Analysis of Biological Structures by Second Harmonic Generation

Paulo A. Ribeiro

Optics and Image Group (GOI)

Departamento de Física, CEFITEC, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, P–2829-516 Caparica, Portugal

pfr@fct.unl.pt

Motivations

- **Share research interests**
- **Intensify future collaboration in new and related scientific areas**

Summary

- **Actual work**
- **Near future developments**
- **Perspectives**

GOI´s Research Interests

- Optics and Instrumentation
- Adsorption of Organic Molecules processes at Solid/Liquid Interfaces
- Devices and Sensors
- Conductive polymers
- Electroluminescent organic materials
- Non-Linear Optics/Photonics
- Biomatter Analysis
- Biomedics
- Biosensors

Emergent Products

- **Flat Panel Displays**
- **Devices for Optical Communications**
- **Data Optical Storage**
- **Solar Cells**
- **Sensors**

Relevant Proprieties for Photonics Applications

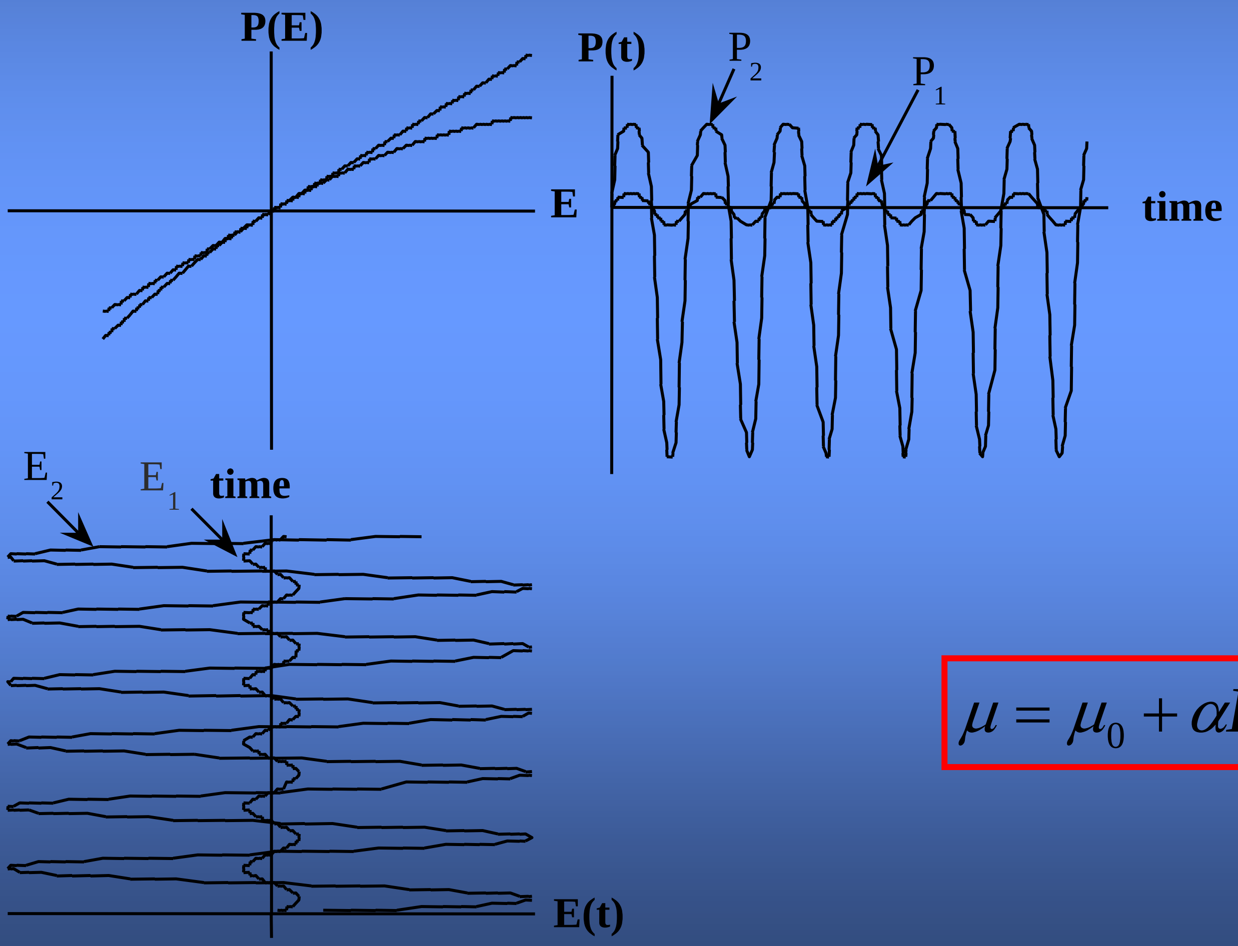
- Linear Electrooptic effect
- Second Harmonic Generation SHG
- Sum Frequency Generation SFG

(Linear Electrooptic effect, SHG, SFG: NLO)

- Quadratic Electrooptic effect
- Foto-refractive effect:
  - Spatial Charge Induction
  - Isomerization

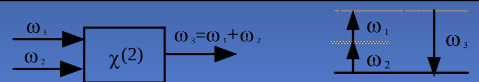
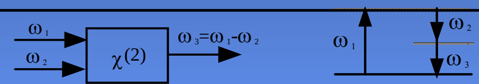
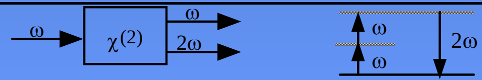
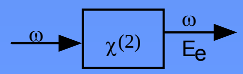
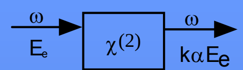
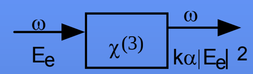
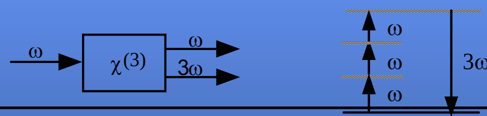
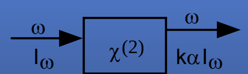
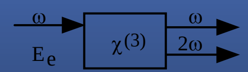
Nonlinear Optics

$$P = P_0 + \varepsilon_0 \left[\chi^{(1)} E + \chi^{(2)} E^2 + \chi^{(3)} E^3 + \ldots \right]$$



$$\mu = \mu_0 + \alpha E + \beta E^2 + \gamma E^3 + \ldots$$

Nonlinear Effects

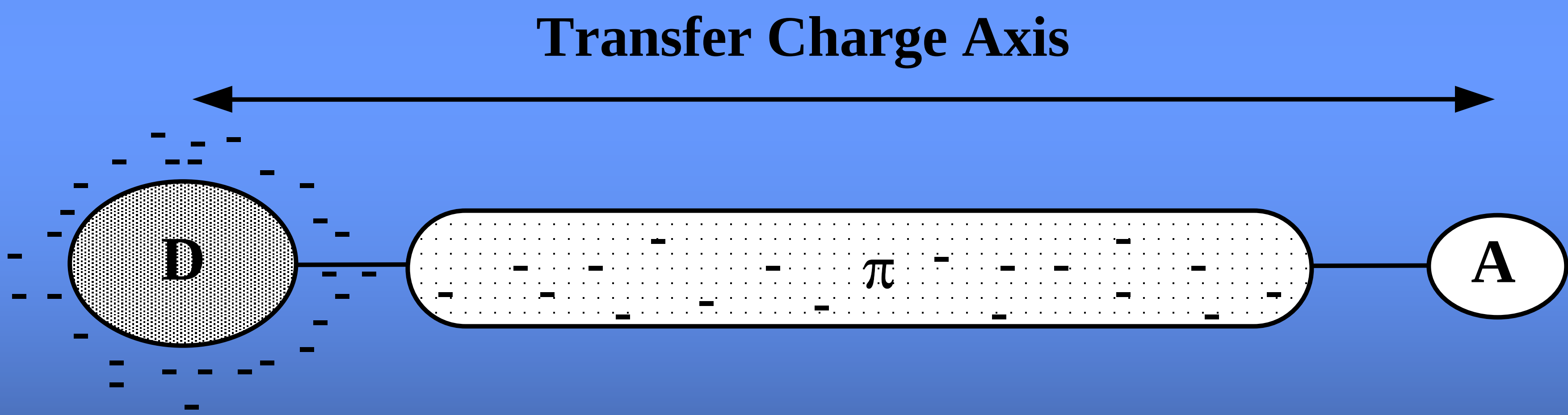
Susceptibility	Description	Applications
$\chi^{(2)}(-\omega_3;\omega_1,+\omega_2)$		Sum and difference Frequency Generation Parametric Amplification
$\chi^{(2)}(-\omega_3;\omega_1,-\omega_2)$		
$\chi^{(2)}(-2\omega;\omega,\omega)$		Second Harmonic Generation
$\chi^{(2)}(0;\omega,-\omega)$		Static Field Generation (Optical Rectification)
$\chi^{(2)}(-\omega;0,\omega)$		Light Modulation (Linear electroptic effect)
$\chi^{(3)}(-\omega;0,0,\omega)$		Light Modulation (Quadratic electrooptic effect or DC Kerr effect)
$\chi^{(3)}(-3\omega;\omega,\omega,\omega)$		Third Harmonic Generation
$\chi^{(3)}(-\omega;\omega,-\omega,\omega)$		Optical commutation, autofocus, (Kerr optical effect)
$\chi^{(3)}(-2\omega;0,\omega,\omega)$		Second Harmonic Generation induced by static field

Molecular Electronic Polarizabilities

Molecular Polarizabilities		Relative Susceptibilities $\chi^{(1)}$
$\alpha \sim 10^{-39}$ $C^2m^2J^{-1}$	$\frac{\alpha}{\varepsilon_o}$ $\sim 10^{-28}$ m^3	$\chi^{(1)} = \frac{N\alpha}{\varepsilon_o}$ ~1
$\beta \sim 10^{-50}$ $C^3m^3J^{-2}$	$\frac{\beta}{\varepsilon_o}$ $\sim 10^{-39}$ $m^4.V^{-1}$	$\chi^{(2)} = \frac{N\beta}{\varepsilon_o}$ $\sim 10^{-11}$ $m.V^{-1}$
$\gamma \sim 10^{-61}$ $C^4m^4J^{-3}$	$\frac{\gamma}{\varepsilon_o}$ $\sim 10^{-50}$ $m^5.V^{-2}$	$\chi^{(3)} = \frac{N\gamma}{\varepsilon_o}$ $\sim 10^{-22}$ $m^2.V^{-2}$

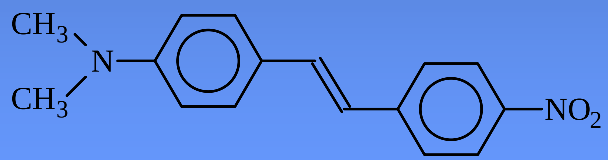
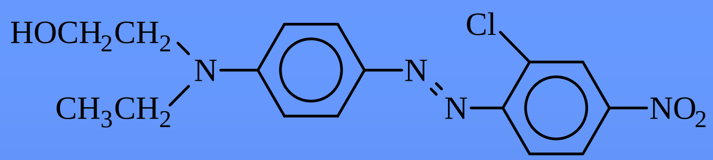
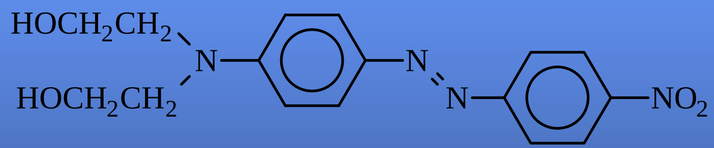
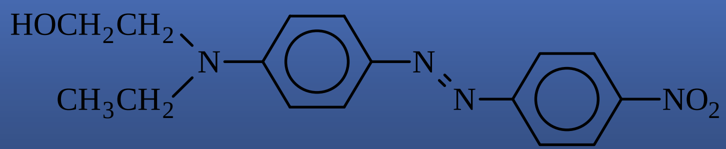
Organic Molecules for NLO Applications

$$\mu = \mu_0 + \alpha E + \beta E^2 + \gamma E^3 + \ldots$$



D-π-A

Examples

Molecule	Designation
	**DR1**
	**DR13**
	**DR19**
	**DANS**

Molecular Polarizabilities

Chromophore	Formulae	Maximum Absorption *(nm)*	Dipole Moment *μ (D)*	2nd order Polarizability *β (m⁴.V⁻¹)*	Melting Point (°C)
DR1	$C_{16}H_{18}N_4O_3$	~502	~8,7	~2x10^{-38}	160-162
DR13	$C_{16}ClH_{17}N_4O_3$	~503	8,6*	2x10^{-38} **	122-129
DANS	$C_{16}H_{16}N_2O_2$	427	7,8	2x10^{-38}	256

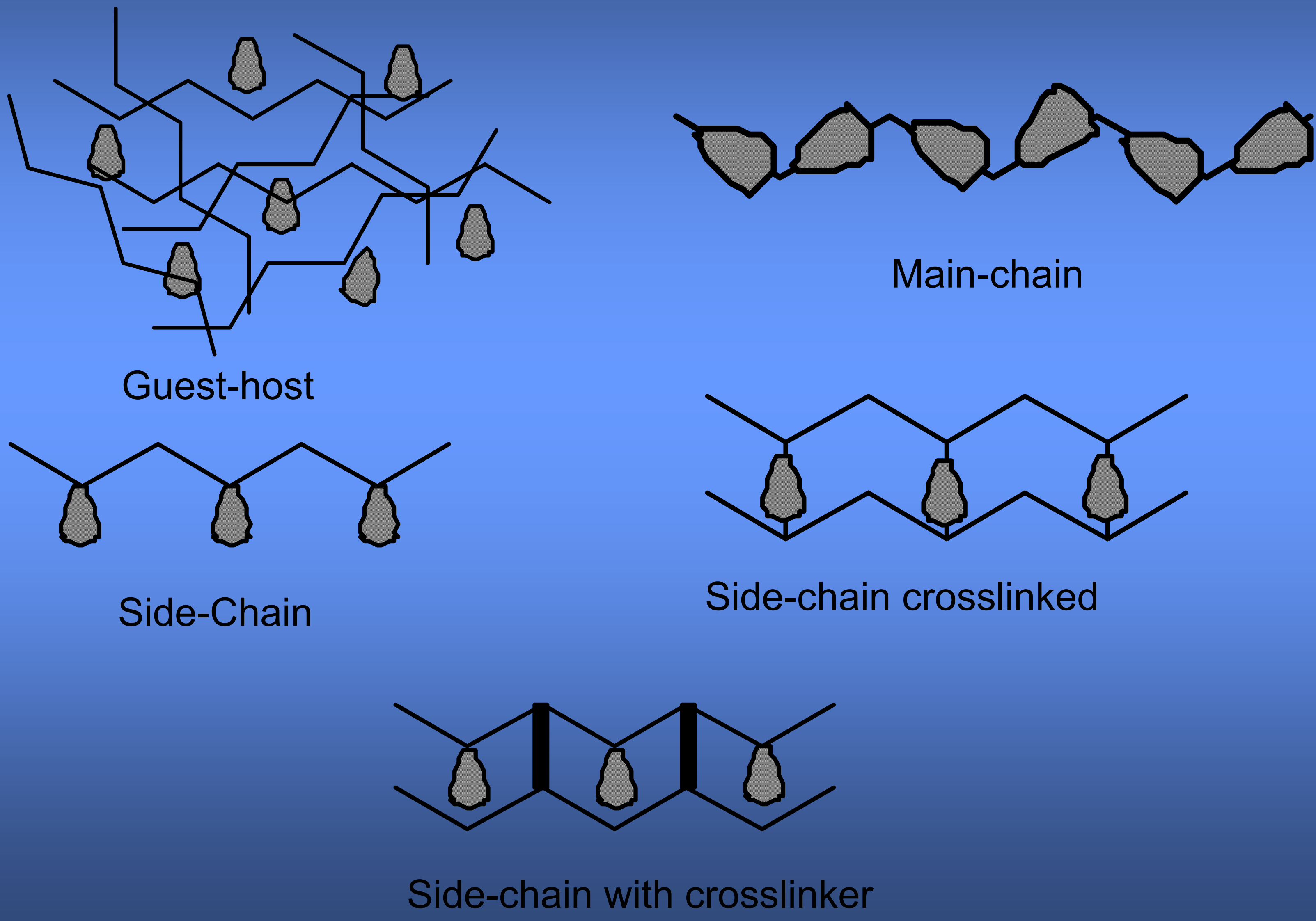
* Obtained value by computational simulation via HyperChem.

