BioPolarimetry

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Introduction

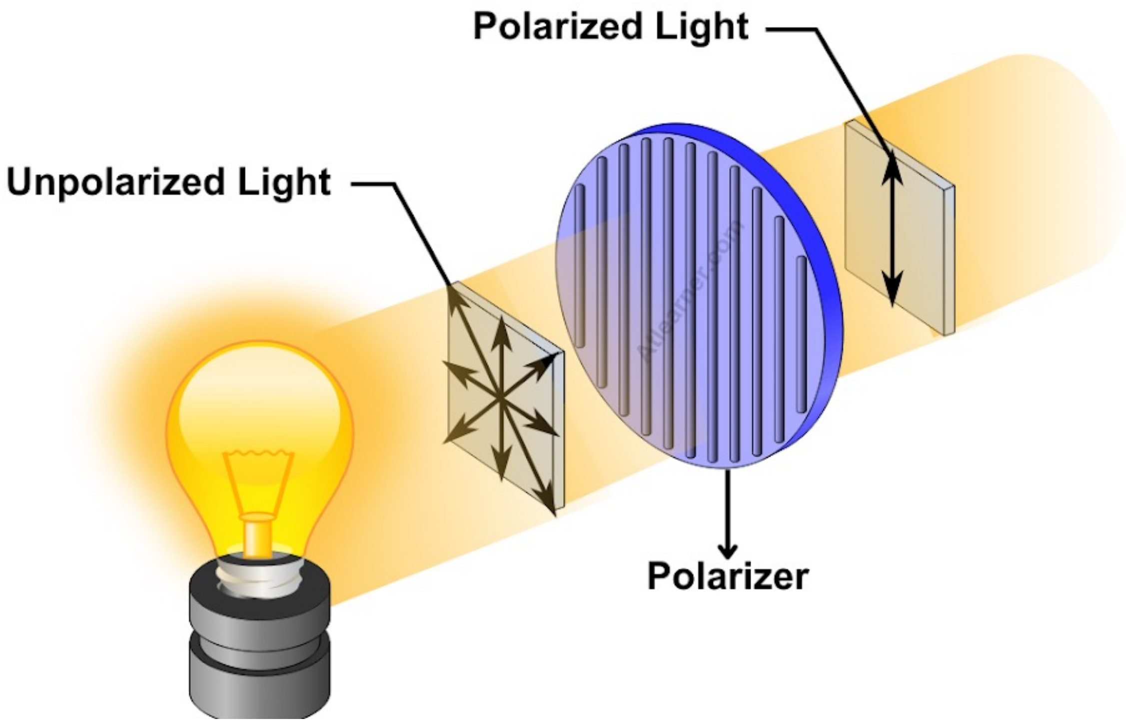
Motivations

- Utilizing the interaction of light with biological tissues and fluids for practical purposes depends on understanding the properties of two large classes of biological media.
- One of them comprises weakly scattering (transparent) tissues and fluids like cornea, crystalline lens, vitreous humor, and aqueous humor of the front chamber of eye.
- The other class includes strongly scattering (opaque or turbid) tissues and fluids like skin, brain, vessel wall, eye sclera, blood, and lymph.

Motivations

- The interaction of light with biological media of the first class can be described by a model of single scattering in an ordered medium with closely packed scatterers that have a complex refractive index.
- Light propagation in tissues of the second class can be described by a model of multiple scattering of scalar or vector waves in a random or ordered low-absorbing medium.

Background

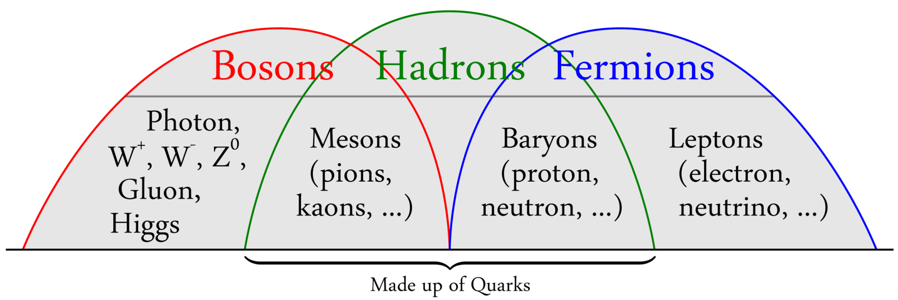


Background

- Photon polarization is the quantum mechanical description of the classical polarized sinusoidal plane electromagnetic wave.
- A photon can be described as having right or left circular polarization, or a superposition of the two. Equivalently, a photon can be described as having horizontal or vertical linear polarization, or a superposition of the two.
- The quantum polarization state vector for the photon is identical with the Jones vector, usually used to describe the polarization of a classical wave through Maxwell's equations .

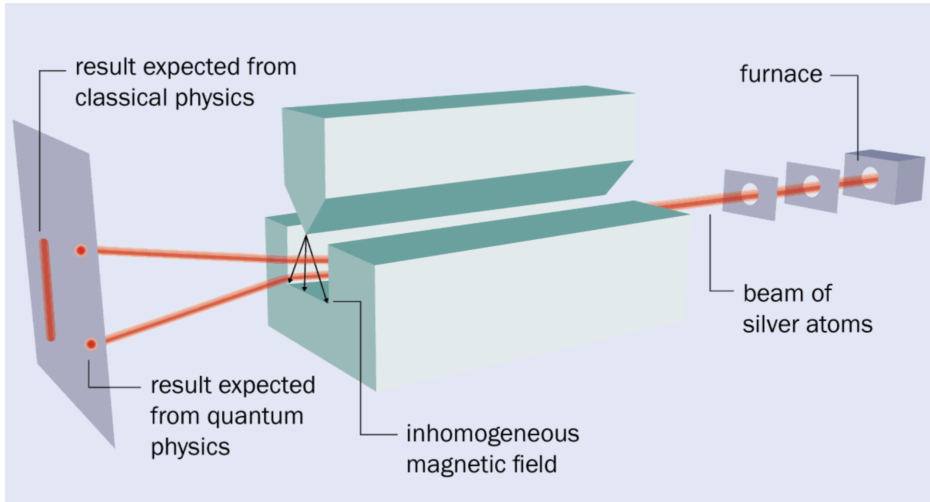
Background

- Spin is the fundamental property that distinguishes the two types of elementary particles: fermions with half-integer spins and bosons with integer spins.
- Photons, which are the quanta of light, have been long recognized as spin-1 gauge bosons. The polarization of the light is commonly accepted as its "intrinsic" spin degree of freedom.
- However, in free space, only two transverse polarizations are allowed. Thus, the photon spin is always only connected to the two circular polarizations.



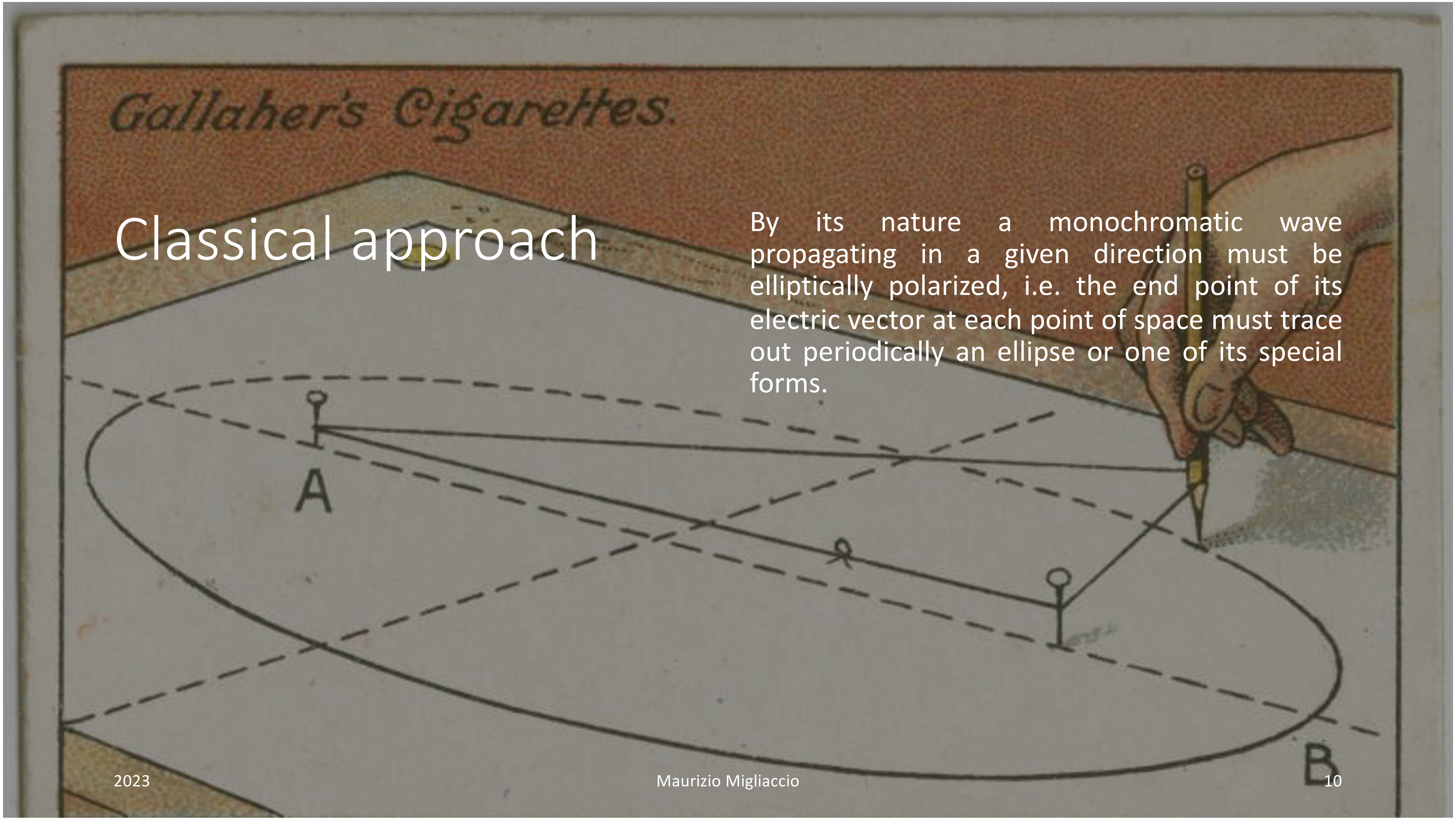
Background

- The Stern–Gerlach experiment demonstrated that the spin of a particle is quantized.
- After its conception by Otto Stern in 1921, the experiment was first successfully conducted with Walther Gerlach in early 1922.



Background

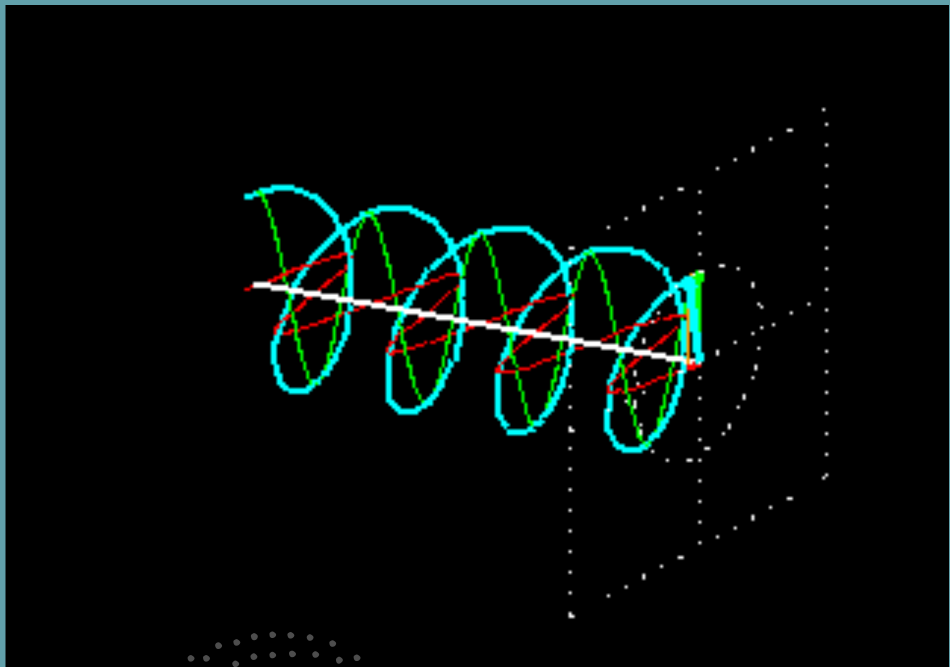
- Light, as an electromagnetic wave, possesses several fundamental properties, which include intensity, wavelength, phase and polarization.
- While the former three are scalar quantities, polarization has vectorial properties; its use has therefore required more advanced optical and computational approaches.
- Hence, studies of either the vector properties of light, described via the state of polarization (SOP) or the full vectorial transformation properties of an object, have a shorter history in biomedical analysis compared with their scalar counterparts, and the extent of their application is still being explored



Classical approach

By its nature a monochromatic wave propagating in a given direction must be elliptically polarized, i.e. the end point of its electric vector at each point of space must trace out periodically an ellipse or one of its special forms.

