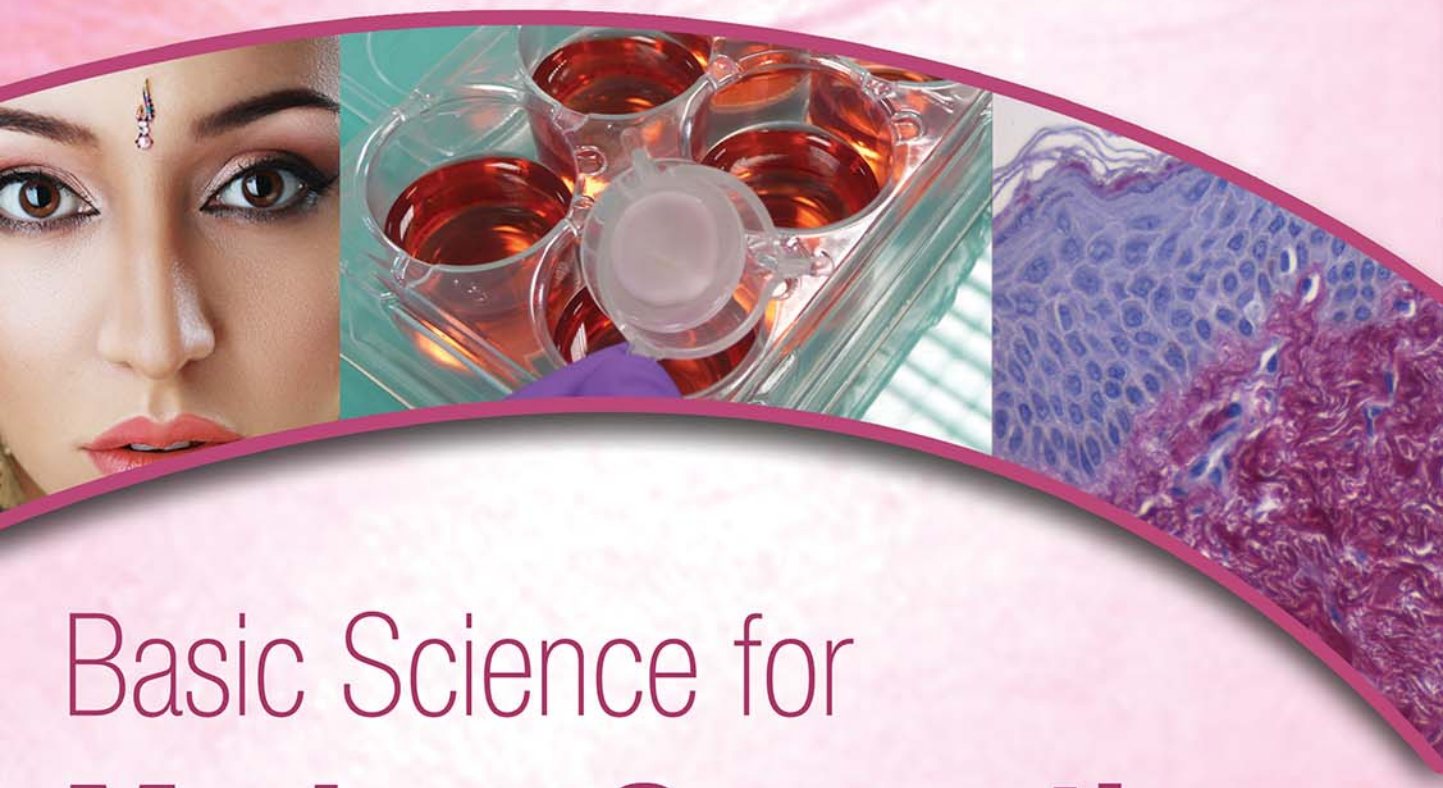

Basic Science for
Modern Cosmetic Dermatology

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Chapter 2

In Vivo Noninvasive Methods and Imaging Techniques for Investigating the Skin

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INTRODUCTION

The human skin is a complex structure that affords protection *vis à vis* the assaults from the external environment. Its outermost layer, the stratum corneum (SC), represents the true interface with the environment and is a clear example of the successful adaptation of a tissue. The stratum corneum is heterogeneous in structure and has been compared to a brick-wall model, in which non-continuous, essentially proteinaceous, terminally differentiated keratinocytes or corneocytes (bricks) are embedded into a continuous matrix of specialized lipids (mortar). The SC consists of 12–16 layers of flattened corneocytes which have a mean thickness of 1 μm and mean surface area of 1,000 μm^2 according to age.

The skin is a sensitive dynamic boundary. It offers protection from infection, ultraviolet rays and trauma. It is essential for controlling water and contributes to the synthesis of essential substances such as vitamin D and other major substances such as collagen, elastin, melanin, etc. The skin is also an important organ of social and sexual contact. Its perception, deeply rooted within cultures of all social groups, is largely driven by its appearance and associated structures. The present chapter deals with novel, non-invasive bioengineering methods of measuring and imaging as tools for evaluating all aspects of the skin. The advantage of bioengineering and imaging techniques is that almost all the properties of the living skin and their changes in healthy (aging) or in pathologic (e.g. dermatitis, psoriasis) conditions are now objectively measurable and quantifiable under non-invasive ways.

These non-invasive methods are by nature objective, quantitative, and generally investigator independent, and can be calibrated and validated *in vivo* and/or *in vitro* on reconstructed skin models. If bioengineering measurements are properly performed under standard conditions, they can also lead to a harmonization of results/data and allow inter-laboratory and inter-observer comparisons to be made.

SKIN MOISTURE ASSESSMENT

Water plays an important role in the complex structure of the SC which influences its elasticity, tensile strength, barrier characteristics and electrical resistance. In the absence of water, the SC is an intrinsically rigid and brittle structure prone to cracking. The state of hydration of the SC is governed by three factors:

1. Water arising from the underlying epidermis.
2. Water loss due to evaporation from the surface.
3. Intrinsic ability of this horny layer to hold water.

The water content of the normal SC should be in the range of 10–20%. Under 10%, the skin looks dry, rough or may even show chaps. To keep the skin with proper moisture level, it is essential to maintain unaltered the physiological environment of skin and promote normal metabolic activity. Moisturizing cosmetic products increase the water content of the SC by their capacity to retain water, mimicking skin natural moisturizing factor (NMF), or by preventing uncontrolled water loss, by reinforcing the SC lipid content. Based on the various modes of action of cosmetics, different techniques can be used to evaluate skin moisturization.

Electrical Properties

The flow of an electrical current (μA range) through the skin surface is related to the water content of the SC. This

directly affects the conductance and capacitance of the skin. However, these parameters are not linearly related to the water content of the skin and are affected by other factors such as the presence of ions, dipolar proteins as well as water bound to keratin.

Conductance Measurements[1,2]

The water present in the SC contains various solutes that conduct electrical current, a capacity used in equipments such as SKICON™. Skin conductance is measured as a high frequency current of 3.5 MHz passes through the skin, using electrodes consisting of a 2 mm diameter disk and a guard ring with an external diameter of 4 mm, creating a 1 mm distance between the inner and outer probe. When the probe is placed onto the measured area, a high-frequency current flows and measurement values are given in micro-Siemens units from 0 µs to 1999 µs.

Capacitance Measurements[2,3]

Skin capacitance, resulting from such a naturally dielectric medium, reflects its hydration state. The higher the water content, the higher the capacitance. Capacitance sensors can then record the dielectric constant of the skin which changes with water content.

The measuring probe (0.65 cm^2) consists of an inter-digitated grid of gold covered electrodes. On the probe head, a thin piece of glass avoids direct galvanic contact between the electrode and skin the surface. An electric scattered field penetrates the very first layers of the skin up to 10–20 µm during measurement and the dielectric constant is determined. One track builds up a surplus of electrons (anode), the other a lack of electrons (cathode), creating an electric field. The repetition frequency varies from 1.15 MHz for a very dry medium to 0.95 MHz for a very hydrated medium, measurements being given in arbitrary units, from 0 AU to 120 AU.

The capacitance method is associated with a short measuring time (seconds) and good reproducibility. To date, the assessment of moisturizing cosmetic products is often performed using Corneometer™ (Courage & Khazaka, Köln, Germany).

Skin Capacitance Imaging (SCI)[4-6]

A special type of non-optical skin surface imaging called SkinChip™ has been developed (Fig. 1). This method is an application of the silicon image sensor (SIS) technology initially developed for recording fingerprints for security reasons. The sensor is composed of 92160 micro-capacitors dispersed onto a 18 x 12.8 mm sensor plate which measures skin capacitance every 50 µm. These micro-capacitors are protected by a thin silicon oxide layer and therefore closely applied to the skin surface for 5 seconds, a short and sufficient time for recording its capacitance profile.



