STRUCTURED GAUSSIAN BEAMS: RAY AND WAVE PICTURES

Miguel A. ALONSO, *,[1],[2]

[1] *Aix Marseille University, CNRS, Centrale Marseille, Institut Fresnel, UMR 7249, 13013 Marseille, France*
[2] *The Institute of Optics, University of Rochester, Rochester NY, U.S.A.*
**miguel.alonso@fresnel.fr*

Keywords: ray optics, wave optics, optical orbital momentum

A simple, intuitive ray-based description of self-similar beams is given in this presentation. Particular emphasis is placed on structured Gaussian beams, which include Hermite-Gauss, Laguerre-Gauss, generalized Hermite-Laguerre-Gauss, and Ince-Gauss beams, as well as many others. These beams can be described through a ray-optical Poincaré sphere, which helps reveal the hidden geometry behind their caustic structure. These beams accumulate in their focal region a Gouy phase, and some of them (the generalized Hermite-Laguerre-Gauss beams) can also accumulate a geometric (Pancharatnam-Berry) phase when subjected to a series of mode transformations. We discuss how the ray-based theory can explain these two types of phase, and even suggests a simple, noninterferometric method for measuring these phases. Finally, the connection to other types of self-similar beam such as Airy, Mathieu and Bessel beams are discussed.

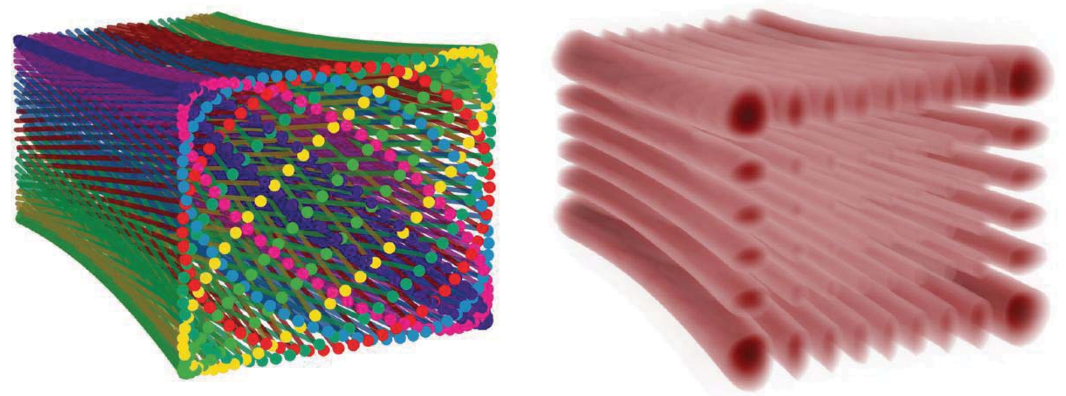

Fig. 1: Ray and wave descriptions of a Hermite-Gauss beam of order 8,5.

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LIGHT PROPAGATION IN SOFT BIO-TISSUES; SYNTHESIS OF RANDOM BEAMS CARRYING OAM

Olga KOROTKOVA,* Xi CHEN, Jia LI, Mohammad S. H. RAFSANJIANI

Department of Physics, University of Miami, 1320 Campo Sano Drive, Coral Gables, FL 33146
* *korotkova@physics.miami.edu*

Keywords: Optical Angular Momentum, random beams, spatial light modulator, power spectrum, anisotropic tissues

In the first part of the talk we consider various scattering and propagation problems arising on interaction of light beams with soft biological tissues, such as epidermis or liver. On following seminal papers by Schmidt and Kumar [1], Xu and Alfano [2] and Sheppard [3] we characterize soft tissues with power spectrum of refractive index having the power-law form, and having three major parameters: power-low constant (balance between small and large contributing inhomogeneites), outer scale (the largest inhomogeneity) and the refractive index structure parameter (local refractive index contrast). In particular, we demonstrate how for tissues with fractal-like power spectrum the direct scattering problem can be analytically solved and the inverse scattering problem of finding the three parameters of the spectrum can be analytically solved from several measurements of light scattered from the tissue to the far zone [4]. We also discuss how the second-order moment (average intensity) and the fourth-order moment (scintillation index) of a light beam propagate in soft bio-tissues [5, 6]. In particular, we show how from the scintillation index analysis the threshold between the weak and strong tissue fluctuations can be determined. The extension of isotropic power spectrum to that with geometrical anisotropy is also considered and possible changes in light statistics due to such anisotropy are outlined [6].

In the second part of the talk we present a recently introduced technique for synthesis of random beams carrying Optical Angular Momentum (OAM) realized with the help of the Spatial Light Modulators (SLM) [7]. This technique is based on relatively low temporal coherence of some lasers (e.g., HeNe) providing with ensembles of realizations having independent phases. We illustrate the technique by using incoherent superposition of the Laguerre-Gaussian mode sequences with tunable weights imposed by the SLM on the lowest-order Gaussian beam leading to the I_0-Bessel correlated beams [8]. These beams can carry any OAM index in their separable phase term and can be tuned to have any spatial coherence state, varying from coherent (single-mode) to virtually incoherent (infinite-mode). Combination of randomness and OAM makes them particularly attractive for interaction with weak random media, such as biological tissues, because of their ability to mitigate scintillations in the rings and have destructive interference effect at the center. We also briefly outline some general trends for the average intensity and the scintillation index obtained on passage of the I_0-Bessel-correlated beams with various OAM through a Spatial Light Modulator with a fractal power spectrum phase screen.

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SPIN ANGULAR MOMENTUM OF NONREGULAR STATES OF POLARIZATION

José J. GIL

Department of Applied Physics, Pedro Cerbuna 12, 50004 Zaragoza, Spain
**ppgil@unizar.es*

Keywords: optics, polarization, spin angular momentum

In general, the polarization ellipse of an electromagnetic wave at a fixed point in space evolves randomly. When the plane containing the polarization ellipse remains fixed during the polarization time [1], the corresponding state of polarization is said to be two-dimensional (2D), while when this does not happen, the polarization state is genuinely three-dimensional (3D) [2].

While 2D polarization states can always be considered as an incoherent superposition of a pure state (fully polarized) and a 2D unpolarized state (the polarization ellipse evolves fully randomly in a fixed plane), in general 3D states cannot be decomposed into a pure state, a 2D unpolarized state, and a 3D unpolarized state. The 3D states that can be decomposed in such a manner are said to be *regular*, which is a limiting case of *nonregular* polarization states [3,4].

A general analysis of the direction of the intensity-normalized spin angular momentum vector $\hat{\mathbf{n}}$ can be carried out from the *characteristic decomposition* [4,5] of the polarization matrix $\mathbf{R}$ associated with the given state of polarization

$$\mathbf{R} = I\left[P_1\hat{\mathbf{R}}_p + \left(P_2 - P_1\right)\hat{\mathbf{R}}_m + \left(1 - P_2\right)\hat{\mathbf{R}}_{u-3D}\right],$$

$$\hat{\mathbf{R}}_p \equiv \hat{\mathbf{u}}_1\hat{\mathbf{u}}_1^\dagger, \quad \hat{\mathbf{R}}_m \equiv \frac{1}{2}\left(\hat{\mathbf{u}}_1\hat{\mathbf{u}}_1^\dagger + \hat{\mathbf{u}}_2\hat{\mathbf{u}}_2^\dagger\right), \quad \hat{\mathbf{R}}_{u-3D} \equiv \frac{1}{3}\left(\hat{\mathbf{u}}_1\hat{\mathbf{u}}_1^\dagger + \hat{\mathbf{u}}_2\hat{\mathbf{u}}_2^\dagger + \hat{\mathbf{u}}_3\hat{\mathbf{u}}_3^\dagger\right) = \frac{1}{3}\mathbf{I}, \qquad (1)$$

where $\hat{\mathbf{u}}_i$ $(i = 1,2,3)$ are the eigenvectors of $\hat{\mathbf{R}} \equiv \mathbf{R}/I$, the dagger stands for conjugate transpose, $\mathbf{I}$ is the identity matrix, and P_1, P_2 are the *indices of polarimetric purity* [6].

The intensity-normalized polarization matrices $\hat{\mathbf{R}}_p$ and $\hat{\mathbf{R}}_{u-3D}$ correspond respectively to a pure state and 3D unpolarized state, and P_1, P_2 are the indices of polarimetric purity of $\mathbf{R}$ [6]. The middle component $\hat{\mathbf{R}}_m$ correspond to a 2D unpolarized state if and only if $\mathbf{R}$ is regular; otherwise $\hat{\mathbf{R}}_m$ corresponds itself to a 3D state with nonzero linear and circular degrees of polarization [4]. In other words, in the case of nonregular states, $\hat{\mathbf{R}}_m$ can always be considered as an incoherent composition of two pure states with different directions of propagation, at least one of them having nonzero degree of circular polarization (i.e., nonzero spin angular momentum [1,7]). It should also be noted that, obviously, the spin angular momentum of the fully random component $\hat{\mathbf{R}}_{u-3D}$ is zero.

For a regular state, $\hat{\mathbf{n}}_m = 0$ and $\hat{\mathbf{n}} = P_1\hat{\mathbf{n}}_p$, so that the directions of $\hat{\mathbf{n}}$ and $\hat{\mathbf{n}}_p$, necessarily coincide. For nonregular states

$$\hat{\mathbf{n}} = P_1\hat{\mathbf{n}}_p + \left(P_2 - P_1\right)\hat{\mathbf{n}}_m, \qquad (2)$$

so that, except for states for which the eigenstates $\hat{\mathbf{u}}_1$ or $\hat{\mathbf{u}}_2$ correspond to a linearly polarized state, vectors $\hat{\mathbf{n}}$ and $\hat{\mathbf{n}}_p$ of a nonregular state have necessarily different directions. In such cases, the lower is the degree of nonregularity of $\mathbf{R}$ [4], the closer are the directions of $\hat{\mathbf{n}}$ and $\hat{\mathbf{n}}_p$.

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USING OPTICAL VORTICES IN SPECKLES FOR COMPRESSED 3D SUPER-RESOLUTION IMAGING

Marc GUILLON[*,1], Ori KATZ[2], Marco PASCUCCI[1], Jérôme GATEAU[1], Hervé RIGNEAULT[3], Gilles TESSIER[1], Valentina EMILIANI[1]

[1]Neurophotonics Laboratory, CNRS UMR 8250, University Paris Descartes, Sorbonne Paris Cité, Paris, France;
[2]The Hebrew University of Jerusalem, Jerusalem, Israel;
[3] Aix-Marseille University, CNRS, Centrale Marseille, Institut Fresnel UMR 7249, Marseille, France
**marc.guillon@parisdescartes.fr*

Keywords: speckles, super-resolution microscopy, compressed sensing

Nonlinear structured illumination microscopy (nSIM) is an effective approach for super-resolution wide-field fluorescence microscopy with a theoretically unlimited resolution. In nSIM, carefully designed, highly-contrasted illumination patterns are combined with the saturation of an optical transition to enable sub-diffraction imaging. While the technique proved useful for two-dimensional imaging, extending it to three-dimensions (3D) is challenging due to the fading/fatigue of organic fluorophores under intense cycling conditions. Here, we present a compressed sensing approach that allows for the first time 3D sub-diffraction nSIM of cultured cells by saturating fluorescence excitation [1]. Exploiting the natural orthogonality of transverse speckle illumination planes, 3D probing of the sample is achieved by a single two-dimensional scan. Fluorescence contrast under saturated excitation is ensured by the inherent high density of intensity minima associated with optical vortices in polarized speckle patterns [2]. Compressed speckle microscopy is thus a simple approach that enables 3D super-resolved nSIM imaging with potentially considerably reduced acquisition time and photobleaching.

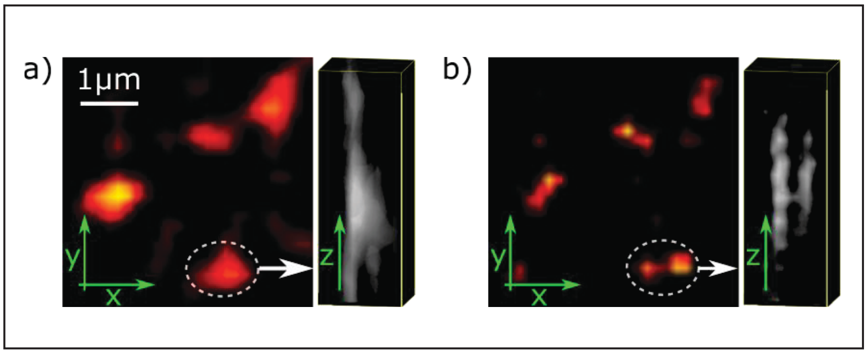

Fig. 1: Fluorescence images of lysosomes in cultured cells. 3D-deconvolved speckle scanning of the object a) and reconstruction of a speckle-saturated fluorescence image by a compressed sensing algorithm.

