

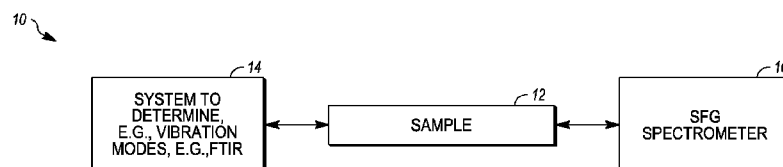


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(54) **Title:** SYSTEM AND METHOD FOR DETERMINATION OF MOLECULAR ORIENTATION AT INTERFACES**FIG. 7**

(57) **Abstract:** Systems and methods are provided for determining aspects of molecular orientation at interfaces. A graphical method is used to extract both mean tilt angle and orientation distributions, i.e., to determine the unique (θ_0, σ) pair for a given D_1 and D_2 . For example, if D_1 and D_2 are determined from SFG measurements, two planes may be drawn that are parallel to the θ_0 - σ plane at the determined points. The projections of both intersection lines on the θ_0 - σ plane are plotted and represent the qualified (θ_0, σ) pairs that have $D_1 = D_1'$ and $D_2 = D_2''$. The intersection point represents the unique (θ_0, σ) pair. When both intersection lines for D_1 and D_2 are determined together, there is only one intersection point, representing the unique (θ_0, σ) pair that satisfies both $D_1 = D_1'$ and $D_2 = D_2''$.

SYSTEM AND METHOD FOR DETERMINATION OF MOLECULAR ORIENTATION AT INTERFACES

STATEMENT OF GOVERNMENT INTEREST

[0001] This invention was made with government support under D15AP000107 awarded by the US Department of Defense (DARPA). The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims benefit of priority of US Provisional Patent Application Serial Number 62/298, 680, filed February 23, 2016, entitled "SYSTEM AND METHOD FOR DETERMINATION OF MOLECULAR ORIENTATION AT INTERFACES", owned by the assignee of the present application and herein incorporated by reference in its entirety.

FIELD

[0003] The invention relates to the determination of molecular orientation at surfaces and interfaces.

BACKGROUND

[0004] Accurately measuring the net orientation of molecules at interfaces is critical for understanding and fabricating molecular mono-layer systems which are used in surface chemical reactions, energy materials, and biological membranes. For instance, in the heterogeneous catalysis for the hydrogenation of acrolein, the orientation of adsorbed acrolein on the Ag(111) surface affects both the reaction activity and selectivity. When the C=C bond of reactants lies nearly parallel to the surface, the bonds are more exposed to H atoms and are therefore more vulnerable toward hydrogenation. Therefore, determining the catalysts' orientation and optimizing the 'good' orientations becomes important. In biological lipid membranes, lipids adopt new configurations and morphologies when new antimicrobial peptides are introduced. Measuring the change of lipid orientation and orientation distribution can provide microscopic pictures of how the membrane morphologies respond to the new peptides.

[0005] However, determining the net orientation of any molecular monolayer, which is an ensemble of molecules, is not a trivial task. To completely quantify the net orientation of the ensemble-averaged molecular monolayer, two physical quantities need to be measured - the mean tilt angle θ_0 and the orientation distribution σ , which is referred to as a (θ_0, σ) pair hereafter.

[0006] Sum frequency generation spectroscopy (SFG) is a technique used to analyze surfaces and interfaces. This nonlinear laser spectroscopy method can deduce the composition, orientation distributions, and some structural information of molecules at gas–solid, gas–liquid and liquid–solid interfaces. In a typical SFG setup, two laser beams mix at a surface and generate an output beam with a frequency equal to the sum of the two input frequencies. SFG has advantages in its ability to be monolayer surface sensitive, its ability to be performed *in situ* (e.g., for aqueous surfaces and in gases), and its lack of causing significant damage to the sample surface. SFG is comparable to second harmonic generation in infrared and Raman spectroscopy. In particular, surface-specific vibrational SFG (referred as 1D VSFG) spectroscopy can selectively probe interfacial molecules, and has therefore been used to probe net molecular orientation at interfaces.

[0007] However, one has to first assume a narrow orientation distribution to estimate the mean tilt angle, since 1D VSFG cannot simultaneously determine the mean tilt angle and the orientation distribution. The consequence of this assumption is that the mean tilt angle determined from 1D VSFG is often inaccurate and the distribution remains unknown, referred as the “magic angle” challenge in surface spectroscopy. This challenge has remained unsolved for more than a decade and has largely limited the applications of using 1D VSFG to quantitatively investigate surface molecular conformations. Such ambiguities also exist in other surface techniques, such as optical ellipsometry, XPS, and FTIR.

[0008] Figs. 1(A) and 1(B) are prior art figures illustrating the so-called “magic angle” challenge in determining molecular orientation. Fig. 1(A) shows orientational parameters D_1 (solid lines) and D_2 (dashed lines) as a function of orientation distribution σ for a series of mean tilt angles θ_0 . D_1 and D_2 are calculated based on a modified Gaussian function.

[0009] This “magic angle” challenge relates to the measurement of molecular orientation using one-dimensional SFG which often assumes a narrow orientation distribution that results in ambiguity in tilt angle measurement.

[00010] For example, using 1D SFG spectroscopy and measuring an effective second-order susceptibility of a molecular interface, an orientational parameter of the interfacial molecules, $D_1 = \langle \cos^3 \theta \rangle / \langle \cos \theta \rangle$, can be determined, where the mean tilt angle θ is the angle between the surface normal and the defined molecular axis, and the bracket means an orientational average, which can be calculated by assuming a narrow distribution ($\sigma=0^\circ$). That is, by assuming a narrow distribution, $\sigma = 0$, the apparent orientation angle is determined based on $D_1 = \cos^2 \langle \theta \rangle$. However, since D_1 is a function of the (θ_0, σ) pair, for any D_1 , there are an infinite number of combinations of θ_0 and σ (solid curves in Fig. 1A).

[00011] Fig. 1(B) shows that when $D_1 = 0.600$, the apparent angle measured is $\theta_0=39.2^\circ$, but it is unknown whether the surface has a uniform orientation distribution with $\theta_0 = 39.2^\circ$, or a broad orientation distribution. Indeed it could be any mean tilt angle with a broad distribution ($\sigma=90^\circ$). The D_2 are different for these two scenarios. This 39.2° ‘magic angle’ represents the extreme case that the θ_0 determined from D_1 does not represent the true molecular net orientation on the surface. This ambiguity also remains for any other D_1 values. Although this mean tilt angle ambiguity is a well-known challenge, there has been little solution to it.

[00012] In other words, using existing techniques, the apparent molecular angle determined from measurement could be far away from the true molecular angle of the surface, and could result in incorrect angular determination for many surfaces.

[00013] US Patent Number 7,787,118, to Klug et al., uses nonlinear optics and Raman spectroscopy to obtain spectral information. However, the same measure and is sensitive to bulk properties, and is not useful for the determination of molecular orientation at interfaces.

[00014] This Background is provided to introduce a brief context for the Summary and Detailed Description that follow. This Background is not intended to be an aid in determining the scope of the claimed subject matter nor be viewed as limiting the claimed subject matter to implementations that solve any or all of the disadvantages or problems presented above.

SUMMARY

[00015] Systems and methods according to present principles meet the needs of the above in several ways.

[00016] In particular, systems and methods according to present principles provide an improved method to determine the net orientation of molecules at interfaces using an advanced

two-dimensional (2D) vibrational SFG (VSFG or just SFG hereafter) spectroscopy, or using both 1D and 2D VSFG spectroscopy, as measuring 2D spectroscopy inherently measures and provides a result for 1D VSFG spectroscopy. This technology unambiguously determines both mean tilt angle (θ_0) and orientation distribution (σ) – from the same measurement—thus improving the fidelity and accuracy of characterizing the surface molecular mean tilt angle distribution. These systems and methods provide a general way to determine the orientations of an ensemble-averaged molecular surface, which can potentially be applied to a wide-range of chemical, material and biological interfaces, such as are commonly found in nano-manufacturing, industrial catalysis, and research in many industries, including the semiconductor and biomedical industry, where molecular surfaces are critical to numerous diagnostic/analytical procedures.

[00017] In one implementation, a graphical method is employed to accurately extract θ_0 and σ .

[00018] As a particular example, an experimentally-measured heterodyned 2D VSFG was employed to apply the technique to a catalyst/electrode interface. In particular, it was found from the measured spectrum that $\text{Re}(4,4'\text{-dicyano-2,2'-bipyridine})(\text{CO})_3\text{Cl}$ self-assembled on a gold slide and formed a relatively-ordered monolayer. The same had a mean tilt angle between the C3 symmetric axis of the catalysts and the surface normal of 52° , with a non-negligible 11° orientation distribution. Although applied to a specific system, this example indicates that the method provides a general way to determine the orientations of an ensemble-averaged molecular surface, which can potentially be applied to a wide-range of chemical, material and biological interfaces.

[00019] In one aspect, the invention is directed towards a method of determining molecular orientation at interfaces, the molecular orientation including mean tilt angle (θ_0) and orientation distribution (σ), including: a. measuring vibrational spectral peak intensities of a sample using 2D heterodyne sum frequency generation spectroscopy; b. calculating surfaces D1 and D2 using a modified Gaussian model, wherein the surface D1 corresponds to mean tilt angle and is a function of tilt angle and orientation, and wherein the surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation; c. calculating values of D' and D'' , where D' and D'' are calculated based at least in part on a measured effective second order susceptibility, a measured effective fourth order

susceptibility, or a combination of the two susceptibilities; d. calculating planes, parallel to a θ - σ plane, corresponding to D' and D'' ; e. determining curves corresponding to the intersection of the planes with the surfaces; and f. determining an intersection of the determined curves, the intersection corresponding to a desired θ - σ pair, whereby the desired θ - σ pair indicates an experimentally measured molecular orientation and distribution of the sample.

[00020] Implementations of the invention may include one or more of the following. The determined D' and D'' may be further based on coefficients that depend on molecular hyperpolarizabilities of the i^{th} vibrational mode. The values of D' and D'' may be based at least in part on a ratio of second and fourth order susceptibilities. The second and fourth order susceptibilities may be calculated by performing spectral fitting to measured 1D VSFG spectra and 2D VSFG spectra. The values of D' and D'' may be based at least in part on an experimental measurement. The effective second order susceptibility and the effective fourth order susceptibility may be expressed as a linear combination of $\langle \cos\theta \rangle$, $\langle \cos 3\theta \rangle$, and $\langle \cos 5\theta \rangle$. The determined D' and D'' may be further based on a ratio of an effective susceptibility between two vibrational modes, or on a ratio of effective susceptibilities for a single vibrational mode under different polarization combinations. The ratio of effective susceptibility between two vibrational modes may be determined by measuring and analyzing or fitting peaks in the 2D heterodyne SFG spectroscopy spectra. The method may further include validating a result by determining if $D_{12} \leq D_2 \leq D_1$, such that if this relationship is satisfied, the result is validated. The method may further include including repeating the steps for a plurality of subsequent time intervals, where the time intervals are spaced to allow a femtosecond time resolution of time-dependent molecular orientation fluctuations.

[00021] In a related aspect, the invention is directed towards a non-transitory computer readable medium, including instructions for causing a computing environment to perform the above method.

[00022] In another aspect, the invention is directed towards a method of determining molecular orientation at interfaces, the molecular orientation including mean tilt angle (θ) and orientation distribution (σ), including: a. using 1D and 2D heterodyne sum frequency generation spectroscopy on a sample, determining surfaces D_1 and D_2 , the surfaces D_1 and D_2 described by 3-D surfaces and calculated using a modified Gaussian model, wherein the surface D_1 corresponds to mean tilt angle and is a function of tilt angle and orientation, and wherein the

surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation, the surfaces D1 and D2 calculated from performing the sum frequency generation spectroscopy at various polarization combinations; b. determining values of D' and D'' from , where the D' and D'' are calculated based at least in part on the ratio of second and fourth order susceptibilities; c. calculating planes, parallel to a θ - σ plane, corresponding to D' and D'' ; d. determining curves corresponding to the intersection of the planes with the surfaces; and e. determining an intersection of the determined curves, the intersection corresponding to a desired θ - σ pair, whereby the desired θ - σ pair indicates a molecular orientation and distribution of the sample.