Figure 1: SkinChip™ measurement
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The SCI images are acquired and displayed in real time on a computer screen where capacitance values are transformed into a range of 256 gray levels to form a non-optical image, where the darker pixels represent high capacitance and the clearer ones, a low capacitance value. The mean gray level of the image then represents the average skin surface hydration. This correlates well with

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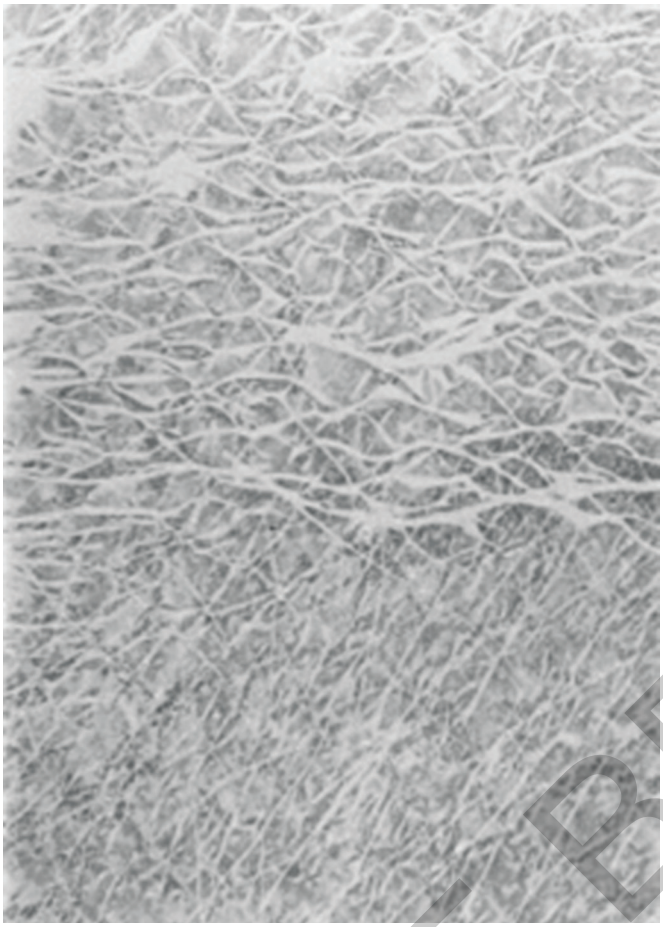

Figure 2: Visualization of a nonhydrated leg skin (upper part) versus hydrated skin (lower part) with Skinchip™
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the average capacitance value of the Corneometer™. The latter averages the capacitance of the contact area while SkinChip™ not only displays a more detailed distribution histogram of the capacitance value but images the skin micro-relief (fine line and cross-line orientations) and its changes with aging. Treating skin with an efficient moisturizer results in a darker capacitance image of the skin with more regular micro-relief network (Fig. 2). Sweating can also be quantitatively measured and visualized.[6] Subcutaneous immunotherapy (SCIT) was also used to study numerous skin lesions: acne pustules and papules, warts, keratoses, pityriasis versicolor, nevi, psoriatic lesions, etc. In some cases, lesions, quite invisible to the naked eye can be easily detected by SCIT because of anhidrotic conditions.[7]

Transepidermal Water Loss[8-10]

Transepidermal water loss refers to the flux of water (in $g/m^2/h$) that continuously diffuses/evaporates from the skin toward the environment, not to be confused with sweat production. Since mostly governed by the structural organization of the SC (and its changes with various disorders) and its thickness, measuring TEWL provides crucial information on the state of the SC. A flaky, disorganized or thinned SC will invariably lead to elevated TEWL values. For example, in the case of specific dermatitis such as psoriasis or ichthyosis or other dry skin diseases, the water level is very low and TEWL is higher than normal. Since TEWL is linked to the hydration level of skin, it is often used together with measurement of water content in horny layer to evaluate the effect of moisturizing cosmetics and to assess and compare the effects of different skincare products.

Since SC quality greatly influences TEWL amplitude, any fluctuations of relative humidity and/or air temperature must be avoided during the measurement; a special room equipped with temperature and humidity control is needed to ensure reproducible results. Two techniques are available for measuring such an important parameter:

1. *Open-chamber technique*: The principle of Open-chamber device is based on Fick's diffusion law. It measures the gradient of water vapor evaporating from the skin surface using an open sensor placed perpendicular onto the skin surface. The probe consists of an open cylinder including two humidity and two thermal sensors located at different distances above the skin surface. From these measurements, the flux of water vapor can be calculated in $mg/cm^2/min$ or $g/m^2/hour$.
2. *Closed-chamber technique*: The difference from Open-chamber technique lies in the closed top of the measuring chamber. A cylindrical hood covers the skin to gather vapor evaporating from the skin surface. One temperature and humidity sensor is used to record the relative humidity. The change in vapor concentration is initially rapid and then gradually decreases as the humidity reaches near 100%. Closed-chamber method does not enable TEWL to be recorded continuously since skin evaporation decreases when the air in the closed chamber reaches water vapor saturation.

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Spectroscopic Properties

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)[11]

This technique is directly used to estimate the skin surface water in vivo. The absorption for amide-I (1,645 cm^{-1}) is influenced by proteins and water while amide-II (1545 cm^{-1}) is restricted to proteins. The water content of the SC can thus be estimated by calculating the ratio between the two attenuation measurements (amide-I/amide-II= Moisture Factor). The depth of measurement varies slightly with the instrument and wavelength, generally considered a few µm below the surface.

Further improvement in this technique using O-H expansion/contraction at 2,100 cm^{-1} has been suggested. The water content of removed SC (using tape strippings) calculated through gravimetry shows good linearity up to 0.2 g H_2O/cm^2 and using such measurements, the estimated moisture content of the skin surface layers is about 9%.

Raman Spectroscopy [12,13]

In this method, the sample of interest is illuminated by a laser beam of a very narrow wavelength band. The electrical component of the electromagnetic field within the laser beam drives the electronic cloud of the molecules present in the sample. The driven and oscillating electronic cloud re-emits most of the collided laser light without energy loss, the only difference being the direction in which the photons are ejected from the molecules. This process is referred to as elastic scattering (Rayleigh scattering), where a very small amount of laser light scatters the ejected photons of a different energy from the injected photon, the difference in energy being released or taken up by the molecules. By measuring the intensity and energy of the re-emitted light of frequencies different than the laser frequency, a Raman spectrum can be obtained.

Raman spectroscopy is a truly non-invasive analytical technique for studying the composition of human SC at the molecular level. The monitoring of topically applied skin penetrating agents can be studied with time along with their interaction with skin components such as water, collagen, lipids, etc.

Near Infrared Spectroscopy[14,15]

The use of near infrared spectroscopy (NIR) to measure SC water content has been reported by de Rigal et al. in 1993. Water molecules have two distinct near infrared absorption bands at 1,450 nm and 1,940 nm. The authors measured the difference between peak absorption of the water molecule at 1,940 nm and the lowest absorption at 1,100 nm for skin and compared it with skin surface conductance and clinical signs. They found that the severity of dry skin more closely corresponded with the difference in absorbance than with skin surface conductance, indicating that NIR measurements extend beyond the SC.

SEBUM LEVEL MEASUREMENT

Sebaceous glands secrete sebum and excrete it onto the skin surface, where sebum diffuses and is emulsified with sweat to form a sebum film that keeps the skins surface smooth and glossy and also prevents water evaporation from the body. Sebum has the function of lubricating skin. Therefore, a certain amount of sebum is necessary for healthy skin, preventing the skin from becoming dry and from fissuring and chapping. Sebum also increases skin elasticity and its capacity to resist stress and insults from the surroundings. However, excess sebum not only results in shiny skin and a dark color, but also favors the development of acne and blemishes that affect the appearance of the skin. Excessive sebum secretion is indeed one major factor that fuels skin diseases such as acne and seborrheic dermatitis.

Some skincare products can alter the sebum level to maintain a normal sebum film. Monitoring sebum excretion on the skin surface may be helpful for studies on sebum-related diseases conducted by clinicians.[16] Accurately measuring the sebum level is of great value for evaluating the effect of these products, understanding the skin type on the face and preventing and mitigating seborrheic diseases. Researchers can choose the most appropriate method based upon the requirements of the study project and thoroughly consider the requirements of each method and the value of information generated by collected data.

