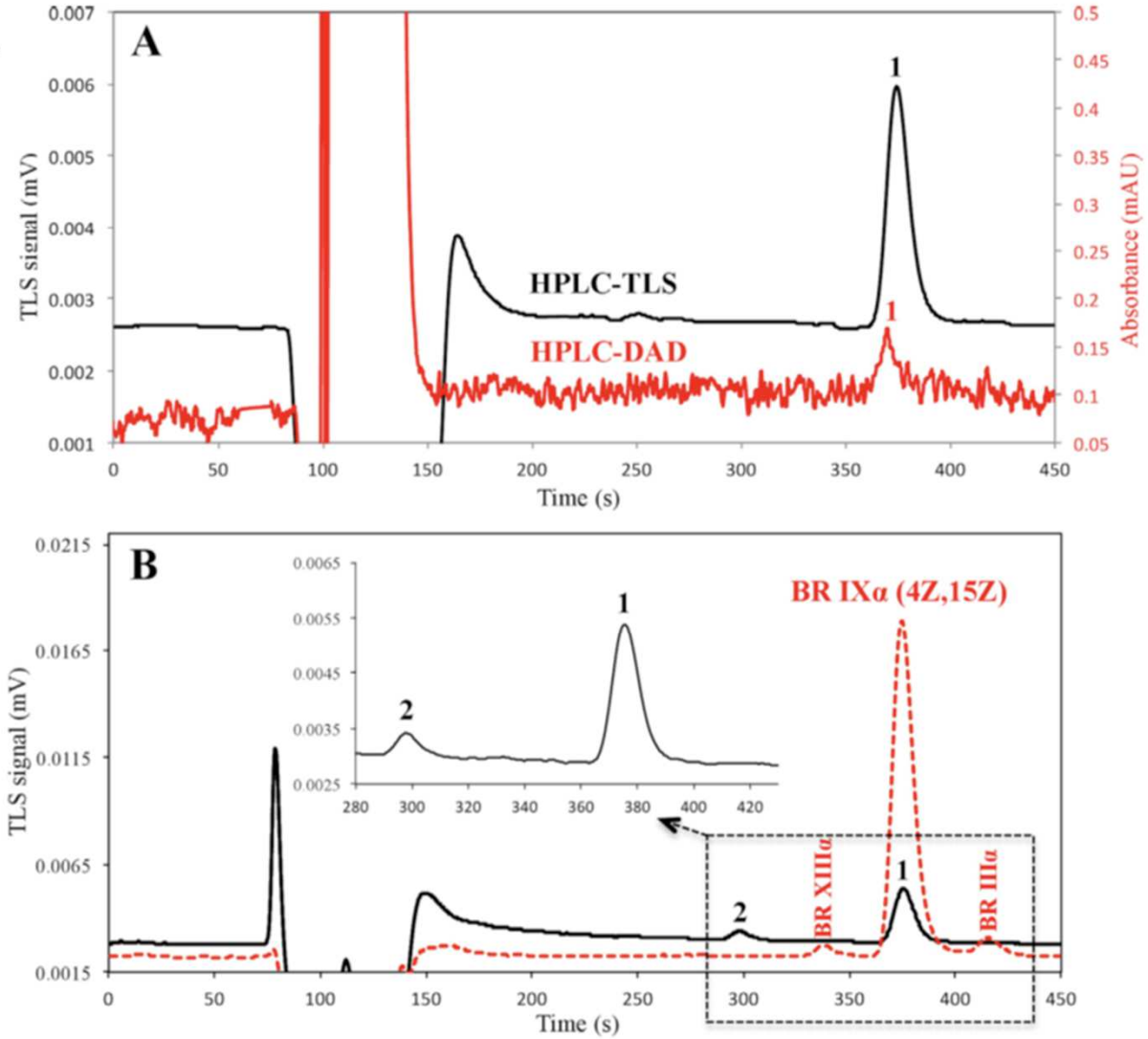
LOD: 90 pM

LOQ: 250 pM

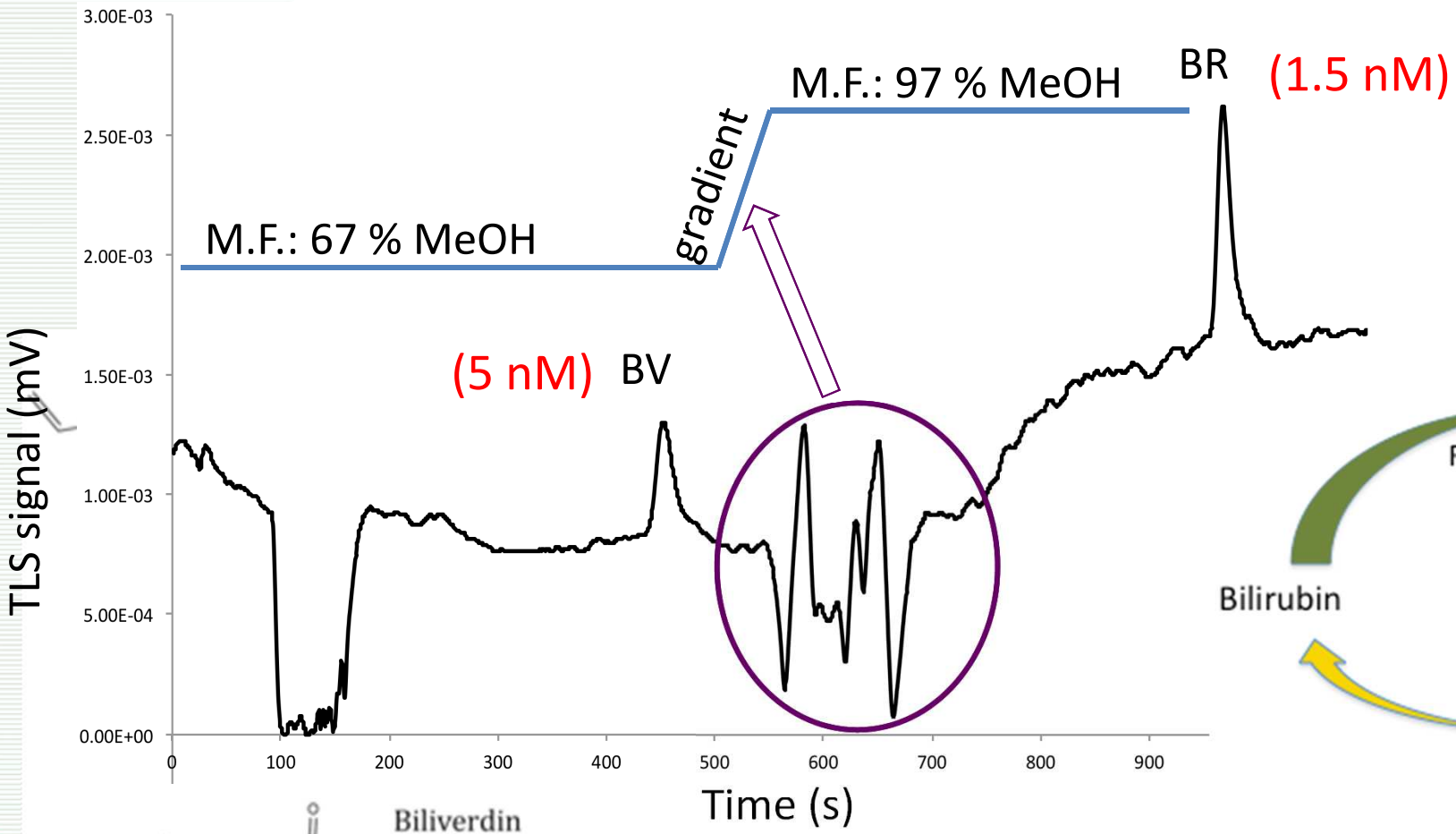
Martelanc M., Žiberna L., Passamonti S., Franko M.:
Anal. Chim. Acta **809**, 2014, 174–182.