[00023] Implementations of the invention may include that the values of D' and D'' may be calculated further based on values from an experimental measurement.

[00024] In a related aspect, the invention is directed towards a non-transitory computer readable medium, including instructions for causing a computing environment to perform the above method.

[00025] In another aspect, the invention is directed towards a method of determining molecular orientation at surfaces or interfaces, the molecular orientation including mean tilt angle (θ) and orientation distribution (σ), including: a. performing 2D heterodyne SFG spectroscopy on a sample to obtain a spectrum including one or more peaks; b. determining two surfaces D1 and D2 using a modified Gaussian model and the obtained spectrum, the surfaces D1 and D2 described by 3-D surfaces, wherein the surface D1 corresponds to mean tilt angle and is a function of tilt angle and orientation, and wherein the surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation; c. performing spectral fitting on the obtained spectrum to determine at least the peak intensity and second order and fourth order nonlinear susceptibilities; d. calculating, using density functional theory, one or more coefficients for determining D' and D'' ; e. determining unique D' and D'' using the calculated coefficients and determined nonlinear susceptibilities; f. calculating planes, parallel to a θ - σ plane, corresponding to the determined unique D' and D'' ; g. determining curves corresponding to the intersection of the planes with the surfaces; and h. determining an intersection of the determined curves, the intersection corresponding to a desired θ - σ pair, whereby the desired θ - σ pair indicates a molecular orientation and distribution of the sample.

[00026] Implementations of the invention may include one or more of the following. The method may further include, prior to the performing 2D heterodyne SFG spectroscopy, measuring or determining one or more vibrational modes. The measuring or determining one or more vibrational modes may be performed using an FTIR technique. The method may further include performing additional spectrum measurements with different polarization combinations.

[00027] In a related aspect, the invention is directed towards a non-transitory computer readable medium, including instructions for causing a computing environment to perform the above method.

[00028] Advantages of the invention may include, in certain embodiments, one or more of the following. Systems and methods according to present principles solve the long-standing “magic angle” challenge. That is, the measurement of molecular orientation using 1D SFG often assumes a narrow orientation distribution that results in ambiguity in tilt angle measurement. In present systems and methods according to present principles, the net orientation (mean tilt angle and orientation distribution) of molecules at interfaces can be unambiguously probed using a new combination of 1D and 2D vibrational SFG.

[00029] Systems and methods according to present principles may be advantageously employed to inspect surface molecular angles and angular distribution, which are the critical parameters for nanomanufacturing, industrial catalysis, biomedical research, and many other fields.

[00030] Other advantages will be understood from the description that follows, including the figures and claims.

[00031] This Summary is provided to introduce a selection of concepts in a simplified form. The concepts are further described in the Detailed Description section. Elements or steps other than those described in this Summary are possible, and no element or step is necessarily required. This Summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended for use as an aid in determining the scope of the claimed subject matter. The claimed subject matter is not limited to implementations that solve any or all disadvantages noted in any part of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[00032] Figs. 1A and 1B are prior art figures illustrating the “magic angle” challenge.

- [00033] Figs. 2A – 2E illustrate determination of a unique (θ_0 , σ) pair.
- [00034] Fig. 3 is a chart illustrating heterodyned 1D [1] and diagonal cut of 2D [2] SFG spectra of Re(4,4'-dicyano-2,2'-bipyridine)(CO)₃Cl monolayer self-assembled on a gold slide.
- [00035] Figs. 4A-4C illustrate three stretching modes related to carbonyls in the Re-complex.
- [00036] Fig. 5 illustrates the relationship between the XYZ (lab) frame and the xyz (molecular) frame.
- [00037] Fig. 6 illustrates polarized Raman spectra of Re(4,4'-dicyano-2,2'-bipyridine)(CO)₃Cl in DMSO (26 mM) acquired with 647.09 nm excitation.
- [00038] Like reference numerals refer to like elements throughout. Elements are not to scale unless otherwise noted.

DETAILED DESCRIPTION

- [00039] Systems and methods according to present principles determine the net molecular orientation (both mean tilt angle and orientation distribution) of molecules at interfaces by using a technique such as advanced heterodyned 2D SFG spectroscopy. Using this method, both mean tilt angle (θ_0) and orientation distribution (σ) can be unambiguously determined, which are the most important parameters describing the orientation of interfacial molecules in biological membrane surfaces, polymer brushes, and catalytic reaction surfaces.
- [00040] In the disclosed method, unlike prior efforts, no assumptions need be made in orientation distribution. Instead, both mean tilt angle and orientation distribution are determined from the parameters D_1 ($\langle \cos^3 \theta \rangle / \langle \cos \theta \rangle$) (also termed a “first parameter”) and D_2 ($\langle \cos^5 \theta \rangle / \langle \cos \theta \rangle$) (also termed a “second parameter”) determined from 2D SFG. In this way, the surface molecular orientation can be accurately measured.
- [00041] As will be described below, the terms D_1 and D_2 are used to represent the general parameters which are functions of θ_0 and σ . For example, the 3-D surface for D_1 and D_2 are in many cases generic surfaces that should apply to many (physical) surfaces for which orientation information is desired. Then, based on a specific 2D SFG measurement, a specific pair of D_1 and D_2 corresponding to the surface, but in this case termed D' and D'' , are determined, to differentiate from the generic D_1 and D_2 , although they are technically still D_1 and D_2 for a specific surface.

[00042] Present systems and methods solve the “magic angle” challenge by determining both mean tilt angle and orientation distribution from the same measurements, using a combination of heterodyned 1D and 2D VSFG spectroscopies. Because 1D VSFG is intrinsically measured at the same time as heterodyne 2D VSFG is performed, the angular determination is done in one single measurement. Heterodyned 1D and 2D VSFG spectroscopies are surface-sensitive vibrational spectroscopies whose signals depend on the molecular orientations and thus the same can be used to extract surface molecular orientations.

[00043] In more detail, the recently-developed heterodyned (HD) 2D VSFG spectroscopy is a core measurement that enables surface molecular orientation heterogeneity characterization. In this technique, fourth-order susceptibilities ($\chi_{eff}^{(4)}$) are measured in HD 2D VSFG. Similar to 1D VSFG, which measures second-order susceptibilities ($\chi_{eff}^{(2)}$), these even-order nonlinear optical signals only survive in non-centrosymmetric environments, such as interfaces. Therefore, as noted above both 1D and HD 2D VSFG spectroscopies are interface-specific vibrational spectroscopies whose signals depend on the molecular orientations.

[00044] The key relationship that enables HD 2D VSFG spectroscopy to measure surface molecular orientation heterogeneity is that $\chi_{eff}^{(2)}$ and $\chi_{eff}^{(4)}$ can be expressed as a linear combination of $\langle \cos \theta \rangle$, $\langle \cos^3 \theta \rangle$ and $\langle \cos^5 \theta \rangle$:

$$\begin{aligned}\chi_{eff,i}^{(2)} &= a_i \langle \cos \theta \rangle + b_i \langle \cos^3 \theta \rangle \\ \chi_{eff,i}^{(4)} &= c_i \langle \cos \theta \rangle + d_i \langle \cos^3 \theta \rangle + e_i \langle \cos^5 \theta \rangle\end{aligned}\tag{0}$$

[00045] To find a unique (θ_0, σ) pair, first and second parameters D1 and D2, expressed as surfaces, are determined. D1 and D2 have different dependences on θ_0 and σ , and therefore a unique pair of θ_0 and σ can be determined from a unique pair of D1 and D2. D1 can be determined from heterodyne 1D VSFG spectra. The orientational parameter D2 = $\langle \cos^5 \theta \rangle / \langle \cos \theta \rangle$ can be accurately determined from HD 2D VSFG measurements, as is illustrated in the details below. It is additionally noted that when $\sigma = 0^\circ$, $D2 = D1^2$. Therefore this relation can also be used to test whether the narrow orientation distribution assumption is valid.

[00046] The different dependence of orientational parameters on θ_0 and σ is clearer when plotted as 3D surfaces, and thus Figs. 2A-2E illustrate a graphical method or solution for searching (θ_0, σ) pairs. That is, the surfaces D1 and D2 are used in the graphical technique to convert experimentally measured D' and D'' to a (θ_0, σ) pair.

[00047] Referring first to Fig. 2D, a 3D surface is plotted of D_1 and D_2 as a function of mean tilt angle θ_0 and orientation distribution σ . As indicated, D_1 is the top surface and D_2 is below D_1 . The projection of these surfaces on the D - σ plane is equivalent to Figure 1A. The D_1 and D_2 surfaces are plotted in the region of $0 \leq \theta_0 < 90^\circ$ and $0 < \sigma \leq 90^\circ$. The D - θ_0 - σ system can be divided into two regions: region I with $\sigma \leq 40^\circ$ and region II with $\sigma > 40^\circ$.

[00048] In region I, because of the unique values of D_1 and D_2 , a (θ_0, σ) pair can be unambiguously determined. For example, Figs. 2A-2C show the determination of a unique (θ_0, σ) pair for $D_1 (D') = 0.407$ and $D_2 (D'') = 0.19$. The way in which these values (0.407 and 0.19) were developed is described below. Two planes that are parallel to the θ_0 - σ plane are drawn at $D' = 0.407$ in the D_1 surface (Fig. 2A) and at $D'' = 0.19$ in the D_2 surface (Fig. 2B). The projections of both intersection lines on the θ_0 - σ plane are plotted in Fig. 2C and represent the qualified (θ_0, σ) pairs that have $D_1 = D'$ and $D_2 = D''$, and the intersection point ($\theta_0 = 52^\circ$, $\sigma = 11^\circ$) represents the unique (θ_0, σ) pair. When both intersection lines for D_1 and D_2 are determined together, there is only one intersection point ($\theta_0 = 52^\circ$, $\sigma = 11^\circ$), which represents the unique (θ_0, σ) pair that satisfies both $D_1 = D'$ and $D_2 = D''$.

[00049] In region II, D_1 and D_2 converge to 0.600 and 0.429 asymptotically, which makes them lose the one-to-one correlation with the (θ_0, σ) pair. Although the (θ_0, σ) pair cannot be uniquely determined in this region, the two asymptotic number pair ($D_1 = 0.600$ and $D_2 = 0.429$) are unique signatures for broad orientation distribution. One important consequence of this asymptotic pair is solving the above-mentioned ambiguity of “magic angle” when only $D_1 = 0.600$ is measured.

[00050] On the other hand, when D_1 and D_2 are both measured, if the interfacial molecules all tilt at 39.2° with a narrow distribution, D_1 should be 0.600 and D_2 should be 0.360; otherwise, with a broad angular distribution, D_1 and D_2 should be close to the signature values of 0.600 and 0.429, respectively (Fig. 1B). Therefore, there is no “magic angle” ambiguity when D_1 and D_2 are measured together.

[00051] In the following, and referring in addition to Fig. 3, it is illustrated how to extract a (θ_0, σ) pair by determining the (θ_0, σ) pair of $\text{Re}(4,4'$ -dicyano-2,2'-bipyridine) $(\text{CO})_3\text{Cl}$ monolayer self-assembled on a gold slide using its experimentally measured heterodyned 1D and 2D VSFG spectra. This example follows the model graphical technique of Fig. 2 and applies the same to the experimentally-measured sample.