** Obtained value using the two level energy model and $\Delta\mu_z = \mu_{eg} \sim 8,6\ D$.

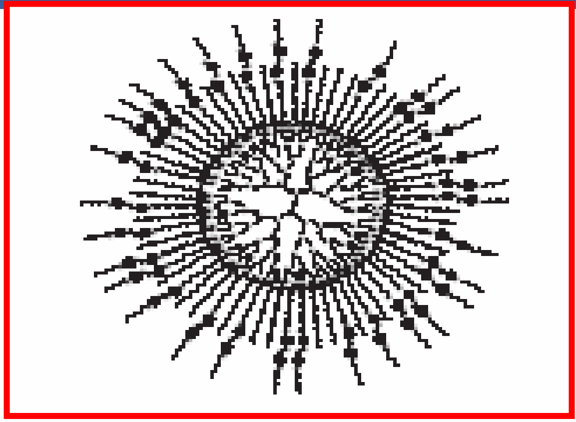
1 D=3,336x10^{-30} $C.m$ $\begin{cases} HCl \sim 1D \\ H_2O \sim 1,9\ D \end{cases}$

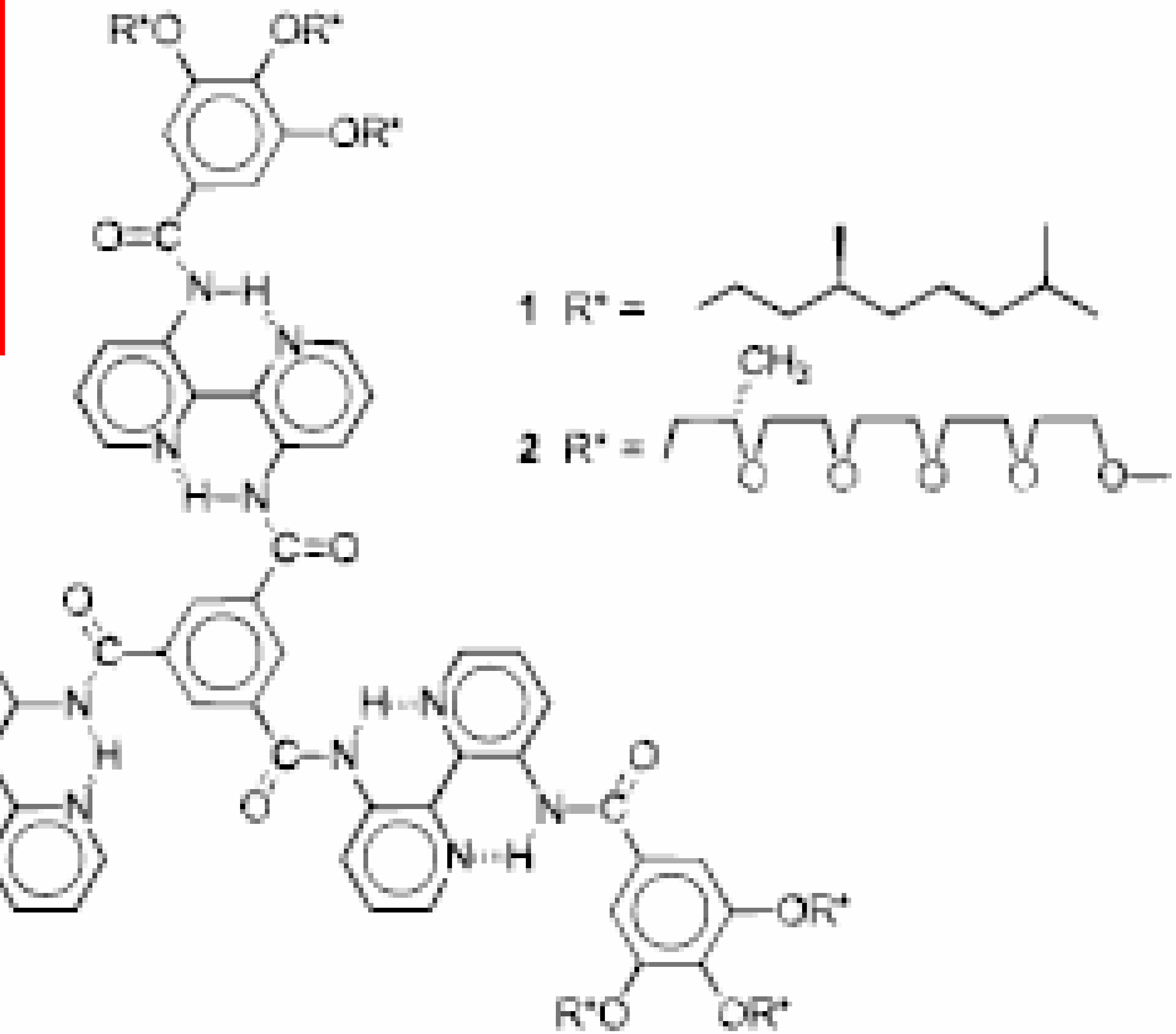
$$\beta_{zzz}(\omega) = \frac{1}{2\varepsilon_o\hbar^2}\Delta\mu_z(\mu_{eg})_z^2 \frac{\left(3\omega_{eg}^2 - \omega^2\right)}{\left(\omega_{eg}^2 - \omega^2\right)^2}$$

NLO Polymeric Materials



Dendrimers





R*O
OR*
OR*
O=C
N–H
N
N
H–N
C=O
O
C
N
H
N
H
N
R*O
R*O
R*O
C
O
H–N
C–N
O
N–C
O
N–H
OR*
R*O
OR*
1 R* =
CH₃
2 R* =

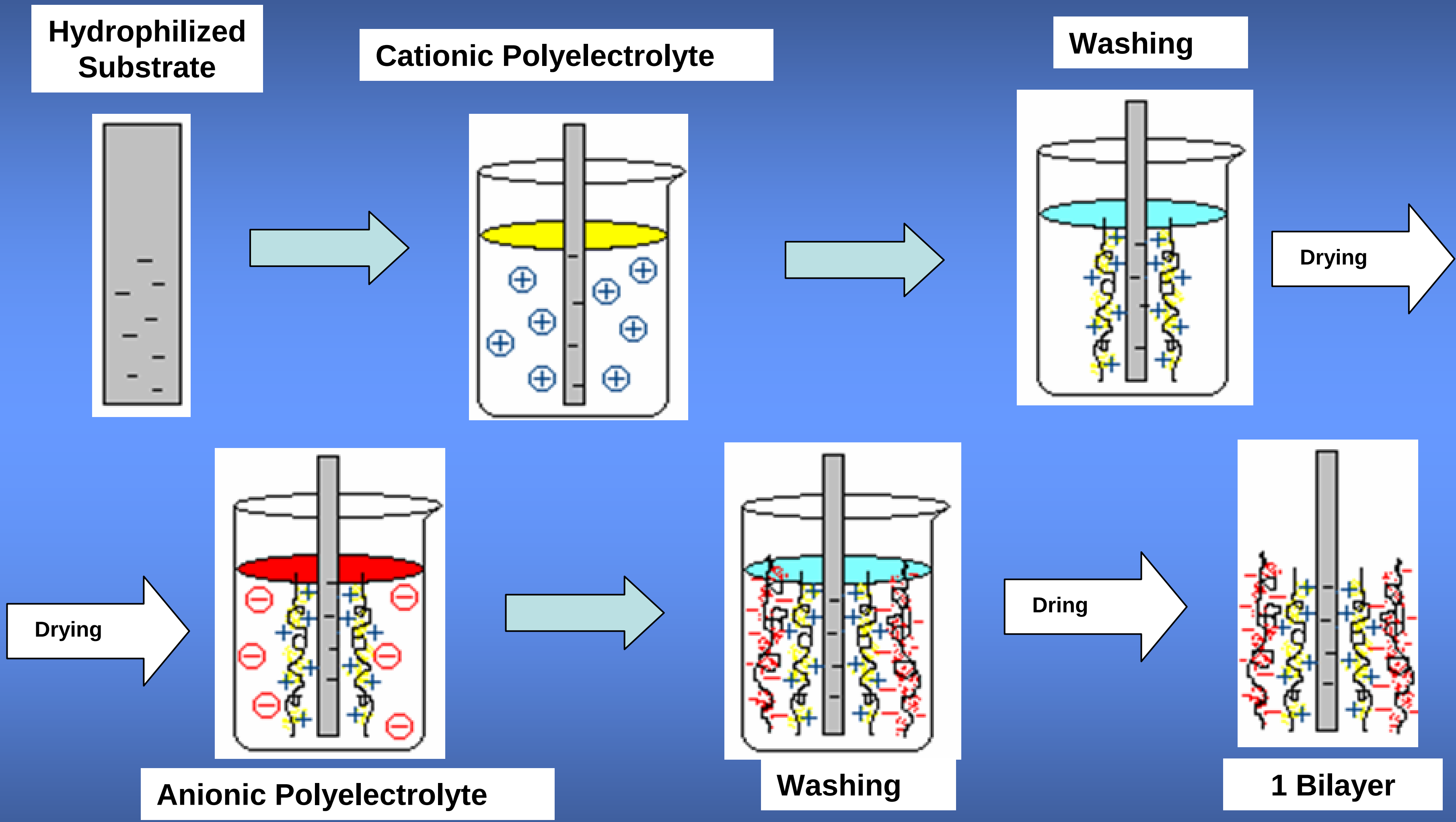
Electrooptic Proprieties

Material	Electrooptic Coeficient (*pm.V*$^{-1}$)	Relative electric Permissivity ε	Refraction index *n*
Inorganic Crystals $LiNbO_3$	r_{33}(633 nm)=30	$\varepsilon_{11}=\varepsilon_{22}=78$ $\varepsilon_{33} = 32$	2,2
KDP (KH_2PO_4)	r_{63}(633 nm)=11	$\varepsilon_{11} = \varepsilon_{22} = 42$ $\varepsilon_{33} = 21$	1,5
KTP ($KTiOPO_4$)	r_{33}(633 nm)=35 r_{23}(633 nm)=13	$\varepsilon_{11} = \varepsilon_{22} = 11$ $\varepsilon_{33} = 78$	1,7
Organic CrystalsCristais MMONS MNA	r_{33}(633 nm)=40 r_{11}(633 nm)=67	2,8 < 3,0	2,0 2,0
Organic Salts DAST	r_{11}(633 nm)= 140 r_{11}(820 nm)=400	$\varepsilon_a = 6,4$ $\varepsilon_b = 4,3$	$n_a = 2,1$ $n_b = 1,6$
Sol-Gel	r_{33}(633 nm)=30 r_{33}(633 nm)=30	~ 3	~1,8
Polymers	30-100	2-3	~1,6

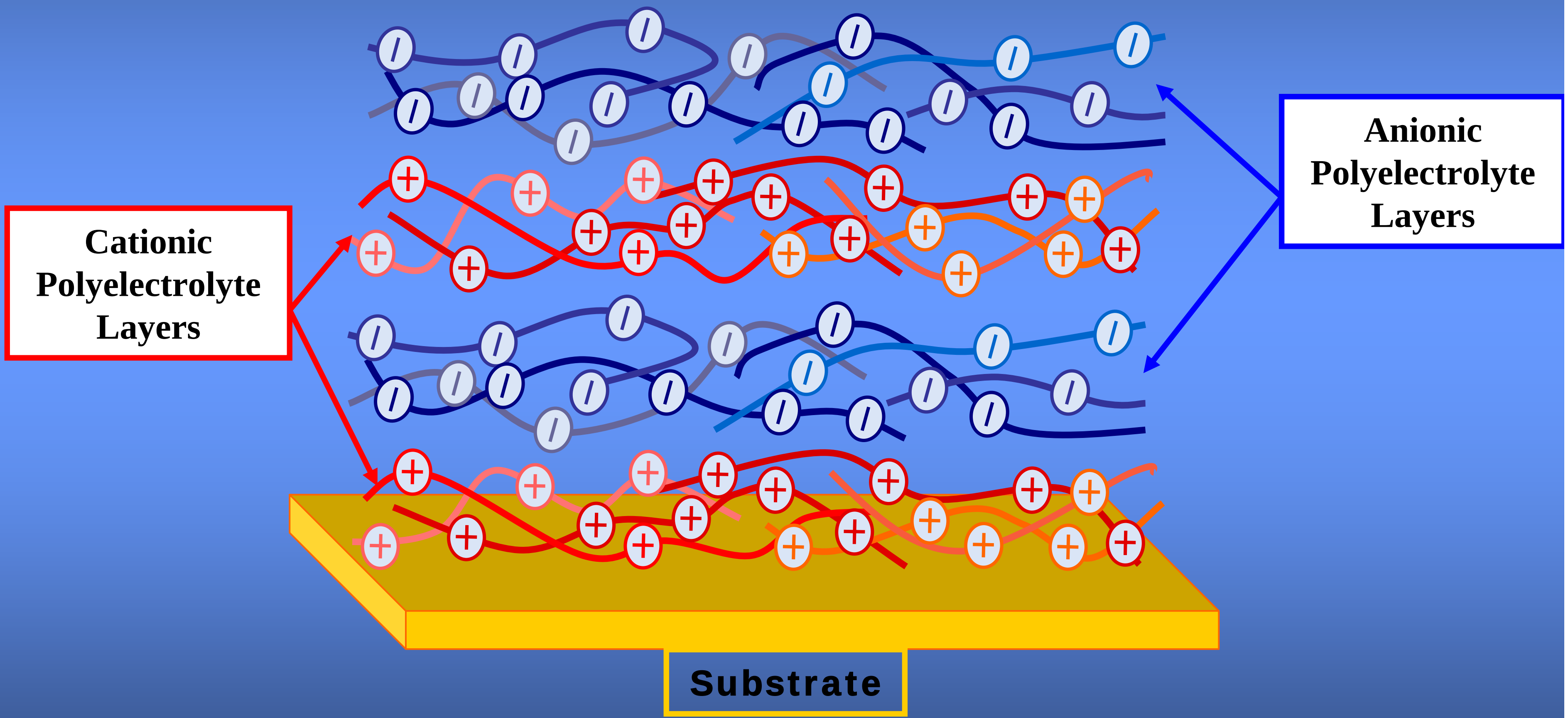
Thin Films Fabrication

- **Spin-coating**
- **Casting**
- **Sol-Gel**
- **Langmuir Blodgett**
- **Layer-by-layer**
- **Self Assembly**

