Instrument for near infrared emission spectroscopic probing of human fingertips in vivo

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We present instrumentation for probing of volar side fingertip capillary beds with free space coupled near infrared light while collecting Raman, Rayleigh, and Mie scattered light as well as fluorescence. Fingertip skin capillary beds are highly vascularized relative to other tissues and present a desirable target for noninvasive probing of blood. But human hands and fingers in particular are also highly idiosyncratic body parts requiring specific apparatus to allow careful and methodical spectroscopic probing. The apparatus includes means for precise and reproducible placement of the tissues relative to the optical aperture. Appropriate means are provided for applying and maintaining pressure to keep surface tissues immobile during experiments while obtaining the desired blood content and flow. Soft matter, e.g., skin, extrudes into the aperture in response to any applied pressure, e.g., to keep the tissue in registration with the optical system, so the position, contact area, pressure, and force are continuously measured and recorded to produce feedback for an actuator applying force and to discern the compliance of the test subject. The compliance strongly affects the reliability of the measurement and human factors must be adequately managed in the case of in vivo probing. The apparatus produces reproducible observations and measurements that allow consistent probing of the tissues of a wide range of skin types. © 2010 American Institute of Physics. [doi:10.1063/1.3314290]

I. INTRODUCTION

In order to access blood and tissue analytes noninvasively in vivo, we probe volar side fingertips with near infrared radiation (NIR). We simultaneously measure the elastically scattered light, referred to as elastic emission (EE) throughout this paper, as well as the undifferentiated inelastic emission (IE) containing both Raman scattered light and fluorescence. Raman scattering1 provides chemically specific and quantitative information concerning molecules in complex mixtures such as blood2 in vivo. EE is a probe based on physical optics3 providing information relating to the presence of red blood cells (RBCs) in the probed volume. To some extent all the tissues in the probed volume fluoresce although the RBCs are the strongest source per unit volume percent followed by the plasma and then the static tissues. In this paper we present a detailed description of instrumentation we found adequate and useful for routinely obtaining reproducible and interpretable spectroscopic data on subsurface tissues in volar side fingertip beds. We do this in the context of earlier studies4 in which we analyzed the sources of variation in glucose concentrations obtained from in vivo Raman spectroscopy of volar side fingertip capillaries.

In that earlier study,4 the distribution of deviations between the noninvasive LighTouch™ measurements and the reference fingerstick measurements reflected various sources of random and systematic error. Better management of the measurement process with regard to human factors emerged as a strategy for improvement with feedback based on applied pressure and tissue placement a specific goal. The present device implements that strategy and is even more advanced since the actual application of pressure is automatic and would work for an unconscious test subject. Moreover, this device produces a digital quantitative record of the measurement cycle so that errors in glucose concentrations can be analyzed in order to improve performance and better understand the total measurement process. We suspect that many of the same ideas in this device would be applicable to spectroscopic probing of other types of soft matter including ex vivo tissue samples for biopsy.

Certainly there are many approaches to in vivo spectral probing in the NIR, particularly in the context of glucose sensing5–8 with the question of whether or not to use a window to attain optical registration with the probed tissue arising immediately.7 When IE is used as a part of the measurement process, any fluorescence or Raman scattering from a window material, or even Rayleigh/Mie process involving the window that produces highly off-axis elastic photon emission,8 adds a background to all measurements and thereby a shot noise contribution that cannot be eliminated. In our experience the depth of focus of the input coupling lens ensures that some of the incident light will be focused
inside the window, at or very near the focus of our very fast emission collection system. For us it was not possible to include any window surface, or wire grid or other approach spanning the orifice, that was not a source of excessive stray light of all kinds, e.g., Raman, Rayleigh, fluorescence.

It is possible to exclude a large proportion of light from the window–laser interaction using more optics and confocal apertures\(^9\) in the collection train but not without sacrifice of simplicity, extreme loss of total signal, and imposition of spatial resolution that does more harm than good to the overall measurement process. We will show how the size of the laser beam compared to the size and spacing of the fingerprint ridges can have important consequences. While we do not rule out the use of windows in the future,\(^7\) the basic features of the LighTouch\(^\text{TM}\) device described in this paper were designed to achieve precise and reproducible treatment of the tissue under spectroscopic scrutiny. Given that capacity, it is possible to methodically and systematically explore the use of different apertures and/or windows in order to achieve specific goals.