[00052] In particular, Fig. 3 illustrates heterodyned 1D (red dots) and a diagonal cut of 2D (blue dots) VSFG spectra of Re(4,4'-dicyano-2,2'-bipyridine)(CO)₃Cl monolayer self-assembled on a gold slide. The 1D spectrum (indicated by the trace [1]) has been significantly broadened by surface inhomogeneity. Solid lines represent theoretical fitting. From the fitting, $\chi^{(2)}_{\text{eff}}$ and $\chi^{(4)}_{\text{eff}}$, can be determined, which are used later to determine experimentally D1 and D2. Inset in Fig. 3 is a depiction of the Re-complex on a gold surface. Capital Z is the surface normal and small z is the molecular C₃ axis. θ is the angle between them. In the Re(4,4'-dicyano-2,2'-bipyridine)(CO)₃Cl monolayer, the mean tilt angle between the surface normal and the C₃ axis of the three carbonyls was found to be $52^\circ \pm 2^\circ$ with an orientation distribution width of $11^\circ \pm 1^\circ$. This result suggests that the Re-complex forms an ordered layer with relative uniform orientations on the surface.

[00053] To determine the (θ_0, σ) pair, first D1 and D2 are evaluated from the effective second-order ($\chi^{(2)}_{\text{eff}}$) / fourth-order ($\chi^{(4)}_{\text{eff}}$) susceptibilities. The susceptibilities can be directly measured from 1D and 2D VSFG spectra, as measured by a VSFG spectrometer. One exemplary formula to extract D1, D2 from $\chi^{(2)}_{\text{eff}}$ and $\chi^{(4)}_{\text{eff}}$ is summarized in Eq.1:

$$\frac{\chi^{(2)}_{\theta 1}}{\chi^{(2)}_{\theta 2}} = \frac{a_1 + b_1 \langle \cos^3 \theta \rangle / \langle \cos \theta \rangle}{a_2 + b_2 \langle \cos^3 \theta \rangle / \langle \cos \theta \rangle} = \frac{a_1 + b_1 \cdot D_1}{a_2 + b_2 \cdot D_1} \quad (1)$$

$$\frac{\chi^{(4)}_{\theta 1}}{\chi^{(4)}_{\theta 2}} = \frac{c_1 + d_1 \cdot \langle \cos^3 \theta \rangle / \langle \cos \theta \rangle + e_1 \cdot \langle \cos^5 \theta \rangle / \langle \cos \theta \rangle}{c_2 + d_2 \cdot \langle \cos^3 \theta \rangle / \langle \cos \theta \rangle + e_2 \cdot \langle \cos^5 \theta \rangle / \langle \cos \theta \rangle} = \frac{c_1 + d_1 \cdot D_1 + e_1 \cdot D_2}{c_2 + d_2 \cdot D_1 + e_2 \cdot D_2}$$

[00054] Where a_i , b_i , c_i , d_i and e_i are constants that depend on molecular hyperpolarizabilities of the i^{th} vibrational mode, which can be determined using *ab initio* calculation or measured from, e.g., the Raman depolarization ratio. The detailed expressions of a_i , b_i , c_i , d_i and e_i in terms of hyperpolarizability are described below. In general, for any polarization combinations, $\chi^{(2)}_{\text{eff}}$ and $\chi^{(4)}_{\text{eff}}$ can be expressed as a linear combination of $\langle \cos \theta \rangle$, $\langle \cos^3 \theta \rangle$, and $\langle \cos^5 \theta \rangle$, and thus from the ratios of χ_{eff} between two vibrational modes, or ratios of χ_{eff} for a single vibrational mode under different polarization combinations, D1 and D2 can be extracted.

[00055] For the Re-complex monolayer, the ratios of χ_{eff} between two vibrational modes were determined by fitting the $A'(1)$ and $A'(2)$ peaks in the VSFG spectra (see Fig. 3). From the fitting, it was found that $\chi^{(2)}_{\text{eff}}[A'(1)]/\chi^{(2)}_{\text{eff}}[A'(2)] = -1.31 \pm 0.04$ and $\chi^{(4)}_{\text{eff}}[A'(1)]/\chi^{(4)}_{\text{eff}}[A'(2)] = -1.3 \pm 0.1$. In this measurement, as all beams were held at p polarization, and as the Fresnel factor on gold is strong in the Z direction, it was found that $\chi^{(2)}_{\text{eff}} \propto \chi^{(2)}_{\text{ZZZ}}$ and $\chi^{(4)}_{\text{eff}} \propto \chi^{(4)}_{\text{ZZZZZ}}$. Using Eq. 1 and the numerical value of hyperpolarizabilities calculated by density functional theory (DFT, B3LYP/LanL2DZ basis set, detailed methods and results below), the numerical relationships were derived between $\chi^{(2)}_{\text{ZZZ},1}/\chi^{(2)}_{\text{ZZZ},2}$, $\chi^{(4)}_{\text{ZZZZZ},1}/\chi^{(4)}_{\text{ZZZZZ},2}$, and D' , D'' . Using this expression, it was found that $D' = 0.407 \pm 0.005$ and $D'' = 0.19 \pm 0.01$, which is as noted above.

[00056] Next, the measured D' and D'' were used to extract all the qualified (θ_0, σ) pairs from the D1 and D2 surfaces using the graphical method described. With $D' = 0.407$ and $D'' = 0.19$, two planes are drawn that are parallel to the θ_0 - σ plane at $D' = 0.407$ and $D'' = 0.19$ to intersect D1 and D2 surfaces (Fig. 2A and 2B), separately. The projections of both intersection lines on that θ_0 - σ plane represent the qualified (θ_0, σ) pairs that have $D1 = D'$ and $D2 = D''$ (Fig. 2C), and the results agree that there are infinite combinations of (θ_0, σ) pairs to match a single D1 value. However, when both intersection lines for D1 and D2 are determined together, there is only one intersection point ($\theta_0 = 52^\circ$, $\sigma = 11^\circ$), which represents the unique (θ_0, σ) pair that satisfies both $D1 = D'$ and $D2 = D''$.

[00057] Taking into account the standard deviation of calculated D' and D'' (details below), the mean tilt angle between the surface normal and the C3 axis of the three carbonyls was $52^\circ \pm 2^\circ$ with an orientation distribution width of $11^\circ \pm 1^\circ$. This result suggests that the Re-complex forms a relatively ordered layer with non-negligible orientation distribution on the surface. In this case, if assuming a narrow orientation distribution, the mean tilt angle is calculated to be 50° , which is not too far from what is determined by systems and methods according to present principles, but there is no orientation distribution learned from the traditional method, which makes it difficult to quantify how organized the monolayer is.

[00058] The approach of current principles is general and can be applied to other polarization combinations. In addition, there are many other ways to extract D1 and D2 ratios, such as measuring 1D and 2D VSFG at various polarization combinations. Therefore, by

combining these established ways of determining D1 and D2, this method can be broadly applied to accurately determine the (θ_0, σ) pair.

[00059] The discrepancy of the mean tilt angle determined by using methods according to present principles as compared to the traditional 1D SFG method with a narrow distribution assumption can be very large. For instance, when $D' = 0.415$ is measured and the narrow distribution is assumed, one gets 50° . However, if with the same sample $D'' = 0.229$, it determines that the angle should be 60° , with distribution of 24° , which means that the angle determined from the narrow distribution assumption has a 17% systematic error.

[00060] An important aspect of retrieving or determining or calculating an accurate (θ_0, σ) pair is the measurement of D' and D'' , which is affected mostly by signal-to-noise ratio in the SFG measurement and molecular hyperpolarizabilities. This becomes more apparent when σ is relatively large, as both D1 and D2 converge to constant values and a small change in the D values could lead to a large uncertainty. However, the relation between D1 and D2 is restricted. For instance, a Gaussian distribution requires that $D1^2 \leq D2 \leq D1$. Therefore for any reliable measurements, this relation has to be satisfied, and measuring D' and D'' indeed provide a way to validate the measurement.

[00061] What has been shown is that, for the first time, the net orientation (mean tilt angle and orientation distribution) of molecules at interfaces can be unambiguously probed using a new combination of 1D and 2D VSFG. In a particular example, it was found that the monolayer formed by $\text{Re}(4,4'\text{-dicyano-2,2'\text{-bipyridine}})(\text{CO})_3\text{Cl}$ on a gold slide is fairly ordered but has a relatively broad distribution that cannot be neglected. This new advancement solves the orientation ambiguity when the net angle is determined only by 1D VSFG spectroscopy – a well-known and important issue for decades. With the growing popularity of 2D VSFG spectroscopy in the surface science community, this new method can contribute significantly in determining the molecular conformations of interfaces in materials, water, biological membranes and many other important interfaces. Furthermore, the intrinsic femtosecond time resolution grants this new method the potential to study time-dependent net orientation fluctuations in the ultrafast regime, which is a critical process for morphology changes in lipid membrane systems.

[00062] In one implementation, and referring to the system 10 diagram of Fig. 7, an exemplary implementation for measuring characteristics such as molecular orientation at a surface or interface of a sample 12 includes a system 14 used to determine a characteristic of the

sample, particularly if the same is unknown. For example, it may be desired to determine vibrational modes of the sample, and to identify the sample or a characteristic of a sample in this way. For example, a Fourier transform infrared spectrometer (FTIR) may be employed for this purpose.

[00063] Subsequent to the determination of the modes, an SFG spectrometer 16 may be employed as noted above. The SFG spectrometer generally includes a 2-D heterodyne SFG spectrometer.

[00064] A flowchart 20 shown in Fig. 8 details a general method of an implementation of a method according to present principles. In a first step, if a material is not known, the same may be partially identified by determining vibrational modes (step 18), e.g., using the FTIR noted above. For example, the infrared spectrum of a sample may be measured. Once this is measured, the sample may be measured by an SFG spectrometer as noted above, e.g., a 2D VSFG spectrometer, and measurements taken (step 22). In some cases, additional spectrum measurements may be performed (step 24), for example, with different polarization combinations.

[00065] Once this is done, spectral fitting may be performed to determine the peak intensity (step 26). Density functional theory (DFT) calculations may be performed to determine one or more coefficients used in subsequent calculations of the first and second parameters, e.g., surfaces, i.e., D' and D'' (step 28).

[00066] Then, the surfaces D and D_2 may be calculated using a modified Gaussian model. The surfaces D_1 and D_2 are generally described by 3-D surfaces and correspond to a mean tilt angle and orientation distribution, respectively.

[00067] To describe the molecular orientation of monolayers, a Gaussian distribution is a common model to use. More complex orientational distribution models may also be employed, but the Gaussian distribution model provides a basic description of the orientation heterogeneity of the molecular monolayers. As noted, in the Gaussian distribution model, two physical quantities need to be measured – the mean tilt angle θ_0 and the orientational distribution σ , which are referred to as the (θ_0, σ) pair or orientation heterogeneity hereafter.

[00068] From the surfaces D_1 and D_2 , the graphical technique described above may be employed. For example, a unique D' and D'' may be determined using Eq.1 (step 34). Planes

may be calculated corresponding to the D' and D'' (step 36). Such planes are generally parallel to the (θ_0, σ) plane.

[00069] Curves may then be determined (step 38) corresponding to the intersection of these calculated planes (the planes corresponding to D' and D'') with the surfaces, i.e., surfaces D1 and D2. An intersection may then be determined (step 42) of the determined curves, the intersection corresponding to the desired (θ_0, σ) pair.

[00070] The below description describes the vibrational modes investigated in this work, a brief description of the Euler transform between the lab and molecular frame, DFT calculations for the transition dipole moment, the Raman Tensor for the coefficient used in Eq. (1), the non-linear susceptibility, and a discussion of how to calculate the errors for mean tilt angle and distribution width.

Three Stretching Modes Related to Carbonyls in the Re-complex.

[00071] The three stretching modes related to carbonyls in the Re(4,4'-dicyano-2,2'-bipyridine)(CO)₃Cl molecule are shown in Figs. 4A-4C, and are labeled as A'(2), A'' and A'(1) with center frequencies of 1911, 1931 and 2020 cm⁻¹ in 2D SFG spectra. A'(2) is an out-of-phase symmetric stretch, A'' is an anti-symmetric stretch, and A'(1) is an in-phase symmetric stretch. In the above description A'(1) and A'(2) modes were primarily analyzed.

