Label Free Imaging and Bioassays

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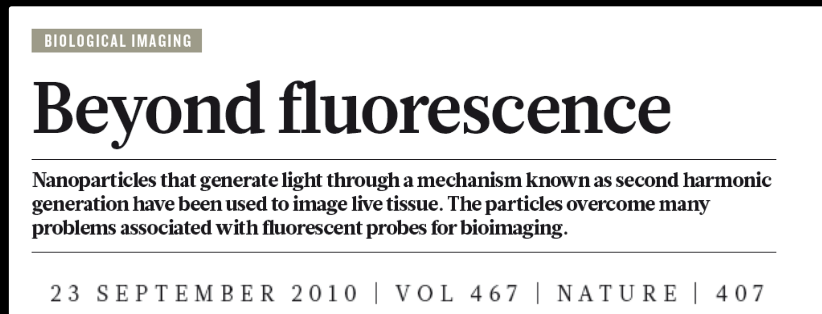
University of Geneva
www.gap.unige.ch/biophotonics

SLN meeting – MUST – 25.11.11

Outline



BIOLOGICAL IMAGING

Beyond fluorescence

Nanoparticles that generate light through a mechanism known as second harmonic generation have been used to image live tissue. The particles overcome many problems associated with fluorescent probes for bioimaging.

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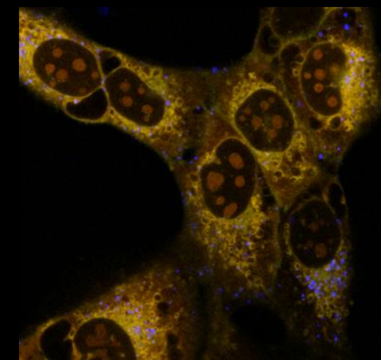
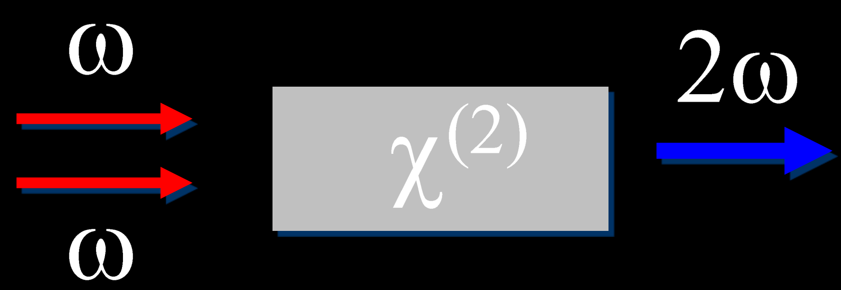


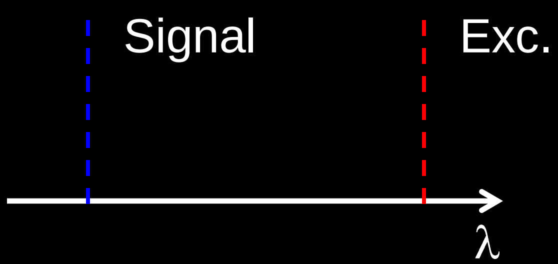
- Nano-doublers
- Imaging
- Towards Label free Imaging: Coherent Control
- Perspectives

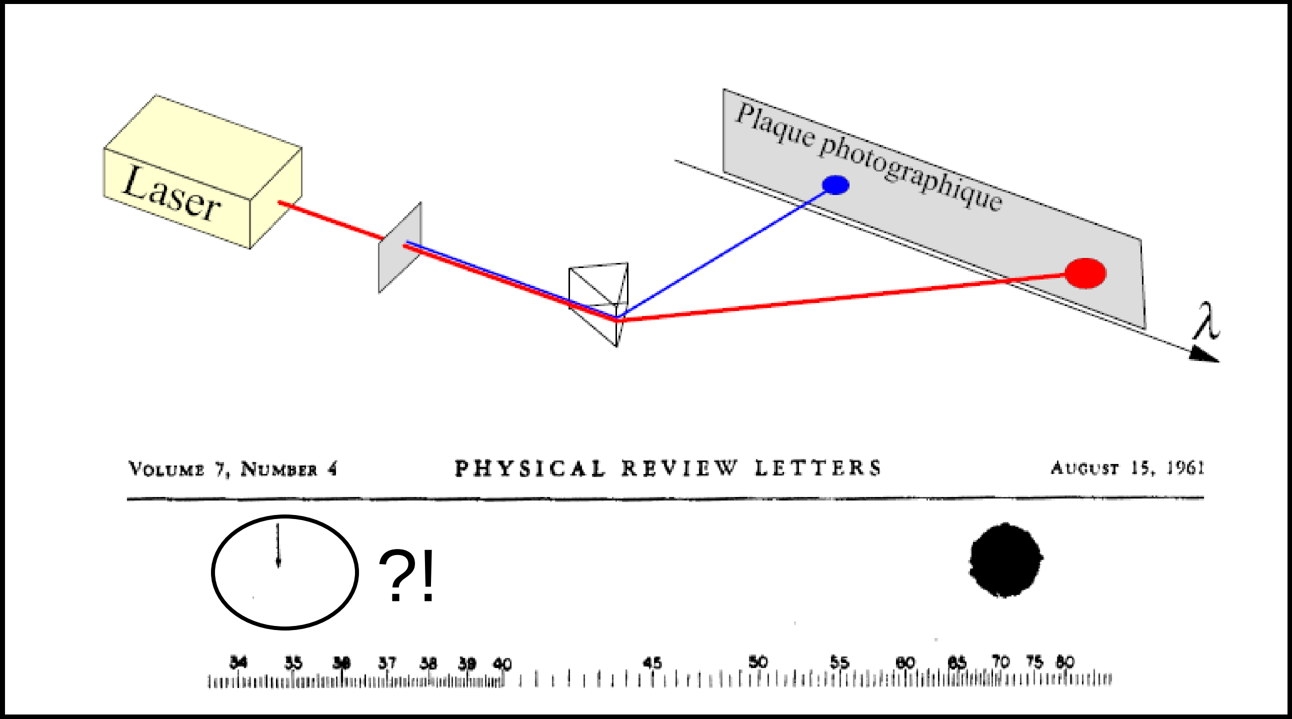
GAP Biophotonics

must

Second Harmonic Generation





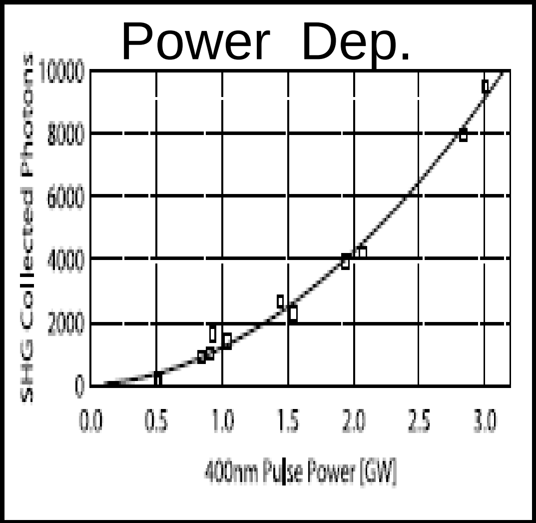


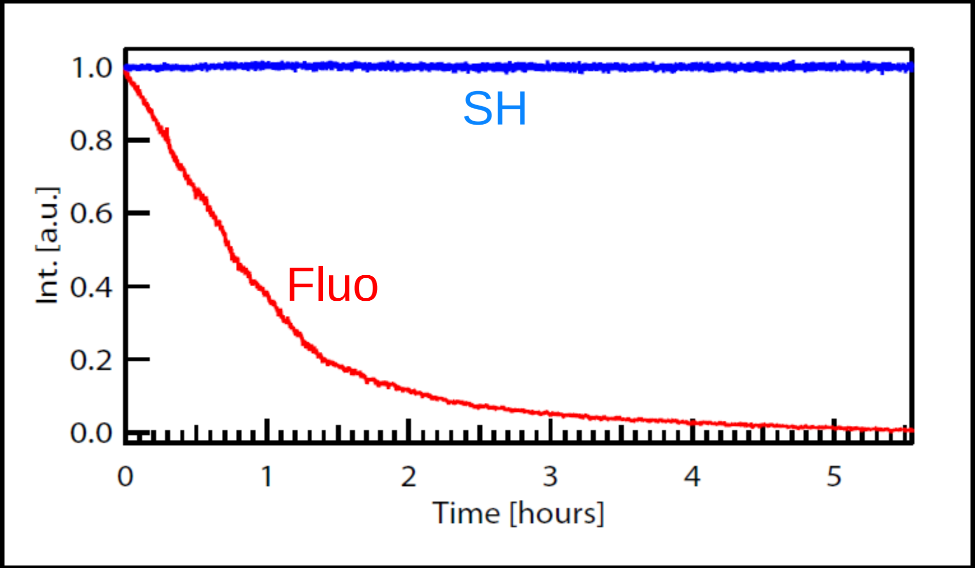
50 YEARS LASER

Since 2007:

$Fe(IO_3)_3$, $KNbO_3$, KTP, ZnO, $BaTiO_3$, SiC, BBO, $LiNbO_3$, and magnetic nanomaterials

Photo-Stability



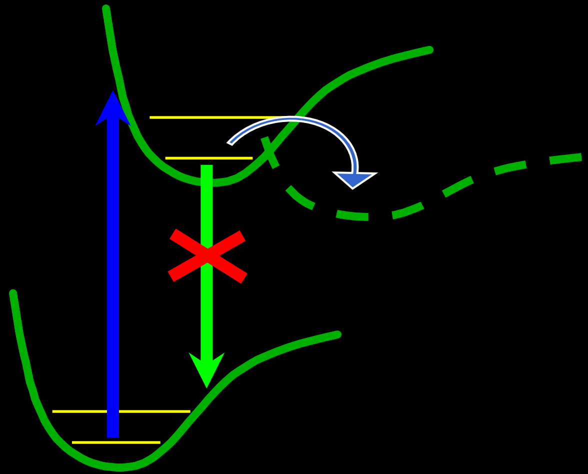


Estimated SHG efficiency of
90 nm $BaTiO_3$: 2000-13000 GM

For comparison:
QD: 47000 GM
GFP: 75 GM
Rhod 6G: 6 GM
(1 GM = 10^{-50} $cm^4 s/photon$)

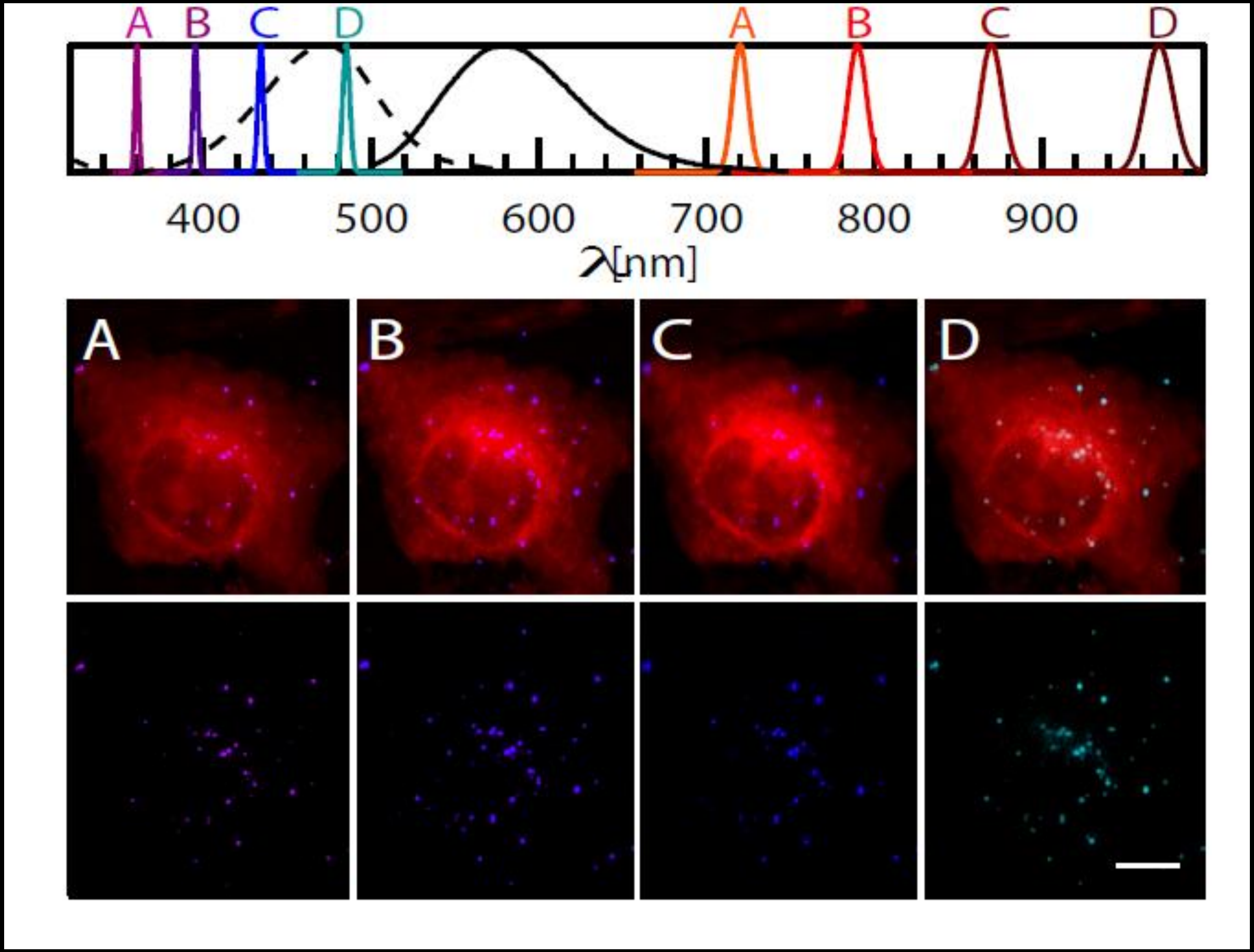
$$W_{2P} = \boldsymbol{\sigma_{2P}}\, I^2$$

C-L Hsieh et al., Opt. Express 17(4), 2880 (2009)



Fluorescence Bleaching

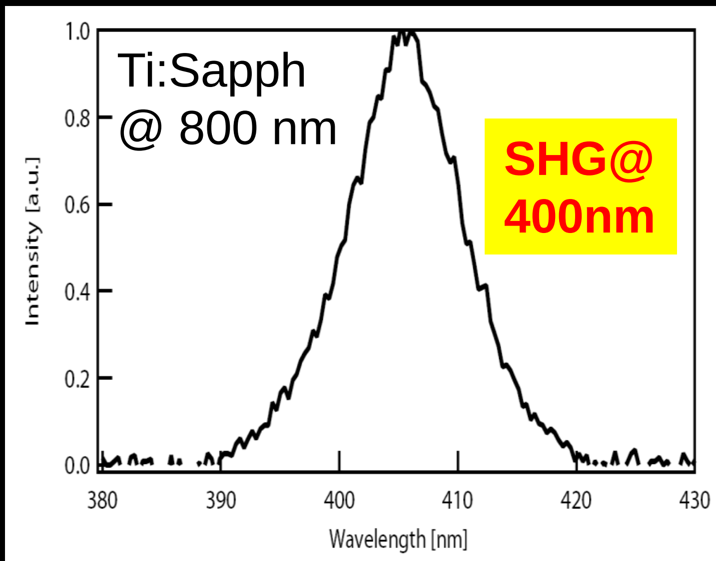
Wavelength Flexibility

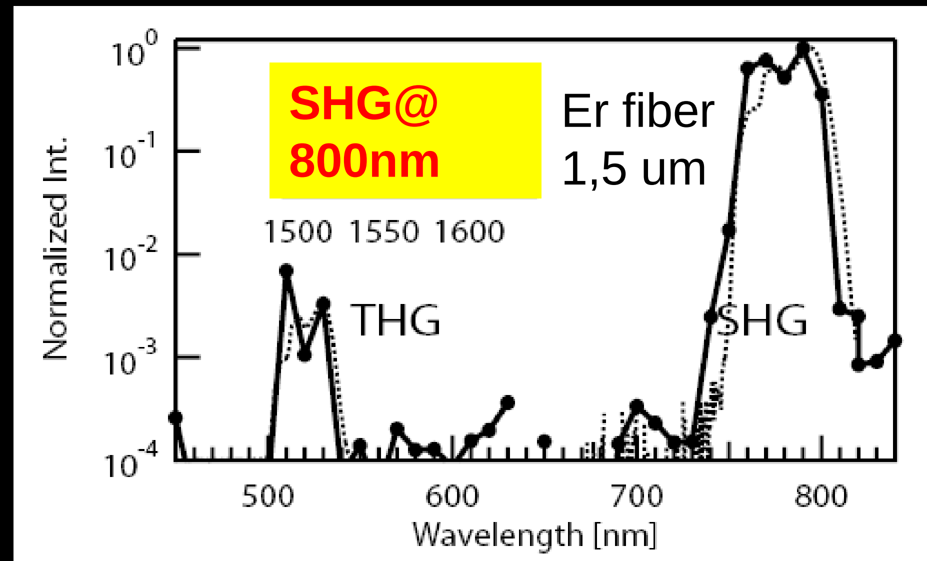


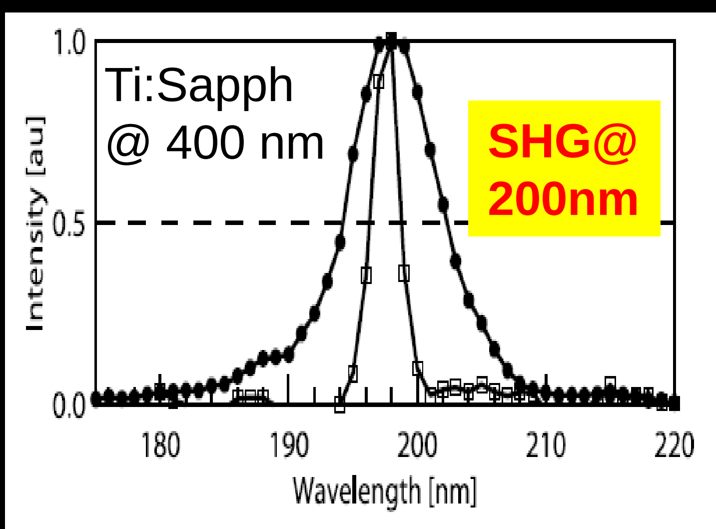
Breast cancer cells (MDA MB 231) - $LiNbO_3$ SHG coated with PEG