Sebumeter

Sebumeter® (Courage & Khazaka, Germany), a commercial device, is now commonly used. It is based on the principle of photometer and measures the change in transmittance of an adhesive tape 0.1 mm thick. The higher the amount

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Figure 3: Sebumeter measurement
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of sebum absorbed by the adhesive tape, the greater the recorded increase in transmittance; thus, the content of skin sebum can be measured indirectly.[17] Sebumeter® has a probe with small volume and is convenient to use; it can measure the sebum at any site (Fig. 3). The result is expressed as μg sebum/cm² and the whole process takes 30 seconds.

Sebutape

Another measurement technique is Sebutape® (CuDerm Corporation, Dallas, USA), which also uses a special sebum-absorbing adhesive tape. However, unlike the above method, it is firstly used to remove fat from the skin area to be tested. The special tape is placed onto the skin. After a contact time of one hour, some transmittance spots may be observed. They correspond to excreting (active) follicles. Excreted sebum is gradually absorbed by the tape. After removing the tape, transmittance spots and their surface area can be measured with a simple image analysis software. The special sebum-absorbing tape is an oil-absorbing micropore film. Since fat does not scatter light, the micropore film becomes transparent when air in the micropores is replaced by absorbed fat. The design characteristics of the micropore film minimize the diffusion of fat on both sides of the micropore so that the outlets of active follicles create a distinct border. The size of the transparent zone can be used to detect the amount of sebum. To perform the measurement, the film is placed over a dark background for convenient visual evaluation and computer-assisted image analysis. The parameters measured with Sebutape include the amount of absorbed sebum, the number of sebum spots, and the largest and smallest sebum spots. Extraordinary large and fusional sebum spots are commonly seen in patients with seborrheic dermatitis. The evaluation must be conducted within 24 hours of removing the tape to avoid lateral spreading. If the sample cannot be assessed immediately, it is suggested to keep it under -30°C.

Other Methods

There are also gravimetric methods that are rarely used today because they are cumbersome and time consuming. They are based on the use of solvents (ethyl ether) to remove sebum from a cigarette paper pressed onto the skin surface for hours.

SKIN AGING ASSESSMENT

All living organisms are subjected to aging, a multifaceted process that differs for various organisms. In humans, it is a physiological process corresponding to a progressive loss in the homeostatic capacity of the organs and tissues. As for skin, two main processes are involved in aging. First, the intrinsic changes induced by chronological aging that progressively affect the skin structure, physiology and mecanobiology. Second, since skin is the organ most exposed to environmental conditions (e.g. sun exposure and related UVB and UVA rays), exposure will lead to deep changes at both cellular and molecular levels, in turn modifying the skin texture and appearance.

A major feature of skin aging is loss of its elasticity. Many different non-invasive instruments are available for evaluating the mechanical properties of the skin, based on various mechanical stresses including tensile, torsional, indentation or suction skin deformations.

Elasticity Measurement

Suction Method[18-20]

In this method, the skin is stretched perpendicularly to its plane by applying a given depression or suction. In the late 1980s, Courage & Khazaka Electronics developed an instrument based on the suction method called

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Cutometer™. A negative pressure is applied to the surface of the skin, using a vacuum pump which draws the skin into the probe. This causes a vertical displacement of the skin to the plane, which is measured by a noncontact optical system. This optical system consists of a light source and a receiver, two glass prisms facing each other and leading the light from the transmitter to the receiver. The reduction in the infrared light beam, depending on the elevation of the skin, is recorded. It can also measure Stress time curve in which the vacuum is suddenly applied and released. From this curve, the pure elastic properties, elastic recovery and viscoelastic properties can be quantified. These have clearly been shown to be related to the degree of aging and skin laxity.

Torsion Method [21,22]

The measurement is performed parallel to the skin surface in order to give a stronger influence to the skin's upper layers (epidermis and dermis versus hypodermis). The most frequently used parameters are extensibility and torsion recovery. The commercial instrument is Twistometer® (or Torquemeter®, Diastron, UK) (Fig. 4). In the torsional method, a central disk is attached to the skin with adhesive tape and rotates under a predetermined torque. The amount of skin under test is limited by a stationary guard ring that is attached by adhesive tape. The gap between the central disk and the concentric guard ring is of critical importance. When measuring the upper layers of the skin, maximum sensitivity is achieved by restricting the gap to 1 mm. For studies of full skin thickness, the gap is increased to 5 mm, allowing the torsional forces to be applied to the deeper layers.

When a torque is applied onto the skin, an initial rapid rotation is followed by a slower exponential phase as the skin progressively resists the torsional forces. The initial rapid phase, which is referred as Ue, is a measure of the extensibility of the skin and has been demonstrated to be a useful measure of SC hydration. The slower phase is due to a creeping effect, which reflects the viscosity and plasticity of skin. When the torque is removed, a progressive recovery from the deformation allows the immediate or delayed recovery (skin elasticity) to be defined.

Sophisticated analysis algorithms have been applied to the curve data with a reported increase in the sensitivity of the method.



Figure 4: Twistometer®
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Indentation Method[23]

This method is based upon the traditional ballistometry principle of impacting an object at a constant force and measuring skin firmness by indentation and dynamic resilience (elasticity) through the degree of rebounds. The torsional ballistometer consists of a 25 cm long slim line probe. The probe contains a rigid low mass arm suspended at its balance point on a torsion wire. A ruby tipped stylus is fixed to one end. The arm is activated by a solenoid that elevates the probe tip from the test surface. When released, the arm oscillates around its balance position and the stylus bounces repeatedly on the test site, up to cessation. The position of the arm is monitored by an optical sensor.

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The peak penetration depth of the probe tip provides indentation: the more the identation, the looser the skin. Greater numbers of rebounds imply higher skin elasticity.

Resonance Methods[24,25]

Measurement of resonance running time (RRT) has been shown to be inversely related to skin stiffness. The probe head contains two sensors that are placed on the skin. The first emits an acoustical shockwave and the other serves as a receiver. Shockwaves propagate differently through the skin according to the state of the elastic fibers and the moisture content. The time needed by the wave to travel from emitter to receiver is the recorded parameter.

Aged skin is characterized, among other symptoms, by profound changes in the orientation of collagen fibers in the dermis. In young skin, these fibers are very small and are oriented in all directions (isotropy). With age, the fibers thicken, with a preferential orientation (anisotropy). The degree of isotropy/anisotropy in dermis can be evaluated using the Reviscometer®. The propagation speed of the shockwave applied to the skin surface at different angles gives the degree of isotropy/anisotropy.

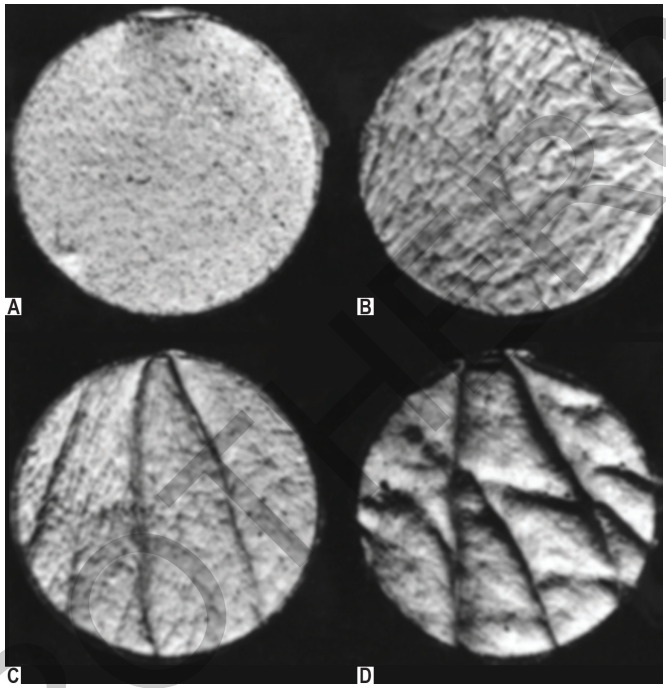

Figure 5: Prints of crow's feet wrinkles from four different ages (from A to D, A being the youngest)
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Skin Surface Microrelief

The skin surface is not perfectly flat, and is characterized by a special relief which probably reproduces the 3D organization of the deeper layers (Fig. 5). A simple examination with the naked eye, or with high microscopic magnification or by hand palpation can give qualitative information.