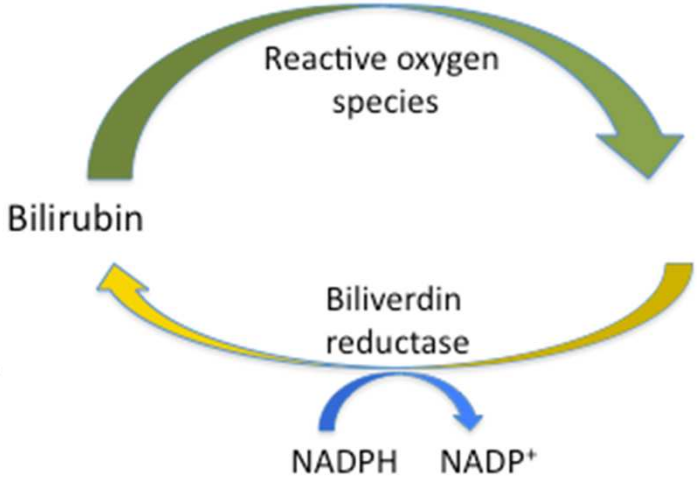
Free bilirubin in blood serum samples

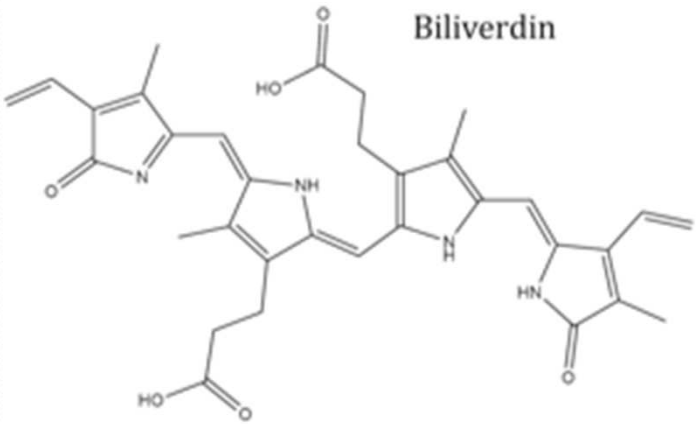
Serum samples	Male (aged 25)	Male (aged 30)	Male (aged 32)	FBSHI	FCSHI
Serum free bilirubin (nM)	13.1 ± 1.1	9.1 ± 0.6	8.5 ± 0.7	13.6 ± 1.2	17.3 ± 1.3



