

A new device for assessing changes in skin viscoelasticity using indentation and optical measurement

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Background/aims: Skin is a viscoelastic material, comprised of fluidic and fibrous components. Changes in viscoelasticity can arise due to a number of conditions including dehydration, swelling (associated with injury or disease), impaired heart function, rehydration therapy, ageing, scarring, sun exposure and genetic conditions affecting connective tissue. Quantification of changes in skin viscoelasticity due to these processes is of great clinical interest in the fields of therapy monitoring, wound healing and disease screening. However, devices currently available to measure aspects of the mechanical properties of skin have limitations in ease-of-use, accessibility, and depth of measurement. This paper describes a new technique to follow changes in the viscoelasticity of the skin, using a novel approach to an indentation manoeuvre. The device is portable, low-cost and easy to use while at the same time providing rich information on the mechanical response of the skin.

Methods: The method proposed optically tracks the skin's recovery from an initial strain, made with a novel linear indenter, using diffuse side-lighting and a CCD video camera. Upon indentation, the skin's elastin fibres are stretched and fluid is displaced from the compressed region. When the indenter is removed, the rate of recovery of the skin from this

imprint is therefore principally dependent on its hydration and elasticity. Using the blue colour plane of the image and polarisation filtering, it is possible to examine the surface topography only, and track the decay of the imprint over time.

Results: The decrease in size of the imprint over time (decay curve) recorded by the device is shown to agree with the theoretical predictions of an appropriate viscoelastic model of skin mechanical behaviour. The contributors to the response measured using the indentation device are fully characterised and evaluated using separate measurement techniques including high-frequency ultrasound, polarisation spectroscopy and optical coherence tomography.

Conclusion: The device developed is capable of tracking the viscoelastic response of skin to minimal indentation. The high precision achieved using low-cost materials means that the device could be a viable alternative to current technologies.

Key words: skin indentation – viscoelasticity – side illumination – skin recovery

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THE SKIN is the largest organ of the body and is frequently used as a 'window' to reveal aspects of the overall health of a person. The classic signs of ill health are often apparent in a person's complexion, especially skin colour; pale skin (corrected for ethnicity), indicates too little haemoglobin (too little blood), vasovagal activity (sickness induced by the vagus nerve) or shock. Other features indicative of illness such as sweating and inflammation can be directly observed by the naked eye.

In addition to the visual assessment of skin health, a 'hands-on' approach can yield more in-depth information. Capillary refilling rates, after application of light pressure with the thumb or finger, can be used as an indicator of circulatory

function. The gathering up of skin between the thumb and index finger to assess its 'turgor' or stiffness and resilience in returning to its original position is often used by clinicians as a reflection of dehydration of the skin and the body as a whole (1). These assessments are, in essence, tests of the mechanical properties of skin with the results used to extract information on its fluid content. The manoeuvres and their assessment are, however, subjective and dependent on the experience of the examiner. While a number of research devices exist for the quantification of specific aspects of skin health (2–7), there is currently no 'gold standard' instrument available to add objectivity to the clinician's qualitative evaluation of skin turgor.

Although pronounced dehydration or overhydration are clinically evident, lesser degrees of both abnormalities and the response of a patient to therapy (intravenous/oral rehydration or diuretics) would be well served by the development of a device for the measurement of skin mechanical properties pertinent to the assessment of dermal and hypodermal water content.

Determinants of Skin Viscoelasticity

The total fluid volume of a 70 kg man is approximately 42 L (60% of total body weight). This is distributed in the intracellular (28 L) and extracellular space [14 L, of which plasma is 3.5 L and interstitial fluid (bathes and surrounds the cells) is 10.5 L] (8, 9). The skin itself acts as a reservoir, containing approximately 20% of all water in the human body (6). The main supply of tissue water comes from the microvasculature; the blood vessels dilate, increasing the permeability of the vessel walls to water molecules, allowing fluid to leak to the interstitial space [a mechanism demonstrated by Ryan (10)].

As well as being filled with elastin and collagen fibres, the extracellular space contains a hydrophilic (water-absorbing) gel known as ground substance. This water-based substance is particularly prevalent in loose connective tissue. The exact composition varies, but contains mucopolysaccharides (glycosaminoglycans) and tissue fluid (11). Of these polysaccharides, the most important is hyaluronic acid. This is a linear chain molecule with a large capacity to absorb water that forms a thick 'gel', impeding the bulk flow of fluid, with occasional gaps within it to allow larger molecules through (12). The mobility of water within this gel meshwork has a large impact on the mechanical properties of the tissue (13–19).

Current Technologies

A number of different technologies exist for the non-invasive assessment of aspects of skin health using electrical, optical and mechanical means. Electrical devices such as the corneometer measure the impedance of the *stratum corneum*, using it as a measure of hydration of this layer (6, 20). Viscoelasticity can be assessed more directly using mechanical devices such as the cutometer, a suction cup technique that measures the force required to raise a pre-defined amount of skin (5,

TABLE 1. Current techniques for non-invasively measuring changes in the mechanical properties of skin

Technique	Measurands	Sampling depth
Corneometer	Water content	<i>Stratum corneum</i>
Cutometer	Elasticity, water content	Full thickness/superficial
Extensimeter	Elasticity	Superficial
Ultrasound	Structure, water distribution	Full thickness
OCT	Structure	Epidermis, upper dermis
Skin indenter	Water content elasticity	Full thickness

21). 'Imaging' of elastic properties can be achieved using ultrasound, and more recently optical coherence tomography (OCT), to track small displacements in skin due to a known applied stress (22–24). Table 1 gives a comparison of some of these technologies on the basis of measurands and penetration depth.

The electrical methods are limited in terms of their depth, only providing information on the *stratum corneum*. The ultrasound and OCT techniques are capable of providing detailed information, but are prohibitively expensive for the vast majority of clinics. Finally, suction cup devices, although sensitive to changes in the viscoelasticity of the full thickness of the skin, are also quite expensive and would certainly be out of reach for most home users. This paper describes a novel device based on an intuitive clinical manoeuvre and constructed from inexpensive materials.

Aims

The aim of this project was to develop a technique that can return information on the viscoelastic properties of skin. The technique should be non-invasive, easily interpretable, simple in its design and have the potential to be implemented easily in a clinical or home-use situation.

Theory

The skin is viscoelastic in nature, having contributions from elastic (collagen and elastin fibres) and viscous (tissue water and ground substance) components. Because of the complexity of human tissue water, its reaction to a mechanical stress involves several factors including fibrous, circulatory and nervous elements.

A mathematical model describing the reaction of skin when it is indented and allowed to recover is discussed. Also, because the device described by this paper uses an optical technique to track the recovery of the imprint in skin, the

principle of measurement is described and the relationship between the size of the imprint and the illumination scheme is derived.

Skin indentation

The mechanical structure of the skin can be simplified as being composed of water and fibre components. Stained histology slides reveal the arrangement of elastin in the epidermis and dermis (Fig. 1a). Using this as a starting point, a schematic of the skin before and after indentation can be drawn (Fig. 1c).

When pressure is applied to the skin, the water directly underneath the point of contact is squeezed to the side. This results in a build-up of fluid to either side of the indenter and a deformation of the vertical elastic fibres as well as the horizontal elastic 'sheet' deeper in the dermis. The displacement of fluid was imaged using high frequency (20 MHz) ultrasound (Dermascan C, Cortex Technology, Denmark) where areas of high fluid content are indicated as areas of low echogenicity (Fig. 1b). When the indenter is removed, the horizontal elastic 'sheet' can recover rapidly (within seconds), but the vertical fibres are slower, their movement hampered by water in the tissue (freely moving and tissue gel) (25, 26). Therefore, the rate of recovery of the skin, and the decay time of the imprint, is

dependent on the elastic properties of the fibres, and the quantity and viscosity of the fluid within the tissue.

Optical imprint tracking

The principle of measurement is the use of side-illumination to reveal the topography of the skin. As illustrated by simple geometric optics in Fig. 2, when a light source is directed at a low angle with respect to the skin's surface, a shadow is formed in areas where the skin deviates from its normal shape, such as an imprint.

As described by the viscoelastic theory, once the indenter is removed, the skin starts to recover and the imprint decreases in size. This results in a decrease in the shadow size that can be explained using a simple geometrical approach.

The light is incident on the imprint as shown and a shadow of width w_s is cast. To see how this is related to the imprint depth d_i , the similar triangles formed by the intersection of the light with the base of the imprint and the undisturbed skin ($\triangle ABC$ and $\triangle CXY$) are examined. The ratio can be written as:

$$\frac{1/2(D - w_i)}{1/2(w_s + w_i)} = \frac{h}{d_i} \quad (1)$$

It should be noted that D , h and w_i are constants and are determined by the lighting ring

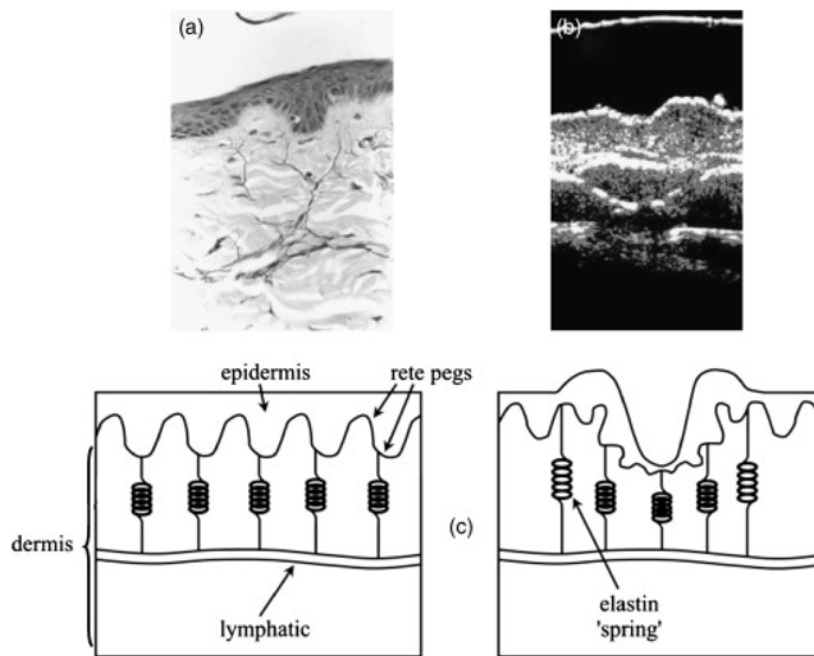


Fig. 1. Stained histology slide (a) showing arrangement of elastic fibres in the epidermis. Ultrasound image of skin (b) showing that after indentation, water is shifted from the compressed area to either side of the imprint (areas of low echogenicity). Vertical elastin fibres act as springs connecting the rete pegs (dermal-epidermal boundary) to fibres in the deeper dermis running parallel to the surface of the skin (c).