According to the technology used, the most frequent methods applied to the study of skin topography can be separated into four subgroups:

1. *Optical profilometry*:[26,27] Optical profilometry is a non-contact interferometric method for characterizing skin surface topography from skin silicone replicas. There are two set ups of this principle:

   a. Autofocusing system: In this system, a diode laser is focused on a point of the replica, the reflected part being focused on a detector which pilots the position of the focusing lens with a motor. When the object is moved, any relief variation loses focus and decreases the intensity of the light received by the detector. This would be followed by focusing lens repositioning at the convergence point of the light beam, and hence recording microrelief profile.

   b. Triangulation system: This method uses three reference points of the fixed source, a laser diode; the point of the surface to be analyzed whose height varies according to the local relief; the image of this point formed by an optical system on the detector plane. When the whole measurement system is displaced relative to the surface, the image formed on the detector changes position and the measurement of this position gives the height of the measured point relative to that of the previous image point.
2. *Mechanical profilometry:*[28] This technique is based on the linear displacement of a stylus along the surface of a skin replica. Its successive vertical movements (i.e. valleys and peaks) alter the voltage of the output signal, which is then digitized to display reconstructed 2D profiles and subsequent 3D images.
3. *Laser profilometry:*[29,30] It is an optical method based upon the amplification and reflection of light from the

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skin replica, depending on the topography. It includes three different techniques. The first uses dynamic focusing, the laser beam being permanently focused on the specimen. The height of a point at its surface is deduced from the position of the mobile lens (Fig. 6). The other two are based upon the theory of triangulation (Fig. 7). Either single or double triangulation can be used. As the height of the specimen varies, the position of the light spot, reflected onto the detector, also varies, enabling the height of the specimen to be determined.

Common to these three techniques, the surface of the specimen is scanned by means of a table moved mechanically in X and Y axes. The recorded variable height (Z axis) therefore allows a 3D representation of the skin surface to be obtained.

4. *Interference fringe profilometry*:[29] This consists in calculating a phase image from interference fringe image projection, giving access to the altitude of each point. The fundamental principle of this system is based upon a projective Moire Profiler that projects the image of a reference grating onto the surface of the object under test. A CCD camera then acquires fringes that are later computerized (Fig. 8). By processing the Moire fringes of the surface of the object under test with the computer-added phase

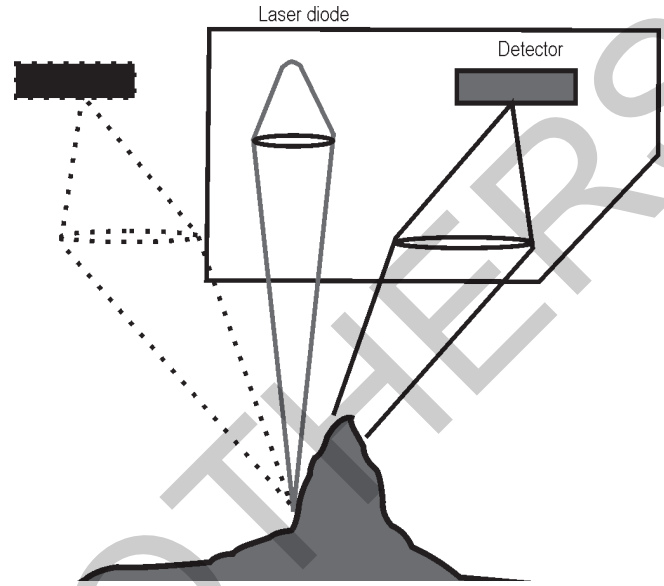

Figure 7: Surface measurement by double triangulation method
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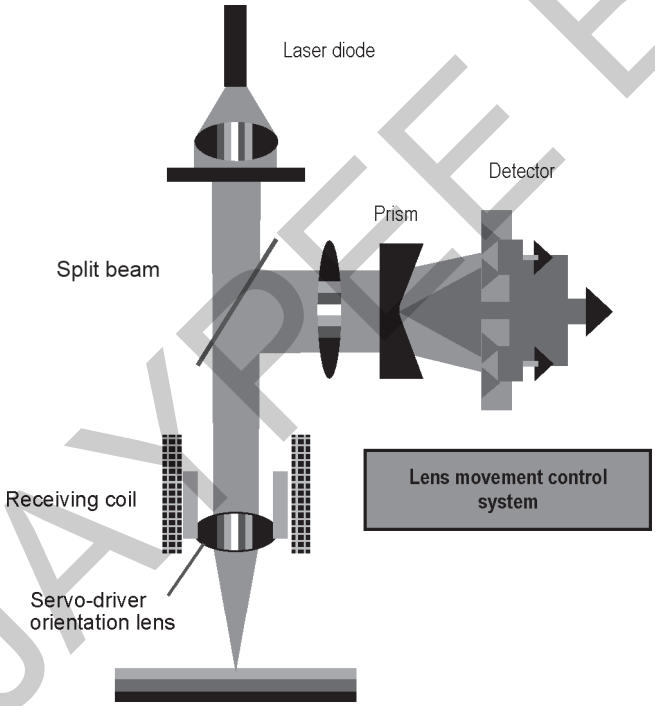

Figure 6: Principle of surface measurement by laser scanning
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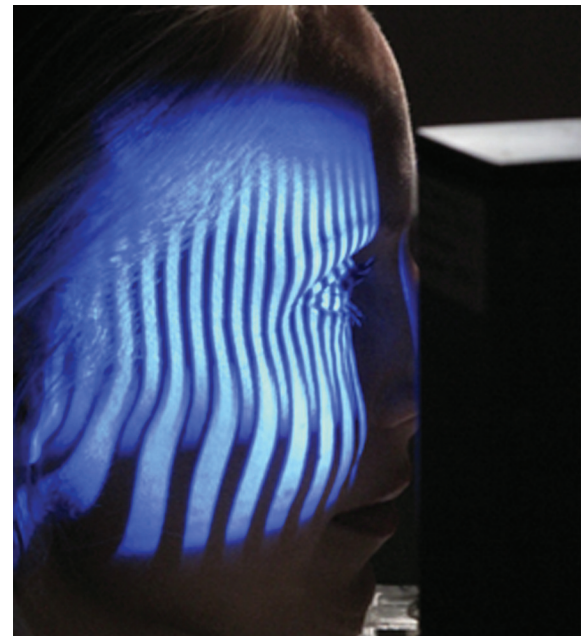

Figure 8: Fringe profilometry measurement
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shifting algorithm, the 3D shape information of the object can be obtained.

SKIN COLOR ASSESSMENT

Human skin is not an opaque surface. Rather, skin has depth and layers, and its appearance is in part determined by the relative proportion between reflected and absorbed light. The amount and color of reflected light is, in turn, determined by the nature and distribution of light absorbing molecules (chromophores) beneath the SC, particularly melanins, hemoglobin and carotenoids.

Facial skin normally allows more than 90% of light to penetrate its surface.[31] White light passing through the skin's transparent surface is scattered back toward the surface by dermal collagen, which acts essentially as a mirror within the skin. The color of light during its journey into the skin becomes modified by the various chromophores, and is reflected back toward the surface. This modified light color is then diffused by the SC, generating a variably luminous glow. Skin color measurements have extensive applications in the fields of skin cosmetology and the skin pigmentation disorders. The classification of skin color is not only helpful to distinguish ethnical–differences, but also to predict the extent of pigmentation and erythema after sunburn. People with darker skin are more likely to develop increased pigmentation after sun exposure, whereas those with lighter skin are more prone to exhibit sunburn.[32,33]

Tristimulus Colorimeter[34,35]

The most used reflectance spectrophotometers for skin color measurement are Chromameter CR™ series. The probe consists of a pulsed xenon arc lamp which illuminates the skin surface. The light reflected by the skin is described in absolute values in a 3D colorimetric system defined by Commission Internationale de l'Eclairage (CIE) that converts it into L*a*b*. The L* value, ranging from the black (0) to white scale (100), is mostly affected by melanin content: the higher the melanin content, the lower the L*. The a* value ranges from red to green region: the higher the a*, the redder the skin. The b* value mainly indicates the yellowness of skin (the higher the b*, the yellower the skin), but is also affected by melanin.

Full Band Reflectance Spectrometer[36]

This technique of skin color measurement is found in instruments like the derma spectrometer or Mexameter™. This skin is illuminated by a light-emitting diode (LED). The reflected light then passes through filters specific to three wavelengths, namely 568 nm (green light), 660 nm (red light) and 870 nm (in the near infrared range). The reflected light at these wavelengths is detected by a photodiode and a built-in microcomputer calculates erythema and melanin indexes.

