

Fundamentals of Raman Polarization Microscopy

Dick Wieboldt, Robert Heintz, Mark Wall, Thermo Fisher Scientific, Madison, WI, USA

Key Words

Raman, Raman Polarization, Spectroscopy

Introduction

Polarized Raman spectroscopy provides vital information about a sample's inherent molecular structure as well as functioning as an effective probe for investigating orientation in highly ordered systems such as crystals, oriented polymers, and carbon nanotubes. This application note will focus upon the fundamental basis behind polarized Raman measurements. In the past Raman point measurements were commonly performed on bulk solids and liquids. Polarized Raman measurements discussed here were collected on a Thermo Scientific™ DXR™2xi Raman imaging microscope and a Thermo Scientific™ DXR™2 Raman microscope. Raman microscopes, like the DXR2 Raman microscope (Figure 1) allow polarized Raman point measurements to be performed on micron sized features. While the latest fast imaging Raman microscopes, like the DXR2xi Raman imaging microscope allows for polarized Raman point measurements to now be extended to polarized Raman images with submicron spatial resolution over large areas. This application note will present polarized Raman point measurements results on both isotropic and anisotropic materials.

Spectroscopy measures the interaction of light with matter. Light is composed of electromagnetic vectors which, under normal circumstances, freely rotate as it propagates. When light impinges upon matter, the interactions are a result of the summation of a mixture of electromagnetic polarization states. When light passes through a polarizing optic, the light is resolved into a polarized beam where the electromagnetic vector propagates



Figure 1: Thermo Scientific DXR2 and DXR2xi Raman microscopes

along a single axis or plane. This is known as linearly polarized light as opposed to freely rotating or depolarized light. When a material is exposed to linearly polarized light, it may interact differently than it does for depolarized light depending on how the material is oriented relative to the polarized light axis.

It is these orientation differences which can provide additional information. For example, a drug may crystallize in different polymorphic forms which have identical spectra when measured with depolarized light. When examined under linearly polarized light, the drug spectra may change providing confirmation of a specific crystalline structure. Polarization measurements are also commonly used to reveal stress in materials such as stretched polymer films, doped silicon structures, or cast materials.

A typical polarization experiment interrogates a sample with linearly polarized light. A spectrum is collected with light polarized along one axis, then a second spectrum is collected with the linearly polarized light rotated perpendicular to the first axis. Various terms are used for these measurements; they all mean the same thing: parallel and perpendicular, vertical and horizontal, X and Y, 0 and 90 degrees, etc. More detailed polarization studies are possible and can become quite complex (see appendix). We will stick to the basic measurements in this article.

Raman spectroscopy is an ideal technique for polarization measurements because the lasers used for excitation are linearly polarized by nature. While this natural polarization can be advantageous (as we shall see), Raman results can be quite misleading if one does not account for laser polarization. For this reason, general purpose instruments, intentionally depolarize the laser excitation beam so as to give consistent results regardless of sample orientation. The spectra using depolarized laser excitation are termed depolarized Raman spectra. Advanced Raman instruments, like the DXR2 and DXR2xi Raman microscopes can be configured to provide both depolarized and polarized Raman measurements. An intuitive software interface provides a simple means to set up the measurement for either depolarized or polarized Raman on a sample.

Porto Notation

When making polarization measurements it is important to keep track of the polarization conditions so that the results can be consistently interpreted. Porto notation was developed as means for recording the orientation of a sample with respect to incident light polarization and outgoing, post sample, analyzer orientation.¹ The notation consists of four terms as follows:²

$$a (b c) d$$

where:

- a = direction of propagation of the incident laser
- b = direction of polarization of the incident laser
- c = direction of polarization of the scattered Raman
- d = direction of propagation of the scattered Raman

The terms outside the parentheses define the direction of propagation of the incident laser beam path and outgoing Raman scattering path. The inner terms describe the polarization direction of the two light paths.

The DXR2 and DXR2xi Raman microscopes use a 180 degree backscatter configuration where the microscope objective focuses the laser onto the sample and also collects the Raman scattering. The propagation axes are the same just with a different direction. This reverse direction is designated by an overbar. The orientation of the DXR2 Raman microscope polarization axes at the sample and the corresponding Porto notation for non-oriented samples is summarized in Figure 2.