Simultaneous determination of bilirubin and biliverdin







Contents lists available at ScienceDirect

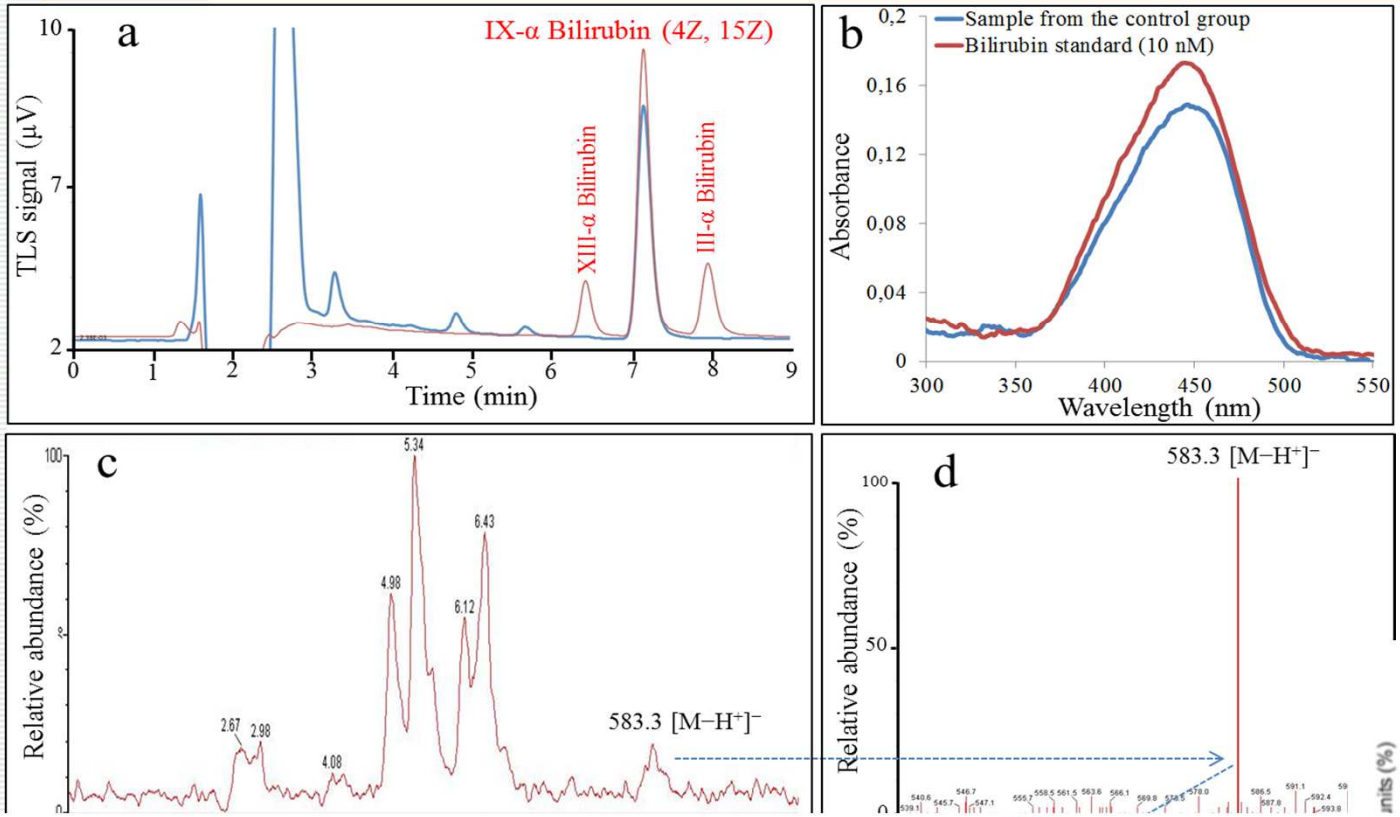
Talanta 154(2016)92–98

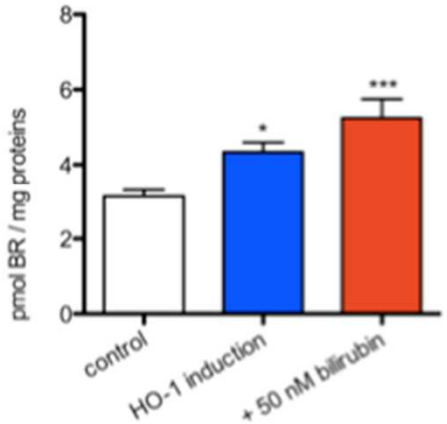
journal homepage: www.elsevier.com/locate/talanta

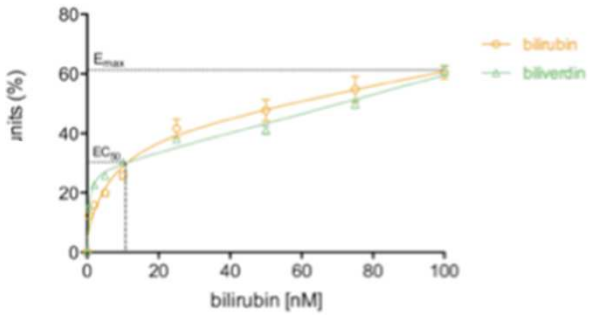
Application of high-performance liquid chromatography combined with ultra-sensitive thermal lens spectrometric detection for simultaneous biliverdin and bilirubin assessment at trace levels in human serum

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

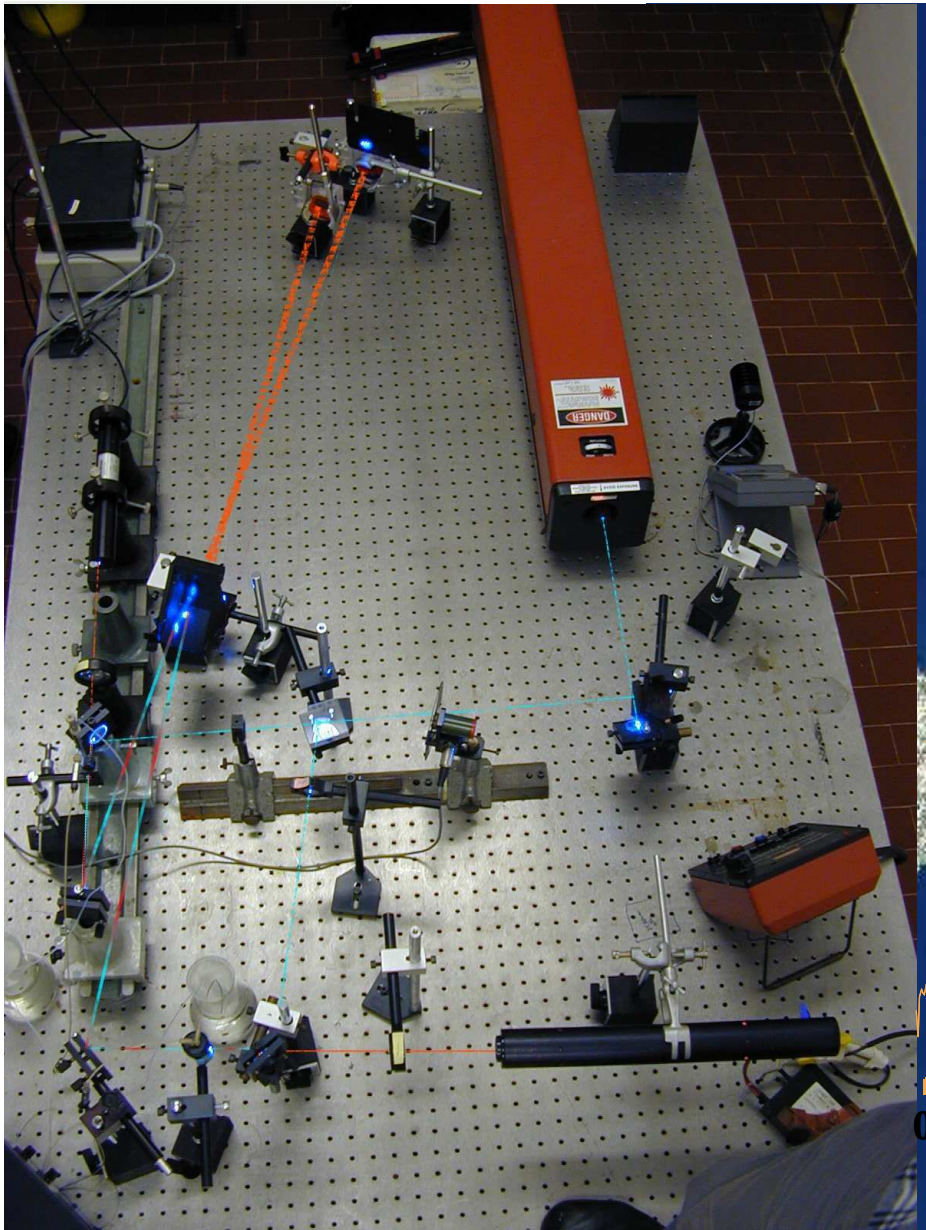
Received: 29 December 2015
Accepted: 14 June 2016