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APPLICATION OF SPIRAL BEAMS IN OPTICAL MICROMANIPULATION AND 3D IMAGING

Svetlana KOTOVA

Lebedev Physical Institute, 221, Novo-Sadovaya Str., Samara, 443011 Russia;
kotova@fian.smr.ru

Keywords: spiral beams, optical micromanipulation, double helix point spread function

Spiral beams belong to the class of fields with phase singularities that retain the intensity distribution shape (except the scaling and rotation) upon propagation and focusing. These beams were first discovered by E. Abramochkin and V. Volostnikov [1, 2] as a class of solutions of the parabolic equation for the light field. The spiral beam optics gives us a new fundamental possibility for forming structured vortical light fields. I present the results of the Lebedev Physical Institute Samara branch research group on the use of spiral beams in two areas: optical micromanipulation and 3D imaging.

Spiral beams can have an intensity distribution described by an arbitrary planar curve. Examples of experimentally obtained images of spiral beams are shown on the Figure.

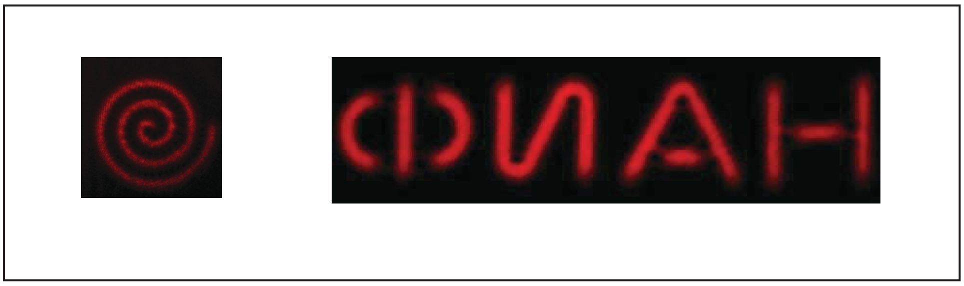

Fig.: Examples of experimentally obtained images of spiral beams

Using the phase part of a spiral beam and the iterative algorithm we obtained phase diffractive elements to shape optical traps in form of arbitrary curves. Owing to the angular momentum of considered fields it is feasible to effectuate the micro-objects motion along a specified trajectory without any mechanical displacement of the device elements, to arrange groups of particles into various configurations, to impose heterogeneous deformation and to force the particles rotation at different velocities. In our experiments the micro-objects motion along the boundaries of a triangle, square and Archimedes spiral was demonstrated, the possibility to deform elongated objects was studied, use of the Archimedes spiral as the micro-mixer was shown.

The theory of spiral beams allows the generation of the two-lobe light fields with the rotating intensity distribution [3, 4]. Using this property, one can obtain a phase mask that transforms the single emitter point spread function of the microscope into double helix point spread function. This allows three-dimensional localization of point light sources [5]. The results of numerical simulation and experiments show that it is possible to create phase filters that form two-lobe light fields with different rotation speeds, with an energy efficiency up to 70%.

This research is financially supported by the RFBR (projects No. 16-29-11809 and No. 16-42-630773).

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SINGLE SHOT POLARIMETRY OF HIGHER ORDER CYLINDRICALLY POLARIZED VECTOR BEAMS

Ignacio MORENO*,[1] María del Mar SÁNCHEZ-LOPEZ,[2] Jeffrey A. DAVIS,[3]
Katherine BADHAM,[3] Don M. COTTRELL[3]

[1]Departamento de Ciencia de Materiales, Óptica y Tecnología Electrónica, Universidad Miguel Hernández de Elche, 03202 Elche, Spain;
[2]Departamento de Física y Arquitectura de Computadores, Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, 03202 Elche, Spain; [1]
[3]Departament of Physics, San Diego State University, California, USA
**i.moreno@umh.es*

Keywords: optics, polarization, optical orbital momentum, vector beams

We introduce a technique to generate polarization diffractive elements by means of liquid crystal spatial light modulators. We illustrate the technique by designing optical elements that produce different vector beams by means of the combination of a polarization diffraction grating (PDG) with encoded q-plate devices. The PDG generates target diffraction orders where the state of polarization at each order can be designed and controlled [1]. The q-plate can be a physical azimuthal retarder, or can be also encoded onto the SLM [2]. The combination of these two elements allows the single-shot generation of different vector beams at different diffraction orders. This element can be used as an element for the detection of vector beams, where the charge and the particular kind of state of polarization can be detected [3].

As an example, the figure shows the diffraction pattern of a one dimensional grating that generates six differet vector beams at the diffraction orders ±1, ±2, and ±3 (Fig. 1(a)). When this PDG is illuminated with a vector beam of charge one, a bright delta function appears on the first order (Fig. 1(b)).

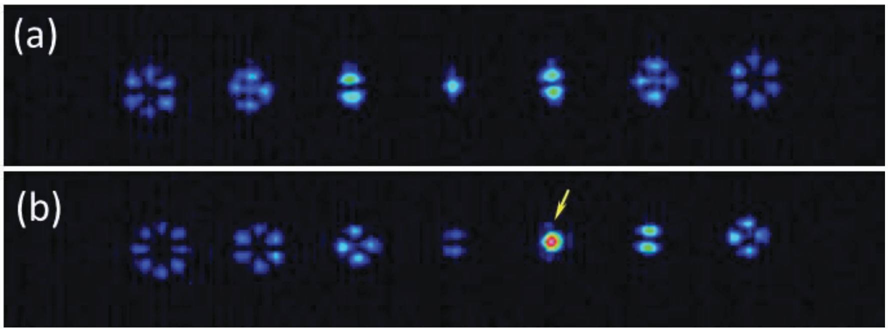

Fig. 1: (a) Experimental diffraction from a PDG generating six vector beams at the diffraction orders ±1, ±2, and ±3. (b) When illuminated with a vector beam, the position of the delta function indicates its charge.

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PERFORMANCE OF GENERAL TIGHTLY FOCUSED BEAMS AFTER LINEAR POLARIZERS

Davd Maluenda,[1] Ignasi Juvells[2], Artur Carnicer,[2] Rosario Martínez-Herrero *,[3]

[1] Consejo Superior de Investigaciones Científicas (CSIC), Centro Nacional de Biotecnología,
Biocomputing Unit, Darwin 3, 28049 Madrid, Spain
[2]Universitat de Barcelona, Departament de Física Aplicada, Martí i Franquès 1, 08028 Barcelona
[2]Universitat Complutense de Madrid, Facultad de Ciencias Físicas, Departamento de Óptica, Ciudad
Universitaria, 208040 Madrid , Spain
**r.m-h@fis.ucm.es*

Keywords: Electromagnetic optics, Polarization, Coherence.

Polarizers are designed to be used in paraxial conditions and with normal incidence. However, the conventional projection character of these devices changes when the beam impinges a polarizer with a certain angle of incidence. This effect is more noticeable if polarizers are used in optical systems with a high numerical aperture because multiple angles of incidence have to be taken into account. Recently, it has been showed that an ideal linear polarizer placed at the focal plane of a high numerical aperture (NA) optical system cannot be considered as a simple projector device [1-4].

In this communication we present analytical expressions for the cross-spectral density function of the electromagnetic field after passing a linear polarizer placed at the focal plane of a high NA lens when the incoming beam is partially coherent and partially polarized. Interestingly, the properties of these beams present some potential interest in bio-optics problems.

The polarization properties of the focused field depend on the spatial distribution, coherence and polarization characteristics of the incident field. The features of the focused field after the polarizer are numerically and experimentally evaluated for some illustrative examples.

The present work has been partially supported by MINECO project number FIS2016-75147-C3-1-P.

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Generating and Multiplexing Highly Selective Orbital Angular Momentum with Multi-Plane Light Conversion

Matthieu MEUNIER[1], Gauthier TRUNET[1], David ALLIOUX[1]*, Pu JIAN[1], Guillaume LABROILLE[1]

[1]*Cailabs, 38 Bd Albert 1er, 35200 Rennes, France ;*
**david@cailabs.com*

Keywords: Multiplexing, Orbital angular momentum, Nonimaging optical systems

Light beams with helical phase profile carrying orbital angular momentum (OAM) [1] are used in a large panel of biological applications. Generating OAMs is now easily accessible by using for instance q-plates, spiral phase plates, holograms or active elements like SLMs [2]. Multiplexing different OAM modes as was demonstrated in telecommunication [3], is an approach that could increase the spatial resolution of microscopy devices [4] and more specifically enable to probe the chirality of multiple molecules inside living organisms [4]. Unfortunately, multiplexing modes often requires the addition of optical elements like beams splitters, inducing high losses and complex alignment when the number of modes increases. In this article we demonstrate highly selective and low loss generation and multiplexing of 7-OAM modes in the near infrared, at 850 nm and 1550 nm, using a Multi-Plane Light Conversion technology.

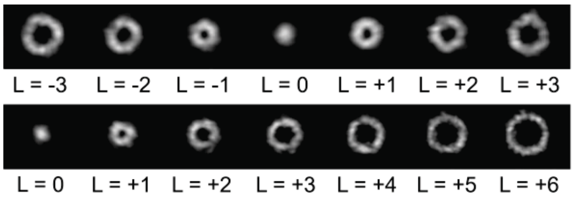

Figure 1: Free-space output of the OAM modes.

Configuration	Insertion losses	Modal crosstalk
850 nm L_1	4.3 dB	- 26.9 dB
1550 nm L_1	3.3 dB	- 30.1 dB
1550 nm L_2	3.1 dB	- 22.9 dB

Table 1: Insertion losses and crosstalk of OAM multiplexers. The values are averaged over the modes.

Multi-Plane Light Conversion (MPLC) is a technique allowing any unitary spatial transform [6]. Theoretically, MPLC enables a passive lossless conversion of a set of N orthogonal spatial modes into another set of N orthogonal modes. The conversion is done through the succession of transverse phase profiles separated by a free space propagation that serves as a fractional Fourier transform operation.

We fabricated three pairs of multiplexers supporting each 7 OAM modes. The first one is at $\lambda = 850$ nm with topological charges $L_1 = \{-3 \text{ to } +3\}$ while the two others are at $\lambda = 1550$ nm with topological charges $L_1 = \{-3 \text{ to } +3\}$ and $L_2 = \{0 \text{ to } +6\}$. 7 SMFs serve as orthogonal input modes, each being converted into one of the desired OAM modes. The profiles of the OAM modes in free space are shown in Figure 1. The multiplexers are characterized using a superluminescent diode source centered at 850 nm or 1550 nm. We measured the transmission matrix of a back-to-back system including a multiplexer and a demultiplexer separated by 500 mm of free space propagation. We show the performances averaged over all modes for a single multiplexer in Table 1. We measured insertion losses ranging from 3.1 dB to 4.3 dB and crosstalk losses from -22.9 dB to -30.1 dB. We believe these highly selective, high number of modes OAM multiplexers could simplify the burgeoning of microscopy technics and molecule chirality exploration.

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MONOCHROMATIC INVERSE SCATTERING WITHOUT PHASE INFORMATION

Roman G. NOVIKOV*[1]

[1]*CMAP, CNRS, Ecole polytechnique, Palaiseau, 91128, France;*
**roman.novikov@polytechnique.edu*

Keywords: scattering, direct and inverse problems

We consider direct and inverse scattering problems of monochromatic wave propagation. Such problems arise, in particular, in different domains of physics, in chemistry, biology and medicine. In addition, in many important cases only scattering data without phase information can be measured directly in practice. In particular, most optical measurement techniques only provide information on the amplitude, but not on the phase of time-harmonic electromagnetic waves.

In this connection we report on non-uniqueness, uniqueness and reconstruction results for inverse scattering without phase information. We are motivated by recent and very essential progress in this domain. For more information we refer to [1], [2] and references therein.

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MULTISPECTRAL AND TEMPORAL CONTROL OF LIGHT IN MULTIPLE SCATTERING MEDIA

Sylvain Gigan

[1]*Laboratoire Kastler Brossel, ENS-Université PSL, CNRS, Sorbonne Université, Collège de France,*
24 rue Lhomond, 75005 Paris, France
**sylvain.gigan@lkb.ens.fr*

Keywords: Wavefront shaping, complex media, pulse-shaping, time-gating

Scattering of light in heterogeneous media, for instance the skin or a glass of milk, is usually considered an inevitable perturbation or even a nuisance. Through repeated scattering and interferences, this phenomenon seemingly destroys both the spatial and the phase information of any laser illumination. At the spatial level, it gives rise to the well-known "speckle" interference patterns. At the temporal (or spectral) level, a short pulse entering a scattering medium will see its length greatly extended due to the multiplicity of possible path length light can take before exiting the medium. From an operative point of view, scattering greatly limits the possibility to image or manipulate an object with light through or in a scattering medium. Multiple scattering is nonetheless an invaluable field of research for experimentalists and theoreticians alike, at the crossing of optics, condensed matter physics, statistical physics, chaos, to name just a few.