SKIN IMAGING TECHNIQUES AND SYSTEMS

Most diagnoses in dermatology rely on visual/clinical examination of the skin, i.e. a non-invasive approach. Most physician diagnosis criteria include anatomic distribution, color, configuration and visible surface changes of a given lesion. In some cases, an invasive (and somewhat painful) punch biopsy has to be performed and further processed histologically to confirm a given diagnosis. In brief, there is a clear need for developing non-invasive methods providing a clear imaging of the skin surface and its deeper structures. Imaging modalities relevant to skin include various modifications of electromagnetic wave imaging (optical, infrared, nuclear magnetic resonance), acoustical wave imaging, mechanical wave imaging. Usually, tomographic images, i.e. 2D cross-sectional images are acquired by a medical imaging system. The 3D tissue volumes are usually imaged by acquiring stacks of consecutive 2D images.[37]

Polarized Imaging Technique [35,36,38,39]

Among the various attributes associated with skin beauty, skin radiance is paramount. The latter is an optical parameter amounted the light emitted from an object as a light source. Although this definition becomes rather vague when applied to the skin, many experiments were conducted to understand this aspect, most using the polarizing image system as an objective tool.

This system uses an instrument called Samba, initially developed by Bossa Nova Technologies™. It consists of an illumination unit equipped with linear polarizing filters and a high resolution digital camera equipped with liquid-crystal polarizer, the polarization angle of which can be electronically flipped from the direction

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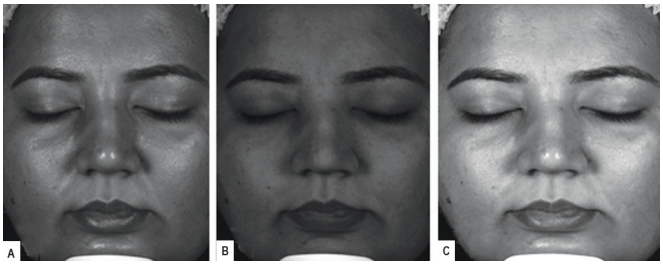

Figure 9: Polarized images using the Samba technique. **A,** P-image giving subsurface and surface reflection. **B,** C-image representing subsurface reflection. **C,** S-image derived by pixel subtraction of P- and C-images
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parallel (P) to the plane of polarization of the polarizing filters of the illumination unit to the one that is at 90˚ to this direction referring to the crossed (C) orientation. When the two polarizing filters are parallel, both the surface and subsurface reflections are present in the resulting image. However, when the two polarizing polarization filters are oriented in a cross-position, surface reflection is excluded and the subsurface reflection is only present in the crossed image (C-image). By subtracting the corresponding pixels in the C-image from those in the P-image, a surface reflectance S-image can be extracted (Fig. 9).

Dermascopy or Dermatoscopy[40-42]

It is a diagnostic technique used worldwide for identifying and diagnosing skin lesions. A dermascope is similar to a magnifying lens, having inbuilt features of an illuminated system, with increased magnification. Structures as deep as the reticular dermis can be assessed. Besides, it enables images to be recorded.

The working principle of dermascopy lies in the transillumination of a lesion with a high magnification to visualize subtle features. Incident light undergoes reflection, refraction, diffraction and absorption, all based on physical properties of the skin. Dermascopy can be done either by the contact technique, which uses non-polarized light source to illuminate the skin surface, or bye the non-contact technique, where a liquid interface is used between the skin and the lens to reduce surface reflection. In the latter, there is no contact of the lens with the skin; the cross-polarized lens absorbs all the scattered light and hence allows only light to pass through a single plane.

A vast amount of software is available for objectively documenting the clinical features of digitized images. Once identified, the computer then extracts features of the lesion important to the physician, e.g. color, hue, texture, degree of asymmetry, degree of border irregularity, diameter, perimeter, length and area, etc.

Multispectral Imaging System[43,44]

Surface-spectral reflectance of an object is an inherent physical property of the object. Spectrophotometric intracutaneous analysis scope (SIAscope™) and Melafinder™ are two dermascopes using multispectral images as inputs for subsequent computer analysis. The SIAscope™ consists of a hand-held imaging probe covering a 12 mm diameter area that is placed in contact with the skin surface. High intensity LEDs illuminate the skin surface with eight consecutive discreet wavebands between 400 nm and 1000 nm, i.e. covering the visible spectrum and a small range of near infrared rays. These wavelength-dependent images provide information on the concentration, distribution and position of skin chromophores. The SIA algorithm determines the amount of collagen within the papillary dermis, using the infrared waveband, the epidermal and/or dermal melanin distribution, vessel distribution and topography of the dermal epidermal junction. This information is then displayed in different SIA graphs and corresponding images to assist the physician in diagnosis.

Ultrasound Imaging System[45-48]

This technique records the reflection of high frequency ultrasonic waves by the various components of the skin. Ultrasonic waves propagate through matter and their velocity is determined by the physicochemical properties of the tissue, among which the presence of water content is a major factor, i.e. a tissue free from water is non-echogenic. They cross skin structures and are reflected by the interfaces between different skin layers and some structural elements such as collagen bundles of the dermis. There are two basic types of ultrasonography with dermatologic applications. The most well established one is 20 MHz scanning, used to measure tumor thickness and /or skin thickness when treating inflammatory diseases such as scleroderma or psoriasis. Real-time sonography with 7.5–10 MHz probes has acquired an increasingly important role, since detecting subcutaneous tumors in

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a variety of clinical settings, including the preoperative stage and follow-up of melanoma surgery.

Ultrasound images are created due to the different acoustic properties of tissues. High-frequency sound impulses are transmitted into the skin and then reflected, refracted, or inflected when a tissue interface with different acoustic impedance is encountered. The intensity of the reflections at different depths in the skin is then plotted onto a display screen to give a one-dimensional graph, known as A-mode. The vertical cross-sectional image, which is the more familiar display of ultrasound data, is called B-mode scanning (brightness display). B-mode scanning uses the brightness level of multiple A-scans to build a 2D image. The current technology also allows 3D C-mode (computed) scanning of structures in skin *in vivo*. C-mode scans, still under development, may provide a better image than that from B-scans. The image of the echogenic structure of the skin can be recorded and the parameters can be measured with automated image processing.

Measuring skin thickness was the first application of this technique. Age, corticosteroids, diseases of connective tissue, skin allergy and irritation induce changes in skin thickness.[48,49] Comparison with histological data is fair and the use of ultrasound imaging for measuring the invasion depth of different types of tumor is often routinely performed before surgery.[50,51] Changes occurring within the skin as a result of aging have been also investigated. Ultrasound imaging showed a Sub-Epidermal Non- (or Low-) Echogenic Band (SENEB or SLEB) in the upper dermis of elderly women which was ascribed to UV-induced alterations in the collagen and elastin network of the upper dermis (Fig.10);[48] a low echogenicity means decreased heterogeneity of the medium, a hallmark of skin photoaging.

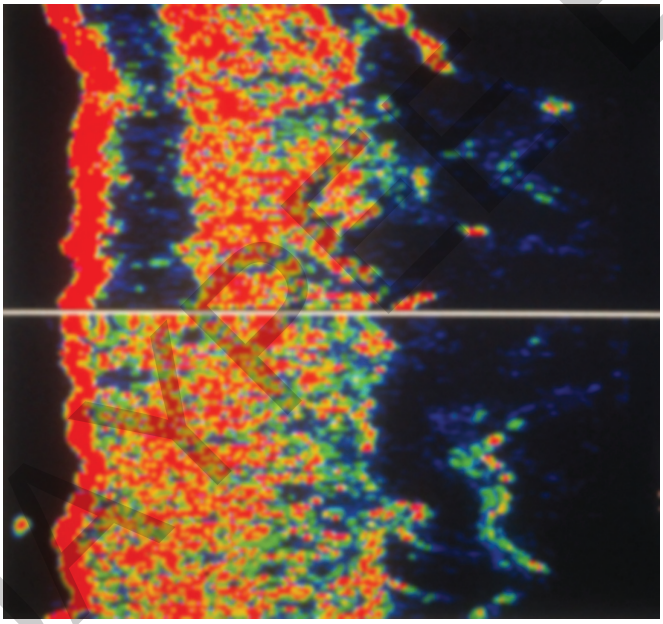

Figure 10: *In vivo* ultrasound image of sun-exposed skin (up) versus sun-protected skin (down): visualization of a Sub epidermal nonechogenic band in sun-exposed skin
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Optical Coherence Tomography[52-54]

Optical coherence tomography (OCT) is analogous to ultrasound B imaging, albeit using light instead of sound waves. It can be described as an intermediate imaging device between ultrasound and Confocal Scanning Laser Microscopy. The OCT technique is based upon the principle of Michelson interferometry. A pulse of near infrared, low coherence (super luminescent diode) light is split in such a way that half of the beam is sent to the specimen and half to a scanning reference mirror system. The light on the specimen is focused on the papillary skin layers, back scattering and recombining with the other reference beam of light reflected from the mirror system. An interference signal is only detected when the optical paths of both arms match with the coherence length of the light source. Measurement of the interference pattern allows the position within the tissue to be recorded where light is reflected. It is the reflectiveness of different components of tissues, such as cell membranes and melanin that provides contrast in the images. A 2D cross-sectional image is built up by lateral scanning across the tissue. Hence, much deeper layers of the skin can be imaged without much scattering as compared to ultrasound techniques. However, acquiring 2D OCT images is not sufficient to fully describe the 3D morphology of biological samples under study.