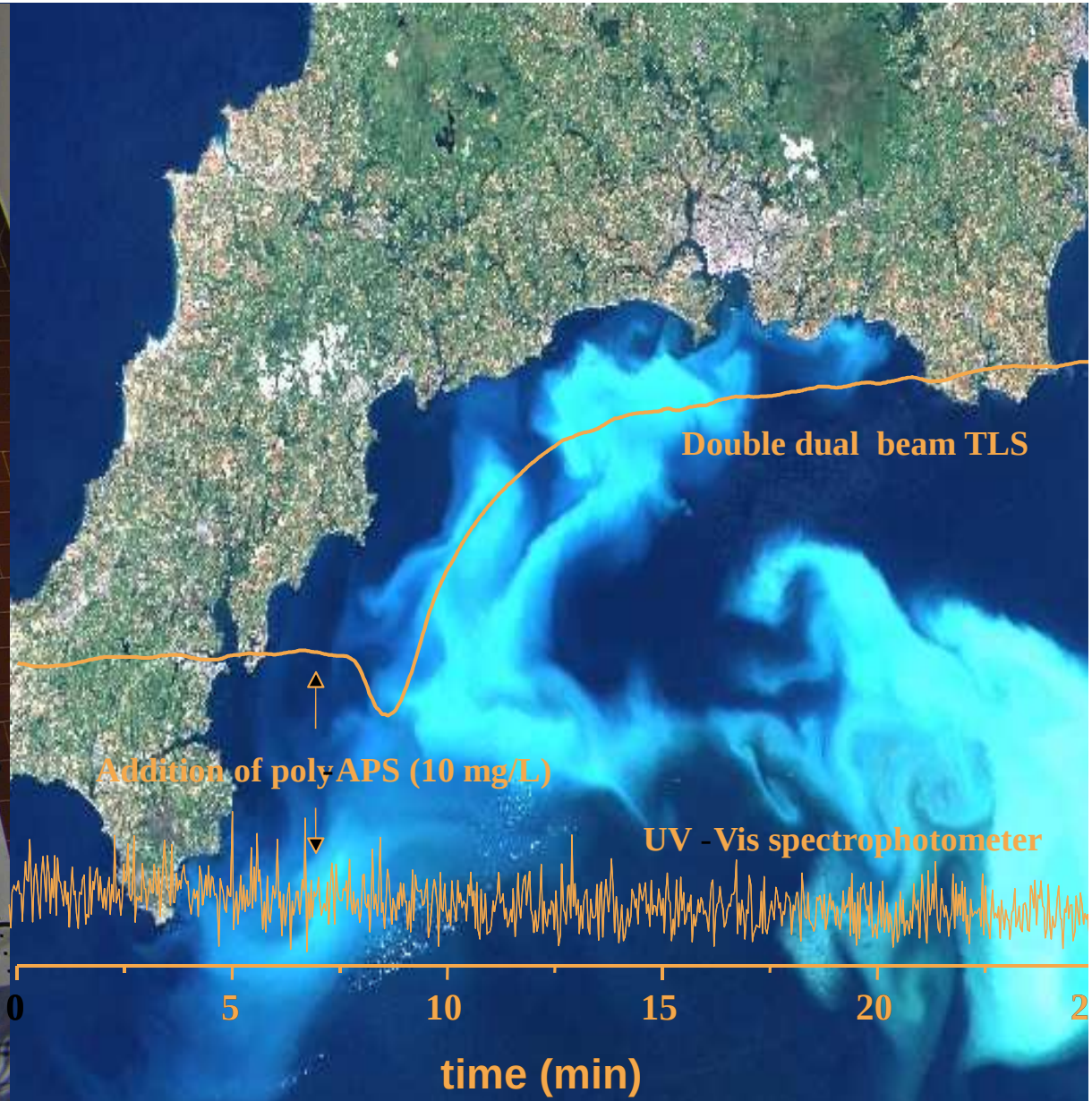
Lovro Ziberna[1,2], Mitja Martelanc[3], Mladen Franko[3] & Sabina Passamonti[1]

Scientific Reports 6:29240
DOI: 10.1038/srep29240

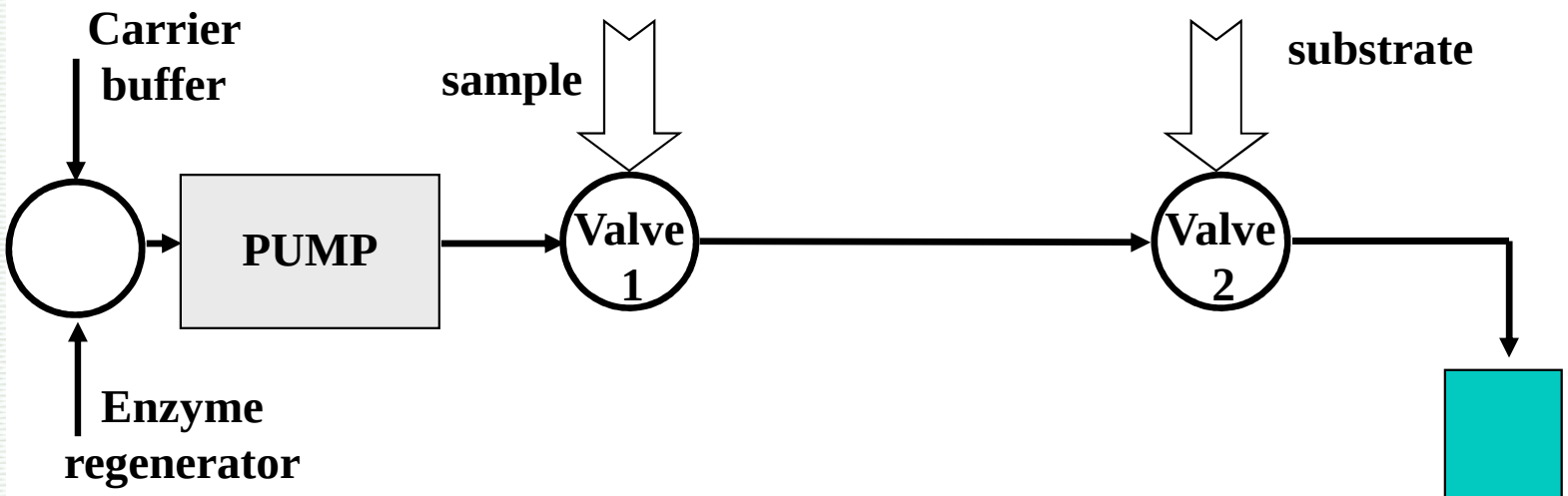
Advantages of TLS: extremely high sensitivity, small sample capability