Multiple scattering is a highly complex but nonetheless deterministic process: it is therefore reversible, in the absence of absorption. Speckle is coherent, and can be coherently controlled. By « shaping » or « adapting » the incident light, it is in principle possible to control the propagation and overcome the scattering process. I will show our recent results on achieving a complete pulse control (spatial and temporal) by means of wavefront shaping.

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SPIN OPTICAL EFFECTS IN PLASMONICS

M. Pal[1], S. Saha[1], S. Chandel[1], S. K. Roy[1], A.K. Singh[1] and Nirmalya Ghosh *,[1]

[1] *Department of Physical Sciences, IISER Kolkata, India;*
**nghosh@iiserkol.ac.in*

Keywords: Polarization, Spin optics, optical orbital momentum, plasmonics

Spin orbit interaction (SOI) dealing with the coupling of spin and orbital degrees of freedom of massive (e.g., electron) and mass-less (e.g., photon) particles has led to several fundamental consequences in diverse fields of physics. Since light can carry both spin (SAM, circular / elliptical polarization) and orbital angular momentum (OAM), on conceptual grounds, coupling and inter-conversion between the spin and orbital AM degrees of freedom of light is expected under certain circumstances. This leads to the SOI of light, and accordingly the evolution of polarized light in a trajectory mimics the SOI effect of a mass-less spin 1 particle (photon) [1]. The SOI of light is typically manifested as two interdependent effects. *(i)* evolution of azimuthal geometric phase due to the effect of the trajectory on the state of polarization of light, leading to intrinsic SAM to intrinsic OAM inter-conversion and its various intriguing manifestations (such as formation of polarization controlled vortices); and *(ii)* the reverse effect of polarization on the trajectory of light, leading to intrinsic SAM to extrinsic OAM inter-conversion and manifesting as the so-called Spin Hall effect (SHE) of light [1]. The photonic SHE, recently observed in various optical interactions, is under recent intensive investigations because of their fundamental nature and potential applications in the development of novel spin-controlled photonic devices. In this talk, the various types of intriguing spin optical effects in micro and nano-optical systems will be reviewed. The recently observed unusual helicity-independent transverse SAM and polarization-dependent transverse momentum (the so-called Belinfante's spin momentum) in structured optical fields will also be discussed. In this regard, our recent results on controlled enhancement of SOI and SHE in plasmonic nanostructures [2-5], giant photonic SHE in spatially tailored inhomogeneous anisotropic medium [6], optimized weak measurements on photonic SHE [7,8], resonant enhancement of transverse SAM and transverse (spin) momentum in plasmonic structures [9-11] will be presented and their implications towards spin-controlled photonic applications will be highlighted. Finally, the results of our ongoing studies on spin-based plasmonics using a recently developed dark-field polarization microscopy / spectroscopy system [12-14], will be discussed.

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Optimal estimation of polarimetric parameters under different types polarimeter architectures and noise sources

François GOUDAIL*,[1] Matthieu BOFFETY,[1] Stéphane ROUSSEL,[1]

[1] *Laboratoire Charles Fabry, Institut d'Optique Graduate School, CNRS, Université Paris-Saclay,*
91127 Palaiseau cedex, France
**francois.goudail@institutoptique.fr*

Keywords: polarimetry, signal processing

Polarimetric imaging consists in measuring the polarization state of the light scattered by each point of a scene. This estimation is done from intensity measurements consisting in projecting the input light on different analysis states. These intensity measurements are then inverted in order to yield estimates of the Stokes vector. Then, it is often necessary to compute from this Stokes vector polarimetric parameters that are more easily related to physical properties, in particular, the degree of polarization (DOP) and the angle of polarization (AOP). However, the intensity measurements that are the starting point of this process are corrupted by measurement noise. There are two main types of noise sources. The most fundamental is Poisson shot noise, due to the discrete nature of light impinging on a sensor. The second one is additive detection noise caused by dark current and read noise. These measurement noise sources have an influence on the estimation precision of the polarimetric parameters.

In this presentation, we will first review recent results about the optimal estimation of the Stokes vector in the presence of these two types of noise sources [1-3]. We will in particular answer to the following important questions: what are the optimal measurement strategies? What is the precision on the Stokes vector that is obtained when they are used?

We will then address estimation precision of DOP and AOP from measurements of polarimetric cameras based on micropolarizer grids placed directly in front of the image sensor (division of focal plane polarimeter). Each pixel of the sensor has a different polarimetric sensitivity linked to the element of the micropolarizer array placed just in front of it. If grouped together, neighbor pixels can sense the whole polarization state of the incoming light. In most existing devices, the array is composed of linear polarizers, so that only the linear characteristics of the polarization states can be measured [4]. Although some micropolarizer arrays featuring circular polarizer have been demonstrated [5], we will focus on linear micropolarizer arrays.

We will present the theoretical expressions of the estimation variances of DOP and AOP in the presence of both additive noise and Poisson shot noise. We will consider and compare ideal configurations of micropolarizers, and real configurations featuring defects due to the manufacturing process [6]. These results are important for engineering and exploiting micropolarizer grid-based polarimetric cameras.

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Implementation of fast polarization-resolved SHG imaging to monitor dynamic collagen reorganization during skin stretching.

Guillaume Ducourthial [1], Jean-Sébastien Affagard [2], Margaux Schmeltz [1], Xavier Solinas [1], Maeva Lopez-Poncelas [2], Jean-Marc Allain [2,3], Emmanuel Beaurepaire [1], Marie-Claire Schanne-Klein [1*]

[1] *LOB, Ecole Polytechnique, CNRS, INSERM, 91128 Palaiseau, France;*
[2] *LMS, Ecole Polytechnique, CNRS, 91128 Palaiseau, France*
3 Inria, Université Paris-Saclay, 91128 Palaiseau, France
**marie-claire.schanne-klein@polytechnique.edu*

Keywords: optics, polarization, multiphoton microscopy, biomechanics, collagen, skin

The mechanical properties of biological tissues are strongly correlated to the specific distribution of their collagen fibers. The 3D architecture of these collagen fibers can be visualized in intact unstained tissues using second harmonic generation (SHG) microscopy. Monitoring the dynamic reorganization of the collagen network during mechanical stretching is however a technical challenge because it requires mapping the collagen fibers orientation in a thick and deforming sample. In this work, a fast polarization-resolved SHG microscope is implemented to map collagen orientation during mechanical assays. This system is based on line-to-line switching of polarization using an electro-optical modulator and it works in epidetection geometry. It is fast enough to obtain reliable orientations in dynamic collagen samples, while slow enough to get a good SHG signal to noise ratio in thick collagenous tissues. We demonstrate that it enables accurate quantitation of dynamic collagen reorganization in murine skin dermis during stretching biomechanical assays. This approach can be generalized to other multiphoton modes of contrast and enable fast imaging of a variety of orientation-sensitive processes.

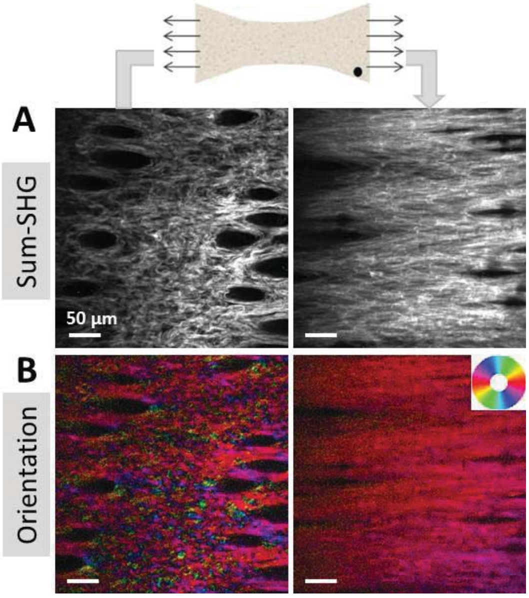

Fig. 1: SHG imaging of *ex vivo* murine skin during stretching assays; left:10 % deformation; right: 40 % deformation. A: Sum-SHG images obtained as the summation of all SHG images recorded for the different linear incident polarizations B: Orientation maps processed from the polarization-resolved SHG images.

FOLDING/UNFOLDING DYNAMICS OF DNA G-QUADRUPLEXES STUDIED BY TIME-RESOLVED CIRCULAR DICHROISM.

Marco SCHMID, Pascale CHANGENET, François HACHE *

Laboratoier d'Optique et Biosciences, Ecole polytechnique/CNRS/INSERM, 91128 Palaiseau cedex
**fancois.hache@polytechnique.edu*

Keywords: circular dichroism, DNA G-quadruplex

Conformational dynamics of biomolecules plays an important role in their biological functions. Among them, G-quadruplexes (G4), resulting from the hydrophobic stacking of a number of guanine quartets stabilized by metal cations are highly polymorphic structures involved in cellular regulation and their folding properties are still under investigation. Experimental studies that have addressed this outstanding question have yielded poor structural information with a time-resolution limited to a few ten microseconds, leading to contradictory pictures of their folding mechanisms [1]. The lack of experimental information has recently stimulated the development of advanced simulations to provide an atomistic description of the folding of short single-stranded G4 structures. These computational studies have suggested that they may follow complex pathways over a large range of time scales, involving the formation of several intermediates [2].

In order to bring some information on these issues, we have carried out experiments aiming at measuring the dynamics of folding of the single-stranded telomeric G4 sequence (Tel21) in the presence of Na+ by implementing a time-resolved circular dichroism experiment with time resolution spanning the microsecond to second range [3]. Fig. 1 displays the CD spectra of Tel 21 for various temperatures and the transient CD signals obtained after a heating/cooling cycle obtained with an IR laser diode (T-jump). Experimental results are discussed in the light of an activation-free downhill diffusion of G4 on a rugged energy surface.

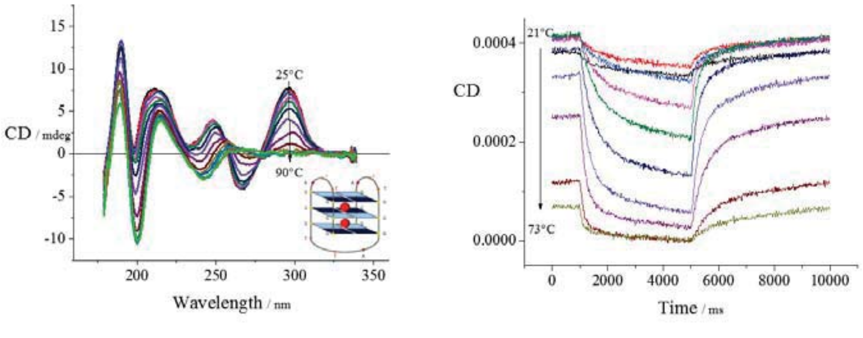

Fig. 1: *CD spectra for temperature varying between 25 and 90°C (left); transient CD signals after a T-jump of 15°C for different initial temperatures (right).*

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The genesis of cancer:
Monitoring of tumor progression by spectro-polarimetric imaging

Jihad ZALLAT*,[1] Briséis VARIN,[1] Jean REHBINDER,[1] Jean DELLINGER,[1] Christian HEINRICH.[1]

Dmonique BAGNARD,[2] Jordan SCHMIDT.[2]

[1]ICube laboratory, University of Strasbourg, Bd Sébastien Brant, 67412 - Illkirch, France;
[2]INSERM U1119 - Labex Medalis, University of Strasbourg, , Bd Sébastien Brant, 67412 - Illkirch, France
**jihad.zallat@unistra.fr*

Keywords: Biomedical optics, polarimetry, optical biopsy, cancer diagnosis.

Minimally invasive Image guided diagnosis will be one of the main evolutions of diagnosis techniques that will provide greater benefit to the patient. To ensure surgical safety, dermatologists will rely on the available instrumentation and imaging device to guide the diagnosis and take important decisions whether to do a biopsy or to remove some tissue zone.

It is important to be capable of quickly distinguishing healthy and abnormal tissues, and, in the latter case, to diagnose the lesion nature. Getting this kind of facilities during an inspection can totally modify the medical care practice.

Many authors in the scientific literature have shown that cancerous tissues are presenting specific polarimetric signatures [1-2]. Consequently, high speed polarization-sensitive imaging apparatus would allow to quickly capture tissues characteristics and could be used as an efficient non-invasive while reliable diagnosis tool.