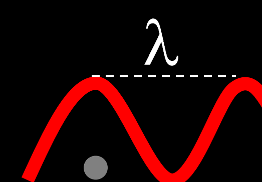
GapBio - unpublished

Wavelength Flexibility









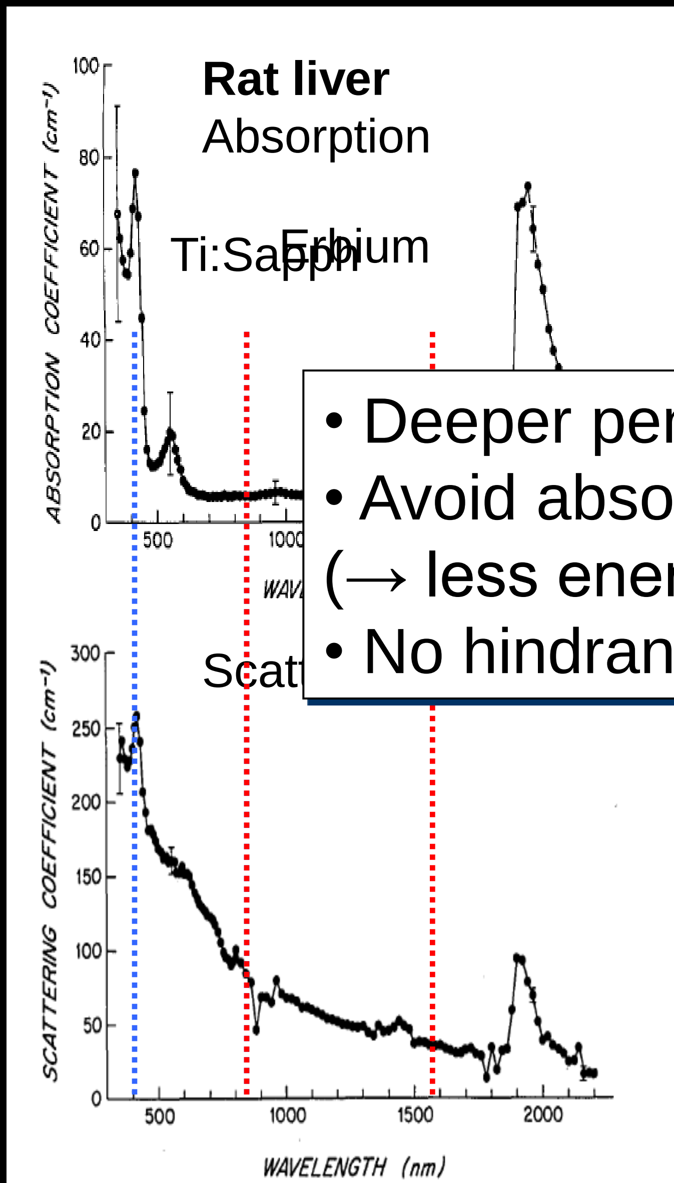
No phase-matching

→Broadband frequency doubling

→Forward and backward emission

J. Extermann et al., Appl. Phys. B, 97, 537 (2009)

Tissue penetration

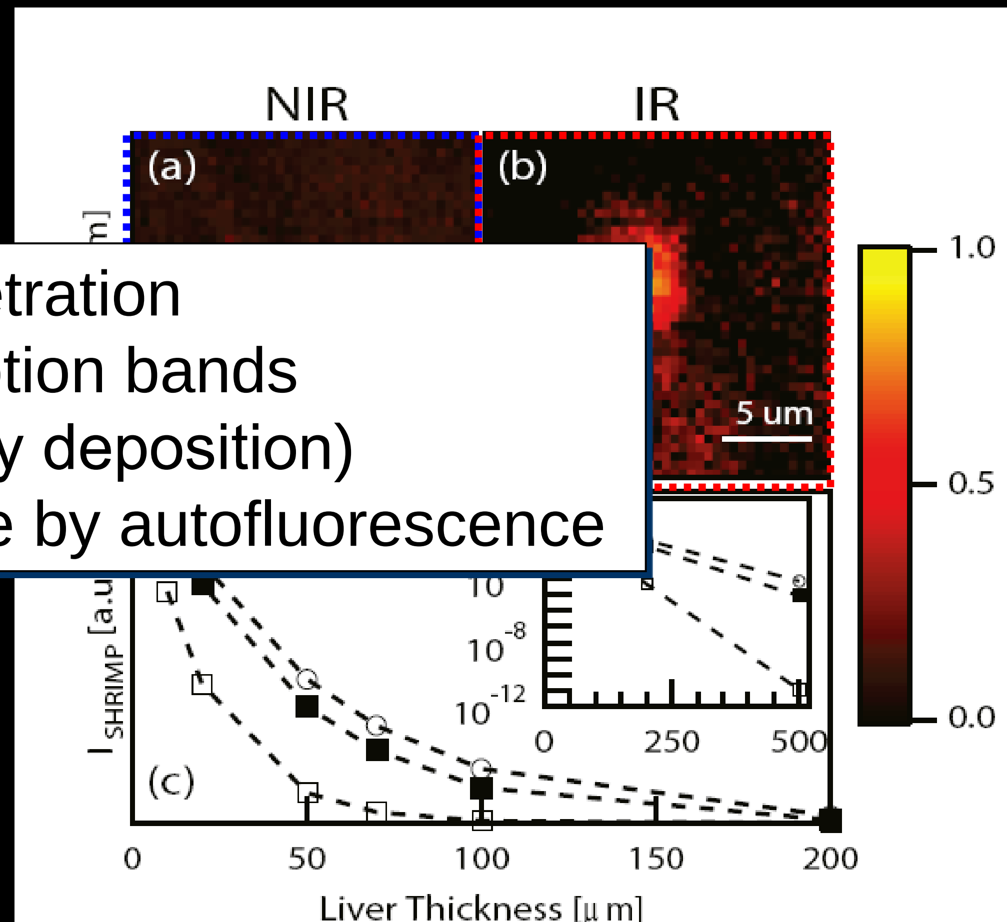


P. Parsa, *Appl.Opt.*, 28, 2325 (1989)

- Deeper penetration
- Avoid absorption bands

(→ less energy deposition)

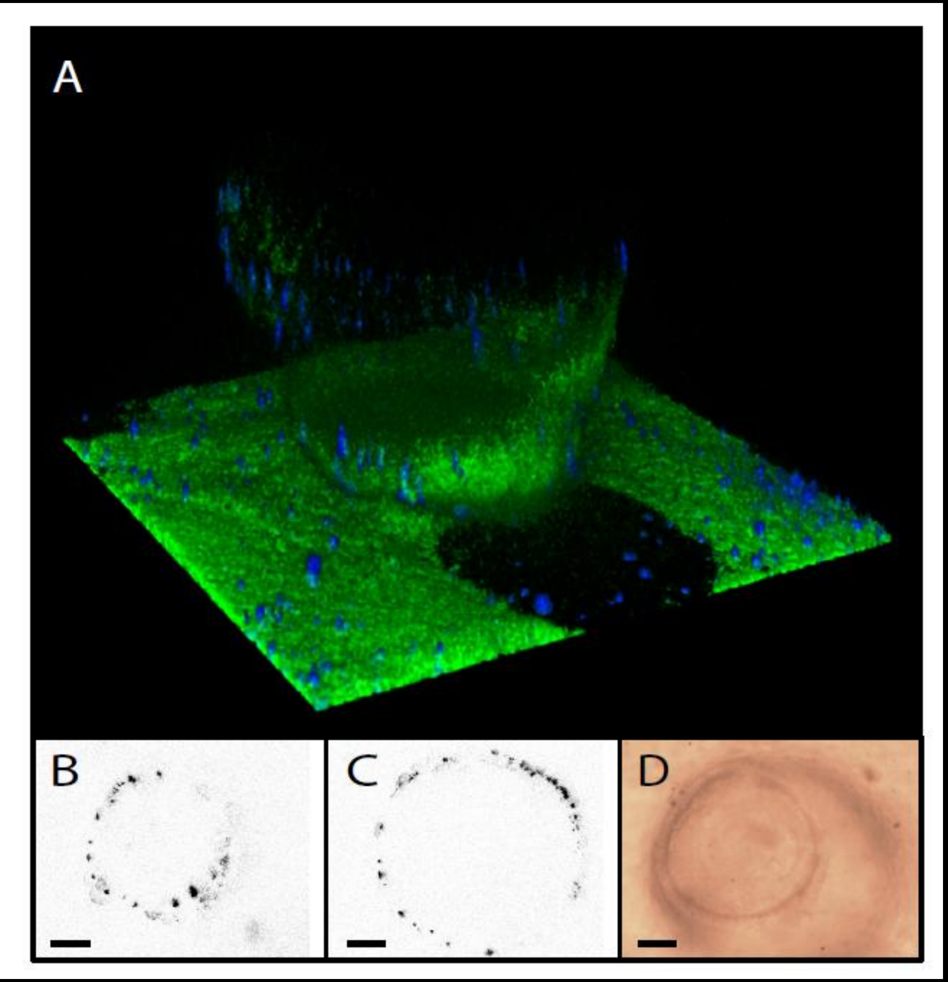
- No hindrance by autofluorescence



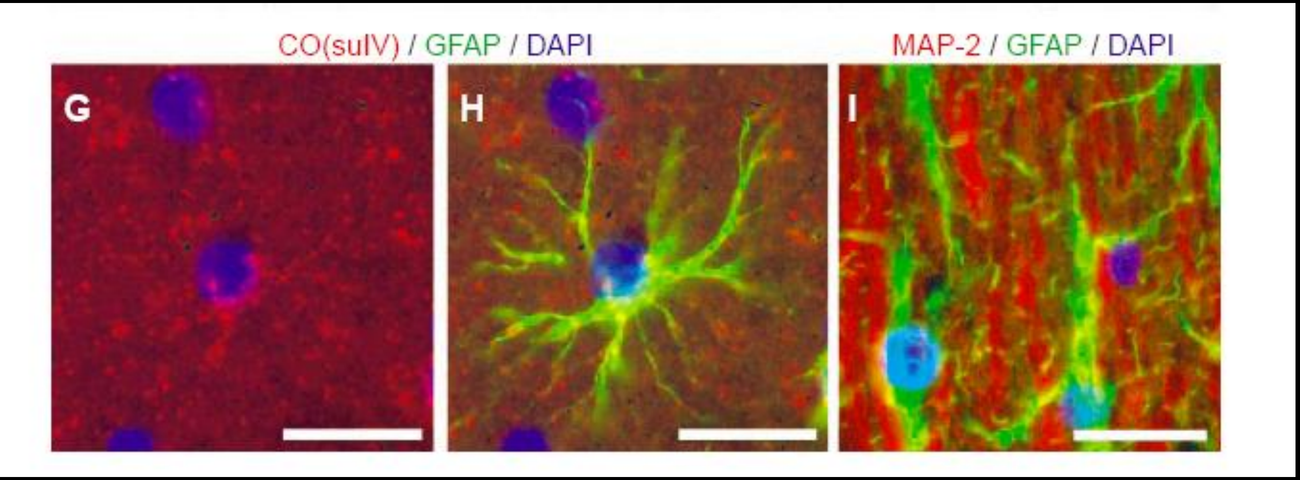
J. Extermann et al., *Optics Express* 17, 15347 (2009)

Cardiac Stem-Cells
"Live" - 4D

$KNbO_3$ SHG coated with PEG



Endo- and Exogenous Fluorescence



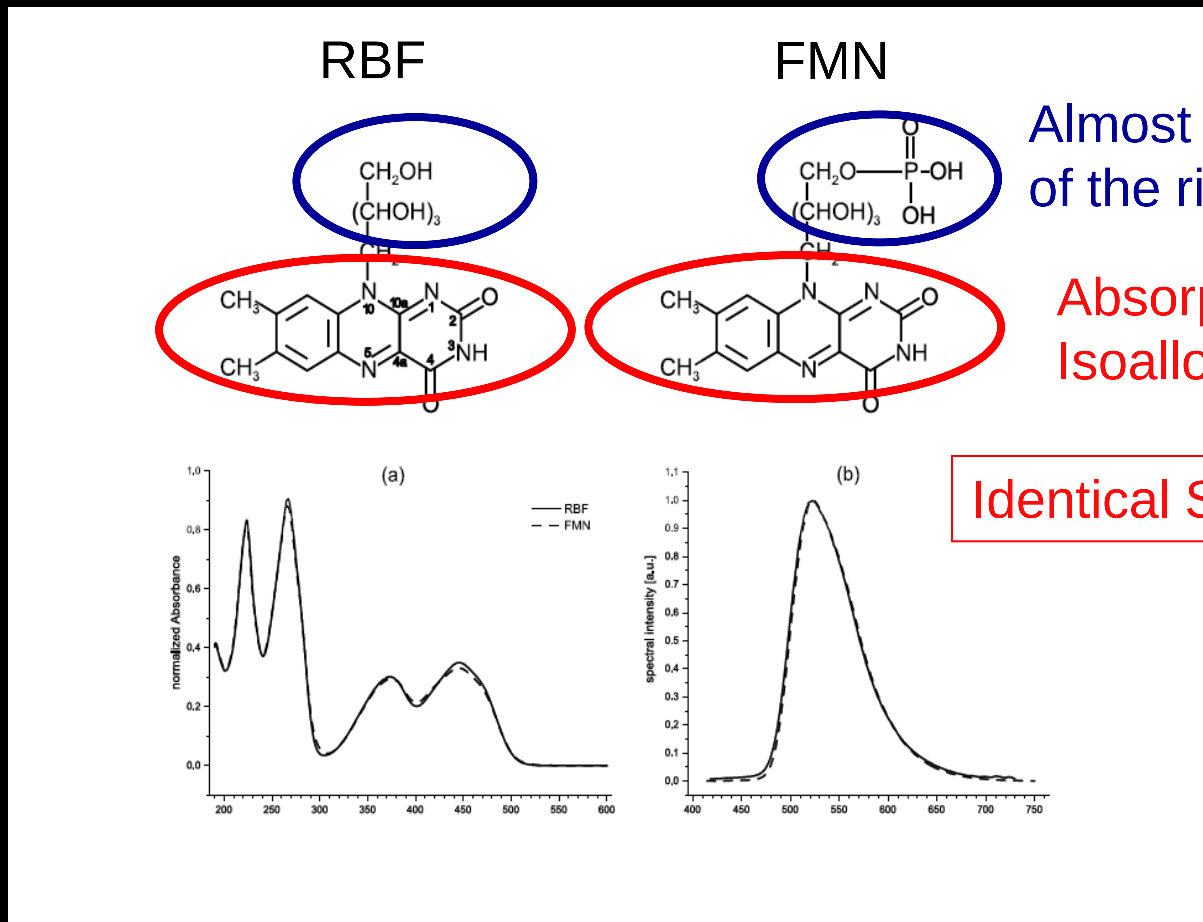
SELECTIVITY = CHEMICAL **PERTURBATION**

LABEL FREE CELL IMAGING AND DIAGNOSTICS

Solution for discrimination : COHERENT CONTROL ?