Classical approach



- The polarimetric ellipse is not equivalent to a geometrical ellipse because we are actually dealing with a dynamic process: namely, the time evolution of the electric field.
- As a matter of fact, beyond the geometrical shape and orientation of the ellipse it must be also specified its sense.

CW vs CCW

- Note the definitions of right-handed circular polarized light (clockwise rotation) and left-handed circular polarized light (anticlockwise rotation) are different in optics books and academic communities.
- It depends on whether the observer 'sees' the light from the source (Convention I), or from the detector (Convention II).
- Institute of Electrical and Electronics Engineers (IEEE) uses Convention I, so it is also widely used in engineering fields; Quantum physicists also use Convention I, to be consistent with the conventions for representing particle spin states.
- However, for numerous optics books such as Principles of Optics (Born and Wolf) and Handbook of Optics, Convention II is used. Hereafter, we use Convention II in order to correspond to such scientific references.

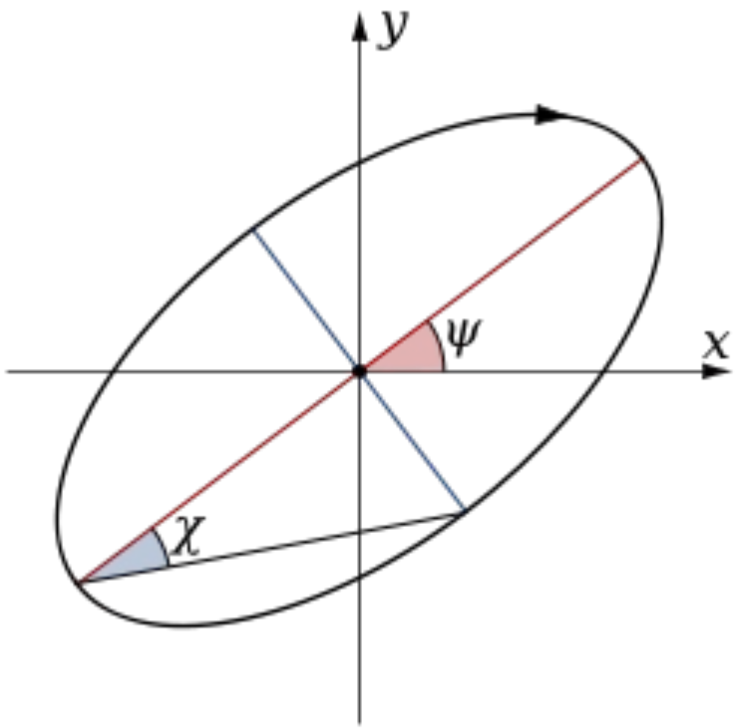
The 2D Jones vector

- The following complex vector representation is known as the 2D Jones vector.

$$\hat{\boldsymbol{E}} = \begin{pmatrix} \hat{E}_x \\ \hat{E}_y \end{pmatrix} = \begin{pmatrix} a_x e^{j\delta_x} \\ a_y e^{j\delta_y} \end{pmatrix} = a_x e^{j\delta_x} \begin{pmatrix} 1 \\ \boldsymbol{\tau} e^{j\delta} \end{pmatrix}$$

- The components of the vector can be expressed in any complex orthogonal base.

The polarization ellipse



- The orientation angle $\psi(-\pi/2<\Psi\leq\pi/2)$ is the inclination of the major axis of the ellipse and the x positive axis of the coordinate system.
- The ellicipity angle χ $(-\pi/4<\chi\leq\pi/4)$ is a measure of the shape of the ellipse. In fact, the ellicipity or axial ratio is the ratio between the major to minor axis and χ is the $\tan^{-1}$ of such ratio.

The Stokes vector

- The four Stokes parameters, collectively known as Stokes vector, for a *deterministic* (*fully polarized*) plane wave are the four quantities:

$$s_o = a_x^2 + a_y^2$$

$$s_1 = a_x^2 - a_y^2$$

$$s_2 = 2a_x a_y \cos\delta$$

$$s_3 = 2a_x a_y \sin\delta$$

- Such representation is in one-to-one correspondence with the 2D Jones vector.
- Note that only the relative phase knowledge $\boldsymbol{\delta}$ is due.
- Of course

$$s_o^2 = s_1^2 + s_2^2 + s_3^2$$

The Stokes vector

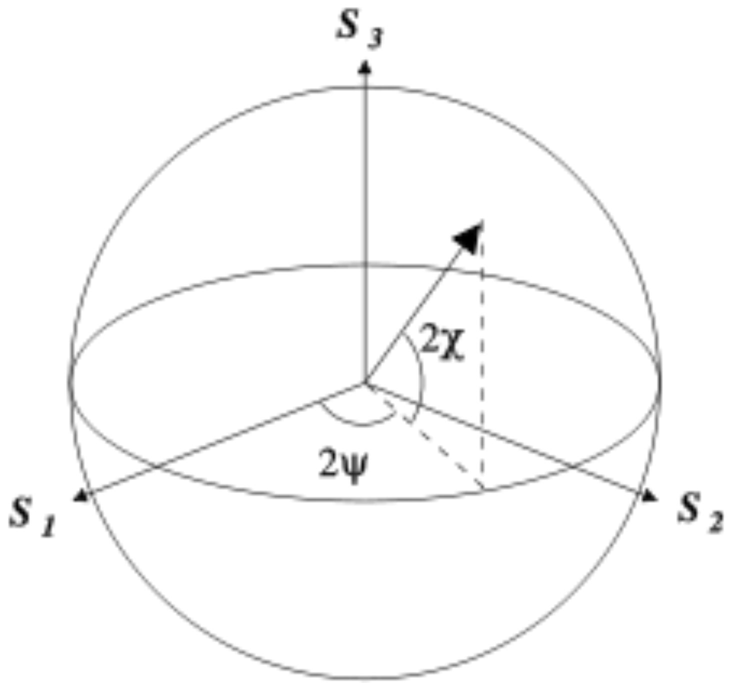
- It can be demonstrated that:

$$s_1 = s_o \cos 2\chi \cos 2\psi$$

$$s_2 = s_o \cos 2\chi \sin 2\psi$$

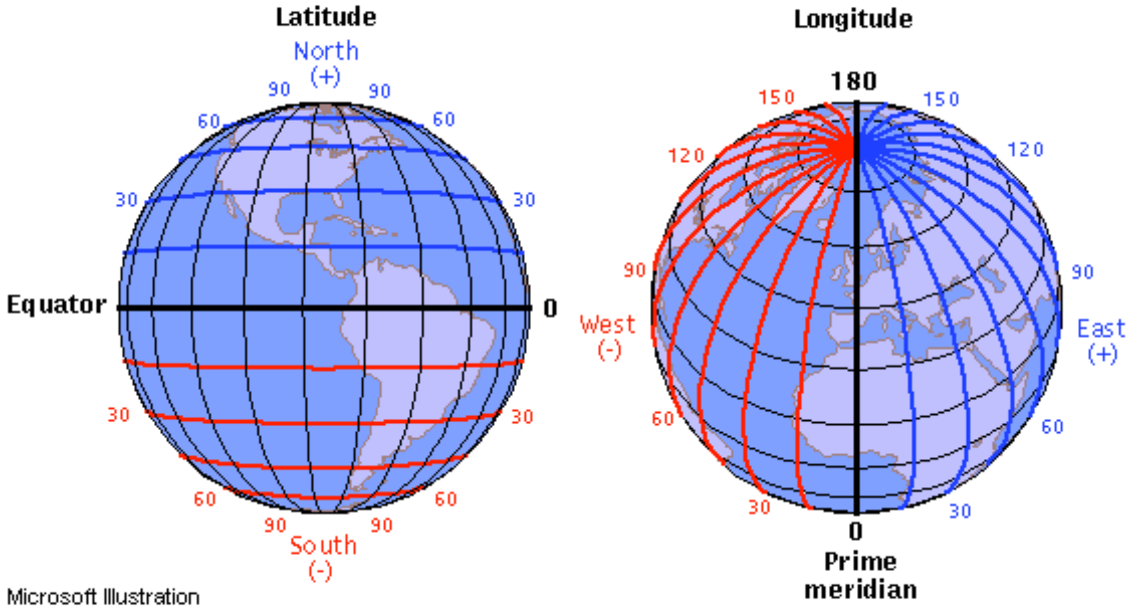
$$s_3 = s_o \sin 2\chi$$

- The longitude and latitude of the point over the Poincaré sphere are 2ψ and 2χ, respectively.



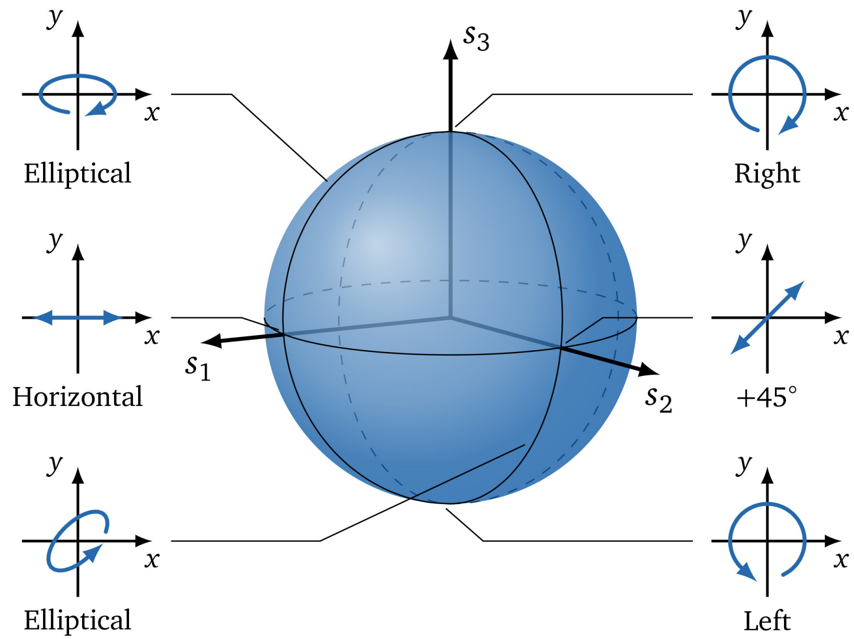
Latitude and Longitude

- The Lat. of a point on the Earth's surface is the angle between the equatorial plane and the straight line that passes through that point. Lines joining points of the same latitude trace circles on the surface of the Earth called parallels.
- The Long. of a point on the Earth's surface is the angle east or west from a reference meridian to another meridian that passes through that point.



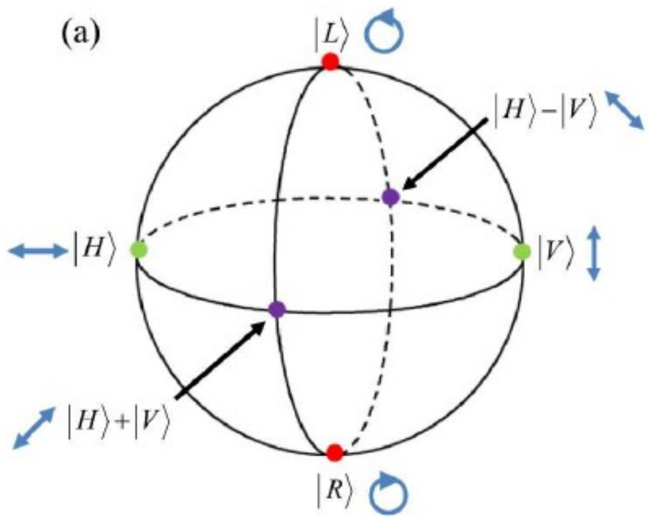
The Poincaré sphere

- *The poles corresponds to circular polarizations while the equator to linear ones.*
- The northern hemisphere corresponds to CW polarizations.
- The surface corresponds to fully-polarized waves.
- Note that aside the optical sense of tracing the ellipse is used !



The Poincaré sphere

- One very important consequence of such mapping is that *orthogonal polarizations* lie diametrically opposite on the sphere and corresponds to antipodal points.
- This is in distinction to the conventional geometrical notion of orthogonality, which involves 90° angular separation between vectors.