Our team developed a new polarization architecture permitting the construction of stable calibration-free spectro-polarimeters compatible with the medical-practice constraints. This instrumentation is one of the very first imaging spectro-polarimeters that will be used outside of an optical laboratory to directly analyze in vivo tissues and real patients in the following, opening a new kind of image-based medical diagnosis.

Our previous studies on "small animals" tissues allowed us to develop an optical-biopsy diagnosis instrumentation (hardware and diagnosis software) to optimize clinical decisions and patient outcome. This instrument is involved in in-vivo small animals trial at the Strasbourg University. The aim of this trial is to monitor the progression of cancerous tissue from its early stages and acquire necessary data to constitute a well-calibrated knowledge database that permits outdoing standard diagnosis rates. Two kinds of cancerous cell lines (pigmented and non pigmented) were inoculated to nude mice and the pathology progression was monitored through spectro-polarimetric imaging over a period of a month for each mouse. We will present here the first results and the design for the next planned trial.

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THE DAWN OF INNOVATIONS IN SCREENING OF BIOLOGICAL TISSUES BY USING STRUCTURED LIGHT WITH OPTICAL ANGULAR MOMENTUM

Igor MEGLINSKI * [1] Alex DORONIN [2], Nicolas VERA[3], Juan Pablo STAFORELLI[3], Tatiana NOVIKOVA[4]

[1]*Laboratory of Opto-Electronics and Measurement Techniques, University of Oulu, Oulu, Finland*
[2]*Computer Graphics Group, School of Engineering and Computer Science, Victoria University, Wellington, New Zealand*
[3]*Facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción, Chile*
[4]*LPICM, CNRS, Ecole polytechnique, University Paris-Saclay, 91128 Palaiseau, France*

* Correspondance: igor.meglinski@oulu.fi

Keywords: optical biopsy, optical angular momentum

In turbid tissue-like scattering medium the conventional polarized light, scattered multiple number of times, is depolarized, and the depolarization rate depends strongly on the size and shape of scattering particles, as well as on the number of scattering events. In fact, the structure of light can be more complicated when the polarization of light across the laser beam can be radially or azimuthally polarized and carry orbital angular momentum. When these structured light beams, such as cylindrical vector beam (CVB) and/or Laguerre-Gaussian (LG) beams, propagates through a turbid tissue-like scattering medium, either anisotropic or inhomogeneous, the spin or angular momentum are changed that leads to spin-orbit interaction. The spin-orbit interaction leads to the mutual influence of the polarization and the trajectory of the light propagation. We investigate the applicability of using CVB and LG beams for optical biopsy. In current presentation propagation of CVB and LG beams in anisotropic turbid tissue-like scattering media is considered in comparison to conventional Gaussian beams. We demonstrate that by applying CVB and LG beams the contrast of visibility becomes at least twice higher in comparison to the conventional tissue polarimetry approach. Both experimental and theoretical results suggest that there is a high potential in application of structured light beams in tissue diagnosis.

APPLICATION OF THE INDICES OF POLARIMETRIC PURITY IN BIOPHOTONICS

Angel LIZANA*,[1] Albert VAN EECKHOUT*,[1] Enric GARCIA-CAUREL,[2] José J. GIL,[3] Razvigor OSSIKOVSKI,[2] Irene ESTÉVEZ,[1] Carla RODRÍGUEZ,[1] Adrià SANSA,[1] Joshua SARRAT,[1] Emilio GONZÁLEZ,[4,5] Juan C. ESCALERA,[1] Ignacio MORENO,[6] Juan CAMPOS[1]

[1]Universitat Autònoma de Barcelona, Grup d'Òptica, Physics Department, 08193, Bellaterra, Spain
[2]LPICM, CNRS, École Polytechnique, Université Paris-Saclay, 91128, Palaiseau, France
[3]Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain
[4]Dpto. de Anatomía, Histología y Neurociencia, Universidad Autónoma de Madrid, 28029, Madrid, Spain
[5]Servicio de Anatomía Patológica, Hospital Universitario de Canarias, 38320, Santa Cruz de Tenerife, Spain
[6]Dpto. de Cienc. de Mat., Ópt. y Tecnol. Electr., Universidad Miguel Hernández de Elche, 03202, Elche, Spain
**angel.lizana@uab.cat*

Keywords: Indices of Polarimetric Purity, Depolarization, Biological tissue, Polarimetric Imaging.

Most common polarimetric methods used for biological samples characterization can be arranged in two main groups: (1) Polarization Gating (PG) methods; and (2) those based on merit functions depending on some coefficients of the Mueller matrix (MM) of studied samples. The former, PG methods, tries to optimize the polarization of the beam illuminating the sample, as well as the polarization analyser detecting the scattered light, in order to maximize the visualization of some structures in the sample. Controversy, MM of a sample codifies its polarimetric content (retardance, diattenuation and depolarization), which can be synthetized by properly arranging the information in the MM coefficients. We have studied the suitability of using these two methods (PG and MM based methods) for biological samples imaging, and we proved how PG methods can be understood as particular cases of a general function based on the MM coefficients of samples [1]. We also studied for the first time the potential of the Indices of Polarimetric Purity (IPPs) for biological applications. First, we provided a physical description of the IPPs through a series of basic experiments [2], experimentally showing how IPPs are related with the intrinsic depolarizing mechanisms in samples and how they allow us to further synthetize the depolarizing information. Ultimately, we used the IPPs to study different tissues present in *ex-vivo* samples, and we observed a significant enhancement in the obtained image contrast, when compared with other commonly used polarimetric images [3].

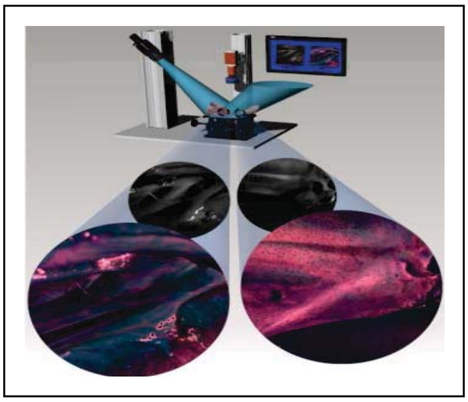

Fig. 1: Imaging polarimetry for the characterization of biological samples based on IPPs.

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ASSESSMENT AND MODELING OF ANISOTROPIC BIOLOGICAL TISSUE PROPERTIES WITH MUELLER MATRIX POLARIMETRY.

Jessica C. RAMELLA-ROMAN,[1,2] Ilyas SAYTASHEV,[2]

[1]*Herbert Wertheim College of Medicine, Florida International University, AHC4, Miami, FL, USA;*
[2]*Department of Biomedical Engineering, Florida International University, AHC4, Miami, FL, USA;*
**jramella@fiu.edu*

Keywords: optics, polarization, Mueller Matrix

Mueller Matrix Polarimetry is becoming a prominent imaging modality in clinical and preclinical applications. It has been shown that Mueller Matrix Polarimetry can enable noninvasive clinical screening of the extracellular matrix (ECM). Given the linear nature of its signal, Mueller Matrix Polarimetry has been challenging to optimize and various models have been proposed to isolate diattenuation, retardance, and depolarization properties of biological samples.

We propose that validation of this imaging modality and inverse models require a new approach. To this end we have developed a new optical system that combines Muller Matrix digital confocal imaging, Muller Matrix reflectance microscopy, and Nonlinear Microscopy. Tomographic images obtained with the confocal Mueller Matrix system can be relayed to the total reflectance Mueller Matrix image. Furthermore, the Second Harmonic Generation imagery can be used to better understand the provenance of the polarimetric signature from anisotropic material within the ECM.

Our results demonstrate positive co-registration of MMP, two-photon imaging and Second Harmonic Generation, as well as matching birefringence measurements, obtained through Mueller Matrix decomposition. We are utilizing this tool to study several ECMs including the one of the eye cornea and uterine cervix.

In this talk we will illustrate the system and the path forward towards validation of reflectance Mueller Matrix Polarimetry in biological media.

Geometric phase flat-optics from inhomogeneous chiral anisotropic media for versatile shaping of polychromatic light fields

Mushegh RAFAYELAN[1], Gonzague AGEZ[2] and Etienne BRASSELET*[,2]

[1] Univ. Bordeaux, CNRS, LOMA, UMR5798, F-33400 Talence, France
[2] CEMES, CNRS, University Paul-Sabatier, F-31055 Toulouse, France
**etienne.brasselet@u-bordeaux.fr*

Keywords: chirality, geometric phase, spin-orbit interaction of light.

Since two decades, substantial research and technological efforts have been made to develop optical elements enabling the versatile manipulation of light fields via geometrical principles, which manifest as the so-called geometric phase that find its root in the optical spin-orbit interaction. Several assets make this approach especially attractive. Indeed, being geometrical by nature, such a beam shaping approach is particularly suitable to process polychromatic light fields. Also, coupling the polarization state of light to the spatial degrees of freedom allow considering the mapping between the two-dimension spin basis and to any two-dimension orbital angular momentum sub-basis, which finds a lot of interests for instance in optical information, optical imaging, optomechanics, optical material processing, and optical sensing. Moreover, technology makes it possible to fabricate flat-optics with versatile beam shaping functionalities.

Still, most of the approach are inherently designed to work efficiently for a discrete set of wavelengths only. In 2016, a novel approach was introduced which consists to combine the intrinsically broadband features of the circular Bragg photonic bandgap of helix-based materials with geometric (Berry) phase arising from space-variant anisotropic optical elements. Here we review our contributions to the development of such reflective Bragg-Berry optical elements [1-4]. An example is shown in Fig.1 where polychromatic vortex beam shaping is demonstrated over the whole visible range.

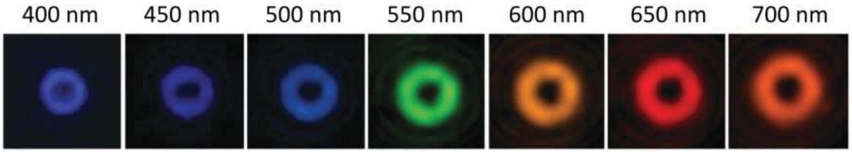

Fig. 1: Ultra-broadband vortex beam shaping from a Bragg-Berry optical element. Adapted from [3].

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INFLUENCE OF THE THIN FILM PARAMETERS ON THE LIGHT BEAM WITH AN ANGULAR MOMENTUM REFLECTION

Maxim BOLSHAKOV[1,2], Nataliya KUNDIKOVA*[1,2]

[1]*South Ural State University, 76 Lenin Av., Chelyabinsk, Russia;*
[2]*Institute of Electrophysics of UD RAS, 106 Amundsen St., Yekaterinburg, Russia*

Keywords: light beams, thin film, orbital angular momentum, spin-orbit interaction of light

One of the light beam three types of angular momentum is the intrinsic orbital angular momentum which is determined by the structure of the light field of the beam [1,2]. The simplest example of the light beam with the intrinsic orbital momentum is the light beam with the topological charge of the different value and sign [1]. The intrinsic orbital momentum can interact with the spin angular momentum associated with polarization [3] and with the extrinsic orbital angular momentum determined by the propagation path of the light beam [4]. The effect of one of the angular momenta on another angular momentum leads to the spin-orbit interactions of light [5]. There is another type of the spin-orbit interaction of light which is connected with the influence of the two type of the angular momentum on another momentum [5]. One effect was observed experimentally under light propagation through a multimode optical fiber [5]. The promising experimental system which can allow finding the different effect is a thin film.

The reflection of the light beam with a topological charge from thin films can also be used for the film parameters determination.