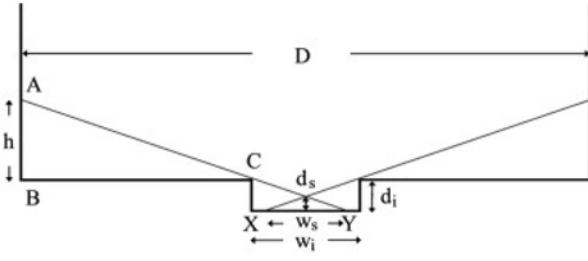


Fig. 2. A simplified picture of imprint illumination. The imprint has depth d_i and width w_i . The light is incident from the lighting ring of height h and inner diameter D forming a shadow of width w_s and 'depth' d_s .

dimensions and indenter thickness, respectively. Equation (1) may be written in terms of the shadow width as:

$$w_s = \frac{d_i(D - w_i)}{h} - w_i \quad (2)$$

From Eq. (2), it can be seen that the shadow width is proportional to the imprint depth. Similarly, the variable d_s (shadow 'depth' i.e., 'darkness') can be related to the imprint depth by considering two different triangles ($\triangle ABC$ and the small triangle of base $0.5 w_s$ and height d_s).

$$\frac{1/2(D - w_i)}{1/2(w_s)} = \frac{h}{d_s} \quad (3)$$

Re-arranging Eq. (3) and substituting the expression for w_s (Eq. (2)):

$$d_s = \frac{d_i(D - w_i) - hw_i}{D - w_i} \quad (4)$$

Equations (2) and (4) show that the area and/or darkness of the shadow in the imprinted area is proportional to the actual imprint depth, and therefore the strain in the skin. If the shadow size is tracked over time after imprinting (using a digital video camera), then some conclusions can be drawn about the mechanical properties of the skin.

The above analysis is based on the comparison of similar triangles. However, it should be noted that the apex of the small triangle in the imprint (of base XY , defining the shadow 'depth') does not extend to the full depth of the imprint. Therefore, when d_s becomes sufficiently small, this triangle approximation breaks down. The limit of the linear relationship defined by Eq. (4) is thus the value of d_i for which d_s is zero. This is simply given by the intercept of the equation and defined by the characteristics of the device:

$$d_{i,MIN} = \frac{w_i h}{D - w_i} \quad (5)$$

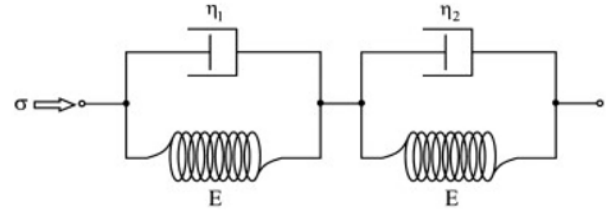


Fig. 3. Adapted Kelvin-Voigt model. Two fluids of viscosities η_1 and η_2 are incorporated, corresponding to fluids of low and high viscosity (interstitial fluid and ground substance) respectively. For simplicity, it is assumed that the bulk elastic properties of the fibres remain constant. Therefore, both springs are assigned identical Young's modulus E . The elements are arranged in series so that when the initial stress σ is removed, the model recovers in a biexponential fashion.

Viscoelasticity

Mechanical analogues of viscoelastic behaviour may be developed in the form of combinations of springs and dashpots (pistons whose movement is damped by a fluid of viscosity η). This has already been used successfully to describe the properties of polymers (27) and applied in stress relaxation and creep experiments in skin (26, 28). In this analogy, the elastic fibres of the skin are represented by the springs and the tissue fluid represented by dashpots.

Because of the effect of absorption by hyaluronic acid, water is present in the skin in varying degrees of viscosity and mobility. Therefore, any model of the skin's behaviour will have to account for this variation. To describe this mathematically, the standard Kelvin-Voigt was modified as indicated in Fig. 3.

When a stress σ is applied to the arrangement shown in Fig. 3, the element will be displaced. If this stress is then removed, the strain will decay as the model returns to its equilibrium condition. In a single Kelvin-Voigt element (spring and dashpot in parallel), this recovery takes the form of an exponential decay in strain (27). For the model above, the total strain is simply the sum of the strains in the individual Kelvin-Voigt elements, and the resulting decay is:

$$\varepsilon(t) = \varepsilon'_1 e^{-\frac{t}{\tau_1}} + \varepsilon'_2 e^{-\frac{t}{\tau_2}} \quad (6)$$

where the time constant is defined as $\tau = \frac{\eta}{E}$ and ε' is the initial strain.

This shows that when the element is deformed and released, it recovers biexponentially with a time constant depending on the viscosity of the fluid and the elasticity of the spring.

The constants ε'_1 and ε'_2 represent the initial conditions (displacements) of the dashpots in their undisturbed state, and as such, the quantity

of fluid in each. Therefore, in the rest of this paper, they are treated as fractional constants giving the relative quantity of one type of fluid with respect to the other and will be referred to as C_1 and C_2 . Application of this curve to experimental data can then be used to follow changes in skin water content and elasticity.

Instrumentation Development

Design

The first task was to find an object capable of making a reproducible imprint to a depth sufficient to cast a detectable shadow that could be easily analysed. The published work on indentation focuses almost entirely on circular indentors (10, 25, 29–32). However, a linear-shaped indenter was chosen based on the predictability of its effect on the tissue and ease of alignment. Also, linear imprints had the advantage of having the ability to be incorporated into a linearly polarised lighting system (not possible with curved geometries).

An indentation time of 20 s was chosen. This was based on previous work in the impression technique using stress relaxation measurements that showed that most fluid translocation and stress relaxation in the skin had occurred after this time (15, 26).

The illumination system consisted of two 'strip lights', each made from four standard LEDs connected in parallel and powered using the 5 V supply from the computer's USB socket. Each LED outputs approximately 5600 mcd at a maximum forward current of 30 mA dispersed over a 20° viewing angle.

Because this measurement technique involves monitoring the changing shape of an imprint in the skin, from an imaging point of view it is the topography, and therefore the uppermost surface of the skin that is of most interest. This means that light that has only interacted with the surface should be included in the analysis. Light in the red region (approximately 600–700 nm) may penetrate several hundred microns or more into the tissue before emerging, as it is in the so-called 'optical window' of low absorption and scattering.

For this reason, light of wavelength < 500 nm is chosen. In practice, this is carried out by manipulating data from the blue colour plane (approximately 400–500 nm) of the camera used to acquire the images. The emission spectrum of the LEDs

was measured using a spectrometer (Ocean Optics HR2000, Dunedin, FL, USA) and found to have strong emission in the blue region, which is suited to this application.

The digital video camera used (Panasonic NV-GS150, Panasonic, Milton Keynes, UK) has three separate CCD arrays (one each for red, green and blue) with two dichroic prisms used to split the incoming beam into three. Image acquisition was performed at 5 frames per second (f.p.s.) with data streamed to the hard disk of a laptop computer via 'Firewire' (IEEE 1394 interface, data transfer rate of approximately 100 Mbit/s) using a frame-grabbing program custom-written in Matlab.

Although an appropriate choice of camera colour plane can ensure that the most superficial data is being looked at, even the heavily absorbed blue light ($\lambda = 400\text{--}500$ nm) still has a finite penetration depth (approximately 150 μm depending on the degree of pigmentation i.e., melanin level) (33). Also, because haemoglobin is a major absorber of light in the blue region, any light that interacts with the microcirculation will be heavily absorbed and sensitive to local changes in blood supply. The penetration depth of blue light is sufficient to reach the superficial dermis and the papillary loops. Indentation results in compression of the capillaries causing a temporary 'blanching' of the skin. Once the blood begins to return to the area in the 10–20 s following the removal of the indenter, an increase in light absorption is seen and the area appears darker. The result is an apparent increase in the size of the shadow. This capillary refilling process is superimposed on the signal due to the changing size of the imprint, rendering those initial seconds useless.

The extent of the flushing effect on the tissue in the vicinity of the imprint was investigated with the TiVi Imager (Wheelsbridge AB, Linköping, Sweden). This is a sub-epidermal imager that uses polarisation gating to extract information on red blood cell (RBC) concentration (34).

The TiVi imager was positioned, using a moveable metal arm, approximately 15 cm above the measurement site (volar forearm) and the skin was imprinted using a linear indenter 15 mm long and 1 mm thick for 20 s. TiVi image acquisition was started 15 s into the indentation so that the skin could be monitored as the indenter was being removed. Total acquisition time was 1 min. The average TiVi value in each image was calculated and plotted as a function of time in Fig. 4.