Layer-by-layer technique



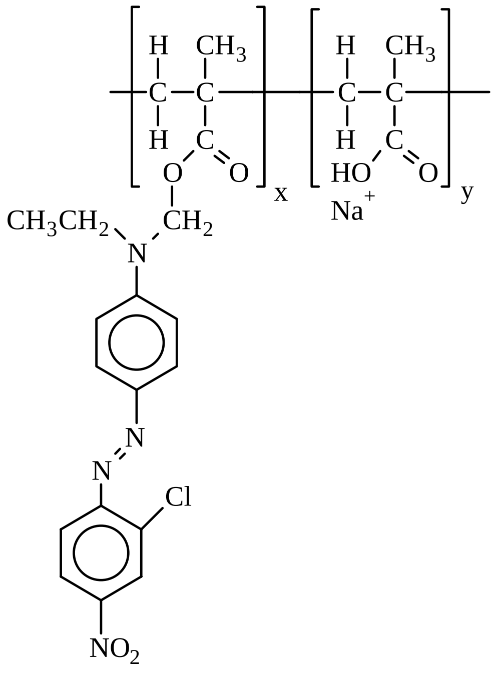
Layer-by-layer Film



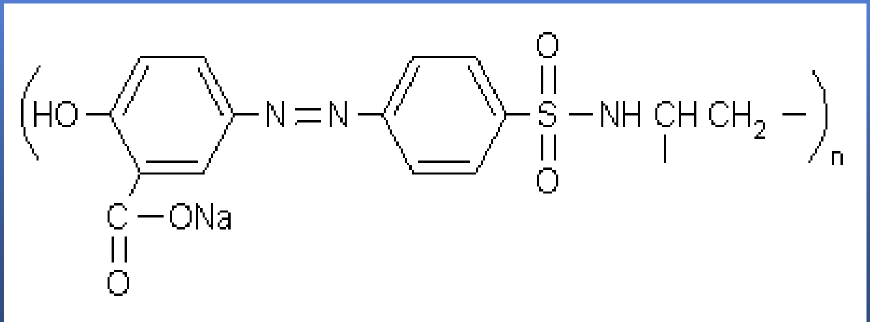
Layer-by-layer technique Advantages

- Simplicity
- Water soluble materials
- Substrate type, shape and size independence
- Heterostructures in layers
- Molecular thickness control
- Film architecture control
- Possible lithography

Polyelectrolytes with Photonics Applications



Methacrylic copolymer functionalized with the azo chromophore 4-[N-ethyl-N-(2-hydroxiethyl)]-amino-2´-chloro-4-nitroazobenzene (MMADR13)

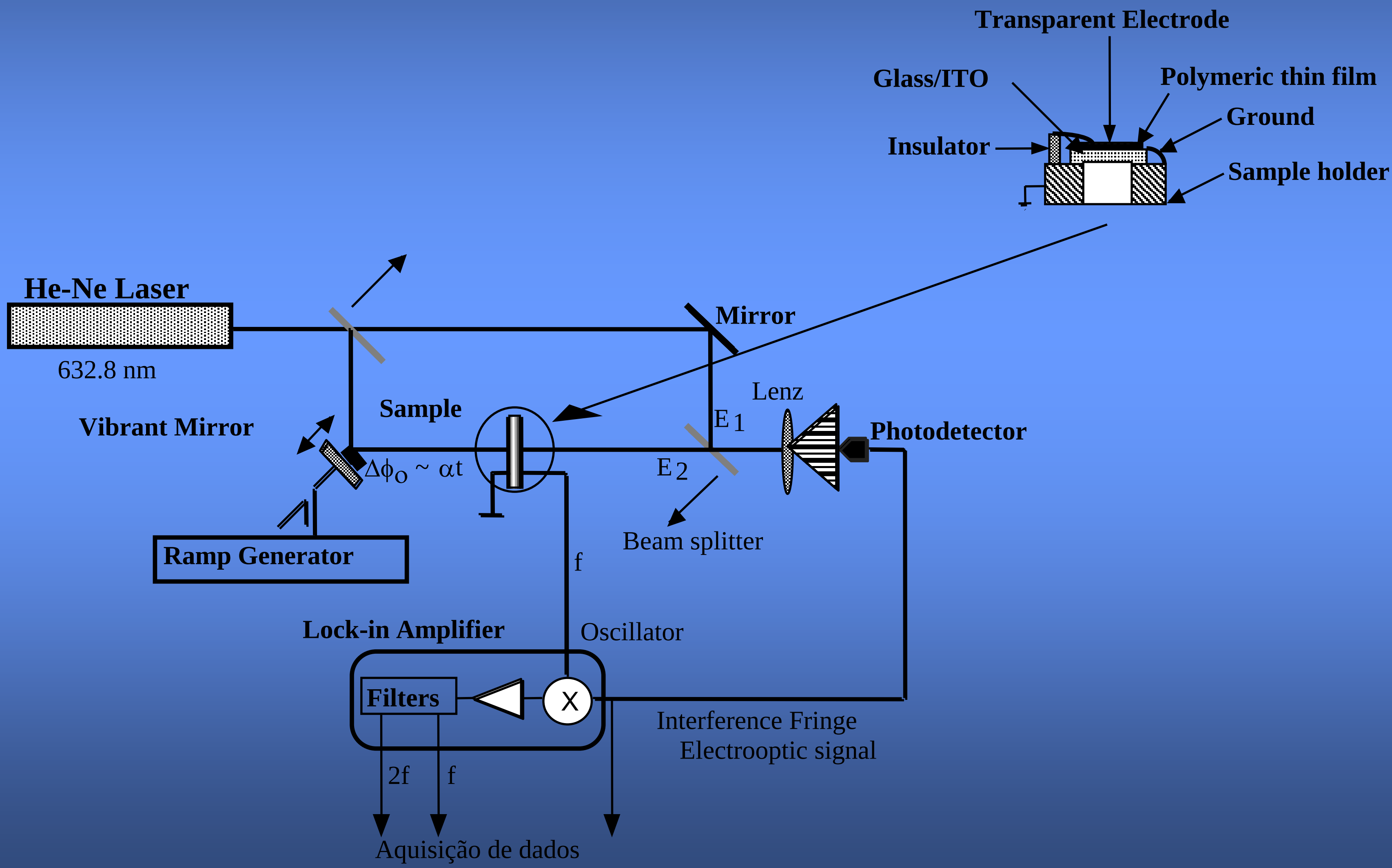


Poly[1-[4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamid]-1,2-ethanediyl, sodium salt] (PAZO)

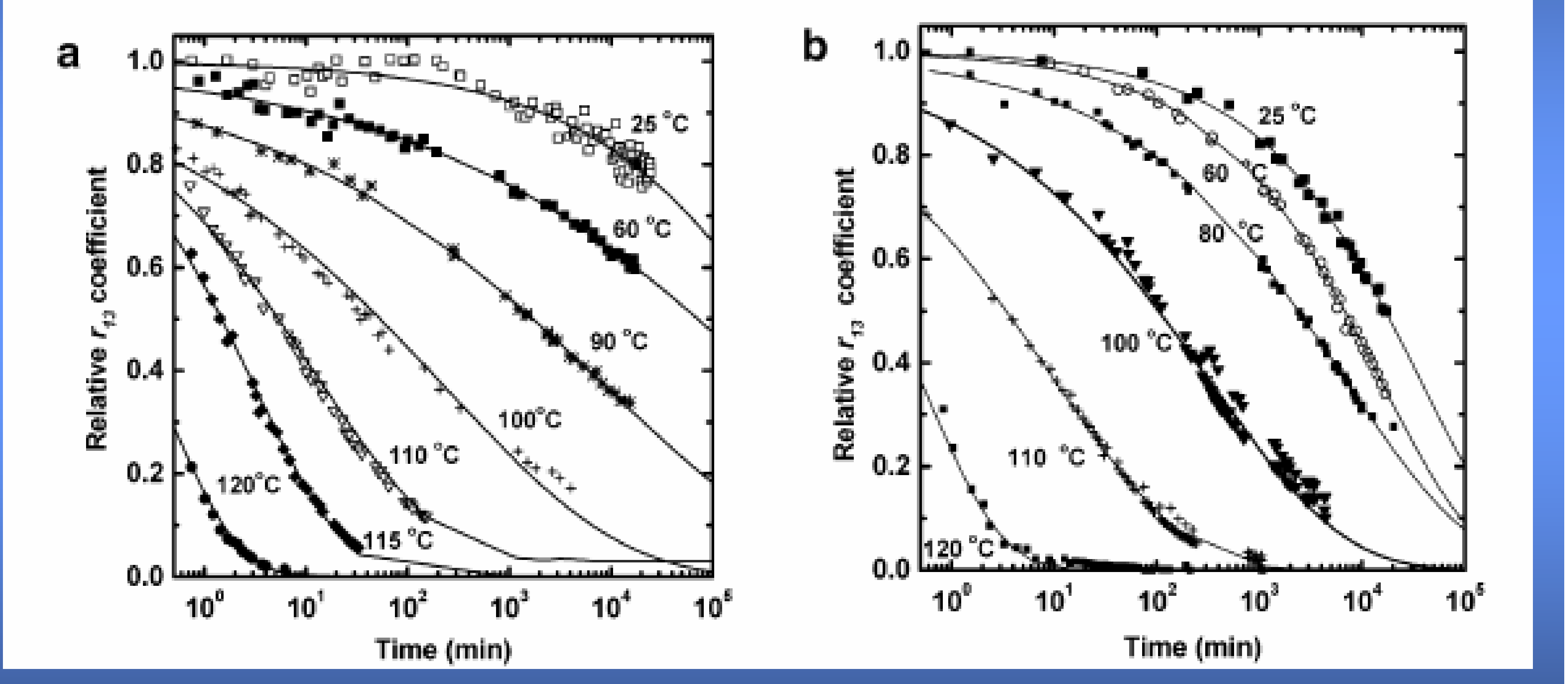
Characterization of NLO LBL Films

- **Electrooptic Effect**
- **Birrefringence induction/measurement**
- **Surface Relief Gratings**
- **Second Harmonic Generation (SHG)**

Electrooptic Effect Experiment



Experimental Results for Electrooptic decay for MMADR13

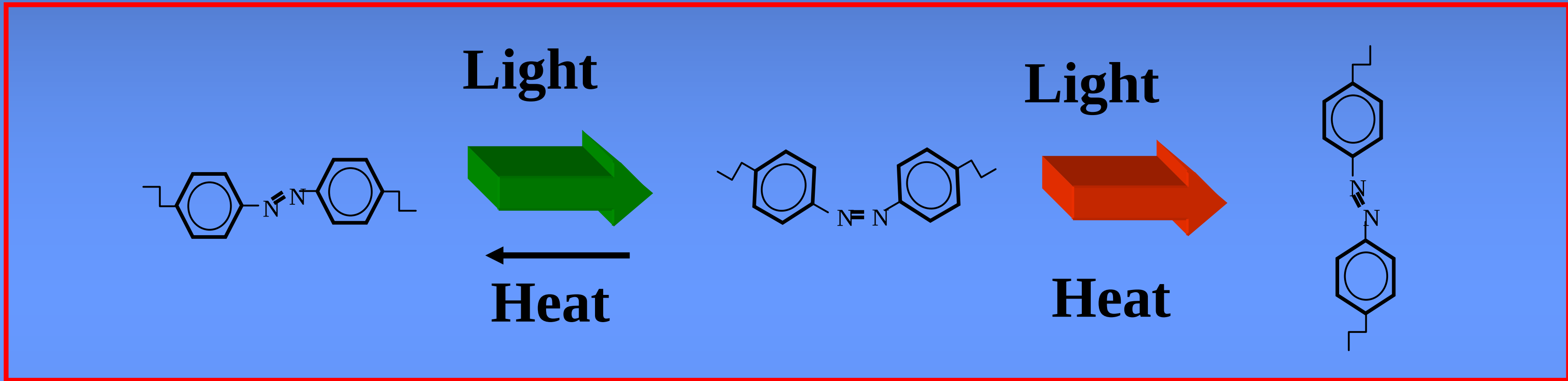


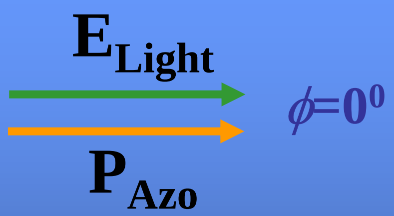
MMA-DR13 (9 wt %) **MMA-DR13 (43 wt %)**

P.A. Ribeiro et al, Macromolecules **2004,** *37*, 2618-2624

Orientation Mechanism by Photo-isomerization

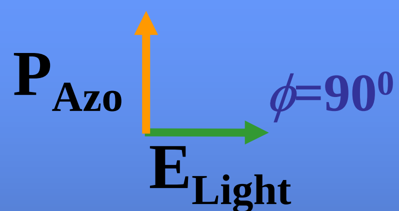
Trans-cis conversion rate: $R = I\cos^2(\phi)$