SFG Spectra Fitting.

[00072] The 1D and 2D SFG spectra may be fitted using the following equation:

$$\chi = \sum_{i=1}^3 \frac{B_i \gamma_i}{(\omega - \omega_i)^2 + \gamma_i^2}$$

Eq. 2

where B_i , ω and γ_i represent the amplitude, center frequency and peak width of the i^{th} vibrational mode, respectively. All the fitting parameters are shown in table 1.

$\chi_{\text{eff}}^{(2)}[A'(1)]/\chi_{\text{eff}}^{(2)}[A'(2)]$ and $\chi_{\text{eff}}^{(4)}[A'(1)]/\chi_{\text{eff}}^{(4)}[A'(2)]$ are calculated using the following equation:

$$\frac{\chi_{\text{eff}}[A'(1)]}{\chi_{\text{eff}}[A'(2)]} = \left(\frac{B[A'(1)]}{\gamma[A'(1)]} \right) / \left(\frac{B[A'(2)]}{\gamma[A'(2)]} \right)$$

Eq. 3

Table 1. Fitting Parameters for 1D and 2D Heterodyned SFG Spectra.

Mode	A'(2)			A''			A'(1)		
	B	ω	γ	B	ω	γ	B	ω	γ
1D	31.3(6)	1912	52.8(7)	11.3(4)	1941	29.7(7)	-48.1(5)	2011	61.8(6)
2D	2.5(2)	1911	10.3(7)	0.7(1)	1931	9(2)	-3.05(9)	2020	9.7(4)

Modified Gaussian Distribution.

[00073] D1 and D2 3D surfaces are calculated by a modified Gaussian model. The representations are shown as follows:

$$f(\theta) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(\theta-\theta_0)^2}{2\sigma^2}}$$

Eq. 4

$$f'(\theta) = \sum_{n=-4}^4 f(2\pi n + \theta) + f(2\pi n - \theta)$$

Eq. 5

where θ_0 is the mean tilt angle and σ describes the distribution width. The net distribution function $f'(\theta)$, which is valid in the range from 0 to π , is developed from the normal Gaussian function $f(\theta)$. The values of $\langle \cos \theta \rangle$, $\langle \cos^3 \theta \rangle$ and $\langle \cos^5 \theta \rangle$ at different (θ_0, σ) pairs are calculated using Matlab through the following equation:

$$\langle \cos^m \theta \rangle = \int_0^\pi \cos^m \theta \cdot f'(\theta) \cdot \sin \theta d\theta$$

Eq. 6

where $\sin \theta$ is a weighting function accounting for the fact that molecules may orient in both the XZ and YZ planes.

Euler Transformation between Laboratory Coordinate Frame and Molecular Coordinate Frame

[00074] The three carbonyls in the Re-complex have an approximated C_{3v} symmetry, and the transition dipole moments of the three stretching modes are approximately perpendicular to

each other. As shown in Figure 5, which shows the relationship between the XYZ frame and the xyz frame, in the molecular coordinate frame, the z axis is defined along the transition dipole moment of the $A'(1)$ mode, which also coincides with the approximated C_3 symmetry axis. Molecular x and y axes are defined along the transition dipole moments of $A'(1)$ and A'' modes, respectively. In the laboratory coordinate frame, the Z axis is defined along the surface normal and the X axis is in the incidence plane perpendicular to Z . The relationship between the XYZ and xyz frame are described by three angles: tilt angle θ , in-plane rotation angle ϕ and twist angle φ .

[00075] In the SFG measurements, all beams are held at p polarization. As the Fresnel factor is much larger in the Z direction compared with those in X and Y directions for all beams, both effective second-order ($\chi_{eff}^{(2)}$) and fourth-order ($\chi_{eff}^{(4)}$) susceptibilities are dominated by a single susceptibility tensor element:

$$\chi_{eff}^{(2)} \approx L_{ZZ}(\omega_1)L_{ZZ}(\omega_2)L_{ZZ}(\omega_3)\sin\beta_1\sin\beta_2\sin\beta_3\chi_{ZZZ}^{(2)}$$

Eq. 7

$$\chi_{eff}^{(4)} \approx L_{ZZ}(\omega_1)L_{ZZ}(\omega_2)L_{ZZ}(\omega_3)L_{ZZ}(\omega_4)L_{ZZ}(\omega_5)\sin\beta_1\sin\beta_2\sin\beta_3\sin\beta_4\sin\beta_5\chi_{ZZZZZ}^{(4)}$$

Eq. 8

where $L_{ZZ}(\omega_i)$ and β_i are the Fresnel factor and incidence/reflection angle of the i^{th} beam. $\chi_{ZZZ}^{(2)}$ and $\chi_{ZZZZZ}^{(4)}$ are related to second-order ($\beta^{(2)}$) and fourth-order ($\beta^{(4)}$) molecular polarizabilities through the following equations:

$$\chi_{ZZZ}^{(2)} = N\langle\beta^{(2)}\rangle = N\sum_{ijk}\langle R_{Zi}R_{Zj}R_{Zk}\rangle\beta_{ijk}^{(2)}$$

Eq. 9

$$\chi_{ZZZZZ}^{(4)} = N\langle\beta^{(4)}\rangle = N\sum_{ijklm}\langle R_{Zi}R_{Zj}R_{Zk}R_{Zl}R_{Zm}\rangle\beta_{ijklm}^{(4)}$$

Eq. 10

where $i, j, k, l, m = x, y, z$; N is the number density of Re-complex on the gold surface, the bracket means taking the orientationally-averaged value, and R is the element of the Euler transformation matrix based on the geometry defined in Figure 5.

[00076] Assuming Re-complex has uniform distribution in the in-plane rotation angle ϕ and twist angle φ , Eq. 9 - 10 can be simplified for mode $A'(1)$ and $A'(2)$, which are employed to determine the orientational parameters D_1 and D_2 . For $A'(1)$ mode,

$$\chi_{zzz}^{(2)}[A'(1)] = N \left[\left(\frac{\beta_{xxz}^{(2)} + \beta_{yyz}^{(2)}}{2} \right) \langle \cos \theta \rangle + \left(\beta_{zzz}^{(2)} - \frac{\beta_{xxz}^{(2)} + \beta_{yyz}^{(2)}}{2} \right) \langle \cos^3 \theta \rangle \right]$$

Eq. 11

$$\chi_{zzzz}^{(4)}[A'(1)] = N \left[\left(\frac{\beta_{xxzz}^{(4)} + \beta_{yyzz}^{(4)}}{2} \right) \langle \cos^3 \theta \rangle + \left(\beta_{zzzz}^{(4)} - \frac{\beta_{xxzz}^{(4)} + \beta_{yyzz}^{(4)}}{2} \right) \langle \cos^5 \theta \rangle \right]$$

Eq. 12

For A'(2) mode,

$$\chi_{zzz}^{(2)}[A'(2)] = N \left[\left(\beta_{xzx}^{(2)} \right) \langle \cos \theta \rangle + \left(-\beta_{xzx}^{(2)} \right) \langle \cos^3 \theta \rangle \right]$$

Eq. 13

$$\chi_{zzzz}^{(4)}[A'(2)] = N \left[\left(\frac{3\beta_{xzxzx}^{(4)}}{4} \right) \langle \cos \theta \rangle + \left(-\frac{3\beta_{xzxzx}^{(4)}}{2} \right) \langle \cos^3 \theta \rangle + \left(\frac{3\beta_{xzxzx}^{(4)}}{4} \right) \langle \cos^5 \theta \rangle \right]$$

Eq. 14

[00077] By rearranging these equations above, Eq.1 can be obtained. The coefficient of Eq.1 dependence on $(\beta^{(2)})$ and $(\beta^{(4)})$, is calculated using the DFT method described below and examined by Raman spectroscopy.

[00078] Calculation of Molecular Hyperpolarizability Tensor Elements. Energy and geometry optimization of Re-complex molecule were performed using the B3LYP functional and the LANL2TZ basis set with the Gaussian 09 software package. Dipole derivatives $(\partial\mu/\partial Q_q)$ and polarizability derivatives $(\partial\alpha/\partial Q_q)$ of each vibrational mode were obtained using the keyword “polar” and “iop(7/33=1)”. The corresponding $\beta^{(2)}$ and $\beta^{(4)}$ is calculated by

$$\beta_{ijk}^{(2)} = -\frac{1}{2\varepsilon_0\omega} \frac{\partial\alpha_{ij}}{\partial Q_q} \frac{\partial\mu_k}{\partial Q_q}$$

$$\beta_{ijklm}^{(4)} = -\frac{1}{2\varepsilon_0\omega^3} \frac{\partial\alpha_{ij}}{\partial Q_q} \frac{\partial\mu_k}{\partial Q_q} \frac{\partial\mu_l}{\partial Q_q} \frac{\partial\mu_m}{\partial Q_q}$$

Eq. 15

Where i, j, k correspond to the x,y,z direction in molecular (xyz) frame in Fig.5. The $\partial\mu/\partial Q_q$ and $\partial\alpha/\partial Q_q$ values for A'(2) and A'(1) modes are shown in Table 2.

[00079] **Table 2.** Dipole Derivatives ($\partial\mu/\partial Q_q$) and Polarizability Derivatives ($\partial\alpha/\partial Q_q$) for A'(1) and A'(2) Modes Determined from *ab initio* Calculation.

Mode 74 (A'(2)):

Dipole derivatives: 3.07650D+01 3.89478D-03 -3.04117D+00

Polarizability derivatives:

-0.988878D+00 0.505546D-03 0.241308D+01

0.505546D-03 0.197090D+01 -0.338132D-03

0.241308D+01 -0.338132D-03 0.228602D+01

Mode 76 (A'(1)):

Dipole derivatives: -4.24616D+00 1.33888D-03 -3.61394D+01

Polarizability derivatives:

-0.970416D+00 -0.419215D-04 0.117419D+01

-0.419215D-04 0.141946D+01 0.173584D-03

0.117419D+01 0.173584D-03 0.440365D+01

Measuring Depolarization Ratio Using Polarized Raman Spectroscopy

[00080] The fidelity of transition dipole moment and the Raman tensor calculation is examined by comparing the calculated depolarization ratio to the experimentally-measured value from Raman spectroscopy. Polarized off-resonance Raman spectra were acquired with 647.09 nm (15,454 cm⁻¹) excitation, produced by a mixed krypton/argon gas-ion laser (Laser Innovations/Coherent; Innova 70C Spectrum). The wavelength was selected with an intracavity prism, and further isolated with a 647.1 nm bandpass filter (Semrock, MaxLine) placed in the beam path near the output of the laser. A right-angle geometry was used for the excitation and detection of scattered light. A spherical lens with focal length 80 mm was used to focus the laser beam into the bottom of a 1×1 cm fluorescence cuvette that contained solvents for calibrations,

or solutions of $\text{Re}(\text{4,4'}\text{-dicyano-2,2'}\text{-bipyridine})(\text{CO})_3\text{Cl}$. Raman scattering was collected and collimated with an F/1.2 camera lens (Canon FD 85 mm). A doublet achromat with 300 mm focal length (CVI-Melles Griot, 01LAO667) focused the light at the entrance slit of the spectrograph. Three optics were placed between the 300 mm lens and the entrance slit. First, a dichroic polarizer (OptoSigma, 069-0120) mounted in a rotation stage was used to analyze the parallel (horizontally-polarized) and perpendicular (vertically-polarized) Raman scattered light. Second, a two-piece quartz wedge (OptoSigma, 068-6770) scrambled the polarization to compensate for different efficiencies of the spectrograph to parallel and perpendicular light. Third, a 647.1 nm long-pass filter (Semrock, RazorEdge) rejected the excitation line. The spectrograph (JY Horiba, iHR320) had a 320 mm focal length, and a single holographic grating with 1200 gr/mm groove density, 500 nm blaze. The detector was an open-electrode CCD (JY Horiba, Synapse). Raman shifts were calibrated based on six emission lines from a neon lamp (Oriol) and several Raman bands, from a 50:50 mixture of acetonitrile and toluene.