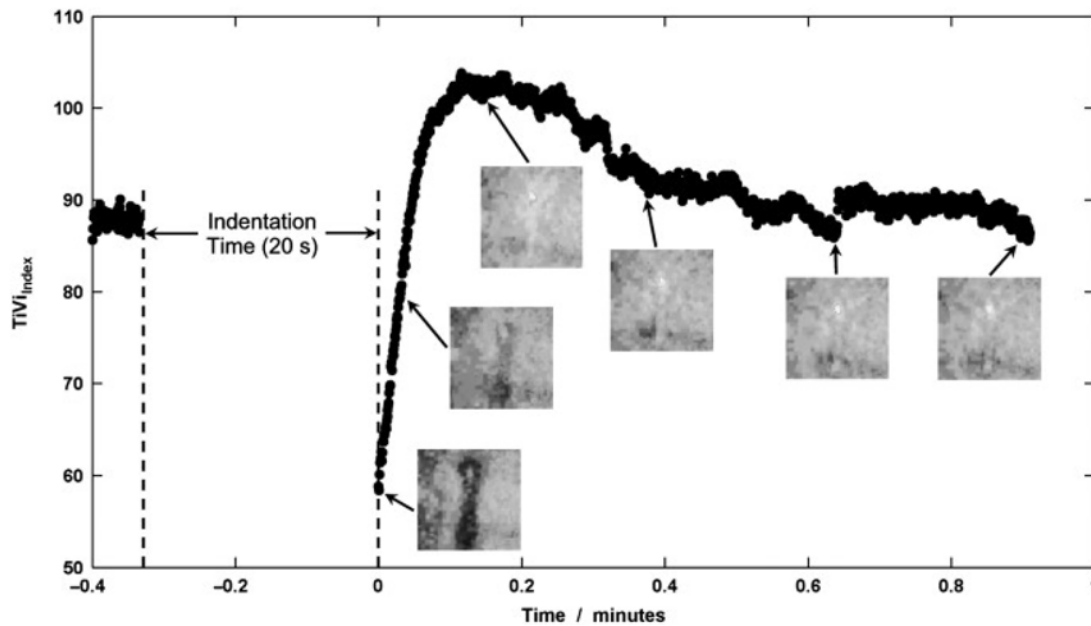


Fig. 4. Average $TiVi_{index}$ of the imprinted region plotted against time showing capillary refilling after removal of the indenter. The indenter was removed from the skin at $t = 0$ min. The images shown are $TiVi_{index}$ maps at six different instances during the test (dark: low concentration of RBCs, bright: high concentration).

The results from the TiVi imager showed that the indentation caused blood to be squeezed from the microvasculature in the compressed area resulting in the dark region shown in the ' $t = 0$ min' image in Fig. 4. The time trace (average $TiVi_{index}$) shows that the concentration of RBCs increases in a reactive hyperaemia as blood flows back into the area, reaching a maximum after approximately 6 s. Baseline levels are reached after approximately 30 s.

To minimise the signal returning from layers deep enough to be influenced by the microcirculation, a polarisation set-up was designed. The side-lighting system was fitted with two linear polarisers so that the transmission axes of each are parallel; therefore, only light reflected from the skin that retains its polarisation state will pass through a third filter (also parallel) on the camera lens. This constitutes approximately 3% of the incident light. In the skin, light becomes depolarised after approximately 10 mean free path-lengths (35). Using measurements of the scattering coefficient of the skin, this corresponds to a depth of $70\text{ }\mu\text{m}$ for blue light ($\lambda = 450\text{ nm}$) (36) and is approximately equivalent to the depth of the epidermis in the forearm. Therefore, the component of the signal that has interacted with the microcirculation will be heavily attenuated while that due to the surface changing topography will not. This serves to enhance the signal by

removing a significant quantity of the interfering deeply penetrating light.

Figure 5 shows the decay of an imprint as measured using OCT. Successive B-scans of a cross-section of the centre of the imprint were acquired using a commercial swept source system (ThorLabs OCS1300SS, central wavelength 1325 nm) and the depth of each was measured using separate software (Fig. 5b). The acquisition speed was set at 5 Hz to match the image acquisition of the indentation device.

The shape of the decay curve matches that predicted by the viscoelastic theory, decaying biexponentially from a maximum at $t = 0$ min. The shape of the first 10 s of the decay is notable for the fact that there is no 'upward' section; thus, this part of the signal observed in the indentation device data is due to a factor unrelated to the mechanical movement of the skin, i.e., capillary refilling, verifying the need to use polarisation filters in the device. Over the course of the 3-minute monitoring period, the imprint decreased in size from 680 to $255\text{ }\mu\text{m}$.

The next step was to determine the optimum placement for the LEDs, in order to ensure the greatest contrast between the imprint shadow and the surrounding skin. This would maximise the dynamic range of the signal between the baseline and the maximum depth of the imprint. Four LEDs were set in 5 mm diameter holes drilled in

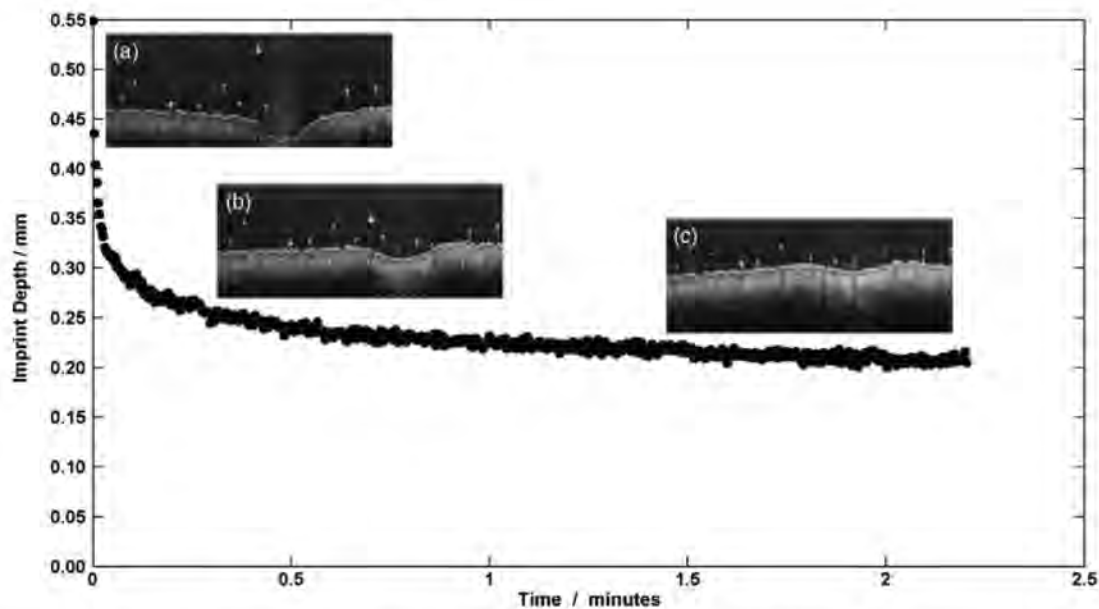


Fig. 5. Plot of imprint depth against time as measured using optical coherence tomography. Inset: optical coherence tomography B-scans showing the imprint cross-section at (a) $t = 0$ min, (b) $t = 0.2$ min, (c) $t = 2$ min. The depth of the imprint is measured using a custom-written software package that allows the user to draw two straight lines and measure the distance between them (b).

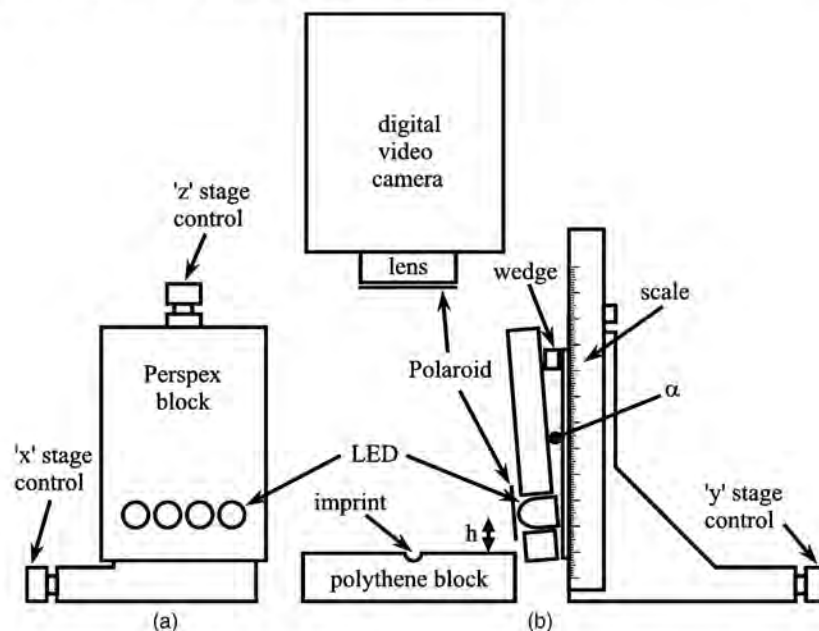


Fig. 6. Test rig to determine the optimum placement of the LED lighting system: (a) Micrometer stage and LED holder and (b) full set-up. The imprint ($15 \text{ mm} \times 1 \text{ mm} \times 0.5 \text{ mm}$) was scratched into the polythene block using a sharp blade. The LEDs were held in the Perspex block in four 5 mm diameter holes. The height, h , of the LEDs was adjusted by the z control of the stage and read off the scale. The inclination, α , of the LEDs was adjusted by changing the wedge size.

a Perspex holder. This was mounted on an XYZ stage with micrometer controls. A diffuser was placed in front of the LEDs in order to avoid spotlighting effects from the individual components. This was achieved by using 12 layers of clouded adhesive tape (optimised experimentally to minimise intensity variations in the field of view). An artificial imprint was created by scratching a

$15 \text{ mm} \times 1.5 \text{ mm}$ channel ($< 1 \text{ mm}$ deep) in a heavy block of white, diffusely reflecting material (polythene). The polythene and lighting rig were set up as shown in Fig. 6.