R=I

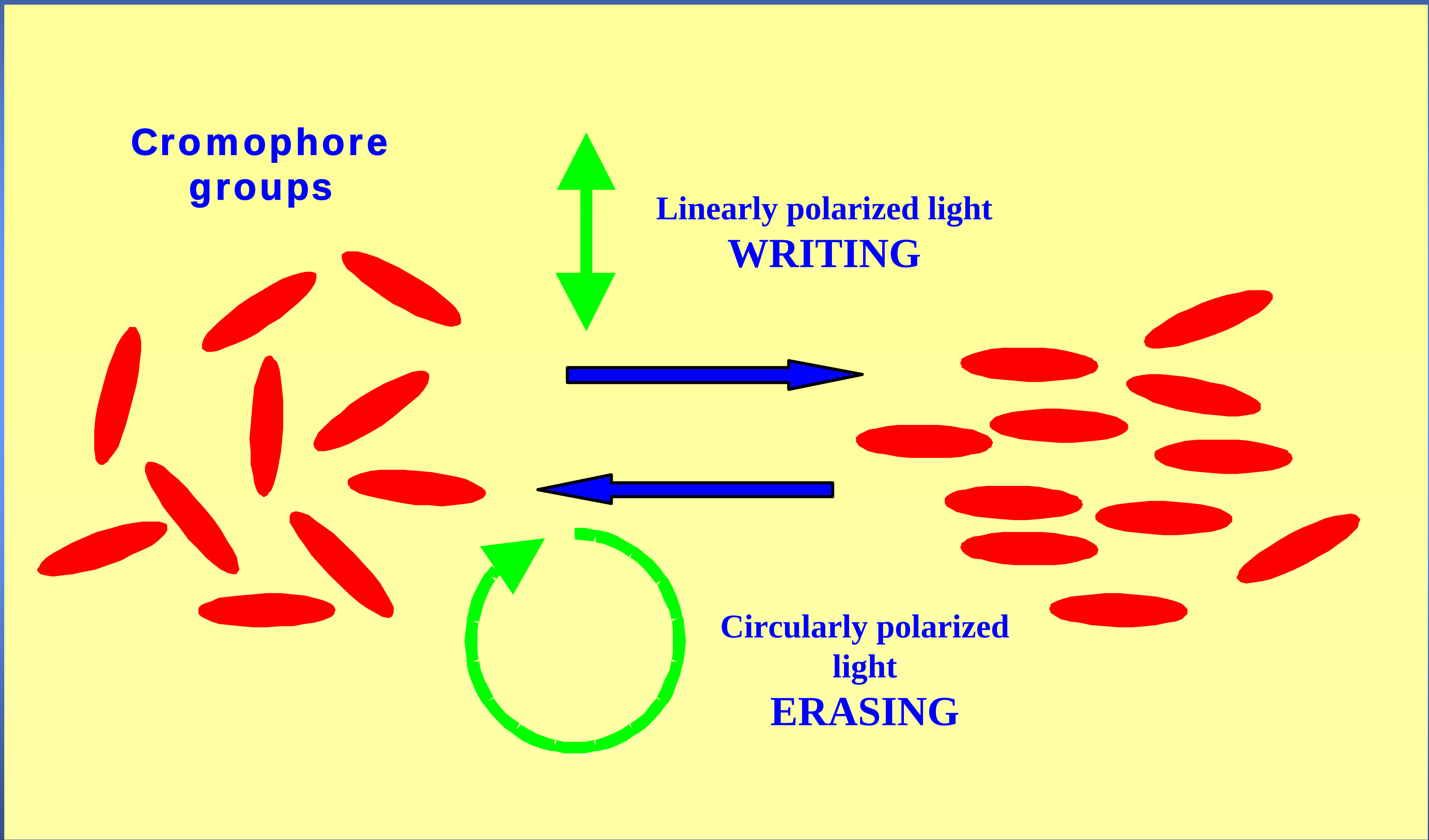
Mobility Induction



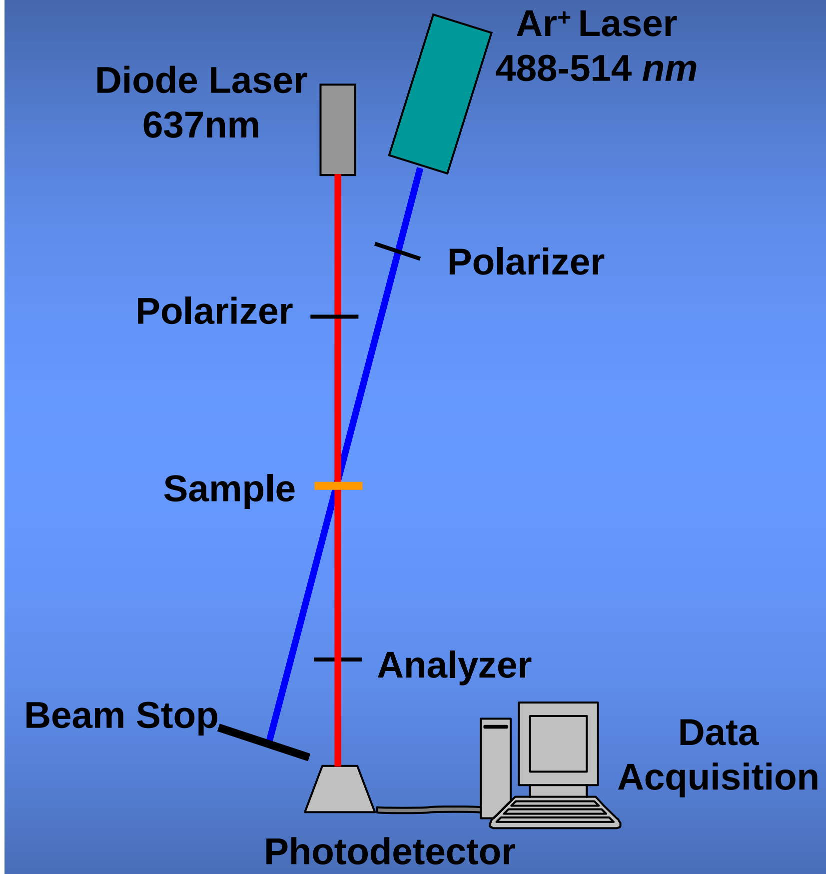
***R*=0**

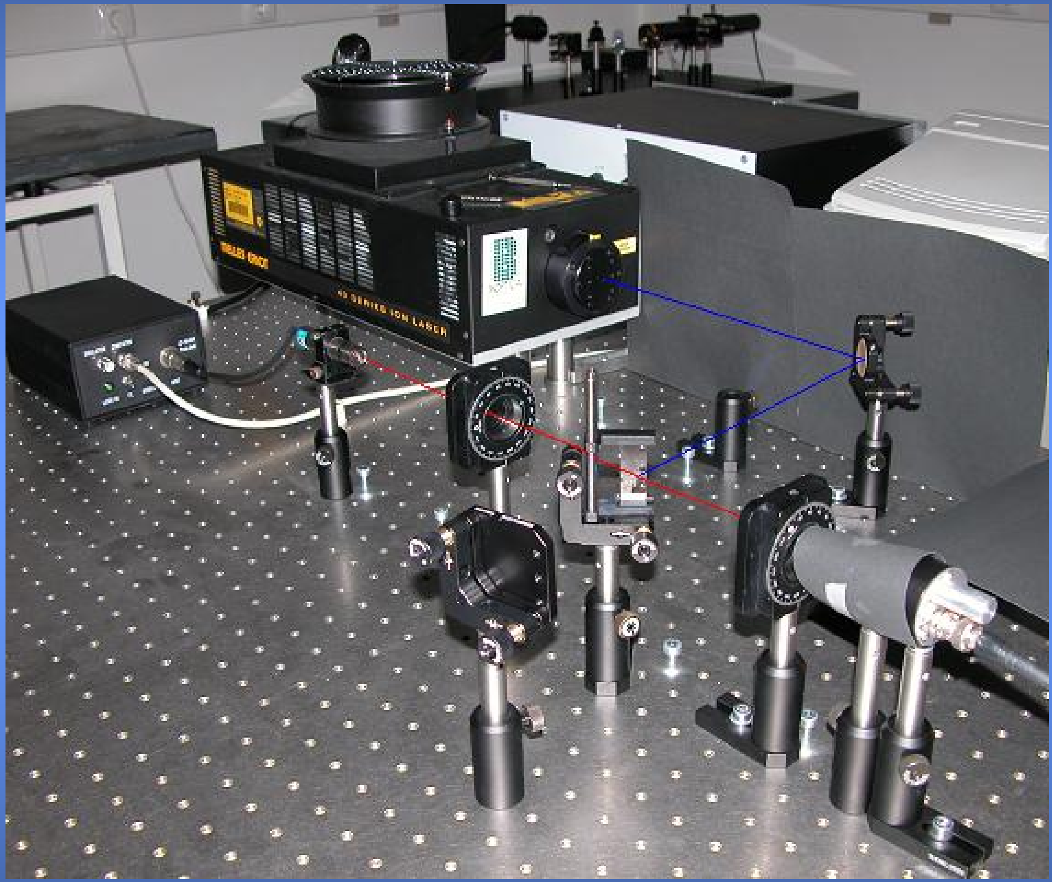
No mobility induction

Write-Erase Cycle



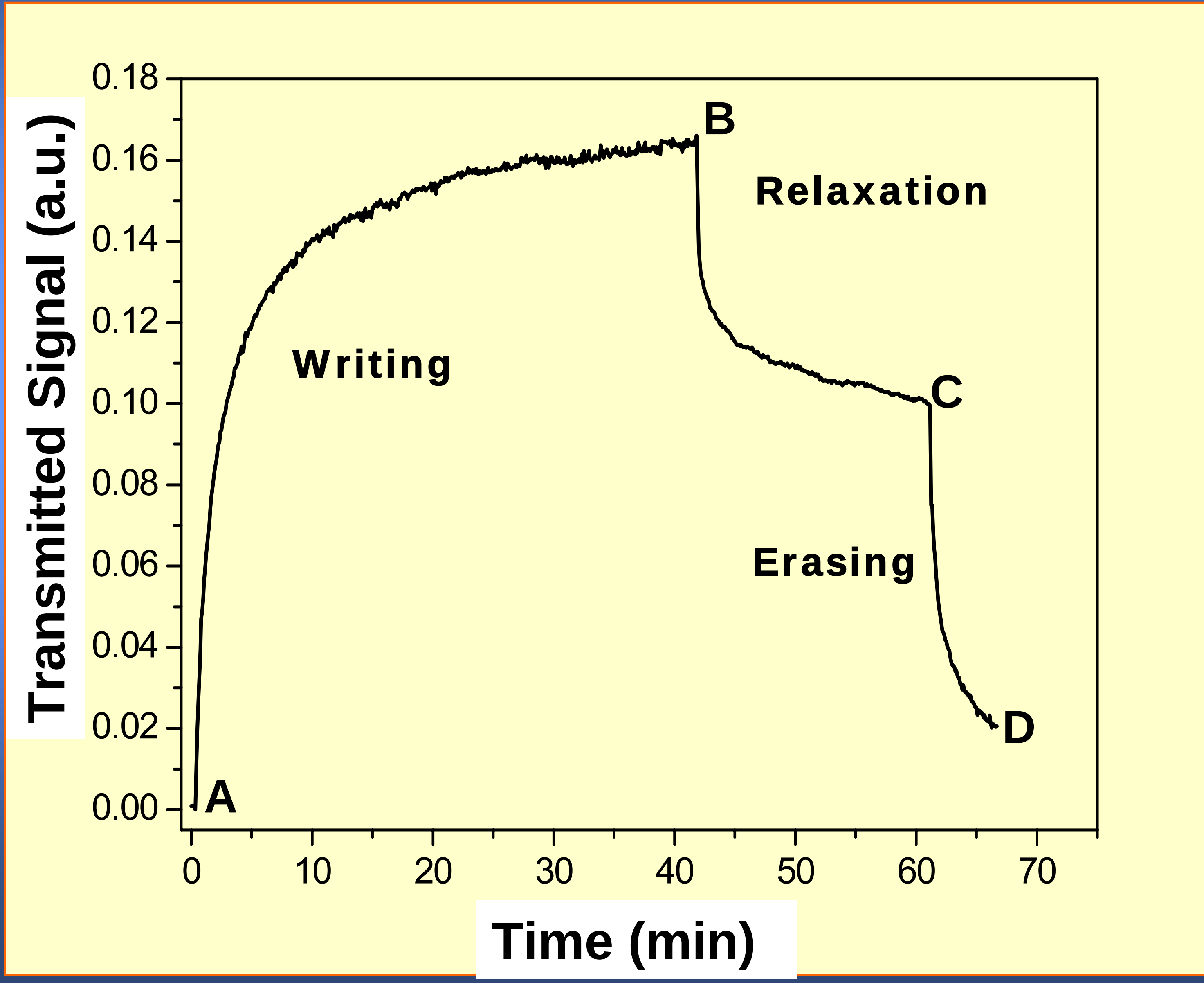
Birrefringence induction





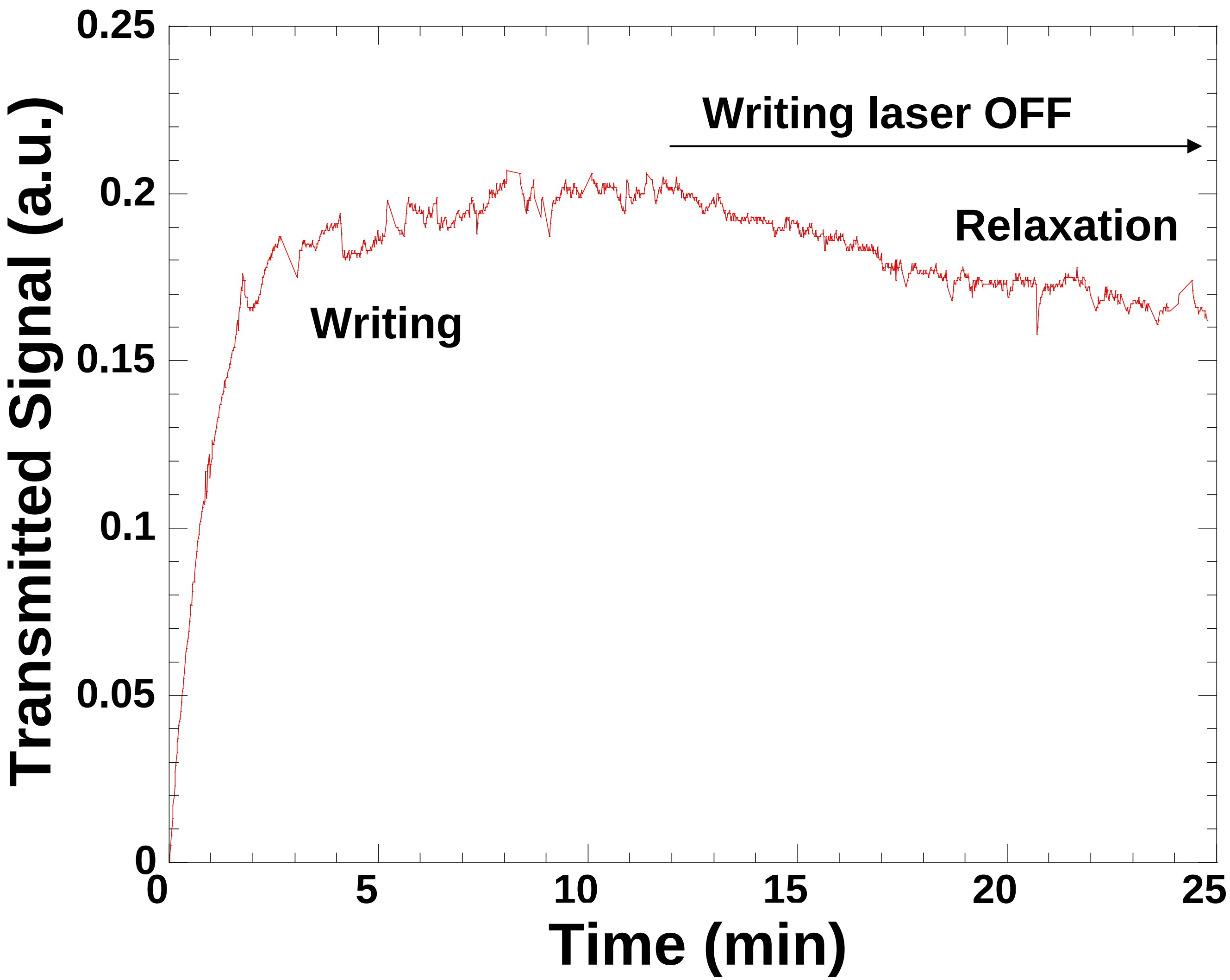
Optical Storage

40 bilayers PAH/MMADR13 LbL film

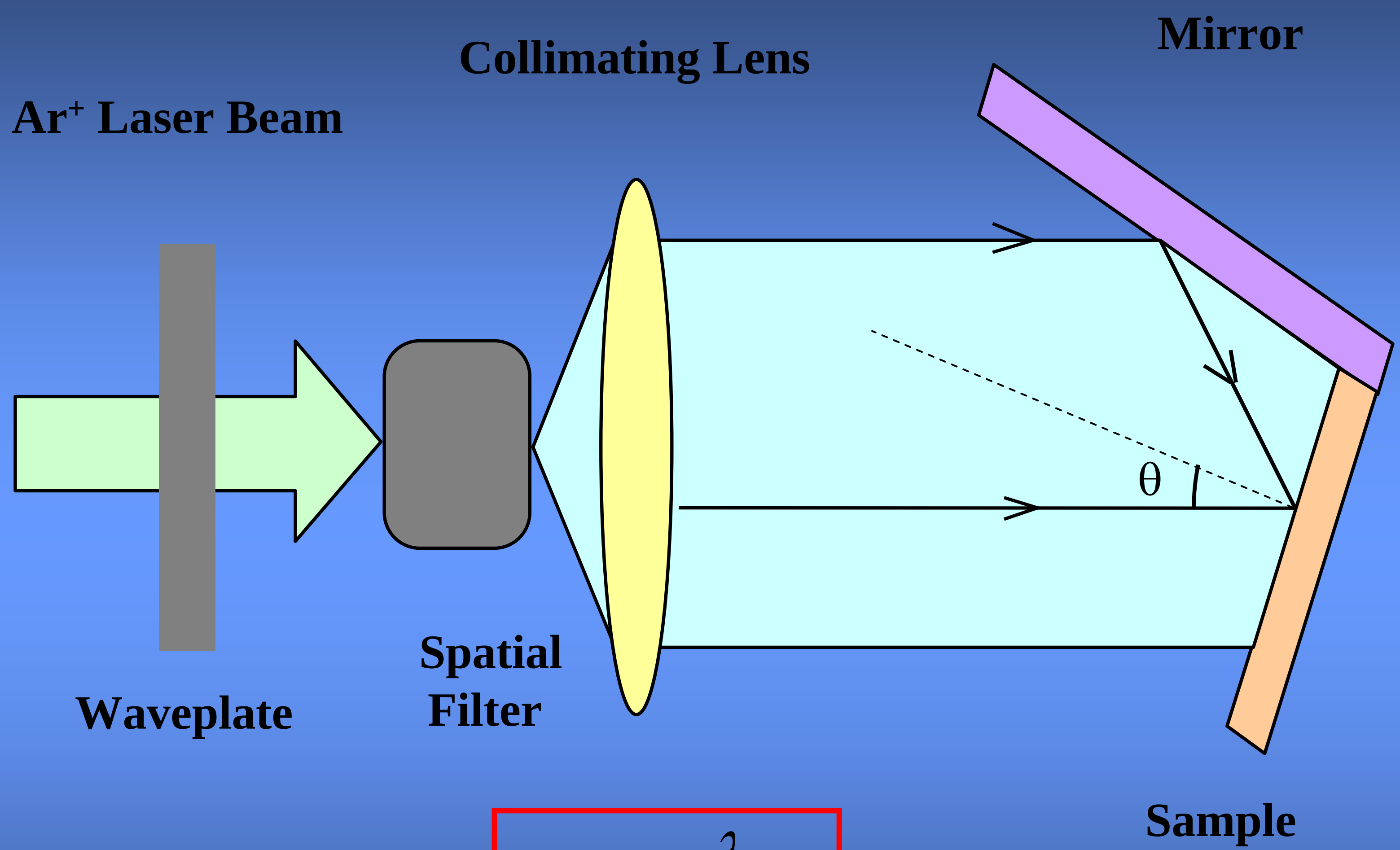


Optical Storage

20 bilayers PAH/PAZO LbL film

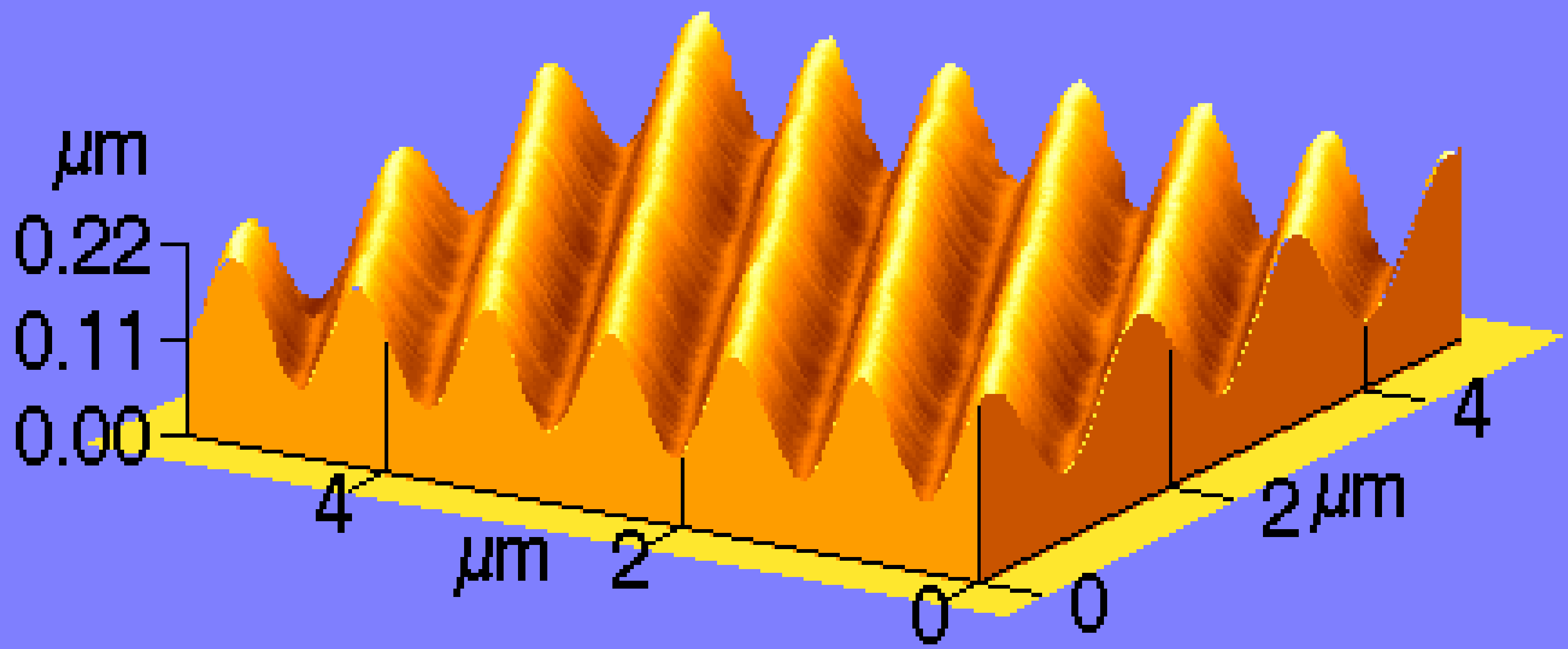


Surface relief Gratings (SRG) Setup



$$\Lambda = \frac{\lambda}{2sen\frac{\theta}{2}}$$

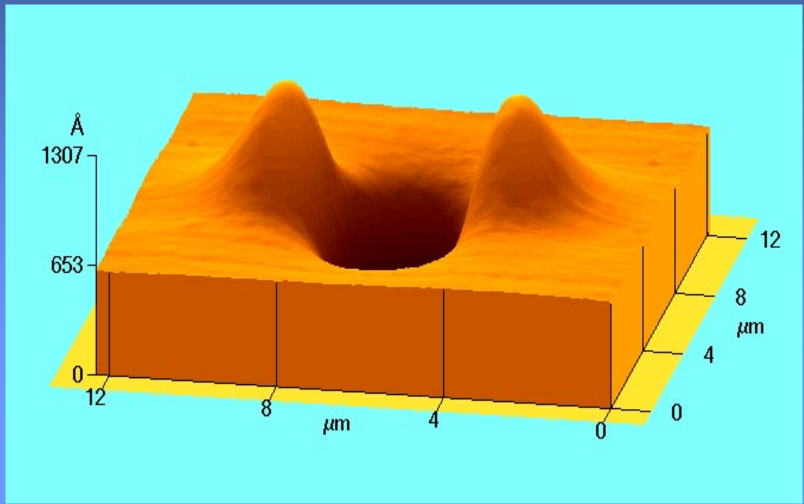
Surface Relief Grating AFM image

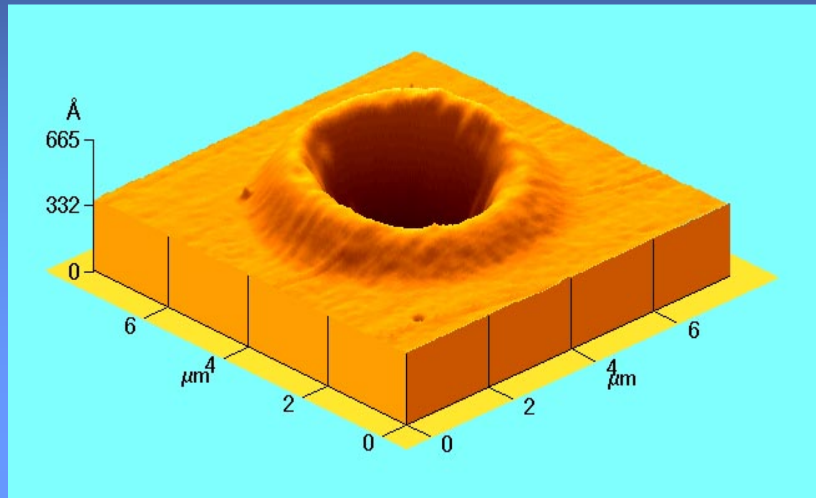


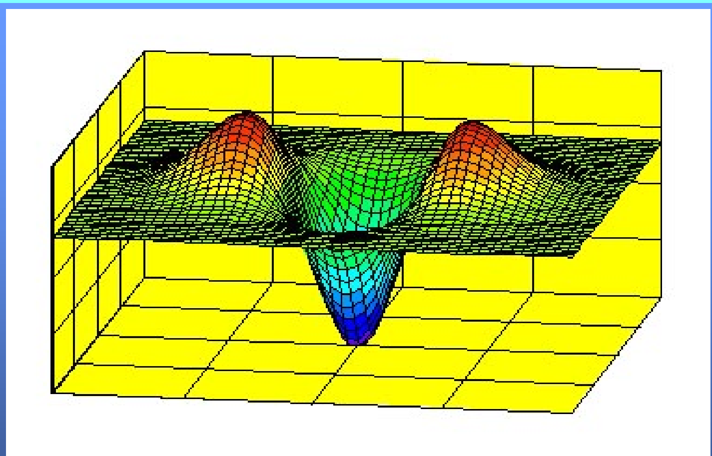
SRGs Main Features

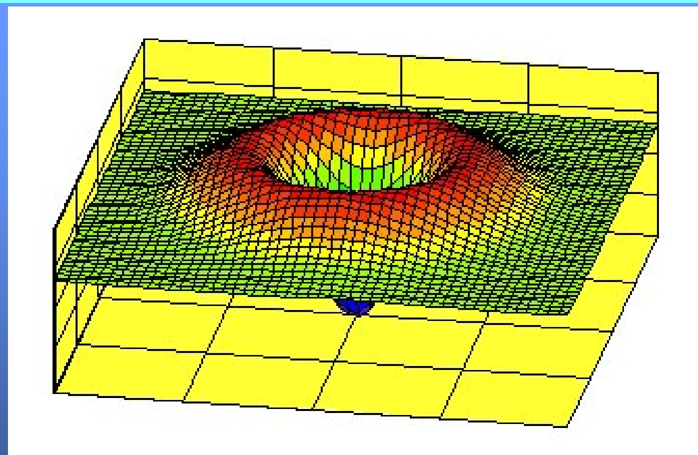
- **Surface modulations have only been found on materials containing azobenzene chromophores**
- **Recording process is polarization dependent (*p* being more efficient)**
- **Surface modulations of 600-1000 nm are easily inscribed on the polymer films**
- **Efficient SRG writing is achieved in polymers with the azobenzene chromophores attached to the polymer chain, either in the main chain or as a side chain**
- **SRGs recorded may be erased optically and thermally**
- **The SRGs are □ shifted with regard to the interference pattern**
- **Light-driven mass transport is involved (for small irradiance** $<1\ W.cm^{-2}$**)**
- **Light-driven mass transport starts at the surface**
- **This phenomena is possible with either continuous or pulsed radiation**
- **Irradiances of 1000** $mW.cm^{-2}$ **(10** mW **in 2** mm **gives 320** $mW.cm^{-2}$**)**

