DOWNLOAD PDF SPECTROSCOPY WITH POLARIZED LIGHT

Chapter 1 : Polarized Raman spectroscopy with differing angles of laser incidence on single-layer graphen

The first book on polarized optical spectroscopy of anisotropic molecules and molecular assemblies is now available in a corrected paperback edition. This work provides a comprehensive study of spectroscopy with polarized light on samples with varying degrees of anisotropy such as liquid crystals, polymers, and membranes.

Introduction to Polarized Light Introduction to Polarized Light Sunlight and almost every other form of natural and artificial illumination produces light waves whose electric field vectors vibrate in all planes that are perpendicular with respect to the direction of propagation. Figure 1 - Polarization of Light The human eye lacks the ability to distinguish between randomly oriented and polarized light, and plane-polarized light can only be detected through an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses. In effect, humans cannot differentiate between the high contrast real images observed in a polarized light microscope and identical images of the same specimens captured digitally or on film , and then projected onto a screen with light that is not polarized. The basic concept of polarized light is illustrated in Figure 1 for a non-polarized beam of light incident on two linear polarizers. Electric field vectors are depicted in the incident light beam as sinusoidal waves vibrating in all directions degrees; although only six waves, spaced at degree intervals, are included in the figure. In reality, the incident light electric field vectors are vibrating perpendicular to the direction of propagation with an equal distribution in all planes before encountering the first polarizer. The polarizers illustrated in Figure 1 are actually filters containing long-chain polymer molecules that are oriented in a single direction. Only the incident light that is vibrating in the same plane as the oriented polymer molecules is absorbed, while light vibrating at right angles to the polymer plane is passed through the first polarizing filter. The polarizing direction of the first polarizer is oriented vertically to the incident beam so it will pass only the waves having vertical electric field vectors. The wave passing through the first polarizer is subsequently blocked by the second polarizer, because this polarizer is oriented horizontally with respect to the electric field vector in the light wave. The first clues to the existence of polarized light surfaced around when Erasmus Bartholin discovered that crystals of the mineral Iceland spar a transparent, colorless variety of calcite produce a double image when objects are viewed through the crystals in transmitted light. During his experiments, Bartholin also observed a quite unusual phenomenon. When the calcite crystals are rotated about a particular axis, one of the images moves in a circle around the other, providing strong evidence that the crystals are somehow splitting the light into two different beams. Figure 2 - Bi-Refraction in Calcite Crystals Over a century later, French physicist Etienne Malus examined images made with light reflected through calcite crystals and noticed that, under certain circumstances, one of the images will disappear. He incorrectly speculated that ordinary daylight is composed of two different light forms that were passed through the calcite crystal in separate paths. It was later determined that the difference occurs due to the polarity of the light passing through the crystal. Daylight is composed of light vibrating in all planes, whereas reflected light is often restricted to a single plane that is parallel to the surface from which the light is reflected. Polarized light can be produced from the common physical processes that deviate light beams, including absorption, refraction, reflection, diffraction or scattering , and the process known as birefringence the property of double refraction. Light that is reflected from the flat surface of a dielectric or insulating material is often partially polarized, with the electric vectors of the reflected light vibrating in a plane that is parallel to the surface of the material. Common examples of surfaces that reflect polarized light are undisturbed water, glass, sheet plastics, and highways. In these instances, light waves that have the electric field vectors parallel to the surface are reflected to a greater degree than those with different orientations. The optical properties of the insulating surface determine the exact amount of reflected light that is polarized. Mirrors are not good polarizers, although a wide spectrum of transparent materials act as very good polarizers, but only if the incident light angle is oriented within certain limits. An important property of reflected polarized light is that the degree of polarization is dependent upon the incident angle of the light, with the increasing amounts of polarization being observed for decreasing incident angles. When considering the incidence of non-polarized light on a flat insulating surface, there is a unique angle at which the reflected light waves are all polarized into a single plane. By examining the equation, it becomes obvious that the refractive index of an unknown specimen can be determined by the Brewster angle. Determining the amount of polarization through reflection techniques also eases the search for the polarizing axis on a sheet of polarizing film that is not marked. The incident ray is drawn with only two electric vector vibration planes, but is intended to represent light having vibrations in all planes perpendicular to the direction of propagation. The incidence plane is defined by the incident, refracted, and reflected waves. The refracted ray is oriented at a degree angle from the reflected ray and is only partially polarized. For water refractive index of 1. Light reflected from a highway surface at the Brewster angle often produces annoying and distracting glare, which can be demonstrated quite easily by viewing the distant part of a highway or the surface of a swimming pool on a hot, sunny day. As discussed above, bright reflections originating from horizontal surfaces, such as the highway or the water in a pool, are partially polarized with the electric field vectors vibrating in a direction that is parallel to the ground. This light can be blocked by polarizing filters oriented in a vertical direction, as illustrated in Figure 4, with a pair of polarized sunglasses. The lenses of the sunglasses have polarizing filters that are oriented vertically with respect to the frames. In the figure, the blue light waves have their electric field vectors oriented in the same direction as the polarizing lenses and, thus, are passed through. In contrast, the red light wave vibration orientation is perpendicular to the filter orientation and is blocked by the lenses. Polarizing sunglasses are very useful when driving in the sun or at the beach where sunlight is reflected from the surface of the road or water, leading to glare that can be almost blinding. Polarizing filters are also quite useful in photography, where they can be attached to the front of a camera lens to reduce glare and increase overall image contrast in photographs or digital images. Polarizers utilized on cameras are generally designed with a mounting ring that allows them to be rotated in use to achieve the desired effect under various lighting conditions. Arago investigated the polarity of light originating from various sources in the sky and proposed a theory that predicted the velocity of light should decrease as it passes into a denser medium. He also worked with Augustin Fresnel to investigate interference in polarized light and discovered that two beams of light polarized with their vibration directions oriented perpendicular to each other will not undergo interference. A majority of the polarizing materials used today are derived from synthetic films invented by Dr. Land in , which soon overtook all other materials as the medium of choice for production of plane-polarized light. To produce the films, tiny crystallites of iodoquinine sulfate, oriented in the same direction, are embedded in a transparent polymeric film to prevent migration and reorientation of the crystals. Land developed sheets containing polarizing films that are marketed under the trade name of Polaroid a registered trademark , which has become the accepted generic term for these sheets. Any device capable of selecting plane-polarized light from natural non-polarized white light is now referred to as a polar or polarizer, a name first introduced in by A. Because these filters are capable of differentially transmitting light rays, depending upon their orientation with respect to the polarizer axis, they exhibit a form of dichroism, and are often termed dichroic filters. Polarized light microscopy was first introduced during the nineteenth