W. Webb et al, Science 305, 100 (2004)

Nearly Identical Cellular Biomolecules: Riboflavin and FMN

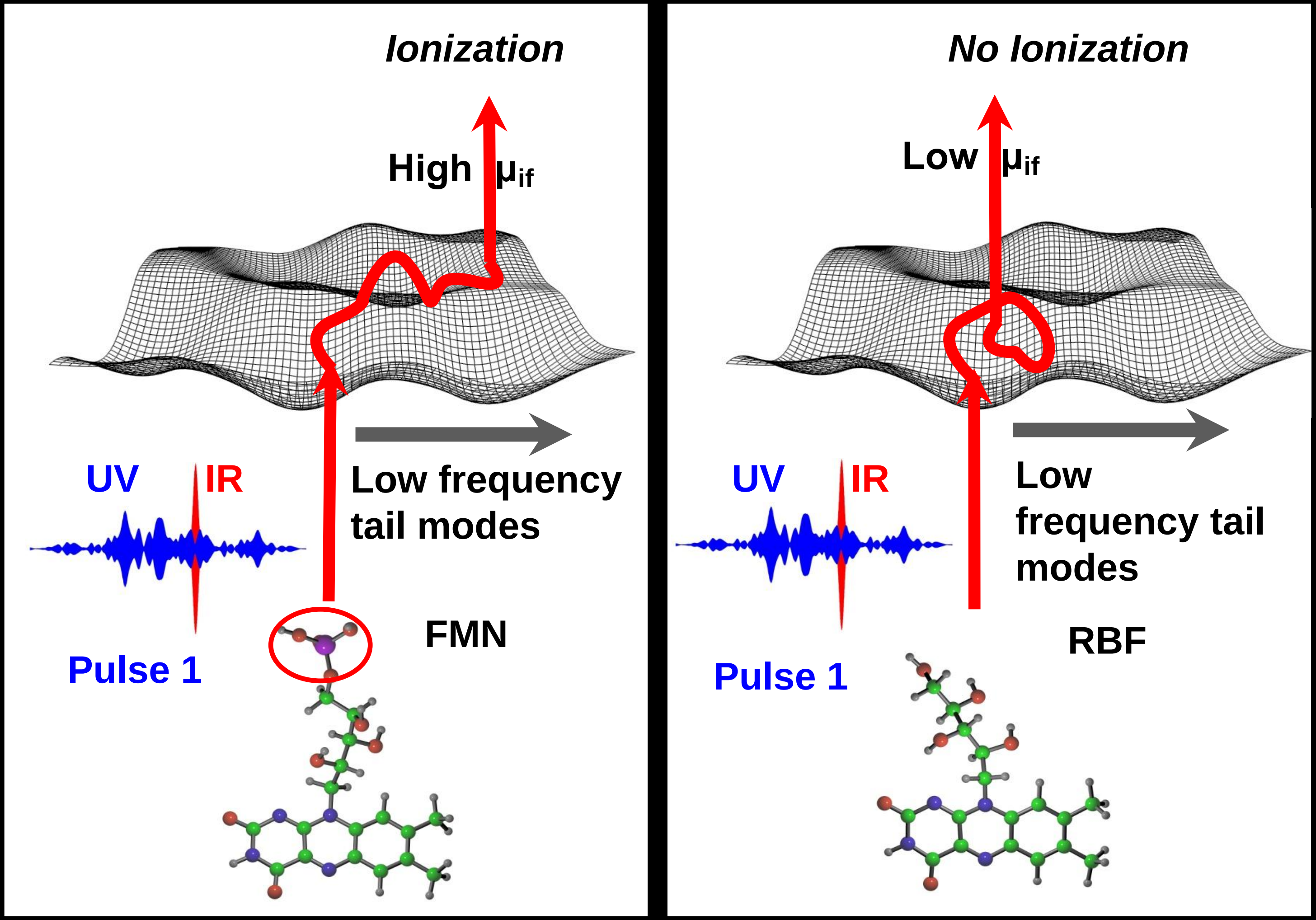


Almost no effect
of the ribityl side chai

Absorption in the
Isoalloxasine ring

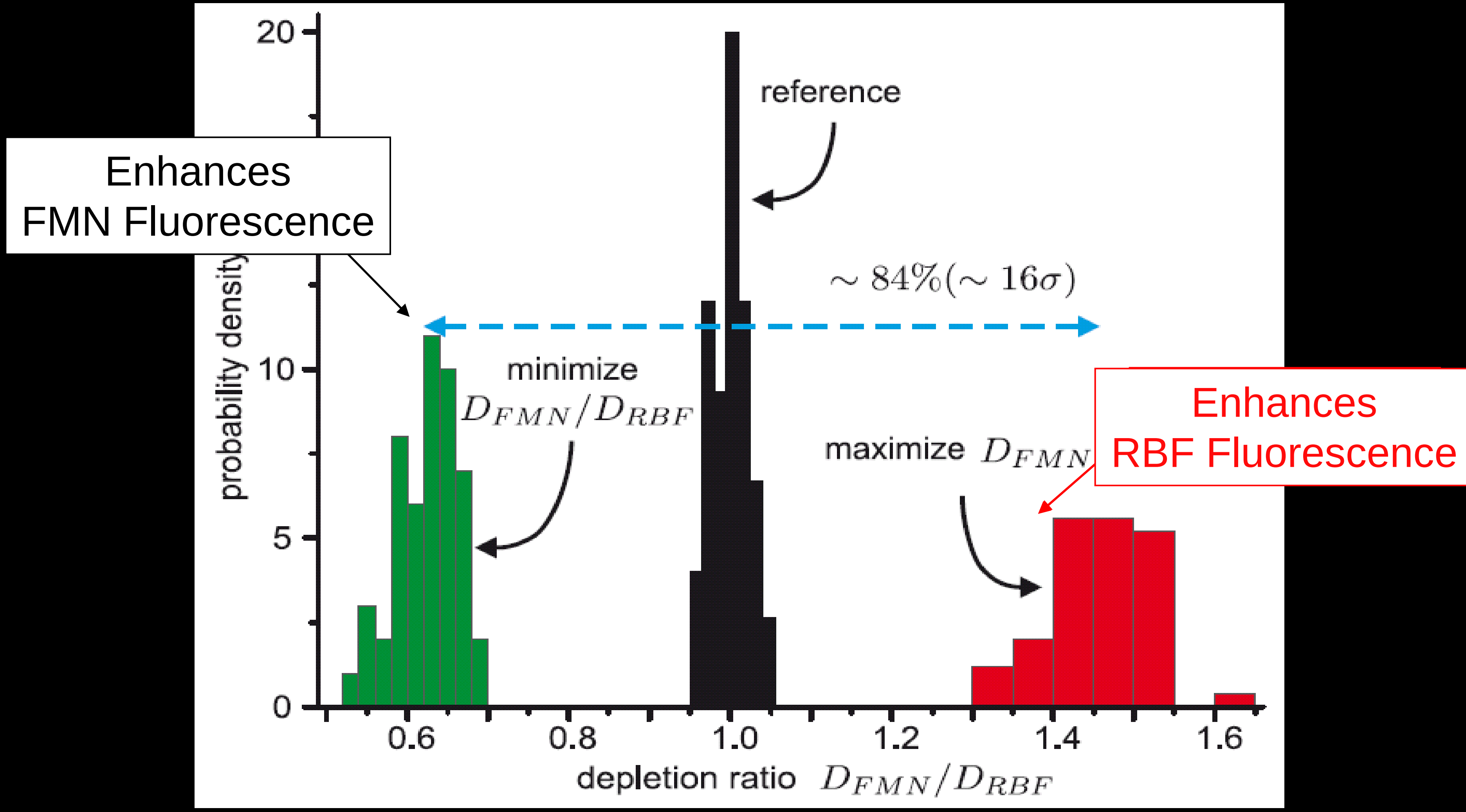
Identical Spectra !

Optimal Discrimination (ODD)



J.Petersen, R. Mitric, V.Bonacic-Koutecky,J.P. Wolf, J.Roslund, H. Rabitz, Phys.Rev.Lett 105, 073003 (2010)

ODD in Flavins



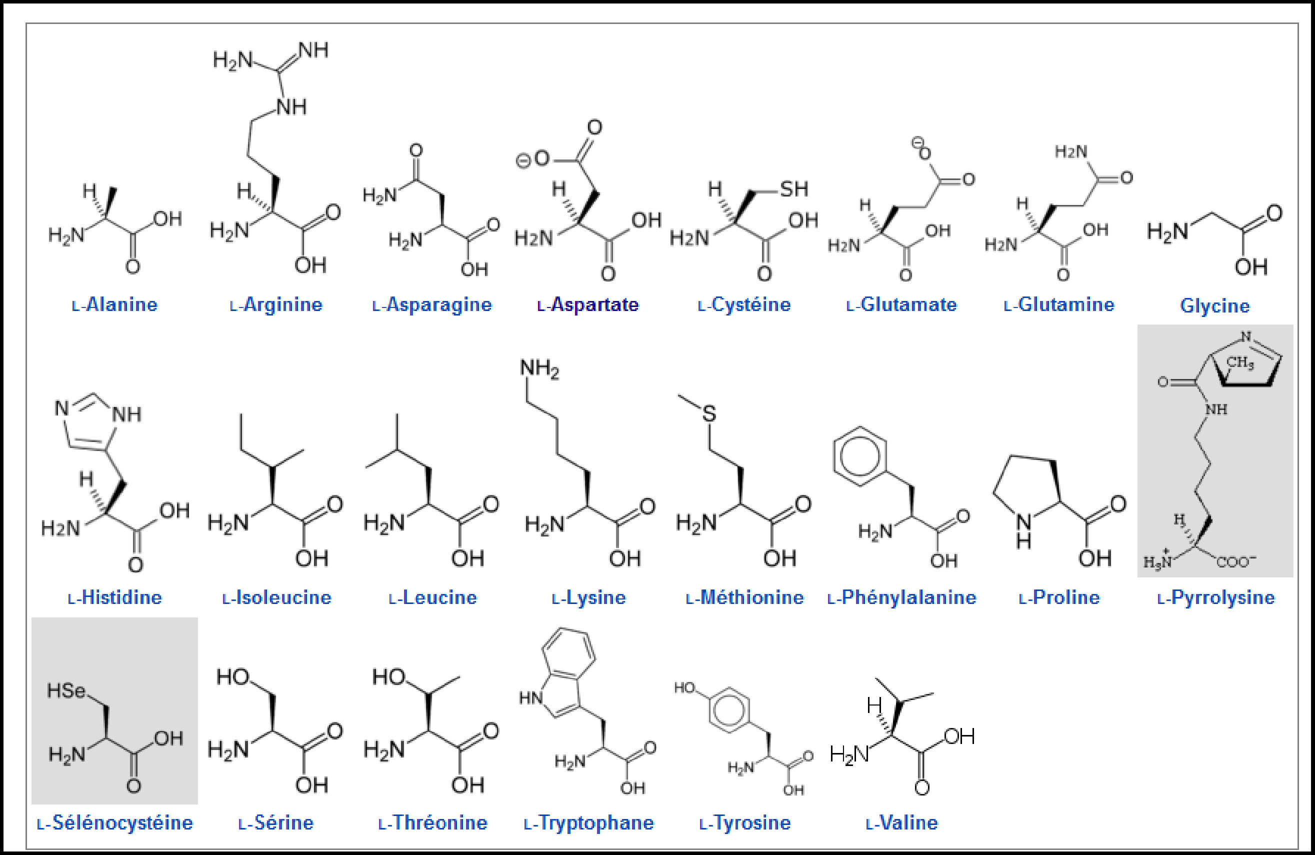
Roth et al, Phys.Rev.Lett 102, 253001 (2009)

Relative Mixing Ratios Determination in the Mixture (same Cell)

	$\bar{c}^*(RBF)$	$\bar{c}^*(FMN)$	$c^*(RBF)$	$\sigma(RBF)$	$c^*(FMN)$	$\sigma(RBF)$
(a)	0.30	0.70	0.24	0.10	0.82	0.19
(b)	0.50	0.50	0.55	0.07	0.47	0.09
(c)	0.33	0.66	0.35	0.04	0.68	0.05

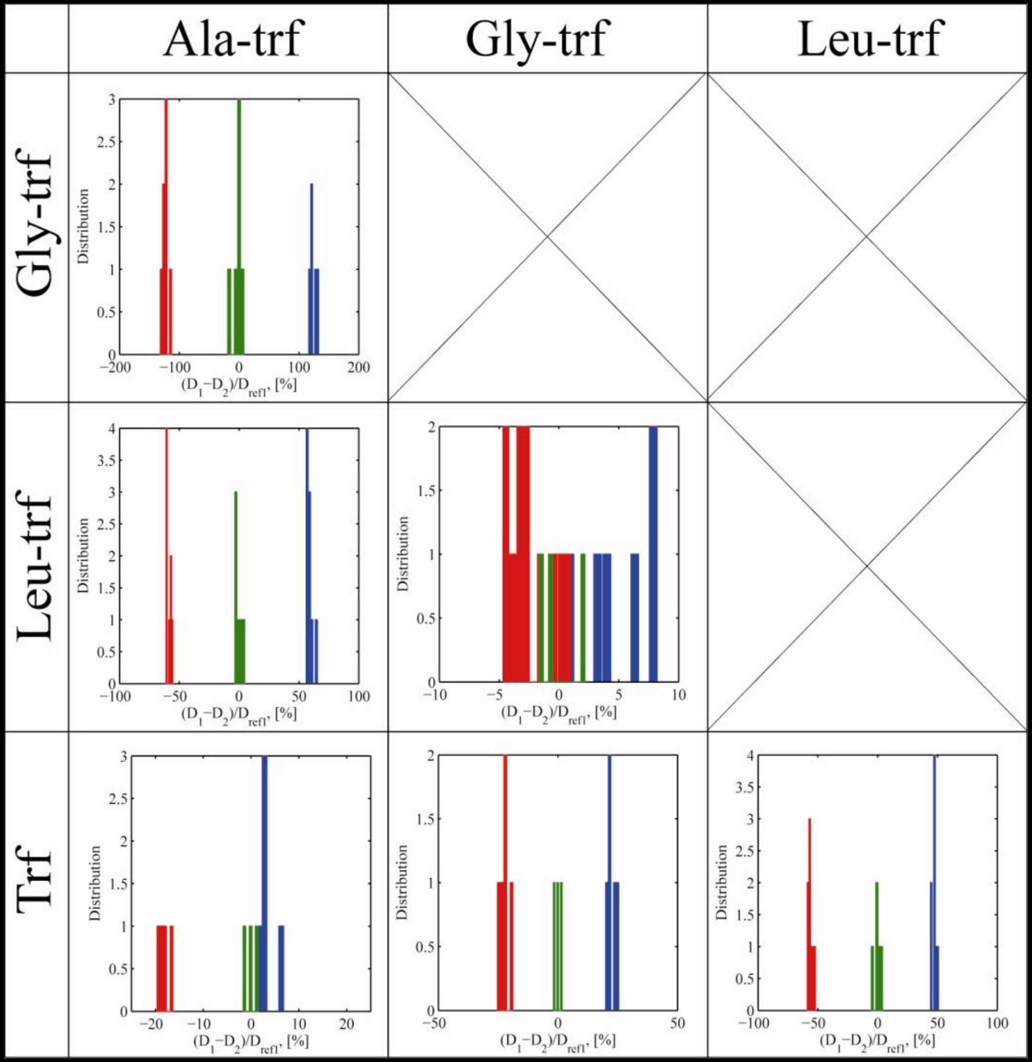
Roth et al, Phys.Rev.Lett 102, 253001 (2009)