Partially polarized waves

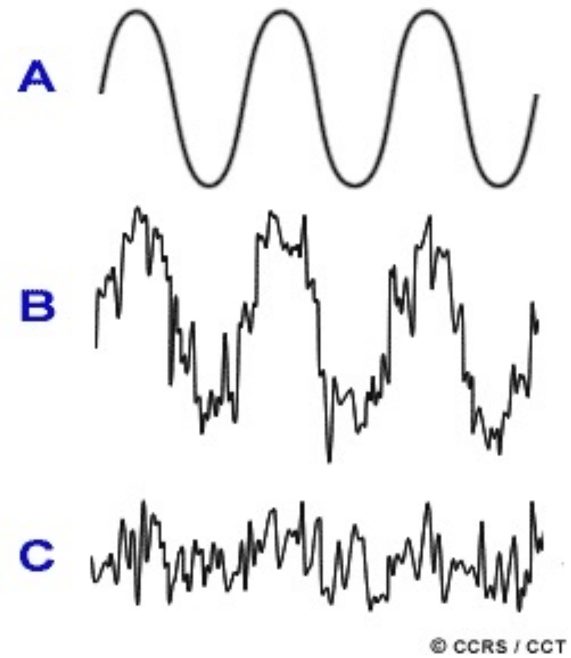
- The Stokes parameters are able to describe partially polarized waves if properly defined:

$$s_o = <\mathscr{E}[a_x^2]> + <\mathscr{E}[a_y^2]>$$

$$s_1 = <\mathscr{E}[a_x^2]> - <\mathscr{E}[a_y^2]>$$

$$s_2 = <2\mathscr{E}[a_x a_y \cos\delta]>$$

$$s_3 = <2\mathscr{E}[a_x a_y \sin\delta]>$$



The Stokes vector

- It can be easily demonstrated that now: $s_o^2 \geq s_1^2 + s_2^2 + s_3^2$
- *Equality holds only in case of fully polarized waves*.
- The Stokes parameters can be uniquely decomposed into two independent parts. A part fully polarized (2 terms) and a part fully unpolarized (1 term), that is:

$$s_o = s_o^{(1)} + s_o^{(2)} \quad s_1 = s_1^{(2)} \quad s_2 = s_2^{(2)} \quad s_3 = s_3^{(2)}$$

- The 2D degree of polarization $P_{(2)}$ is:

$$0 \leq P_{(2)} = \frac{s_o^{(2)}}{s_o} \leq 1$$

The Stokes vector

- Note that sometimes the four Stokes parameters are labelled as I,Q,U and V and are collectively known as Stokes (column) vector (I, Q, U, V)^T

$$I = \left|\hat{E}_x\right|^2 + \left|\hat{E}_y\right|^2; \quad Q = \left|\hat{E}_x\right|^2 - \left|\hat{E}_y\right|^2$$

$$U = 2\Re(\hat{E}_x\hat{E}_y^*); \quad V = -2\Im(\hat{E}_x\hat{E}_y^*)$$

- In these equations T stands for the transposed operator, * for the complex conjugate, $\mathscr{R}$ for the real part and $\mathscr{I}$ for the imaginary part.
- With temporal dependence exp(-jωt), since $\delta \rightarrow -\delta$ then $V = 2\Im(\hat{E}_x\hat{E}_y^*)$

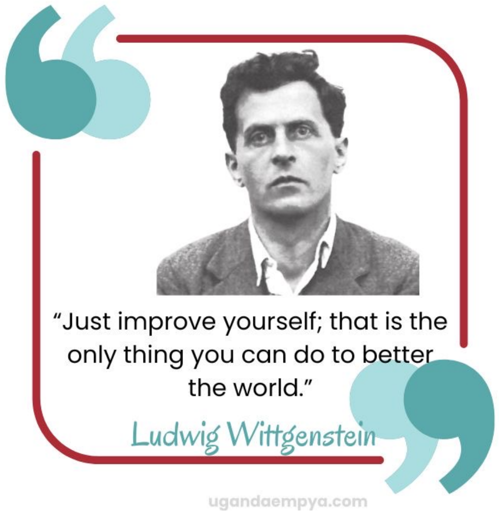
The Stokes vector

- The Stokes vector can be expressed with the format shown where I_0 , I_{90} , I_{45} , I_{45} are the projection intensities (different linear components in directions of 0°, 90°, 45°, −45° with respect to the local coordinate system) of a light beam, I_{cL} and I_{cR} are components of left/right-handed circular polarized light, respectively.

$$\begin{pmatrix} I = I_{0^\circ} + I_{90^\circ} \\ Q = I_{0^\circ} - I_{90^\circ} \\ U = I_{45^\circ} + I_{-45^\circ} \\ V = I_{CR} - I_{CL} \end{pmatrix}$$

Some remarks

- We see that the Stokes vector can be calculated via intensity measurements that can be readily performed in an experiment.
- It is a more general descriptor with respect to Jones vector that can only represents fully polarized waves.



Note

- The Jones vector is a first order descriptor.
- The Stokes vector is a second order descriptor.

DOP

- An intrinsic parameter of a (partially) polarized wave is its Degree of Polarization (DOP):

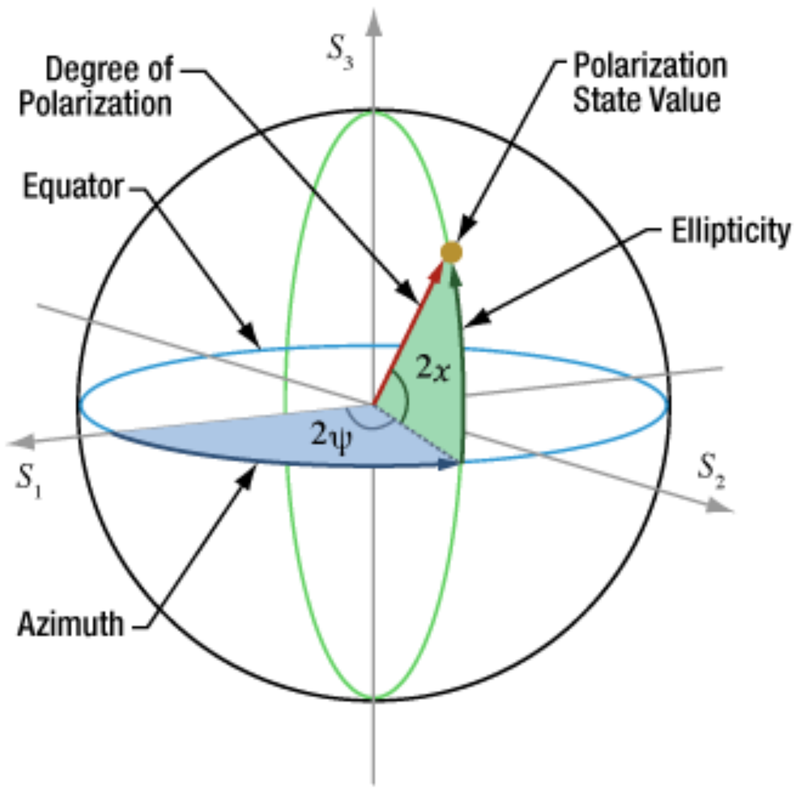
$$DOP = \sqrt{Q^2 + U^2 + V^2}/I$$

- Further it is defined the Degree of Linear Polarization (DOLP):

$$DOLP = \sqrt{Q^2 + U^2}/I$$

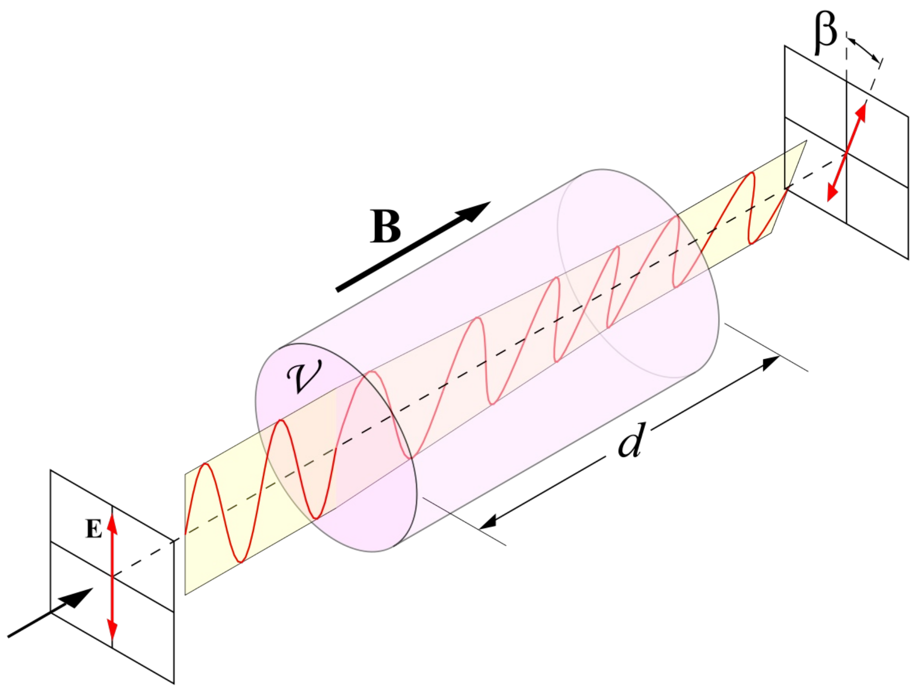
and the Degree of Circular Polarization (DOCP):

$$DOCP = \sqrt{V^2}/I$$

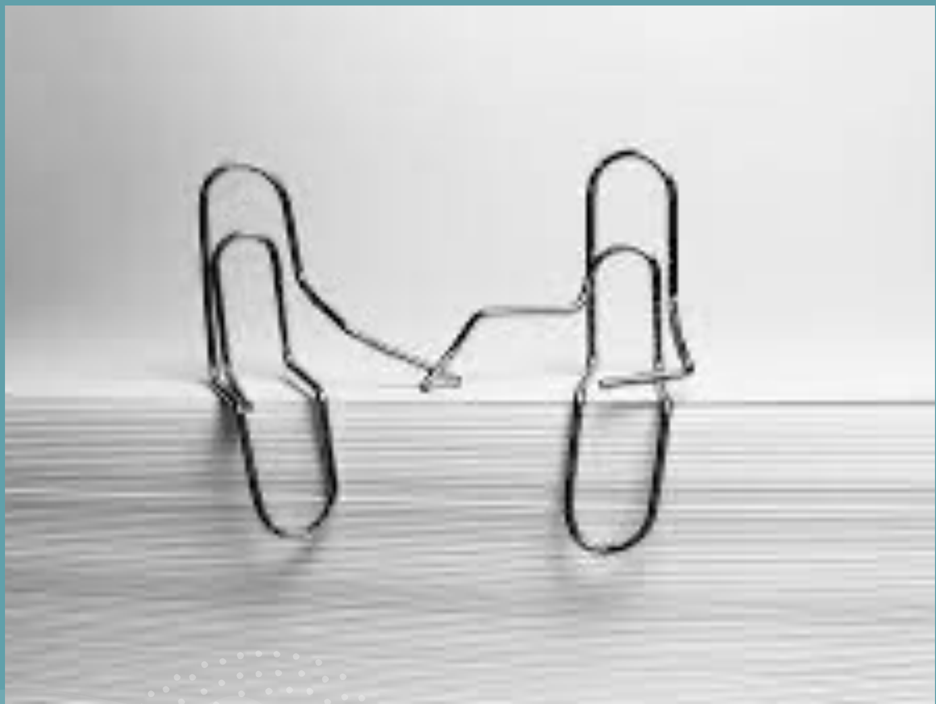


Depolarization vs Repolarization

- A fully polarized wave is depolarized if a physical process changes its unitary degree of polarization.
- A fully polarized wave is repolarized if a physical process changes the components values without altering its unitary degree of polarization.



Polarimetric input-output relationships



- Two main relationships are here relevant.
- The first relationship is based on the input and output polarimetric *Jones vector descriptors*.
- The second relationship is based on the input and output polarimetric *Stokes vector descriptors*.

Ellipsometry

- The transformation of an arbitrary polarized light (linear, circular, or elliptical) by a weakly scattering media can be described using a linear relationship between the incident and the scattered field components described by their Jones vectors.
- Such a relationship is known as the scattering matrix (S-matrix) or *Jones matrix* (2x2 complex matrix):

$$\begin{pmatrix} \hat{E}_x^s \\ \hat{E}_y^s \end{pmatrix} = \begin{pmatrix} \dot{S}_{xx} & \dot{S}_{xy} \\ \dot{S}_{yx} & \dot{S}_{yy} \end{pmatrix} \begin{pmatrix} \hat{E}_x^i \\ \hat{E}_y^i \end{pmatrix}$$

Robert Clark Jones

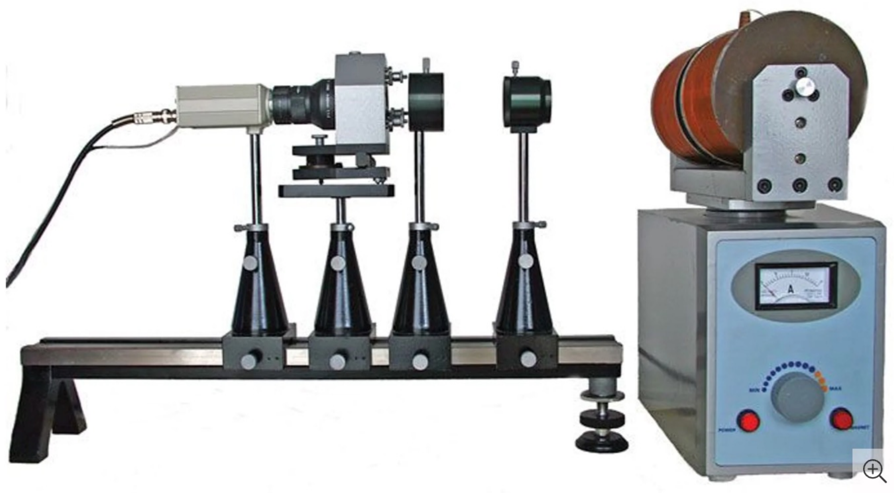
- R. Clark Jones (June 30, 1916 – April 26, 2004) was an American physicist working in the field of optics.
- He studied at Harvard University and received his PhD in 1941. Until 1944 he worked at Bell Labs, later until 1982 with the Polaroid Corporation.
- In a sequence of publications between 1941 and 1956 he demonstrated a mathematical model to describe the polarization of coherent light, the Jones calculus.