The imprint was offset 20 mm from the LED strip and the camera was positioned 80 mm overhead. The z control of the stage was adjusted so that the central axes of the LEDs were level with

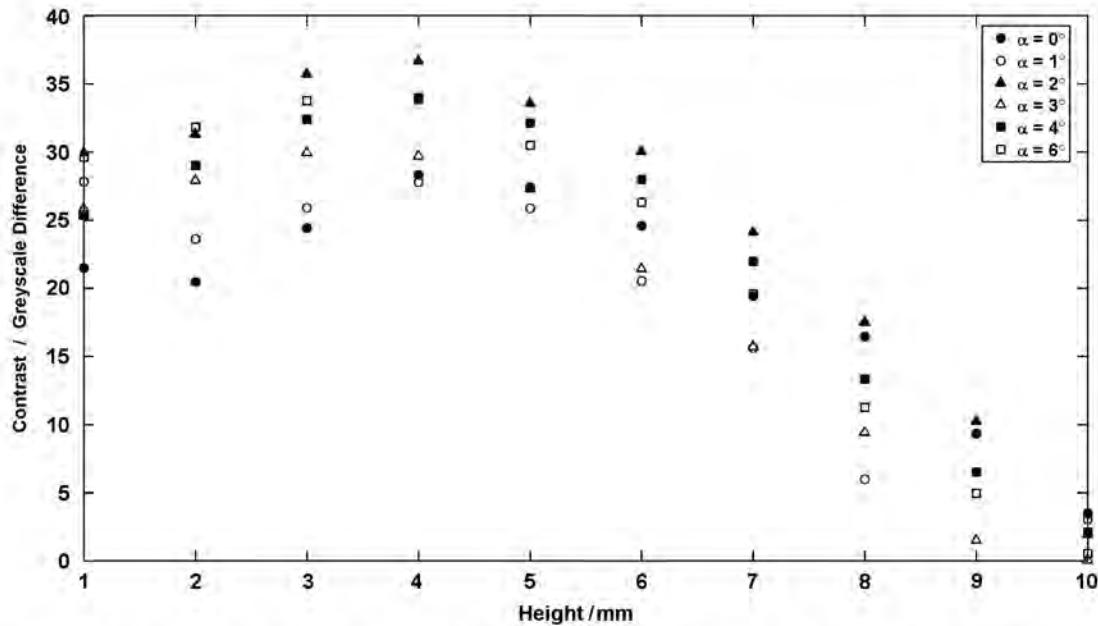


Fig. 7. Plot of contrast as a function of height and tilt (α). The highest point on the plot represents the optimum configuration.

the top of the polythene block. This was the starting point for measurement of the height, h . The angle of inclination, α , of the lights was varied by inserting wedges of varying size as shown in Fig. 6. For each specific value of h and α , 25 images of the imprint were acquired using the Matlab frame-grabbing program. α was varied from 0° to 6° in steps of 1° , and h was varied from 1 to 10 mm in steps of 1 mm. A program was written in Matlab to process the images and calculate a measure of the contrast between the imprint and the unimprinted area. Regions of interest (ROI) corresponding to these areas were selected and the average greyscale value calculated. The contrast then, was simply the difference between the average greyscale values for each ROI. This was plotted against h for each value of α (Fig. 7).

At each value of α that was tested, the contrast increased with h , reaching a maximum at 4 mm above the imprint. After this point, light striking the imprint from above began to reach the inside of the imprint and 'wash out' the shadow resulting in a decrease in contrast. At $h = 4$ mm, the corresponding contrast maximum for α was found at 2° .

The final prototype was constructed using the design parameters calculated for the new lighting system: $h = 4$ mm, $\alpha = 2^\circ$, diffuser thickness = 12 layers, and illumination strip width = 5 mm. The final design is illustrated in Fig. 8. The walls of the main housing were made from Perspex and

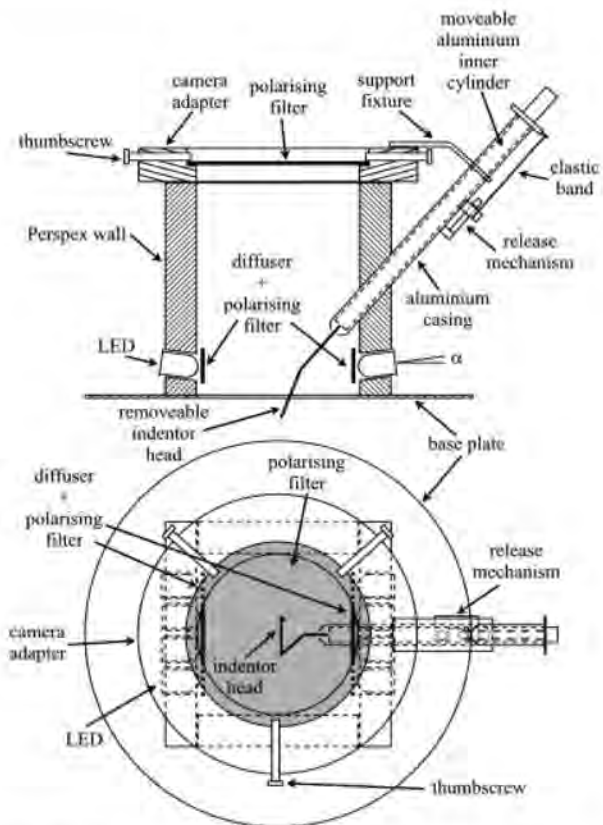


Fig. 8. Indentation device final design. α was set to 2° , the distance from the central axis of the LEDs to the bottom surface of the base plate was 4 mm and the diffuser was placed between the LEDs and the polarising filter.

the adapter (allowing connection to the camera) was heavy polythene. The base plate was used to spread the weight of the device, ensuring a stable

measurement environment and minimising discomfort to the test subject. The indenter mechanism was constructed entirely from aluminium, making the structure robust and reducing the risk of indenter misalignment (lateral movement of the indenter head) upon contact with the skin during a test. The release mechanism consists of a 'C-shaped' piece of aluminium, which grasps a pin attached to the inner cylinder of the indenter shaft. This locks the indenter in the 'down' position and when turned, releases the indenter rapidly. The indenter head itself is made from heavy gauge (1 mm diameter) steel wire, bent into shape. The entire device can be completely disassembled to allow access for maintenance.

The finished prototype is attached to the camera lens assembly and secured to an adjustable tripod. To conduct an indentation test, the indenter is locked in the 'down' position before making contact with the skin. Using the tripod controls, the device is then lowered against the skin until contact is made, and allowed to come to rest under its own weight. After the imprint time has elapsed, the release mechanism is activated and the indenter recoils from the skin. The camera then records images of the imprint over the following 3 min.

Image processing

An algorithm based on calculating the correlation between successive images and a reference value has been developed. This so-called correlation coefficient (CC) algorithm computes a pixel-by-pixel comparison between the images of interest and a reference image, tracking the changing size of the imprint over time.

The CC is used to indicate the strength of a linear relationship between variables and may vary between -1 and 1 . Mathematically, the CC of one variable X , with another variable Y , is defined as their covariance divided by the product of their standard deviations. This can be expanded to two dimensions to compute a CC between two $m \times n$ matrices, A and B :

$$CC(2-d) = \frac{\sum_m \sum_n (A_{mn} - \bar{A})(B_{mn} - \bar{B})}{\sqrt{\left(\sum_m \sum_n (A_{mn} - \bar{A})^2\right) \left(\sum_m \sum_n (B_{mn} - \bar{B})^2\right)}} \quad (7)$$

where $\bar{A}$ and $\bar{B}$ are the average values in each matrix. This concept can therefore be applied to

images, which are two-dimensional arrays of greyscale values. The one-dimensional CC provides a measure of how strongly one variable is related to another; similarly, the two-dimensional CC provides a measure of how strongly one matrix is related to another. This can be loosely interpreted as how 'similar' one image is to another. Following from this, the coefficient can quantify how 'different' a particular image is from some reference. This means that for an image sequence showing the decay of an imprint (shadow of decreasing size), the CC between one particular image and a steady reference image will provide a variable that changes with imprint size.

If the reference chosen is the undisturbed skin (before indentation), the CC of each image in an indentation test with this reference should show a value of zero (at $t = 0$ min) when the imprint is at its maximum size, which gradually increases as the skin recovers and approaches its initial state. Therefore, with a small alteration, a CC index can be defined that varies from '1' when the imprint is at its maximum, to '0' when it has disappeared:

$$CC\ Index = 1 - CC_{i,ref} \quad (8)$$

where $CC_{i,ref}$ is the two-dimensional CC between an image i , in an imprint decay series, and a reference image ref as defined by Eq. (7).

Before an imprint is made, three sets of 50 images (each representing a 10 s period) of the imprint site are acquired. The reference image is then calculated as the average of these 150 images. This average is necessary to account for possible placement error as the device has to be removed from the skin before the indenter is placed in the 'down' position and ready for indentation (to ensure that the skin is compressed only in the direction perpendicular to its surface).