century, but instead of employing transmission-polarizing materials, light was polarized by reflection from a stack of glass plates set at a degree angle to the plane of incidence. Later, more advanced instruments relied on a crystal of doubly refracting material such as calcite specially cut and cemented together to form a prism. A beam of white non-polarized light entering a crystal of this type is separated into two components that are polarized in mutually perpendicular orthogonal directions. One of the light rays emerging from a birefringent crystal is termed the ordinary ray, while the other is called the extraordinary ray. The ordinary ray is refracted to a greater degree by electrostatic forces in the crystal and impacts the cemented surface at the critical angle of total internal reflection. As a result, this ray is reflected out of the prism and eliminated by absorption in the optical mount. The extraordinary ray traverses the prism and emerges as a beam of linearly-polarized light that is passed directly through the condenser and to the specimen positioned on the microscope stage. Several versions of prism-based polarizing devices were once widely available, and these were usually named after their designers. The most common polarizing prism illustrated in Figure 5 was named after William Nicol, who first cleaved and cemented together two crystals of Iceland spar with Canada balsam in Nicol prisms were first used to measure the polarization angle of birefringent compounds, leading to new developments in the understanding of interactions between polarized light and crystalline substances. Figure 5 - Nicole Polarizing Prism Presented in Figure 5 is an illustration of the construction of a typical Nicol prism. A crystal of doubly refracting birefringent material, usually calcite, is cut along the plane labeled a-b-c-dand the two halves are then cemented together to reproduce the original crystal shape. A beam of non-polarized white light enters the crystal from the left and is split into two components that are polarized in mutually perpendicular directions. One of these beams labeled the ordinary ray is refracted to a greater degree and impacts the cemented boundary at an angle that results in its total reflection out of the prism through the uppermost crystal face. The other beam extraordinary ray is refracted to a lesser degree and passes through the prism to exit as a plane-polarized beam of light. Other prism configurations were suggested and constructed during the nineteenth and early twentieth centuries, but are currently no longer utilized for producing polarized light in modern applications. Nicol prisms are very expensive and bulky, and have a very limited aperture, which restricts their use at high magnifications. Instead, polarized light is now most commonly produced by absorption of light having a set of specific vibration directions in a filter medium such as polarizing sheets where the transmission axis of the filter is perpendicular to the orientation of the linear polymers and crystals that comprise the polarizing material. In modern polarizers, incident light waves having electric vector vibrations that are parallel to the crystal axis of the polarizer are absorbed. Many of the incident waves will have a vector orientation that is oblique, but not perpendicular to the crystal axis, and will only be partially absorbed. The degree of absorption for oblique light waves is dependent upon the vibration angle at which they impact the polarizer. Those rays that have angles close to parallel with respect to the crystal axis will be adsorbed to a much greater degree than those having angles close to the perpendicular. The most common Polaroid filters termed the H-series transmit only about 25 percent of the incident light beam, but the degree of polarization of the transmitted rays exceeds 99 percent. A number of applications, most notably polarized optical microscopy, rely on crossed polarizers to examine birefringent or doubly refracting specimens. When two polarizers are crossed, their transmission axes are oriented perpendicular to each other and light passing through the first polarizer is completely extinguished, or absorbed, by the second polarizer, which is typically termed an analyzer. The light-absorbing quality of a dichroic polarizing filter determines exactly how much random light is extinguished when the polarizer is utilized in a crossed pair, and is referred to as the extinction factor of the polarizer. Quantitatively, the extinction factor is determined by the ratio of light that is passed by a pair of polarizers when their transmission axes are oriented parallel versus the amount passed when they are positioned perpendicular to each other. In general, extinction factors between 10, and , are required to produce jet-black backgrounds and maximum observable specimen birefringence and contrast in polarized optical microscopy. Figure 6 - Transmission of Polarized Light Through an Analyzer The amount of light passing through a crossed pair of high-quality polarizers is determined by the orientation of the analyzer with respect to the polarizer. When the polarizers are oriented perpendicular to each other, they display a maximum level of extinction. However, at other angles, varying degrees of extinction are obtained, as illustrated by the vector diagrams presented in Figure 6. The analyzer is utilized to control the amount of light passing through the crossed pair, and can be rotated in the light path to enable various amplitudes of polarized light to pass through. In Figure 6 a , the polarizer and analyzer have parallel transmission axes and the electric vectors of light passing through the polarizer and analyzer are of equal magnitude and parallel to each other. Rotating the analyzer transmission axis by degrees with respect to that of the polarizer reduces the amplitude of a light wave passing through the pair, as illustrated in Figure 6 b. In this case, the polarized light transmitted through the polarizer can be resolved into horizontal and vertical components by vector mathematics to determine the amplitude of polarized light that is able to pass through the analyzer. The amplitude of the ray transmitted through the analyzer is equal to the vertical vector component illustrated as the yellow arrow in Figure 6 b. Continued rotation of the analyzer transmission axis, to a degree angle with respect to the transmission axis of the polarizer, further reduces the magnitude of the vector component that is transmitted through the analyzer Figure 6 c. When the analyzer and polarizer are completely crossed degree angle , the vertical component becomes negligible Figure 6 d and the polarizers have achieved their maximum extinction value. In this case, light passed by the polarizer is completely extinguished by the analyzer. When the polarizers are partially crossed at 30 and 60 degrees, the light transmitted by the analyzer is reduced by 25 percent and 75 percent, respectively. Polarization of Scattered Light Gas and water molecules in the atmosphere scatter light from the sun in all directions, an effect that is responsible for blue skies, white clouds, red sunsets, and a phenomenon termed atmospheric polarization. The amount of light scattered termed Rayleigh scattering depends upon the size of the molecules hydrogen, oxygen, water and the wavelength of light, as demonstrated by Lord Rayleigh in Longer wavelengths, such as red, orange, and yellow, are not scattered as effectively as are the shorter wavelengths, such as violet and blue. Figure 7 - Polarization of Scattered Sunlight Atmospheric polarization is a direct result of the Rayleigh scattering of sunlight by gas molecules in the atmosphere. Upon impact between a photon from the sun and a gas molecule, the electric field from the photon induces a vibration and subsequent re-radiation of polarized light from the molecule illustrated in Figure 7. The radiated light is scattered at right angles to the direction of sunlight propagation, and is polarized either vertically or horizontally, depending upon the direction of scatter. A majority of the polarized light impacting the Earth is polarized horizontally over 50 percent , a fact that can be confirmed by viewing the sky through a Polaroid filter. Reports have surfaced that certain species of insects and animals are able to detect polarized light, including ants, fruit flies, and certain fish, although the list may actually be much longer. For example, several insect species primarily honeybees are thought to employ polarized light in navigating to their destinations. Yellow pigment proteins, termed macula lutea, which are dichroic crystals residing in the fovea of the human eye, are credited with enabling a person to view polarized light. Elliptically and Circularly Polarized Light In linearly polarized light, the electric vector is vibrating in a plane that is perpendicular to the direction of propagation, as discussed above.