The Cartesian coordinate system (Figure 2 inset right) shows the axis notation used by the Thermo Scientific™ OMNIC™xi (and OFDR OMNIC or OMNIC for Dispersive Raman) software for selecting polarization optic for both the laser excitation and post sample analyzer. Incoming laser excitation coming from the microscope objective propagates along the z axis. Raman scattering is collected using the same microscope objective and along the same z axis but in the opposite direction. This is known as a 180 degree backscattering configuration and the convention is to use a overbar on the z axis notation (\bar{Z}) to designate the reverse direction.

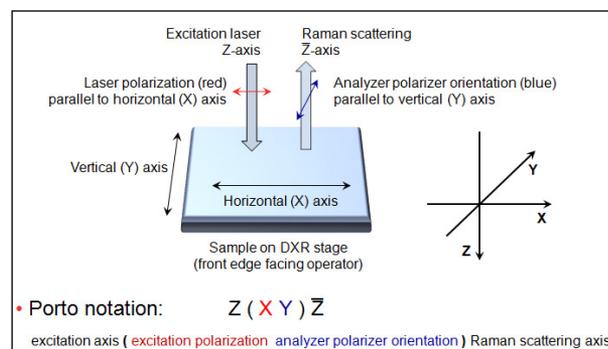


Figure 2: Orientation of DXR2xi Raman microscope polarization axes at the sample and the corresponding Porto notation for non-oriented samples

The polarization axis parallel to the front edge of the stage (left-to-right) as you face the instrument is the Horizontal axis or x axis or 0 degrees. The Vertical or y axis or 90 degree polarization axis is perpendicular to the horizontal x axis and parallel to the edge of the stage going front-to-back as you face the instrument.

Experimental

All experiments were performed on either a DXR2 Raman microscope or a DXR2xi Raman imaging microscope. Each instrument was configured with a fully automated polarization option which can be used with 455, 532, 633, and 785 nm laser excitation. This option allows for operation of the instrument in several different modes: 1) Depolarized Raman – circularly polarized light is delivered to the sample and subsequent scattered light is collected and detected 2) Polarized Raman – software selectable delivery of linearly polarized to the sample that is oriented either vertically or horizontally, post sample polarization analyzer can be set to be either parallel or perpendicular to the excitation polarization. 3) Angle Resolved Polarized Raman – linearly polarized light is delivered to the sample and the polarization analyzer is set to a user defined angle between 0 and 180 degrees.

Results and Discussion

Isotropic and Anisotropic Samples

Not all materials are sensitive to the orientation of polarized light. The term *isotropic* means the properties of the sample (e.g., its Raman spectrum) are not sensitive to sample *orientation*. Examples include liquids, powders, and randomly oriented polymers. While these samples are not sensitive to orientation, they may still interact with polarized light and cause the polarization of the scattered Raman beam to be rotated relative to the incident laser polarization.

In contrast, samples such as crystals or strained films are typically very sensitive to sample orientation and are called anisotropic. Oriented or *anisotropic* samples have an axis of symmetry or optical axis. The alignment of this axis relative to the incident laser polarization can result in quite different spectra.

For isotropic samples it is sufficient to make just two polarization measurements:

1) Analyzer aligned parallel to the laser polarization axis, $Z(XX)\bar{Z}$ or $Z(YY)\bar{Z}$

and

2) Analyzer perpendicular to the laser polarization, $Z(XY)\bar{Z}$ or $Z(YX)\bar{Z}$

To understand why this is true, consider the four possible polarization orientations by their Porto notations shown above. The first two terms describe the incident laser polarization: $Z(X$ looks the same as $Z(Y$ to the sample. The last term, \bar{Z} , is the collection axis and does not change. As long as the inner terms are the same (XX) or (YY) it doesn't matter. The spectra will be identical because any polarization interaction will be the same for both.

The same reasoning applies to the second case where the laser polarizer and analyzer are crossed. As long as the inner terms are perpendicular, (XY) or (YX), the spectra will be the same for isotropic samples.