Ellipsometry

- The majority of tissues and cells can be represented as particle systems composed of optically soft scattering particles (with a low degree of refractive index mismatch between the scatterers and ground medium). That allows one to restrict the description of light propagation to a single scattering approximation in a number of cases.
- Such media are considered to be weakly scattering ones.
- Polarized light interactions with a scattering medium are displayed as a transformation of the polarization state (linear, circular, or elliptical) when the light beam propagates within the medium. To correctly exploit a single scattering approximation, the optical thickness of the object under study must be quite small.

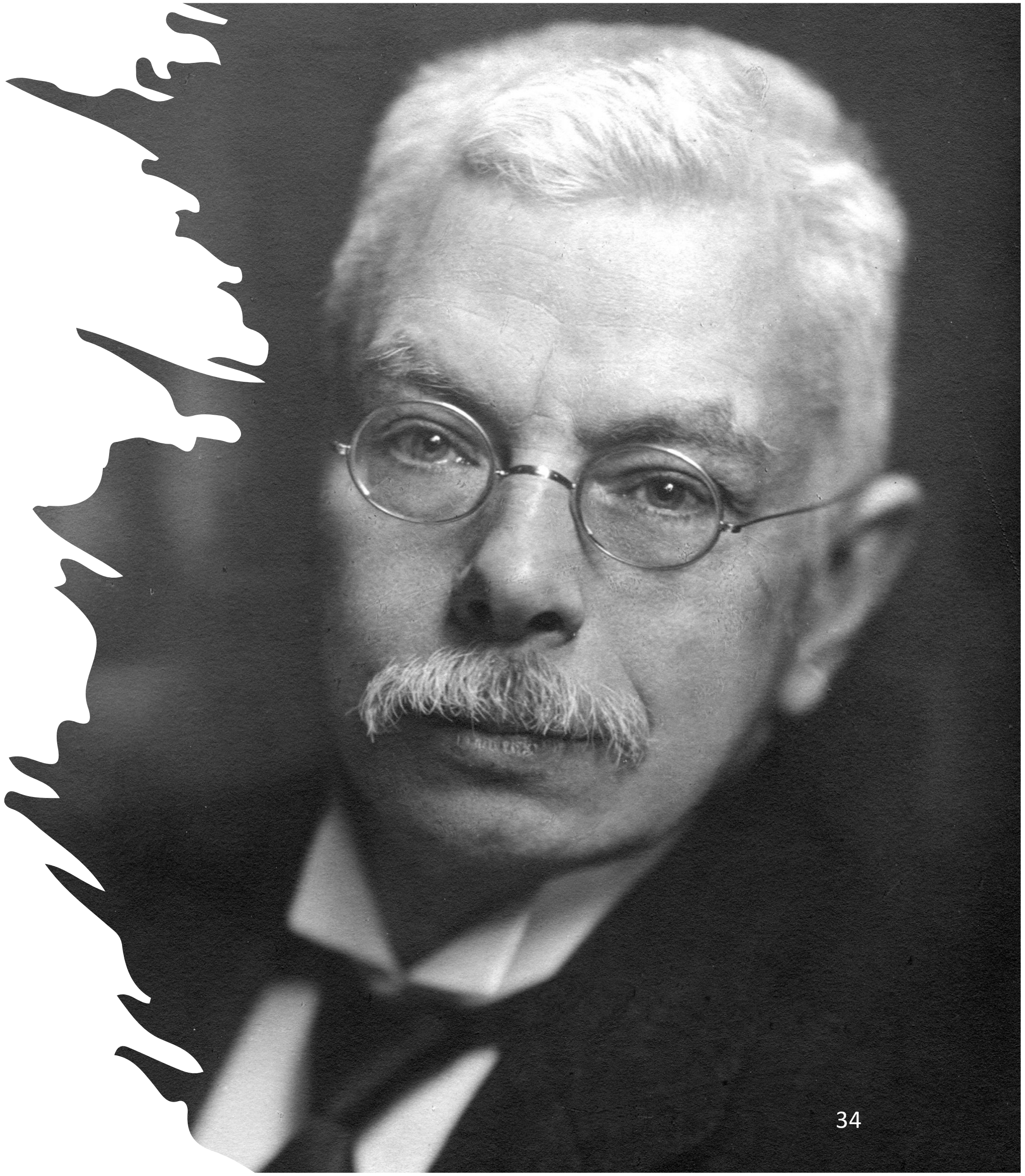
Ellipsometry

- Polarimetric experimental methods are available to determine the Jones matrix elements of transparent optical materials.
- For scattering tissues, the direct measurements of Jones matrix elements can be done using a two-frequency Zeeman laser, which produces two laser lines with a small frequency separation (∼250 kHz) and orthogonal linear polarizations, or by the OCT technique.



Pieter Zeeman

- Pieter Zeeman (25 May 1865 – 9 October 1943) was a Dutch physicist who shared the *1902 Nobel Prize in Physics* with Hendrik Lorentz for his discovery of the Zeeman effect.
- The Zeeman effect is the spectral line splitting of a light source into several components when in the presence of a static strong magnetic field.

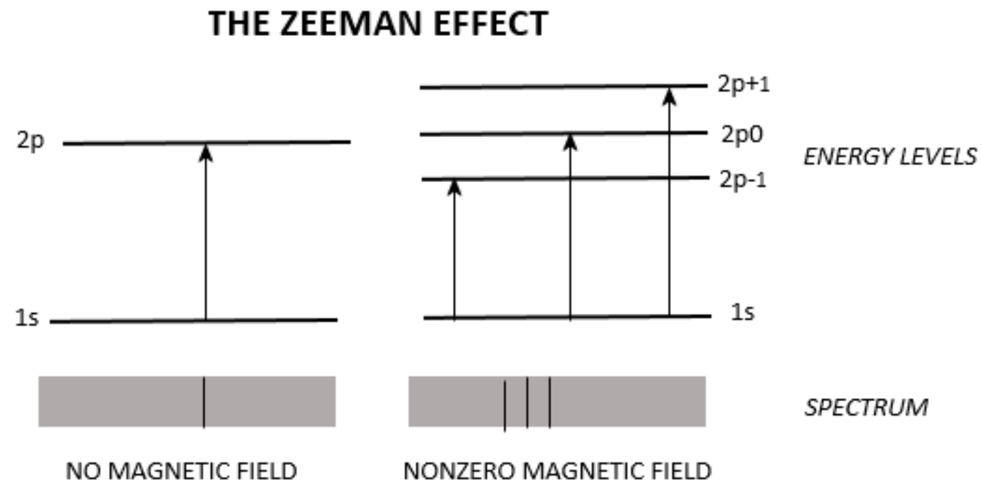


Zeeman effect

Since the distance between the Zeeman sub-levels is a function of magnetic field strength, this effect can be used to measure magnetic field strength, e.g. that of the Sun and other stars or in laboratory plasmas.

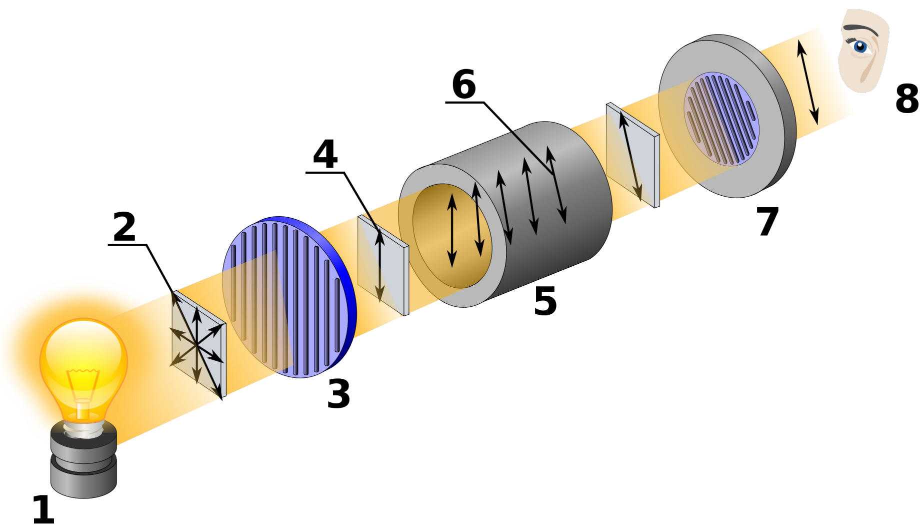
The Zeeman effect is very important in applications such as nuclear magnetic resonance spectroscopy, electron spin resonance spectroscopy, magnetic resonance imaging (MRI) and Mössbauer spectroscopy. It may also be utilized to improve accuracy in atomic absorption spectroscopy.

A theory about the magnetic sense of birds assumes that a protein in the retina is changed due to the Zeeman effect.



Polarimeter

- A polarimeter is a scientific instrument used to measure optical rotation: the angle of rotation caused by passing linearly polarized light through an optically active substance.
  - Operating configuration of an optical polarimeter:
    1. Light source;
    2. Unpolarized light;
    3. Linear polarizer;
    4. Linearly polarized light;
    5. Sample tube containing *chiral* molecules under study
    6. Optical rotation due to molecules;
    7. Rotatable linear analyzer;
    8. Detector.