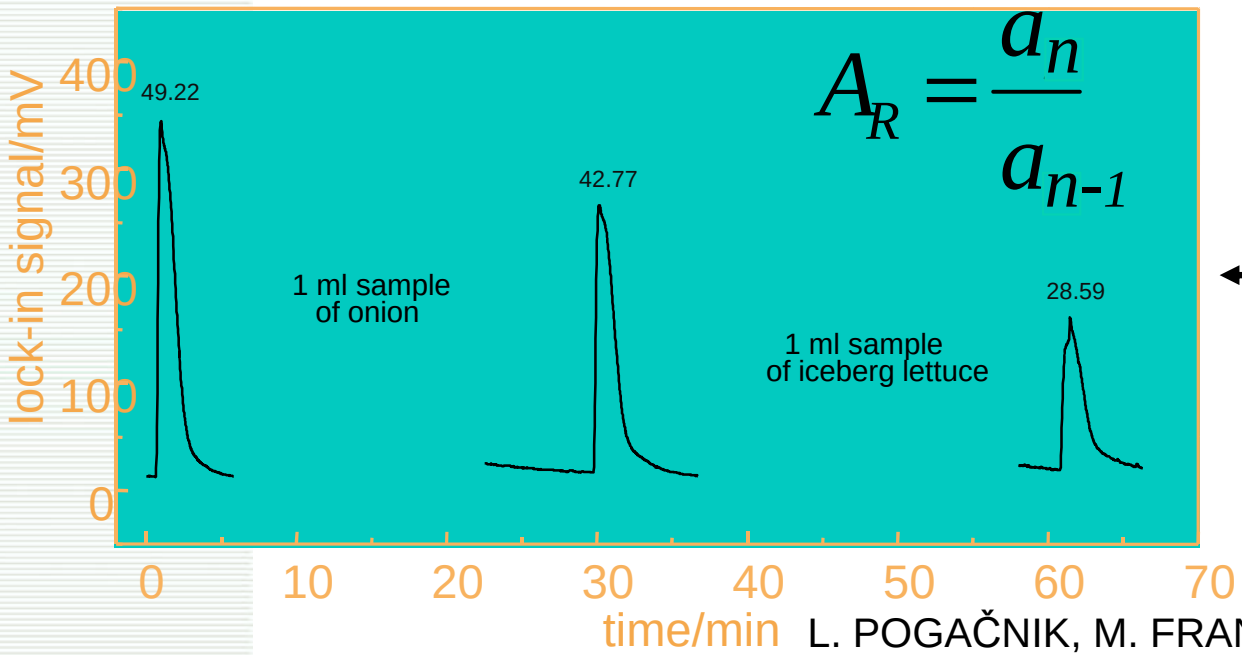
(100 – 1000 times lower LOD than SF)