In this case where two polarization measurements are sufficient, you may see a notation convention based on H and V where H refers to horizontal and V to vertical. Thus, HH means the incident laser polarization is oriented horizontally and the analyzer polarizer (for the scattered Raman) is oriented horizontally. HV means the analyzer polarizer is oriented vertically (perpendicular to the incident laser). There are numerous variations of this type of notation (H_H and H_V, 0 and 90 degrees, parallel and perpendicular, etc.). These all mean the same thing: an isotropic sample with arbitrary and orthogonal polarization axes.

For oriented, anisotropic samples (crystals, stretched films or fibers, etc.), it is important to document how sample axis is aligned with the instrument polarization axis. The convention is to use the coordinate system of the sample for the Porto notation rather than the instrument's coordinate system. This convention allows other researchers to align the oriented sample on a different instrument and measure the same polarization results.

If it is not possible to identify the sample axis or if the sample properties are unknown, it is advisable to capture a micrograph of the sample to record its orientation. A rotatable sample holder is useful for allowing easier alignment of a known axis of the sample with the instrument polarization axis.

There are only four polarization tensors that can be analyzed with a Raman microscope. They are:

$Z(XX)\bar{Z}$ laser polarized parallel to an x axis;
analyzer set to pass x axis polarized light

$Z(YY)\bar{Z}$ laser polarized parallel to a y axis;
analyzer set to pass y axis polarized light

$Z(XY)\bar{Z}$ laser polarized parallel to an x axis;
analyzer set to pass y axis polarized light

$Z(YX)\bar{Z}$ laser polarized parallel to a y axis;
analyzer set to pass x axis polarized light

For anisotropic or unknown samples, it is recommended to make all four of these measurements. It is a good idea to also measure the depolarized (non-polarized) spectrum to completely document the sample.

Raman Polarization for Isotropic Samples

The classic example for Raman polarization measurements is carbon tetrachloride (CCl₄). CCl₄ is an isotropic liquid with bands at 459, 314, and 218 cm⁻¹ Raman shift. The 459 cm⁻¹ band is due to the totally symmetric vibrational mode A₁ and is termed totally polarized. This means that it emits Raman scattering in the same polarization orientation as the incident light. When the analyzer is aligned with the laser polarization, the maximum Raman scattering is observed. When the analyzer is crossed, the intensity of the linearly polarized Raman scattering is extinguished. The ratio of the crossed intensity divided by the aligned intensity is called the depolarization ratio and will approach zero.

Unlike the 459 cm⁻¹ band, the bands at 314 and 218 cm⁻¹ are not as sensitive to polarization. The 314 cm⁻¹ band is due to the non-totally symmetric vibrational mode E₂. This mode has a symmetric scattering tensor and is termed depolarized. The band at 218 cm⁻¹ is also depolarized.

Depolarized bands have a ratio $Z(XY)\bar{Z}/Z(XX)\bar{Z}$ of 0.75. This information can be used to determine which bands are depolarized in the sample spectrum. For example, if a peak height varies when comparing the crossed (perpendicular) and aligned (parallel) polarization spectra it is possible to confirm that this is a depolarized peak by calculating the peak height ratio. If it is approximately 0.75 (\pm experimental variation), the peak is depolarized.

Figure 3 shows an example of how to calculate the depolarization ratios from the four polarized Raman spectra of carbon tetrachloride. While the ratio could be based on just one pair of measurements (YX/YY) or (XY/XX), it is a good practice to measure both and use the average. This minimizes any bias introduced by the polarization optics due to the inevitable variation between instruments.