The Amino-Acids



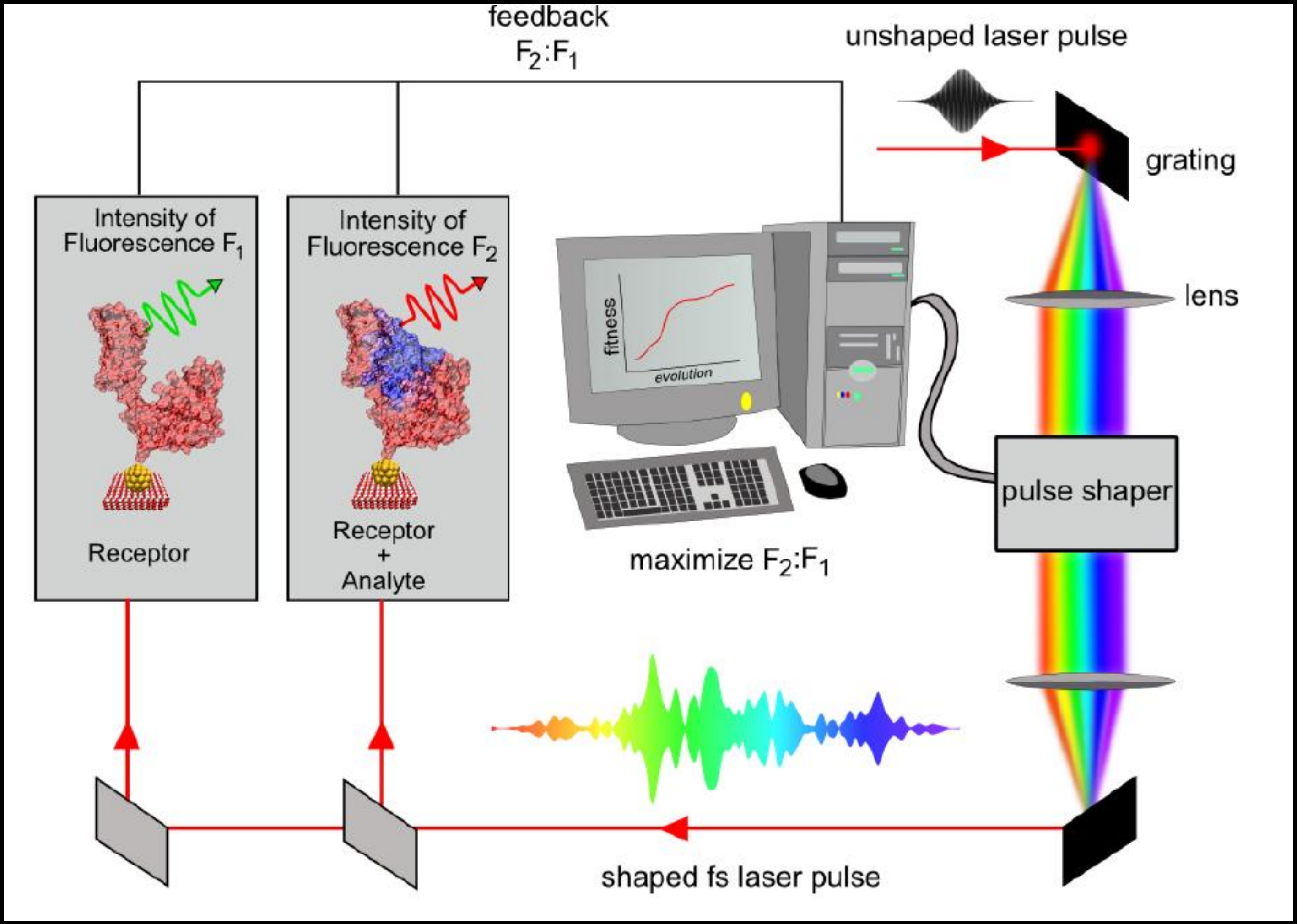
Peptidic Bonds between AAs make Proteins

Discrimination of Peptides

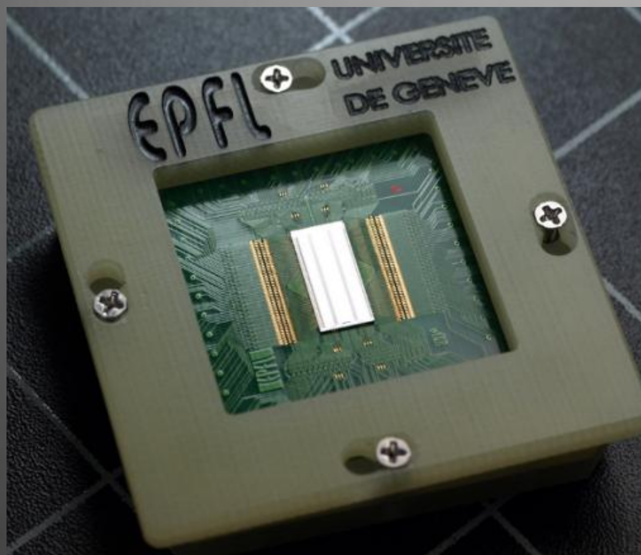


GapBio - unpublished

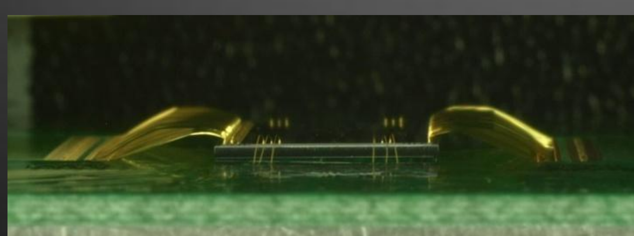
Next Step: Label-Free Bioassays

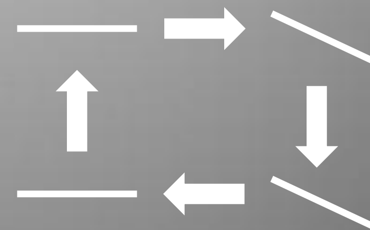


MEMS Laser Pulse Shaper
(UniGe-IMT-EPFL)

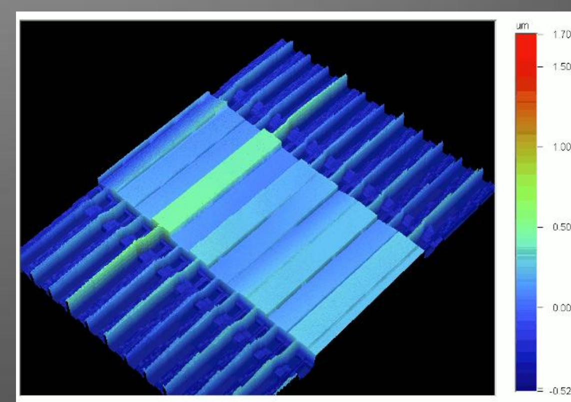


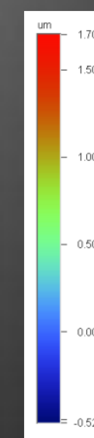
S. M. Weber *et al.*, *Proc. SPIE* **7594**, 75940J (2010)





Piston- and Tilt-Loop

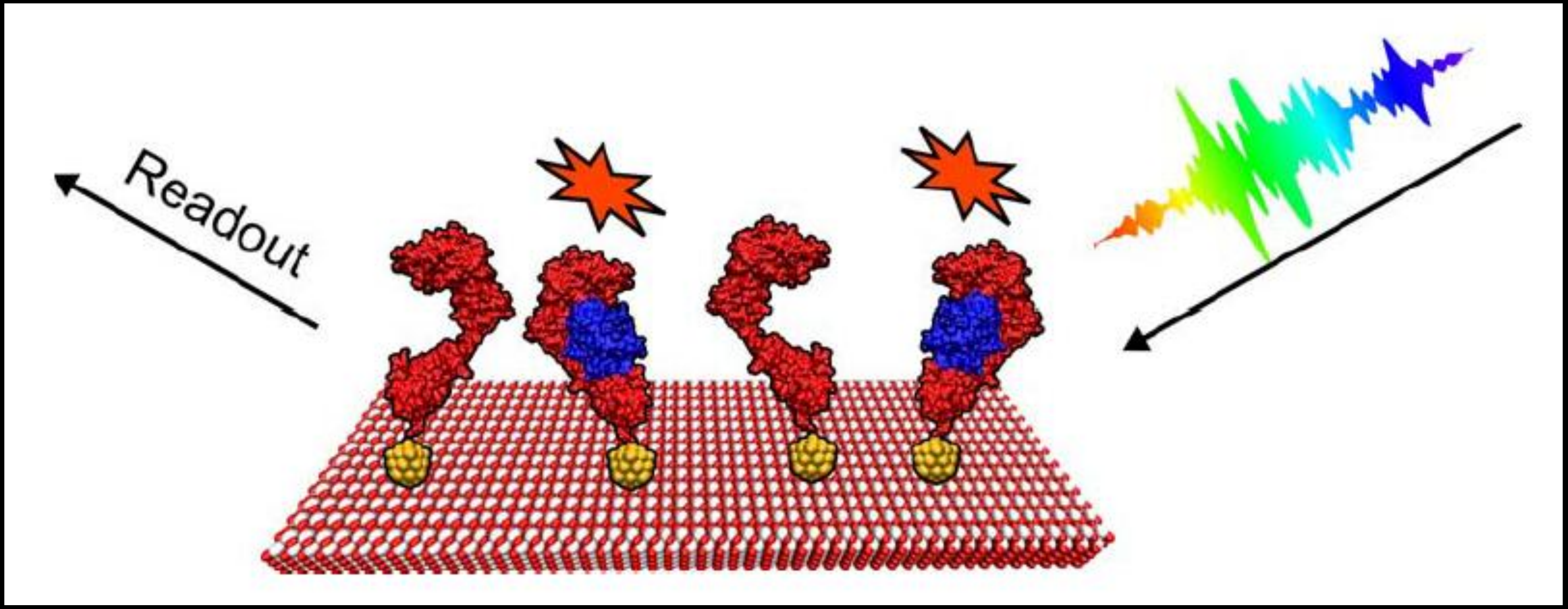




0 → 60 V piston and tilt for two mirrors independently

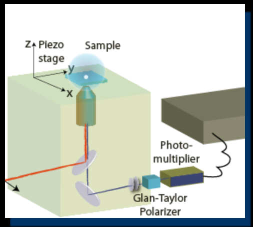
White-Light Interferometry

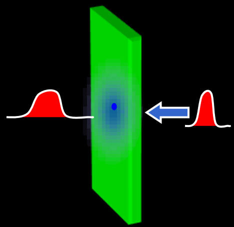
Next Step: Label-Free Bio-Chips

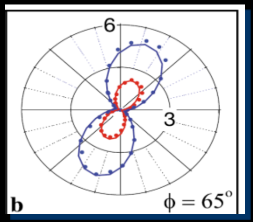


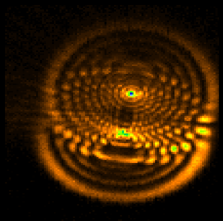
« Label-Free Bioassays », US Patent, Pending (2011)

Conclusions









- Nano-doublers in strong development: FP7: characterized, fonctionalized, tested for toxicity, applied to actual cancer and stem cell research
- Collaboration with Nikon Microscopes
- Multimodality

-Label free discrimination by Coherent Control demonstrated on peptides
- Imaging to be tested
- Label free immuno-assays, Patent
- Industrial interest (Innanovate, UK)

THANK YOU

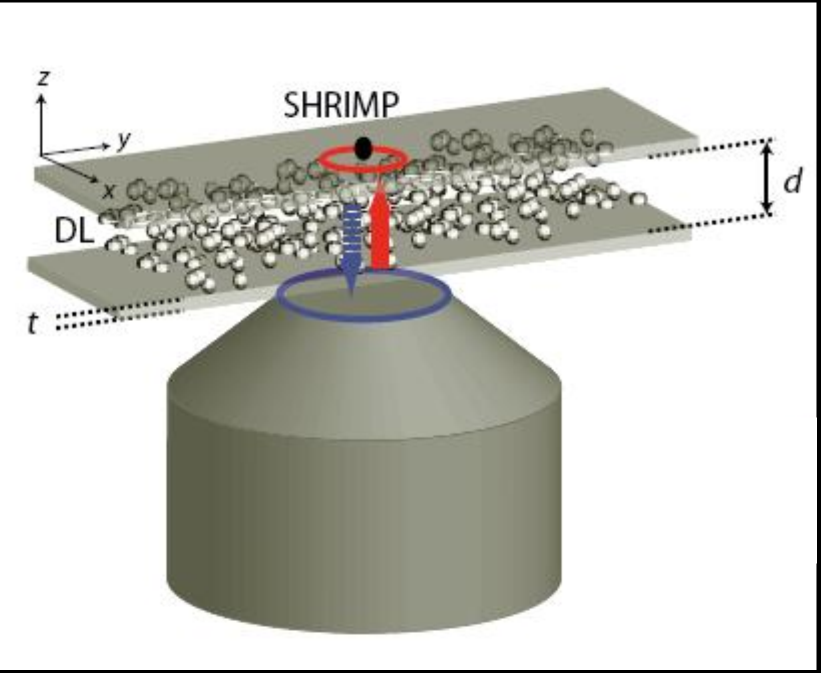
Group Leader :	J.P. Wolf
Senior Scientists:	J. Kasparian
	L. Bonacina
	A. Stepanov
Post-Docs:	**F. Courvoisier**
	S. Weber
	J. Extermann
	P. Bejot
	M. Petrarca
Biologist**:**	**C. Kasparian**
PhD Students:	**A. Rondi**
	S. Henin
	D. Kiselev
	T. Magouroux
	S. Afonina
	O. Nenandl
Engineer:	M. Moret
Master Students:	**S. Courvoisier**
	D. Mohit

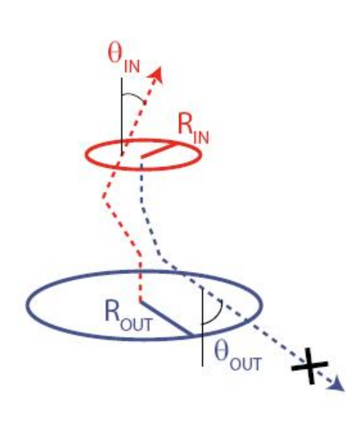
COLLABORATIONS

IMT-EPFL Neuchatel
H. Rabitz group, Princeton
FP7: Namdiatream
NCCR : MUST Consortium

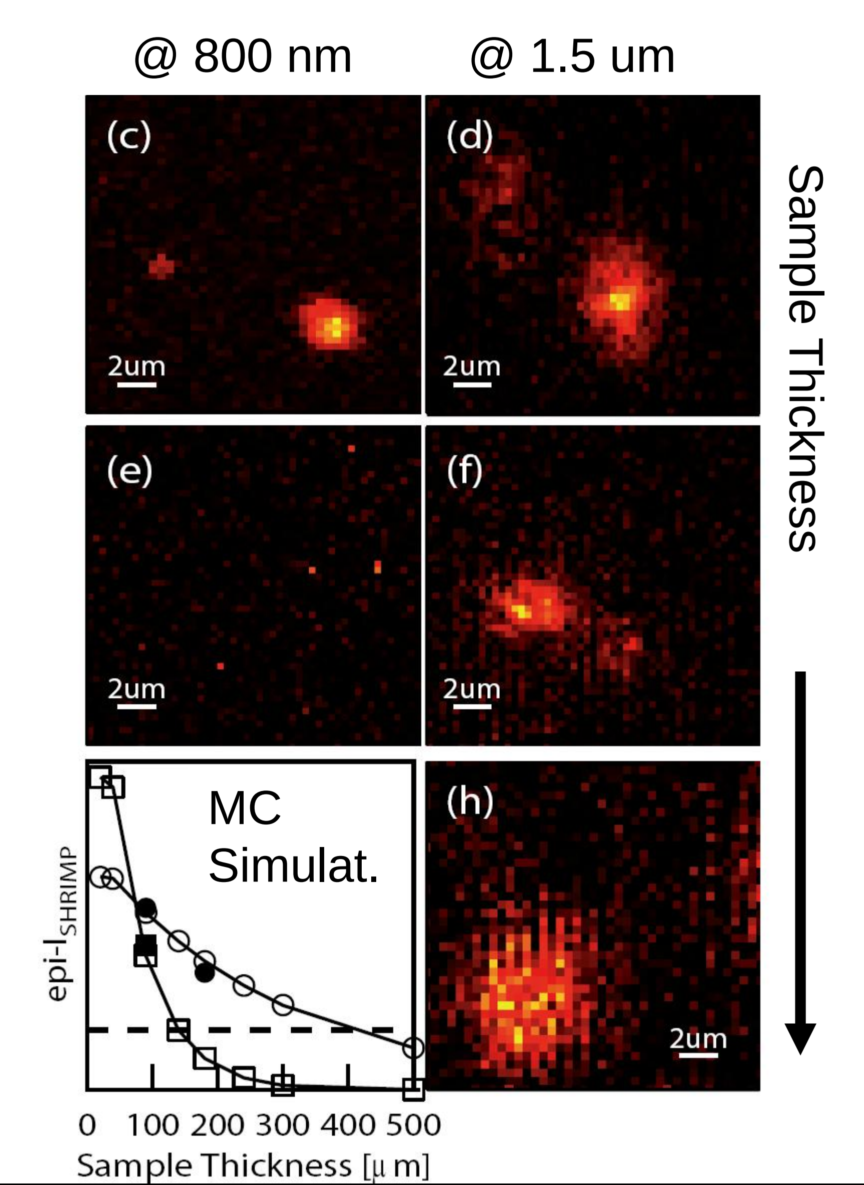
THEORY
V. Bonacic-Koutecky, HU Berlin
R. Mitric, FU Berlin