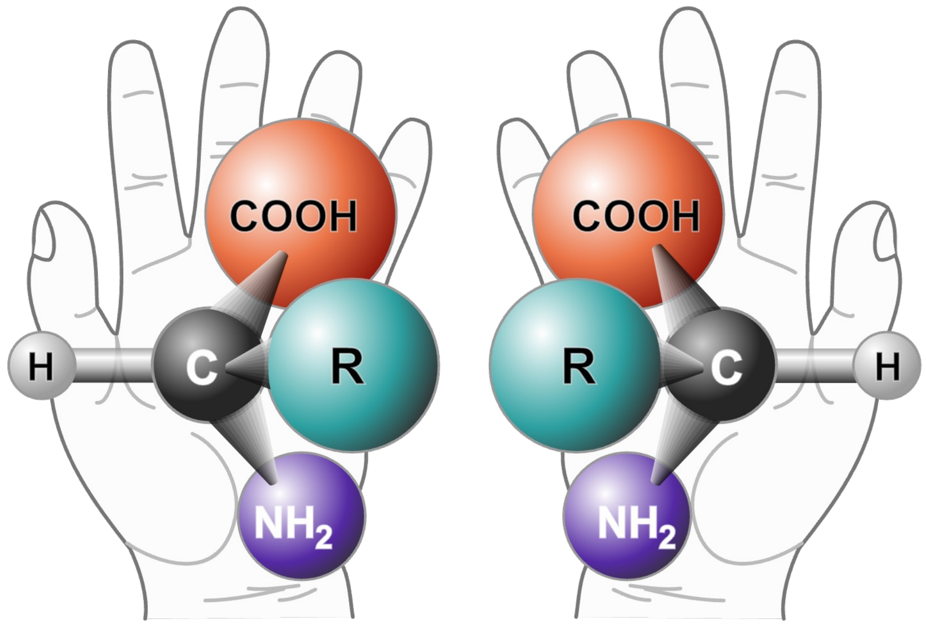
Polarimeter

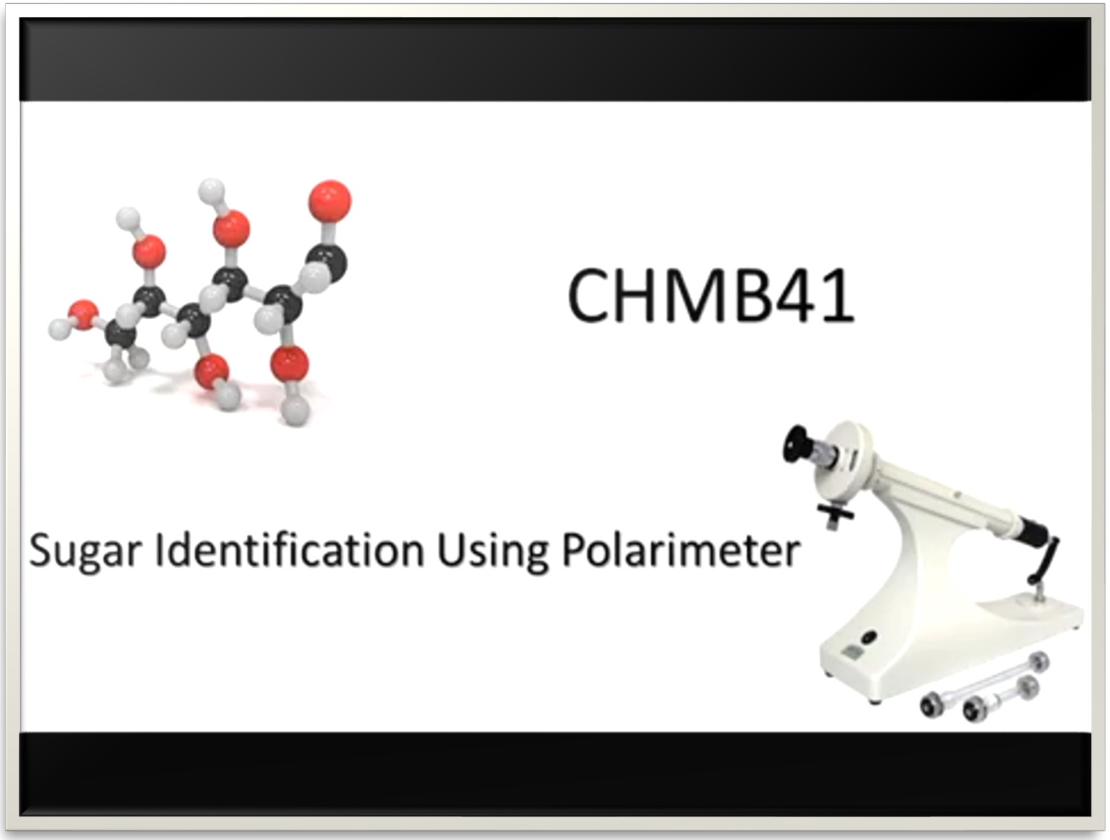
- Some chemical substances are optically active, and linearly polarized light will rotate CW or CCW when passed through these substances.
- The angle of rotation direction (CW/CCW) and its magnitude reveals information about the sample's chiral properties such as the relative concentration of enantiomers present in the sample.
- The cost is about 500 €

Chirality

- In mathematics, a figure is chiral (and said to have chirality) if it cannot be mapped to its mirror image by rotations and translations alone.
- Many biological molecules are chiral, including the naturally occurring amino acids (the building blocks of proteins) and sugars.

Two enantiomers of a generic amino acid that is chiral

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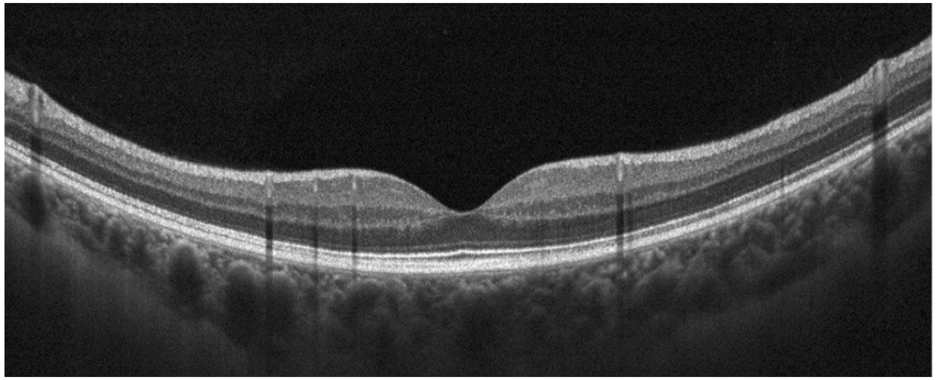
Sugar Identification Using Polarimeter

Look Inside

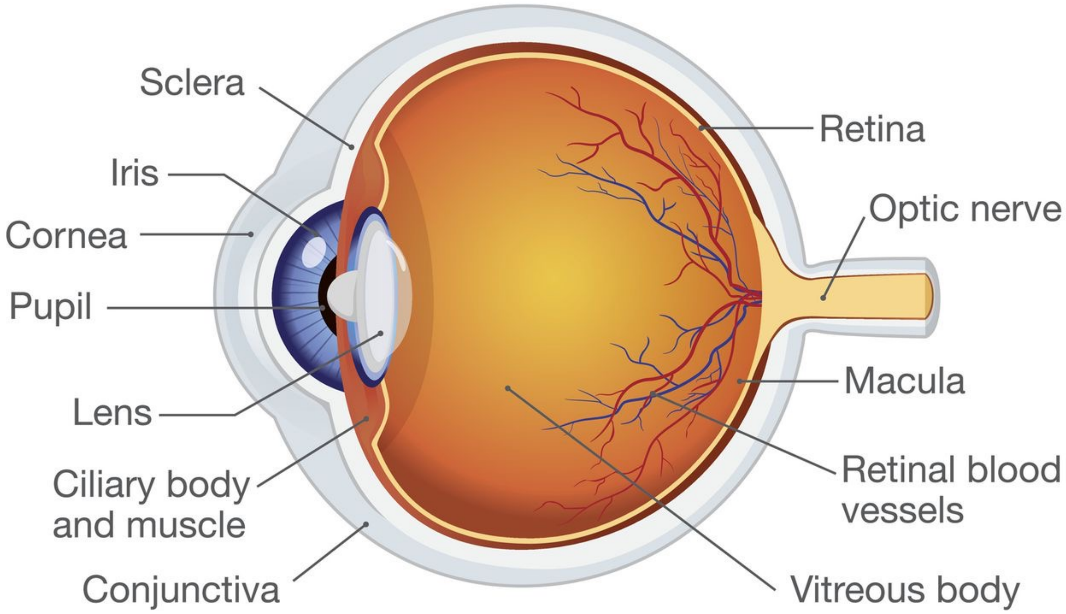
- Since 1895 it has been possible to look "inside" the human body to diagnose dysfunctions and diseases.
- Ultrasound probes and magnetic resonance imaging have been added to the X-rays, discovered by the German physicist Wilhelm Conrad Roentgen and perfected by CT scanning.
- But certain parts of the body and certain organs have remained invisible: soft tissues for example or the capillaries of the heart or retina are, or rather were, almost impossible to visualize from the outside.

OCT

- As a result of its noninvasive characteristic, its high spatial resolution and its easy optical fiber implementation, optical coherence tomography (OCT) is emerging as an important optical imaging modality.

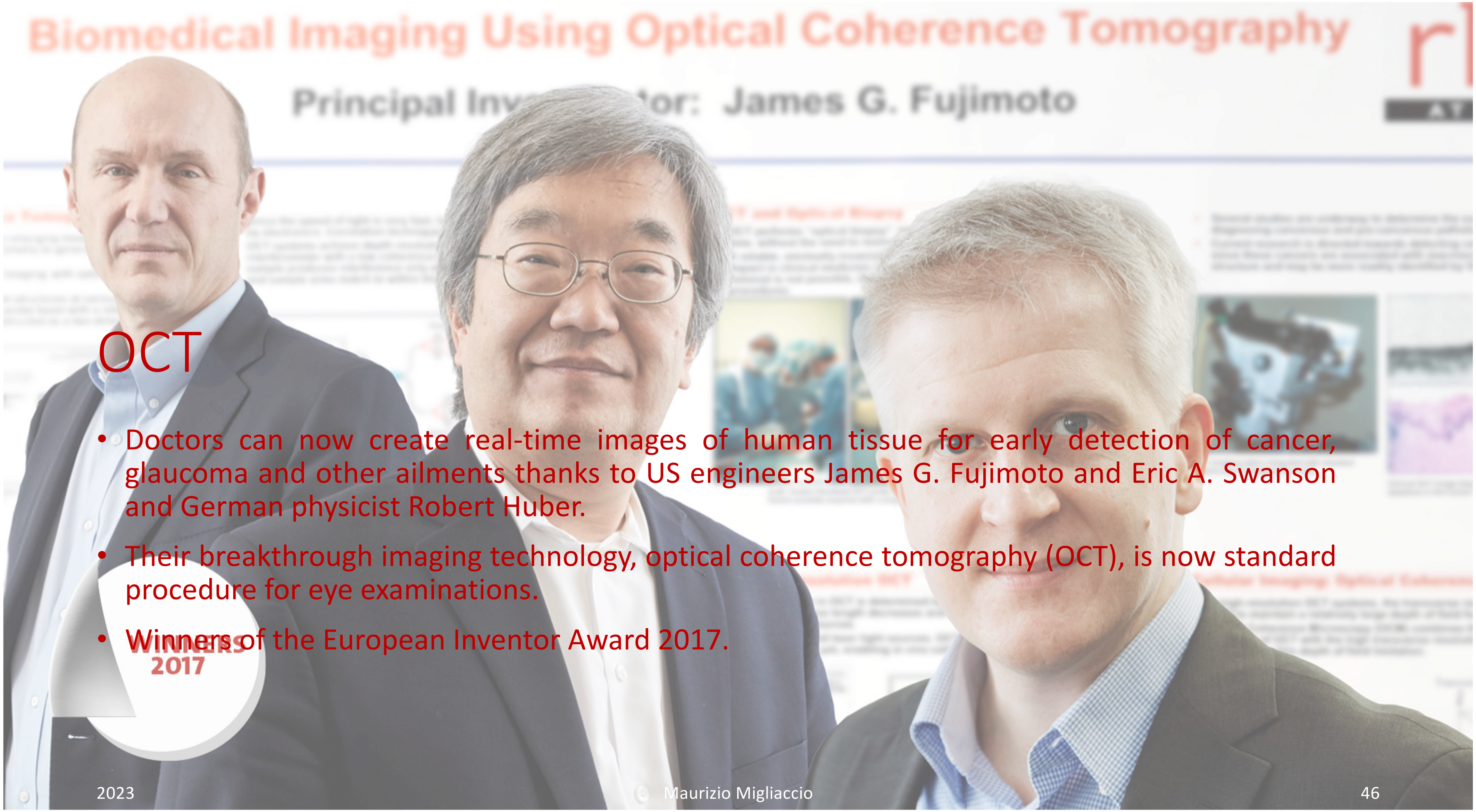


Human Eye Anatomy



OCT

- Ocular (or ophthalmic) OCT (Optical Coherence Tomography) is used heavily by ophthalmologists and optometrists to obtain high-resolution images of the retina and anterior segment.
- Owing to OCT's capability to show cross-sections of tissue layers with micrometer resolution, OCT provides a straightforward method of assessing cellular organization, photoreceptor integrity, and axonal thickness in glaucoma, macular degeneration, diabetic macular edema, multiple sclerosis, optic neuritis, and other eye diseases or systemic pathologies which have ocular signs.
- Polarization-sensitive OCT was recently applied in the human retina to determine optical polarization properties of vessel walls near the optic nerve.



OCT

- Doctors can now create real-time images of human tissue for early detection of cancer, glaucoma and other ailments thanks to US engineers James G. Fujimoto and Eric A. Swanson and German physicist Robert Huber.
- Their breakthrough imaging technology, optical coherence tomography (OCT), is now standard procedure for eye examinations.
- Winners of the European Inventor Award 2017.

OCT

- To completely retrieve the information carried by backscattered light fields, both amplitude and polarization information need to be recorded.
- Conventional OCT systems record the amplitude but not the polarization information from scattered light.
- In contrast, polarization-sensitive OCT can capture the polarization states of backscattered light and, as a result, can reveal the polarization properties, such as birefringence, of a sample, which cannot be recovered by conventional OCT.
- Birefringence is related to various biological components such as collagen, muscle fibers, myelin, retina, keratin, and glucose. Consequently, polarization enhances imaging, diagnosis, and sensing.

Rasmus Bartholin

- Birefringence was first described in calcite crystals by the Danish scientist Rasmus Bartholin (13 August 1625 – 4 November 1698) in 1669.
- Rasmus Bartholin is remembered especially for his discovery of the double refraction of a light ray by Iceland spar (calcite).
- He published an accurate description of the phenomenon, but since the physical nature of light was poorly understood at the time, he was unable to explain it.
- It was only after Thomas Young proposed the wave theory of light, c. 1801 that an explanation became possible.

Birefringence

Birefringence is the optical property of a material having a refractive index that depends on the polarization and propagation direction of light.