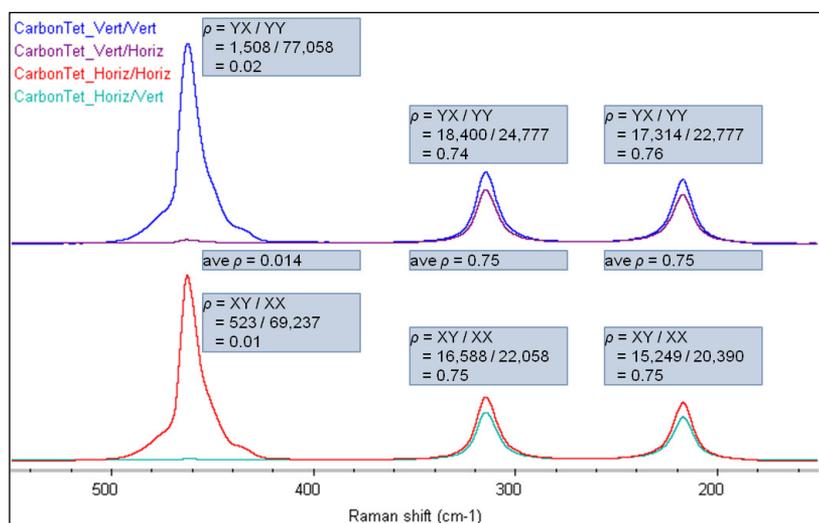


Figure 3: Example showing the calculation of depolarization ratio (ρ) from spectra of CCl_4 collected on a DXR2xi Raman imaging microscope with 785 nm laser excitation. Values are baseline corrected peak area measurements.

Raman Polarization for Anisotropic Solids

One of the challenges of spectroscopic analysis is identifying spectral features which are relevant to a sample property of interest. Raman polarization can be particularly useful for this type of application. For example, the depolarization ratio for a spectral peak may be indicative of a specific sample morphology. This might not have been apparent from the depolarized Raman spectra alone.

As you explore the application of Raman polarization, you will find that sample orientation is important. Whether you are aligning the laser polarization to a specific crystal axis in a known sample or exploring a sample of unknown symmetry, document your work. This will make it easier to compare results between samples and with other laboratories. A micrograph taken with the DXR2 Raman microscope video camera is a good means for this.

LiNbO₃ Example

In the previous section carbon tetrachloride was used as a good example for isotropic Raman polarization. In this section lithium niobate will be used as an example of an anisotropic sample having known crystallographic symmetry.

LiNbO₃ has a hexagonal crystal structure with three-fold symmetry and is grown along its optical axis or c axis. By convention, a Cartesian coordinate system is used to describe the LiNbO₃ crystal with the z axis parallel to the optical c axis. This vertical axis is shown in Figure 4.³

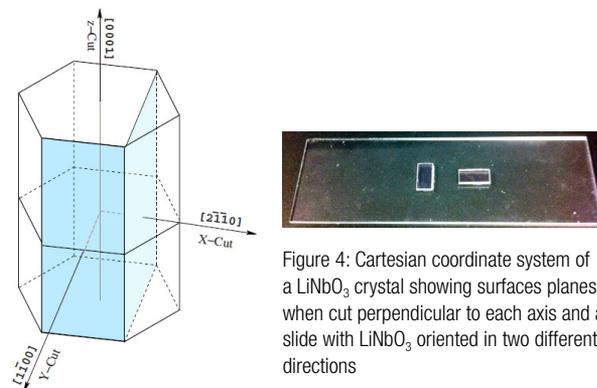


Figure 4: Cartesian coordinate system of a LiNbO₃ crystal showing surfaces planes when cut perpendicular to each axis and a slide with LiNbO₃ oriented in two different directions

LiNbO₃ can be prepared in the following way for polarization measurements. The LiNbO₃ is X-cut, meaning it is cut perpendicular to the crystal x axis. The plane of this surface (light blue in Figure 4) is the known as $[2\bar{1}10]$ for trigonal or hexagonal representations, respectively. As shown mounted on the slide in Figure 4, the longer dimension is the crystal z axis and the shorter is the crystal y axis. The crystal x axis is perpendicular to the surface and is parallel to the incident laser light.

The use of Porto notation for oriented solid samples is important because it gives a consistent mechanism for reporting results from different instruments. Recall that we chose instrument coordinates for Porto notation when working with isotropic samples like liquids. For oriented samples, the convention is to use the Cartesian coordinates of the crystal axes in the Porto notation.

For the LiNbO₃ example, the Porto notation outer terms (incident and scattered light) are parallel to the crystal x axis. The inner terms indicate the orientation of the polarized light in terms of the crystal axes. For the chip mounted with the long axis front-to-back on the sample stage and the laser polarization set to Vertical, the first inner term will be Z indicating the incident polarization is aligned with the crystal z axis. A value of Y for the second inner term means the analyzer polarizer is oriented along the crystal y axis or perpendicular to the polarization of the incident light.