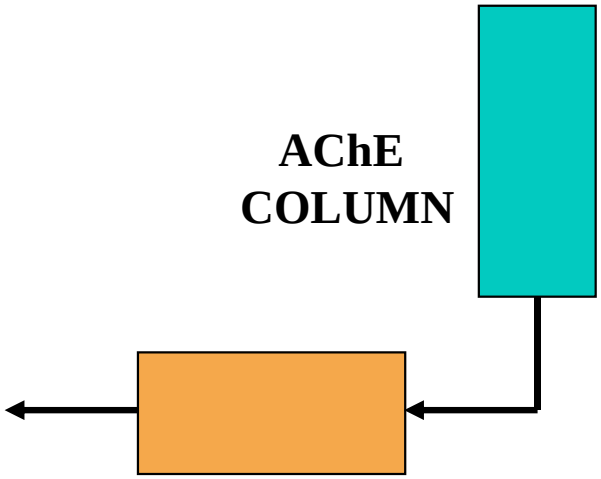


Bioanalytical FIA system



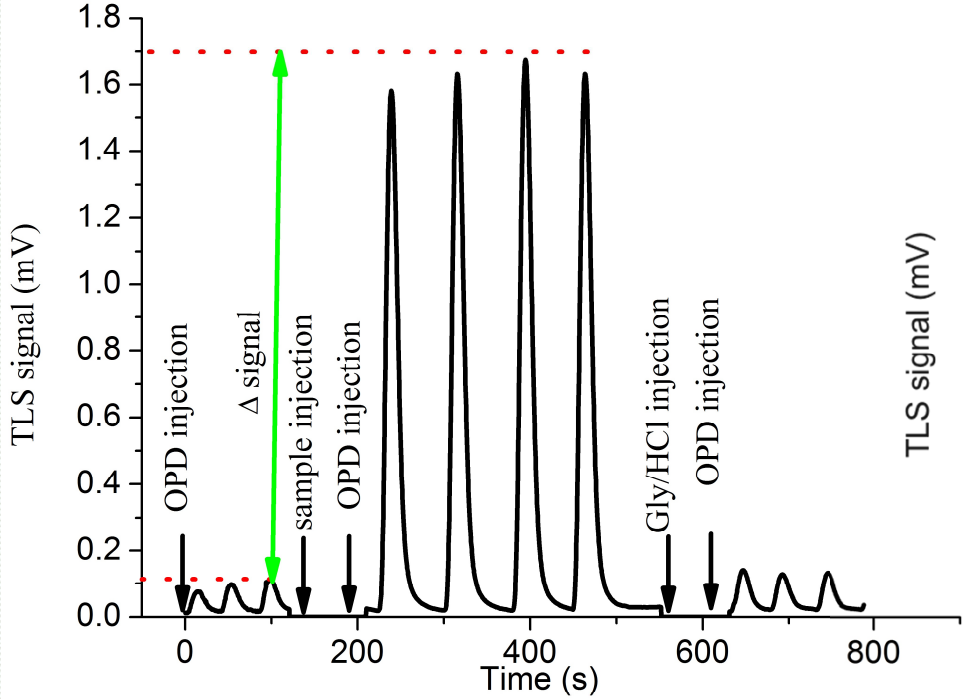


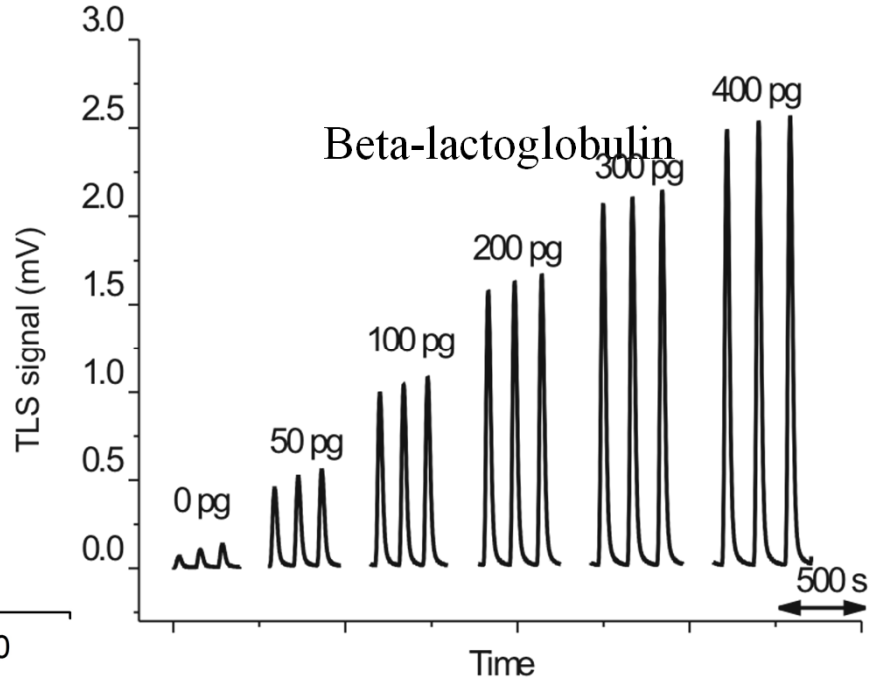
$$A_R = \frac{a_n}{a_{n-1}}$$



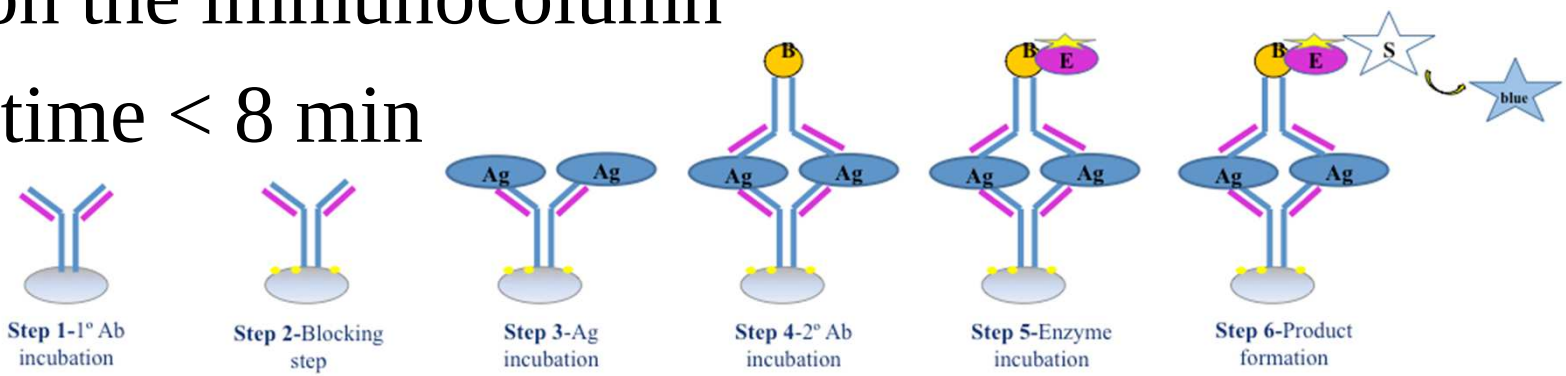
L. POGAČNIK, M. FRANKO, Biosens. Bioelectron.. **2003**, 18, 1-9

FIA-ELISA-TLS Detection of Food Allergens

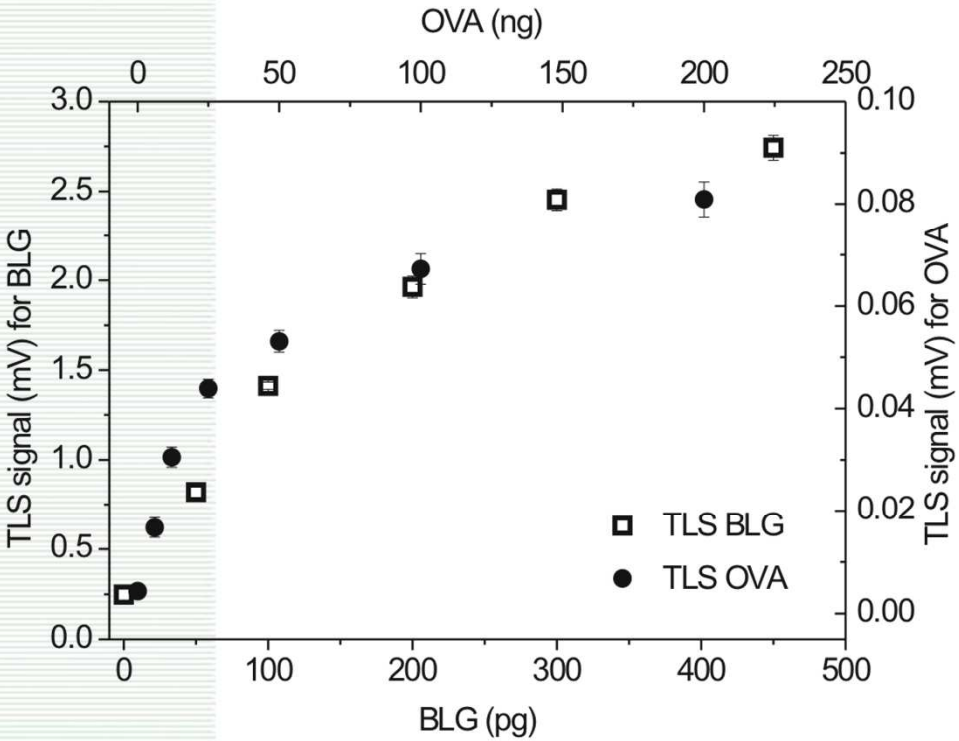


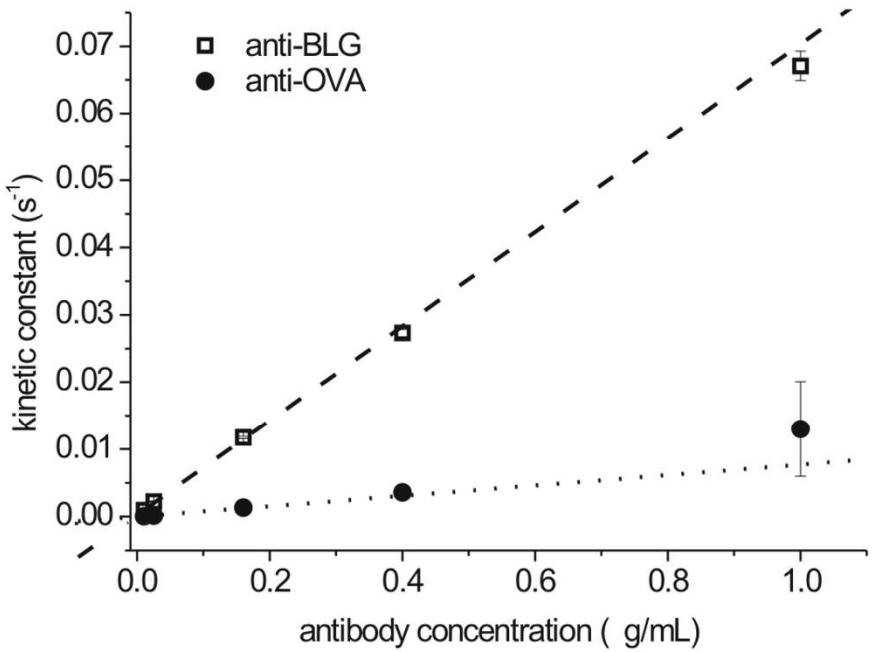


- TLS signal proportional to the amount of allergen retained on the immunocolumn
- Analysis time < 8 min