SRGs obtained with Gaussian Beams



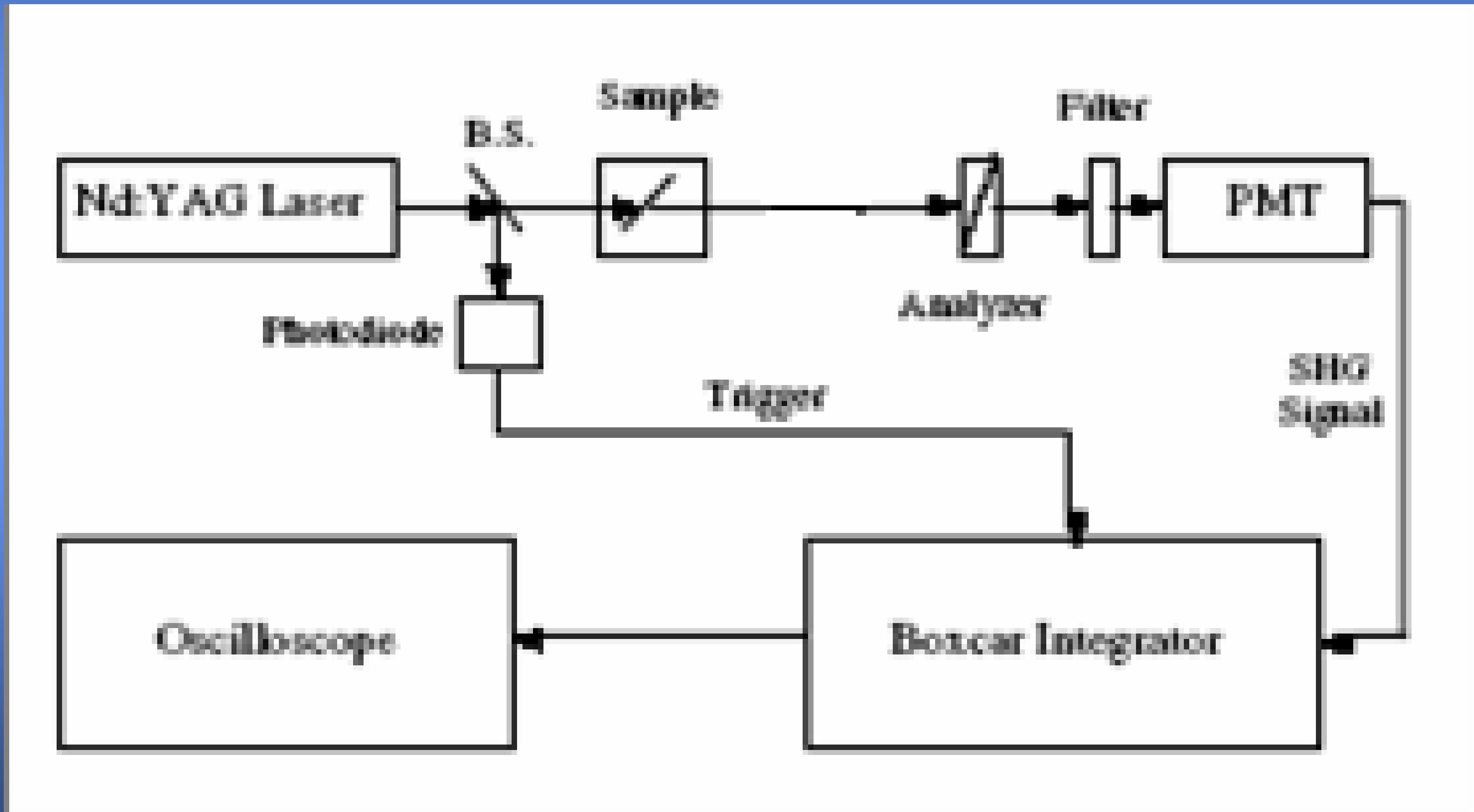






Tripathy et al. – UMass. EUA

Second Harmonic Generation (SHG) Experimental Setup

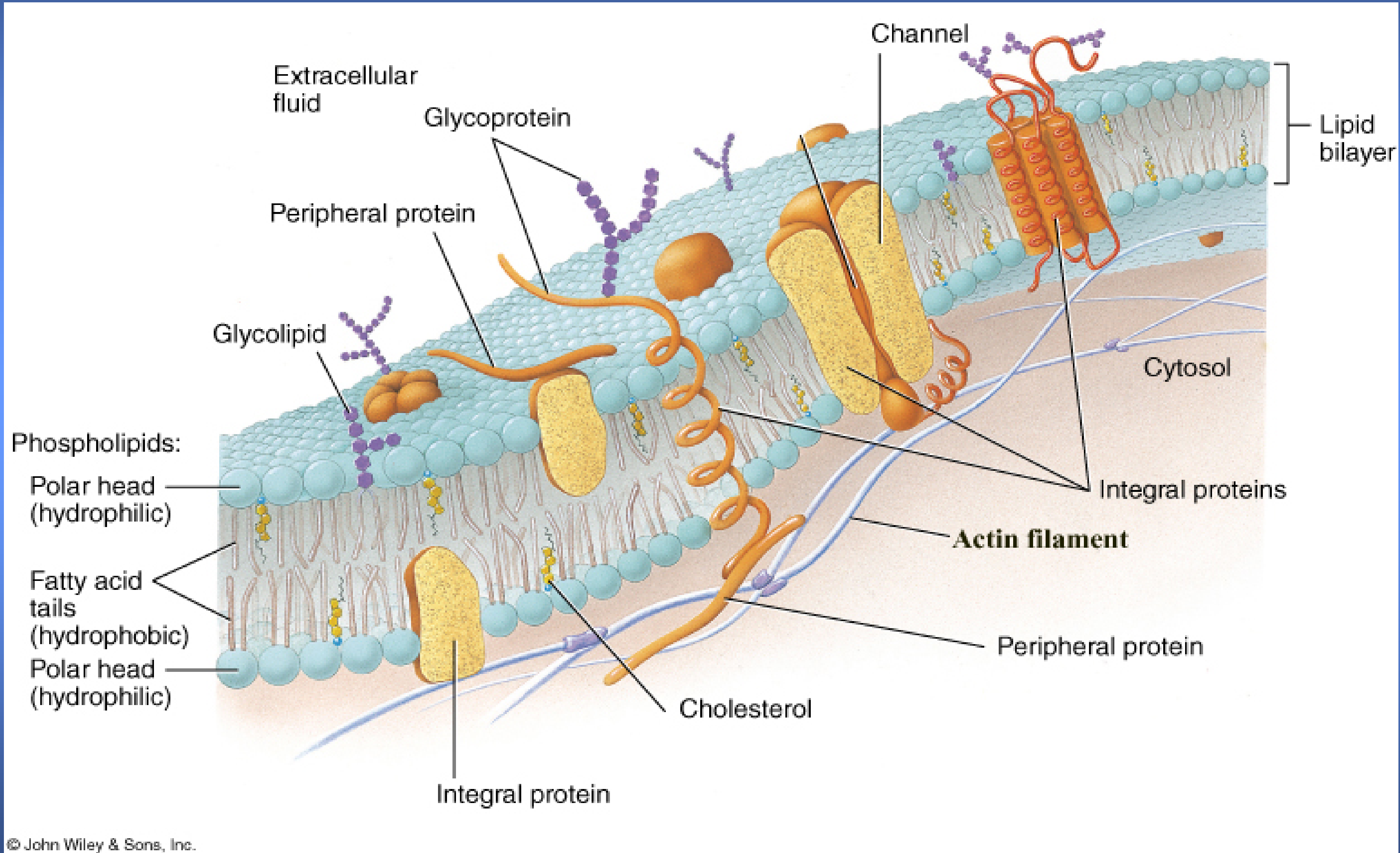


SRGs Applications

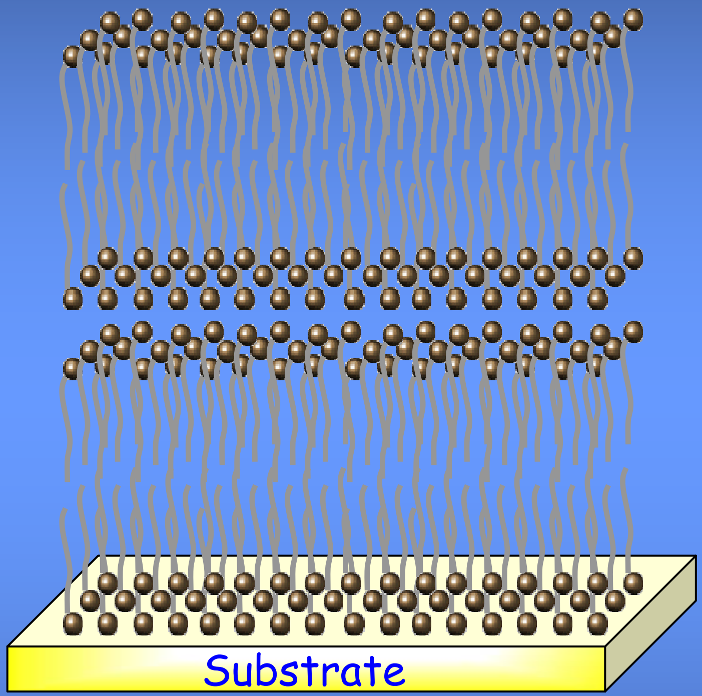
- Optical Storage
- Relief patterning
- Holography
- Wave Guides
- Liquid Crystal Alignment
- Nanomachines
- Patterns of SHG

Analysis of Biological Structures by Second Harmonic Generation

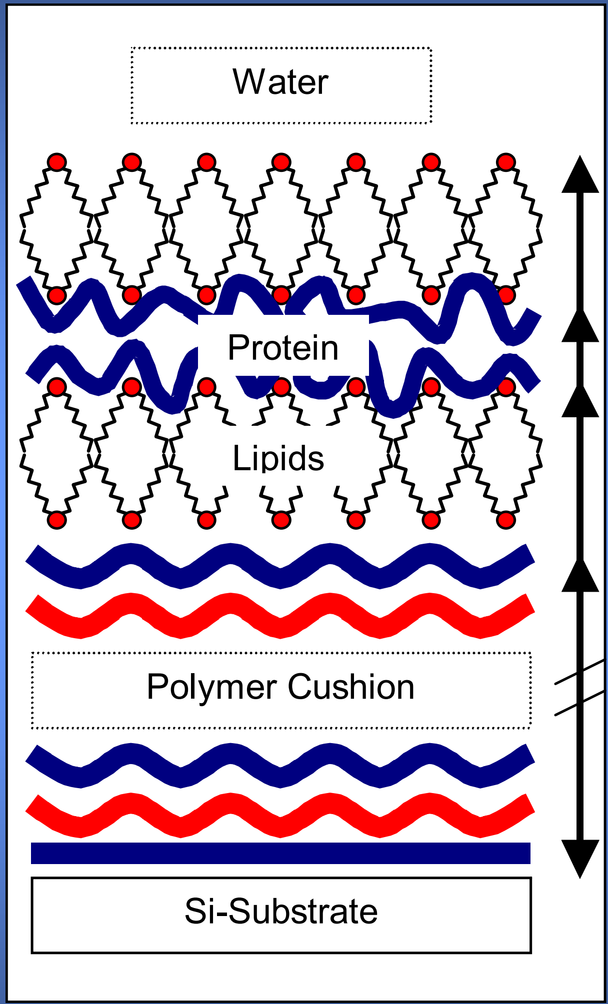
If one looks at ...



Thin Films Membranes



Langmuir- Blodget membrane



Molecular organization of the supported membrane prepared by LbL, such as derived from the neutron reflectivity data.

Analyzing Interfaces…

- Difficult to bring powerful methods of spectroscopy
- Traditional absorption, emission and Raman Scattering are generally not able to diferenciate optical signal from surface versus bulk

Signal will come essentially from the bulk

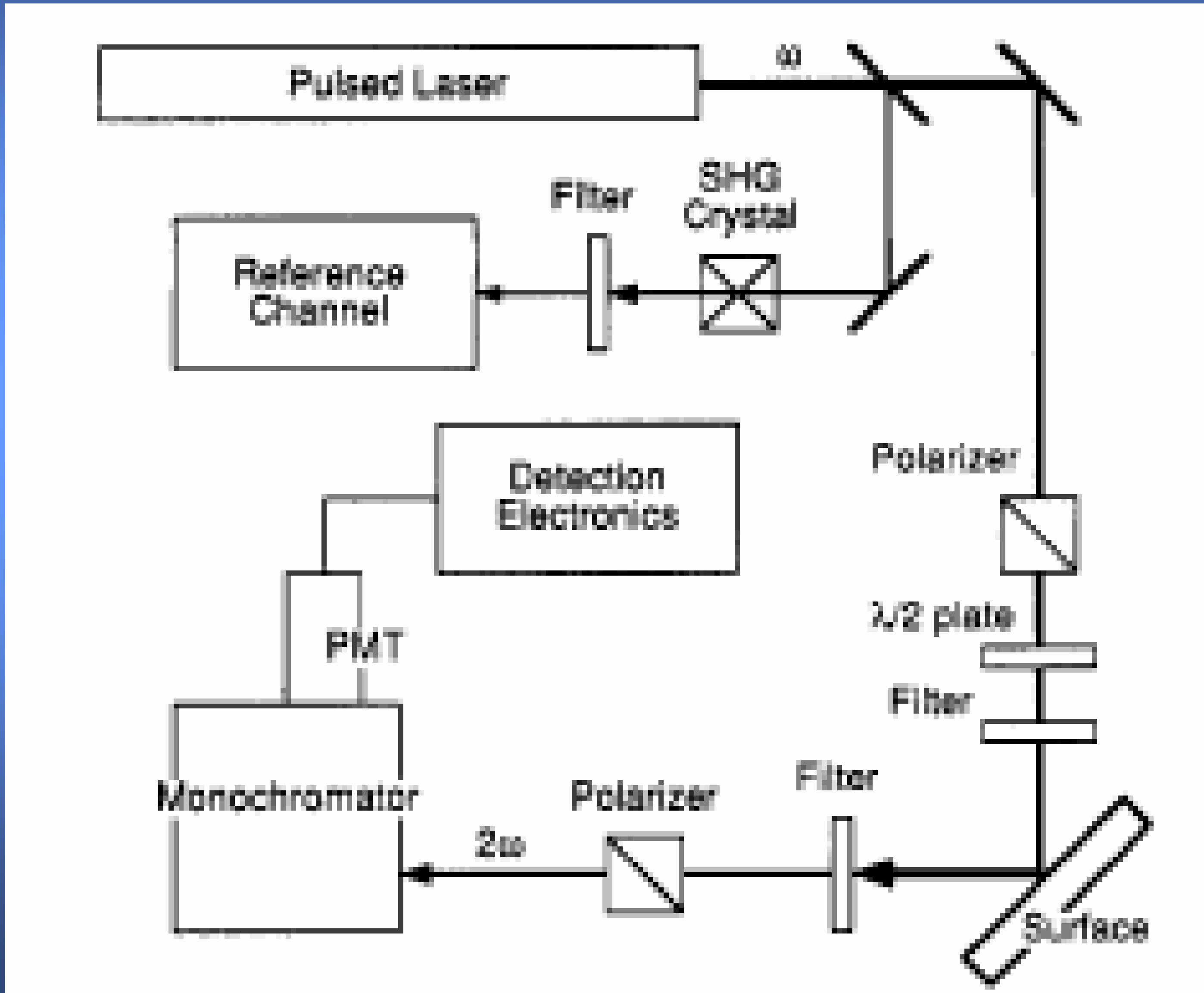
What about?

- Only noncentrosymetric systems will give a second-harmonic response.