X(ZY)X̄

If the Lithium niobate sample that has been x cut is oriented sequentially along both the x and y instrument axes and the four combinations of laser/analyzer polarizer positions are measured for each orientation, then eight polarized LiNbO₃ spectra will be collected as shown in Figure 5.

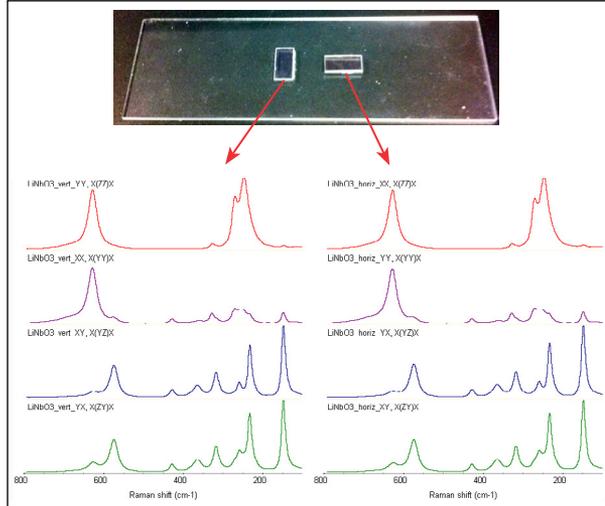


Figure 5: Polarized Raman spectra of LiNbO₃ samples mounted on the slide vertically (left side with _vert in title) and horizontal (right) with their Porto notation

The polarizer settings are captured in the spectrum collection information. The _XY notation appended to the sample name refers to the instrument polarization setting for laser polarizer and analyzer respectively.

Note the spectra having the same Porto notation are identical even though they have different physical orientation and polarizer optics settings. Tying the Porto notation to the sample axes establishes a common reference for making these types of comparisons.

If the spectra are plotted on the same vertical axis scale and offset (Figure 6), it is possible to visually look for depolarized peaks as was done for CCl₄.

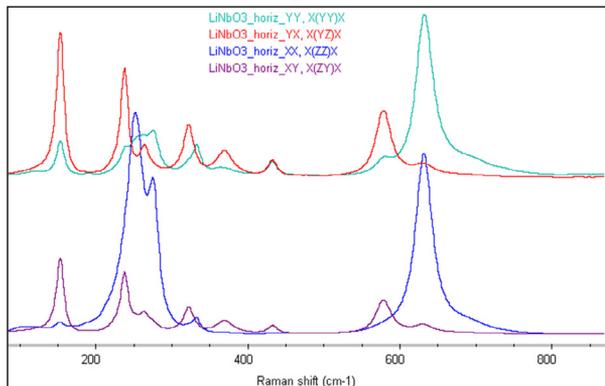


Figure 6: LiNbO₃ spectra plotted as cross-polarized pairs. All spectra are on the same Y-axis scale and offset for comparison. Spectra were measured over the full range (50–3300 cm⁻¹ Raman shift); the only bands observed are the ones shown.

It is easy to see that the different polarizations yield a rich set of spectral features and all bands are sensitive to polarization. In this case, the bands can be assigned to specific Raman polarization tensors (see appendix) because we have a known sample and crystal axis aligned in a specific orientation to the instrument polarization axes.

For unknown samples, the same set of spectral measurements can be measured and compared. While the details of crystal symmetry might not be known, if the sample is oriented consistently the spectral differences can be correlated with sample properties.

Conclusion

The fundamental basis for Raman polarization measurements were presented in this application notes. Porto notation was introduced so as to have a means for recording the orientation of a sample with respect to incident light polarization and outgoing post sample analyzer orientation, which allows for consistent interpretation of Raman polarization measurements. Raman point polarization results were presented on a couple of types of sample, specifically, isotropic (CCl₄) and anisotropic (LiNbO₃) samples. Raman polarization measurements provide information that allows the nature of the vibrations to be revealed as well as aiding in the assignment of these bands. Subsequent application notes will extend these measurements from single point measurements to Raman polarization imaging

Appendix

The remainder of this document provides a more detailed explanation of Raman polarization. There are additional resources in textbooks and on the web to help explain this topic.

