



ILLUSTRATION BY MICHAEL GIBBS

Doubling Up

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Dual polarization interferometry determines protein structure and function.

Proteins, the building blocks of life, are wonderfully complex molecules whose correct function is essential to all biological processes and whose malfunction lies at the heart of almost every disease. Part of the complexity and subtlety of their operation rests in the fact that it is not merely their chemical composition but their shape that determines their characteristics. In response to pH, ionic strength, or other molecules binding to them, proteins can open and close, changing shape and, as a result, the manner in which they perform their allocated function.

For many years, biochemists and pharmacologists have worked to determine precisely the consequences of a protein's 3-D shape, in order to gain a better understanding of how they operate or how drug molecules could be designed to assist in function or mediate in malfunction. Images of protein crystal structure are of intense interest to the pharmaceutical industry, but these are a static snapshot of what is an elegantly mobile mechanism and provide only one fragment of the information required to unravel the mys-

teries of proteins. Furthermore, imaging a protein using x-ray diffraction requires first crystallizing the protein, an extremely difficult process (fortunately for the existence of life!) that has seriously restricted the number of useful images that have been obtained to date.

Researchers require an analytical approach with which they can study a protein in a more native environment, measure protein structure in real time and at a resolution likely to yield information about its function, and directly correlate any structural changes with protein function. Dual-polarization interferometry (DPI) provides just such a method.

DPI measures the structure of a protein in one dimension (i.e., its diameter or size) and the density (i.e., its mass per unit volume or how tightly folded it is) by coupling the protein to a glass slide and probing its structure using non-diffractive optics. The method resolves protein structure to subatomic dimensions (well below 0.1 Å) in real time and has a growing acceptance among researchers in the field of protein characterization, an essential discipline in the science of proteomics.

How DPI Works

In any waveguide structure, light is not wholly confined within the physical boundaries on the guiding medium but, rather, decays exponentially at the boundaries. Changes in the refractive index of materials within this decaying, or evanescent, field alter the speed of propagation of the field (the optical path length within the waveguide). DPI leverages this phenomenon to probe the optical properties of proteins that have been covalently, hydrophobically, or electrostatically attached to the sensing surface.

Interferometers detect the change in optical path length experienced by an optical field passing through the sensing path of the interferometer. Sensitivity is governed by, among other things, the interaction length and the signal-to-noise ratio of the detection scheme. Typically, integrated optical interferometers are configured in the Mach-Zehnder format by creating channel waveguiding regions in the top surface of an optical dielectric stack.¹

DPI uses a much-simplified interferometer based on slab waveguides, with the reference arm buried beneath the sensing arm. Coherent light broadly illuminates the stack, traversing both waveguides, and upon exiting the structure diffracts into free space. Because the waveguides are so close together, even within a few hundred micrometers of the end of the stack the diffracted wavefront generates the well-known pattern of Young's interference fringes in the far-field. Changes in the optical properties of proteins on the surface of the sensing waveguide translate into variations in the interference pattern, as captured by a high-resolution camera.

The optical tolerances are so forgiving that macroscopic movements of the input coupling beam on the order of hundreds of micrometers cause no change in the interference pattern. These loose tolerances allow the stack to be inserted and removed from the optical train without alignment, an essential characteristic for a disposable measurement platform.

For a given measurement of the interference pattern, one can determine an optogeometric parameter for a layer of protein. This parameter will not, however, unambiguously resolve the size or any other structural signature of the protein: A large protein in small number density will produce the same signal as a small protein in large number density. Resolving this ambiguity requires two simultaneous measurements of the optical properties of the protein in two orthogonal polarizations (TE and TM) which, because the evanescent fields have two different profiles, can be mathematically converged on a single solution for the protein size

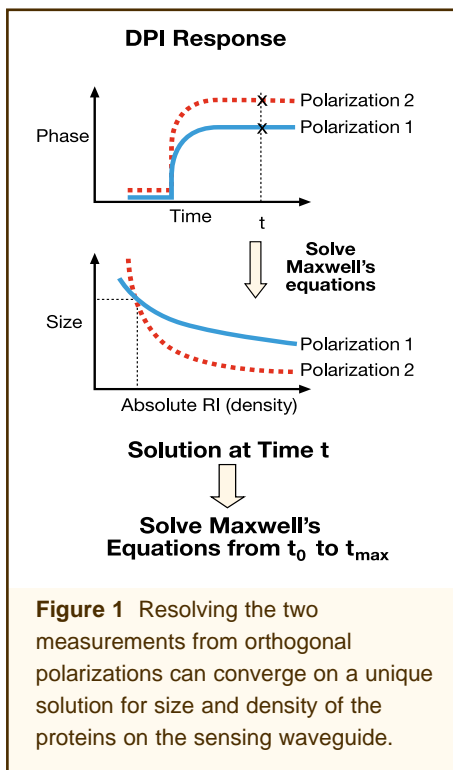


Figure 1 Resolving the two measurements from orthogonal polarizations can converge on a unique solution for size and density of the proteins on the sensing waveguide.