[00081] The rotation of the polarizer was optimized while collecting one-second spectra of carbon tetrachloride (Sigma Aldrich, $\geq 99.9\%$ pure). The entrance slit of the spectrograph was set to 50 μm (resolution $\sim 4\text{ cm}^{-1}$). After optimized positions were found, perpendicular and parallel spectra were recorded. The depolarization ratio of the 314 cm^{-1} and 459 cm^{-1} bands were determined by integrating the bands (perpendicular/parallel) and were 0.75 and <0.01 , respectively. These depolarization ratios matched known values. Repeat measurements of CCl_4 before and after collection of the rhenium samples yielded consistent depolarization ratios. Additionally, to check the performance of the system further in the red ($\sim 720\text{ nm}$), Raman spectra of benzene were collected and the $1585+1604\text{ cm}^{-1}$ bands were analyzed. This pair of bands had a depolarization ratio of 0.75, as expected.

[00082] The depolarization ratios of $\text{Re}(\text{4,4'}\text{-dicyano-2,2'}\text{-bipyridine})(\text{CO})_3\text{Cl}$ were recorded in DMSO solution at two concentrations, 2 mM and 26 mM (Table 3). The laser power at the sample was 45 mW, and the entrance slit was 100 μm . Each polarization was collected for a total of 10 – 20 minutes, and solvent-only spectra were also acquired for the same time. Spectra were processed using Igor Pro (WaveMetrics). Peaks caused by cosmic rays were removed manually from 1- or 2-minute acquisitions, and the acquisitions for the full collection time were then averaged. The solvent bands were subtracted from the spectra of $\text{Re}(\text{4,4'}\text{-dicyano-2,2'}\text{-bipyridine})(\text{CO})_3\text{Cl}$ solutions by appropriate scaled subtraction (Figure 6). A broad luminescence

background was removed from the solvent-free spectra using a spline fit. In particular, Fig. 6 illustrates polarized Raman spectra of $\text{Re}(4,4'\text{-dicyano-2,2'}\text{-bipyridine})(\text{CO})_3\text{Cl}$ in DMSO (26 mM) acquired with 647.09 nm excitation. Spectra are shown after subtraction of solvent bands and a broad luminescence background. The y-axis units are counts per 120-second acquisition time, and each of the two spectra are the average of 10 acquisitions (total collection time 20 minutes per spectrum). The perpendicular spectrum is offset +90,000 counts.

[00083] The B3LYP/LanL2TZ functional/basis set calculates the depolarization ratio of the $A'(1)$ and $A'(2)$ modes to be 0.34 and 0.50, respectively, which matches with the experimental measured ratios : 0.44 and 0.55, best.

[00084] **Table 3.** Depolarization Ratios of Selected Bands of $\text{Re}(4,4'\text{-dicyano-2,2'}\text{-bipyridine})(\text{CO})_3\text{Cl}$ at Two Different Concentrations. Values are determined by integrating the bands of polarized Raman spectra acquired with 647.09 nm excitation. A depolarization ratio of 2mM was used above.

	$A'(2)$	A''	$A'(1)$
2 mM	0.55	0.58	0.44
26 mM	0.59	0.57	0.45

[00085] Additional details of HD 2D SFG spectroscopy are provided below.

[00086] HD 2D SFG spectrum is collected in a pump-probe geometry, with additional narrow band (fwhm~1.5nm) 800nm pulse for the SFG process. Three mid-IR pulses are sent to interact with the molecular sample, where two vibrational coherences are created during t_1 and t_3 period, and the picosecond 800nm pulse is used to interact with the second vibrational coherence for sum frequency generation process. During the mid-IR pulse interactions, two vibrational coherences are generated during t_1 and t_3 periods, respectively. The first coherence is measured by scanning the t_1 time from 0 to 2500 fs in steps of 20 fs using the pulse shaper (PhaseTech), where a rotating frame at $f_0=1583\text{ cm}^{-1}$ is used to shift the oscillation period to 80 fs, so that the scanning step can meet with the Nyquist frequency requirement. To remove scatter and 1D SFG signal, instead of taking the difference between pump on and off SFG spectra, the difference SFG spectra at the same t_1 but with different pump pulse phase are recorded, which is known as phase cycling. The second vibrational coherence is upconverted to a virtue state by a picosecond 800 nm pulse and subsequently emits visible signals through sum frequency generation process. Since the 800 nm serves as a window function, the t_3 time delay is simultaneously covered by the

upconversion process and the 800 nm pulse duration determines how long t_3 is “scanned”. The SFG signals are heterodyned by the local oscillator from non-resonance signal from gold surface and experimentally Fourier transformed by a spectrograph and detected by a CCD camera (400×1,340, Andor). To get full 2D absorptive SFG spectra, the first vibrational coherence is numerical Fourier transformed into frequency domain. The HD 1D SFG spectra can be extracted by adding the two phase cycled pump probe spectra together, where the 4th order signals cancel out and only leaves the 2nd order HD 1D SFG spectra. SFG of bare gold was also collected as the reference for phase calibrations. The HD 2D SFG signal is measured at ppppp polarization, where the polarizations of all pulses are set to be p (in plane with the surface normal-incidence beam plane) to the sample, by pairs of waveplates and polarizers, and only p-polarized signal is detected. Gold surfaces are used as the substrate, which generate the non-resonance SFG signal. The gold SFG signal can interfere with the SFG signal of interests. Therefore, in principle, both experiments are self-heterodyned. The samples are constantly rastered between each scan (~10 min) to avoid sample damaging. To improve signal to noise ratio, multiple scans are averaged for each time step.

[00087] It is noted that although the orientational distribution of the Re-catalysts studied here is narrow and the mean tilt angle measured using HD 2D VSFG is essentially the same as the result from heterodyne 1D VSFG when a narrow distribution is assumed, systems and methods according to present principles are further able to make the experimental determination that the Re-catalysts form a well-ordered monolayer on the surface, which is a new capability complementary to 1D VSFG. In addition, as noted above, the discrepancy of the mean tilt angle determined using the systems and methods and that determined using 1D SFG method can be large, and systems and methods according to present principles can unambiguously resolve the error.

[00088] It is noted that there are at least three important aspects that are influential to the orientation heterogeneity measurement. First, to properly measure surface molecular orientation heterogeneity, it is important to implement heterodyne detection, rather than homodyne detection. In heterodyne and homodyne detections, the measured 2D VSFG signals can be expressed as:

$$\begin{aligned} S_{\text{heterodyne}} &\approx E_{2DVSFG} * E_{LO} \propto \chi_{\text{eff}}^{(4)} \\ S_{\text{homodyne}} &\approx E_{2DVSFG} * E_{1DVSFG} \propto \chi_{\text{eff}}^{(4)} * \chi_{\text{eff}}^{(2)} \end{aligned} \quad (16)$$

[00089] When heterodyne detection is used, the measured signal is proportional to $\chi^{(4)}_{eff}$, but when homodyne detection is used, the 1D VSFG signal essentially acts as a local oscillator to heterodyne the 2D VSFG signal. Therefore, the measured signal is proportional to $\chi^{(4)}_{eff} * \chi^{(2)}_{eff}$. As a result, it is difficult to disentangle these two terms and determine the $\chi^{(4)}_{eff}$ ratio from homodyne 2D VSFG.

[00090] Second, the values of D_1 and D_2 are the important aspects in retrieving an accurate (θ, σ) pair, which is affected mostly by the signal-to-noise ratio (S/N) in the measurements. This becomes most apparent when σ is relatively large, making both D_1 and D_2 converge and a small change in the D values could lead to a large difference. In measurements described here, the S/N of HD 2D VSFG is about 20, which leads to the same order of magnitude of uncertainty in the spectral fitting mentioned above. Therefore, the experimental noise does not lead to additional uncertainty in the orientation heterogeneity measurements. It is reiterated, however, that the relation between D_1 and D_2 is restricted. For instance, a Gaussian distribution requires that $D_1^2 \leq D_2 \leq D_1$. This relation has to be satisfied if a Gaussian distribution is appropriate to describe the orientation heterogeneity of the interfacial molecules; otherwise, other models have to be employed.

[00091] Third, another source of uncertainty comes from the value of hyperpolarizability. Here, high level DFT calculations were used to determine the hyperpolarizability, which is a common approach used in Raman and 1D VSFG spectroscopic studies. The accuracy of hyperpolarizability and the resulted (θ, σ) pair depends on the choice of basis sets. To evaluate that, basis sets were tested at different levels. The results of hyperpolarizability, Raman depolarization ratio, and corresponding (θ, σ) pair is summarized in Table 4. It was found that the calculated hyperpolarizability converges to a level where the variation of hyperpolarizabilities from different basis sets does not make significant changes to the (θ, σ) pair. Another alternative for determining hyperpolarizability is to derive it from the experimental Raman depolarization ratio. However, this method is limited to symmetric vibrational modes with perfect C_{3v} or C_{∞} symmetry. Since the vibrational modes of Re-complex studied here do not have a perfect C_{3v} symmetry, the Raman depolarization approach cannot be directly applied for this study. Nevertheless, a comparison between the experimental measured and the DFT calculated depolarization ratios could indicate the accuracy of the hyperpolarizability calculation. In this work, it was found that among all functional/basis set combinations tried, the

B3LYP/LanL2TZ functional/basis set calculates the depolarization ratio of the A'(1) and A'(2) modes to be 0.34 and 0.50, respectively, which matches with the experimental measured ratios : 0.44 and 0.55, best. (Table 4). Other implementations may include determining hyperpolarizabilities of complex molecules that lack rigorous symmetries.

[00092] **Table 4.** D_1 , D_2 , θ_0 and σ Determined Using Different Functionals and Basis Sets.

	Experimental value	B3LYP/LanL2TZ	B3LYP/Def2QZVP	B3LYP/Def2TZVP	M06L/LanL2TZ	M06L/Def2QZVP	M06L/Def2TZVP
$\rho_{A'(1)}^*$	0.44	0.35	0.32	0.33	0.33	0.30	0.31
$\rho_{A'(2)}^*$	0.55	0.50	0.49	0.50	0.51	0.51	0.51
D_1	/	0.364	0.335	0.337	0.336	0.331	0.331
D_2	/	0.14	0.13	0.13	0.14	0.12	0.13
θ_0	/	53°	56°	56°	58°	56°	57°
σ	/	5°	9°	9°	12°	7°	10°

*Depolarization ratio.

[00093] What has been shown includes that the surface molecular mean tilt angle and orientation distribution can be determined from the same measurement, which largely improves the fidelity and accuracy of the measurement. Such is far more accurate and unambiguous than existing techniques, which have to assume a narrow angular distribution of interfacial molecules to determine the surface orientations, and which result in ambiguity in tilt angle measurement.

[00094] While a specific implementation has been described above, variations and applications of systems and methods according to present principles will be understood to one of ordinary skill in the art given this teaching. For example, systems and methods can benefit nanometal level thin film surface characterization fields in the semiconductor industry, as well as surface molecular level diagnostic/analytical procedures in biomedical fields and research, as well as numerous other fields.