Chapter 2 : Circular Dichroism - Chemistry LibreTexts

Different analytical techniques are introduced and compared and introductions to the use of polarized light in various forms of spectroscopy are provided. Show less This comprehensive introduction to polarized light provides students and researchers with the background and the specialized knowledge needed to fully utilize polarized light.

Circular Dichroism CD Spectroscopy Circular Dichroism CD is observed when optically active matter absorbs left and right hand circular polarized light slightly differently. The instrument needs to be able to measure accurately in the far UV at wavelengths down to nm. In addition, the difference in left and right handed absorbance A l - A r is very small usually in the range of 0. The CD is a function of wavelength. CD spectra for distinct types of secondary structure present in peptides, proteins and nucleic acids are different. The analysis of CD spectra can therefore yield valuable information about secondary structure of biological macromolecules. See reference [1] for a review. Physics of CD and ORD Linear polarized light can be viewed as a superposition of opposite circular polarized light of equal amplitude and phase. A projection of the combined amplitudes perpendicular to the propagation direction thus yields a line figure 1a. When this light passes through an optically active sample with a different absorbance A for the two components, the amplitude of the stronger absorbed component will smaller than that of the less absorbed component. The consequence is that a projection of the resulting amplitude now yields an ellipse instead of the usual line draw on a sheet of paper and check. Note that the polarization direction has not changed. The occurrence of ellipticity is called Circular Dichroism - it is not the same as optical rotation. Rotation of the polarization plane or the axes of the dichroic ellipse by a small angle a occurs when the phases for the 2 circular components become different, which requires a difference in the refractive index n. This effect is called circular birefringence. The change of optical rotation with wavelength is called optical rotary dispersion, ORD. For more details on the physics see reference [3]. Figure 1 a Linear polarized light can be viewed as a superposition of opposite circular polarized light of equal amplitude and phase. The actual effect is minute and using actual numbers the ellipse would still resemble a line. We have a Jasco model with a temperature controller and sample changer for rapid denaturation studies. The air cooled W Xenon lamp does not necessitate water cooling, and the whole optics design and the piezo-mechanic modulator are a great advantage over the old, floor-space space hogging Cary with its Pockels-cells. You still need to purge with ample nitrogen to get to lower wavelengths below nm. Jasco with Windows 95 data acquisition and processing computer. The annoying character blocking the view is graduate student Chris Barry. Most of the Carys are upgraded in some way. Any compound which absorbs in the region of interest - nm should be avoided. A buffer or detergent or other chemical should not be used unless it can be shown that the compound in question will not mask the protein signal. For instance imidazole cannot be used below nm because it overwhelms the spectrum from then on. Therefore ensure that only the minimum concentration of additives are present in the protein solution. From the above follows that the protein solution should contain only those chemicals necessary to maintain protein stability, and at the lowest concentrations possible. The protein itself should be as pure as possible, any additional protein or peptide will contribute to the CD signal. Unfolded protein, peptides, particulate matter scattering particles , anything that adds significant noise or artifical signal contributions to the CD spectrum must be avoided. Filtering of the solutions 0. Initial experiments are useful to establish the best conditions for the "real" experiment. Changing this has a profound effect on the data, so small increments or decrements are called for. If that does not produce reasonably good data, a change in buffer composition may be necessary. It would also be a good idea to check the sample for unforeseen aggregation via Dynamic Light Scattering DNA repair enzymes are an especially good example of this behavior. If absorption poses a problem, cells with shorter path 0. CD data analysis As mentioned in the introduction, the difference in absorption to be measured is very small. The raw data plotted on the chart recorder represent the ellipticity of the sample in radians which can be easily converted into degrees To be able to compare these ellipticity values we need to convert into a normalized value. The unit most commonly used in protein and peptide work is the mean molar ellipticity per residue. We need to consider path length l, concentration c , molecular weight M and number of residues in proper units

CD spectroscopists use decimol which finally reduces to The values for mean molar ellipticity per residue are usually in the As we mentioned already, each of the three basic secondary structures of a polypeptide chain helix, sheet, coil show a characteristic CD spectrum. A protein consisting of these elements should therefore display a spectrum that can be deconvoluted into the tree individual contributions. This has been realized quite early after CD was introduced and the standard curves shown to the right were published in by Greenfield and Fasman [2]. Although those are actually for poly-lysine only in different conformations, only little improvement in the accuracy of fits has been achieved by attempting to generate other standard data sets from protein spectra of known structure [5]. There are many limitations inherent in the method such as the lack of consideration of chromophore interaction between different structural regions and neglect of other elements, helices etc. The method is, however, very reliable for monitoring changes in the conformation of proteins under different conditions denaturation studies, unfolding experiments etc, helix induction by TFE [4], see poly-glutamine example. Certain backbone conformations can reveal quite different spectra - click highlight for an example of an extended sheet of poly-Q. Note that the results can be different depending on the region of fit [1] - a clear indication that such fits must be treated with care. Rupp, CD raw data file: RES Standard data file:

Chapter 3 : Raman spectroscopy - Wikipedia

Polarized light is used in electronic spectroscopy not only to obtain information about the positions. Intensities, and fine structures of absorption and luminescence bands, which can be obtained.