Imaging through scattering tissues



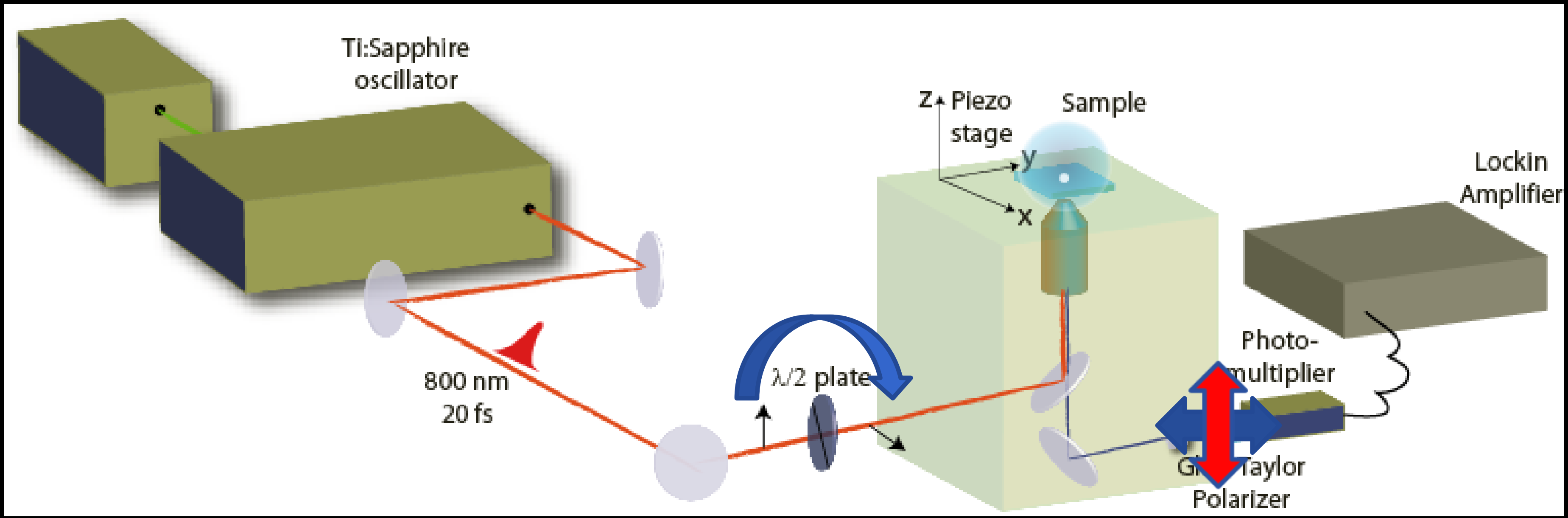


Monte Carlo simulation
Multiple scattering
Mie theory

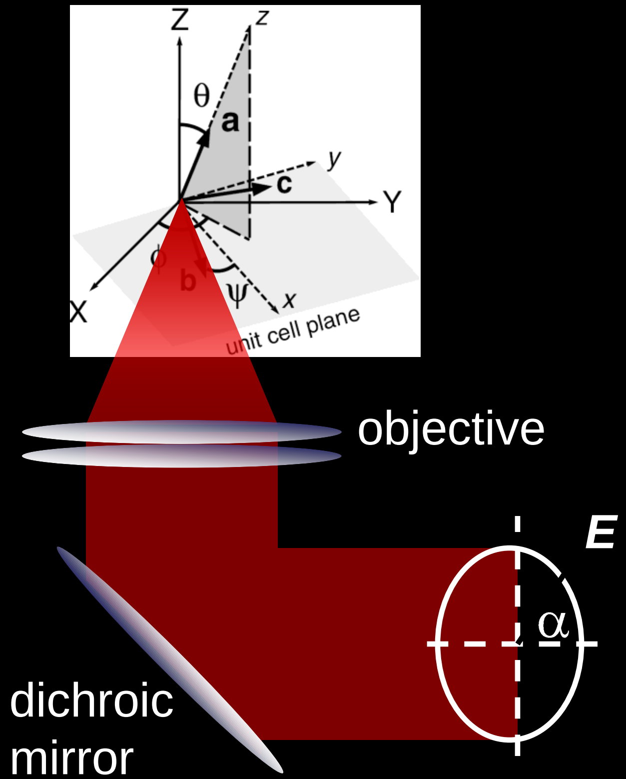


J. Extermann et al., *Optics Express* 17, 15347 (2009)

Polarization: *nanoCompass*



Model / laser excitation

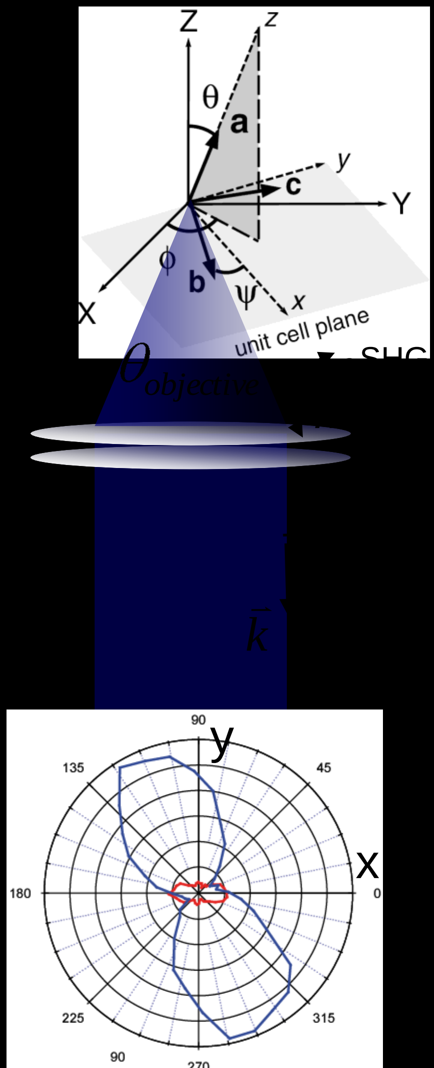


Polarization at the focal point:

$$\vec{E}(\alpha,\delta,\gamma,\omega t) = \frac{E}{\sqrt{1+(1-\gamma)^2}} \begin{bmatrix} \cos\alpha\cos(\omega t) \\ (1-\gamma)\sin\alpha\cos(\omega t+\delta) \\ 0 \end{bmatrix}$$

V. Le Floc'h et al., J. Phys. Chem B 107, 12403 (2003)

Model / emission & detection



Crystal frame
→ lab referential

$$\vec{\varepsilon}^{SHG} = \vec{k} \wedge (\vec{P}^{(2)} \wedge \vec{k})$$

Objective

$$\mathbf{B}\vec{\varepsilon}^{SHG} = \begin{bmatrix} B_{xxx} & B_{xyy} & B_{xxy} \\ B_{yxx} & B_{yyy} & B_{yxy} \\ B_{zxx} & B_{zyy} & B_{zxy} \end{bmatrix} \begin{bmatrix} E_x^{\ 2} \\ E_y^{\ 2} \\ E_x E_y \end{bmatrix}$$

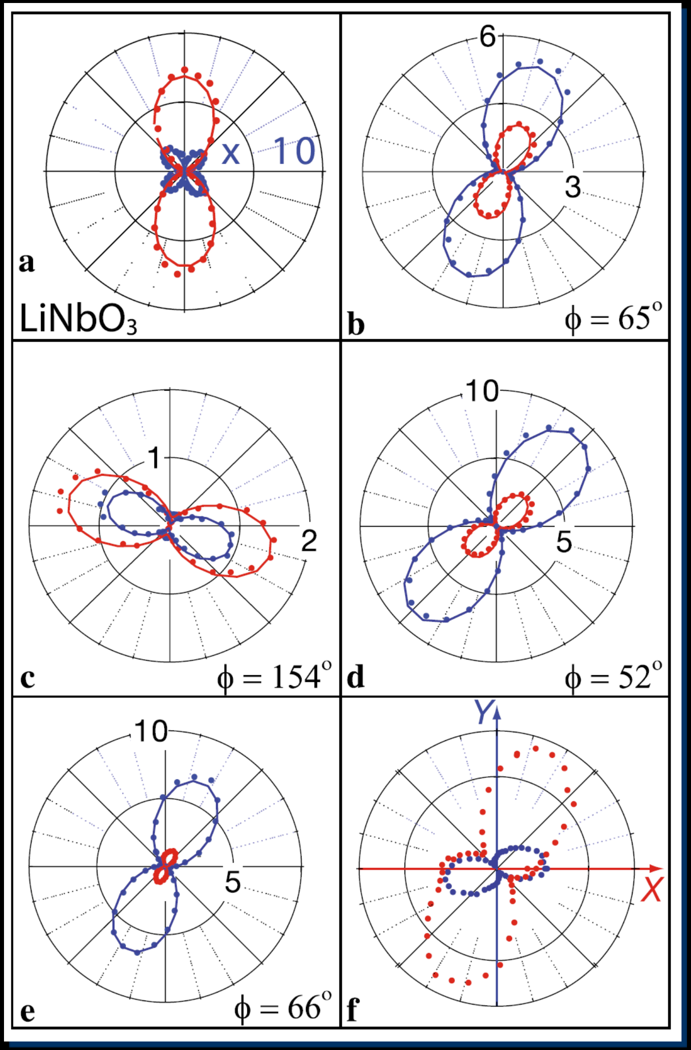
Integration over
angular variables
(coherent process)

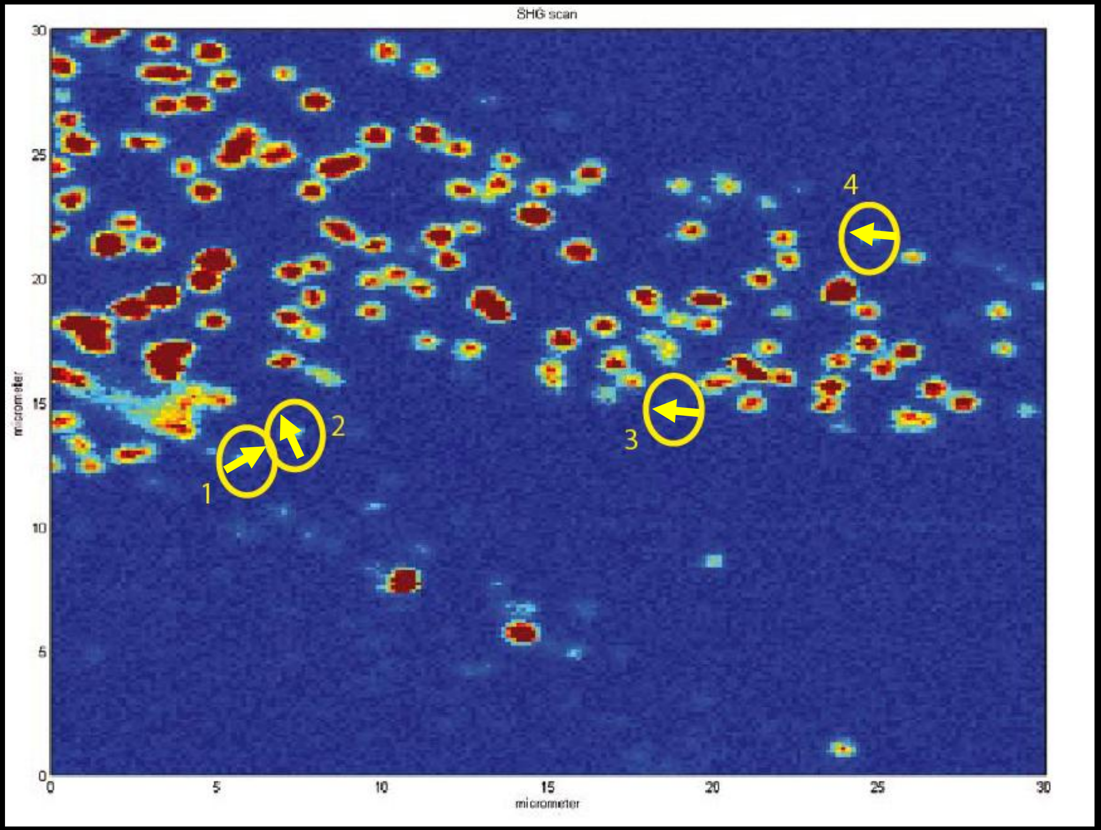
$$b_{ijk} = \int_0^{2\pi} \int_0^{\theta_{obj}} B_{ijk} \sin u \, \mathrm{d}u \, \mathrm{d}v$$

$$I_{i=x,y}^{SHG} = b_{ixx}^2 \overline{E_x^4} + b_{iyy}^2 \overline{E_y^4} + 2\left(b_{ixx} b_{iyy} + b_{ixy}^2\right) \overline{E_x^2 E_y^2} + \\ + 2 b_{ixx} b_{ixy} \overline{E_x^3 E_y} + 2 b_{iyy} b_{ixy} \overline{E_x E_y^3}$$

V. Le Floc'h et al., J. Phys. Chem B 107, 12403 (2003)

Polarization: *nanoCompass*





→ individual nanocrystal orientation
→ probes for local ***E*** field

Polarization: *nanoCompass*

Name	Point Group
$LiNbO_3$	3m
BBO	3m
KTP	mm2
$KNbO_3$	mm2
$BaTiO_3$	4mm
ZnO	6mm
$Fe(IO_3)_3$	6