Optical coherence tomography images of healthy skin show some similarities with ultrasound images: a strong entrance signal corresponds to the amount of light reflected by the skin. Depending on the resolution level of the OCT device used, some other details of skin structure can be distinguished, e.g. the SC layer (two parallel lines) and the interface between the epidermis and the dermis

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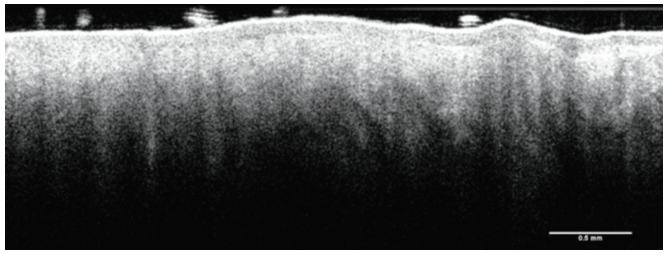

Figure 11: Optical coherent tomography (OCT) image of the crow-pad skin: Stratum corneum, epidermis and upper dermis may be distinguished
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owing to different gray levels (Fig. 11). Epidermis signal is less intense than that of the dermis one.[55] Due to a higher thickness, the SC and living epidermis are easily visible on the fingertips. In this area, sweat ducts can be recognized. The effects on skin of treatment by some drugs and cosmetics can be investigated using OCT imaging and have been reported. Other studies characterize irritant dermatitis, psoriasis, wound healing and skin tumors.[53] Effect of aging on the dermal-epidermal junction in women has been recently published.[52]

Magnetic Resonance Imaging

Developed in the early seventies, magnetic resonance imaging (MRI) is based on the interaction of an applied magnetic field with the spin of protons. The method is now routinely used in hospitals for clinical diagnosis. Magnetic resonance imaging is a highly flexible technique for making images of the whole human body. A Hydrogen (proton) nucleus behaves like a small magnet, implying that when the body is exposed to a strong external magnetic field, its own protons will align with the magnetic field. When properly excited, the protons (spin momentum) rotate around the direction of the applied magnetic field, producing a measurable signal in a nearby detector coil. The frequency of precession movement being proportional to the local magnetic field, making the field varying across the body, the signals arising from different locations are distinguished according to their frequency.

There are three basic components in a MRI system: (1) a large, static magnetic field (e.g. 3 Tesla for the human imagers at the Center; (2) radiofrequency (RF) coils that are used as a transmitter to excite the MR signal, and as a receiver to detect the MR signal; and (3) gradient coils that are pulsed on and off to produce linear gradients of the magnetic field for imaging. MRI techniques involve pulsing currents through the RF and gradient coils, explaining that this particular technique is often referred as a pulse sequence. By varying the pulse sequence, one can produce a huge range of images of different spatial and temporal resolutions and with a substantially high contrast between tissues in the image.

Magnetic resonance imaging displays good contrast in soft tissue. The increase in total proton density associated with changes in water structure alters free mobile protons in the epidermis, dermis, and subcutaneous tissue. These changes lead to increased contrast and brightness in MRI images (Fig. 12).

The major advantage of MRI is that it makes it possible to characterize the skin in a given region of interest by measuring the density of protons and T1 and T2 relaxation times. This was applied to skin aging studies.

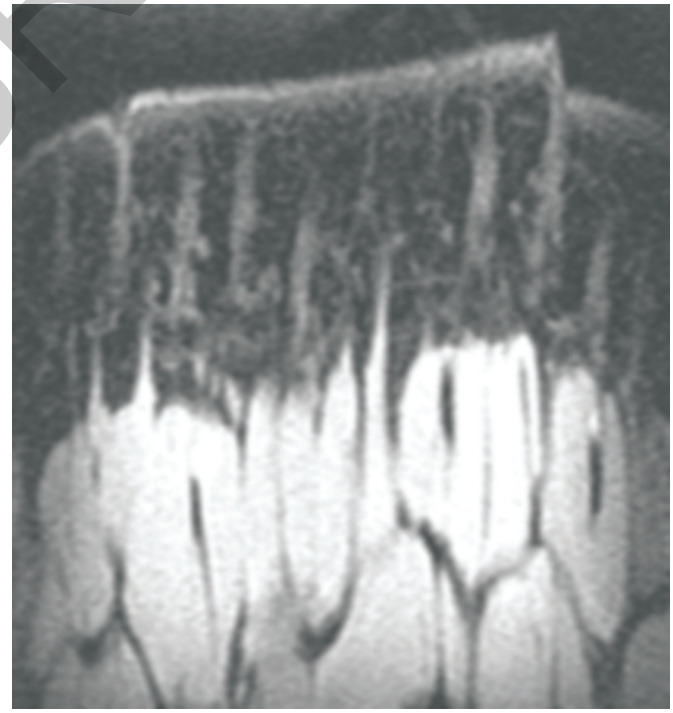

Figure 12: Magnetic resonance imaging of back skin: The epidermis with its pilosebaceous units can be distinguished. Some invasions of hypodermis into the dermis can also be noticed
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It was demonstrated that free water was higher in the epidermis than in the dermis irrespective of age and that free water was higher in the upper dermis of elderly (more than 70 years) as compared to fairly young (less than 40 years) adults.[56] Other published work deals with the measurement of the hydration gradient in the callus of the foot and the effect of a cosmetic treatment.[57] MRI was also used to study the precise location of hyaluronic acid in the upper dermis and its time-course following injection.[58]

Confocal Laser Microscopy[59,60]

The confocal laser microscope is a novel and interesting non-invasive tool for imaging skin lesions and subsurface skin lesions that are not visible to the naked eye or by dermatoscopy. It is based on the principle that when a diode laser beam crosses the skin, the reflected light is used to construct detailed images of optical sections through the tissue. A laser provides excitation light of high intensity. The laser light is reflected from a dichroic mirror. From there, it hits two mirrors which scan the laser light across the sample. Emitted light from the sample gets de-scanned by the dichroic mirror and is focused onto the pinhole after being measured by a detector (photomultiplier tube). As a limiting factor, any slight movement of the skin or body prevents the correct acquisition of images. The detector attached to a computer helps to build up the image one pixel at a time. The detection of backscattered light along with differences in the tissue refractive index helps in high resolution of cellular detail and contrast when examining thin sections, thus making staining unnecessary.

Nowadays, two different imaging modes are available for application in dermatology: (1) the reflectance mode; and (2) the fluorescence mode. In the first mode, contrast comes from changes in the refractive index of skin structural components. It is well established as a clinical diagnostic tool for the diagnosis of melanoma and non-melanoma skin cancers, and also for a wide range of other skin diseases. In the fluorescence mode, only fluorescent reemitted light (at a longer wavelength than that of the excitation light) is recorded. Contrast is achieved by utilizing the inherent refractive index properties of the various cellular microstructures. In vivo, reflectance mode is the most routinely used.

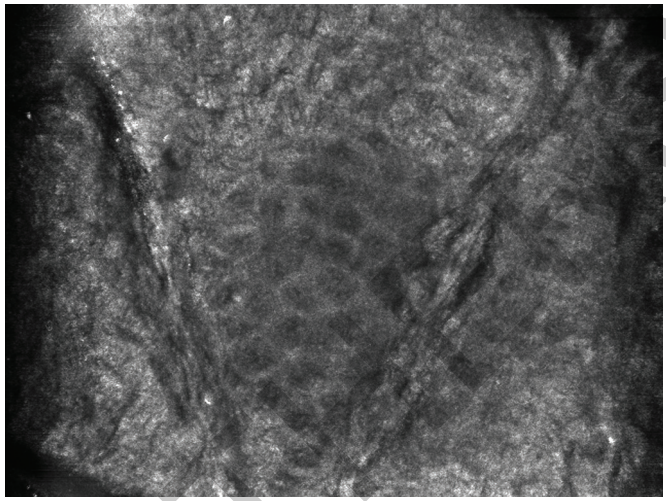

Figure 13: Confocal image of forearm skin (Horizontal section along the dermal epidermal junction)
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This type of measurement is quite accurate but is eventually possible on a very tiny surface area only. SC thickness as well as that of living epidermis can be measured with a very high accuracy.[47,61] Single cells can also be observed in their natural environment with quite good contrast, they can be counted and their diameter precisely measured. The dermal epidermal junction can also be easily studied due to the high reflectivity of melanin (Fig. 13). By counting the number of papillae in a given unit area, the inter-digitation between epidermis and dermis can be estimated. Data on the effects of repeated sun exposure, photoaging and aging have been reported.[62]