With regard to our principal goal, spontaneous Raman scattering is generally a millimicron detection limit technique. Applied to quantifying glucose concentrations below 5 mM in volumes that are only partially filled with glucose containing materials, it has been surprising to us\(^2,4,10,11\) that Raman spectroscopy in volumes that are only partially filled with glucose containing materials quickly become coated with sebaceous secretions and sweat, in spatially inhomogeneous and sometimes reproducible patterns, i.e., fingerprints. Clearly the same materials are always on the skin but failure to clean the window between uses, a human factors issue, means that on a given measurement cycle the materials on the skin and the materials on the dirty window contribute to variability. Choosing to probe through an open aperture avoids this problem and all the ones described above but at the expense of having to manage the surface topography of the tissue being probed.

All skin has surface topography, even relatively smooth forearm skin, and the effect on optical probing can be anticipated\(^12\) using Fresnel’s equations. Depending on the relative size of the probing light beam and the scale of surface topographical features, the presence of topography may be of little consequence whether a window is used or not. Note that, now, in the context of probing skin through an open aperture, the depth of focus of the incident laser beam can be an advantage. Being much longer than any motion or extrusion of tissue in the direction of propagation of the incident laser, the properties of the incident laser can be taken as constant regardless of the motion of the tissue. As will be seen in the results presented in this paper, having studied unperturbed skin surface topography using an aperture and not a window, and with control of the applied pressure so that subsurface blood motion can be discriminated from the effect of surface topography, it is relatively easy to devise means to automatically place and maintain the laser position with respect to skin surface topographical features.

Ridged skin\(^13\) such as is found on volar side fingertips provides an important thermoregulation function and so is highly vascularized, presenting a blood rich tissue to probe. Such volar side ridged skin is only weakly pigmented but the dimensions of the surface ridges can be commensurate with
the diameter of the laser beam, leading the results to have a strong dependence on the placement of the laser beam on the skin surface. In any optical instrument the sample, e.g., the tissue, must be brought into reproducible registration with an optical system. In this case, an external isobaric servo driven actuator presses the fingertip against a circular aperture in a thin spring steel sheet and a NIR laser beam penetrates the skin at the exposed surface. The aperture and the fingertip can be moved together as a unit with an electromechanical actuator so that using the various forms of emission measured simultaneously with a charge coupled device (CCD) detector, the laser can be brought to a reproducible registration with the fingerprint ridges.

It is an empirical fact that pressure on the fingertip, even constant pressure, has at least two effects. As mentioned above, the pressure causes “doming” or extrusion of tissues into the aperture, i.e., the exposed skin surface is not flat, independently of the presence or lack of ridges. In addition, over time, constant pressure causes some fluid, e.g., blood, to flow out of the irradiated region, without much changing the static tissue. The fact that the blood transmits a local pressure field into the probed volume intended to maintain homeostasis, i.e., appropriate mm Hg of diastolic and systolic pressure, in addition to the local hydrostatic and oncotic pressures, dictates that the applied pressure be the figure of merit for describing the physical state of tissue under observation. Note that as fluid moves in response to the applied pressure, the contact area between the tissue and the surface containing the aperture, i.e., the “modulating surface,” must increase necessitating an external real-time servo system(s) in order to produce consistent results.

We are certainly not the first\(^1\) to attempt to use forms of difference spectroscopy in concert with tissue modulation\(^2\) in order to obtain chemical information concerning subsurface tissues. Earlier instruments\(^4,8\) we employed muscle memory, visual memory and visual and audio cues in order to direct the test subject him/herself to apply the appropriate force and maintain an appropriate finger posture in order to modulate the blood content of the capillaries. Perhaps, not surprisingly, even when humans are given very accurate and precise feedback, they vary in their competence to execute the required actions. In addition to focusing our attention on the optical and spectroscopic issues of a particular experiment, it is helpful to have a record of the test subject’s actual compliance (or noncompliance) during the measurement process in order to help sort out motion and placement artifacts from actual spectroscopic content. Experience with each of these and the desire to have a device that would be applicable to unconscious people led us to seek a way to modulate the skin and blood automatically under external control.