A Matlab program with a graphical user interface (GUI) was written to implement the algorithm, incorporating a number of pre-processing steps. Each image was read and processed individually. The initial steps, with each image, are to select the blue colour plane (superficial data) and cut out the ROI surrounding the imprint. This reduces the image from a $256 \times 256 \times 3$ matrix to an $m \times n$ matrix, where m and n are the dimensions of the ROI specified by the user. This image is then equalised by normalising each pixel value in the image by the average for the whole image. Note that these initial pre-processing steps apply to the reference image as well as

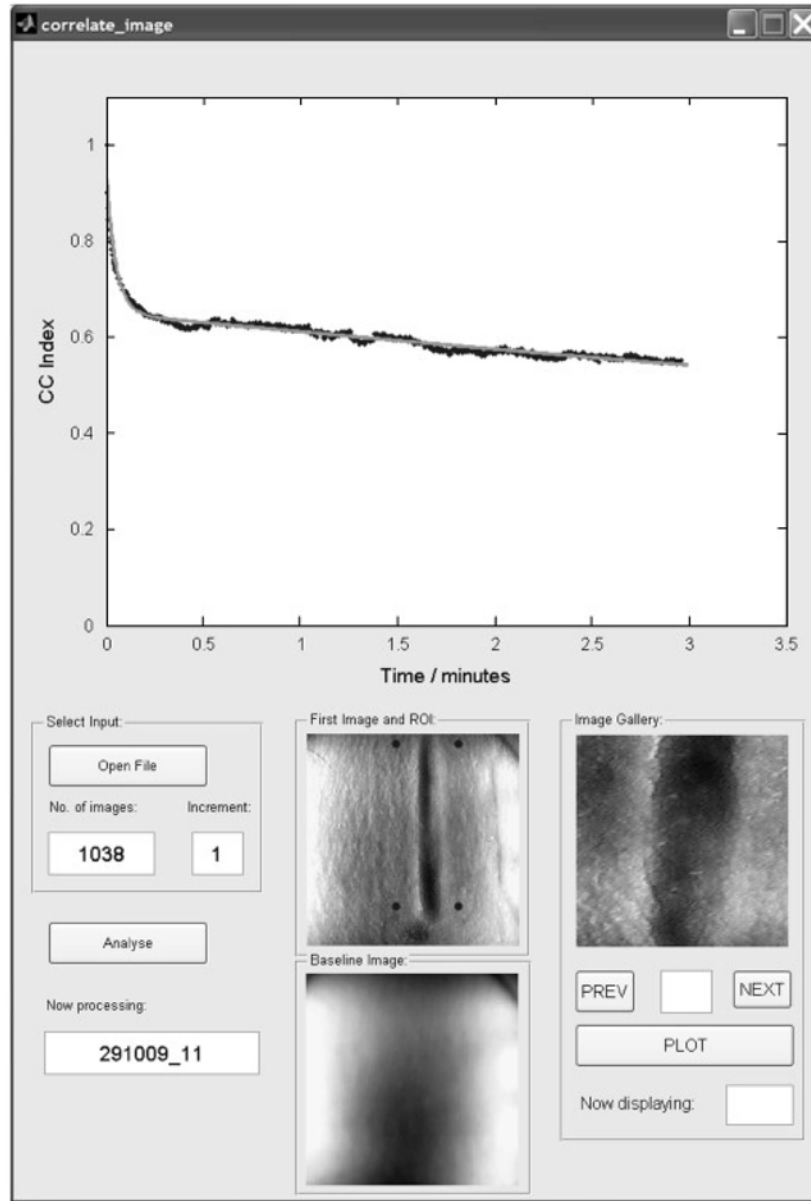


Fig. 9. Graphical User Interface written in Matlab to implement the CC algorithm. The main panel shows the decay data with its corresponding biexponential fit, while the panels underneath show (clockwise from top left) the first image and the regions of interest (ROI), gallery of normalised regions of interest in each image in the set, and the reference image.

the test images. Finally, the CC Index is calculated for the image by comparing it with the reference using Eq. (8). The time stamp is calculated based on the frame rate of the data (f.p.s.), each frame in the sequence representing $[\text{frame no.}/(\text{f.p.s.} \times 60)]$ minutes. The decay curve is then plotted simply as the CC Index against time. The front panel for the finished GUI is shown in Fig. 9.

Validation

Physical model and algorithm evaluation

To assess the performance of the device to controlled changes in imprint depth, a physical

model was devised using 'Blu-Tack[®]' (a mouldable adhesive made from synthetic rubber and polymers). A small flattened sample of the material was laid out on a Teflon block, acting as a base, covering an area of approximately $4\text{ cm} \times 4\text{ cm}$ at an even thickness of approximately 3 mm. OCT was used to ensure that the surface of the Blu-Tack[®] was as flat as possible.

The indentation device was mounted on an adjustable tripod and the control software was set to acquire images at 5 f.p.s. Using the indentation device, 20 sets of reference images of the undisturbed Blu-Tack[®] were acquired. Each 'set' con-

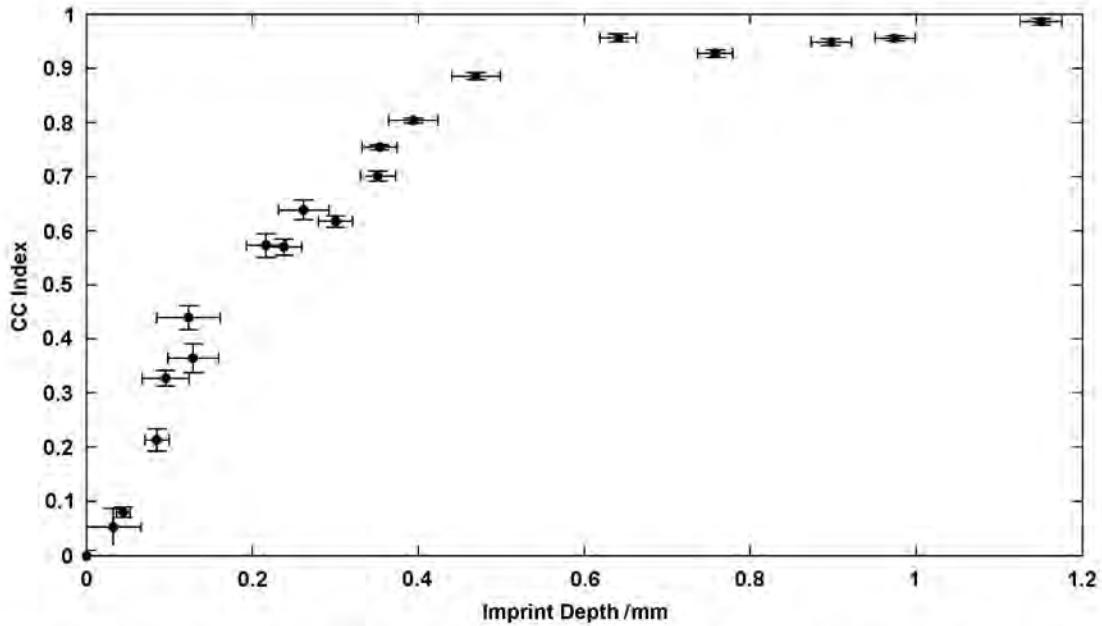


Fig. 10. Output of the CC algorithm (CC Index) plotted against imprint depth in a Blu-Tack model. The error bars represent ± 1 standard deviation.

sisted of 1 s of data (five images) and between each set, the model was removed and replaced under the indentation device (to simulate placement error and/or patient movement). A steel linear indenter head, measuring 30 mm long and 1 mm wide and mounted on a 'z' micrometer stage, was used to make controlled imprints of increasing depth in the surface of the Blu-Tack[®]. The exact depth of each imprint was measured at 15 different cross-sections along its length using OCT. After each round of OCT measurements, the indentation device was used to record 20 sets of images of the imprint in the same manner as for the reference images. The process was repeated to obtain results at 21 different imprint depths. The indentation device images were then processed using the CC algorithm. The output of the device was plotted against the average imprint depth (average of the 15 OCT 'slices') (Fig. 10).

The CC algorithm shows an increase in its output with increasing imprint depth. This appears to rise linearly until imprint depths of approximately 0.5 mm, where a saturation effect is observed, increasing at a much slower rate thereafter. The reason for this saturation is due to the greyscale values recorded by the camera and the relationship between the darkness of the shadow and its depth. As the imprint increases in depth, the shadow becomes darker, but at a certain point, further increases in depth do not

result in corresponding decreases in the greyscale value recorded by the camera.

The saturation effect is predicted by Eq. (2), which defines a linear relationship between imprint depth and shadow width between two limiting values. The lower limit is the shallowest imprint that will generate a shadow and is defined by the physical characteristics of the device. This is calculated by setting w_s equal to zero in Eq. (2):

$$d_{i,\text{MIN}} = \frac{w_i h}{D - w_i} \quad (9)$$

The applicability of this equation is demonstrated by considering a situation where the imprint is illuminated from above. In this case, the height of the light source is infinite, therefore requiring an imprint of infinite depth to generate a shadow. Consequently, no shadow is formed unless there is a side-illumination of a finite height.

The upper limit is simply the width of the imprint, because the shadow cannot extend beyond it. The imprint depth at which this occurs is calculated, therefore, as the value of d_i for which w_s is equal to w_i :

$$d_{i,\text{MAX}} = \frac{2w_i h}{D - w_i} \quad (10)$$

To test this relationship, OCT was used to measure the imprint width and a thresholding

algorithm was used to measure the number of pixels across the width of the shadow in the indenter images. An experimentally measured calibration constant was then used to convert the width of the shadow to millimetres. For the indentation device, the parameters D and h were measured as 33 and 6 mm, respectively, while the imprint width, d_i , was a constant 1.048 ± 0.059 mm. The lower and upper limits were calculated as 0.197 and 0.393 mm, respectively. This theoretically predicted linear operating range of the device is narrow compared with the experimentally measured one (0–0.5 mm). This discrepancy may be due to the assumptions made in the theory that the imprint has a perfectly rectangular cross-section.

When the 0–0.5 mm range of imprint depths is considered, the algorithm has a linear response to changing imprint size, having a CC of 0.97. A straight line fitted to the data using the least-squares method results in a slope of 1.93 mm^{-1} (intercept = 0.06) and an r^2 value of 0.95.

In the linear range of the device, the precision is high, showing a capability of detecting changes in imprint depth of $50 \text{ }\mu\text{m}$. To obtain a measure of the accuracy of the device, the calibration relation above was used to convert the output of the CC algorithm to a reading in millimetres. The output of the indentation device could then be compared with that of the OCT.