[00095] The system and method, including data analysis to determine plane placement, plotting of the projected intersection line, determination of intersection of D1 and D2, and other processing steps may be fully implemented in any number of computing devices. Typically, instructions are laid out on computer readable media, generally non-transitory, and these instructions are sufficient to allow a processor in the computing device to implement the method of the invention. The computer readable medium may be a hard drive or solid state storage having instructions that, when run, are loaded into random access memory. Inputs to the application, e.g., from the plurality of users or from any one user, may be by any number of appropriate computer input devices. For example, users may employ a keyboard, mouse,

touchscreen, joystick, trackpad, other pointing device, or any other such computer input device to input data relevant to the calculations. Data may also be input by way of an inserted memory chip, hard drive, flash drives, flash memory, optical media, magnetic media, or any other type of file – storing medium. The outputs may be delivered to a user by way of a video graphics card or integrated graphics chipset coupled to a display that may be seen by a user. Alternatively, a printer may be employed to output hard copies of the results. Given this teaching, any number of other tangible outputs will also be understood to be contemplated by the invention. For example, outputs may be stored on a memory chip, hard drive, flash drives, flash memory, optical media, magnetic media, or any other type of output. It should also be noted that the invention may be implemented on any number of different types of computing devices, e.g., personal computers, laptop computers, notebook computers, net book computers, handheld computers, personal digital assistants, mobile phones, smart phones, tablet computers, and also on devices specifically designed for these purpose. In one implementation, a user of a smart phone or WiFi – connected device downloads a copy of the application to their device from a server using a wireless Internet connection. An appropriate authentication procedure and secure transaction process may provide for payment to be made to the seller. The application may download over the mobile connection, or over the WiFi or other wireless network connection. The application may then be run by the user. Such a networked system may provide a suitable computing environment for an implementation in which a plurality of users provide separate inputs to the system and method. In the described system where various quantities including tilt angle and distribution are calculated, the plural inputs may allow plural users to input relevant data at the same time.

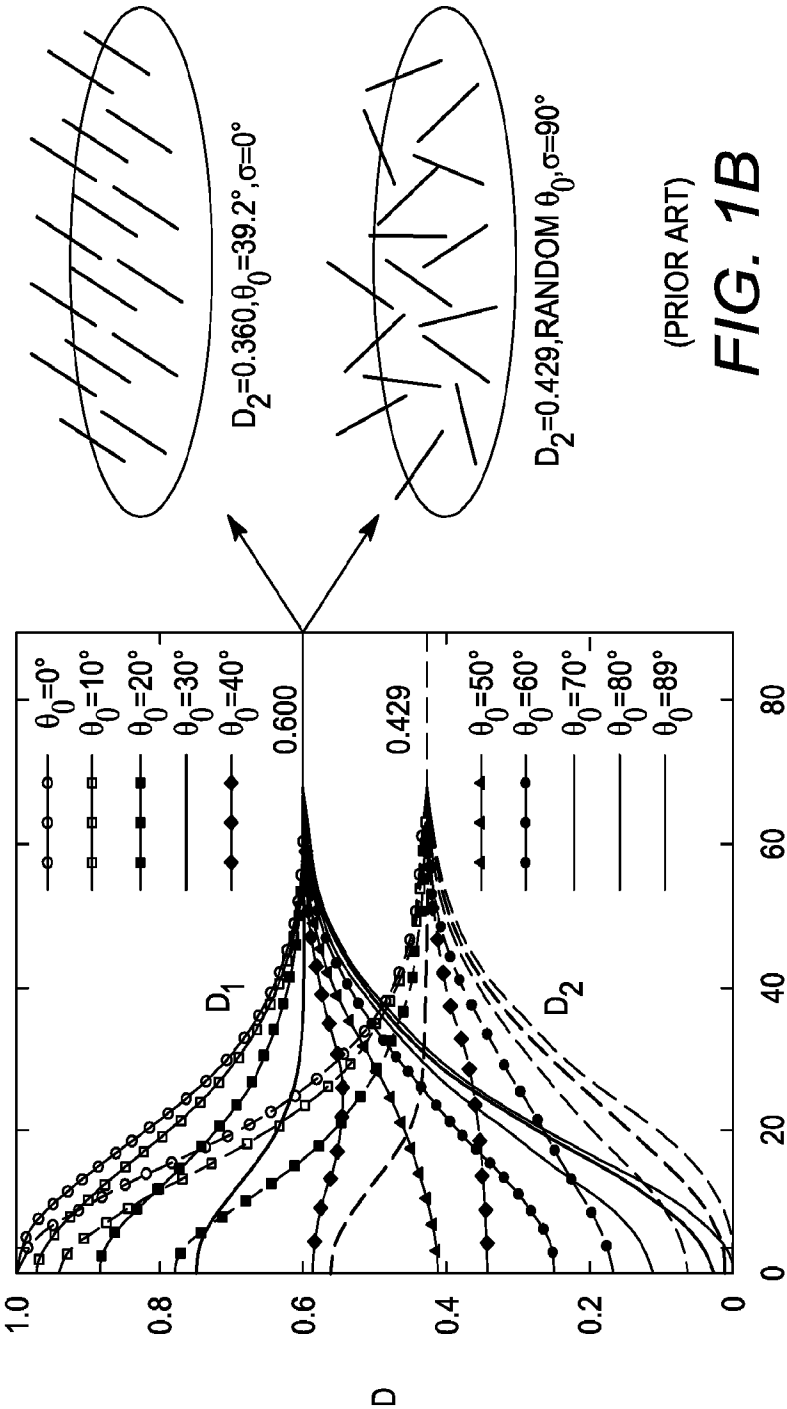
CLAIMS

1. A method of determining molecular orientation at interfaces, the molecular orientation including mean tilt angle (θ_0) and orientation distribution (σ), comprising:
 - a. measuring vibrational spectral peak intensities of a sample using 2D heterodyne sum frequency generation spectroscopy;
 - b. calculating surfaces D1 and D2 using a modified Gaussian model, wherein the surface D1 corresponds to mean tilt angle and is a function of tilt angle and orientation and wherein the surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation;
 - c. calculating values of D' and D'', wherein D' and D'' are calculated based at least in part on a measured effective second order susceptibility, a measured effective fourth order susceptibility, or a combination of the two susceptibilities;
 - d. calculating planes, parallel to a θ_0 - σ plane, corresponding to D' and D'';
 - e. determining curves corresponding to the intersection of the planes with the surfaces; and
 - f. determining an intersection of the determined curves, the intersection corresponding to a desired θ_0 - σ pair, whereby the desired θ_0 - σ pair indicates an experimentally measured molecular orientation and distribution of the sample.
2. The method of claim 1, wherein the determined D' and D'' are further based on coefficients that depend on molecular hyperpolarizabilities of the ith vibrational mode.
3. The method of claim 1, wherein the values of D' and D'' are based at least in part on a ratio of second and fourth order susceptibilities.
4. The method of claim 1, wherein the second and fourth order susceptibilities are calculated by performing spectral fitting to measured 1D VSFG spectra and 2D VSFG spectra.
5. The method of claim 1, wherein the values of D' and D'' are based at least in part on an experimental measurement.

6. The method of claim 1, wherein the effective second order susceptibility and the effective fourth order susceptibility are expressed as a linear combination of $\langle \cos\theta \rangle$, $\langle \cos^3\theta \rangle$, and $\langle \cos^5\theta \rangle$.
7. The method of claim 1, wherein the determined D' and D'' are further based on a ratio of an effective susceptibility between two vibrational modes, or on a ratio of effective susceptibilities for a single vibrational mode under different polarization combinations.
8. The method of claim 7, wherein the ratio of effective susceptibility between two vibrational modes is determined by measuring and analyzing or fitting peaks in the 2D heterodyne SFG spectroscopy spectra.
9. The method of claim 1, wherein further comprising validating a result by determining if $D1^2 \leq D2 \leq D1$, wherein if this relationship is satisfied, the result is validated.
10. The method of claim 1, further comprising repeating the steps for a plurality of subsequent time intervals, wherein the time intervals are spaced to allow a femtosecond time resolution of time-dependent molecular orientation fluctuations
11. A non-transitory computer readable medium, comprising instructions for causing a computing environment to perform the method of claim 1.
12. A method of determining molecular orientation at interfaces, the molecular orientation including mean tilt angle (θ_0) and orientation distribution (σ), comprising:
 - a. using 1D and 2D heterodyne sum frequency generation spectroscopy on a sample, determining surfaces D1 and D2, the surfaces D1 and D2 described by 3-D surfaces and determined using a modified Gaussian model, wherein the surface D1 corresponds to mean tilt angle and is a function of tilt angle and orientation, and wherein the surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation, the surfaces

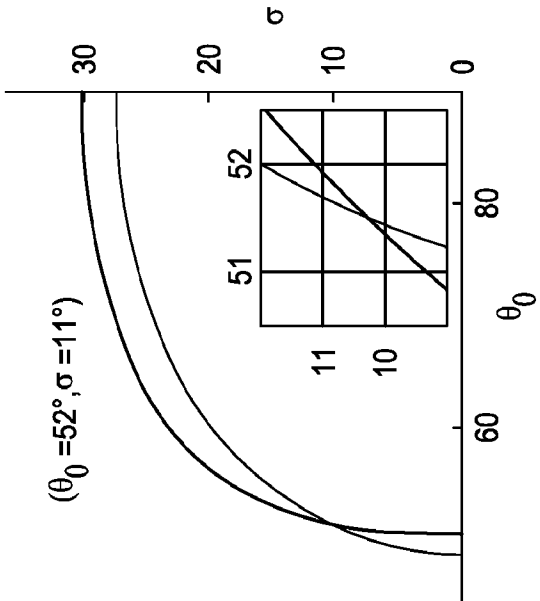
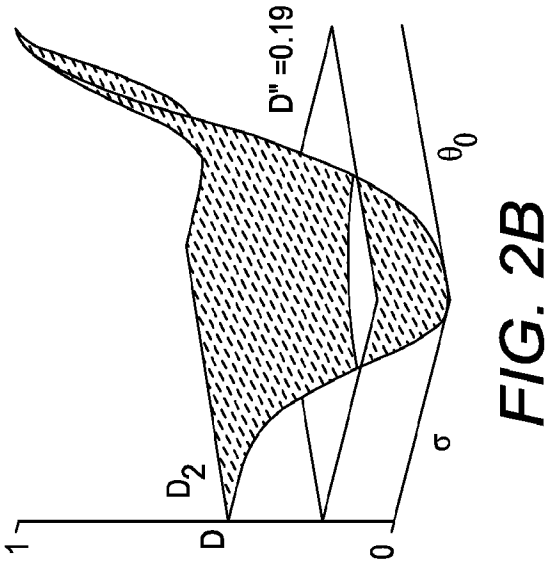
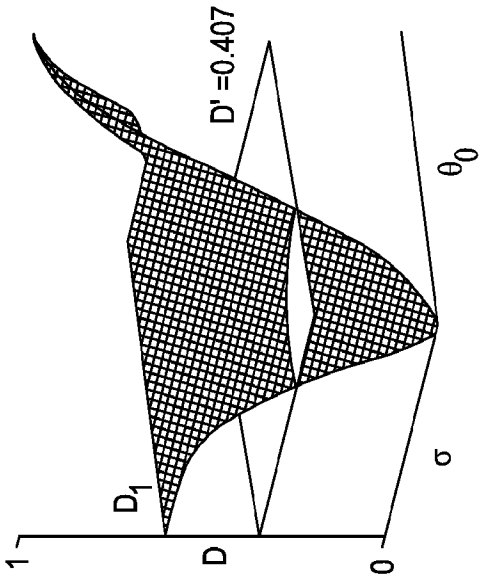
- D1 and D2 calculated from performing the sum frequency generation spectroscopy at various polarization combinations;
- b. determining values of D' and D'' , wherein the D' and D'' are calculated based at least in part on the ratio of second and fourth order susceptibilities;
 - c. calculating planes, parallel to a θ_0 - σ plane, corresponding to D' and D'' ;
 - d. determining curves corresponding to the intersection of the planes with the surfaces; and
 - e. determining an intersection of the determined curves, the intersection corresponding to a desired θ_0 - σ pair, whereby the desired θ_0 - σ pair indicates a molecular orientation and distribution of the sample.
13. The method of claim 12, wherein the values of D' and D'' are calculated further based on values from an experimental measurement.
14. A non-transitory computer readable medium, comprising instructions for causing a computing environment to perform the method of claim 12.
15. A method of determining molecular orientation at surfaces or interfaces, the molecular orientation including mean tilt angle (θ_0) and orientation distribution (σ), comprising:
- a. performing 2D heterodyne SFG spectroscopy on a sample to obtain a spectrum including one or more peaks;
 - b. determining two surfaces D1 and D2 using a modified Gaussian model and the obtained spectrum, the surfaces D1 and D2 described by 3-D surfaces, the surfaces determined at least in part using a modified Gaussian model, wherein the surface D1 corresponds to mean tilt angle and is a function of tilt angle and orientation, and wherein the surface D2 corresponds to both mean tilt angle and orientation distribution and is a different function of tilt angle and orientation;
 - c. performing spectral fitting on the obtained spectrum to determine at least the peak intensity and second order and fourth order nonlinear susceptibilities;
 - d. calculating, using density functional theory, one or more coefficients for determining D' and D'' ;