In clinical research, confocal microscopy seems to be a very promising technique for differentiating in vivo between equivocal lesions such as melanomas and other melanocytic lesions.[63] Other studies have been conducted for a better characterization of lentigines.[64]

Multiphoton Imaging of the Skin

Unlike confocal microscopy, when used in the fluorescent mode, multi- or two-photon microscopy (TM) produces fluorescence as a result of simultaneous absorption of two photons having the same energy. When a chromophore is excited, part of its energy is dissipated through the sample, while another part is emitted as a fluorescent photon with the energy lower than the total absorbed energy. Besides

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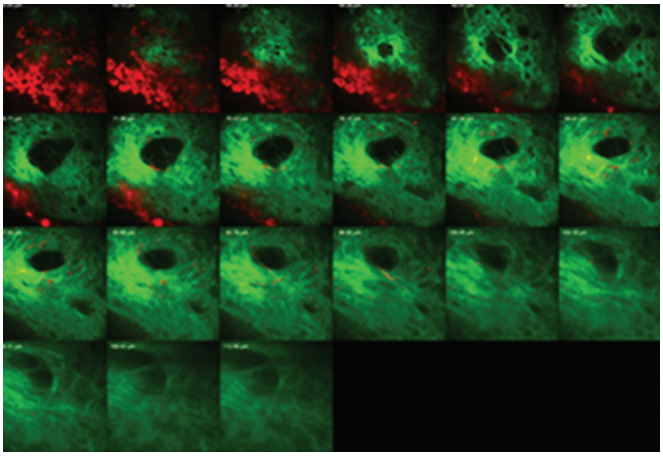

Figure 14: *In vivo* multiphoton images of forearm skin corresponding to 21 successive sections from upper down to mid-dermis. Elastic fibers are coded in red, collagen in green ©L'Oréal Research and Innovation

this two-photon auto-fluorescence (TPA) however, another type of deactivation of the chromophore takes place: some photons are emitted and their energy is the total amount of energy of the two incident photons. The latter emission type is called "second harmonic generation (SHM)". It is of particular relevance for looking at collagen which generates "second harmonic" photons at 410 nm corresponding to the simultaneous absorption of two photons with a wavelength of 820 nm.[65,66] The only way to produce simultaneous absorption of several photons by the same molecule is to use an ultrafast (femtosecond) laser in the infra-red range (tuning possibility 730–960 nm: MaiTai, Spectra Physics, Coherent, Chameleon). Such a laser can deliver a high power in a very short period (about 10–15 p-seconds). The total energy focused at a given point into the skin (the focal point) is minimized by modulating the laser impulse at 80 MHz. A commercially available TM device is DermalInspect (JenLab GmbH, Jena, Germany).

TM technique is quite recent. Masters et al. demonstrated the ability to quantify NADPH autofluorescence for assessing the metabolic states of human epidermal cells *in vivo*.[67] Other authors showed that TM enabled "optical biopsies" of inner epidermis and upper dermis to be recorded.[68] For instance, cells of the stratum granulosum, the stratum spinosum and the stratum basale could be differentiated according to the fluorescence level of their nuclei and their shape. An interesting application of TM is assessment both of the elastin and the collagen content on the same optical section (Fig. 14).[69,70] Elastin is evaluated from (TP) and collagen from (SH). Such studies demonstrate a progressive decrease in collagen and a relative increase in elastin with aging. The ratio of SHG to TPA could provide a quantified index of photoaging.[71]

TM was also used to study dermatological disorders such as psoriasis and skin cancer within pigmented area. Further studies in these fields, however, are needed before reaching any final conclusion. TM technique was used for screening the effects of drug *in situ*.[70] When a topical cream contains an active fluorescent ingredient, the penetration level of the ingredient into a given skin layer can be estimated.[71]

REFERENCES

1. Leveque JL, De Rigal J. Impedance method for studying skin moisturization. J Soc Cosmet Chem. 1983;34:419-25.
2. Fluhr JW, Gloor M, Lazzerini S. Comparative study of 5 instruments measuring stratum corneum hydration. Skin Res Tech. 1999;5:161-70.
3. Kazaka G, Courage W. Courage Kazaka Electronics, Köln, Germany, technical information concerning the digital measuring probes of Corneometer CM. 2004; 825.
4. Leveque,JL, Querleux B. Skin Chip©, a new tool for investigating the skin surface in vivo. Skin Res Technol. 2003;9:343-7.
5. Pierard GE. Leveque JL. What is Skin Chip? From silicon image sensor to skin chip. Dermatology. 2004;208:291-2
6. Batisse D, Giro, F, Leveque JL. Capacitance imaging of the skin surface. Skin Research Technol. 2006;12:99-104.
7. Thune P, Nilsen T, Hanstad IK, et al. The water barrier function of the skin in relation to the water content of stratum corneum, pH and skin lipids. The effect of alkaline soap and syndet on dry skin in elderly, non-atopic patients. Acta Derm Venereol. 1988;68:277-83.
8. Tagami H, Kobayashi H, Kikuchi K. A portable device using a closed chamber system for measuring Transepidermal water loss: comparison with the conventional method. Skin Res Technol. 2002;8:7-10.
9. Barel AO, Clarys P. Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments Evaporimeter and Tewameter. Skin Pharmacol. 1995;8:186-90
10. Wa CV, Maibach HI. Mapping the human face: biophysical properties. Skin Res Technol. 2010;16:38–54.
11. Gloor M, Hirsch G, Willebrandt U. Use of infrared spectroscopy for the in vivo measurement of water content of the horny layer after application of dermatologic ointments. Arch Dermatol Res. 1981;271:305-13
12. Caspers PJ, Lucassen GW, Puppels GJ. Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin. Biophys J. 2003;85:572-80.
13. Chrit L, Hadjur C, Morel S, et al. In vivo chemical investigation of Human skin using confocal Raman fiber optic microprobe. J Biomed Optics. 2005;10:1-11.