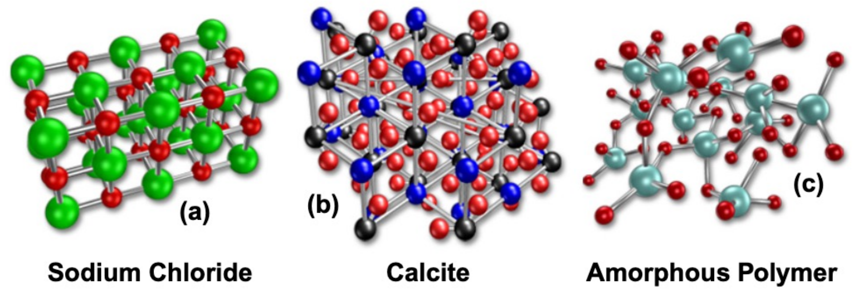
These optically anisotropic materials are said to be birefringent (or birefractive).

The letters on a paper covered by a calcite crystal show up as doubled. This is an example of the phenomenon of birefringence (double refraction).

Birefringence

- Birefringence, or double refraction, is the splitting of a ray of light into two rays when it passes through certain types of material, such as calcite crystals (b). The two rays, called the ordinary ray and the extraordinary ray, travel at different speeds.
- This effect can occur only if the structure of the material is anisotropic, so that the material's optical properties are not the same in all directions.

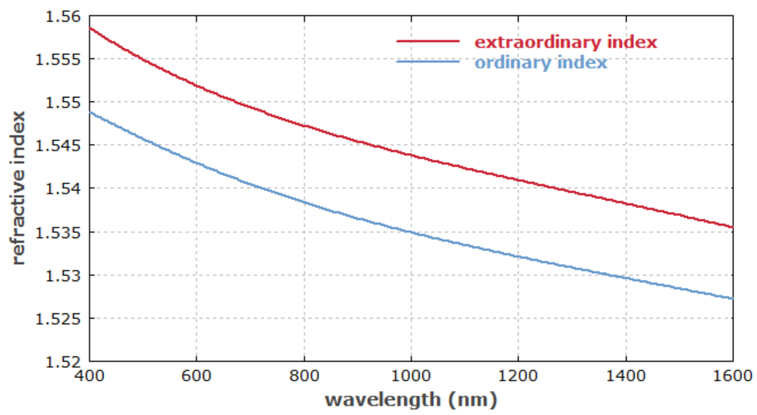
Figure 1 - *Crystalline Structure of Isotropic and Anisotropic Materials*



Birefringence

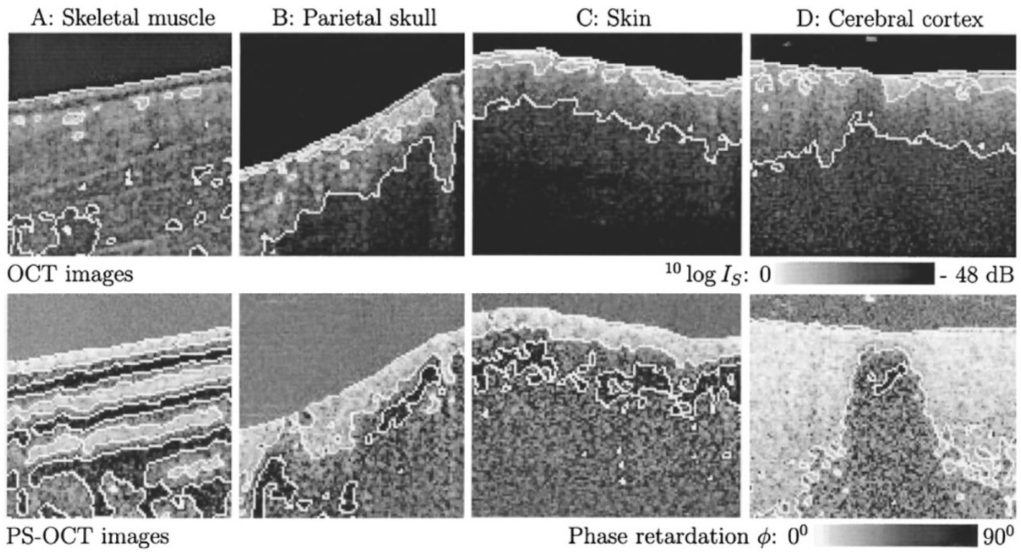
- Birefringence is the property of some transparent optical materials that the refractive index depends on the polarization direction – which is defined as the direction of the electric field.
- For example, it is observed for crystalline quartz, calcite, sapphire and ruby, also in nonlinear crystal materials like $LiNbO_3$, LBO and KTP. Figure shows that for α-quartz.

Refractive indices of α-quartz vs. wavelength. For this positive uniaxial material, the extraordinary index is higher.



OCT vs PolOCT

De Boer et al., “Polarization Effects in Optical Coherence Tomography of Various Biological Tissues”, *IEEE J Sel Top Quantum Electron. 1999*; 5(4): 1200–1204. doi:10.1109/2944.796347



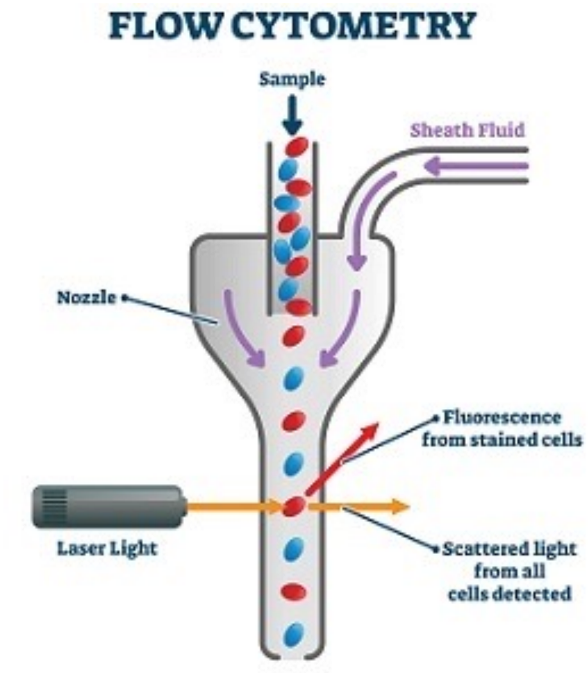
OCT

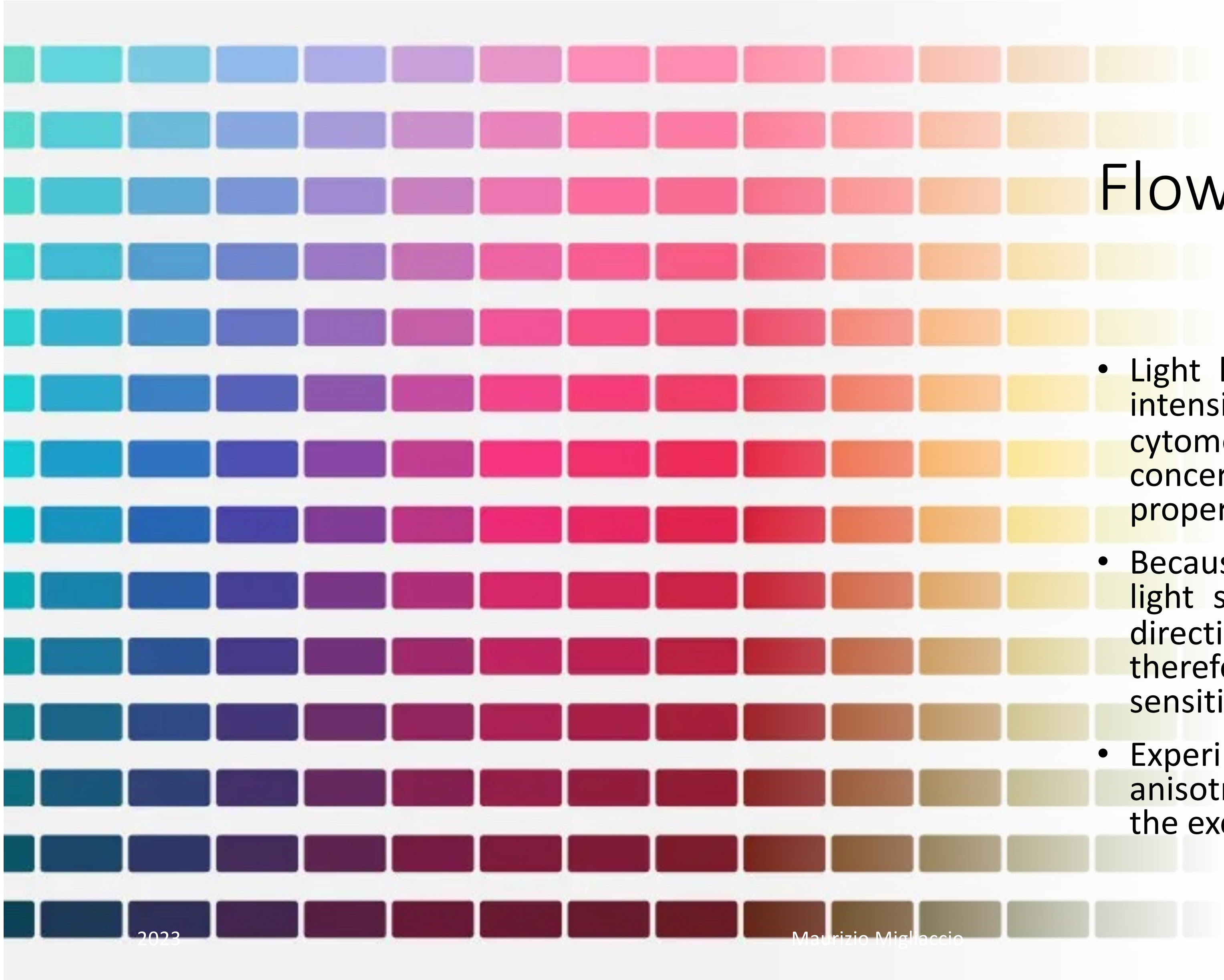
Jiao et al. demonstrated that the degree of polarization (DOP) of backscattered light measured by OCT is unity throughout the detection range and that a DOP of unity indicates that the measured Mueller matrix is nondepolarizing. This conclusion allows the use of a *Jones matrix*, instead of a *Mueller matrix*, in OCT.

S. Jiao, G. Yao, and L.-H.V. Wang, "Depth-resolved two-dimensional Stokes vectors of backscattered light and Mueller matrices of biological tissue measured with optical coherence tomography," Appl. Opt. 39(34), 6318–6324 (2000)

Flow cytometry

- Flow cytometry is a technology that rapidly analyzes single cells or particles as they flow past single or multiple lasers while suspended in a buffered salt-based solution.
- Polarization measurements can be collected for flow cytometry.





Flow cytometry

- Light has three qualities: color, intensity, and polarization. In cytometry, we are usually concerned with the first two properties only.
- Because of particle anisotropy light scattering do depends on direction of incoming light and therefore are polarization sensitive.
- Experiments indicate that anisotropy is the rule rather than the exception !

StarCellBio

presents

FLOW CYTOMETRY

Mueller Matrix Polarimetry

MUELLER

Hans Mueller

Hans Mueller (27.10.1900–10.6.1965) was a Swiss physicist and professor at the Massachusetts Institute of Technology (MIT).

Mueller matrix

- A Jones matrix (J is sometimes used in place of S) transforms an input Jones vector into an output Jones vector while a *Mueller matrix* (M) transforms an input Stokes vector into an output Stokes vector, thus:

$$\begin{bmatrix} I_s \\ Q_s \\ U_s \\ V_s \end{bmatrix} = \begin{bmatrix} M_{11} & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{bmatrix} \begin{bmatrix} I_i \\ Q_i \\ U_i \\ V_i \end{bmatrix}$$

- Elements of matrix depend on the scattering angle θ, the wavelength, and the geometrical and optical parameters of the object.

Mueller matrix

- Depolarization is intrinsically associated with scattering and a loss of coherence in the polarization state.
- If the degree of polarization *DOP* of a light field remains at unity after transformation by an optical system, this system is nondepolarizing; otherwise, the system is depolarizing.
- In general, all 16 elements of the M are nonzero.
- For nondepolarizing systems, the number of independent M elements cannot be more than *seven* since both the Mueller matrix and the Jones matrix can represent the system.