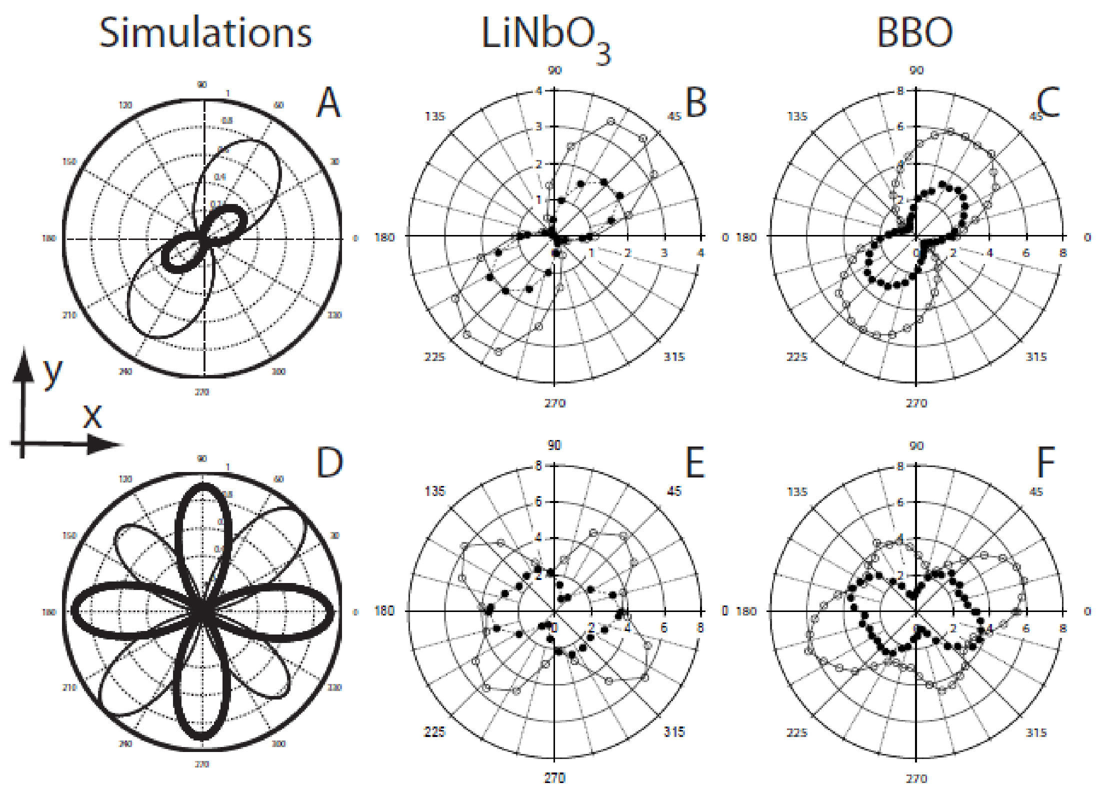
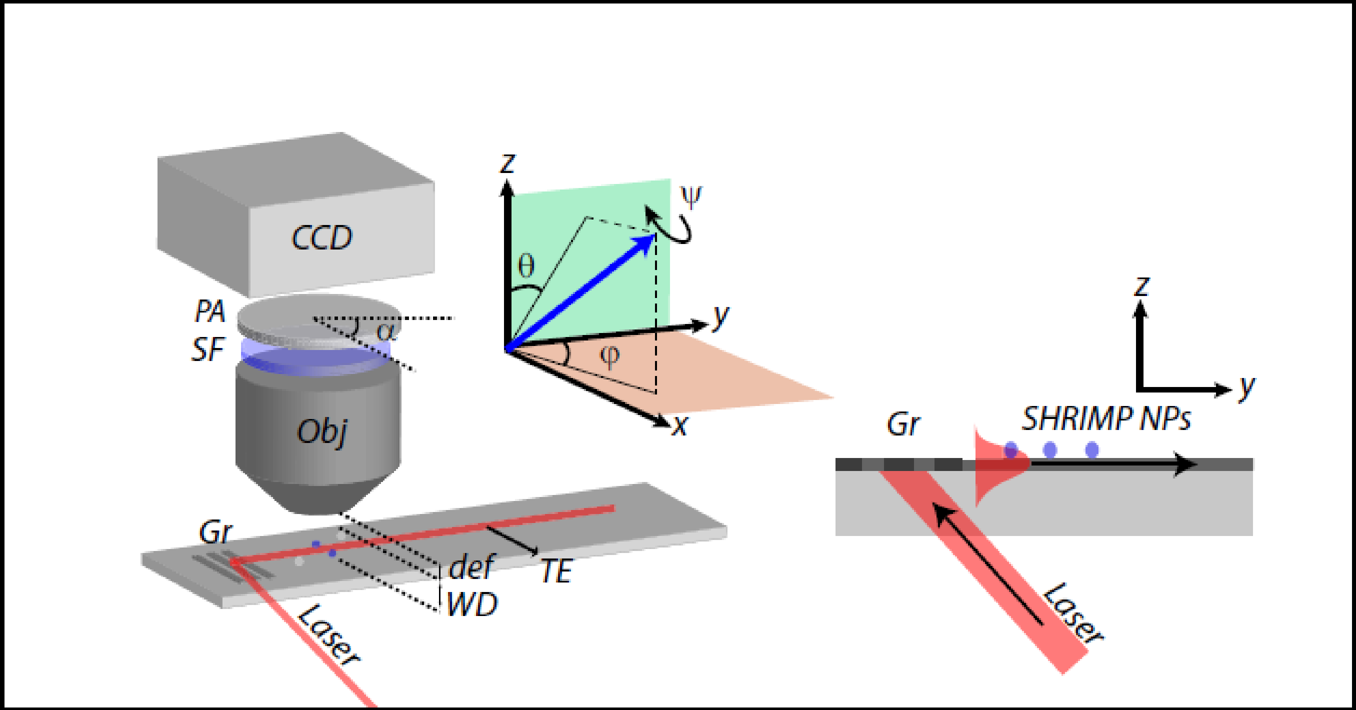


Figure 3.4: Polarization emission for 3m crystals. .

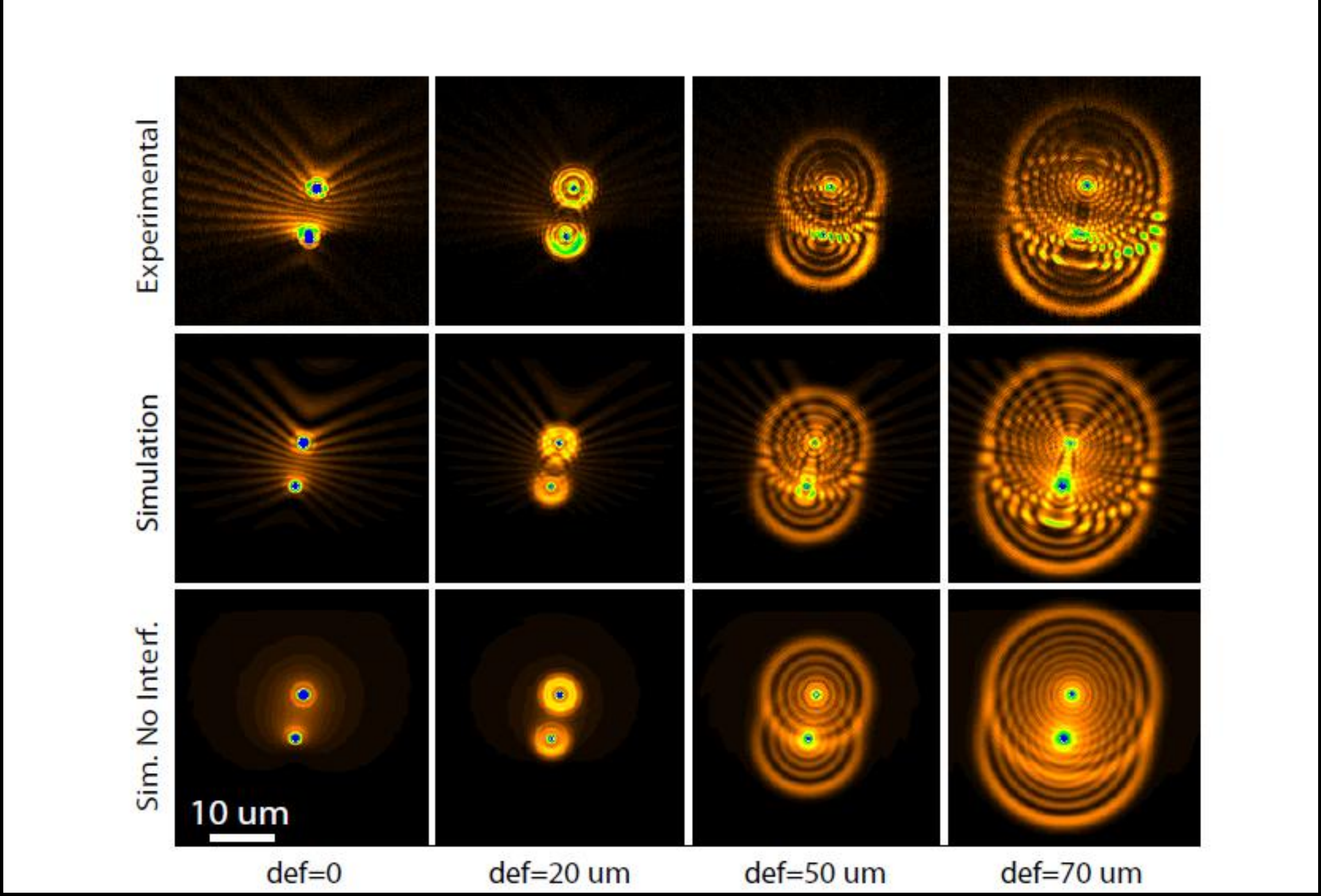
Coherence



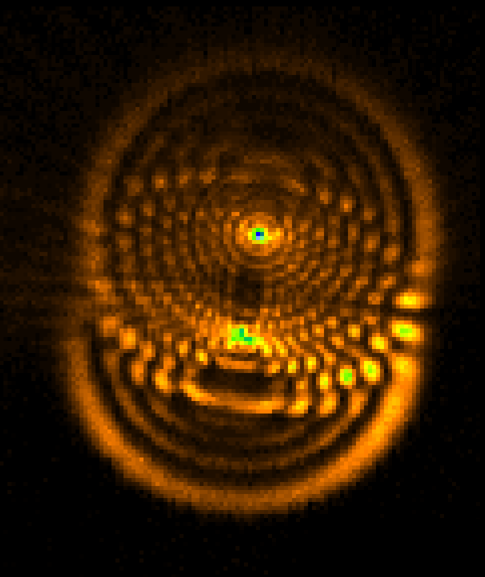
In collaboration with Laser-Laboratorium Göttingen GmbH and *J. Enderlein*

R. Baumner *et al.* submitted

Coherence


Experimental
Simulation
Sim. No Interf.
10 um
def=0
def=20 um
def=50 um
def=70 um

Coherence



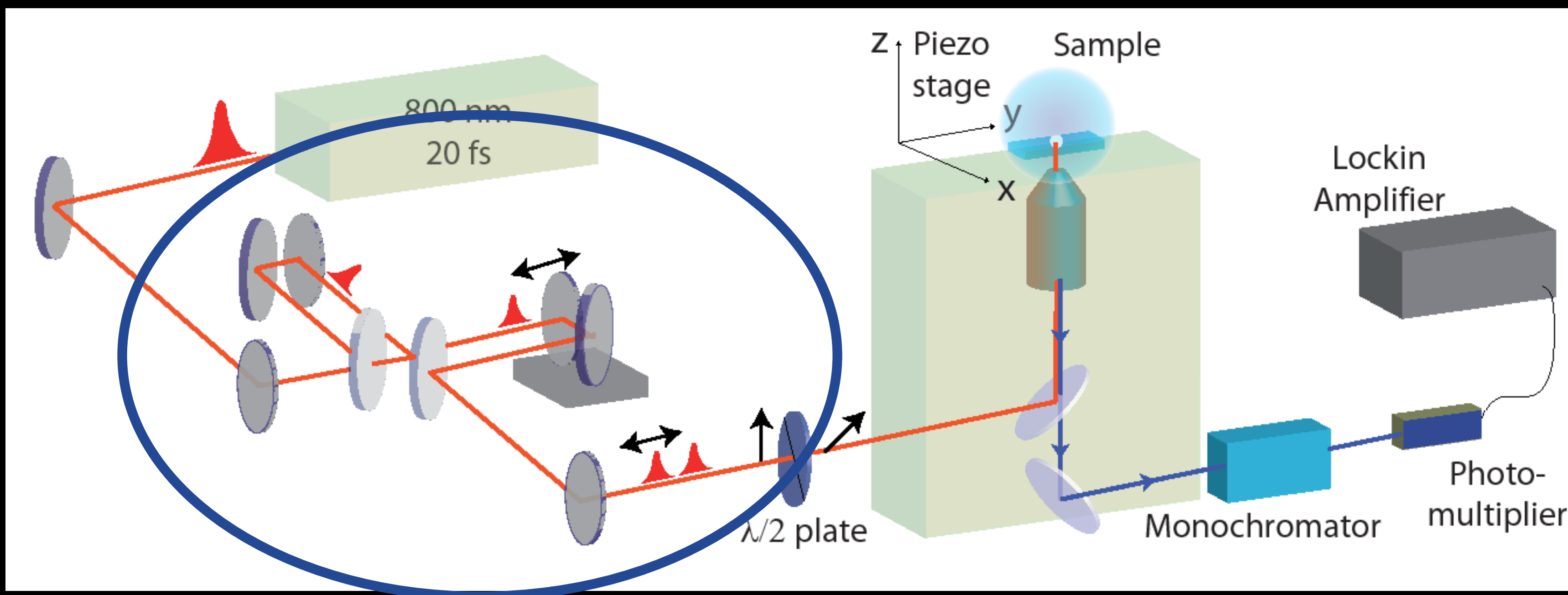
External Field Interference:

• Homodyne Detection (increase sensitivity) *Roch*

• Harmonic Holograpy (no scan axial position) *Psaltis*

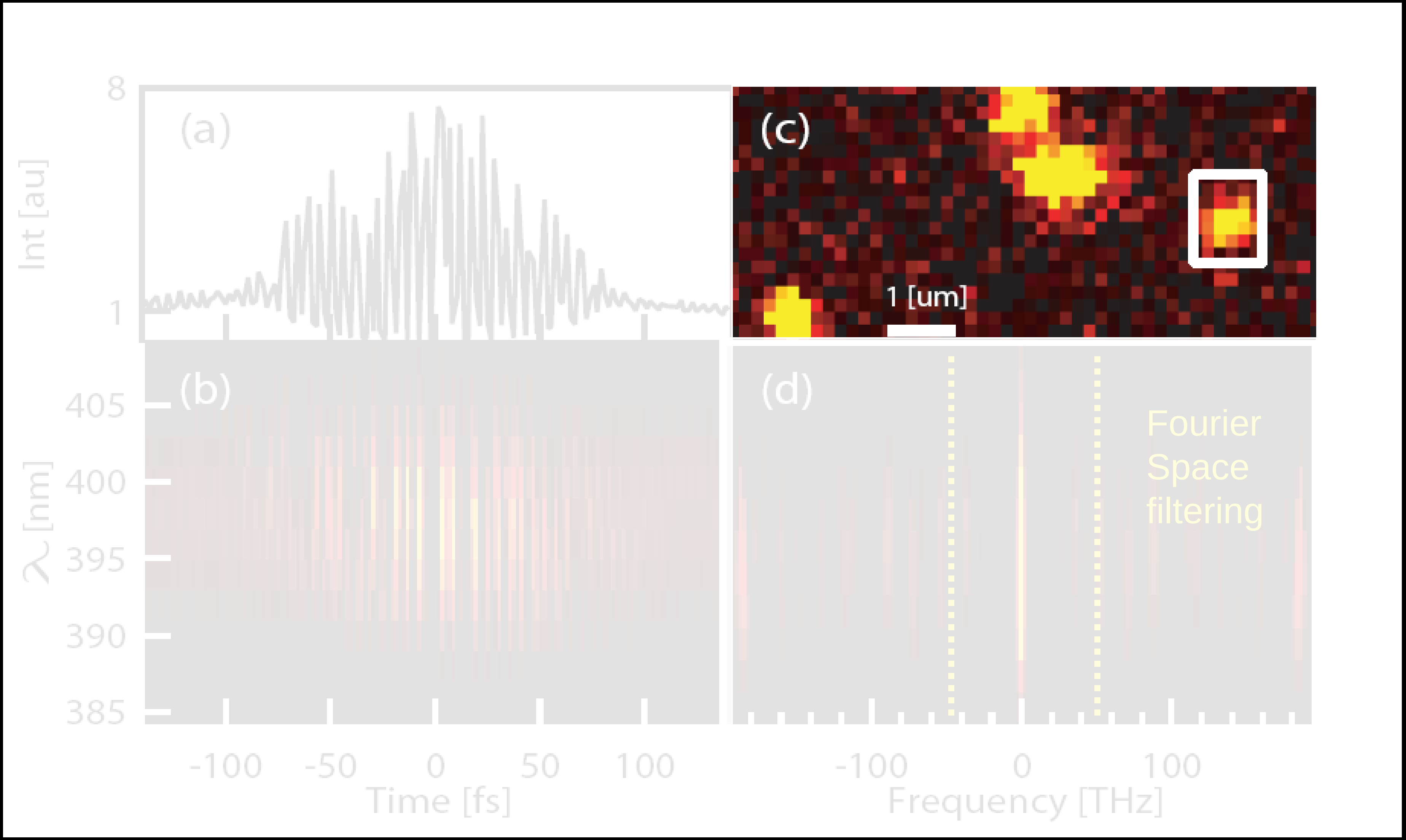
• Digital Phase Conjugation (optical turbidity suppression) *Psaltis (June 2010)*

nano-FROG* (Self Referenced)