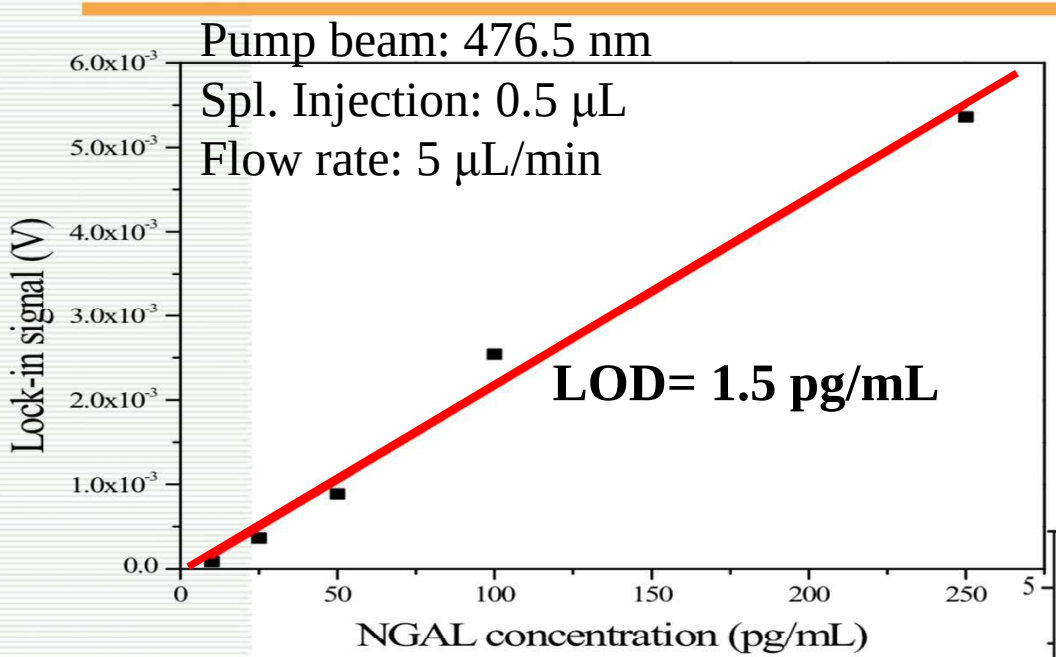
Determination of BLG and OVA by FIA-ELISA-TLS



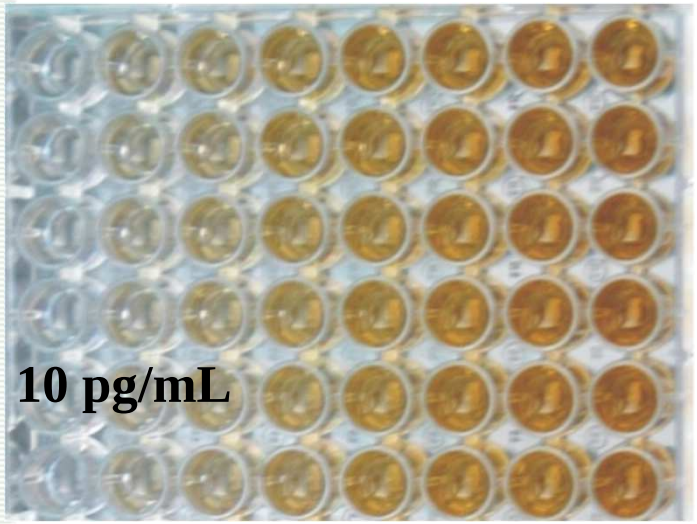


LOD for beta-lactoglobulin (BLG) =2.3 pg/ 100 µL (190 pg by ELISA – Bethyl)
LOD for ovalbumin (OVA) =1 ng/ 100 µL (1 µg by ELISA– Abcam)

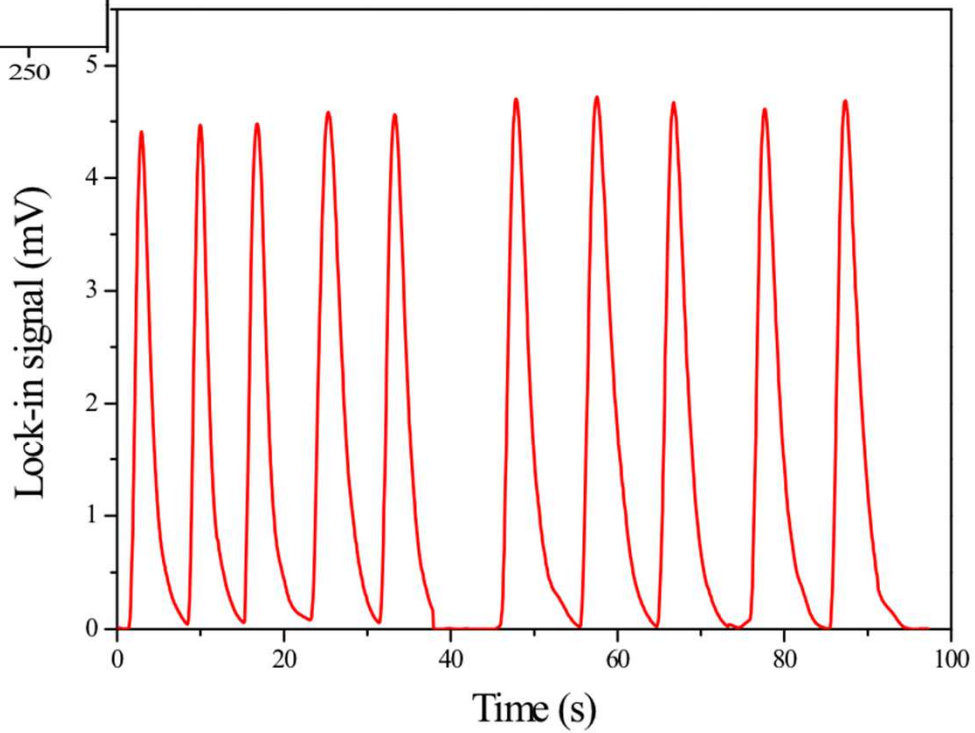
Determination of NGAL - a biomarker of acute kidney injury