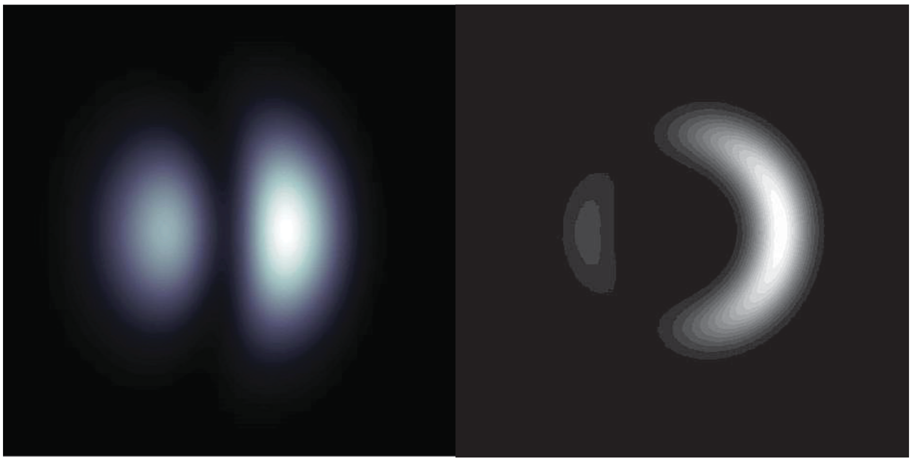

Fig. 1: deformation of the light beam intensity under reflection. Left, topological charge is 0; right, topological charge is +1

We were successful to observe the intensity distribution distortion and the influence of the value of the topological charge on the intensity distribution (Fig. 1) by numerical simulation. This work was partly carried out within the scope of the topic of State Assignment No. 0389-2016-0003

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Separating photons by spin with the method of A. Fresnel

Oriol ARTEAGA,[1,2,*] Enric GARCIA-CAUREL,[2] Razvigor OSSIKOVSKI,[2]

[1]Dep. de Física Aplicada, IN2UB, Feman Group, Universitat de Barcelona, C/ Martí i Franquès 1, Barcelona 08030, Spain
[2]LPICM, CNRS, Ecole Polytechnique, Université Paris-Saclay, 91128 Palaiseau, France
**oarteaga@ub.edu*

Keywords: spin angular momentum, circular polarization, circular birefringence

This work considers a method proposed by Augustin Fresnel in 1822 [1] to produce selective deflection of photons according to their spin angular momentum. This experiment is the most clear and visual demonstration that linearly polarized light can be decomposed in the sum of right- and left-circularly polarized components. Fresnel's method is based on the fact that right- and left- circularly polarized waves propagating in a chiral medium travel at different speeds. This effect, today well-known as optical rotation or circular birefringence, usually does not produce any visible spatial separation of right- and left- circularly polarized waves, but in Fresnel's experiment it is capable of separating photons macroscopically according to their spin. In this presentation we will show a practical implementation of this experiment with modern tools and we will discuss its analogy with quantum mechanics.

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Determination of the pseudo-gyration tensor of KTP by ellipsometry

Chris STURM*, Vitaly ZVIAGIN, Marius GRUNDMANN

Felix-Bloch-Institut für Festkörperphysik, Linnéstr. 5, Universität Leipzig, Germany
**csturm@physik.uni-leipzig.de*

Keywords: ellipsometry, dielectric tensor, pseudo-gyration tensor, singular optic axes

The presence of optical activity of a material leads to the rotation of the plane of polarization of linear polarized light. This effect cannot be described with the dielectric function only, and a pseudo-gyration tensor has to be taken into account. Typically, the magnitude of the components of the pseudo-gyration tensor is determined by means of polarized transmission techniques. However, this technique is limited to the transparent spectral region of a material. Here we show exemplarily on potassium titanyl phosphate ($KTiOPO_4$, KTP), that generalized ellipsometry can be used in order to determine the pseudo-gyration tensor of anisotropic materials in their absorption region.

KTP is an optically active biaxial material with an orthorhombic crystal structure. It is widely used for second harmonic generation (SHG) in particular in diode pumped sold state lasers. The optical properties in the transparent spectral range are well known. There exist only few reports for the absorption spectral range, which are limited to the onset of the absorption. By means of generalized ellipsometry we determined the full dielectric tensor and the pseudo-gyration tensor in a spectral range from 0.5eV up to 8.4eV (Fig. 1). From a line shape analysis of the dielectric tensor components, the properties of the electronic transitions were deduced and the fundamental band gap energy was determined to 4.2eV for dipoles polarized along the c-axis. For the transitions polarized along the a and b direction, the gaps are blue-shifted by 0.70eV and 0.05eV, respectively. The magnitude of the pseudo-gyration tensor in the transparent spectral region is within the experimental uncertainty negligible and became well pronounced as soon as the absorption sets in. Based on the determined dielectric and pseudo-gyration tensor, we also discuss the orientation and dispersion of the (singular) optic axes of KTP.

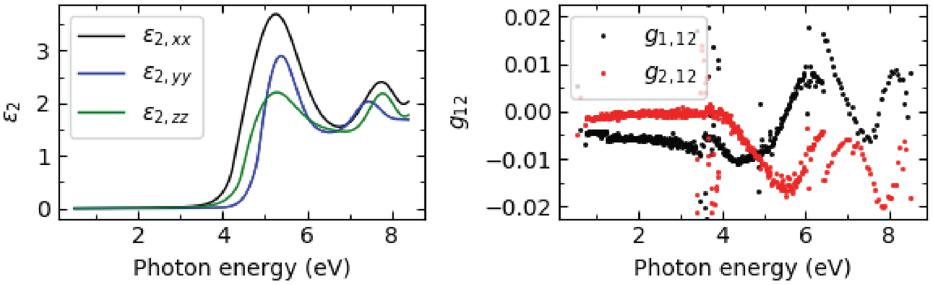

Fig. 1: (left) Imaginary part of the dielectric function in the absorption spectral range. (right) The real and imaginary part of the pseudo-gyration tensor component g_{12}.

DIFFUSE REFLECTANCE SPECTROSCOPY WITH POLARIZATION GATING

Anabela Da Silva,* Susmita Shridar, Hind Oulhaj, Callum M. Macdonald, Ugo Tricoli, Vadim Markel
[1]*Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel UMR 7249, 13013 Marseille, France*
**anabela.dasilva@fresnel.fr*

Keywords: polarization gating imaging, Monte Carlo simulations

Spectrometry involves the analysis of chromophore content by observing the wavelength dependent attenuation of light which has propagated through a sample (Beer-Lambert law). Knowledge of the pathlength through which light has travelled is needed to treat this problem. In the presence of scattering, as in biological tissues, the optical pathlength depends non-trivially on the wavelength. As a result, the relationship between the measured attenuation, and the medium absorption, becomes non-linear. Polarization filtering to linearize this problem in the transmission geometry [1-2] allows simple models, such as the modified Beer-Lambert law to still be applied. We are interested in solving this problem in the backscattering geometry, using wide field illumination, and wide field detection (Fig.1).

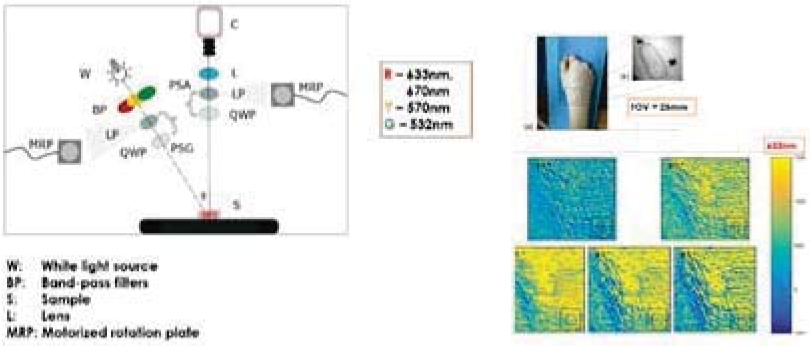

Fig. 1 : Left : Polarization gating Diffuse Reflectance setup ; Right : Examination of a burn scar, examples of measurements obtained with different ellipticities of polarization.

If a constant pathlength can be achieved through polarization modulation, then consistency of the probing volume will also likely improve, reducing errors incurred by mesoscopic inhomogeneities in the medium under investigation. In previous work, we have shown that modulation of the ellipticity of polarization, from linear to circular, allowed to sense the tissues at increasing depth [3-5]. Here, with the development of an optimized Monte Carlo simulation software [6-7], we investigate the use of polarization filtering with improved combinations of linear, elliptical, or circular, in order to improve this selectivity of the effective optical pathlength.

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TOWARDS SCREENING OF BRAIN MALFORMATIONS WITH CIRCULARLY POLARIZED LIGHT

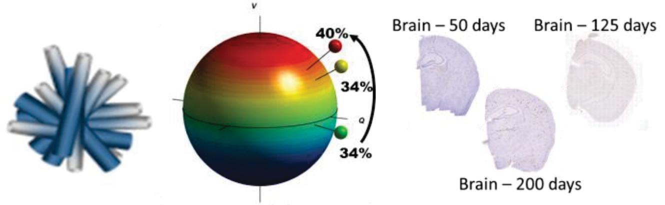
Mariia BOROVKOVA *,[1] Alexander BYKOV[1], Alexey POPOV[1], Jens PAHNKE[2], and Igor MEGLINSKI[1]

[1] University of Oulu, Pentti Kaiteran katu 1, Oulu 90570, Finland;
[2] University of Oslo / Oslo Universiy Hospital, 4950 Nydalen, Oslo 0424, Norway
** mariia.borovkova@oulu.fi*

Keywords: optics, polarization, optical orbital momentum

Polarized light is extensively used in various biomedical applications to provide valuable information about biological tissues and their abnormalities changes associated with different diseases [1]. It has been demonstrated that circularly and/or elliptically polarized light scattered within the biological tissues is highly sensitive to the presence of cancer cells and their aggressiveness [2]. In this study, we investigate the applicability of the circularly polarized light for the quantitative assessment of the accumulation of amyloid plaques (Fig.1-a) between nerve cells (neurons) in the brain, as one of the hallmarks of Alzheimer's disease.

In the experiment, the right-hand circularly polarized light was directed at 55^{o} towards a sample of mice brain (Fig.1-c) tissue in vitro. The state of polarization of the light scattered back from the brain sample was analyzed by the Stokes vector polarimeter. The Poincare Sphere (Fig.1-b) is used as a convenient tool for analysis of the state of polarization of the light scattered through biological tissues with various amount of amyloid plaques.



(a) (b) (c)

Fig.1 (a) Distribution of the fibrils composing the neuritic plaques [3]; (b) measured Stokes vectors mapped on the Poincare sphere (green color corresponds to the 50-days old brain, yellow shows the 125-days old brain, and red stands for the 200-days old brain; the degree of polarization is specified next to each experimental point; the arrow shows the trend of the state of polarization which correlates with the disease progression); (c) – correspondent images of the brain tissue slices (50, 125, and 200 days old) with amyloid plaques.

The black arrow in Fig.1-b demonstrates the significant up-trend of the V Stokes parameter associated with the growing presence of amyloid plaques and the disease progression. The current study aims to establish the proof of concept of using fundamental properties of light, namely circular polarization, for automatic digital tissue biopsy for characterization of brain tissue abnormalities. The results show that circularly polarized light can be effectively utilized for the non-invasive screening of brain tissues influenced by Alzheimer's disease. The developed technique provides the foundation for future work implementing circularly polarized light for non-invasive assessment of the presence of amyloid plaques in brain tissues and their growing associated with the progression of Alzheimer's disease.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713606.

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MODELLING THE ADHESION OF A SPHERICAL PARTICLE TO A SUBSTRATE WITH GEOMETRIC OPTICS AND POLARIMETRY

Andrea FERNÁNDEZ,[1] Thomas Sang Hyuk YOO,[2] José Luis FERNÁNDEZ-LUNA,[3]
Fernando MORENO,[1] Enric GARCÍA-CAUREL,[2] José María SAIZ VEGA*,[1]

[1] *Dpto. de Física Aplicada, Universidad de Cantabria, Av. Los Castros 48, 39005, Santander, Spain;*
[2]*LPICM, CNRS, Ecole polytechnique, Université Paris-Saclay, Palaiseau 91128, France;*
[3]*Unidad de Genética HUMV, 39008, Santander, Spain*
** josemaria.saiz@unican.es*

Keywords: geometric optics, polarization, adhesion, cell.

Polarimetry has shown to be a successful method for analyzing light-matter interaction. This is supported by its many successful applications in a variety of fields, like astronomy, agriculture, weather radar, environmental science, etc. [1]. More recently, it is being employed in medicine and biology, given its non-invasive character. When light interacts with matter its polarization state changes. One of the most accepted mathematical methods to analyze these changes is through the Mueller matrix formalism. The Mueller matrix is a 4x4 matrix, **M**, that fully characterize the polarimetric response of a given medium. Its physical interpretation is not a straightforward task, so a mathematic decomposition is convenient to obtain more physically understandable parameters [2].

Our model of adhesion starts with a latex sphere of $r = 5$ μm and $n = 1.59$. The spheres are deposited on a glass substrate and then heat is applied, so that different degrees of melting are achieved. This will somehow mimic the geometry acquired during the adhesion process of a living cell to a flat substrate [3].

The experimental measurements of the matrix **M** are performed with a transmission Mueller polarimeter [4]. Some further matrix decompositions are applied to the matrix. Linear dichroism and birefringence seem to be the most sensitive parameters to the adhesion state of the sphere.
Experimental measurements are compared to numerical simulations of the Mueller matrix of spheres and spherical cups with a light model based on coherent ray tracing, wave optics (Fresnel theory) and polarization. Good agreements are found between experimental results and simulations.