Mueller matrix

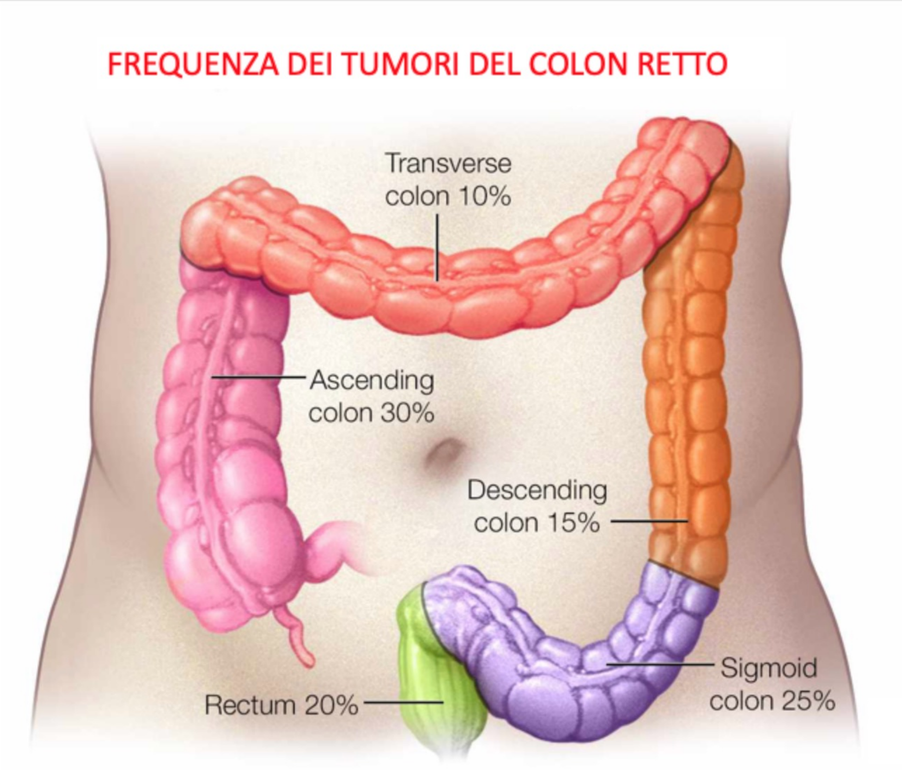
- Once that the Mueller matrix has been measured it is possible to determine the scattering Degree of Polarization *P*:

$$P = \sqrt{\frac{1}{3}\left(\frac{Tr\left(M^T M\right)}{M_{11}^2} - 1\right)}$$

- This invariant non-dimensional parameter is bounded to the interval 0 ≤ P ≤ 1. The lower bound is reached in case of a completely depolarizing scattering process, whereas the upper bound corresponds to a non-depolarizing scattering process.

Mueller imaging

- Cancerous and healthy human colon samples have been analyzed ex-vivo using a multispectral imaging Mueller polarimeter operated in the visible (from 500 to 700 nm) in a backscattering configuration with diffuse light illumination.
- Three samples of Liberkühn colon adenocarcinomas have been studied: common, mucinous and treated by radiochemotherapy. For each sample, several specific zones have been chosen, based on their visual staging and polarimetric responses, which have been correlated to the histology of the corresponding cuts.



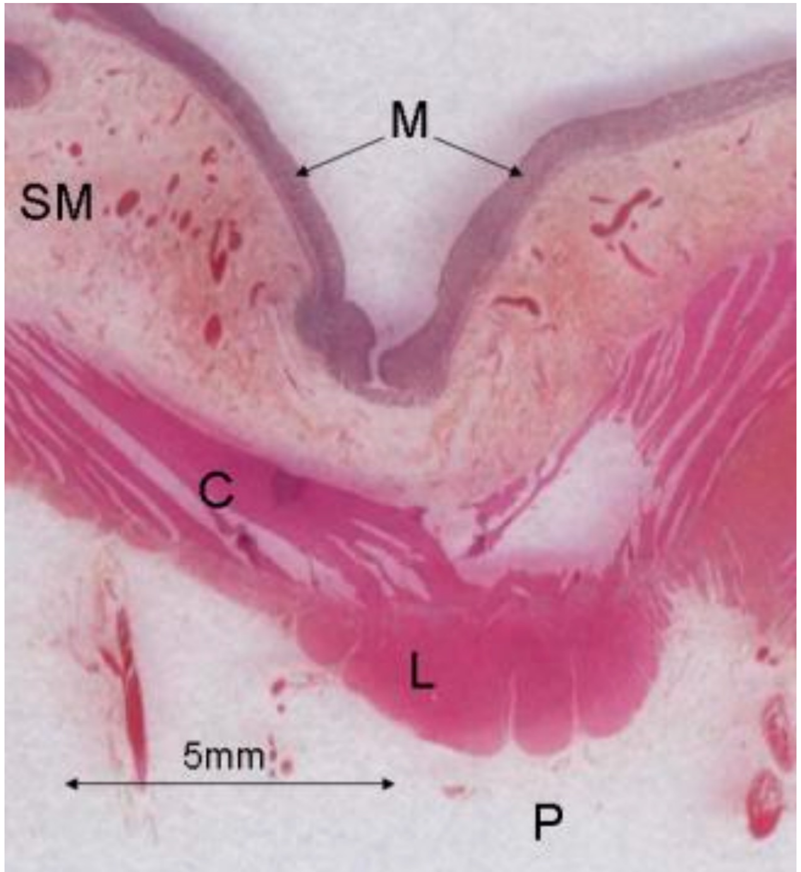
Mueller imaging

- There is an emerging interest in the applications of polarized light for biomedical diagnostics.
- Antonelli et al. demonstrated that cancerous zones at early stages of development are less depolarizing than surrounding healthy tissue for human colon - M.R.Antonelli, A.Pierangelo, T.Novikova, P.Validire, A.Benali, B.Gayet, and A.De Martino, “Mueller matrix imaging of human colon tissue for cancer diagnostics: how Monte Carlo modeling can help in the interpretation of experimental data,” Opt. Express 18(10), 10200–10208 (2010).

Colon cancer

Healthy colon tissue has an ordered microscopic structure. We can distinguish between different tissue layers, starting from the innermost layer: the mucosa, the submucosa, the muscularis externa (formed by circular muscular tissue and longitudinal muscular tissue), the pericolic tissue and the serosa.

Microscopic structure of a healthy colon sample, with its different layers: the mucosa (M), the submucosa (SM), the circular muscular tissue (C), the longitudinal muscular tissue (L) and the pericolic tissue (P).

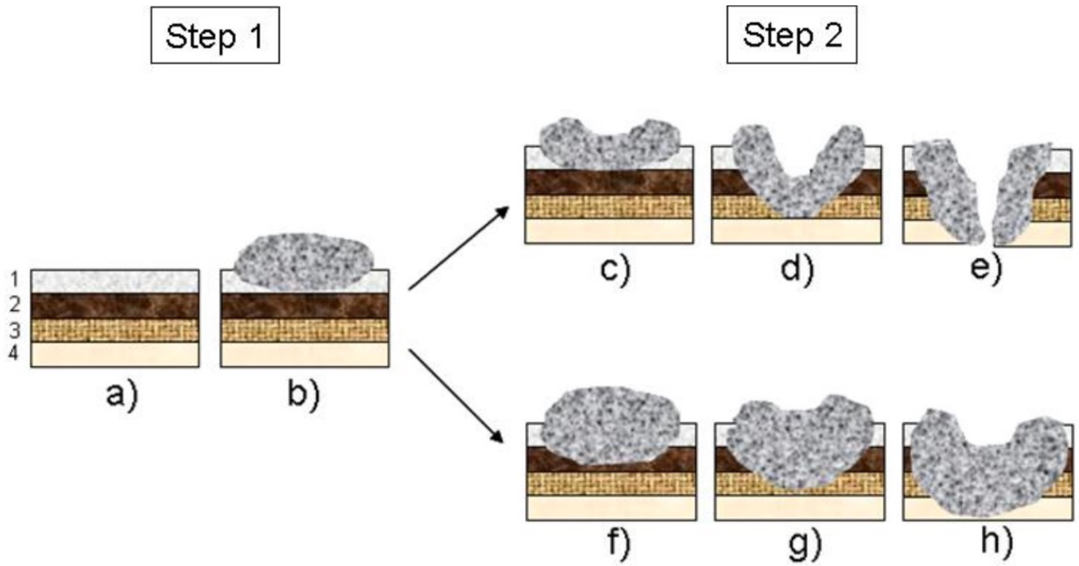


Colon cancer

The development of colon cancer can be summarized in two steps (see Figure).

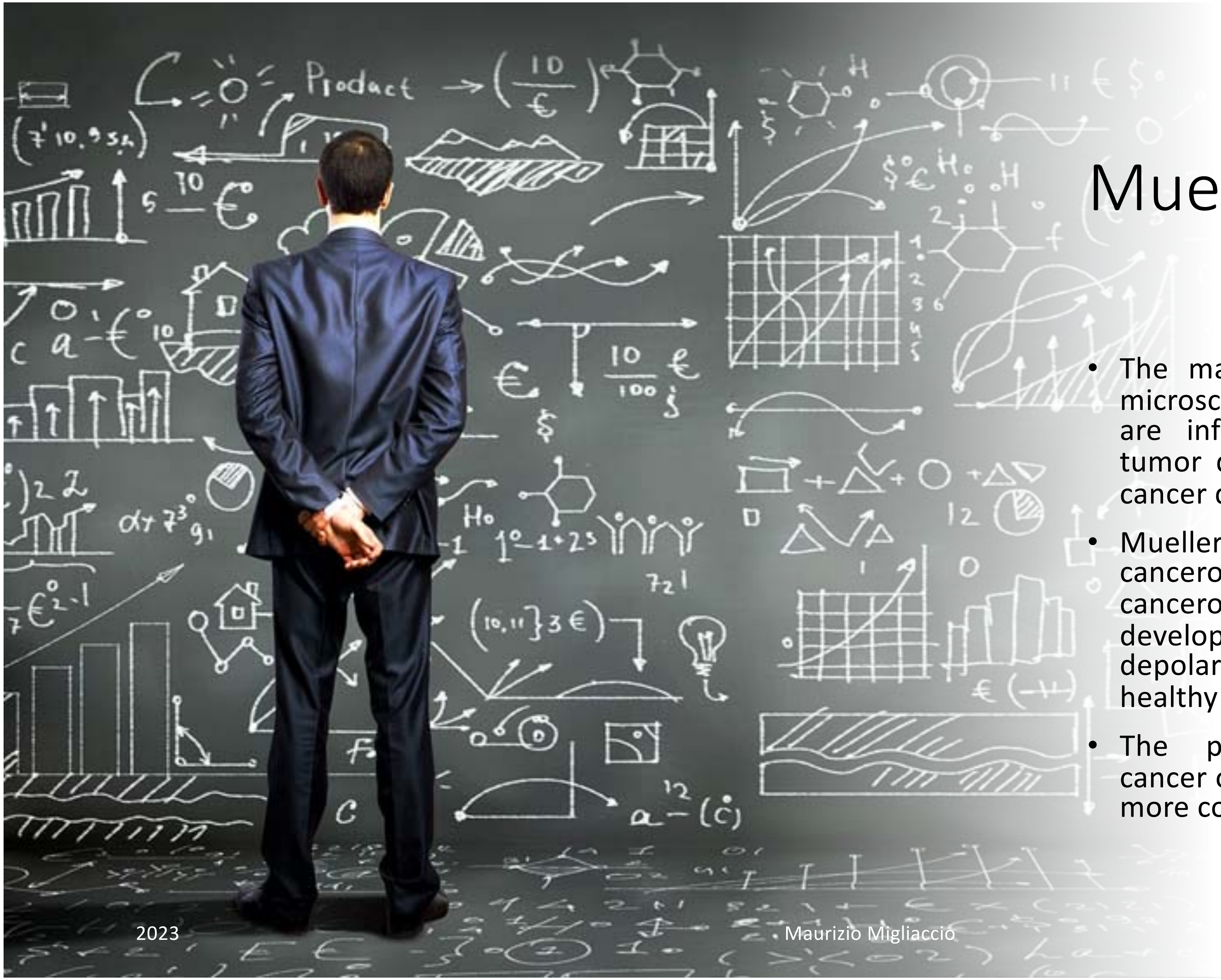
Initially an uncontrolled growth of the epithelial cells occurs, with a consequent increase of the epithelial layer thickness and an invasion of the mucosa layer down to the muscularis mucosa. This first stage of the disease is named carcinoma in situ (Tis). Usually, at this stage the cancer appears as a uniformly exophytic/budding cellular growth with predominantly intraluminal aspect (see b). The abnormal cells are confined to the mucosa layer and muscularis mucosa.

The penetration of abnormal cells in deeper colon layers begins on the second step. The lesion is named T1 when the submucosa is invaded, T2 when the abnormal cells spread into the muscularis externa and T3 when the tumor spreads into the pericolic tissue or serosa. Finally, the lesion is named T4 when cancer spreads to other organs or structures.



Colon cancer

- Generally, the tumor proliferation into deeper colon layers is accompanied by surface ulceration (a decrease of tissue thickness due to the loss of its superficial part) of the cancerous zone.
- Sometimes the ulceration and the penetration processes do not progress with the same speed.
- Histological examination of typical surgical samples show that during the second step of cancer growth a strong penetration of abnormal cells in deeper layers may occur, with either deep (see former Fig. c-e) or shallow ulceration (see former Fig. f-h) on the surface.



Mueller imaging

- The macroscopic aspects and the microscopic features of colon cancer are influenced by the phase of tumor development at the time of cancer detection.
- Mueller polarimetric imaging of cancerous colon shows that cancerous zone at the first step of development (see Fig. b) is less depolarizing than the surrounding healthy tissue.
- The polarimetric signatures of cancer on more advanced stages are more complex.