We became aware of other studies into these issues but in other contexts. Asada and co-workers\(^8,16\) studied optically the application of pressure by a human test subject onto a window surface so as to aid in the design of “virtual reality gloves” and joy-stick applications. The movement of blood in the capillary beds is measured optically in order to quantify and spatially discriminate the test subject’s tactile intentions. Similarly, calibration type measurements and analysis have been made by Jones\(^17\) in terms of determining the ability of humans to apply, discern, or maintain specific force levels with their hands and fingers. Such information gathered in real time would be used to direct other apparatus ranging from games to the operation of heavy machinery. We found these studies very useful and relevant but not adequate for our specific purposes. Even if compliance requires the test subject to be passive, as for an unconscious patient, the probability of involuntary tremor and the known occurrence of inadvertent or unconscious pressing or unpressing against the aperture by the test subject require the instrumentation to play an active role in producing, measuring, and recording the actual position and actual applied stress field in the test subject.

In what follows we describe instrumentation and experimental methods used to obtain reproducible and reliable measurements on finger tip capillary beds. The entire apparatus is coordinated using a C++ based graphical user interface, i.e., the graphical user interface (GUI), with embedded software in the placement and force measurement system [position detector-pressure monitor (PDPM)] and in the CCD camera. All transducers are commercial-off-the-shelf and the circuitry needed to implement them is available from their manufacturers. Initially we give an overview of apparatus and procedures before specifically discussing in more detail placement or positioning and contact area function and then force and pressure measurement and application. We then present some measurements that demonstrate the utility of the apparatus.

II. OVERVIEW AND GENERAL DESCRIPTION

We provided optical apparatus diagrams in our earlier publications\(^2,8\) and a schematic of the current apparatus is shown in Fig. 1 along with corresponding photographs and drawings in subsequent figures. Data are obtained using external cavity diode lasers (Process Instruments, Salt Lake, UT, USA and Sacher Lasertechnik Tiger Model, Marburg, Germany) with a clean-up filter (Semrock, Rochester, NY, USA). The excitation is free space coupled from the laser to the fingertip using a 15 cm focal length lens. The angle of incidence of the light at a flat surface in the plane of the back surface of the spring steel was 53°. Zap-it paper and knife-edge experiments estimated the laser diameter to be elliptical with a 100 \(\mu m\) minor axis and a 237 \(\mu m\) major axis. All optics must be thin and fused silica, e.g., Corning HPFS series, particularly in the excitation side, to reduce back-

![FIG. 1. (Color online) Schematic diagram of apparatus and layout.](image-url)
ground fluorescence and Raman originating with the lenses. In this connection we stress that the optics can be thick on the collection side of the optical system because the total signal power incident on the first collection lens is very small, i.e., approximately equal to four to five orders of magnitude smaller than the incident laser power.

The assembly containing the aperture is mounted on a stage that can be driven electromechanically using a Newport NSA12 micropositioning system. The collection train consists of a custom triplet collection lens, followed by Semrock Razor Edge filter to remove the laser line, and then a custom doublet refocusing lens. A 61-fiber bundle presents an anti-reflection coated circular target to the refocusing lens and a line configuration at the spectrograph entrance slit (Process Instruments, Salt Lake, UT, USA). The effective slit width is 70 μm and the net collection and spectrograph system is approximately f/2.0. The wavelength dispersed light is imaged onto either an Andor DU420-BR-DD CCD camera operating at −65 °C or a Critical Link MityCCD-E3011-BI CCD camera, cooled to −45 °C. Our IRB allowed use of 200 mW cw power at 830 nm for as much as 200 s although others have been allowed to use as much as 300 mW on forearm tissue.

In order to achieve reliable and reproducible optical registration the volar side of a fingertip is placed in contact with a rigid flat stiff surface having a = 2 mm hole, chamfered (15°) on the side facing the incident light. The size of this hole and the chamfering are chosen to minimize extrusion of tissue and to minimize the scattered light produced by interaction of the laser with the edges of the hole. To prepare to apply pressure after insuring appropriate placement with respect to the aperture, an actuator is brought to the threshold of contact with the dorsal surface of the distal phalanx coarsely using a stepping motor. When the load cell response signals the threshold, the stepping motor stops and back steps a predetermined amount relative to the dorsal skin surface. The solenoid can apply maximum force and fastest response, i.e., display optimum performance, when the shaft is positioned within plus or minus a few millimeters of the center of its range of motion. The stepping motor leaves the solenoid so that it can remake skin contact in the optimal shaft position.