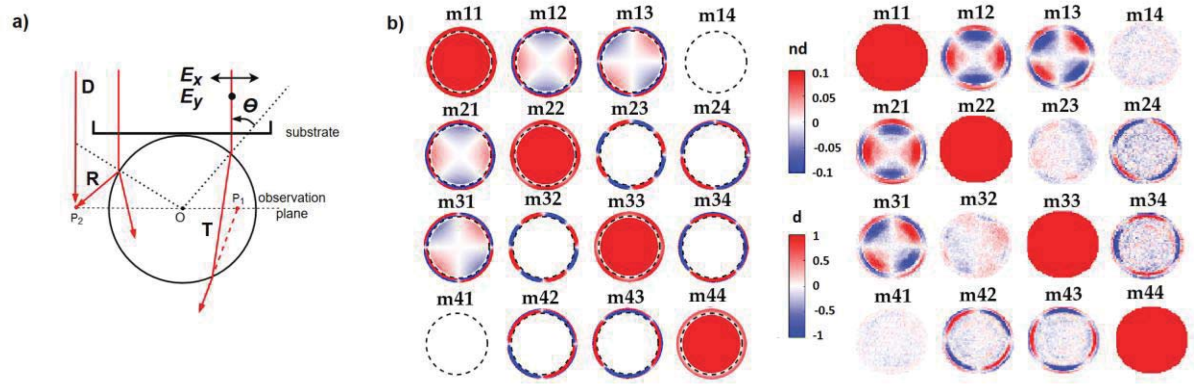

Fig. 1: schematics of the model used for the simulations combining geometric optics and polarization (a). Simulated (left) and measured (right) Mueller matrix of a 5 μm latex sphere.

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POLARIMETRIC DATA POST-PROCESSING FOR PRE-CANCER DETECTION FROM UTERINE CERVIX SPECIMENS

Meredith KUPINSKI *[,1,2], Matthieu BOFFETY [3], François GOUDAIL [3], Razvigor OSSIKOVSKI [1], Angelo PIERANGELO [1], Jean REHBINDER [1], Jérémy VIZET [1], Tatiana NOVIKOVA [1]

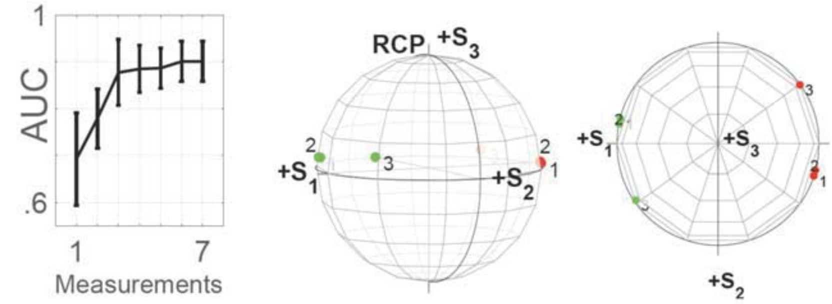
[1]LPICM, CNRS, Ecole polytechnique, Palaiseau, France;
[2] University of Arizona, College of Optical Sciences, Tucson, USA
[3] Institut d'Optique Graduate School, Paris-Saclay University, Palaiseau, France
**meredith@optics.arizona.edu*

Keywords: polarimetric imaging, probability theory, stochastic processes, biomedical diagnostics

The non-linear and linear post-processing compressions of the full Mueller matrices of cervical specimens, measured in visible wavelength range with imaging Mueller polarimeter in backscattering configuration, were examined in terms of the diagnostic performance for the detection of cervical intraepithelial neoplasia CIN2-3 (pre-cancer conditions).

Area Under the receiver operating characteristic curve (AUC) is the gold standard metric to quantify detection performance in medical applications, $0.5 \leq \text{AUC} \leq 1$, being equal to 1 for an ideal observer perfectly detecting all healthy and diseased pixels on the image and 0.5 for guessing. Our prior studies [1] have shown that using scalar retardance (calculated from non-linear Lu-Chipman decomposition of Mueller matrix [2]) as a decision variable yields an average AUC of 0.94. Applying non-linear Cloude decomposition of Mueller matrix [3] and using 3 smallest eigenvalues of the coherency matrix (representing the depolarizing properties of tissue) as the decision variables yields an average AUC of 0.93. When scalar retardance and 3 smallest eigenvalues of the coherency matrix are used simultaneously for the diseased zones detection the average AUC is 0.95.

The linear post-processing compressions were performed in order to understand polarimetric measurement utility for the optimization of future imaging protocols. The J-optimal Channelized Quadratic Observer (J-CQO) method [4] for optimizing polarimetric measurements demonstrates equivalent AUC values for the full Muller matrix and 6 J-CQO optimized measurements. The advantage of this optimization is that only 6 measurements, instead of 16 for the full Mueller matrix, are required to achieve equivalent AUC, thus, preserving detection performance [5].



(a) J-CQO Detection Performance (b) Side-view Poincaré sphere (c) Top-view Poincaré sphere

Fig. 1 (a) AUC of J-CQO versus number of measurements. Error bars are ± one standard deviation around mean; (b,c) J-CQO optimal PSA/PSG measurements states on the Poincaré sphere, red (PSA) and green (PSG).

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DIGITAL HISTOLOGY WITH MUELLER POLARIMETRY

Heeryung LEE[1*], Thomas Sang Hyuk Yoo[1], Pengcheng Li[2], Christian Lotz[3], Florian Kai Groeber- Becker[3,4], Sofia Dembski[3,4], Enric Garcia-Caurel[1], Razvigor Ossikovski[1], and Tatiana Novikova[1]

[1]LPICM, CNRS, Ecole polytechnique, University Paris-Saclay, Palaiseau 91128, France;
[2]Department of Physics, Tsinghua University, Beijing 100084, P. R. China
[3]Department of Tissue Engineering & Regenerative Medicine TERM, University Hospital Würzburg, Würzburg 97070, Germany
[4]Translation Center Regenerative Therapies, branch of Fraunhofer Institute for Silicate Research ISC, 97082 Würzburg, Germany

**hee-ryung.lee@polytechnique.edu*

Keywords: Mueller polarimetry, differential Mueller matrix formalism, skin tissue models

For advanced optical characterization of anisotropic scattering media, the approach combining theory (differential Mueller matrix formalism) and experiments (Mueller polarimetry) was tested on the models of human skin tissue. These models were grown from epidermal keratinocytes forming a multilayered epidermis on top of collagen I hydrogel with dermal fibroblasts. The set of fixed unstained histological cuts of artificial skin of varying thickness (5 - 30 μm) was prepared. The samples were measured by custom-built Mueller polarimetric microscope in transmission mode, then the values of polarization and depolarization parameters were calculated by applying pixel-wise the logarithmic decomposition of Mueller matrices. The obtained results confirmed the linear dependence of polarization parameters and parabolic dependence of depolarization parameters on the thickness of the samples. It proves that phenomenological modeling of complex anisotropic scattering medium (e.g., biological tissue) may effectively disentangle the polarization and depolarization properties of a system and might be used for histological analysis and diagnostics of tissue.

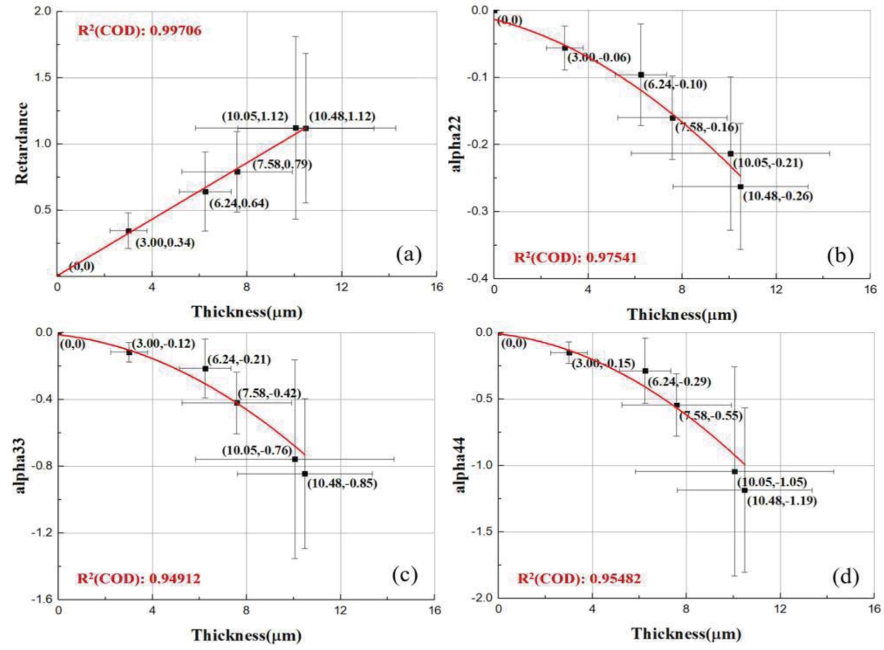

Fig. 1: Thickness dependence plots of (a) retardance (in radians), (b-d) depolarization coefficients (α_{22}, α_{33}, α_{44}). Black squares indicate experimental data, and red solid lines show the linear (a) and parabolic (b-d) fit curves.

Innovative and high-performance instrumentation for biomedical Mueller polarimetric imaging *in vivo*

Arvid Lindberg,[1] Camille Gennet[1], Jérémy Vizet[1], Jean rehbinder[2], Jean-Charles Vanel[1] and Angelo Pierangelo[1]

[1]*Ecole Polytechnique, LPICM, CNRS, Palaiseau, 91128, France;*
[2]*Université de Strasbourg, iCube, CNRS, Illkirch, 67412, France*
**arvid.lindberg@polytechnique.edu*

Keywords: optics, polarization, Mueller polarimetric imaging, optimization, Biomedicine

Mueller Polarimetric Imaging (MPI) showed promising results in biomedical applications, especially for early detection of precancerous lesions on biological tissues [1], [2]. The technique is label free, non-invasive and can be implemented with off-the-shelf biomedical equipment. The development of innovative MPI systems able to analyze biological tissues in vivo on human patients in hospital settings remain an instrumental challenge because of the slowness of MPI, the light absorption of tissues and the need for multispectral data.

Our goal is to build full-field MPI systems based on Ferroelectric Liquid Crystals (FLCs) capable of fast multispectral (in the visible range) analysis of biological tissues in vivo, with high accuracy, while using compact components.

In this work an innovative and pragmatic approach is showed to realize an optimized and fast FLC based MPI able to perform multispectral full-field acquisitions in 0.6 seconds in the spectral range 450 to 700 nm with error less than 1% on all the elements of the measured Mueller matrices. This system can be easily calibrated by using the Eigenvalue Calibration Method also in presence of increased residual instrumental depolarization, up to 40 %, at all operation wavelengths.

Fig. 1: Mueller polarimetric colposcope

This approach enables us to realize compact MPI machines which can be easily integrated into existing instruments currently used in medical practice. We constructed a first simultaneous multispectral (450, 550 and 650 nm) MPI colposcope for the analysis of the uterine cervix in vivo in the framework of cancer screening [3], as well as a first multispectral MPI laparoscope for use in minimally invasive surgery.

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GENERATION OF POLYCHROMATIC VECTOR BEAMS WITH A TUNABLE *Q*-PLATE

David MARCO*,[1] María del Mar SÁNCHEZ-LOPEZ,[1] Pascuala GARCÍA-MARTÍNEZ,[2] Ignacio MORENO[3]
[1]Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, 03202 Elche, Spain
[2]Departamento de Óptica, Universidad de Valencia, 46100 Burjassot, Spain;
[3]Departamento de Ciencia de Materiales, Óptica y Tecnología Electrónica, Universidad Miguel Hernández de Elche, 03202 Elche, Spain;
**dmarco@umh.es*

Keywords: *q*-plate, optical retarders, vector beams, polarization, orbital angular momentum

Q-plates are linear retarders with an azimuthal distribution of the principal axis. These devices have received much attention because they generate vector beams with cylindrically symmetrical polarization [1]. They can be fabricated using liquid crystals (LC), where the LC director follows a fraction *q* of the azimuth angle. Thus, the retardance can be tuned by applying a voltage.

In this work, we present a spectral calibration of a *q*=1/2 LC tunable *q*-plate from ArcOptix [2]. The experimental set-up is shown in figure 1(a). We use a white broadband light source and analyze the device spectral transmittance between circular polarizers, following the procedure reported in [3]. The retardance is obtained as a function of wavelength and voltage in order to identify the optimal operation of this *q*-plate for a given wavelength. These results are shown in Fig. 1(b). The simultaneous generation of different types of vector beams could be achieved by illuminating this *q*-plate device simultaneously with various wavelengths where the device shows different retardance. Figure 1(c) shows four birefringent colours for different applied voltages when the *q*-plate is placed between linear crossed polarizers. These colours agree with the transmittance spectrum measured at the corresponding voltage.