and density. Knowing size and density, one can also trivially calculate the total mass, surface concentration, number of protein molecules, molecular footprint, and a host of other useful parameters.

Operating Principles

The phase changes of interest, $\Delta\phi$, involve changes to the effective refractive index N_s of the mode in the upper (sensing) waveguide. Changes occurring at the surface do not alter the effective index of the lower (reference) mode N_r , because the evanescent field of this mode decays rapidly in the region between the two guiding layers. The phase difference is given by

$$\Delta\phi = k_0 L \Delta N_s \quad (1)$$

where k_0 is the free space wave number, L is the interaction length, and

ΔN_s is the effective index change in the upper waveguide mode. Direct measurement of $\Delta\phi$ is obtained by continuously monitoring the relative phase position of the fringe pattern by performing a Fourier transformation relating intensity to position. The path length is fixed, so one can thus easily convert the experimental data to changes in effective refractive index.

A standard transfer matrix approach provides evaluation of the guided modes for the structure. This allows inclusion of an arbitrary number of layers in a model, each of which is represented by its own layer matrix. Within each layer, with propagation in the z-direction being parallel to the layers and the x-direction being perpendicular, the electromagnetic fields are proportional to

$$(Ae^{ik_x} + Be^{ik_x}) e^{i(k_z z - \omega t)} \quad (2)$$

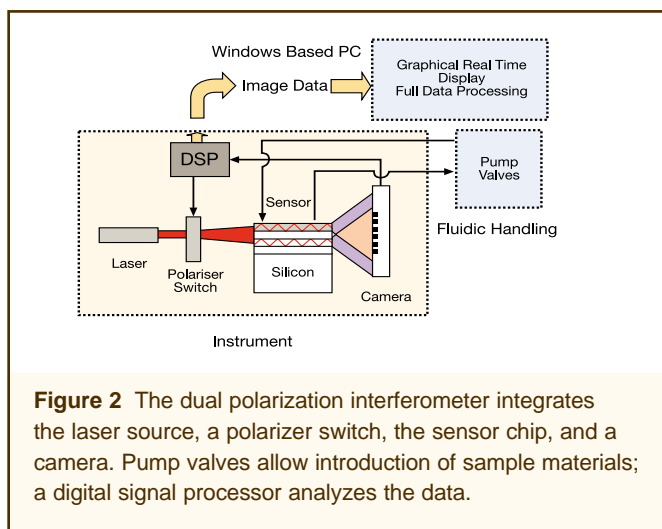


Figure 2 The dual polarization interferometer integrates the laser source, a polarizer switch, the sensor chip, and a camera. Pump valves allow introduction of sample materials; a digital signal processor analyzes the data.

Measuring Success

Although he runs a company that works in the life sciences, Gerry Ronan started out as an electrical engineer.

A native of Northern Ireland, Ronan studied electronics at the Owens University of Manchester (Manchester, UK), where he graduated with First Class honors in 1980. He completed an MS in electronics at the University of Manchester (Manchester, UK) the following year, and then headed to Japan to work as a research associate at Kyushu University (Fukuoka, Kyushu, Japan). He returned to the University of Manchester, where he earned a PhD in 1986. He is a Chartered Engineer, a fellow of the Institute of Physics, and an SPIE member.

Working on his PhD, Ronan studied plasma physics, which got him interested in instrumentation. Shortly after completing a stint as a Royal Society University Research Fellow, he took a job with VG Instruments—which was later absorbed by Thermo Electron (Waltham, MA)—designing plasma ion sources for use in analytical applications.

“Since then,” Ronan says, “I have worked for and run several instrumentation companies and have commercialized 50 or more analytical techniques, including MRI systems, a variety of mass spectrometers, particle sizers, neutron and x-ray analyzers, and, more recently, biosensors and analytical tools for protein characterization.”