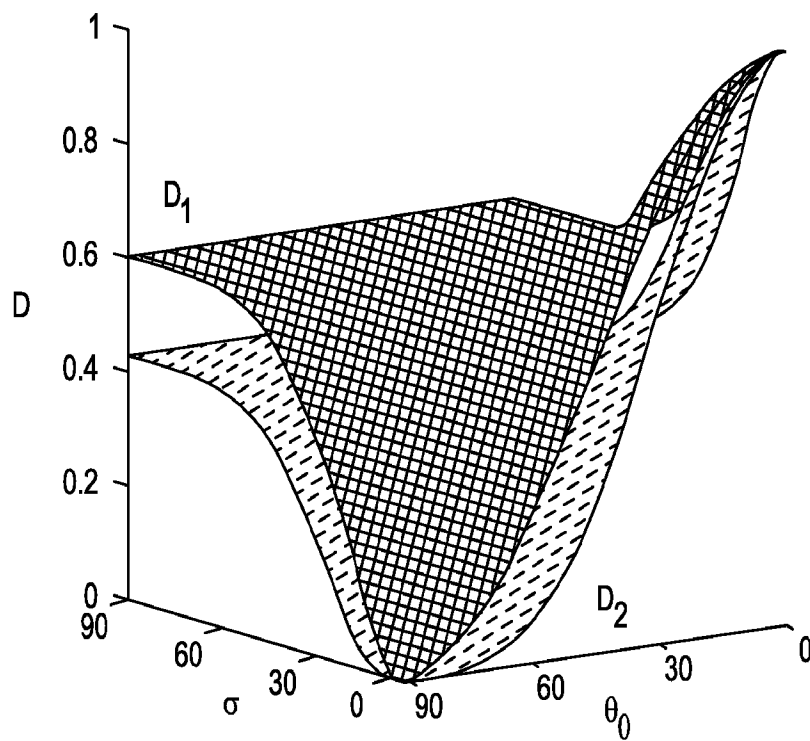
- e. determining unique D' and D'' using the calculated coefficients and determined nonlinear susceptibilities;
 - f. calculating planes, parallel to a θ_0 - σ plane, corresponding to the determined unique D' and D'' ;
 - g. determining curves corresponding to the intersection of the planes with the surfaces; and
 - h. determining an intersection of the determined curves, the intersection corresponding to a desired θ_0 - σ pair, whereby the desired θ_0 - σ pair indicates a molecular orientation and distribution of the sample.
16. The method of claim 15, further comprising, prior to the performing 2D heterodyne SFG spectroscopy, measuring or determining one or more vibrational modes.
17. The method of claim 16 wherein the measuring or determining one or more vibrational modes is performed using an FTIR technique.
18. The method of claim 15, further comprising performing additional spectrum measurements with different polarization combinations.
19. A non-transitory computer readable medium, comprising instructions for causing a computing environment to perform the method of claim 15.



(PRIOR ART)
FIG. 1B

(PRIOR ART)
FIG. 1A



*FIG. 2D*

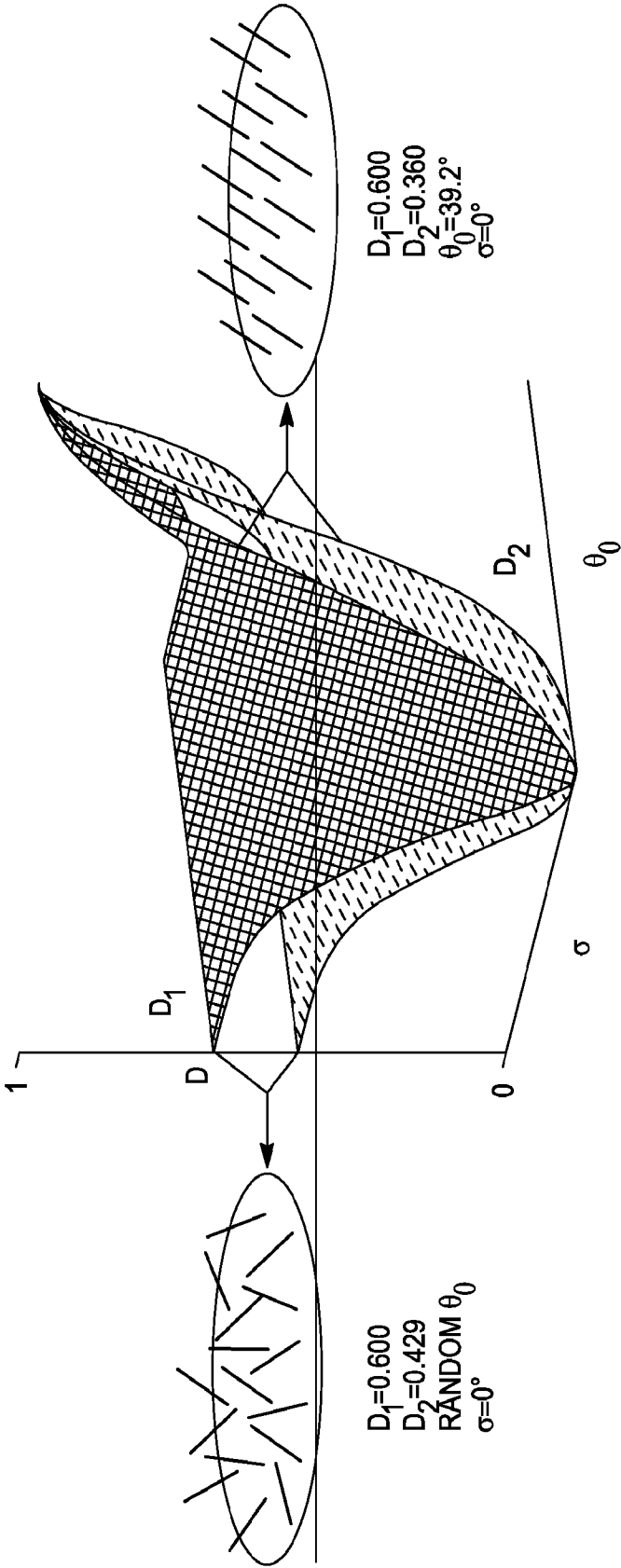
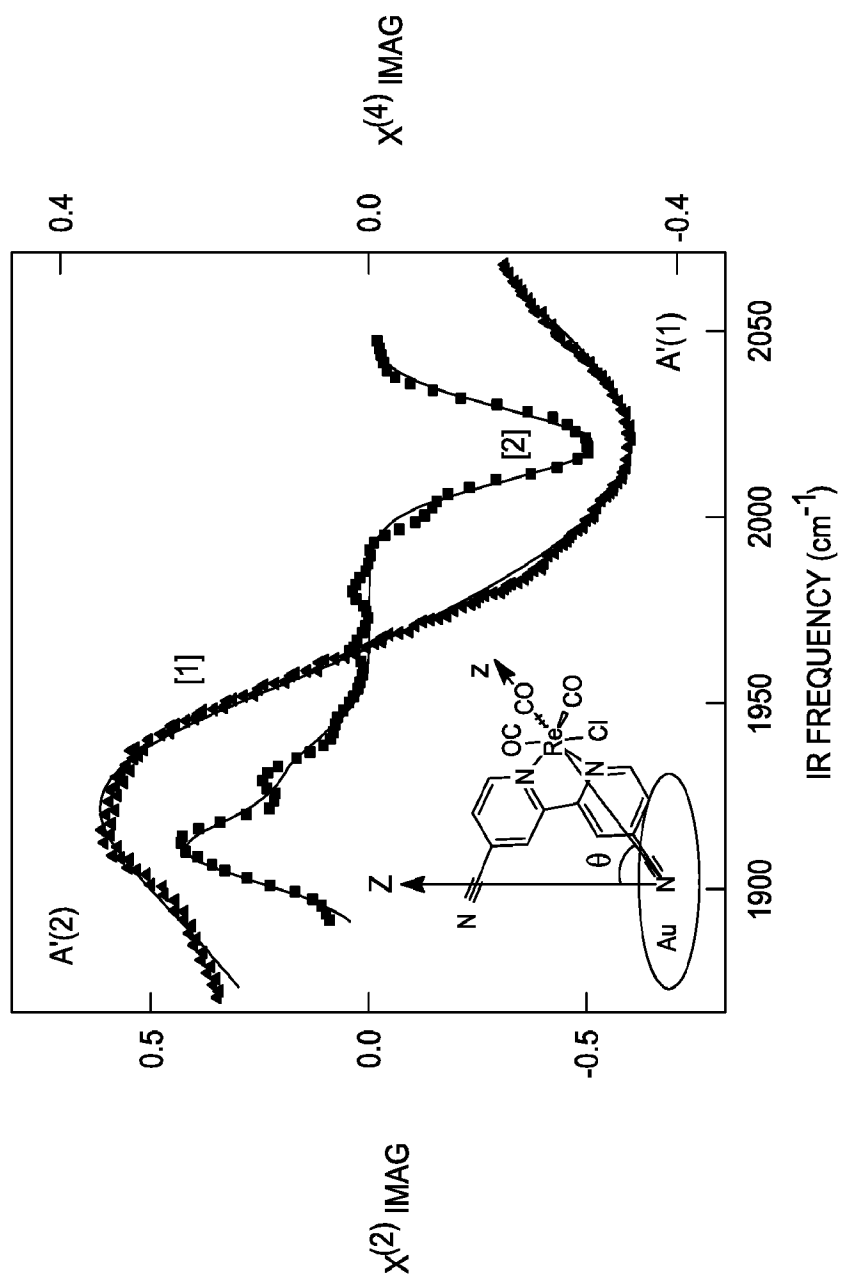
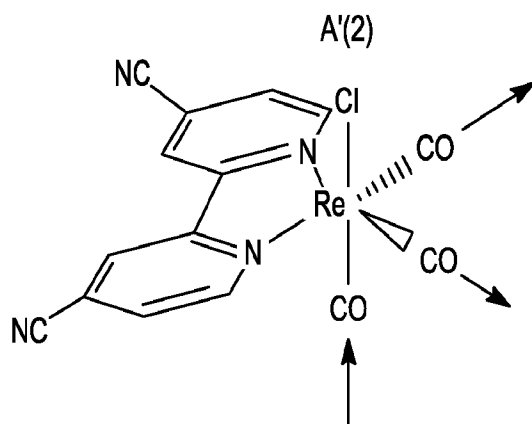
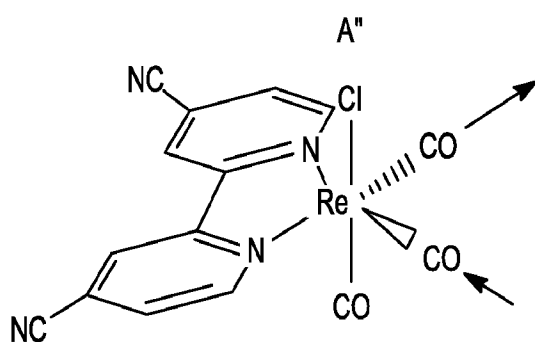
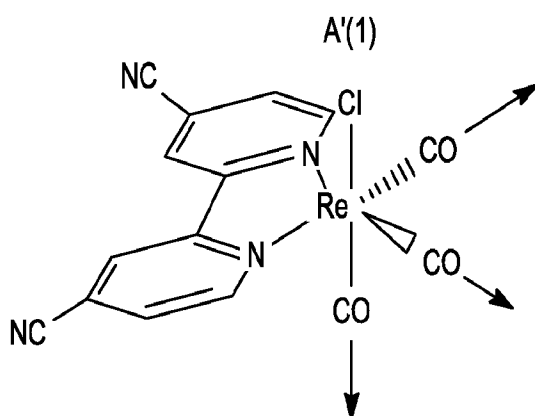
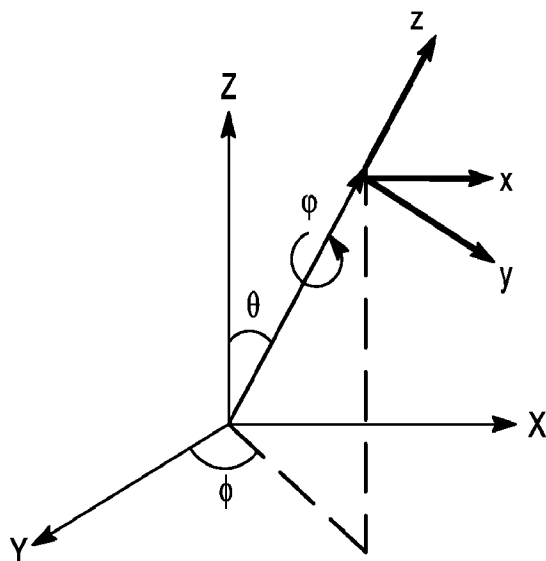
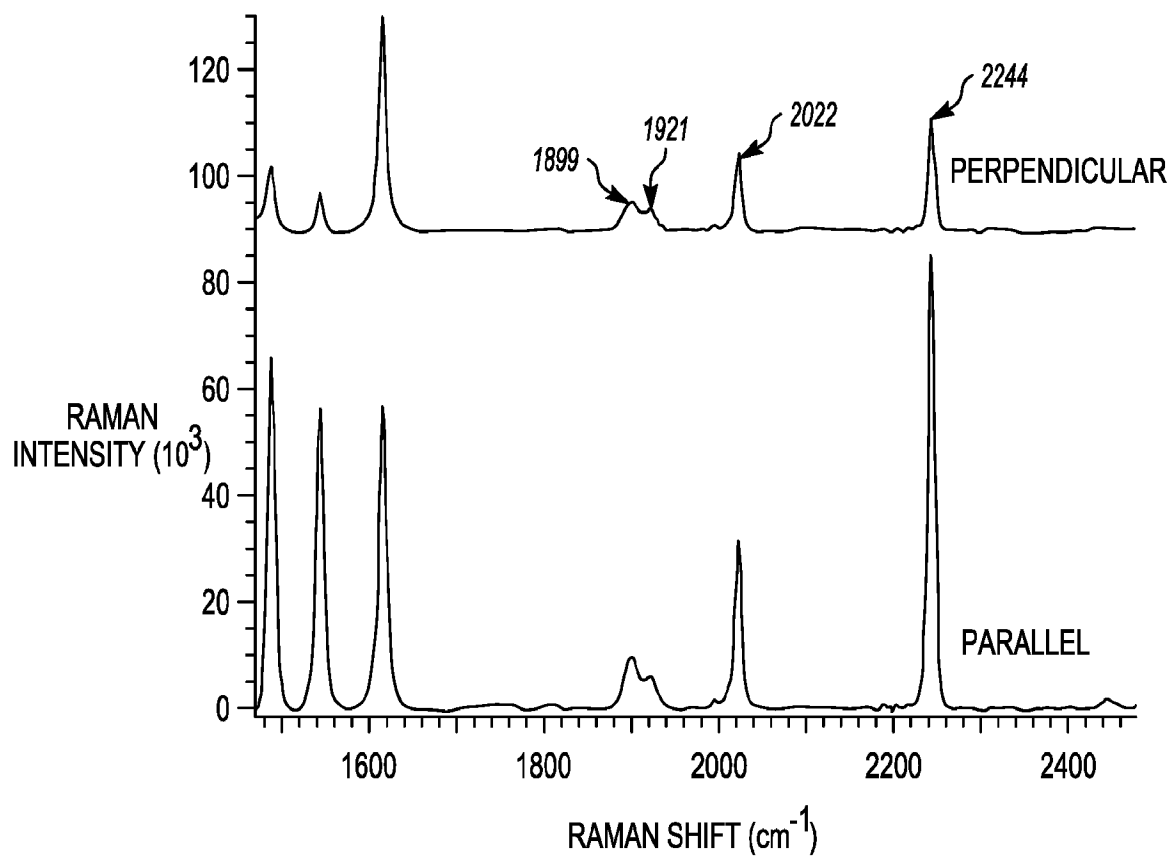
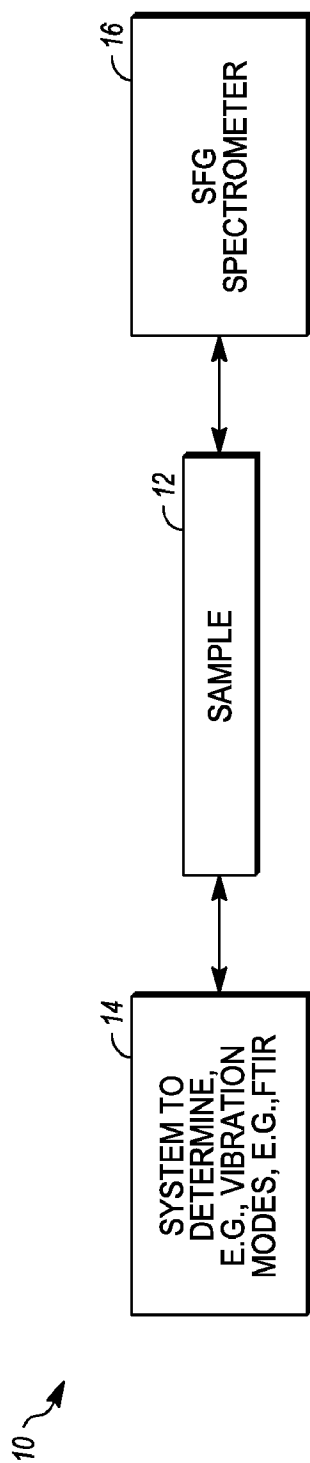