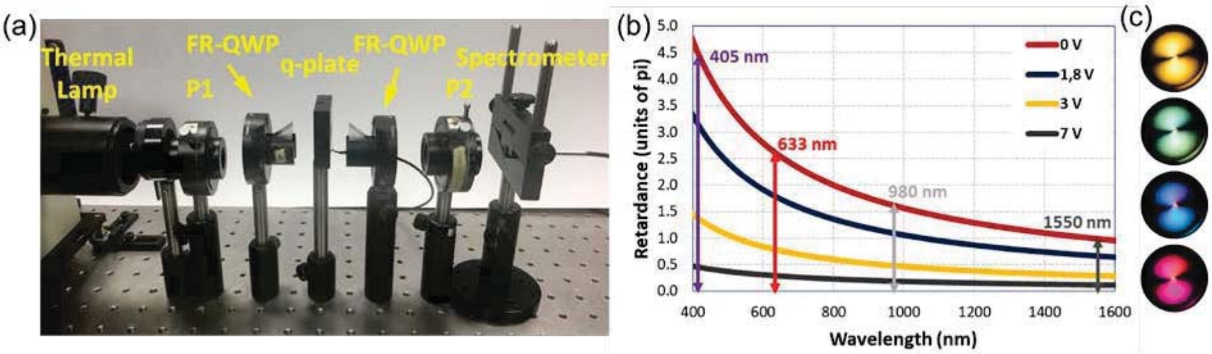

Fig. 1: (a) Picture of the experimental calibration setup, where quarter-wave Fresnel rhombs (FR-QWP) and Glan-Taylor linear polarizers (P) were used to build the broadband circular polarizers. (b) Spectral retardance for voltages: 0 V, 1.8 V, 3 V and 7 V. (c) Image of the *q*-plate between linear crossed polarizers for voltages 0 V, 1.5 V, 1.7 V and 1.9 V from top to bottom.

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HIGH-SPEED POLARIZATION-RESOLVED THIRD HARMONIC GENERATION MICROSCOPY APPLIED TO THE CHARACTERIZATION OF MOLECULAR ORDER IN LIPID ASSEMBLIES AND IN BIOMATERIALS

Joséphine MORIZET,*,[1] Guillaume DUCOURTHIAL,[1] Marie-Claire SCHANNE-KLEIN,[1]
Chiara STRINGARI,[1] Emmanuel BEAUREPAIRE[1]

[1]Laboratoire d'optique et biosciences, Ecole Polytechnique, CNRS, INSERM, Palaiseau, France
josephine.morizet@polytechnique.edu

Keywords: non-linear microscopy, polarization

Multiphoton microscopy is used to probe nonlinear optical properties of label-free tissues with micrometric three-dimensional resolution. It has been applied in various fields ranging from developmental biology to neuroscience. Remarkably, third harmonic generation microscopy (THG) is sensitive to third-order non-linear susceptibility and to refractive index [1]. The contrast mechanism of THG microscopy is rather peculiar as no signal is detected when the excitation volume probes an isotropic homogeneous medium with normal dispersion; however the THG signal emerges in the two following cases: (i) on an interface between two different media (water/ lipid interface) and (ii) in strongly birefringent media. Finally, these two types of signals are strongly influenced by the polarization state of the excitation beam. By recording several images with different linear polarization states, we may extract information on molecular order and orientation in multi-lamellar lipid assemblies [2].

A practical difficulty to apply this approach to the characterization of biological structures is that the samples usually move or become distorted during image acquisition. We have overcome this issue by developing a THG microscope integrating an electro-optical modulator (EOM) to perform fast polarization-resolved third harmonic generation microscopy. We have also compensated for the polarization distortions induced by scanning microscope components, and obtained a residual ellipticity of less than 6% in the case of linear polarization. It is now possible to switch the polarization between each line of the image at kHz rate using the EOM. Finally, we have also developed a method of data analysis based on Fourier transform of the stack of polarization-resolved images to extract molecular information at pixel size. We have demonstrated the principles and the performances of the first system fast p-THG microscopy. We have also performed advanced applications of this method through characterization of multi-lamellar lipid assemblies undergoing phase-transition and detection of crystallinity of flowing endogenous microparticles in the inner ear of zebrafish embryo (Fig. 1). We have also developed a method to reveal birefringence at micron scale in moving biomaterials while shifting rapidly from linear to circular polarization states.

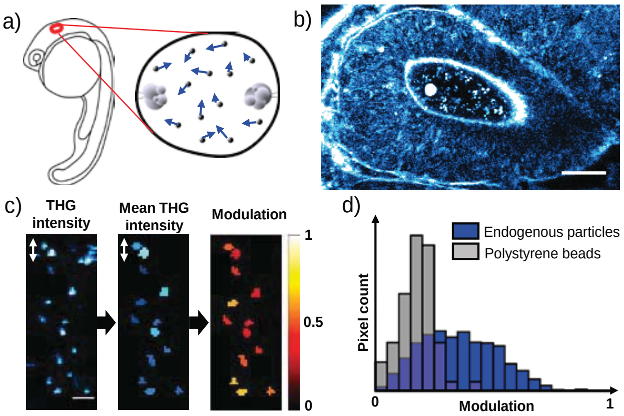

Fig. 1. *In vivo* detection of anisotropy of flowing particles in the zebrafish inner ear. (a) Schematic of the otolith cavity in a 22 hours post fertilization (hpf) zebrafish. (b) Time-lapse THG imaging of the cavity with flowing microparticles. Scale bar 20 µm. See Visualization 3. (c) Extraction of the microparticles mean P-THG modulation. (d) Histogram of THG modulation for endogenous otolith microparticles (blue) and injected polystyrene beads (grey). The data identifies that the microparticles are made of anisotropic material.

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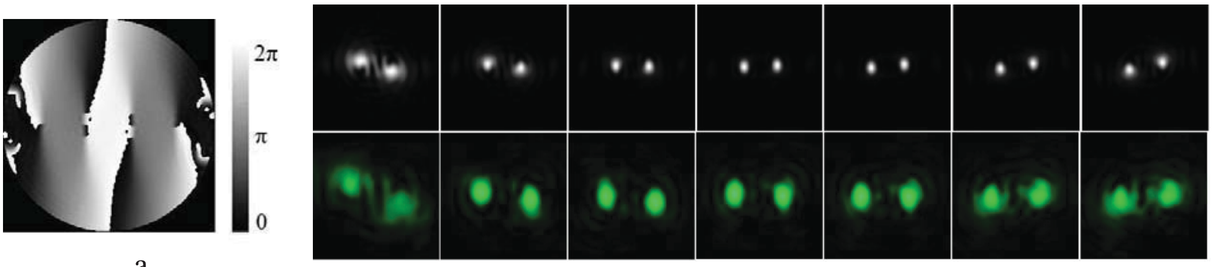
OPTIMIZATION OF PHASE MASKS FOR 3D FLUORESCENCE NANOSCOPY

Darya PROKOPOVA[1,2,*], Svetlana KOTOVA[1,2], Nicolay LOSEVSKY[1], Sergey SAMAGIN[1],
Vladimir VOLOSTNIKOV[1], Evgeny VORONTSOV[1], Ivan EREMCHEV[3],
Aleksei GORSHELEV[3], Sergei KULIK[3], Andrei NAUMOV[3]

[1]Lebedev Physical Institute, 221, Novo-Sadovaya Str., Samara, 443011 Russia;
[2]Samara National Research University, 34, Moskovskoye shosse, Samara, 443086 Russia
[3]Institute of Spectroscopy RAS, 108840 Troitsk, Moscow, Russia
**prokopovadv@gmail.com*

Keywords: spiral beams, double helix point spread function, super-resolution microscopy

To develop the methods of 3D nanoscopy [1] is currently important taking into account the evolution of spectroscopy of single molecule and systems with quantum dots. Increasing the longitudinal resolution of fluorescence optical microscope is possible with the use of phase mask, which transforms point spread function (PSF) so, that it takes the form of two bright spots revolving around the common center during the process of defocusing. Similar masks were made active use of after a phase mask of high energy efficiency (56%) had been submitted in paper [2]. Phase masks forming two-lobe light fields of different rotation rate (DH PSF) can be created applying methods of spiral light beam optics. We obtained masks for homogeneous distribution of illuminating beam intensity. These masks are used for determination of the position of a point light source on the Z-axis to within 30 nm. However we observed that in the microscopic system a beam illuminating a phase mask is not homogeneous and it depends on the type of a microscope objective. Phase masks figured for a particular microscope objective were created. The energy efficiency of the masks amounted to: 40% with rotation angle of intensity distribution 200° which corresponds to longitudinal shift in the microscopic system $\Delta Z=3$ μm; and 72% with rotation angle 60° which corresponds to $\Delta Z=2$ μm. The last masks can be used for solving problems that requires accurate determination of a particle position in a thin layer on the Z-axis. In figures the phase mask (fig. a) with 72 % efficiency and its field (fig. b) are given.



a

b

Fig.: a) Phase mask profile in grayscale b) Intensity distribution in sections perpendicular to the axis of propagation (top row – numerical simulation, bottom row – experiment results)

This research is financially supported by the RFBR (projects No. 16-29-11809).

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POLARIZATION MEMORY IN INHOMOGENEOUS ELLIPTICAL BIREFRINGENT MEDIUM

First name LAST NAME,[1] First name LAST NAME,[2] First name LAST NAME *,[2]
Sergey SAVENKOV,[1]* Thomas SANGHYUK YOO,[2] Enric GARCIA CAUREL,[2] Yevgen OBEREMOK,[1] Ivan KOLOMIETS,[1] Tatiana NOVIKOVA,[2] Razvigor OSSIKOVSKI,[2] and Alexander KLIMOV[1]

[1]*Taras Shevchenko National University of Kyiv, Radiopysics Department, Vladimirskaya str. 64, 01 033 Kiev, Ukraine*
[2]*LPICM, CNRS, Ecole Polytechnique, Universite Paris—Saclay, 91128 Palaiseau, France*
**sns@univ.kiev.ua*

Keywords: polarization, degree of polarization, polarization memory, birefringence

The main goal of the paper is to provide rigorous analysis of polarized light scattering by inhomogeneous elliptical birefringent medium in single scattering case. The object under consideration is a crystalline slab characterized by elliptical birefringence with surface roughness. We study the output light parameters depending on roughness and the direction of light propagation in the crystal

We show that for all possible directions of light propagation in the crystal there are only two polarization states (they also depend on the direction of light propagation in a crystal) for which the output light is completely depolarized. In these cases, it is said that the medium forgets these input polarizations. The exception is two directions of propagation: when light propagates along and orthogonally to the optical axis of the crystal, i. e., when the crystal under consideration is characterized either by only circular or by only linear birefringence. In these two cases, there are infinitely many input polarizations, for which the output light is completely depolarized.

The input polarizations for which the output light has the maximum degree of polarization, as in the cases of pure circular and pure linear birefringence, are only two. It is important, however, that these input polarizations are not orthogonal and depend on the direction of propagation in the crystal. As it turned out, the degree of polarization of the output light depends on the direction of propagation in the crystal as well.

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Quantitative assessment of parchment degradation by Polarization-resolved Second Harmonic Microscopy

Margaux SCHMELTZ,[1] Laurianne ROBINET,[2] Sylvie Thao-Heu,[2] Claire TEULON,[1]
Guillaume DUCOURTHIAL,[1] Marie-Claire SCHANNE-KLEIN,[1] Gaël LATOUR *[3]

[1] Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS, INSERM, Université Paris-Saclay, Palaiseau, France
[2] Centre de Recherche sur la Conservation, Sorbonne Universités, Ministère de la Culture et de la Communication, Museum National d'Histoire Naturelle, CNRS, Paris, France
[3] Laboratoire Imagerie et Modélisation en Neurobiologie et Cancérologie, Université Paris-Sud, CNRS, Université Paris-Saclay, Orsay, France
**gael.latour@u-psud.fr*

Keywords: polarization, second harmonic generation, multiphoton microscopy, collagen, parchment

Parchments are major artefacts in cultural heritage. They were the main writing surfaces in Europe until the 10th century and they provide precious indications on human history. These artefacts are made of untanned animal skin (dermis layer) and are mainly composed of fibrillar collagen. Unfortunately, they can suffer from degradation, mainly due to heat and/or water. It is very important for museum curators and restorers to be able to characterize the exact alteration state of every manuscript, with a non-invasive nor destructive method.