Raman polarization

The three dimensional frame of reference shown in Figure 7 can be used to describe the orientation of light incident on the sample.

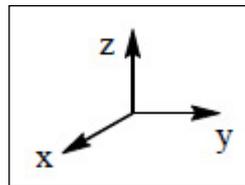


Figure 7: Frame of reference for Raman microscope polarization described in the Appendix

A ray of light can be resolved into three component electrical vectors E_x , E_y , and E_z which point along each of the three axes. We can use similar vectors P_x , P_y , and P_z to describe the dipole induced by this ray of light. These two vectors are expressed by a 3×3 matrix called the polarization tensor:⁴

$$\begin{bmatrix} P_x \\ P_y \\ P_z \end{bmatrix} = \begin{bmatrix} a_{xx} & a_{xy} & a_{xz} \\ a_{yx} & a_{yy} & a_{yz} \\ a_{zx} & a_{zy} & a_{zz} \end{bmatrix} \begin{bmatrix} E_x \\ E_y \\ E_z \end{bmatrix} \quad (\text{Equation 1})$$

where the notation α_{xx} is the polarizability component for incident light polarized in the x axis and the molecule polarization along the x axis.

This matrix can be expanded out to give equations for P_x , P_y , and P_z :

$$P_x = \alpha_{xx}E_x + \alpha_{xy}E_y + \alpha_{xz}E_z \quad (\text{Equation 2})$$

$$P_y = \alpha_{yx}E_x + \alpha_{yy}E_y + \alpha_{yz}E_z$$

$$P_z = \alpha_{zx}E_x + \alpha_{zy}E_y + \alpha_{zz}E_z$$

From these equations, you can see the induced dipole in one direction depends on all three electrical vectors. This is why polarization measurements in Raman spectroscopy are somewhat complex.

An oscillating dipole cannot scatter light along the same axis as itself. Because we are observing along the z axis, $E_z = 0$, and only light scattered due to P_x and P_y are collected. Therefore, the above equations can be reduced to:

$$P_x = \alpha_{xx}E_x + \alpha_{xy}E_y \quad (\text{Equation 3})$$

$$P_y = \alpha_{yx}E_x + \alpha_{yy}E_y \quad (\text{Equation 4})$$

The intensity of light is proportional to the square of the induced dipole. The intensity of scattered light excited with an electric vector along the x - and y -axes is as follows:

$$I_x = \alpha_{xx}^2 E_x^2 + \alpha_{xx}\alpha_{xy}E_xE_y + \alpha_{xy}\alpha_{xx}E_yE_x + \alpha_{xy}^2 E_y^2 \quad (\text{Equation 5})$$

$$I_y = \alpha_{yx}^2 E_x^2 + \alpha_{yx}\alpha_{yy}E_xE_y + \alpha_{yy}\alpha_{yx}E_yE_x + \alpha_{yy}^2 E_y^2 \quad (\text{Equation 6})$$

When we use a laser that is linearly polarized along one axis, yet another term goes to zero. For example, if the laser is polarized along the y axis:

$$E_z = 0, E_x = 0, \text{ and } E_y \neq 0$$

Equations 5 and 6 reduce to the following (along with their Porto notations):

$$I_x = \alpha_{xy}^2 E_y^2 \quad z (y x) \bar{z} \quad (\text{Porto notation}) \quad (\text{Equation 7})$$

$$I_y = \alpha_{yy}^2 E_y^2 \quad z (y y) \bar{z} \quad (\text{Equation 8})$$

If the incident laser polarization is flipped to the x axis, $E_z = 0$, $E_y = 0$, and $E_x \neq 0$ and we have the following equations:

$$I_x = \alpha_{xx}^2 E_x^2 \quad z (x x) \bar{z} \quad (\text{Equation 9})$$

$$I_y = \alpha_{yx}^2 E_x^2 \quad z (x y) \bar{z} \quad (\text{Equation 10})$$

These are the four geometrical measurements available for a Raman microscope system. These equations apply to rigid samples whose molecules are fixed in space.