Contact between the actuator and the skin surface is always chosen to occur between the cuticle and the distal joint such that the actuator does not make direct contact with the fingernail. The exact point of contact determines the type of modulation that will occur and certain characteristics of the compliance information that will be available. Application of pressure onto the fingernail itself produces a reproducible modulation effect in the volar side capillary bed that is essentially the opposite effect of pressing on the volar side soft tissue. We contacted the nail bed capillary blood through the fingertip for spectroscopic purposes before but the difficulty in that approach is bringing the rigid nail surface into reproducible registration with the aperture. Thus, in the present context making contact between the cuticle and the joint ensures that the induced stress field does not produce opposing effects on the volar side.

Trial and error has shown that, once a measurement cycle has begun, the aperture must be stationary with respect to the collection optics and the laser delivery lens to at most ±25 μm throughout any experiment to obtain consistent results. It appears that inconsistent aperture movement combined with inconsistent extrusion of tissue into the aperture produces an unmanageable spectroscopic response. All force transducers (in our case an Omega LCKD-150 g transducer with 12 bit A-D) require motion of some kind to produce an electrical signal. We employ a separate National Institute of Standards and Technology traceable (Sensotec, Honeywell) readout device with a separate identical transducer to calibrate our instrument. We use a lever system, a simplified nonscale drawing is given in Fig. 2 to magnify the motion of our modulating surface at the transducer contact point and minimize the motion at the optical aperture so as to achieve sufficient sensitivity and stability with respect to knowing the total applied force at the aperture. Consistent with the specifications of the transducer we detected no hysteresis in this system so the design seems acceptable.

The position of the aperture with respect to the fulcrum determines the largest finger size that can be used in this type of apparatus. Related to this is the fact that the fingertip is not a point load. The perceived force applied to a specific point on the fingertip increases as the distance between the fulcrum and the point of interest increases. The load cell does not detect any force applied to a region of a fingertip that overlaps the fulcrum. Thus, since the fingertip must overlap the aperture, the distance between the aperture and the fulcrum is the largest width of finger that can be accommodated. In calibrating the force measurements of this design, it is important that any applied load, i.e., known test loads, have a contact area with the spring steel plate about half the maximum and be centered at the aperture. We designed a fixture that mounts the second reference load cell to the end of the solenoid shaft so that a chosen force/pressure setting can be tested in the instrument for independent calibration.

Various designs of this system have all required some temperature control of the modulating surface (spring steel) and nearby connected materials (aluminum and stainless steel).
steel) to control potentially random but usually periodic variation of the transducer output associated with 0 applied force. The apparent 0 of applied force can drift due to differential expansion of materials with respect to ambient temperature drift and, often, differential warming of the modulating surface itself by the in vivo tissue being modulated. Choosing to maintain the modulating system at or near 37 °C is advantageous and a thermistor driven freestanding analog proportional-integral-derivative feedback loop is sufficient for this purpose.

The side of this metal surface facing the collection system is painted black and the thinner the material, we currently use 625 μm thick spring steel, the easier it is to direct the incident laser through the aperture while producing acceptably low scattered/reflected light. As the sheet material (spring steel) forming the aperture is made thicker, the aperture turns into a tunnel until the laser cannot get through the aperture at any angle relative to the tunnel axis without contacting the edges. When this occurs depends on the diameter of the laser and the exact thickness of the sheet material. Since a Gaussian never goes to zero, this is a question of extent when trying to achieve maximum throughput with the least background. With the dimensions given above, “acceptably low” = 0 counts for a 20 ms timescale can be achieved reproducibly.

We used a number of different materials but we prefer spring steel for its superior stiffness. At the particular load cell we specify above, the contact point is very small so the contact area increases with longer time pressing. The dynamic range of the transducer/analog to digital converter set defines a range of applicable pressures. The “optimal degree of sensitivity” will allow application of pressures from about 10–150 g/cm² in steps of about ±5–10 g/cm² for fingers with a contact area from 0.5–2.75 cm², consistent with a range of reasonably normal blood pressures and finger dimensions.

The time responses of the pulses evident in the force and pressure records are a definite function of the hardware and accessories constituting the method of mechanically coupling the finger tissue to the solenoid/actuator. When a splint is employed in addition to the plastic shaft, such as described below, the pulses are wider in time. The heavier the coupling, i.e., total weight of whatever is mounted to the end of the solenoid shaft and whatever splint attached to the dorsal side of the finger may be employed to mate to the solenoid shaft fixture, the more damped the response of the force and pressure.