To compare the output of the indentation device with OCT results in a more quantitative manner, the data were analysed using the Bland–Altman method, the standard for this type of analysis. The technique involves plotting the difference in output between two instruments (where one is a gold standard) against the output from the gold standard instrument (in cases where neither is a gold standard, the average output of the instruments is used instead) (37). This was carried out for the linear range of the instrument and is plotted in Fig. 11. Overlaid on each plot are horizontal bars representing the 'bias' (mean difference) and the 'limits of agreement' (bias $\pm 1.96 \times$ standard deviation of the differences) where 95% of the differences are expected to lie. Each bar has an associated precision represented by a 95% confidence interval (38). The limits of agreement in this case represent a measure of the accuracy of the device.

There is an even scatter of data about the x -axis and no appreciable bias in the output. Because of the low number of samples, the confidence intervals for each of the limits of agreement are relatively wide, with ranges of ± 0.031 and ± 0.018 mm for the upper/lower limits of agreement and the bias, respectively. However, a high level of accuracy (equivalent to the separation of the limits of agreement) is achieved by the device (± 0.066 mm).

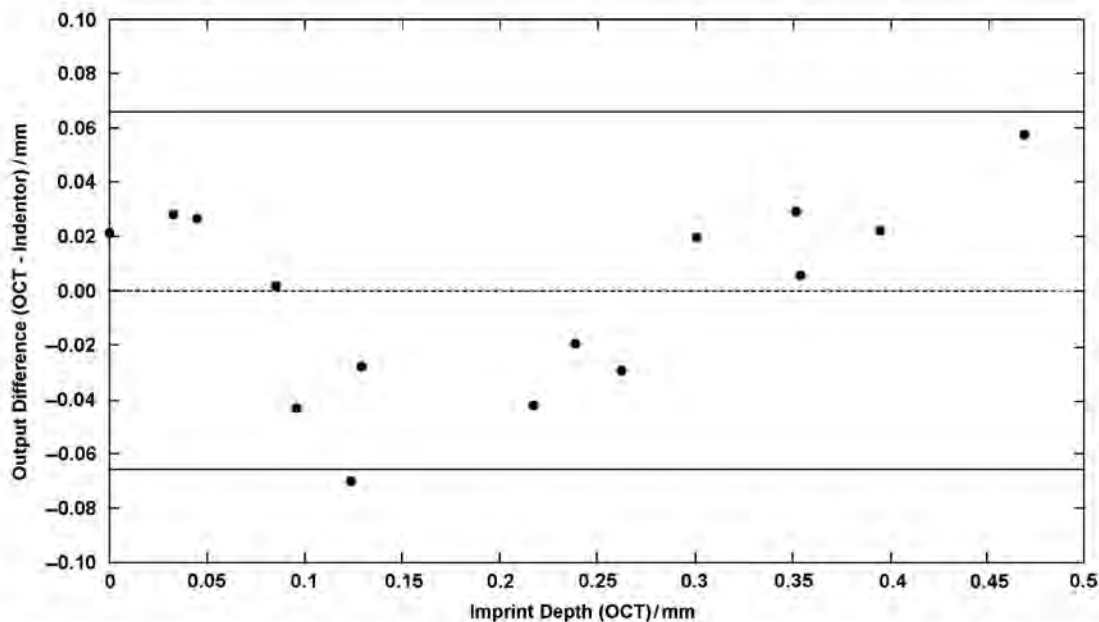


Fig. 11. Bland–Altman plot showing the difference between the calibrated output of the indentation device and the optical coherence tomography (OCT)-measured imprint depth plotted against the OCT measured imprint depth. The horizontal bars represent $\pm 1.96 \times$ standard deviation, and the bias (dashed).

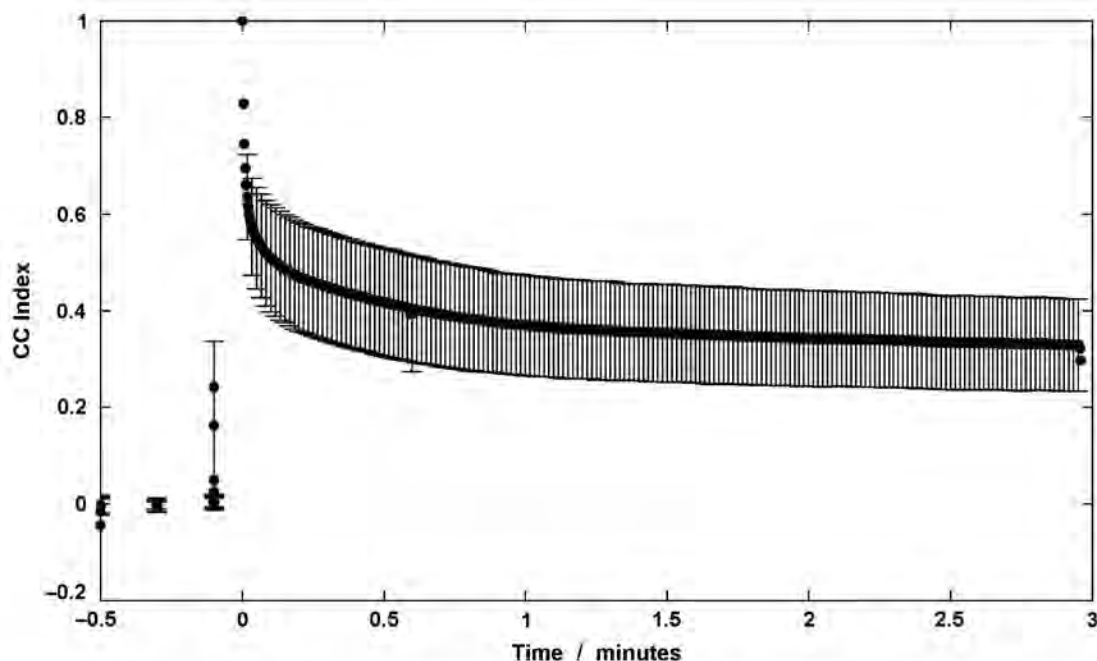


Fig. 12. Average decay data for all 55 tests. The CC Index plotted as a function of time (error bars represent ± 1 standard deviation). Variability is at a maximum between 0.12 and 0.50 min where the standard deviation is approximately 0.11. In the tail of the curve, the standard deviation is steady at 0.10. The points at the negative side of the Time axis correspond to the reference images acquired before the imprint was made.

Device noise and repeatability

Noise present in the signal includes electrical noise and thermal drift in the CCD of the camera, intensity fluctuations in the light source and biological noise due to motion of blood in the skin.

Noise in the CCD and intensity variations due to instability in the LED are inherently coupled. To examine these sources, a static (non-biological) sample was used. A piece of brown cardboard (close to skin colour) was placed underneath the device and the illumination was switched on. Images were acquired over a period of 5 min and relative intensity fluctuations were calculated by computing the 'image average' (the numerical average of the greyscale values of the pixels in the image) of the blue colour plane in each frame. The effect of thermal fluctuations was also investigated by heating the camera (blowing hot air on it) beyond the normal operating laboratory temperature. Noise (including thermal drift) in the CCD was found to be negligible, accounting for a variation of $<0.5\%$ of the total signal.

It has already been shown in this paper that capillary refilling plays a significant role in the shape of the early part of the decay curve unless a polarisation filter is used. However, other biological temporal variations before an imprint is made may also contribute to the signal. For

example, vasomotion may result in a rhythmic variation in intensity of any light that has interacted with the microcirculation.

To test for this biological noise, a series of measurements were carried out on the undisturbed skin of the forearm. A healthy 27-year-old volunteer was seated at a bench-top and the device placed on the volar forearm. The device was allowed to 'settle' for 20 s (mimicking the indentation time taken up during a normal test) before image acquisition started. Images were acquired at 25 Hz over 3 min and analysed in the same manner as the non-biological samples. This process was repeated three times on the forearm. Variations in the signal were small in each of the three tests, with standard deviations in the greyscale value representing a variation of 0.3% to 1% of the total signal.

The device has been shown to generate reproducible measurements on static controlled samples ($<3\%$ error in readings on a Blu-Tack[®] imprint). However, it would also be necessary to have a clear understanding of the reliability of the device in making measurements in the much more complex environment of human skin where biological variations play a large role.

The test subject used for this reproducibility study was a healthy 27-year-old male. Five sites were chosen on the forearm for the study: Site 1 was 4 cm from the elbow crease, with each sub-

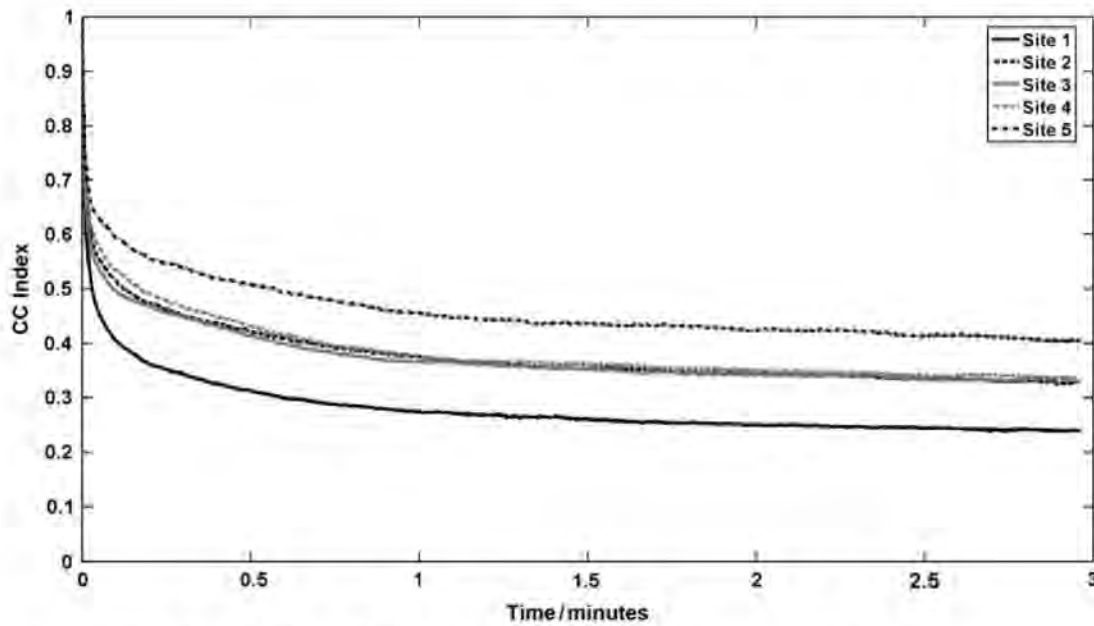


Fig. 13. Decay data sorted by test site. CC Index plotted against time for each of the five measurement sites.