Depolarization Ratio

The ratio of the spectral band intensities measured along two axes is called the depolarization ratio. By convention, the ratio is the perpendicular polarized intensity divided by the parallel intensity.

For laser light polarized along the y axis, the equation for depolarization ratio is as follows:

$$\rho = \frac{I_{\perp}}{I_{\parallel}} = \frac{I_x}{I_y} = \frac{\alpha_{xy}^2 E_y^2}{\alpha_{yy}^2 E_y^2} \quad (\text{Equation 11})$$

A first approximation of the theoretical value of ρ is:

$$\rho = \frac{3\beta^2}{45\alpha^2 + 4\beta^2} \quad (\text{Equation 12})$$

where α is known as the spherical polarizability and β is called the first anisotropy.⁵ Spherical polarizability is equivalent to the diagonal terms of the polarizability tensor and has the value:

$$\alpha = \frac{1}{3} (\alpha_{xx} + \alpha_{yy} + \alpha_{zz}) \quad (\text{Equation 13})$$

The first anisotropy is calculated from the off diagonal terms and has the following value:

$$\beta = \sqrt{(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2 + 6(\alpha_{xy}^2 + \alpha_{yz}^2 + \alpha_{xz}^2)} \quad (\text{Equation 14})$$

Measuring the depolarization ratio for a Raman band yields information about the values of α and β . From this, we can deduce symmetry information for the vibration causing the Raman band.

For example, a vibration mode which is degenerate (not totally symmetric) produces values of $\alpha = 0$ and $\beta \neq 0$. This gives a depolarization ratio value of $\frac{3}{4}$.

For modes which are totally symmetric and of cubic or icosahedral symmetry, the value of $\beta = 0$ and the value of the ratio = 0. Other modes have symmetry yielding non-zero values of α ($\alpha \neq 0$). This leads to depolarization ratios between the values of zero and $\frac{3}{4}$.^{4,6}

Isotropic Materials

Isotropic materials are liquids or gases where the sample molecules are constantly tumbling relative to our fixed laboratory frame of reference. In this situation, we measure an average value of the polarizability: $\bar{\alpha}$.

The equation for depolarization ratio is the same as Equation 12 except the average polarizability is calculated:

$$\rho = \frac{3\beta^2}{45\bar{\alpha}^2 + 4\beta^2} \quad (\text{Equation 15})$$

References

1. Damen, T. C.; Porto, S.P.S.; Tell, B. *Phys. Rev.*, **1966**, *142*, 570.
2. Bilbao Crystallographic Server, <http://www.cryst.ehu.es> .
3. Sanna, phy rev B 2010 v81 p214116, “Lithium niobate X-cut, Y-cut, and Z-cut surfaces from ab initio theory”.
4. Hendra, P.; Jones, C.; Warnes, G. “The vibrational behaviour of molecules”, in *Fourier Transform Raman Spectroscopy Instrumentation and Chemical Applications*; Ellis Horwood Ltd.: Chichester, England, 1991.
5. Diem, M. “Raman Spectroscopy – Polarizability”, in *Modern Vibrational Spectroscopy and Micro-Spectroscopy*, John Wiley & Sons, Ltd: Chichester, UK, 2015, chapter 4.2.
6. Gilson, T. R.; Hendra, P. J. “Raman intensities and depolarization ratios”, in *Laser Raman Spectroscopy*; Wiley-Interscience: New York, 1970.

www.thermoscientific.com

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Africa +43 1 333 50 34 0	Denmark +45 70 23 62 60	India +91 22 6742 9494	New Zealand +64 9 980 6700
Australia +61 3 9757 4300	Europe-Other +43 1 333 50 34 0	Italy +39 02 950 591	Russia/CIS +43 1 333 50 34 0
Austria +43 810 282 206	Finland/Norway/Sweden +46 8 556 468 00	Japan +81 45 453 9100	Spain +34 914 845 965
Belgium +32 53 73 42 41	France +33 1 60 92 48 00	Latin America +1 561 688 8700	Switzerland +41 61 716 77 00
Canada +1 800 530 8447	Germany +49 6103 408 1014	Middle East +43 1 333 50 34 0	UK +44 1442 233555
China +86 21 6865 4588		Netherlands +31 76 579 55 55	USA +1 800 532 4752