III. PLACEMENT AND CONTACT AREA

We previously described various means for reproducibly and precisely placing the volar side fingertip skin surface in contact with the modulating surface. The current PDPM system automatically measures the test subject’s volar side contact area with the “tissue-modulating surface,” i.e., the flat spring steel region surrounding the aperture, while providing feedback for initial placement and later a record of the actual placement during a measurement sequence.

The placement and contact area measurement needed as feedback for an actuator to maintain constant pressure is accomplished using an array of metallic gold spots on an insulating substrate as shown in Fig. 3. This gold metallization pattern is deposited on a flexible Mylar™ circuit board that is glued to the spring steel surface. There is also adhesive on the Mylar™ surface between the metallic spots, so that when final placement and contact is achieved, the skin in contact with the adhesive cannot easily move parallel to the modulating surface. This raises a human factors issue since this flexible circuit board must be changed regularly due to fouling of the spots by skin borne fluids.
The electrical resistance between the gold annulus enclosing the optical aperture and each of the gold spots is relatively low when skin is in contact with both conductors and infinite otherwise. Each spot in a corresponding pattern displayed by the GUI is colored green or red depending on the resistance state sampled in real time every 20 ms. There are also two sliders produced in the same GUI window (not shown) depicting in real time the actual applied pressure to the modulating surface and the target pressure for the modulating cycle. Based on which and how many spots are in the low resistance state (green), and the spacing and dimensions of the spots, we devised an algorithm to calculate the contact area between the modulating surface and the fingertip. The spot pattern, contact area, force, and pressure can be “played back” to allow examination of the measurement process frame by frame.

The electrical resistance of the skin perceived by the spots depends on many factors that we distill into two major but not completely orthogonal categories: hydration and turgor. Possibly there are two categories because there are two properties of the tissues, electrical and mechanical, that determine the response of the gold spot system. Considerable experimentation with senior citizens shows that it is possible to have well hydrated skin and still have low net turgor causing the spot system to fail. Considerable experimentation with young people shows that it is also possible to have very high turgor but sufficiently dry skin so as to cause the spot system to fail. All tissues in the mechanical train that starts with the spring steel and terminates at the opposing distal phalange bone surface inside the finger contribute to the apparent turgor of the whole assembly. Each tissue in the train has its own turgor properties but so long as the net turgor allows good mechanical contact between the skin and spots, only the outermost surface skin, i.e., the stratum corneum, contributes to the apparent electrical impedance sensed by the spot system.

“Hydration” is more than the simple presence of water. There is water in the stratum corneum, the interstitial spaces, and the cells themselves that affect the mechanical properties of the tissues as well as the electrical properties. In our experience, regardless of turgor, most commercial skin lotions and similar substances used regularly but not within a few hours of executing a placement will cause the apparent electrical resistance to decrease and the placement spots to work better than topical application of water or even electrolytes much closer in time to the initiation of the actual measurement cycle.

Turgor relates to water content of each cell and the oncotic pressure that keeps the water inside. This determines the apparent electrical resistance in that the skin must be brought into sufficient mechanical contact with the metal regardless of the availability of charge carrying capacity. Mechanical contact requires the tissues between the bone, i.e., the distal phalanx and the modulating surface, resist further compression at some applied pressure. When the oncotic pressure\(^8\) is sufficiently low, all the tissues between the bone and the Mylar\(^{TM}\) surface are so compressible that the mechanical contact is insufficient to allow adequate electrical current to flow, even though the stratum corneum is sufficiently well hydrated to make the spots respond. Although better approaches to this problem may emerge, with regular usage of almost any over-the-counter skin lotion, the approach presented here allows a very wide variety of test subjects to easily achieve proper placement and reliable contact area measurements without any apparent spectroscopic consequences.