FIG. 2E

**FIG. 3**

**FIG. 4A****FIG. 4B****FIG. 4C**

**FIG. 5****FIG. 6**

*FIG. 7*

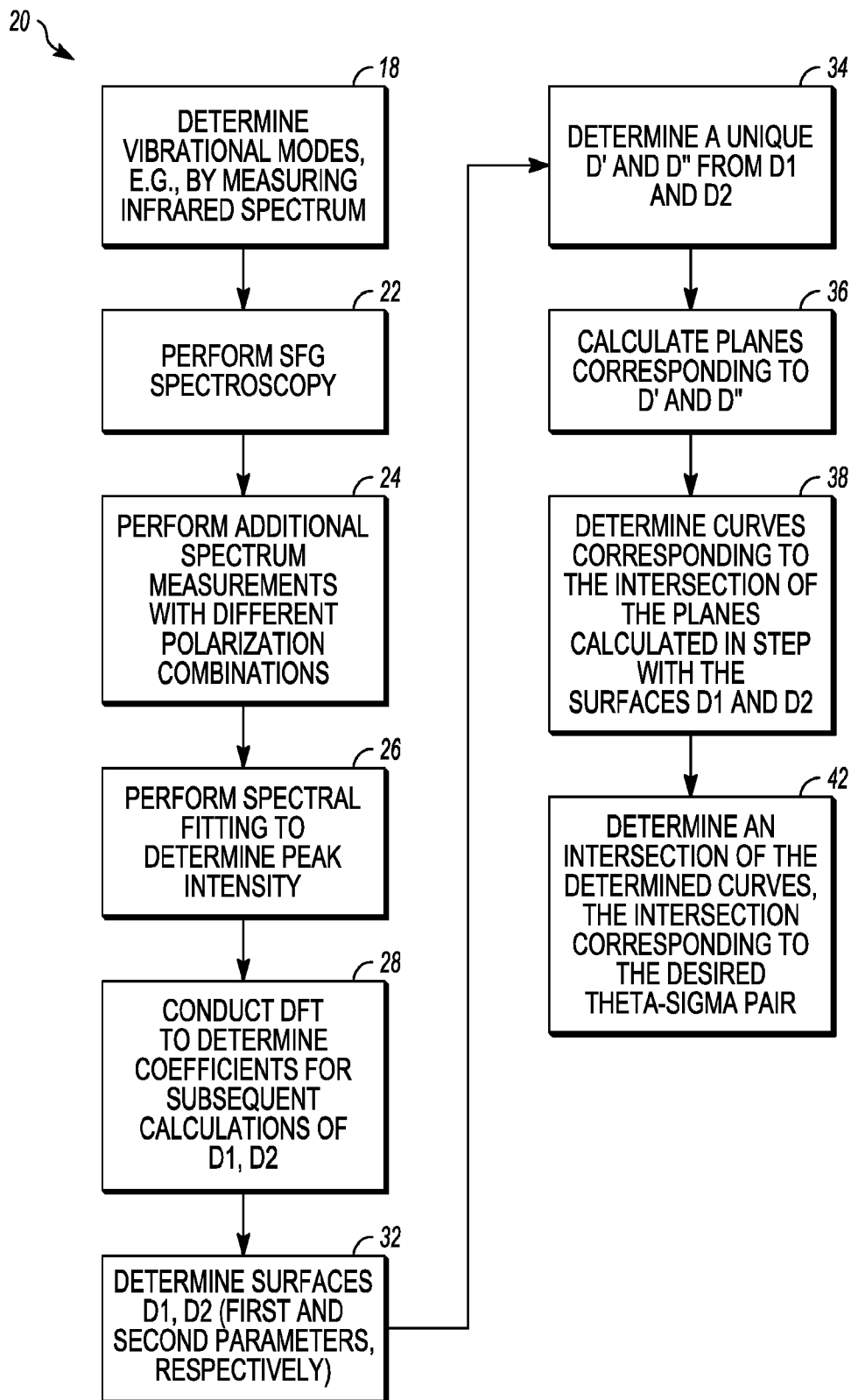


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/19152

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01J 3/28, G01J 3/10 (2017.01)

CPC - G01J 3/453, G01N 21/636, G01J 3/4338, G01N 21/45, G01J 3/45, G01J /433, G01J 3/2803, G01J 3/457

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAMAGUCHI "Heterodyne-detected electronic sum frequency generation: "Up" versus "down" alignment of interfacial molecules." THE JOURNAL OF CHEMICAL PHYSICS 129, 101102 (2008)	1-19
A	US 2010/0265501 A1 (BENDERSKII et al.) 21 October 2010 (21.10.2010),	1-19
A	SUNG et al., "Surfaces of Alcohol-Water Mixtures Studied by Sum-Frequency Generation Vibrational Spectroscopy." J. Phys. Chem. B 2005, 109, 18507-18514 (2005),	1-19
A	HARP et al., "Infrared-Visible Sum Frequency Generation Spectroscopic Study of Molecular Orientation at Polystyrene/Comb-Polymer Interfaces." J. AM. CHEM. SOC. 2003, 125, 11283-11290	1-19
A	US 2007/0152154 A1 (DECAMP et al.) 05 July 2007 (05.07.2007), entire document	1-19

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
19 April 2017

Date of mailing of the international search report
11 MAY 2017

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