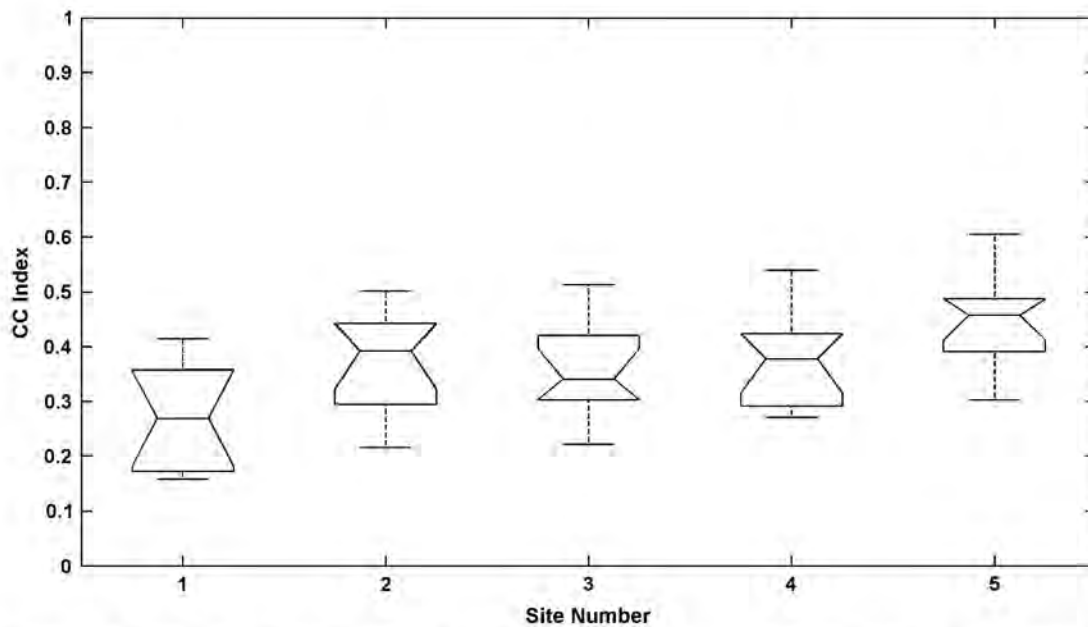


Fig. 14. Box plot of CC Index values reached after 1 min. The overlapping notches indicate that the results from sites 2, 3 and 4 are statistically similar. There is very little overlap between the notches of boxes 1 and 5, showing that there is a statistically significant difference between them.

sequent site located in steps of 2 cm towards the distal end of the arm.

A total of 55 sets of decay data were obtained over six different days. The average decay curve is shown in Fig. 12 along with its standard deviation. The standard deviation varied between 0.10 ('tail' of the decay) and 0.11 at its maximum (0.12–0.50 min). The results were normally distributed, with 95% of the values found within ± 2 standard deviations.

When the data were sorted and averaged by test site, the curves shown in Fig. 13 were obtained. Each group showed a similar level of variability (maximum standard deviations between 0.09 and 0.10; minimum standard deviations between 0.07 and 0.08), but at a level that was less than the 'bulk' variability of all 55 tests together.

The second major observation from Fig. 13 is the alignment of the curves. The data from sites 2–4 overlap strongly while sites 1 and 5 are

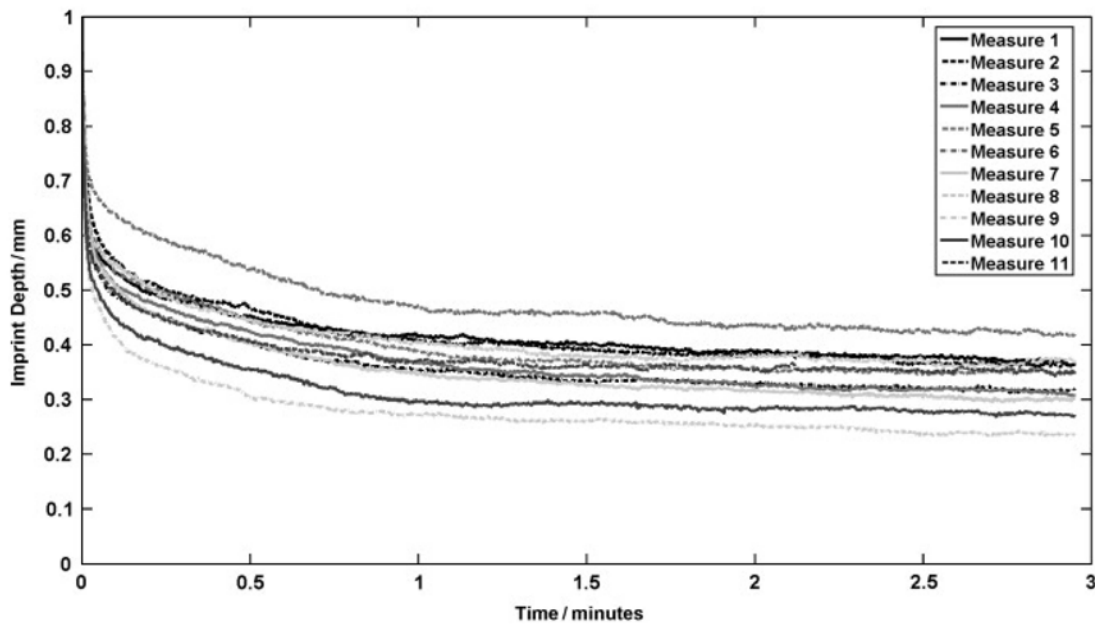


Fig. 15. Variation of decay data when the average over the three central sites is used.

displaced either side. This may be due to differences between the proximal and distal ends of the arm. The data suggest that the skin at the proximal end (site 1) recovers more quickly, while at the distal end the imprint is held for longer.

The percentage coefficient of variation (defined as the standard deviation normalised by the mean) for the reproducibility data shows that when all five sites are considered together, it reaches a maximum of 30%. However, when the peripheral sites are omitted, this drops to <20%.

Figure 14 shows box plots of the range of CC Index values reached 1 min after removal of the indenter (the notches correspond to a 95% confidence interval about the median), which yield extra statistical information on the results. Firstly, the variation within each set of results at the individual sites is lower than that of the full set. Secondly, the centre three measurements show greatest similarity in the position of the median and the upper and lower limits of the error bars. More significantly, however, the notches of these three sets of results overlap strongly meaning that they are similar in a 95% confidence interval. Finally, the results from sites 1 and 5 differ significantly in terms of the position of the notches, showing little or no overlap with each other or the three central measurements.

The significant differences between the central and extreme proximal and distal sites led to the conclusion that the most suitable measurement site is that area spanned by the three central sites.

This average was calculated for the three central imprints in each group of five (Fig. 15). It is proposed that a single 'measurement' from the device should consist of the average of three indentations in this region (6–10 cm from the elbow crease). When the biexponential model is applied, the average parameters for C_1 , τ_1 , C_2 and τ_2 are 0.28 ± 0.03 , 0.13 ± 0.05 min, 0.42 ± 0.06 and 11.77 ± 3.59 min, respectively.

Comparison with OCT in vivo

While there is currently no 'gold standard' specialist instrument for measuring this type of skin recovery from indentation, OCT represents a gold standard in measurement of skin thickness, by producing high resolution B-scans. A comparative experiment was conducted to investigate the performance of the prototype indenter against a recovery test performed using the OCT system. The OCT scanner head was mounted on a moveable arm and a Perspex ring (diameter 25 mm) attached to the head so that it was positioned close to the focal plane of the system. Using this set-up, it was possible to secure a section of the forearm while scans were acquired. Once the arm was in position, the skin could be indented using a hand-held indenter head, identical to that used in the prototype device.

Measurements using the OCT system were performed on a separate day as parallel use of the devices was not possible. To avoid any

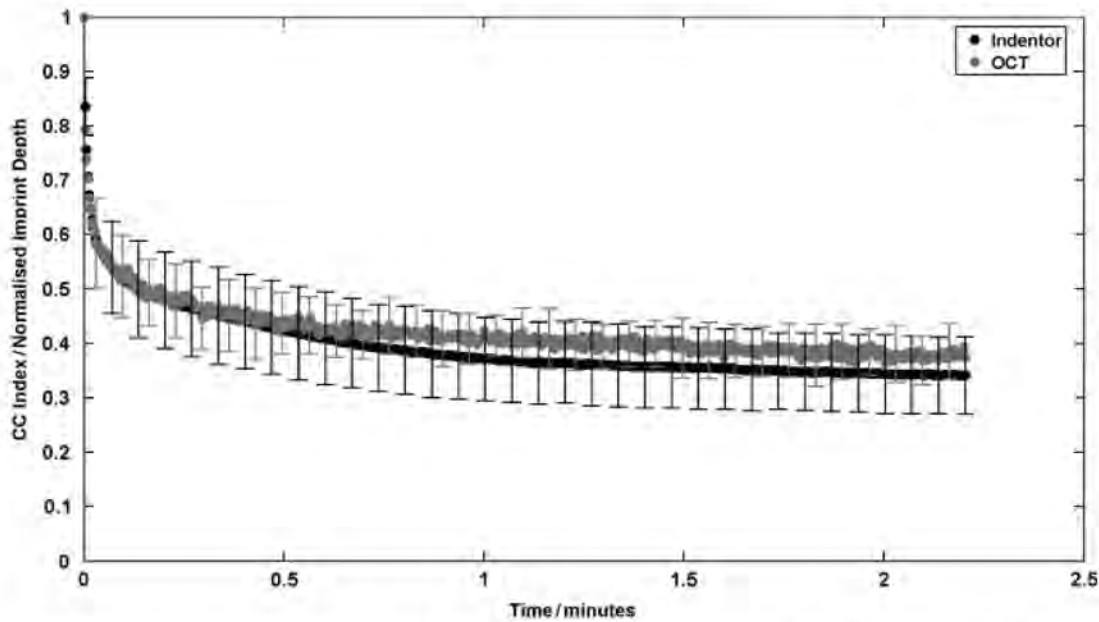


Fig. 16. Average decay curve data from the indentation device (black) and optical coherence tomography (OCT) scanner (CC Index and Normalised Imprint Depth, as measured using OCT, against time). Each shows a biexponential decay from a maximum at the instant that the indenter is released. At the end of the measurement period, the imprint has not disappeared completely. The error bars represent ± 1 standard deviation.