IV. FORCE, PRESSURE, AND THE MEASUREMENT CYCLE

There are two modes for (1) positioning the fingertip in proximity to the aperture and (2) moving the actuator into proximity to the fingertip thereby initiating a measurement cycle. In one the test subject places his/her own fingertip against the modulating surface, shown in close-up in Fig. 3, achieving desired placement by visual feedback using the GUI provided colored spot pattern. Or as shown in Fig. 4, a fixture is mounted on the solenoid, the finger is placed in a splint outside the device, and then the finger with the splint attached is slid into the fixture all as described in greater detail below. In this case positioning is followed by the “set up” process, i.e., the stepper motor/solenoid moves the fixture holding the passive splint-mounted fingertip toward the aperture. If positioned without the splint, pressing a button in the GUI causes the actuator to approach the stationary finger from the dorsal side using the stepping motor.

In either case, upon first simultaneous three-way contact between the modulating surface, the finger and the actuator surface, the stepping motor stops and then retracts a few millimeters. The actuator position is then further adjusted using a solenoid to achieve a pre-unsupported pressure between actuator, finger, and spring steel of \(\approx 10\) g/cm\(^2\) pressure in the feedback system. This is very light pressure that most test subjects cannot discern. Utilizing the solenoid from this point forward allows continuous adjustment of actuator force and displacement, in order to keep the measured pressure, i.e., the ratio of the total applied force and the measured contact area, constant.

The actuator for applying force can be made to contact the dorsal side of the distal phalanx between the nailfold and the first joint in a few different ways. This can be as basic as a simple cylinder, or a foam-pad-tipped cylinder attached to
with the dorsal side of the finger to achieve and maintain the preset “unpressed” target pressure. Since the contact area between the volar side skin and the modulating surface increases in time as the blood leaves the capillary bed, the force must be increased in proportion to maintain constant target pressure. The placement spots and the load cell are polled at 20 Hz giving the approximate bandwidth of the pressure servo. The initial target pressure is achieved and the system becomes mechanically stable in about 100 ms. When a tissue modulation cycle is required, after a suitable “unpressed” collection period, the target pressure is changed to reflect the transition to the “pressed” state, the actuator applies more force to achieve the new target pressure and 20 ms CCD frames are collected for an equal time period as the unpressed state. Afterwards, the actuator is retracted, and the CCD frames are stored as a record of the applied force, applied pressure, net contact area, and contact history of the individual placement spots. In a separate step, the CCD frames are processed for calculation of glucose or bicarbonate concentrations or hematocrit.

V. SYSTEM ALIGNMENT

Leaving out obvious steps we note that to achieve reproducible results, the optical system is aligned in the following manner. First, the laser is directed into the center of the aperture at the appropriate angle, 53°. This is Brewster’s angle for an air-water interface. Since the skin index of refraction is roughly that of water and we free space couple the light into the tissue, at Brewster’s angle the transmission across the interface is maximized thereby coupling the most of the (appropriately polarized) incident laser light into the probed volume. A tightly pressed pellet, pressure >5000 lbs., of pure glucose or some other material with suitable Raman and mechanical properties is positioned against the aperture in place of a finger. It is placed and held at the same total force as is midrange for fingertip spectroscopy; the contact area can be assumed to be 1 cm² since that is about the average. The laser is incident on the pellet surface very near the center of the aperture and depending on the thickness of the spring steel may be further adjusted to pass through the modulating surface as close to the center of the aperture as possible. The main issue here is that the pellet surface is at the backside of the modulating surface and this initial alignment is with respect to that surface.

The pellet is then removed and a finger is positioned so that in real time the CCD response can be observed. Usually the position is already optimal at this point because the inevitable doming causes the light to impact the tissue just above and to the side of the level that it impacted the flat tightly pressed pellet. But the position of the collection cone relative to the dome is unchanged, because it is already aligned normal to the dome top. Thus there is a very small distance between the center of the laser and the center of the collection cone of the first lens. The raw wavelength shifted CCD output contains fluorescence, Raman scattered light, and stray light from all other sources that enter the spectrograph at sufficiently high angles. As shown in Fig. 6, at this point, in our apparatus, roughly 200 to 300 counts of
(Andor) CCD output can be observed across 800 cm$^{-1}$ of Raman shift in a single 20 ms frame. Roughly 20–40 counts above the fluorescence baseline comprising amide I, amide III, and CH$_2$ deformation modes at roughly 1670, 1310, and 1450 cm$^{-1}$ Raman shift, respectively, are easily seen on a single frame.