Applications[edit] Raman spectroscopy is used in chemistry to identify molecules and study chemical bonding and intramolecular bonds. In solid-state physics , Raman spectroscopy is used to characterize materials, measure temperature , and find the crystallographic orientation of a sample. As with single molecules, a solid material can be identified by characteristic phonon modes. Information on the population of a phonon mode is given by the ratio of the Stokes and anti-Stokes intensity of the spontaneous Raman signal. Raman spectroscopy can also be used to observe other low frequency excitations of a solid, such as plasmons , magnons , and superconducting gap excitations. Distributed temperature sensing DTS uses the Raman-shifted backscatter from laser pulses to determine the temperature along optical fibers. In nanotechnology, a Raman microscope can be used to analyze nanowires to better understand their structures, and the radial breathing mode of carbon nanotubes is commonly used to evaluate their diameter. Raman active fibers, such as aramid and carbon, have vibrational modes that show a shift in Raman frequency with applied stress. Polypropylene fibers exhibit similar shifts. In solid state chemistry and the bio-pharmaceutical industry, Raman spectroscopy can be used to not only identify active pharmaceutical ingredients APIs , but to identify their polymorphic forms, if more than one exist. For example, the drug Cayston aztreonam , marketed by Gilead Sciences for cystic fibrosis , [10] can be identified and characterized by IR and Raman spectroscopy. Using the correct polymorphic form in bio-pharmaceutical formulations is critical, since different forms have different physical properties, like solubility and melting point. Raman spectroscopy has a wide variety of applications in biology and medicine. It has helped confirm the existence of low-frequency phonons [11] in proteins and DNA, [12] [13] [14] [15] promoting studies of low-frequency collective motion in proteins and DNA and their biological functions. Multivariate analysis of Raman spectra has enabled development of a quantitative measure for wound healing progress. This is a large advantage, specifically in biological applications. Raman spectroscopy is an efficient and non-destructive way to investigate works of art. It also gives information about the original state of the painting in cases where the pigments degraded with age. Raman spectroscopy has been used in several research projects as a means to detect explosives from a safe distance using laser beams. Raman4Clinic is a European organization that is working on incorporating Raman Spectroscopy techniques in the medical field. They are currently working on different projects, one of them being monitoring cancer using bodily fluids such as urine and blood samples which are easily accessible. This technique would be less stressful on the patients than constantly having to take biopsies which are not always risk free. Please help improve this article by adding citations to reliable sources. Unsourced material may be challenged and removed. Since it is a scattering technique, specimens do not need to be fixed or sectioned. Water does not generally interfere with Raman spectral analysis. Thus, Raman spectroscopy is suitable for the microscopic examination of minerals , materials such as polymers and ceramics, cells , proteins and forensic trace evidence. A Raman microscope begins with a standard optical microscope, and adds an excitation laser, a monochromator , and a sensitive detector such as a charge-coupled device CCD , or photomultiplier tube PMT. FT-Raman has also been used with microscopes. Ultraviolet microscopes and UV enhanced optics must be used when a UV laser source is used for Raman microspectroscopy. In direct imaging, the whole field of view is examined for scattering over a small range of wavenumbers Raman shifts. For instance, a wavenumber characteristic for cholesterol could be used to record the distribution of cholesterol within a cell culture. The other approach is hyperspectral imaging or chemical imaging , in which thousands of Raman spectra are acquired from all over the field of view. The data can then be used to generate images showing the location and amount of different components. Taking the cell culture example, a hyperspectral image could show the distribution of cholesterol, as well as proteins, nucleic acids, and fatty acids. Sophisticated signal- and image-processing techniques can be used to ignore the presence of water, culture media, buffers, and other interference. Raman microscopy, and in particular confocal microscopy , has very high spatial resolution.

Since the objective lenses of microscopes focus the laser beam to several micrometres in diameter, the resulting photon flux is much higher than achieved in conventional Raman setups. This has the added benefit of enhanced fluorescence quenching. However, the high photon flux can also cause sample degradation, and for this reason some setups require a thermally conducting substrate which acts as a heat sink in order to mitigate this process. Another approach called global Raman imaging [31] uses complete monochromatic images instead of reconstruction of images from acquired spectra. This technique is being used for the characterization of large scale devices, mapping of different compounds and dynamics study. It has already been use for the characterization of graphene layers, [32] J-aggregated dyes inside carbon nanotubes [33] and multiple other 2D materials such as MoS2 and WSe2. Since the excitation beam is dispersed over the whole field of view, those measurements can be done without damaging the sample. By using Raman microspectroscopy, in vivo time- and space-resolved Raman spectra of microscopic regions of samples can be measured. Sampling is non-destructive and water, media, and buffers typically do not interfere with the analysis. Consequently, in vivo time- and space-resolved Raman spectroscopy is suitable to examine proteins , cells and organs. In the field of microbiology, confocal Raman microspectroscopy has been used to map intracellular distributions of macromolecules, such as proteins, polysaccharides, and nucleic acids and polymeric inclusions, such as poly-B-hydroxybutyric acid and polyphosphates in bacteria and sterols in microalgae. Combining stable isotopic probing SIP experiments with confocal Raman microspectroscopy has permitted determination of assimilation rates of 13C and 15N-substrates as well as D2O by individual bacterial cells [34]. Using confocal Raman microspectroscopy essentially as a single-cell mass spectrometer is enabled by the fact that the vibrational frequency of any molecular bonds is a function of the masses of the bound atoms. Thus, incorporation of heavy isotopes will cause quantitative "red shifts" in diagnostic Raman peaks. YAG are especially common. The use of these lower energy wavelengths reduces the risk of damaging the specimen. Recently advances were made which had no destructive effect on mitochondria in the observation of changes in cytochrome c structure that occur in the process of electron transport and ATP synthesis. Raman microscopy of inorganic specimens, such as rocks and ceramics and polymers, can use a broader range of excitation wavelengths. While conventional Raman spectroscopy identifies chemical composition, polarization effects on Raman spectra can reveal information on the orientation of molecules in single crystals and anisotropic materials, e. Polarizationâ€"dependent Raman spectroscopy uses plane polarized laser excitation from a polarizer. The Raman scattered light collected is passed through a second polarizer called the analyzer before entering the detector. The analyzer is oriented either parallel or perpendicular to the polarization of the laser. Spectra acquired with the analyzer set at both perpendicular and parallel to the excitation plane can be used to calculate the depolarization ratio. Typically a polarization scrambler is placed between the analyzer and detector also. For isotropic solutions, the Raman scattering from each mode either retains the polarization of the laser or becomes partly or fully depolarized. If the vibrational mode involved in the Raman scattering process is totally symmetric then the polarization of the Raman scattering will be the same as that of the incoming laser beam. In the case that the vibrational mode is not totally symmetric then the polarization will be lost scrambled partially or totally, which is referred to as depolarization. Hence polarized Raman spectroscopy can provide detailed information as to the symmetry labels of vibrational modes. In the solid state, polarized Raman spectroscopy can be useful in the study of oriented samples such as single crystals. The polarizability of a vibrational mode is not equal along and across the bond. Therefore the intensity of the Raman scattering will be different when the lasers polarization is along and orthogonal to a particular bonds axis. This effect can provide information on the orientation of molecules with a single crystal or material. The spectral information arising from this analysis is often used to understand macromolecular orientation in crystal lattices, liquid crystals or polymer samples. Each mode is separated according to its symmetry.