Mueller imaging

- The experimental Mueller matrices of images of the samples are essentially diagonal, where only the M_{22}, M_{33} and M_{44} do not vanish.
- Moreover, for both cancerous and healthy zones, we observe that

$$|M_{22}| = |M_{33}| > |M_{44}|$$

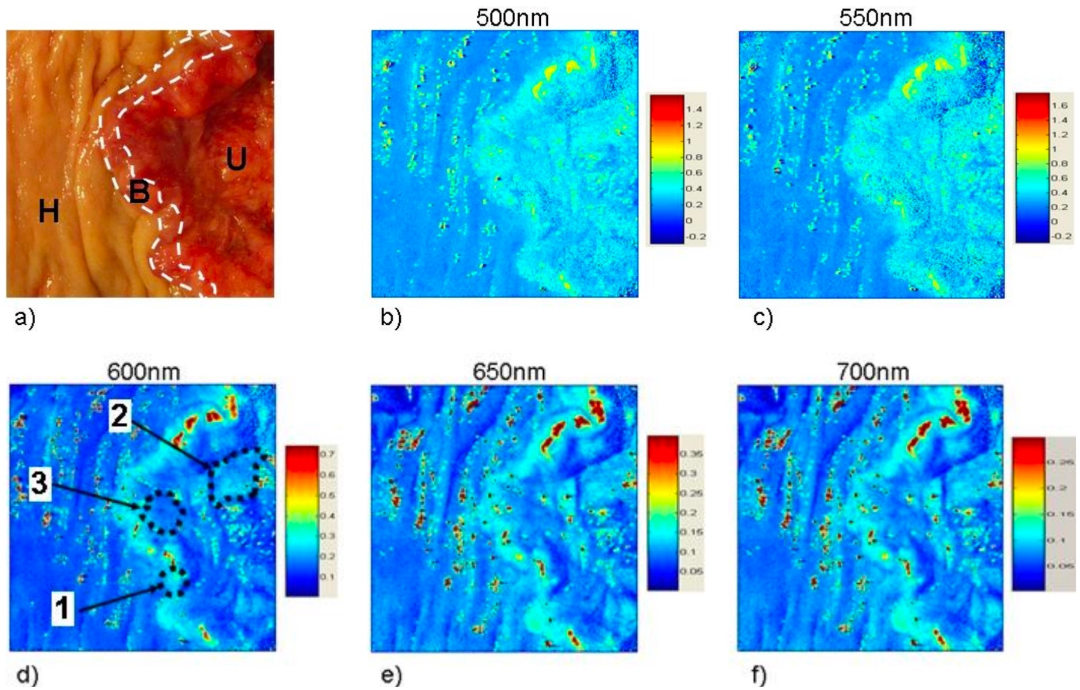
- This result indicates that the backscattered light is less depolarized when the incident light is linearly rather than circularly polarized.
- The Mueller matrix images of any given sample taken at 500, 550, 600, 650, 700 nm exhibit depolarization which increases with the wavelength.

Mueller imaging

The aside sample under study is a 60 mm diameter Lieberkühn adenocarcinoma, which occupied 90% of the colon circumference.

a) Photo of the first colon sample. H, B and U respectively identify healthy, budding and ulcerated regions. b) – f) Corresponding polarimetric images (element M_{22}) taken at different wavelengths. Depolarization increases with decrease of M_{22} values.

The values of M_{22} larger than 1 are unphysical, they are due to intense specular reflections which locally saturate the camera.

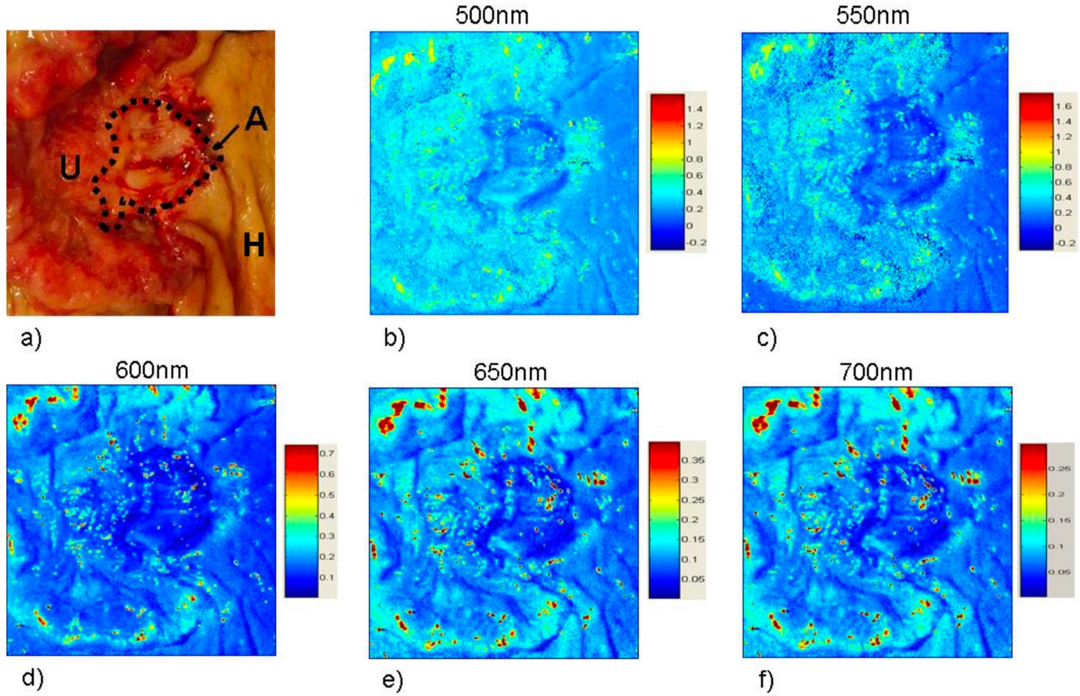


Mueller imaging

- The polarimetric images of this sample (diagonal element M_{22} of Mueller matrix) taken at different wavelengths are shown in Fig. 3b-f.
- At 500 and 550 nm both ulcerated and budding cancerous zones depolarize less than the healthy tissue.
- In contrast, at 600, 650 and 700 nm at first sight the ulcerated zone appears rather similar to the healthy tissue while the exophytic zone remains less depolarizing.
- However, careful examination of Figs. 3d-f shows that at these wavelengths the depolarization is not homogeneous in the ulcerated zone: part 3 seems almost identical to the healthy zone H while part 2 is less depolarizing and more similar to the part 1 in the exophytic zone B.

Mueller imaging

Figure 4a is a photo of the same sample, shifted to the left in the field of view. The cancerous part now covers three quarters of the image on the left side. A biopsy was taken in the part A (the region is delimited by the dotted curve), where all the layers above pericolic tissue were intentionally removed. This part is clearly more depolarizing than both the healthy and ulcerated zones for all investigated wavelengths.



Mueller imaging

- By a critical analysis supported by histological examination the study showed that the development of colon cancer is characterized by increased cellular density, modified morphological characteristics of the cells, increased vascularisation, formation of stroma and destruction of the natural order of tissue.
- The polarimetric images of a sample of common Liberkühn adenocarcinoma suggest that the polarization response in the abnormal zone is predominantly determined by the thickness of cancerous layer, and secondarily by the nature of the underlying tissues, with a significant increase in depolarization when only the serosa is left beneath a thin layer of cancerous tissue.

Lesson learnt

More details can be found in A.Pierangelo et al., “*Ex-vivo* Characterization of Human Colon Cancer by Mueller Polarimetric Imaging”, *Optics Express*, 2011.

Mueller imaging

- Healthy colon possesses an ordered and complex microscopic structure. The polarimetric response of healthy colon is the sum of contributions of its constituent layers (mucosa, submucosa, muscularis externa, pericolic tissue and serosa).
- The proliferation of cancer destroys this natural order by an uncontrolled cellular growth, an increase of cellular density, morphological and biochemical mutations of the cells with possible secretion of mucus and development of an inter-cellular substance (stroma) which supports the cancerous cells growth.

Mueller imaging

- The interaction of polarized light with cancerous and healthy zones is very different.
- The results of ex-vivo measurements of three surgical colon samples performed with a multi-spectral Mueller matrix imaging polarimeter show that the polarimetric signature of the sample is determined by the cellular density, the thickness of the cancerous layer, the degree of surface ulceration and the light penetration depth.
- When the thickness of the cancerous layer is large enough, the light interacts predominantly with this layer at all studied wavelengths.
- When its thickness is smaller, the light (with wavelength 600 nm and longer) penetrates deeper and interacts also with the healthy underlying layers.

Mueller imaging

- Though still preliminary, our data show that multi-spectral Mueller matrix imaging polarimetry may provide enhanced contrasts to differentiate types of cancer (common and mucinous) and their stage of advancement and penetration, which are normally visible only with histological examination.
- Moreover, this technique may also be useful to quickly verify the presence of residual cancer in colon samples treated using radiochemotherapy.
- Of course, this “optical biopsy” is not likely to replace classical histology, but it may provide useful information to better manage the choice of the cut placements to be studied in more detail and thus improve the overall efficiency of the work of pathologists.

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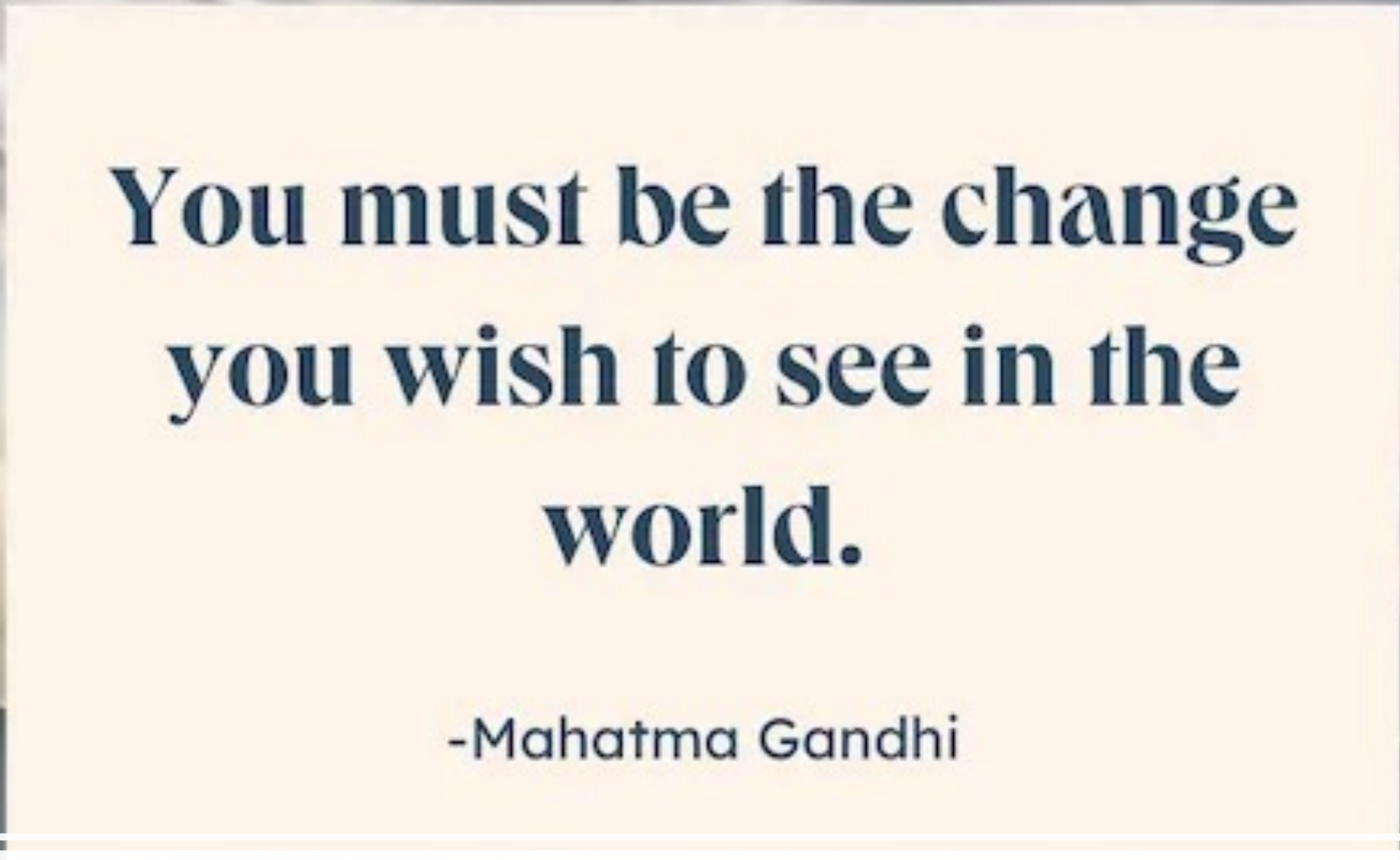

Conclusions

Innovons ensemble au service du vivant

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