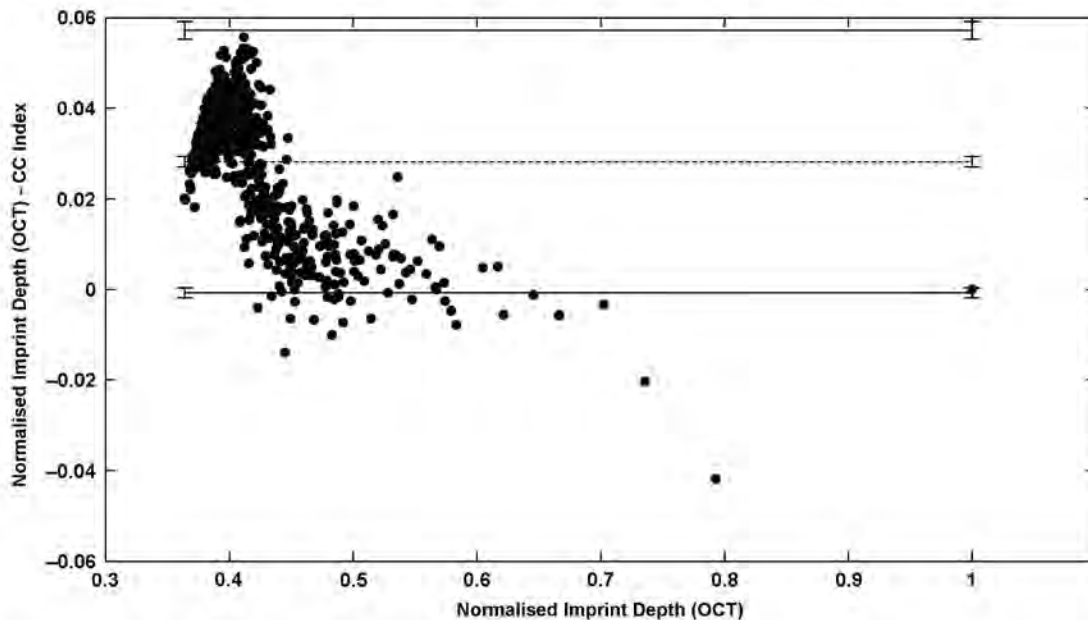


Fig. 17. Bland-Altman plot showing the difference in output between the indentation device and the optimal coherence tomography (OCT) scanner. The limits of agreement show that 95% of the differences are within a margin of ± 0.03 . The 'error bars' represent the confidence intervals for the bias and limits of agreement.

'memory effects' in the skin, it was given 24 h allowing it to recover fully and ensuring that the next measurement would be performed at the same time of day, eliminating the likelihood of diurnal variations in water content. A total of seven OCT measurements were made. The acquisition speed of the OCT system was set to 5 Hz to match that of the indentation device (5 f.p.s.). To make a comparison between the two measurement techniques, the OCT data were averaged

and normalised by its maximum so that the scales of both had a range of '0' to '1'. The data from the indentation device was the average decay curve of the three central sites obtained during the repeatability study. The resulting average decay curves are plotted in Fig. 16.

To assess the agreement between the techniques, a Bland-Altman test was carried out (Fig. 17). Because the OCT is an 'effective gold standard' in this case, the Bland-Altman plot consists

of a plot of the difference in output (39) (*CC Index-Normalised Imprint Depth*) against the *Normalised Imprint Depth* (as measured by OCT).

Figure 17 shows that there is a slight bias in the differences between the instruments, with the indentation device underestimating the size of the imprint by 0.03 on average. This bias is heavily dependent on the differences that arise between the instruments towards the tail end of the decay curve when the normalised indices fall to approximately 0.4 (corresponding to an actual imprint depth of 0.220 mm). In deeper imprints, the differences are smaller, as shown by the clustering of points about the x -axis for normalised index values >0.4 . Overall, the limits of agreement show that 95% of the differences between the instruments lie within a margin of ± 0.03 , representing a variation of just 6% over the entire measurement scale of the instrument.

Discussion

The development and testing of a skin indentation device has been described in this paper. A novel approach to skin indentation has been introduced, which adds to the fundamental understanding of the mechanical behaviour of skin and the time course of an imprint in it.

The device described in this paper tracks the skin's recovery after an imprint is made. This method differs from existing measurement devices that use stress relaxation manoeuvres, lateral stretching of the skin, suction cup techniques and electrical provocation. This new method is intuitive from a clinical perspective, complementing the existing qualitative tests that clinicians routinely perform to detect 'pitting' oedema.

The linear shape of the indenter head is the first of its kind, differing from the more common circular or hemispherical shapes seen in the literature (10, 13, 25). This shape offers the advantage of having a very predictable effect on the tissue and the ease of mathematical description. The shadows cast by the illumination system are more easily defined theoretically than for a circular imprint. The linear arrangement facilitates polarisation by allowing parallel orientation of the filter transmission axes and lighting. Finally, the shape offers flexibility in that it can be easily 'sliced' using OCT, as its cross-section is uniform along its length. In future applications, it also offers the potential to imprint across a provoked/normal boundary in the skin, record-

ing provoked and reference information simultaneously.

A side-lighting technique has been used to follow changes in imprint size in the skin. This approach has not been used before for dynamic *in vivo* measurements. A high level of sensitivity and precision has been achieved using a carefully chosen set of design parameters with the system capable of resolving depth changes of approximately 50 μm . After calibration of the output, it was shown to be accurate to within 60 μm of the value measured using OCT. Polarisation gating was successfully used to acquire data from the superficial layers of the skin and minimise the effect of microcirculatory variations.

The device developed is highly portable, simple in its construction, using low-cost materials, giving it the potential to be highly accessible. However, the data acquired using the customised illumination and processing software provide rich information on the mechanical response of the skin.

The imprint manoeuvre

Once the indenter is removed from the skin, the imprint changes size rapidly in the first 30 s as the elastic fibres recoil and the low viscosity fluid starts to move back into the imprinted region. After 3 minutes, the imprint is still visible as the high viscosity fluid has not fully returned to the imprinted area. A commercial OCT imager has confirmed the prediction of viscoelastic theory that the depth of the imprint decays in a biexponential fashion from its maximum depth (450 μm on average).

Because of the inherent coupling of the fibrous and fluidic components of the skin's response, it is difficult to deduce absolute measurements of tissue water or elasticity from the decay curves. Therefore, the main strength of this device is expected to lie in following changes in one property when the other is known to be constant, for example, tracking changes due to varying hydration within a single patient where it can be assumed that elasticity does not change.

Applications

Quantification of the degree of oedema (swelling) is one of the principle applications of a device capable of assessing the viscoelastic properties of skin. This would be important in patients suffering from heart failure, where swelling in the legs

and ankles results (18). A reliable measurement of skin hydration as part of a regular check-up has the potential to help in the diagnosis of conditions like heart failure by detecting changes in the viscoelasticity of skin even in mild or subclinical oedema and allowing a more timely treatment before the oedema causes discomfort and more significant effects on cardiac function. This could be an extremely important advance as heart failure affects a significant fraction of the population and is still poorly managed, with a 15–20% mortality rate (40).

Possibly, more common than the assessment of cardiac failure is the evaluation of the degree of hydration in a patient. A fast quantitative method of monitoring the fluid balance of a patient would be a useful tool in the hospital emergency room to help avoid the risks associated with intravenous rehydration of the dehydrated patient [previous work has shown that oedema due to over-hydration can lead to venous eczema and ulceration, as the excess fluid increases the distance between tissue cells (41), reducing oxygen consumption (42, 43)]. The proposed device could fulfil this requirement by tracking changes in the viscoelastic properties in a particular patient over time. Because the elasticity of the connective fibres would not be expected to change over the duration of treatment, any changes observed can be assumed to be due to varying fluid levels. The device developed in this paper aims to assist the clinician in making the decision on when to reduce the administration of fluid to the patient.

Bioengineering techniques have made significant advances over recent decades in the measurement of *stratum corneum* hydration and water barrier function using devices such as the corneometer and evaporimeter. Methods for the assessment of water content in the dermis and hypodermis at a skin functional level need further development. Cutaneous microdialysis and the testing of inflammatory response can serve as examples in which changing fluid levels in the skin due to inflammation can occur (44, 45) and changes other than erythaema have not been quantified previously although they can influence the interpretation of data.

Conclusion

The indentation device developed and described in this paper is capable of generating and tracking the recovery of an imprint made in skin. It

succeeds in its aims to be portable, low-cost and have a simple operating procedure. Using inexpensive materials in a novel design, a high degree of accuracy and precision has been achieved, comparable with results obtained using the more expensive and sophisticated OCT scanner. Future work will focus on the *in vivo* testing of the device and detecting controlled changes in the skin's viscoelastic properties.

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