Chapter 4 : Circular Dichroism Spectroscopy

For example, chapter 6 deals with the physics of spectroscopy and polarized light, chapter 7 deals with orientation and photoselection effects, and chapter 8 (the last chapter) covers polarized light in condensed phases.

Circular Dichroism, an absorption spectroscopy, uses circularly polarized light to investigate structural aspects of optically active chiral media. It is mostly used to study biological molecules, their structure, and interactions with metals and other molecules. Introduction Circular Dichroism CD is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light. Optically active chiral molecules will preferentially absorb one direction of the circularly polarized light. The difference in absorption of the left and right circularly polarized light can be measured and quantified. UV CD is used to determine aspects of protein secondary structure. Circular Polarization of Light Electromagnetic radiation consists of oscillating electric and magnetic fields perpendicular to each other and the direction of propagation. Most light sources emit waves where these fields oscillate in all directions perpendicular to the propagation vector. Linear polarized light occurs when the electric field vector oscillates in only one plane. In circularly polarized light, the electric field vector rotates around the propagation axis maintaining a constant magnitude. When looked at down the axis of propagation the vector appears to trace a circle over the period of one wave frequency one full rotation occurs in the distance equal to the wavelength. In linear polarized light the direction of the vector stays constant and the magnitude oscillates. In circularly polarized light the magnitude stays constant while the direction oscillates. Diagram of linearly polarized and circularly polarized light As the radiation propagates the electric field vector traces out a helix. The magnetic field vector is out of phase with the electric field vector by a quarter turn. When traced together the vectors form a double helix. Light can be circularly polarized in two directions: If the vector rotates counterclockwise when the observer looks down the axis of propagation, the light is left circularly polarized LCP. If it rotates clockwise, it is right circularly polarized RCP. If LCP and RCP of the same amplitude, they are superimposed on one another and the resulting wave will be linearly polarized. The superposition of LCP and RCP light of the same amplitude produces linearly polarized light Interaction with Matter As with linear polarized light, circularly polarized light can be absorbed by a medium. Any absorption of light results in a change in amplitude of the incident wave; absorption changes the intensity of the light and intensity of the square of the amplitude. In a chiral medium the molar absorptivities of LCP and RCP light are different so they will be absorbed by the medium in different amounts. This differential absorption results in the LCP and RCP having different amplitudes which means the superimposed light is no longer linearly polarized. The resulting wave is elliptically polarized. On the left the two circular waves red and green have the same amplitude which produces linearly polarized light blue. On the right the LCP red has a larger amplitude than the RCP green , the superposition of the two waves blue forms an ellipse. Applications Instrumentation Most commercial CD instruments are based on the modulation techniques introduced by Grosjean and Legrand. Light is linearly polarized and passed through a monochromator. The single wavelength light is then passed through a modulating device, usually a photoelastic modulator PEM , which transforms the linear light to circular polarized light. As the incident light swtches direction of polarization the absorption changes and the differention molar absorptivity can be calculated. Biological molecules The most widely used application of CD spectroscopy is identifying structural aspects of proteins and DNA. The peptide bonds in proteins are optically active and the ellipticity they exhibit changes based on the local conformation of the molecule. Secondary structures of proteins can be analyzed using the far-UV nm region of light. These unique spectra form the basis for protein secondary structure analysis. It should be noted that in CD only the relative fractions of residues in each conformation can be determined but not specifically where each structural feature lies in the molecule. In reporting CD data for large biomolecules it is necessary to convert the data into a normalized value that is independent of molecular length. To do this the molar ellipticity is divided by the number of residues or monomer units in the molecule. The real value in CD comes from the ability to show conformational changes in molecules. It can be used to determine how similar a wild type protein is to mutant or show the extent of denaturation with a change in temperature or chemical environment. It can also provide information about structural changes upon ligand binding. In order to interpret any of this information the spectrum of the native conformation must be determined. Some information about the tertiary structure of proteins can be determined using near-UV spectroscopy. Absorptions between nm are due to the dipole orientation and surrounding environment of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, and cysteine residues which can form disulfide bonds. Near-UV techniques can also be used to provide structural information about the binding of prosthetic groups in proteins. Metal containing proteins can be studied by visible CD spectroscopy. Visible CD light excites the d-d transitions of metals in chiral environments. Free ions in solution will not absorb CD light so the pH dependence of the metal binding and the stoichiometry can be determined. VCD is still a relatively new technique and has the potential to be a very powerful tool. Resolving the spectra requires extensive ab initio calculations, as well as, high concentrations and must be performed in water, which may force the molecule into a nonnative conformation. Methods in Enzymology , Protein secondary structure and circular dichroism: Polarisation modulation-the measurement of linear and circular dichroism. Journal of Physics E: Kinetic and spectroscopic studies of NC lipoxygenase:

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Chapter 5 : Introduction to Polarized Light | MicroscopyU

This book deals with polarized optical spectroscopy of partially oriented fluid or rigid solutions. Starting from elementary concepts and relying on numerous illustrations, it provides an.