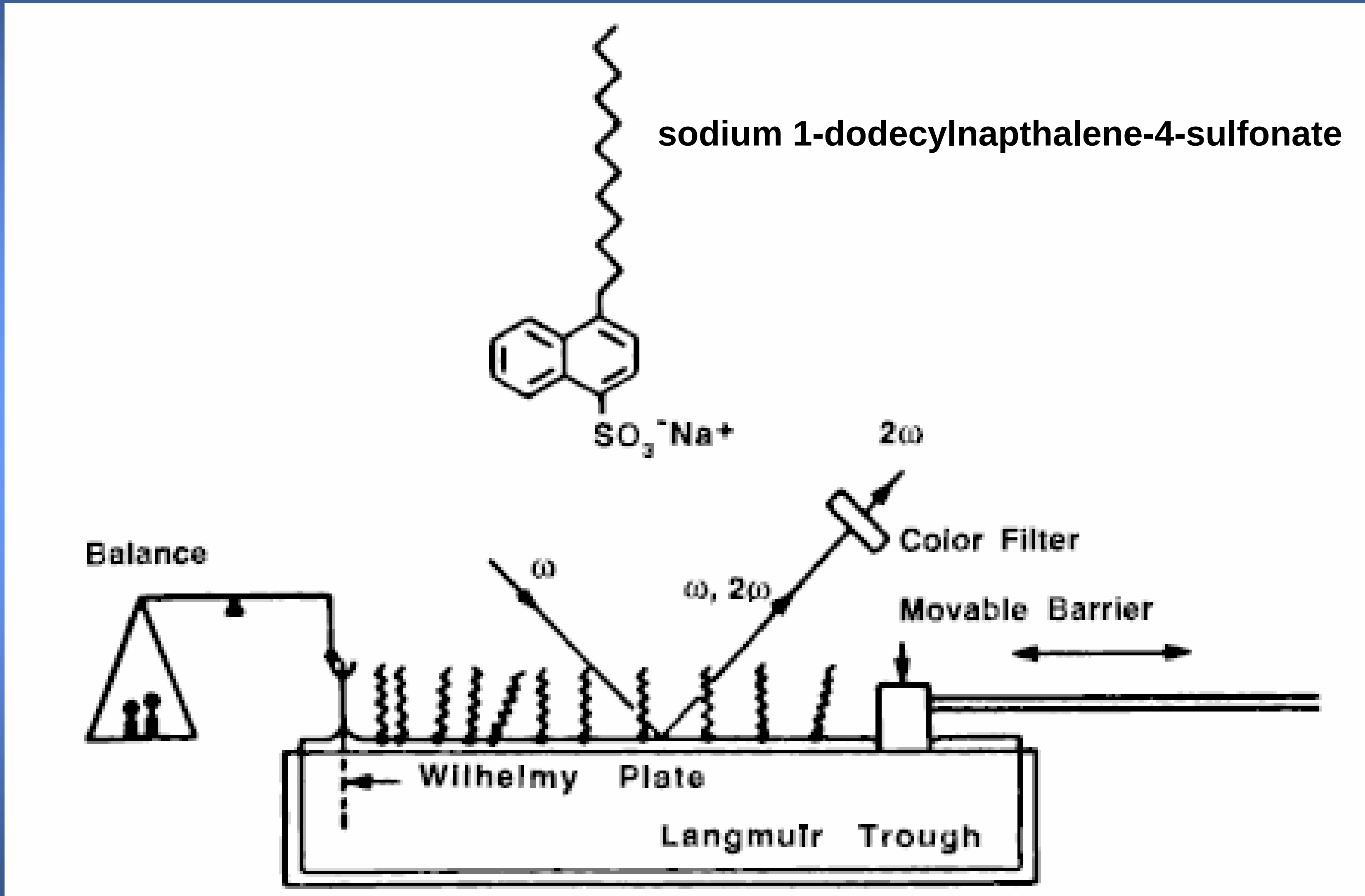
- Center of symmetry is naturally broken at a surface or interface

- Large Nonlinear response
- Distinguish bulk from surface

SHG Experimental Setup for Surface Analysis

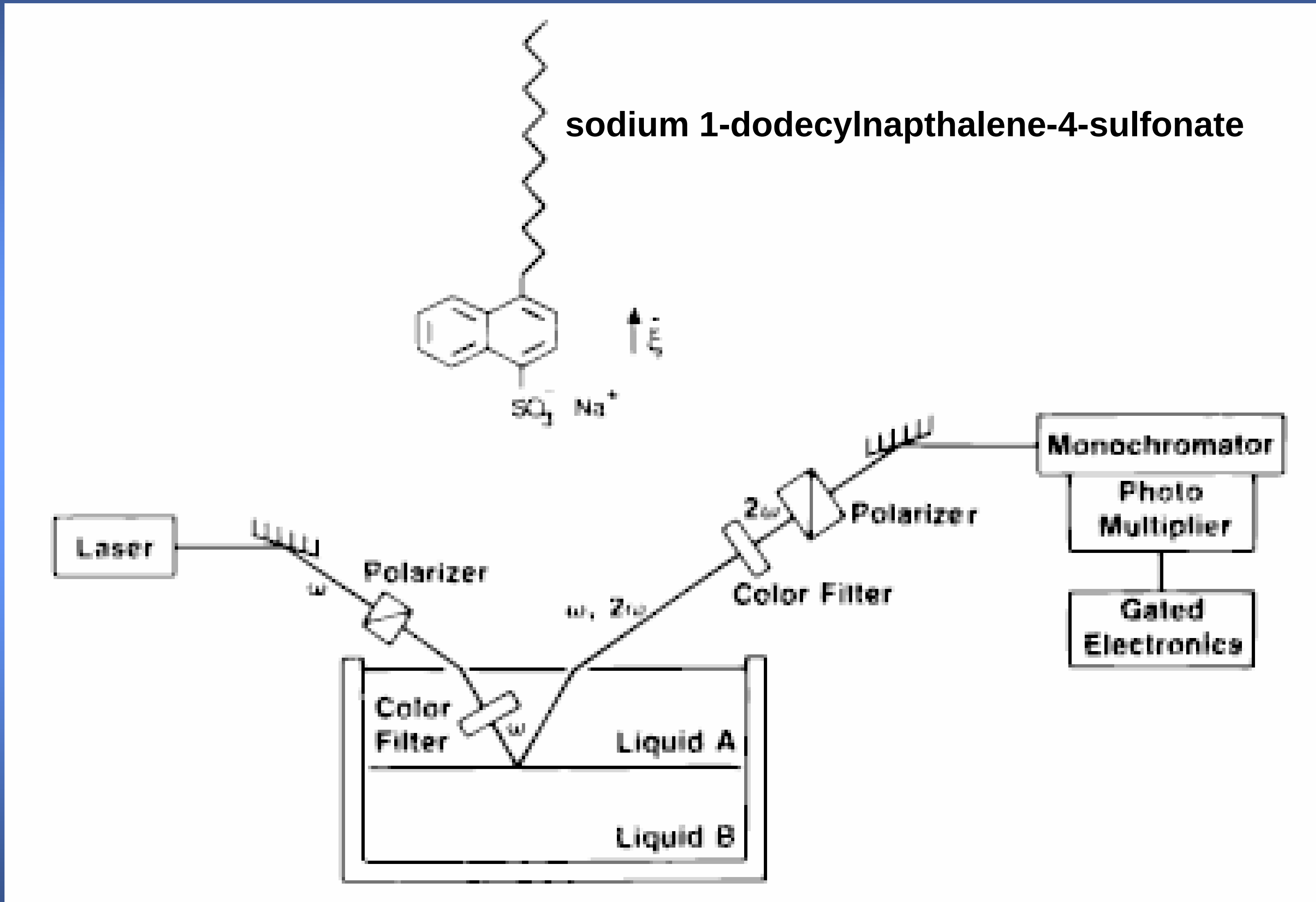


Kinetics of Langmuir monolayer



(K.B. Eisenthal, *Chemical Reviews*, 1996, 96,1345)

Monolayers at a liquid/liquid interface

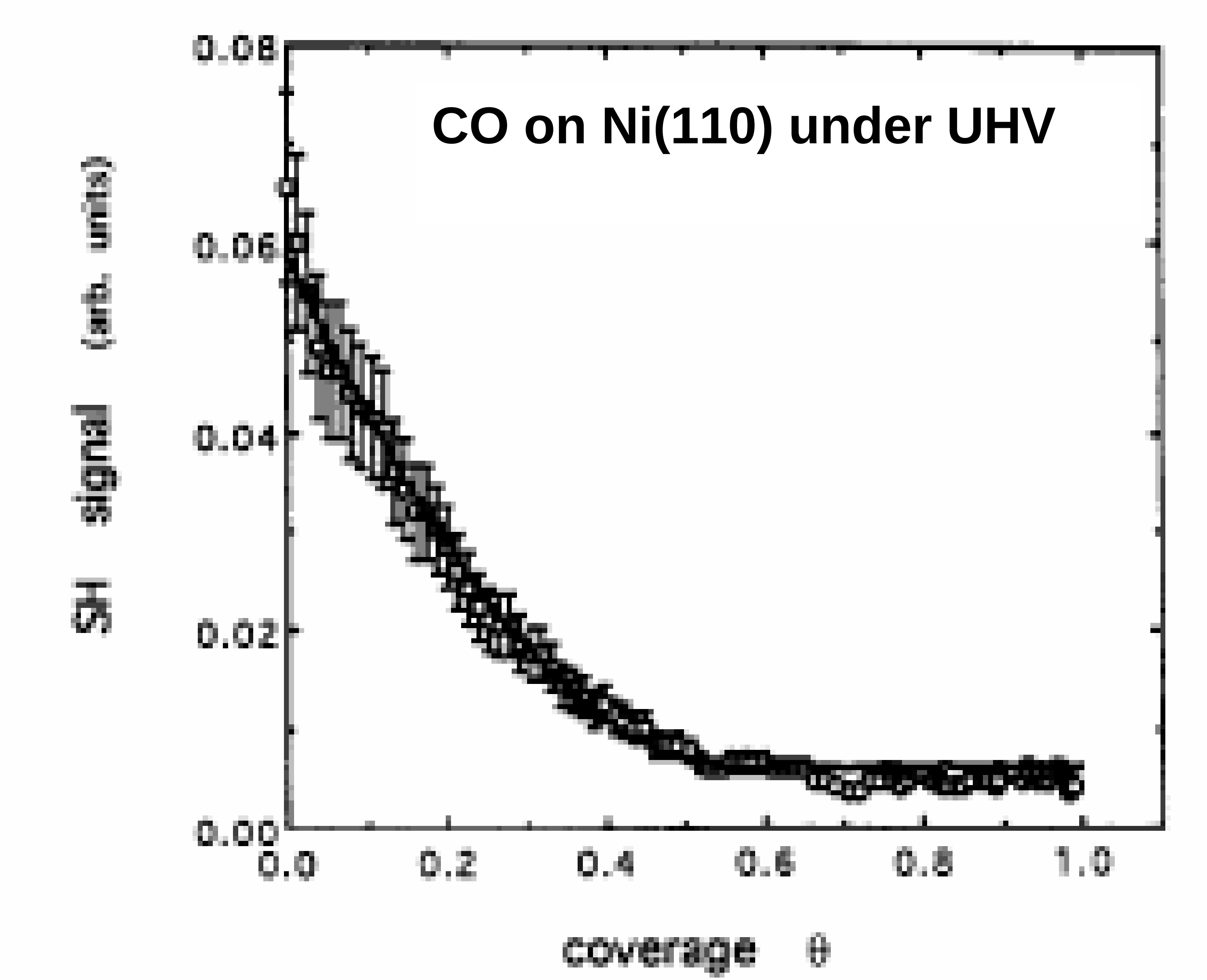


(K.B. Eisenthal, *Chemical Reviews*, 1996, 96,1345)

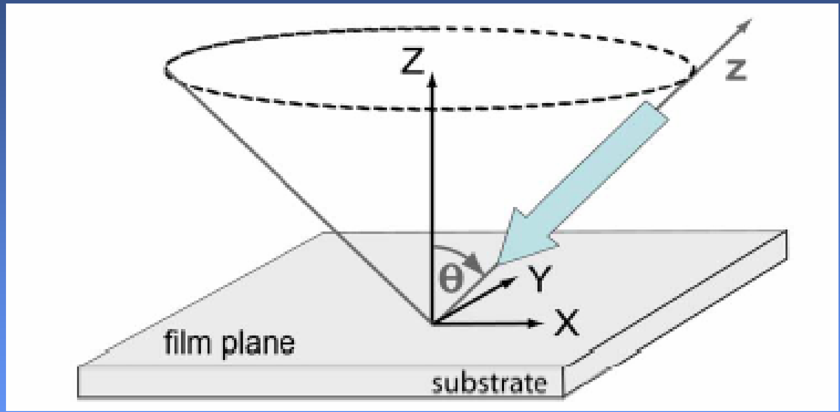
What it can be obtained from experiments

- Adsorption strength and surface coverage
- Molecular average orientation
- Surface symmetry
- Interfacial electric field strength
- Reaction kinetics and surface diffusion

Adsorption kinetics



Molecular orientation

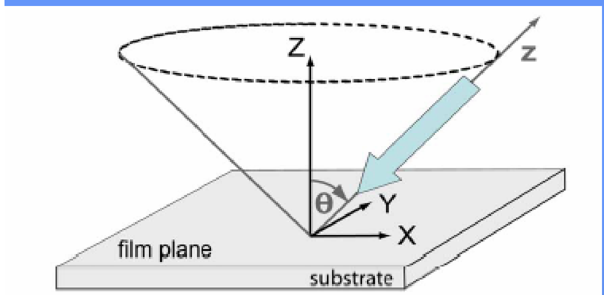


Measure

- Measurement of $\chi^{(2)}$ magnitudes
- Relative $\chi^{(2)}$ phases with respect to the fundamental light field (eventually)
- Response of individual molecule calculated or assumed
- Determination of average molecular orientation

Susceptibilities at an interface

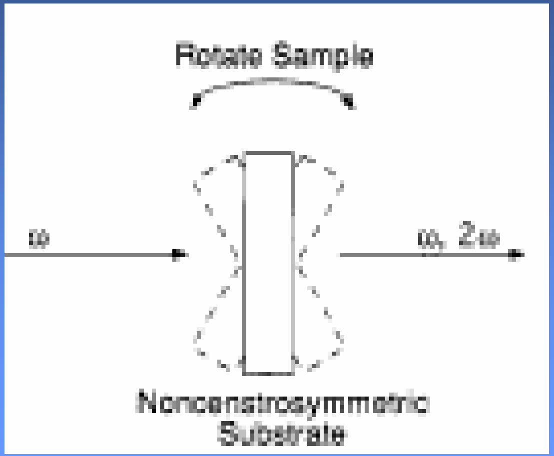
Two of the three non-vanishing allowed components of $\chi^{(2)}$ at an interface bounded by a bulk media