JAYPEE BROTHERS

14. Walling PL, Dabney JM. Moisturization in skin by near infrared spectroscopy. J Soc Cosmet Chem. 1989;40:151-71.
15. De Rigal J, Losch MJ, Bazin R, et al. Near-infrared spectroscopy: A new approach to the characterization of dry skin. J Soc Cosmet Chem. 1993;44:197-209
16. Charakida A, Charakida M, Chu AC. Double-blind, randomized, placebo-controlled study of a lotion containing triethyl citrate and ethyl linoleate in the treatment of acne vulgaris. Br J Dermatol. 2007;157:569–74.
17. Nouveau-Richard S, Zhu W, Li YH, et al. Oily skin: specific features in Chinese women. Skin Res Technol. 2007;13:43–8.
18. Kazak G, Courage W. Courage Kazaka Electronics, Köln, Germany, technical information concerning the Cutometer. 2004.
19. De Rigal J, Lévêque JL. In vivo measurement of the *stratum corneum* elasticity. Bioeng Skin. 1985;1:13–23.
20. Ohshima H, Kinoshita S, Oyobikawa M, et al. Use of Cutometer area parameters in evaluating age-related changes in the skin elasticity of the cheek. Skin Res Technol. 2013;19: e23–e242.
21. Griffiths CEM. The clinical identification and quantification of photodamage. Br J Dermatol. 1992;127:37-42.
22. Murray BC, Wickett RR. Correlation between dermal torque meter, cutometer and Dermal phase measurements of human skin. Skin Res Technol. 1997; 3;10106
23. Tosti A, Compagno G, Fazzini ML, et al. A ballistometer for the study of the plasto-elastic properties of skin. J Invest Dermatol. 1997;69:315–7.
24. Paye M, Mac-Mary S, Elkhyat A, et al. Use of Reviscometer for measuring cosmetic induced skin surface effects. Skin Res Technol. 2007;13 343-9
25. Reviscometer: Courage-Khazaka Electronics GmBH, Köln, Germany, 2008.
26. Grove GL, Grove MJ, Leyden JJ, et al. Optical profilometry: an objective method for quantification of facial wrinkle. J Am Acad Dermatol. 1989:21:631–7.
27. Leveque JL. EEMCO guidance for the assessment of skin topography. J Eur Acad Dermatol. 1999;12:103-14
28. Couturaud V, Zahouani H, Broucho G, et al. Measurement of skin topography imaging by defocalization laser device. Proceedings of the 20th IFSCC Congress. 1998
29. Lagarde JM, Rouvrais C, Black D, et al. Skin topography measurement by interference fringe projection: a technical validation. Skin Res Technol. 2001;**7**:112–21.
30. Corcuff P, de Rigal J, Leveque JL. Skin relief and aging. J Soc Cosm Chem. 1983;3:177190.
31. Griffiths CEM. The clinical identification and quantification of photodamage. Br J Dermatol. 1992;127:37-42.
32. Latreille J, Gardinier S, Ambroisine L, et al. Influence of skin colour on the detection of cutaneous erythema and tanning phenomena using reflectance spectrophotometry. Skin Res Technol. 2007;13:236-41.
33. Liu W, Lai W, Wang XM, et al. Skin phototyping in a Chinese female population: analysis of four hundred and four cases from four major cities of China. Photodermatol Photoimmunol Photomed. 2006;22:184-8.
34. Moncrieff M, Cotton S, Claridge E, et al. Spectrophotometric Intracutaneous Analysis: a new technique for imaging pigmented skin lesions. Br J Dermatol. 2002;146:448–57.
35. Petitjean A, Sainthillier JM, Macmary S, et al. Skin radiance how to quantify? Validation of an optical method. Skin Res Technol. 2007;13:28.
36. Matsubara A, Liang Z, Sato Y, et al. Analysis of human perception of facial skin radiance by means of image histogram parameters of surface&and subsurface reflections from the skin. Skin Res Technol. 2012;18:265-71.
37. Wilhelm P, Enzo B, Peter E, et al. Bio Engineering of Skin: Skin Imaging and Analysis. Dermatology: Basic science series. 2007; 423-43.
38. Anderson RR. Polarized light examination and photography of the skin. Arch Dermatol 1991;127:1000–5.
39. Muccini JA, Kollias N, Phillips SB, et al. Polarized light photography in the evaluation of photoaging. J Am Acad Dermatol. 1995;33:76-–9
40. Guillard G, Lagarde J. Skin lesions segmentation and quantification from 3D body's models. Skin Res Technol. 2005;11:123–31.
41. Nischal KC, Khopkar U. Dermoscope. Indian J Dermatol Venereol Leprol. 2005;71:300-3.
42. Benvenuto-Andrade C, Dusza SW, Agero AC, et al. Differences between Polarized Light Dermoscopy and Immersion Contact Dermoscopy for the Evaluation of Skin Lesions. Arch Dermatol. 2007;143:329-38.
43. Moncrieff M, Cotton S, Claridge E, et al. Spectrophotometric Intracutaneous Analysis: a new technique for imaging pigmented skin lesions. Br J Dermatol. 2002;146:448–57.
44. Guillard G, Lagarde J. Skin lesions segmentation and quantification from 3D body's models. Skin Res Technol. 2005;11:123–31.
45. Mlosek RK, Ebowska RM, Lewandowski MI et al. Imaging of the skin and subcutaneous tissue using classical and high-frequency ultrasonographies in anti-cellulite therapy. Skin Res Technol. 2011;17:46–8.
46. Wortsman XC, Holm EA, Wulf HC. Real-time spatial compound ultrasound imaging of skin. Skin Res Technol. 2008;10:23–31.
47. Nouveau-Richard S, Monot M, Bastien P, et al. In vivo epidermal thickness measurement: ultrasound vs. confocal imaging. Skin Res Technol. 2004;10:13-–40.
48. De Rigal J, Escoffier C, Querleux B, et al. Assessment of aging of the human skin by in vivo ultrasonic imaging. J Invest Dermatol. 1989;93:621-25
49. Richard S, Duteil L, Poli F, et al. Echographic assessment of corticosteroid-induced skin thinning, including echogenicity measurement. Skin Res Technol. 1996;2:18-22.
50. Matthes U, Hoxtermann S, Hoffman K, et al. Acoustic microscopy in dermatology: normal skin structure and tumours, In: Altmeyer P, el Gammal S, Hoffman K, (eds). Ultrasound in dermatology. Berlin, Germany: Springer-Verla,; 1992.
51. Vilana R, Puig S, Sanchez M, et al. Preoperative assessment of cutaneous melanoma thickness using 10-MHz sonography. AJR Am J Roentgenol. 2009;193:639-43.
52 Querleux B, Baldeweck T, Diridollou S, et al. Skin from various ethnic origins and aging: an in vivo cross-sectional multimodality imaging study. Skin Res Technol. 2009;15:306-13.
53. Welzel J. Optical coherence tomography in dermatology: a review. Skin Res Technol. 2001;*7*:1-9.
54. Pierce MC, Strasswimmer J, Park BH, et al. Advances in optical coherence tomography imaging for dermatology. J Invest Dermatol. 2004;123:458-63.
55. Weissman J, Hancewicz T, Kaplan P. Optical coherence tomography of skin for measurement of epidermal thickness by shapelet-based image analysis. Optics Express. 2004;12: 5760-9.
56. Richard S, Querleux B, Bittoun J, et al . Characterization of the skin in vivo by high resolution magnetic resonance imagin : water behavior and age related effects. J Invest Dermatol. 1993;100:705-9.

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57. Querleux B, Richard S, Bittoun J, et al. In vivo hydration profile in skin layers by high-resolution magnetic resonance imaging. Skin Pharmacol. 1994;7:210-6.
58. Gensanne D, Josse AM, Schmidt AM, et al. In vivo visualization of hyaluronic acid injection by high-resolution T2 parametric magnetic resonance images. Skin Res Technol. 2007;13:385-9.
59. Rajadhyaksha M, González S, Zavislan JM, et al. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J Invest Dermatol. 1999;113:293-303.
60. González S, Swindells K, Rajadhyaksha M, et al. Changing paradigms in dermatology: confocal microscopy in clinical and surgical dermatology. Clin Dermatol. 2003;21:359-69.
61. Sauerman K, Clemann S, Jaspers S. Age related changes of human skin investigated with histometric measurements by confocal laser scanning microscopy in vivo. Skin Res Technol. 2002;8:52-6.
62. Gamblicher T, Sauerman K, Altintas MA. Effects of repeated sunbed exposures on the human skin. In vivo measurements with Confocal Microscopy. Skin Res Technol. 2004;20:27-32.
63. Pellacani G, Guitera P, Longo C, et al. The impact of in vivo reflectance microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol. 2007;127:2759-65.
64. Nakajima A, Funasaka Y, Kawana S. Investigation by in vivo reflectance confocal microscopy: melanocytes at the edges of solar lentigines. Exp Dermatol. 2012;21:18-21.
65. Konig K, Riemann I. High resolution multiphoton tomography of human skin with subcellular spatial resolution and picosecond time resolution. J Biomed Opt. 2003;8:432-9.
66. Zipfel WR, Williams RM, Christie R, et al. Live tissue intrinsic microscopy using multiphoton excited native fluorescence and second harmonic generation. Proc Natl Acad Sci USA. 2003;100:7075-80.
67. Masters BR, So PT, Kim KH, et al. Multiphoton excitation microscopy, confocal microscopy, and spectroscopy of living cells and tissues; functional metabolic imaging of human skin in vivo. Methods Enzymol. 1999;307:513-36.
68. Lin SJ, Wu RJ, Tan HY, et al. Evaluating cutaneous photoaging by use of multiphoton fluorescence and second harmonic generation microscopy. Optics Letters. 2005;30:2275-7.
69. Decencière E, Tancrède-Bohin E, Dokladal P, et al. Automatic 3D segmentation of multiphoton images: A key step for the quantification of human skin. Skin Res Tech. 2013;19:115124.
70. El Madani AH, Tancrède-Bohin E, Bensussan A, et al. In vivo multiphoton imaging of human skin: assessment of topical corticosteroid-induced epidermis atrophy and depigmentation. J Biomed Opt. 2012;17:026009.
71. Konig K, Ehlers A, Stracke F,Iet al. In vivo drug screening in human skin using femtosecond laser multiphoton tomography. Skin Pharm Physiol. 2006;19:78-88.

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Basic Science for
Modern Cosmetic Dermatology

Cosmetic Dermatology is a science that endeavors to define the most appropriate preventive and therapeutic measures for skin and hair care. With the increase in life expectancy worldwide, a preventive approach with cosmetics is particularly important, and is sustained by numerous scientific works.

This book gathers together advances in the basic science in cosmetic dermatology for a better understanding of physiology that underlies the most relevant prevention strategies for a maintenance of healthy hair and skin, with a specific focus for Indian population.

Tremendous progress has been made in recent years in developing new tools for research, especially in molecular biology, genomics and proteomics. These breakthroughs afford a better knowledge of the skin and hair diversity, taking into account innate characteristics and environmental influences, such as weather variations and cultural habits.

The authors, clinical dermatologists and scientists, both Indian and international, have made these innovations in cosmetic science relevant to all Indian dermatologists.

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