Polarized Light Microscopy Polarized Light Microscopy Polarized light is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials when compared to other techniques such as darkfield and brightfield illumination, differential interference contrast, phase contrast, Hoffman modulation contrast, and fluorescence. Polarized light microscopes have a high degree of sensitivity and can be utilized for both quantitative and qualitative studies targeted at a wide range of anisotropic specimens. Qualitative polarizing microscopy is very popular in practice, with numerous volumes dedicated to the subject. In contrast, the quantitative aspects of polarized light microscopy, which is primarily employed in crystallography, represent a far more difficult subject that is usually restricted to geologists, mineralogists, and chemists. However, steady advances made over the past few years have enabled biologists to study the birefringent character of many anisotropic sub-cellular assemblies. Figure 1 - Polarized Light Microscope Configuration The polarized light microscope is designed to observe and photograph specimens that are visible primarily due to their optically anisotropic character. In order to accomplish this task, the microscope must be equipped with both a polarizer, positioned in the light path somewhere before the specimen, and an analyzer a second polarizer; see Figure 1 , placed in the optical pathway between the objective rear aperture and the observation tubes or camera port. Image contrast arises from the interaction of plane-polarized light with a birefringent or doubly-refracting specimen to produce two individual wave components that are each polarized in mutually perpendicular planes. The velocities of these components, which are termed the ordinary and the extraordinary wavefronts Figure 1 , are different and vary with the propagation direction through the specimen. After exiting the specimen, the light components become out of phase, but are recombined with constructive and destructive interference when they pass through the analyzer. These concepts are outlined in Figure 1 for the wavefront field generated by a hypothetical birefringent specimen. In addition, the critical optical and mechanical components of a modern polarized light microscope are illustrated in the figure. Polarized light microscopy is capable of providing information on absorption color and optical path boundaries between minerals of differing refractive indices, in a manner similar to brightfield illumination, but the technique can also distinguish between isotropic and anisotropic substances. Furthermore, the contrast-enhancing technique exploits the optical properties specific to anisotropy and reveals detailed information concerning the structure and composition of materials that are invaluable for identification and diagnostic purposes. Isotropic materials, which include a variety of gases, liquids, unstressed glasses and cubic crystals, demonstrate the same optical properties when probed in all directions. These materials have only one refractive index and no restriction on the vibration direction of light passing through them. In contrast, anisotropic materials, which include 90 percent of all solid substances, have optical properties that vary with the orientation of incident light with the crystallographic axes. They demonstrate a range of refractive indices depending both on the propagation direction of light through the substance and on the vibrational plane coordinates. More importantly, anisotropic materials act as beamsplitters and divide light rays into two orthogonal components as illustrated in Figure 1. The technique of polarizing microscopy exploits the interference of the split light rays, as they are re-united along the same optical path to extract information about anisotropic materials. Interactive Tutorial - Birefringent Crystals in Polarized Light Explore how birefringent anisotropic crystals interact with polarized light in an optical microscope as the circular stage is rotated through degrees. Polarized light microscopy is perhaps best known for its applications in the geological sciences, which focus primarily on the study of minerals in rock thin sections. However, a wide variety of other materials can readily be examined in polarized light, including both natural and industrial minerals, cement composites, ceramics, mineral fibers, polymers, starch, wood, urea, and a host of biological macromolecules and structural assemblies. The technique can be used both qualitatively and quantitatively with success, and is an outstanding tool for the materials sciences, geology, chemistry, biology, metallurgy, and even medicine. Figure 2 - Conoscopic Interference Patterns Although an understanding of the analytical techniques of polarized microscopy may be perhaps more demanding than other forms of microscopy, it is well worth pursuing, simply for the enhanced information that can be obtained over brightfield imaging. An awareness of the basic principles underlying polarized light microscopy is also essential for the effective interpretation of differential interference contrast DIC. Basic Properties of Polarized Light The wave model of light describes light waves vibrating at right angles to the direction of propagation with all vibration directions being equally probable. This is referred to as "common" or "non-polarized" white light. In plane-polarized light there is only one vibration direction Figure 1. The human eye-brain system has no sensitivity to the vibration directions of light, and plane-polarized light can only be detected by an intensity or color effect, for example, by reduced glare when wearing polarized sun glasses. Polarized light is most commonly produced by absorption of light having a set of specific vibration directions in a dichroic medium. Certain natural minerals, such as tourmaline, possess this property, but synthetic films invented by Dr. Land in soon overtook all other materials as the medium of choice for production of plane-polarized light. Tiny crystallites of iodoquinine sulfate, oriented in the same direction, are embedded in a transparent polymeric film to prevent migration and reorientation of the crystals. Any device capable of selecting plane-polarized light from natural unpolarized white light is now referred to as a polar or polarizer, a name first introduced in by A. Today, polarizers are widely used in liquid crystal displays LCDs , sunglasses, photography, microscopy, and for a myriad of scientific and medical purposes. There are two polarizing filters in a polarizing microscope - termed the polarizer and analyzer see Figure 1. The polarizer is positioned beneath the specimen stage usually with its vibration azimuth fixed in the left-to-right, or East-West direction, although most of these elements can be rotated through degrees. The analyzer, usually aligned with a vibration direction oriented North-South, but again rotatable on some microscopes, is placed above the objectives and can be moved in and out of the light path as required. When both the analyzer and polarizer are inserted into the optical path, their vibration azimuths are positioned at right angles to each other. In this configuration, the polarizer and analyzer are said to be crossed, with no light passing through the system and a dark viewfield present in the eyepieces. For incident light polarized microscopy, the polarizer is positioned in the vertical illuminator and the analyzer is placed above the half mirror. Most rotatable polarizers are graduated to indicate the rotation angle of the transmission azimuth, while analyzers are usually fixed into position although advanced models can be rotated either 90 or degrees. The polarizer and analyzer are the essential components of the polarizing microscope, but other desirable features include: Specialized Stage - A degree circular rotating specimen stage to facilitate orientation studies with centration of the objectives and stage with the microscope optical axis to make the center of rotation coincide with the center of the field of view. Many stages designed for polarized light microscopy also contain a vernier scale so that rotation angle can be measured to an accuracy of 0. For advanced studies of conoscopic images, a universal stage having multiple axes of rotation can also be employed to enable observation