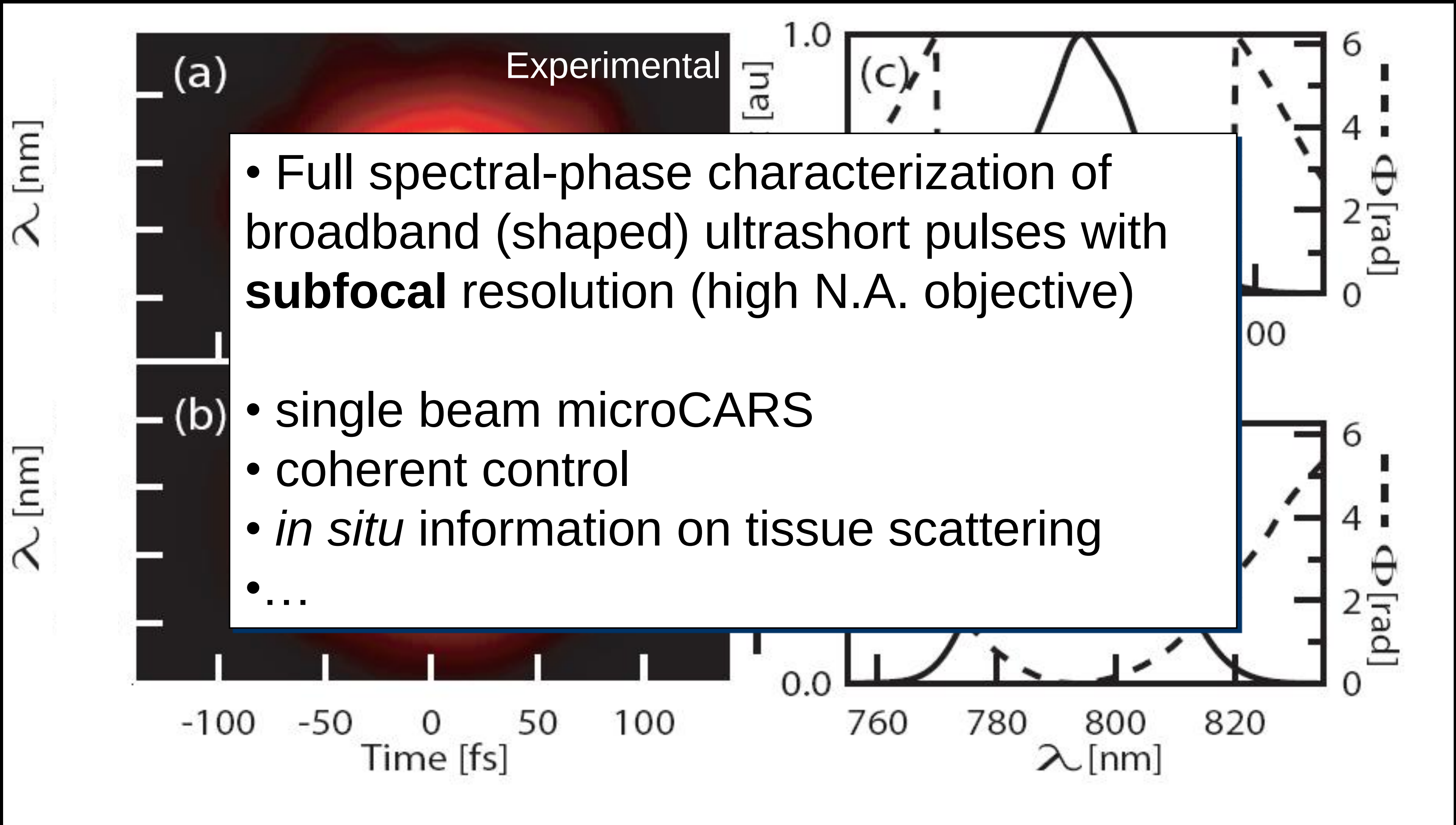
* Frequency
Resolved
Optical Gating

nano-FROG



J. Extermann et al., Optics Express 16,10405 (2008)

nano-FROG



- Full spectral-phase characterization of broadband (shaped) ultrashort pulses with **subfocal** resolution (high N.A. objective)

- single beam microCARS
- coherent control
- *in situ* information on tissue scattering
- …

J. Extermann et al., Optics Express 16,10405 (2008)

GAP
Biophotonics
http://www.gap.unige.ch/biophotonics/

GAP Biophotonics – Uni Geneva

J. Extermann, C. Kasparian, Prof J.-P. Wolf

Since July 2010

Namdiatream

Health-EU
The Public Health Portal of the European Union

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH
THE UNIVERSITY OF DUBLIN

SEVENTH FRAMEWORK PROGRAMME

Nikon

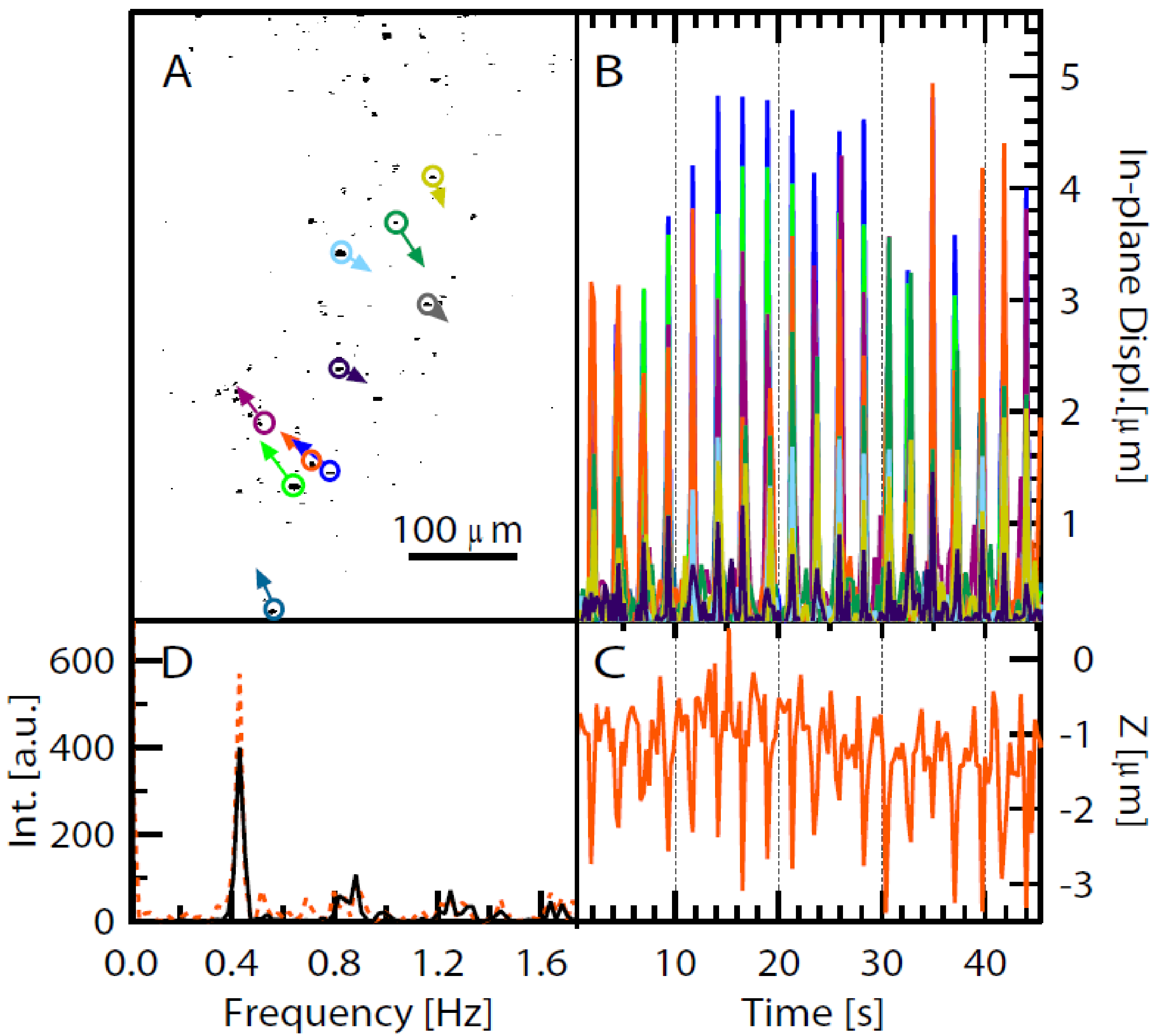
BD Chemical

Since March 2010

Interreg
FRANCE-SUISSE

MP0604
Optical Micro-Manipulation by Nonlinear Nanophotonics

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

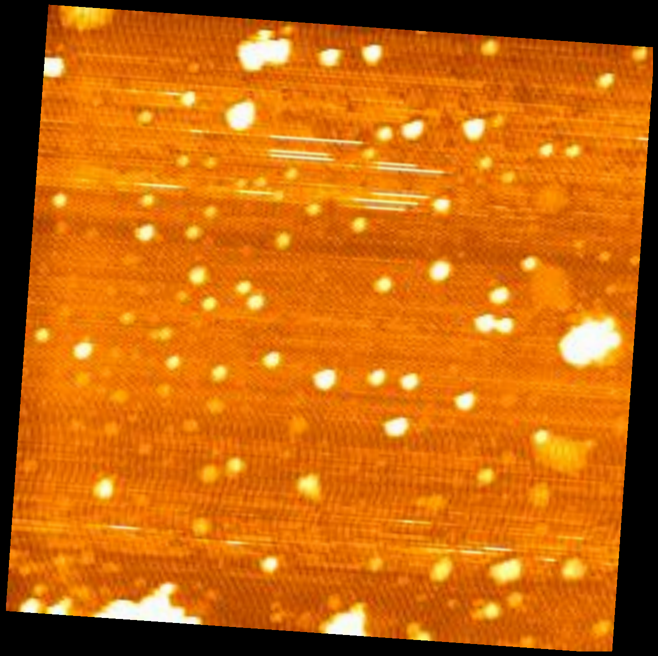


A
100 μm
B
In-plane Displ.[μm]
1
2
3
4
5
C
Z [μm]
0
-1
-2
-3
10
20
30
40
Time [s]
D
Int. [a.u.]
0
200
400
600
0.0
0.4
0.8
1.2
1.6
Frequency [Hz]

SHRIMPs

Second Harmonic Radiation IMaging Probes

(Inorganic) non-centrosymmetric nanoparticles



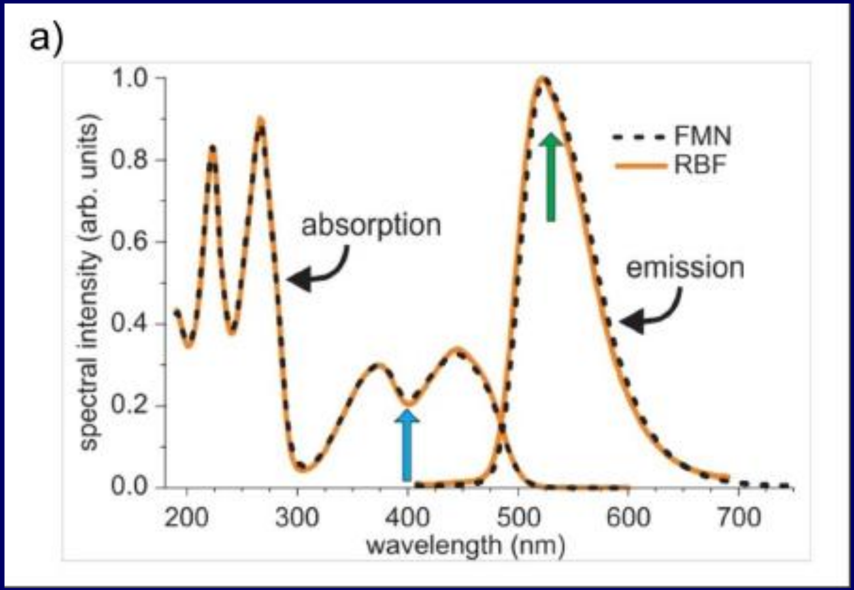
Since 2007:

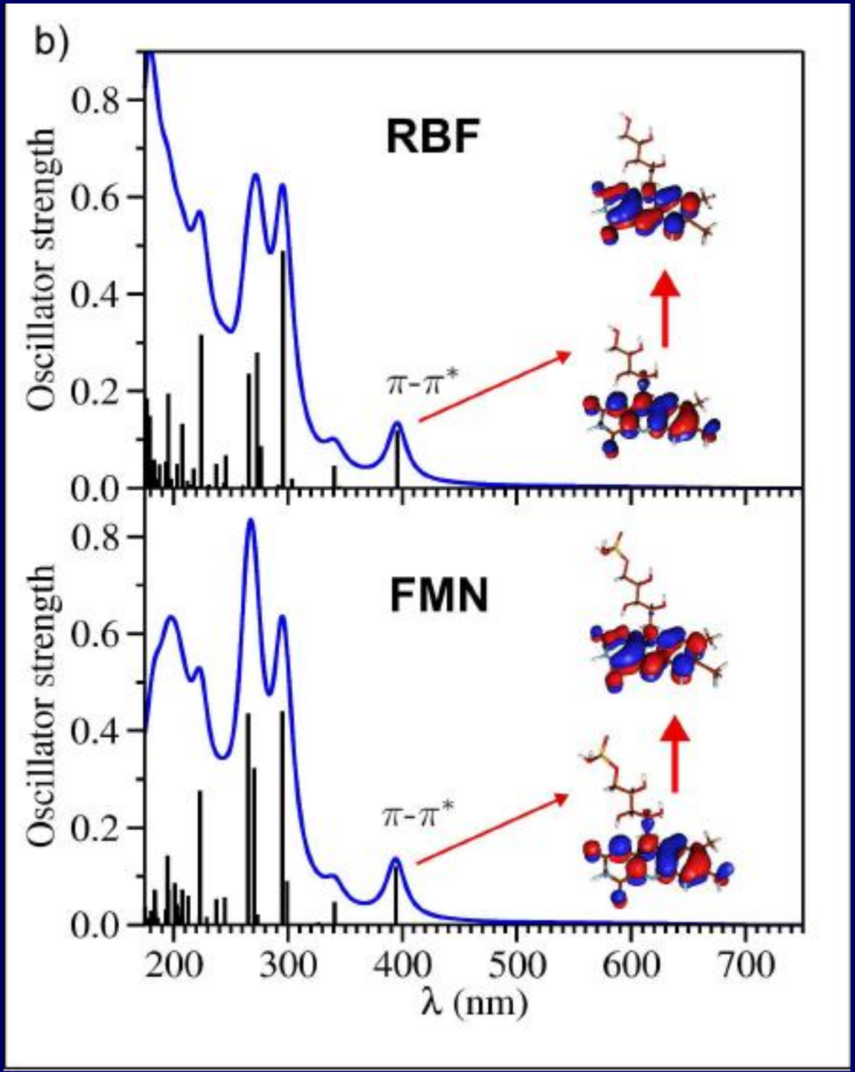
$Fe(IO_3)_3$ (*GAP Bio*), $KNbO_3$ (*Saykally, GB*), KTP (*Roch, GB*), ZnO (*Prasad, GB*), $BaTiO_3$ (*Psaltis*)

Now SiC, BBO, $LiNbO_3$, and magnetic nanomaterials (MRI, NL)