As we have noted earlier, if the finger is pressed relatively hard against the modulating surface; the overall signal level can be seen to decrease significantly and the position of the irradiated spot on the finger tip moves very slightly, usually <20 µm away from the center of the aperture. Because the hole is so small, and the spot diameter is long >200 µm in that dimension, there is considerable overlap of the irradiated spots in the pressed and unpressed states. For applied pressures in the range of normal blood pressures, there is virtually no movement at all. It is important that the pressure be well defined to define a spectroscopic experiment that can be executed reproducibly. We found it useful to independently measure and record the blood pressure and pulse rate of all test subjects at least once during a testing session and to define the applied pressure in terms of these values.

The orientation of the collection cone, i.e., optical axis of the first collection lens, and the entrance point of the laser into the stratum corneum is shown in Fig. 1 and the alignment position is arrived at by the procedure described above. The Raman and fluorescence is optimized by translating the aperture very slightly in the plane of the incoming laser and the symmetry axis of the collection system, keeping the irradiated spot close to the center of the aperture. The tissue modulating surface containing the aperture is oriented normal to the collection system optical axis and is mounted on a micrometer driven translation stage so that it can be moved while keeping the laser and collection optics stationary.

VI. EXPERIMENTAL RESULTS

The regions used to calculate the integrated inelastic signal (IE) and the integrated elastically scattered radiation (EE) are shown in Fig. 6. Mie and Rayleigh scatterings from all the tissues in the irradiated volume dominate the elastic signal but in practice it also contains light reflected from the skin surface. In order to probe the skin topography, we can translate the skin relative to the laser beam, either (1) by moving the finger relative to the stationary aperture with the aperture stationary relative to the excitation and collection optics, or (2) by moving the aperture relative to the excitation and collection optics with the skin stationary relative to the aperture. In the latter case, the amount of detectable off-axis elastic light depends on the location of the edges of the aperture relative to the incoming laser and that is clearly not the case with the former approach.

Figure 7 shows data obtained by the former approach. Here a test subject scrapped his fingertip across the aperture moving in the plane of the incoming laser and the symmetry axis of the collection system. The design of this instrument ensures that the part of the fingertip probed always has ridges that run perpendicular to the direction of motion. While observing the color changes of the placement spots and the pressure slider on the PDPM feedback window, the fingertip was moved through a known distance in a known amount of time with relatively constant applied pressure. The IE and EE integrals are plotted as a function of distance translated. Both fluctuate by roughly 20% with a spacing of about 500 µm, characteristic of fingerprint ridges. Interestingly, the fluctuations in the EE integral and the IE integrals are complementary, one increasing when the other decreases, as is observed in pulse modulated time dependent measurements.

Since the aperture is large enough, extrusion of the fingertip skin to form a dome in the aperture can occur using either approach. When the test subject is being relied upon to move the finger relative to the aperture and the optics, it is difficult to control the position and extrusion of the tissue.
or Mi

Fig. 8. A wide variation in what and the EE little or not at all. This photobleaching effect will be discussed thoroughly in another paper but for now we note that it corresponds to observations we previously described incorrectly as a “settling effect.”

Figure 7 shows an example of typical data obtainable by a skilled test subject using the former approach to show the qualitative features of such data.

For comparison with theory, we mostly employ data obtained using the second approach, in which the fingertip is stationary relative to the moving aperture. This can be successfully executed by anyone including an unconscious test subject. In the second approach the aperture position is known and reproducible to 0.1 μm with the applied force servo working as described above maintaining constant pressure to ±10 g/cm². Figure 8 shows data obtained in this way in which the aperture was moved in 20 μm steps and held motionless for a second at each position. While the position is stationary we observe that the IE decays somewhat and the EE little or not at all. This photobleaching effect will be discussed thoroughly in another paper but for now we note that it corresponds to observations we previously described incorrectly as a “settling effect.”

As described in detail elsewhere, the observations in Fig. 8 are well described by theory. When the tissue extrudes a small dome is formed in the aperture and much of the difference between the Figs. 7 and 8 occurs because the position of the collection and excitation optics is locked on the same part of the dome for the Fig. 7 but the incoming laser scans across the dome in the Fig. 8. A wide variation in turgor, blood pressure, finger sizes, and skin tones are spanned by the selected test subjects with qualitatively similar results observed for all. The pressure used to establish and maintain registration of the fingertips with the aperture was chosen in each case to be the average of the systolic and diastolic blood pressures of each individual test subject. Figure 8 shows that using this instrument we obtain consistent results from a wide variety of test subjects. The consistency of these observations indicates that monitoring the variation in EE and IE with fine adjustment of the aperture position after the setup procedure described above could allow the device to automatically find and interrogate the tissues at a location of consistent, predetermined topography and signal content. Specifically, positions of maximized IE and minimized EE are preferred because they correlate to the tops of primary fingerprint ridges.