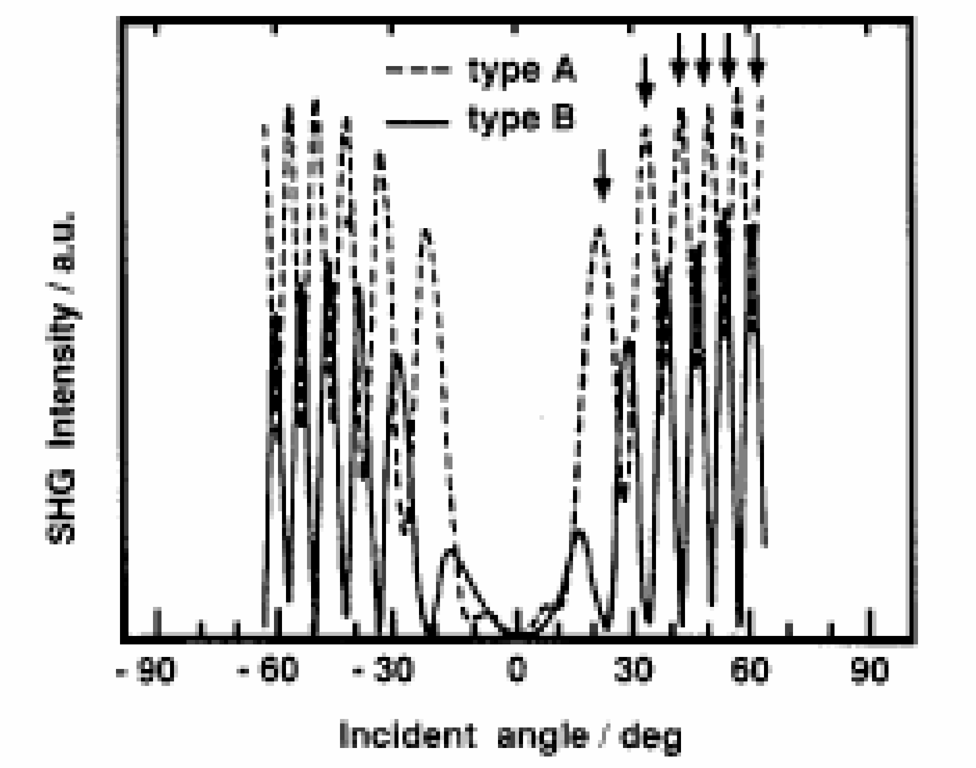


$$\begin{cases} \chi^{(2)}_{ZZZ} = N_s \beta_{ZZZ} \langle \cos^3 \theta \rangle \\ \chi^{(2)}_{XYZ} = \frac{1}{2} N_s \beta_{ZZZ} \langle \cos\theta sen^2\theta \rangle \end{cases}$$

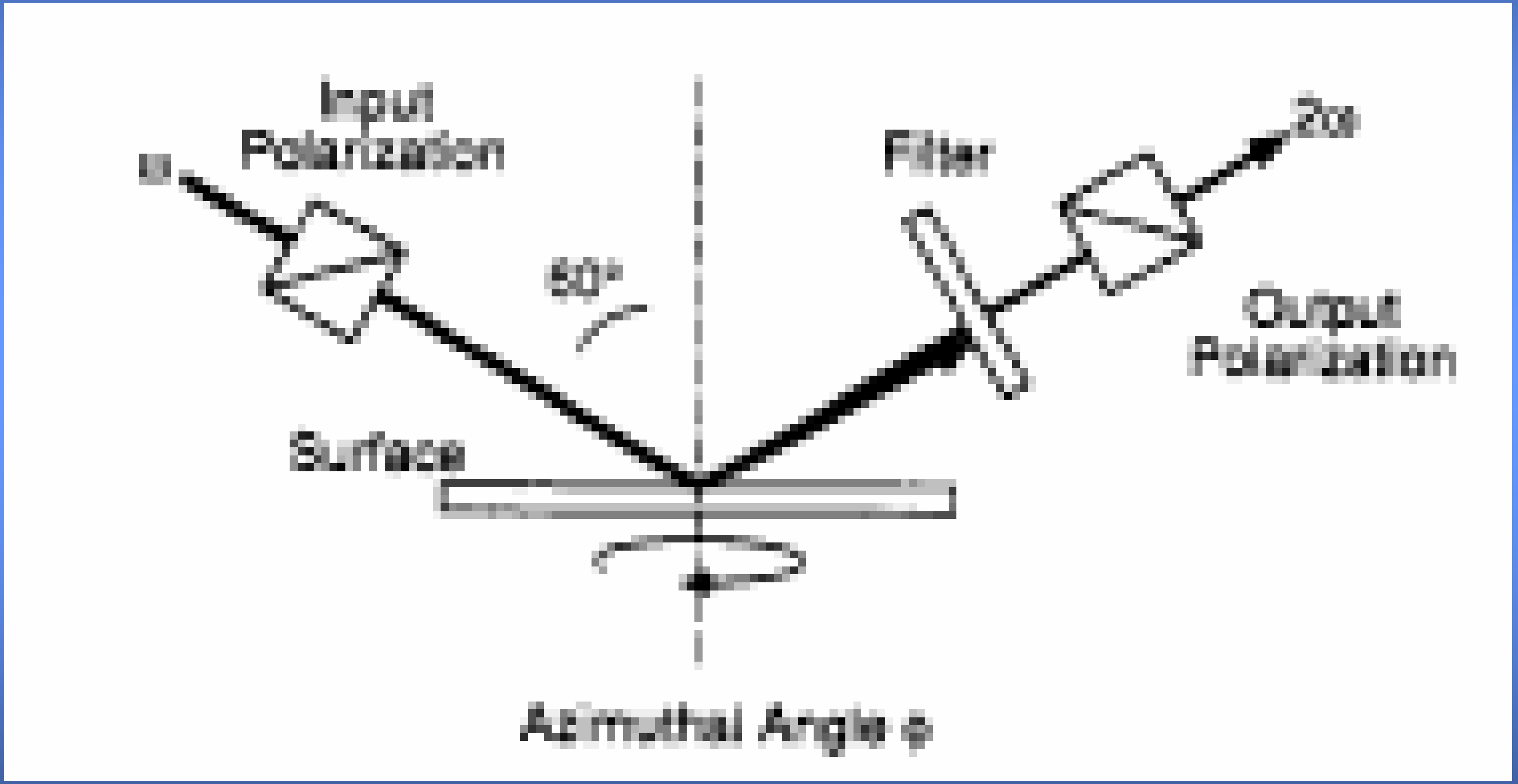
Molecular dynamics simulation allows the determination of the orientational distribution

Molecular absolute orientation





Surface Symmetry

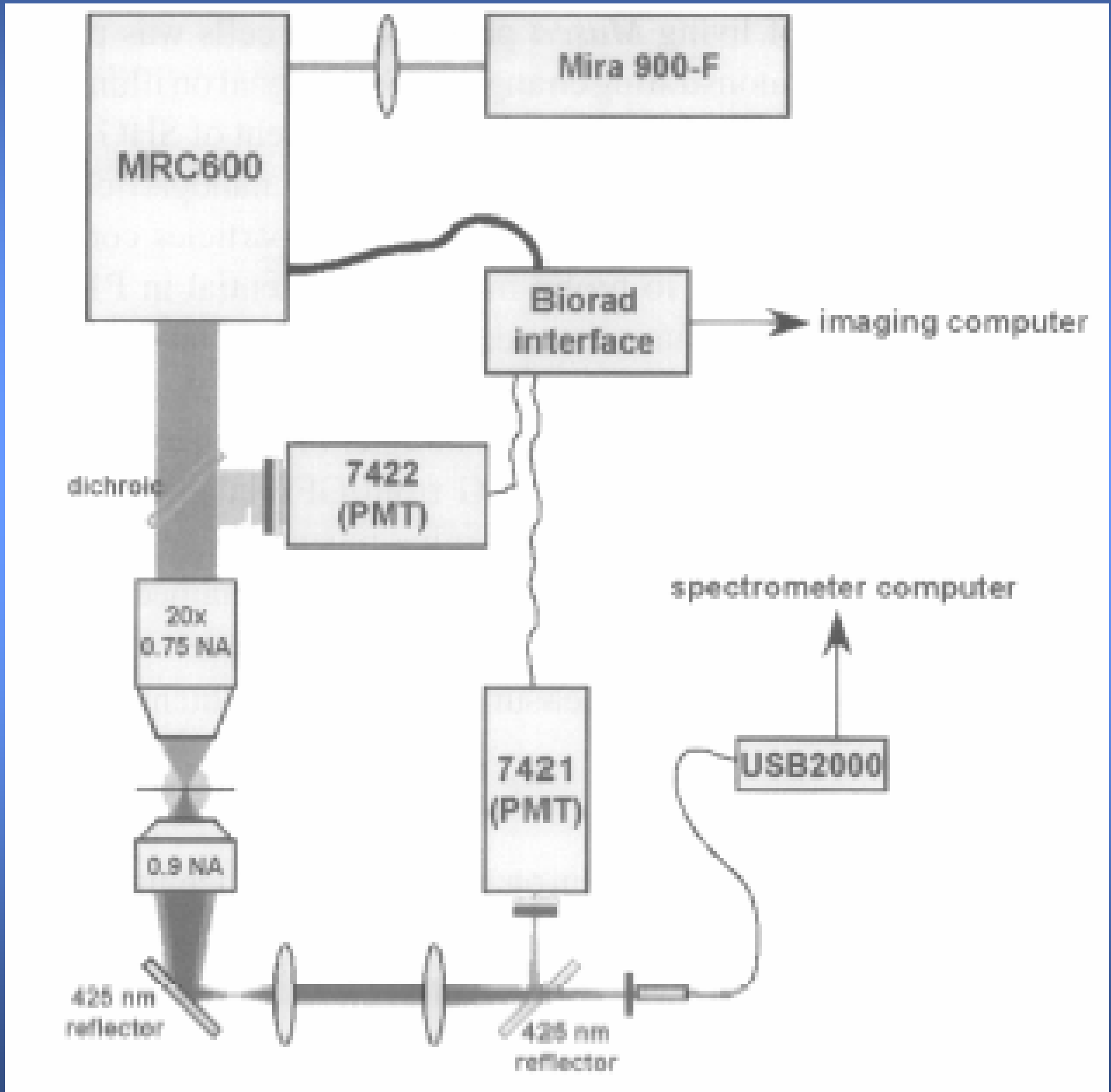


Bio-matter Analysis

Generally bio-matter is basically a set of interfaces

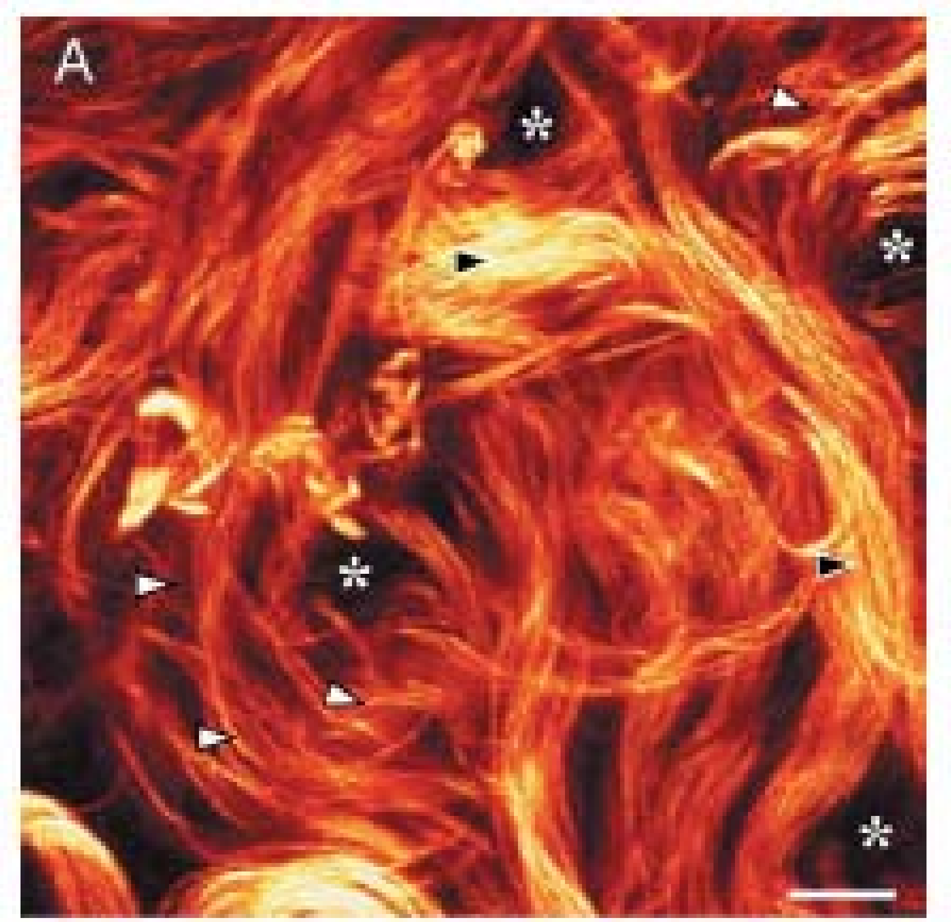
- Cellular membranes
- Supramolecular structures within cells and tissues
- Exploit the membrane biophysics
- Detect optical activity and probe chirality (much larger in NLO experiments)

Second Harmonic Generation Imaging Microscopy (SHIM)



A.C. Millard et al, Methods in Enzimology, 2003, 361,47-69.

3D reconstruction of human dermis and dermal blood vessels by SHIM



SHG imaging of the native collagen fiber network in the human dermis achieved at 880 nm for the detection of the SHG emission signal at 440 nm (25µm in depth). Open spherical spaces (asterisks) represent the location of vertical blood vessels that are not detected by SHG at 880 nm.

(P. Friedl, Histochem Cell Biol (2004) 122:183–190)

SHG Applications

- **Engineering:** laser and photonics
- **Biophysics, Medicine:** membrane binding and transport, imaging
- **Environmental Sciences**: air/water; soil/water and air atmospheric ice interfaces

Research Team

- Maria Raposo
- Paulo Limão-Vieira
- Pedro Vieira
- João Lourenço
- Quirina Ferreira
- Manuel Maneira
- Paulo J. Gomes
- MárciaTeruya

Other Collaborators

- **Portugal**
  - Ana Maria Botelho do Rego (IST/UTL)
  - Carlos Nunes de Carvalho DM/(FCT/UNL)
  - Madalena Dionísio (DQ/FCT/UNL)
- **United Kingdom**
  - N.J. Mason (Open University)
- **Brazil**
  - José Alberto Giacometti (FCT/UNESP)
  - Osvaldo Novais Oliveira Jr.(IFSC/USP)
  - Débora Gonçalves (IFSC/USP)
  - Débora Balogh (IFSC/USP)
  - Nara C. de Souza(FCT/UNESP)
  - David S. dos Santos (IFSC/USP)
- **Germany**
  - Roland Steitz (HMI)
  - Manuel Gonçalves (Ulm University)
- **Spain**
  - António de Saja (Valladolid University)