NEW (MUST) : Underlying Mechanism

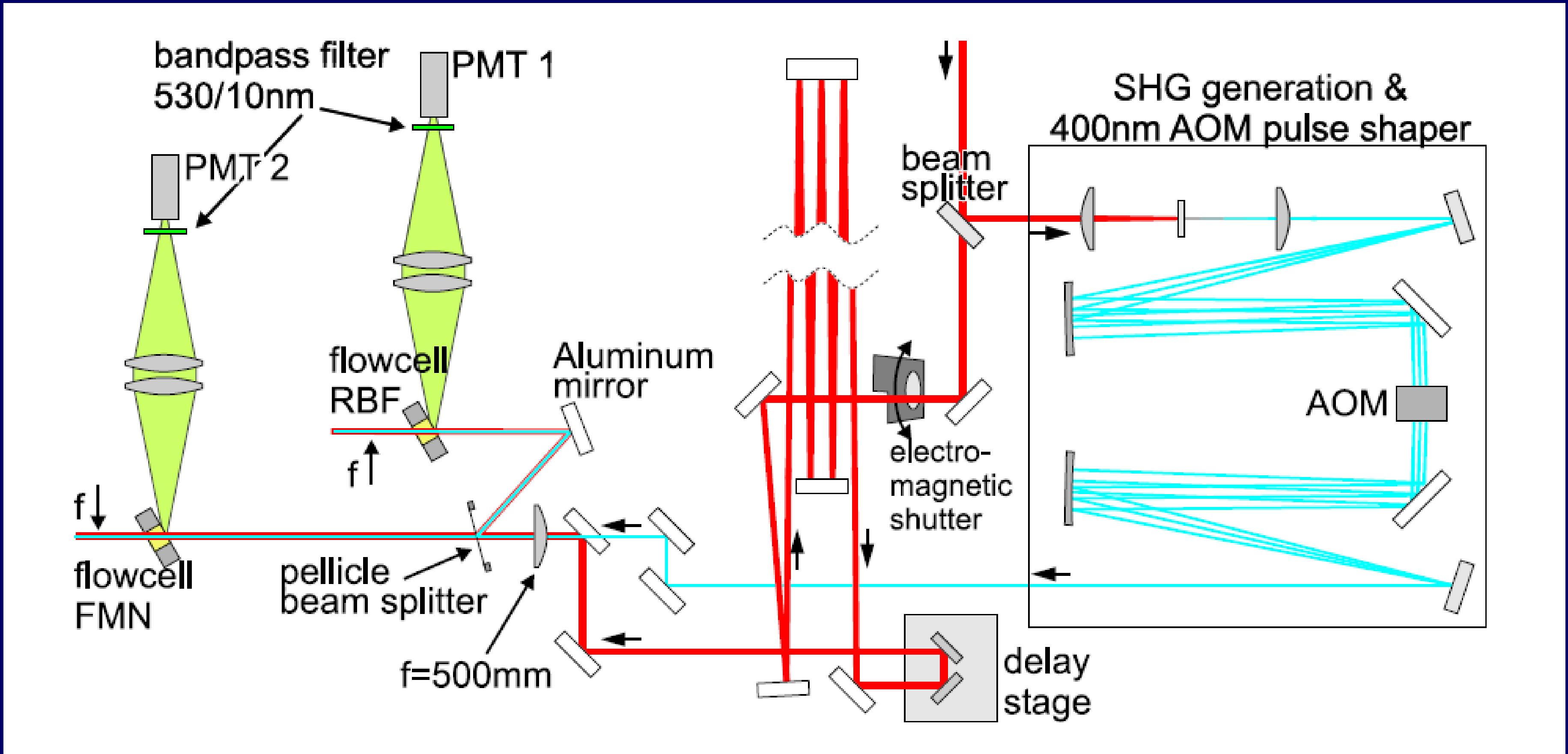
ab-initio Calculations (V. Bonacic Koutecky)





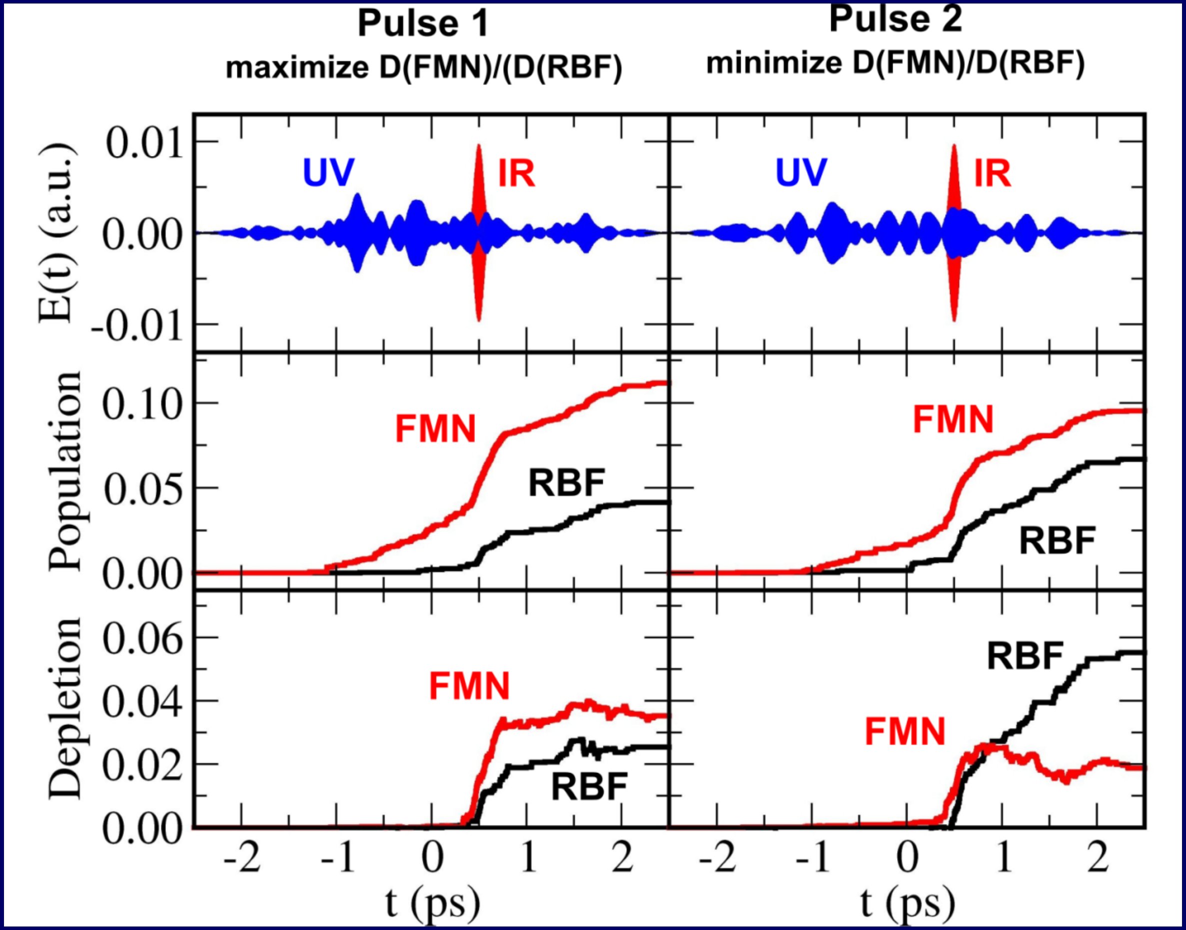
J.Petersen, R. Mitric, V.Bonacic-Koutecky,J.P. Wolf, J.Roslund, H. Rabitz, Phys.Rev.Lett 105, 073003 (2010)

Shaped Pulse Excitation



M.Roth et al, App.Phys. B80, 441 (2005)
M.Roth et al, Rev.Sci.Inst.77, 083107 (2006)

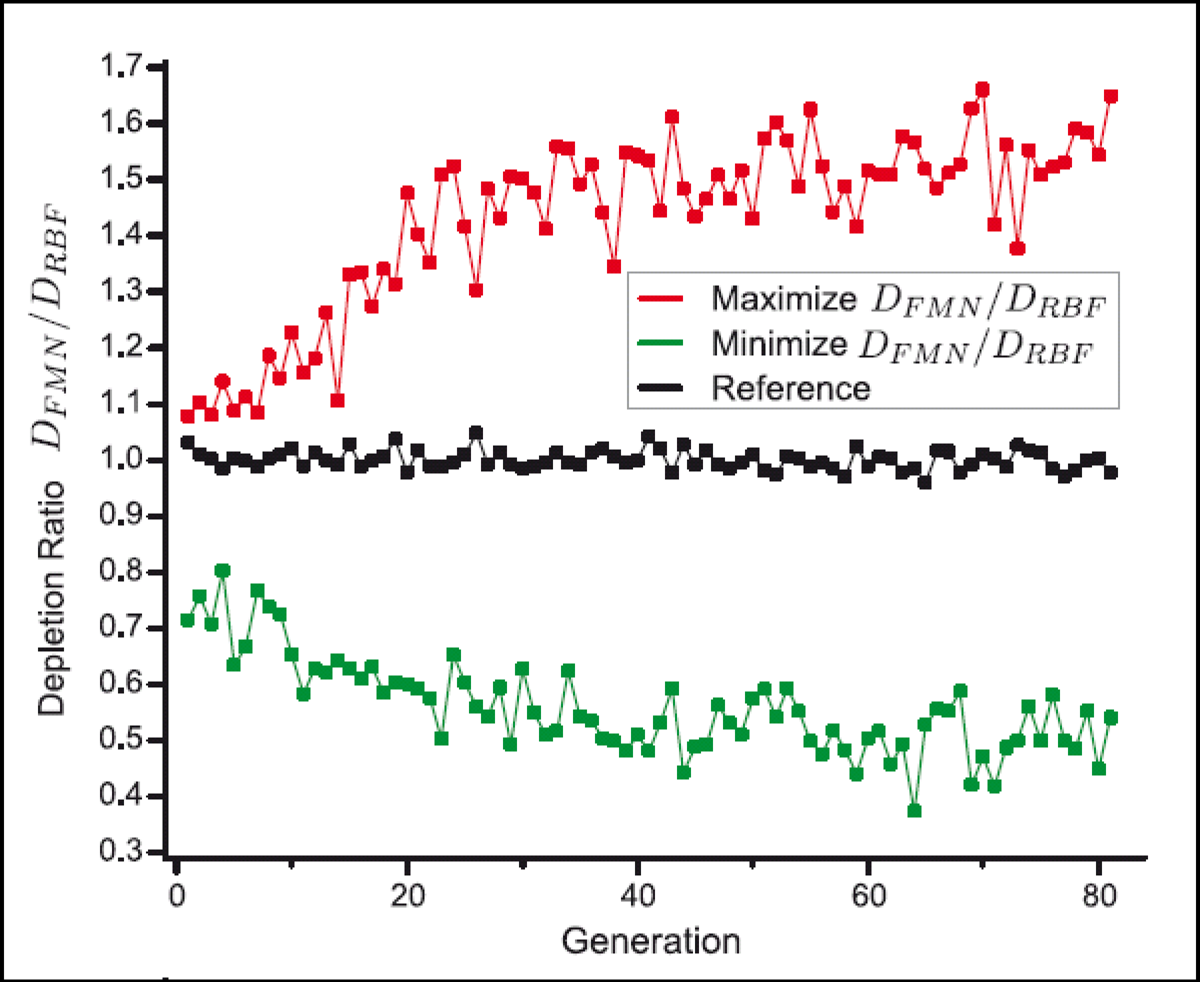
Field-Induced Surface-Hopping (FISH)



FMN Depletion with pulse 1 increases and with pulse 2 decreases

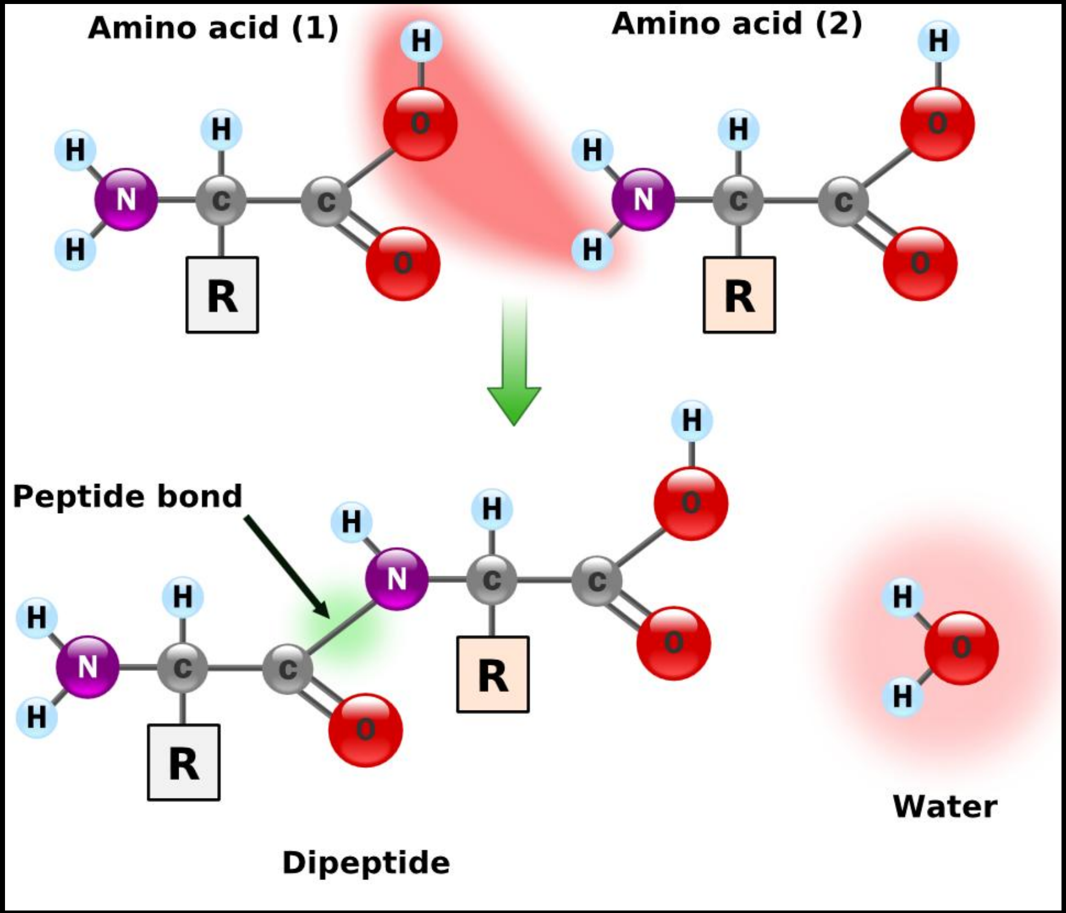
J.Petersen, R. Mitric, V.Bonacic-Koutecky,J.P. Wolf, J.Roslund, H. Rabitz, Phys.Rev.Lett 105, 073003 (2010)

Optimal Control Learning: Optimal Dynamic Discrimination (ODD)



Roth et al, Phys.Rev.Lett 102, 253001 (2009)

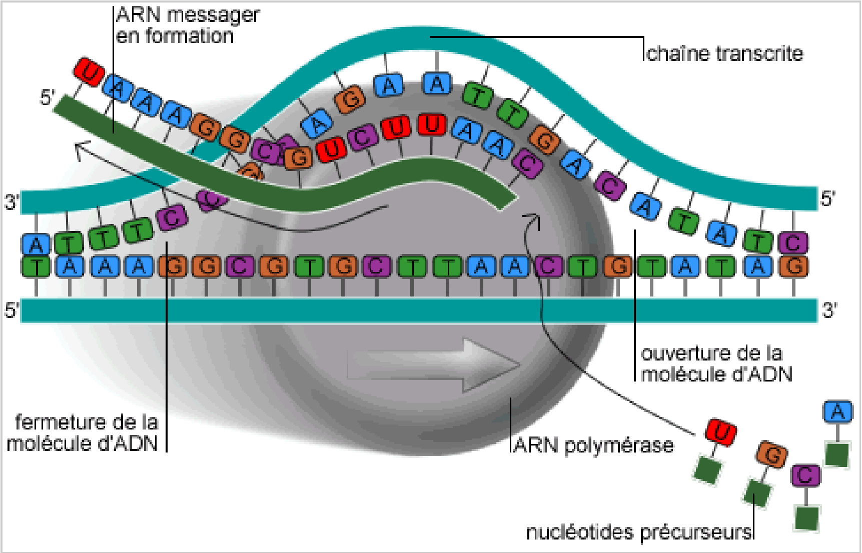
Peptide Bonds



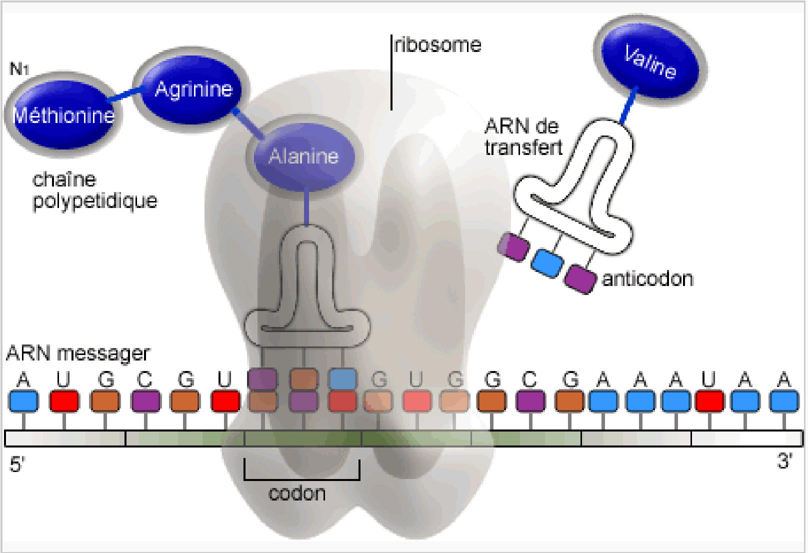
GAP Biophotonics

must

Protein Synthesis



Transcription de l'ADN en ARN messager.



Traduction de l'ARN messager en polypeptide.

Spectra of Dipeptides