“I’ve always been fascinated by pushing the boundaries of what’s possible in measurement,” he says, adding that life sciences provides the most demanding measurement problems.

Now, Ronan is CEO of Farfield Sensors Ltd. (Manchester, UK), which makes instruments for determining how the structure of a protein relates to its function. Since the completion of the Human Genome Project, protein analysis has become a promising area for understanding disease and developing new drugs. —Neil Savage



With ω fixed at the laser frequency, a scan through the propagation constant k_z determines the transverse wave vector k_x for each sub-layer via

$$k_x^2 = n_i^2 k_0^2 - k_z^2 \quad (3)$$

where n_i is the refractive index of the i^{th} sublayer ($k_y = 0$). Beginning with an evanescent solution in the region above the top layer of the waveguide structure, one can enforce matching of the tangential components of \mathbf{E} and \mathbf{H} for the TE and TM modes at each successive interface of the structure. This information yields the values of the coefficients A and B for each layer. A successive approximation method provides the optimum refractive-index value and the thickness of the thin protein

layer on the surface of the waveguide (see figure 1).

Instrumentation and Results

The 26 mm \times 6 mm slab waveguide is housed in a dual-zone, temperature-controlled enclosure that maintains thermal stability to within 1 mK (see figure 2). The clamping system also provides a fluidic interface to the sensing surface with a dead volume of approximately 2 μL , allowing introduction of test materials as required. A helium neon laser ($\lambda = 632.8$ nm) illuminates the end facet of the slab and a ferroelectric liquid crystal (FLC) halfwave plate switches the state of polarization of the input beam between TE and TM at typical frequencies of 50 Hz. The high tolerance to relative movement

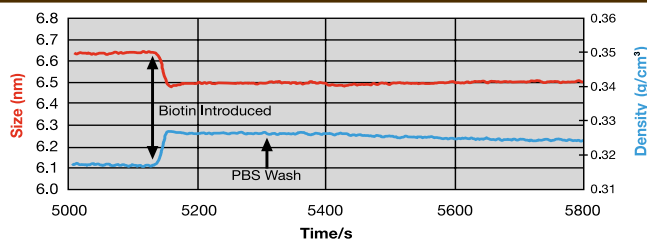


Figure 3 The data plot shows that a small molecule binding to a large protein induces a conformational change in the protein of just over 1 Å (left vertical axis). Simultaneously, the plot shows an increase in density (right vertical axis), as there is now higher mass in a smaller volume.

of the incident beam comes into play here—the FLC plate produces small refractive displacements during switching but the output image remains stationary throughout.

A 1024×1024 -element imaging device captures the diffraction fringes and passes the output to a digital signal processor. The image device output is synchronized with the FLC drive signal and sampled. The relative phase position is updated every 2 ms using a spatial Fourier transform method. Further analysis by PC displays the data and processes it into thickness and refractive index values for the protein system. Millikelvin thermal control using an autonomous dual-zone control system and the opportunity for averaging yields an rms noise level of around ± 0.1 mrad, equivalent to about 0.1 pg/mm^2 mass loading or less than 0.1 \AA dimensional change.

DPI has been used to study surfactants, lipids, proteins, and many other interfacial chemistry systems. Researchers have verified the method against other physical measurements such as crystal structures or neutron methods.^{2,3} One brief example involves a protein called streptavidin (Mwt 55,000 Da), which was immobilized to the sensing waveguide and challenged with biotin (Mwt 244 Da), a known small molecule binding partner with one of the strongest binding strengths known in biology. DPI analysis revealed that the protein (plus immobilizing linker chemistry) has a size of just over 66 Å, which is in good agreement with what would be expected from its crystal structure

(see figure 3). When challenged by biotin, the streptavidin undergoes a reduction in size of 1.3 Å; simultaneously, as there is now a larger mass in a smaller volume, the density increases, providing an unambiguous marker of the protein function at a previously unimaginable fidelity.

In more broad terms, the slab waveguide provides a convenient and disposable optical bench on which non-diffractive optics can probe a 100-nm region above the slab surface. A multitude of optical methods compatible with this device could yield additional high-resolution measurements to further characterize the molecular system of interest. Already absorption studies (spectroscopy) have been observed, as have scattering signatures (morphology). The combination of wavelength and polarization (dichroism) promise to deliver both compositional and structural information highly relevant to the life scientist in years to come. We expect a portfolio of new analytical techniques to emerge, truly illuminating the molecular world. **oe**

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