of the specimen from any direction. Strain Free Objectives - Stress introduced into the glass of an objective during assembly can produce spurious optical effects under polarized light, a factor that could compromise performance. Objectives designed for polarized light observation are distinguished from ordinary objectives with the inscription P, PO, or Pol on the barrel. The performance of an objective is limited by several factors, including the anti-reflection coatings used on lens surfaces, and the refractive properties due to angle of incident light on the front lens. In addition, lens strain can be introduced at the cement junction between elements in a lens group or from a single or group of lenses that has been mounted too tightly in the frame. Centerable Revolving Nosepiece - Because the objective optical axis position varies from one assembly to another, many polarized light microscopes are equipped with a specialized nosepiece that contains a centering mechanism for individual objectives. This enables each objective to be centered with respect to the stage and microscope optical axis so that specimen features remain in the center of the viewfield when the stage is rotated through degrees. Strain Free Condenser - Condensers designed for polarized light microscopy have several features in common, including the use of strain free lenses. Some condensers are equipped with a receptacle for the polarizer or have the polarizing element mounted directly into the condenser, beneath the aperture diaphragm. Many polarized light condensers have a top lens that can be removed a swing-lens condenser from the light path to generate nearly parallel illumination wavefronts for low magnification and birefringence observations. Eyepieces - Polarized light microscope eyepieces are fitted with a cross wire reticle or graticule to mark the center of the field of view. Often, the cross wire reticle is substituted for a photomicrography reticle that assists in focusing the specimen and composing images with a set of frames bounding the area of the viewfield to be captured either digitally or onto film. Orientation of the eyepiece with respect to the polarizer and analyzer is guaranteed by a point pin that slides into the observation tube sleeve. Bertrand Lens - A specialized lens mounted in an intermediate tube or within the observation tubes, a Bertrand lens projects an interference pattern formed at the objective rear focal plane into focus at the microscope image plane. The lens is designed to enable easy examination of the objective rear focal plane, to allow accurate adjustment of the illuminating aperture diaphragm and to view interference figures, similar to the ones presented in Figure 2. Note that in Figure 2 a and 2 b , the interference patterns represent those observed with a uniaxial crystal in polarized light, while the pattern in Figure 2 c is typical of a uniaxial crystal with a first order retardation plate inserted into the optical pathway. In most modern microscope designs, this slot is placed either in the microscope nosepiece or an intermediate tube positioned between the body and eyepiece tubes. Compensation plates inserted into the slot are then situated between the specimen and the analyzer. Polarized light microscopy can be used both with reflected incident or epi and transmitted light. Reflected light is useful for the study of opaque materials such as ceramics, mineral oxides and sulfides, metals, alloys, composites, and silicon wafers see Figure 3. Reflected light techniques require a dedicated set of objectives that have not been corrected for viewing through the cover glass, and those for polarizing work should also be strain free. Figure 3 - Reflected Polarized Light Microscopy Illustrated in Figure 3 is a series of reflected polarized light photomicrographs of typical specimens imaged utilizing this technique. On the left Figure 3 a is a digital image revealing surface features of a microprocessor integrated circuit. The blemished surface of a ceramic superconducting crystal bismuth base is presented in Figure 3 b , which shows birefringent crystalline areas with interference colors interspersed with grain boundaries. Metallic thin films are also visible with reflected polarized light. Figure 3 c illustrates blisters that form imperfections in an otherwise confluent thin film of copper about 0. Careful specimen preparation is essential for good results in polarized light microscopy. The method chosen will depend on the type of material studied. In geological applications, the standard thickness for rock thin sections is micrometers. Specimens can be ground down with diamond impregnated wheels and then hand finished to the correct thickness using abrasive powders of successively decreasing grit size. The final specimen should have a cover glass cemented with an optically transparent adhesive. Softer materials can be prepared in a manner similar to biological samples using a microtome. Slices between one and 40 micrometers thick are used for transmitted light observations. These should be strain-free and free from any knife marks. Biological and other soft specimens are mounted between the slide and the cover glass using a mounting medium whose composition will depend on the chemical and physical nature of the specimen. This is particularly significant in the study of synthetic polymers where some media can chemically react with the material being studied and cause degrading structural changes artifacts. Manifestations of Polarized Light in Optical Microscopy Different levels of information can be obtained in plane-polarized light analyzer removed from the optical path or with crossed polarizers analyzer inserted into the optical path. Observations in plane-polarized light reveal details of the optical relief of the specimen, which is manifested in the visibility of boundaries, and increases with refractive index. Differences in the refractive indices of the mounting adhesive and the specimen determine the extent to which light is scattered as it emerges from the uneven specimen surface. Materials with high relief, which appear to stand out from the image, have refractive indices that are appreciably different from the mounting medium. Immersion refractometry is used to measure substances having unknown refractive indices by comparison with oils of known refractive index. Examinations of transparent or translucent materials in plane-polarized light will be similar to those seen in natural light until the specimen is rotated around the optical axis of the microscope. This pleochroism a term used to describe the variation of absorption color with vibration direction of the light depends on the orientation of the material in the light path and is a characteristic of anisotropic materials only. An example of a material showing pleochroism is crocidolite, more commonly known as blue asbestos. The pleochroic effect helps in the identification of a wide variety of materials. Figure 4 - Michel-Levy Birefringence Interference Color Chart Polarization colors result from the interference of the two components of light split by the anisotropic specimen and may be regarded as white light minus those colors that are interfering destructively. Figure 2 illustrates conoscopic images of uniaxial crystals observed at the objective rear focal plane. Interference patterns are formed by light rays traveling along different axes of the crystal being observed. Uniaxial crystals Figure 2 display an interference pattern consisting of two intersecting black bars termed isogyres that form a Maltese cross-like pattern. When illuminated with white polarized light, birefringent specimens produce circular distributions of interference colors Figure 2 , with the inner circles, called isochromes, consisting of increasingly lower order colors see the Michel-Levy interference color chart, Figure 4. A common center for both the black cross and the isochromes is termed the melatope, which denotes the origin of the light rays traveling along the optical axis of the crystal. Biaxial crystals display two melatopes not illustrated and a far more complex pattern of interference rings. The two orthogonal components of light ordinary and extraordinary waves travel at different speeds through the specimen and experience different refractive indices, a phenomena known as birefringence.