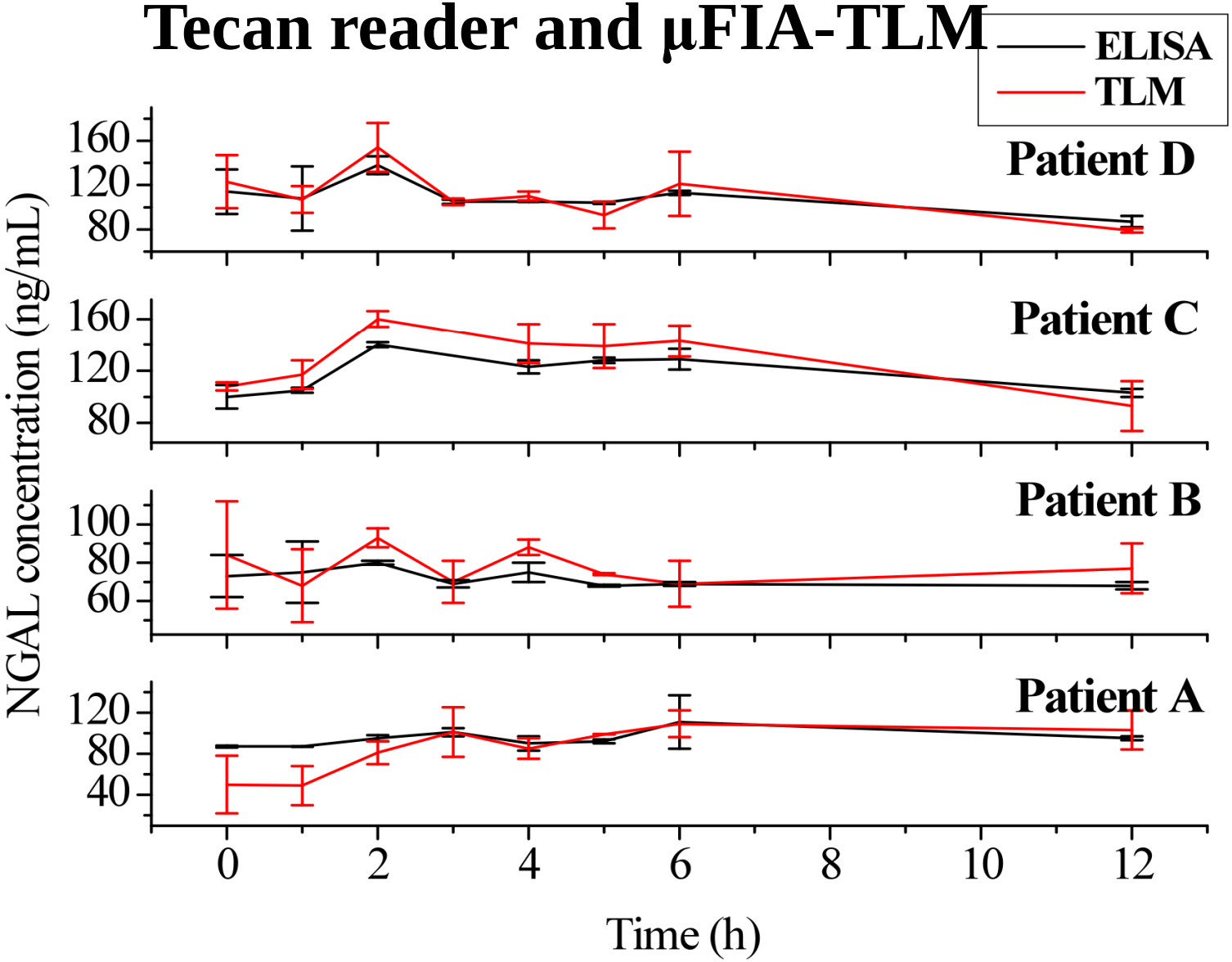
TLS signals for replicate injections of two aliquots of 500-times diluted blood plasma sample (217 pg/mL)

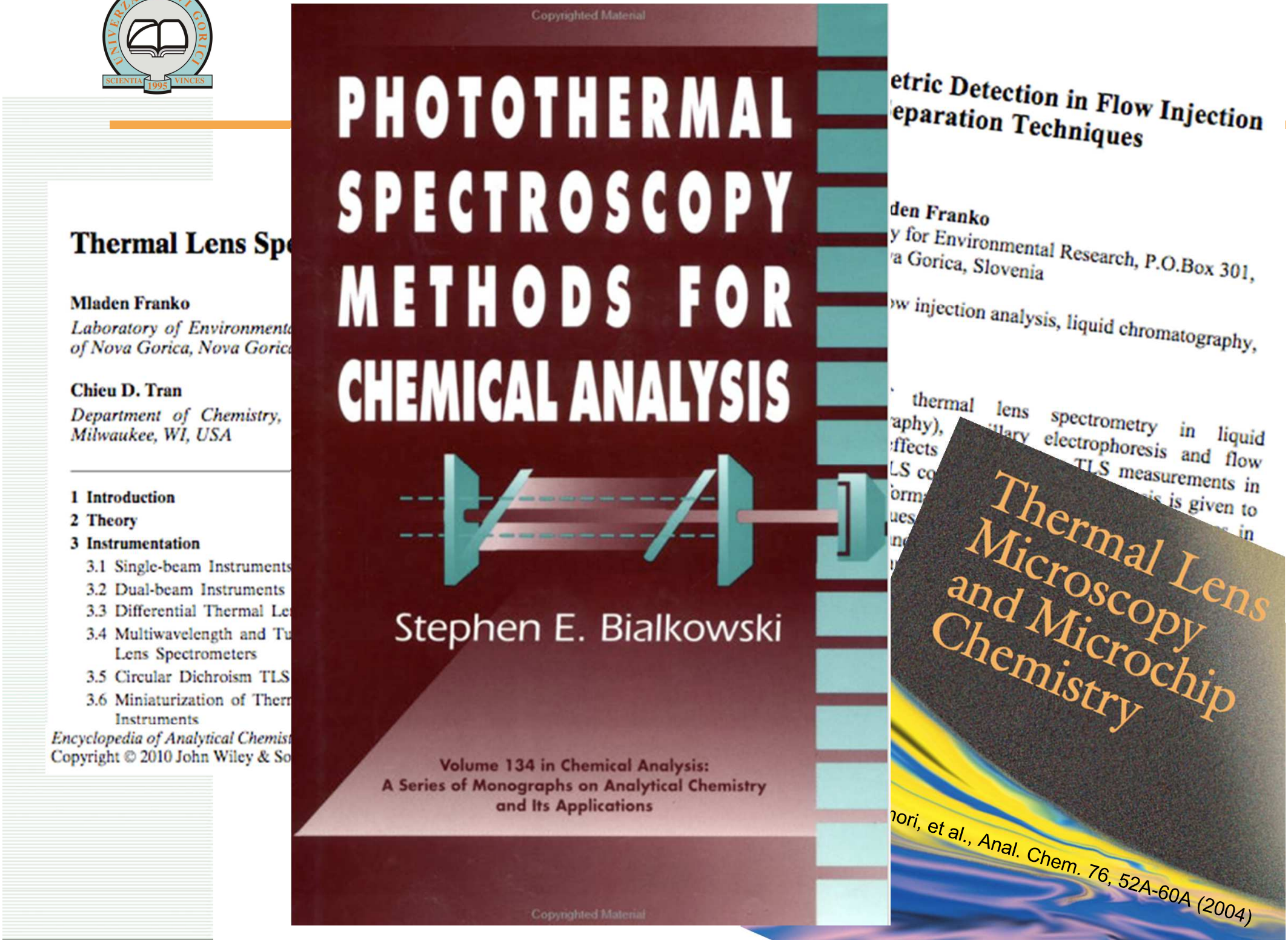


LOD= 10 pg/mL



Detection of NGAL in blood plasma of patients after percutaneous coronary angiography (injection of contrast agents) by Tecan reader and µFIA-TLM




Thermal Lens Sp
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