We point out that despite the idiosyncratic aspects of in vivo human spectroscopic probing, the gross anatomy of ridged fingertip skin is constant across all but the most abnormal test subjects. We suggest that the results are reasonably consistent because the treatment of the different tissues samples, i.e., test subjects, has been relatively consistent. This general consistency suggests that the pressure is a universal variable appropriate for characterizing the state of perfused tissue and soft matter generally during spectroscopic probing. Thus it is important that the pressure be well defined to define a spectroscopic experiment that can be executed reproducibly.

Perhaps under appropriate constant pressure conditions, the turbidity and relative density of the tissues in the probed volume do not vary much across individuals. Using this instrument, except for pulse driven motions and acoustic waves, the tissues can be isobaric and motionless during the measurement process. This does not necessarily mean that further processing of data acquired using this instrument for purposes of a turbidity correction to the quantitative analysis of blood and other tissues is unnecessary. This must be ascertained with more experimentation but using this instrument, data collected for different test subjects or at different times for the same test subject can be compared and reproduced in an objective and quantitatively consistent manner.

The instrument described in this paper was developed subsequent to a previous small study of noninvasive glucose using “tissue modulated,” i.e., “difference spectra.” In this case there was considerable correlation between the external fingerstick data and the corresponding LightTouch™ measurements but there were some inaccurate data that apparently resulted from inconsistent application of pressure during the measurement cycle. Because of the observed correlation with external glucose measurements, we surmise that most of that data were obtained when the test subject compliance was adequate but there was no quantitative record of each measurement process for confirmation.

While there are many types of motion and placement artifacts, Fig. 9 shows data demonstrating one type of motion artifact made detectable by this instrumentation. Figure 9 shows the sensor record of an intentional “rolling motion” of the type described by Asada. The record of the measure-
ment sequence provided by the device allows deduction of the course of events. In this case the initial placement of the finger is skewed (uneven number of spots top and bottom, i.e., quadrants 1 and 0, at \( \tau=0 \)) and apparently becomes acceptable (not equal but stable numbers of spots for each) for the last third of the experiment.

But the asymmetry evident in the quadrant dot pattern in the middle graph shows that the finger rolled somewhat from top to bottom during the first part of the measurement. The placement was skewed initially and remained slightly skewed after the rolling motion occurred, i.e., no twisting motion occurred simultaneously. We know it was a rolling
motion and not a scraping motion because the quadrants 2 and 3 (spots are on either side of aperture along the axis of the finger) are relatively constant. On this basis alone the data should be rejected. The change in contact area was abrupt, so we know there was gross motion and not normal subsurface fluid movement due to the applied pressure. This record was generated intentionally but it also corresponds to the situation when the well-meaning test subject suspected that something was wrong after the measurement cycle started and tried to correct the placement without informing anyone.

Since only a simple cylinder was used to contact the bare finger, i.e., no dorsal splint was employed, there are sharp spikes in the optical record corresponding to the spikes in the force and pressure records due to the pulses, and they are very weak using this particular transducer and coupling. Together with appropriate criteria based on general quantitative experience, any specific human factor data can be processed to allow objective rejection or acceptance of associated spectroscopic data before calculation of, e.g., a glucose concentration, without operator involvement.

VII. SUMMARY AND CONCLUSIONS

We described a versatile instrument for probing human volar side fingertip capillary beds with NIR while collecting Raman, Rayleigh/Mie, and fluorescence simultaneously. The probing can be executed under constant pressure conditions, and a complete record of the placement, motion and behavior under compression of the tissue in question is collected and stored throughout the measurement process. This apparatus should allow methodical and systematic experimentation with noninvasive blood and tissue analysis in vivo. This instrument and the same spectroscopic tools may also be applicable to a variety of other animate and inanimate soft matter samples.

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3 V. Tuchin, Tissue Optics (SPIE, Bellingham, 2000).
7 See, for example, J. Chaiken, U.S. Patent No. 6,223,063 (24 April 2001).