In this study, we show that Polarization-resolved Second Harmonic Generation (P-SHG) microscopy is an efficient tool to address these needs. First, SHG signals are very specific to fibrillar collagen in parchments because it is a dense and non-centrosymmetric structure, with no counterpart in usual (linear) optical techniques. As a consequence, well-preserved parchments exhibit strong SHG signals whereas SHG signals vanish in case of high degradation where fibrillar collagen is denatured into gelatin (centrosymmetric structure).[1] Second, two complementary quantitative information at different scales are extracted with P-SHG technique: (i) the mean **orientation** of the collagen fibrils within the focal volume, which provides an orientation mapping in the field of view of the SHG image (0.1 - 1mm); (ii) the SHG anisotropy parameter, which is related to the contrast of the polarimetric response in the focal volume and provides the **degree of disorder** at a submicrometer scale.

We first performed P-SHG analyses on a set of model parchments, artificially degraded by exposure to dry heat at 100°C for increasing duration. The level of degradation was assessed by using Differential Scanning Calorimetry (DSC) on a micro-sample, as it is the gold-standard technique to measure collagen denaturation. At high levels of degradation, SHG signals decreased as expected, while fluorescent signals appeared. In the early stages of degradation, the SHG intensity level was preserved but the SHG anisotropy parameter increased, which revealed a disorganisation at the fibrillary scale. We found a very good correlation between DSC and P-SHG results, which validated P-SHG as a quantitative probe of parchment degradation. We then took advantage of the non-invasivity of this technique, which does not require any sampling or contact, in contrast to DSC that is destructive, to assess the conservation state of historical manuscripts from Chartres library.

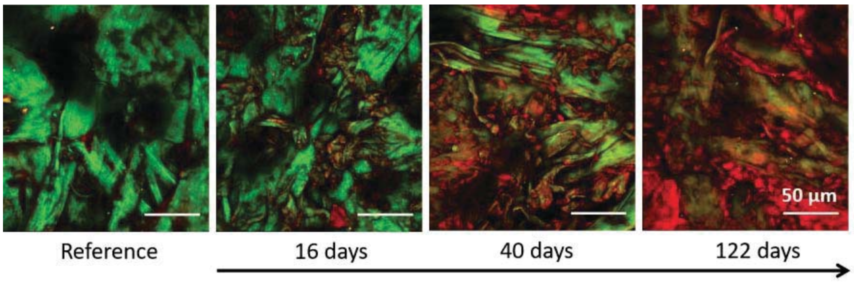

Fig. 1: SHG (green) and fluorescence (red) signals in model parchments at various degradation states

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Dermapol,
an innovative spectro-polarimetric optical biopsy tool for real-time skin cancer diagnosis

Briséis VARIN,[1] Jean REHBINDER,[1] Jean DELLINGER,[1] Christian HEINRICH,[1]
Jihad ZALLAT*

[1]ICube laboratory, University of Strasbourg, Bd Sébastien Brant, 67412 - Illkirch, France
** jihad.zallat@unistra.fr*

Keywords: Biomedical optics, polarimetry, optical biopsy, cancer diagnosis

The diagnosis of skin cancer and skin pathologies is a delicate matter for dermatologists, who only have a little number of tools they can rely on. Up to now, the standard technique has been visual examination followed by anatomo-pathology on biopsied sections, the suspicious tissue being sent to a lab before treatment decision. Patients with multiple suspicious zones suffer a lengthy analysis process of limited reliability compared to a possible full-body biopsy-capable system. The aim of our project is to develop a new optical biopsy noninvasive instrument to help the dermatologists make the important decision whether to do a biopsy or to remove some tissue zone.

Our instrument uses the fact that cancerous tissues present specific polarimetric signatures [1-3]. It measures the polarimetric properties of skin tissue via a Mueller spectro-polarimeter. Our instrument is innovative in several ways: it is a stable handheld device that requires no calibration once properly built. Two prototypes of this instrument have been assembled and characterized, one in the visible (470-660 nm) and one in the near-infrared (680-880 nm) spectral ranges.

Both prototypes have been used in a pilot study monitoring tumor growth of melanoma cells injected in mice. This study prepares the clinical validation of the technique through the characterization of the polarimetric signature of various benign and pathological skin lesions at the Strasbourg University Hospital.

Fig. 1: "Dermapol", our handheld instrument prototype

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Effect of Geometric Phases in the Polarimetric Response of Small Particles Illuminated at Oblique Incidence

Thomas Sang Hyuk YOO,[1] Andrea FERNANDEZ,[2] Fernando MORENO,[2] José Maria SAIZ,[2] Tatiana NOVIKOVA,[1] Razvigor OSSIKOVSKI,[1] Enric GARCIA-CAUREL*,[1]

[1]LPICM, CNRS, Ecole polytechnique, Université Paris-Saclay, Palaiseau 91128, France;
[2]Dpto. de Física Aplicada, Universidad de Cantabria, Avda. Los Castros s/n, 39005, Santander, Spain
**enric.garcia-caurel@polytechnique.edu*

Keywords: optics, polarization, geometric phase, light scattering

In this work we discuss the angle-resolved polarimetric response of light scattered by small particles when they are illuminated by a collimated beam at oblique incidence. Particles are positioned between the two objectives of a polarimetric microscope developed in-house which is able to make measurements in real and reciprocal space modes in transmission configuration. The microscope design allows a complete control of the direction and aperture of both the illumination and the imaged beam. In standard measurements, the proved scattering objects are illuminated with a beam parallel to the optic axis of the microscope. The novelty of this work consisted of illuminating the particles with a collimated beam in oblique incidence respect to the optic axis of the microscope. In these conditions, an effective optical activity becomes apparent in the polarimetric response of the probed particle. The angular distribution of the effective optical activity is highly dependent on the oblique angle of incidence of the illumination beam, the direction at which the scattered light is observed, and, the shape of the probed particle. For small spherical particles, when Mie resonances are not active, the observed optical activity can be fully accounted by a geometric phase resulting from the closed loop in the k-space that the direction of light encompasses across its propagation between the illumination and the imaging microscope objective. When the direction of the illumination beam is parallel to the optic axis of the microscope, the geometric phase is null. The latter explains why no optical activity has been observed by other authors in previous works, because non-oblique illumination was systematically used. In the present work we show the dependence of the geometric phase on the illumination conditions and the shape of the proved particles.

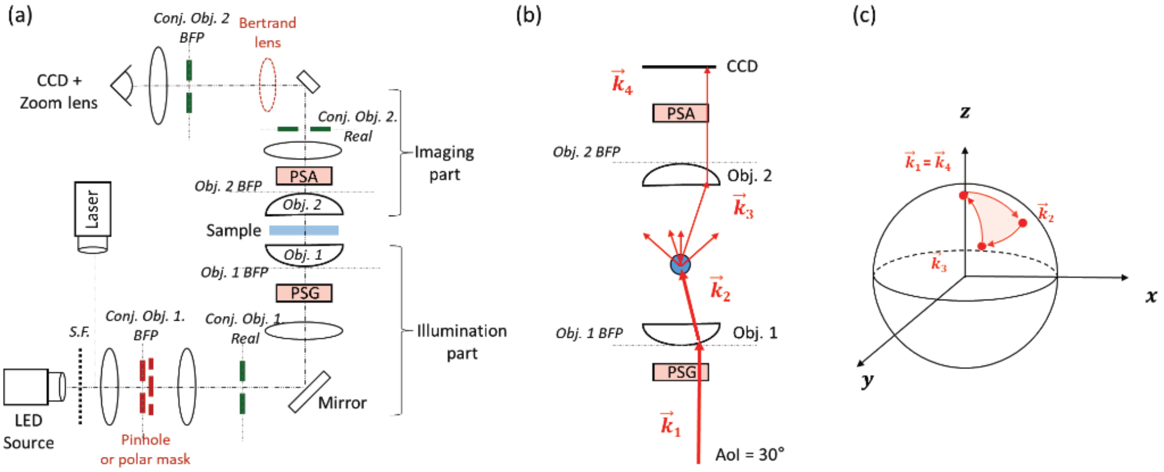

Fig. 1: Schematic diagram of the in-house polarimetric microscope showing the imaging optics, the PSG / PSA used for polarization control, and the sample position between two microscope objectives (a), trajectory of the incident wave vector of the illuminating beam and that of one of the multiple plane waves scattered by the proved micro-particle until that it reaches the imaging microscope objective (b), representation in the k-space of the closed loop trajectory followed by the k-vector of the incident light, and that of one of the plane waves scattered by the probed particle. The area of the spherical triangle enclosed by the closed-loop is related to the geometric phase associated to the given scattered plane wave (c).

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USE OF IPPS INDICATORS TO IDENTIFY AND CLASSIFY TISSUES

Albert VAN EECKHOUT*,[1] Angel LIZANA,[1] Enric GARCIA-CAUREL,[2] José J. GIL,[3] Irene ESTÉVEZ,[1] Carla RODRÍGUEZ,[1] Adrià SANSA,[1] Joshua SARRAT,[1] Emilio GONZÁLEZ,[4,5] Juan Carlos ESCALERA,[1] Ignacio MORENO,[6] Juan CAMPOS[1]

[1]Universitat Autònoma de Barcelona, Grup d'Òptica, Physics Department, 08193, Bellaterra, Spain
[2]LPICM, CNRS, École Polytechnique, Université Paris-Saclay, 91128, Palaiseau, France
[3]Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain
[4]Dpto. de Anatomía, Histología y Neurociencia, Universidad Autónoma de Madrid, 28029, Madrid, Spain
[5]Servicio de Anatomía Patológica, Hospital Universitario de Canarias, 38320, Santa Cruz de Tenerife, Spain
[6]Dpto. de Cienc. de Mat., Ópt. y Tecnol. Electr., Universidad Miguel Hernández de Elche, 03202, Elche, Spain
**albert.vaneeckhout@uab.cat*

Keywords: Depolarization, Biological tissue, Polarimetry, Imaging, Tissue characterization

In the last decade, several works have shown the interest of using polarimetric images in the characterization and visualization of biological tissues structures, which can be applied, for example, in the detection of several types of cancers. Several studies reveal that retardance and depolarization are the most sensitive polarization channels in terms of image contrast when dealing with biological tissues. In the depolarization case, the most used channel is the so-called depolarization index, P_Δ, which gives a global measure of the depolarization introduced by a sample (i.e., as far as it is from an ideal depolarizer). Other indicators of great interest are the so-called Indices of Polarimetric Purity (IPPs), proposed by J.J. Gil. et al. [1, 2], being three mathematical indicators that allow a greater synthesization of the polarimetric content associated to a sample. The IPPs are 3 easy-to-use mathematical criteria that can be calculated from the Mueller matrix of the sample, this being the polarimetric transfer matrix that relates the polarizations illuminating and exiting the sample. In this context, we used IPPs for the first time to the analysis of biological tissues, and several combinations of them that provide further contrast [3]. Moreover, IPP channels are more sensitive than P_Δ allowing to identify and classify different tissue structures. The experimental results obtained in several *ex-vivo* tissues demonstrate the potential of these indicators for their use in biomedical applications.

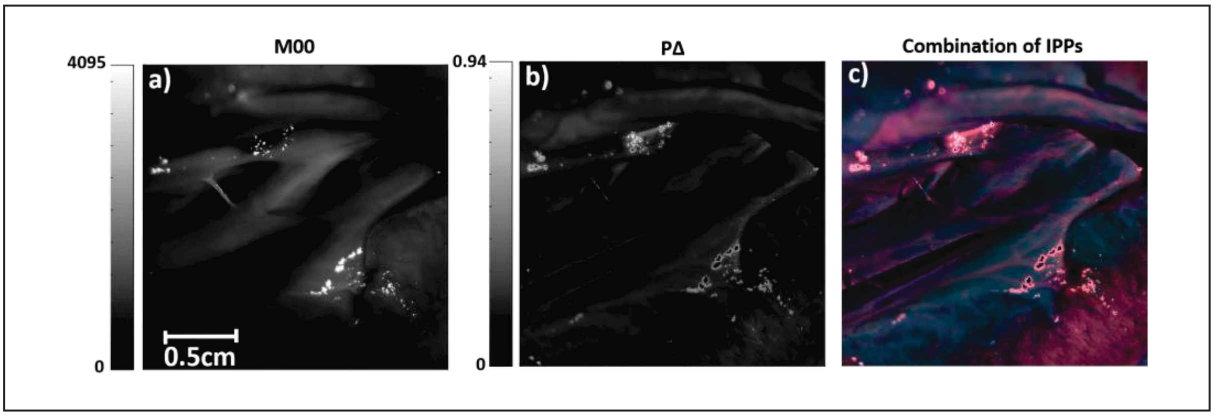

Fig. 1: Image enhancement provided by IPPs: (a) intensity image of a lamb's heart section; (b) polarimetric image obtained from the P_Δ depolarization index; and (c) pseudo-colored image obtained from a combination of the polarimetric images based on the IPPs.

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