Chapter 6 : Polarization spectroscopy - Wikipedia

This comprehensive introduction to polarized light provides students and researchers with the background and the specialized knowledge needed to fully utilize polarized light. It provides a basic introduction to the interaction of light with matter for those unfamiliar with photochemistry and.

Home Polarized Light Microscopy Adapting the Intel Play QX3 microscope for use with plane-polarized light is one of the simplest and most useful configurations available for this amazing microscope. Polarized light microscopy allows the student to explore the birefringent properties of common household items such as sugar, epsom salts, Kool Aid, vitamins, aspirin, and moth balls. The basic setup for transmitted polarized light using the QX3 microscope is illustrated in Figure 1. A polarizer is added to the optical pathway somewhere beneath the specimen, either on the stage or within the mixing chamber Figure 4. Another polarizer, termed the analyzer is placed over the front lens of the objective in the body of the microscope Figure 3. An external tungsten-halide light source with a fiber optics light guide is used to provide auxiliary illumination Figure 1 , because polarizers can block up to 90 percent of the light passing through them. A quarter-inch hole is drilled at the base of the substage mixing chamber to allow light from the illuminator to enter. The mixing chamber will assist in diffusing the light and minimize any problems with bright spots in the image. Light passing through a polarizer is polarized in a single vibrational plane by a polymer film that is highly oriented within the polarizer. Interactive Java Tutorial Polarized Light Microscopy Explore how a real polarized light microscope works to create beautiful kaleidoscopic colors from vitamins and other chemicals Heavy Download. The polarizers must be installed with a specific orientation in the microscope to ensure their polarization vectors are crossed perpendicular. Two sets of polarizers in different orientations are illustrated in Figure 2. Black arrows on the polarizers indicate the direction of the polarization vector. The pair of hands to the left in Figure 2 are holding two polarizers that have the polarization vectors aligned parallel and the pair of hands on the right are holding the polarizers with their vectors perpendicular crossed polarizers. It is the arrangment of crossed polarizers on the right-hand side of Figure 2 that is useful for polarized light microscopy. When two polarizers are held together with their polarization vectors parallel to each other as shown in Figure 2, then light polarized and passed by the first filter will be in the correct orientation to pass through the second. The polarizers will appear transparent when held before a strong light source. However, when the polarizers are held together with their polarization vectors perpendicular, the second polarizer will no longer pass light polarized by the first. In this case, holding the crossed polarizers before a strong light source will demonstrate that no light passes through. After the vibrational planes have been identified for the polarizers, the next job is to attach them in a fixed position on the microscope. Carefully cut one of the polarizers into the shape depicted in Figure 3 for placement in front of the objective lenses. The white arrow in Figure 3 indicates the correct direction of the vibrational plane for the polarizer placed in the microscope body. This polarizer, termed the analyzer and having a specific orientation by convention, is placed with the vibrational plane in a North-South front to back direction. The next step is to place a polarizer either on top of the diffusion screen in the stage or beneath the screen in the mixing chamber. Illustrated in Figure 4 is a polarizer secured in the mixing chamber and showing the orientation of the vibrational plane of the polarizer white double-headed arrow. This polarizer is the one responsible for the initial polarization of white light before it illuminates the sample. Also by convention, this polarizer is oriented in an East-West left to right direction with respect to the microscope body. It is often more convenient, especially when frequently interchanging illumination modes, to simply place the polarizer on the stage underneath the specimen Figure 1. When correctly oriented, the Live View window should be very dark or black when the microscope is illuminated but no specimen is on the stage. Once the polarizers are installed, the microscope is ready to image birefringent specimens. Candidates for polarized light microscopy include any number of common chemicals found around the home or in the supermarket or drug stores. Many vitamins can be recrystallized to form colorful patterns when imaged between crossed polarizers. Likewise, sugar can be dissolved in water and the solution sandwiched between a microscope slide and coverslip, then allowed to slowly evaporate. For more suggestions, visit our section on Specimen preparation for polarized light microscopy. Interactive Java Tutorial Examine how a birefringent crystal behaves when rotated between crossed polarizers. The photomicrograph depicted in Figure 5 illustrates birefringent crystallites of the caffeine, a naturally occuring chemical found in coffee and added to many soft drinks. The specimen was prepared through the melt-recrystallized technique in which a few micrograms of pure crystalline power are placed on a microscope slide and melted with a Bunsen burner. After melting, the slide is placed on the counter top and the crystals are allowed to slowly form from the melt. After the melted caffeine has completely recrystallized and the slide has cooled to room temperature, it is placed on the microscope stage and digitally imaged under polarized light. Vitamin C, aspirin, and acetamidophen Tylenol are also good candidates for quick melt-recrystallization experiments in polarized light. Only those specimens that display birefringence will be visible under polarized illumination. This includes most crystals such as sugar, drugs, and vitamins, some spices and herbs, rock thin sections, bone sections, feathers, butterfly wings, fish scales, and a wide spectrum of other specimens. Part of the fun of polarized light microscopy is to determine which specimens are birefringent. For instance, table salt is one of the most readily available crystals, but the chemical nature of the salt crystal makes it optically isotropic, or invisible in polarized light. Hairs and fibers are often birefringent and make excellent candidates for polarized light microscopy, but materials like wood, metals, and opaque plastics are not. A careful examination of specimens immediately available will help initiate the course towards successful polarized light microscopy. You can learn more about birefringence and polarized light , along with a number of other topics in the physics of light and color and optical microscopy in our Molecular Expressions Microscopy Primer website. Send us an email. Davidson and The Florida State University. The material contained in this website is solely the opinion of the authors and is intended for eduational use only. Last Modification Friday, Nov 13, at Visit the websites of our partners in education:

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The excitation in Raman spectroscopy is usually linearly polarized monochromatic light from a laser. The Raman scattered light can be polarized parallel or perpendicular with respect to the incident laser polarization depending on the symmetry species of the vibrational modes.