Abstracts

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Abstracts

Oral Session
Molecular imaging of skin I

1(*Invited lecture*)
In-vivo non-linear spectral microscopy of mouse skin

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Background: In Multi-photon excitation, fluorescent molecules having absorption bands in the visible or UV are excited by the simultaneous absorption of two or more Infra Red (IR) or Near Infra Red (NIR) photons. The non-linear dependency of the two-photon absorption probability on the excitation intensity results in the confinement of the excitation volume. This behavior can be employed for 3-D imaging without a pinhole in multi-photon excitation microscopy. Non linear excitation lifts some of the problems related to conventional autofluorescence spectroscopy and microscopy. This technique affords excitation of autofluorescing molecules with one photon absorption bands in the UV without the complications related to the use of UV light. Importantly, Multi-photon excitation microscopy enables high resolution imaging deep inside tissues. This makes this technique a powerful tool for the study of skin.

Purpose: In this work a dedicated multi-photon spectral imaging was employed to study the intrinsic emission of mouse skin tissues. The purpose of the research was to investigate the potential of non-linear excitation methods in tissue research. Challenges include 1) determination of intrinsic emission based 'optical signatures' of skin and evaluate their potential for diagnosing diseases and 2) In depth imaging of the skin morphology based on intrinsic emission alone.

In this study we focused on the spectral imaging of the intrinsic emission from mouse skin post mortem biopsies, thin sections, and *in vivo* tissue samples.

Methods: The home made microscope was equipped with a tunable (700 nm - 1000 nm) mode-locked titanium: sapphire (Ti:Sa) laser with typical pulse widths of 70 to 100 fs and a repetition rate of 82 MHz. The setup was optimized for transmission over a broad wavelength range by employing UV-VIS-IR achromat lenses, equipped with both a galvanometer mirror scanner and an XYZ piezo translation (sample) stage. The results reported here were acquired in the inverted geometry using air (20×/0.75) and oil-immersion (40×/1.30) objectives (160mm tube length, Fluor, Nikon, Japan) and an excitation wavelength of 764 nm. The resolution for the two objectives was estimated to be 0.62 μm and 0.36 μm laterally, and 1.4 μm and 0.68 μm axially for the two objectives respectively. The emission passed through a dichroic mirror and was filtered by a set of BG-40 short-pass filters before entering the spectrograph. The spectrograph consisted of two prisms and a high sensitivity CCD camera (Princeton Instruments, Spec-10:2KBUV,). Only a small section of the camera was used to record the emission spectra and in addition on-chip binning was employed. Effectively, 100 wavelength channels were recorded with a spectral resolution of about 5 nm at a maximum rate of 1000 spectra per second. Total image acquisition time for 220x220 pixel images was about 2 minutes.

Results: Mouse skin post mortem biopsies, thin sections, and *in vivo* tissue samples were investigated. Although only the intrinsic emission of the skin was imaged, the different layers of skin could be clearly distinguished based on both their spectral signature and morphology. Auto fluorescence images were recorded that contain signals from cellular and extra cellular structures. The fluorescence arises from native chromophores such as NADPH, elastin, collagen and flavins. Collagen in the dermis could be identified based on the strong second harmonic signal that is characteristic for type 1 collagen.

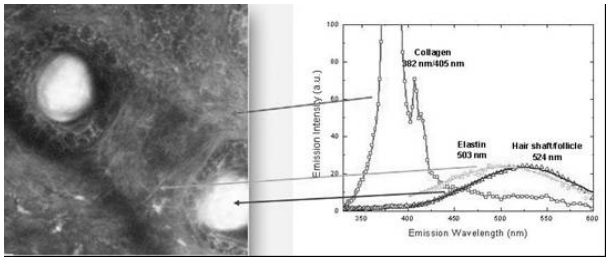
Visualization of the spectral images using a 'true color' visualization method allowed us to identify different cell types including epidermal cells, lipid-rich keratinocytes and dermal cells (fibroblasts). In addition intercellular structures such as hair follicles, collagen and elastin could be observed, Deeper layers in the skin also revealed fluorescence and structures that are related to the presence of sebaceous glands.

The spectral imaging system can typically image as deep as 100 μm. The system's ability to image deep structures is predominantly limited by the index of refraction mismatch between the sample and the (oil-immersion) objective. Individual cells could be observed starting in the *stratum spinosum* at about 5 μm below the surface of the *stratum corneum*. The blue fluorescence of these cells *in vivo* mainly originates from the cytoplasm. Previous studies revealed that the main source of intracellular autofluorescence is NAD(P)H. It is fluorescent only when reduced and has a characteristic blue fluorescence peak at around 460 nm. Another source of redox related autofluorescence comes from cellular flavins. In contrast to NAD(P)H, these molecules are fluorescent in their oxidized state and has a characteristic yellow fluorescence peak at around 535 nm. In the post mortem biopsies and thin sections this distinct blue color was absent.

Our results showed morphological and spectral differences between the mouse skin post mortem biopsy and *in vivo* samples which explained by biochemical differences, specifically of NAD(P)H.

Comparison of the post mortem biopsies and the thin

sections enabled us to determine the wavelength dependent scattering of the tissue. This, in principle, affords correction of the measured spectra for scattering effects.
In addition to two-photon excited fluorescence other non-linear signals were observed. SHG from collagen and a narrowband spectral emission band related to collagen were observed. The narrowband emission shifts with excitation wavelength and is therefore related to Raman scattering.



Conclusions: Non-linear excitation methods provide a powerful tool for the imaging of skin. Both morphology and biochemical aspects can be imaged without application of dyes. Besides clear autofluorescence signals also strong second harmonic signals were observed in the dermis that are characteristic for collagen type 1. Overall, spectral imaging provided a wealth of information not easily obtainable with present conventional multi-photon imaging methods.
More information about this work can be found in:
J. A. Palero, H. S. de Bruijn, A. van der Ploeg-van den Heuvel, H. J. C. M. Sterenborg, H. C. Gerritsen, *Optics Express* **14**, 4395 (2006).
J. A. Palero, H. S. d. Bruijn, A. v. d. P. v. d. Heuvel, H. J. C. M. Sterenborg, and H. C. Gerritsen, Bioph. J. **93**(3), 992-1007(2007).
Keywords: *In Vivo*, Non-invasive imaging, Multi-photon microscopy, Spectral imaging, Second harmonic generation, Autofluorescence, Skin

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The imaging of melanin distribution using multiphoton autofluorescence deday curves

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Background/Purpose: Multiphoton fluorescence lifetime imaging (FLIM) is a technique for producing an image based on differences in the decay rate of the fluorescence from a sample. Based on this method, the DermaInspect (JenLab, Jena, Germany) was developed to observe human skin components non-invasively. In this study, we applied the DermaInspect to the study of melanin in skin. The validity of FLIM was verified by comparison with data obtained from a conventional histochemical staining method.

Methods: A human 3D skin model containing melanocytes (MEL-300 MatTeck corporation, MA, U.S.A) was embedded in O.C.T.compound, frozen and sectioned at 10-µm. Melanin distribution in each section was visualized by the DermaInspect, using time-resolved single photon counting and near-infrared femotosecond lasor pulse excitation. Melanin distribution of the same sample was then visualized by the Fontana-Masson staining method.
Results: High-resolution images were generated from the ratio of a1/a2 ($a_1e^{-t/\tau 1}+ a_2e^{-t/\tau 2}$ was chosen to express the exponential fluorescent decay curve) by the DermaInspect. Granules with high a1/a2 ratio, approximately 1µm in diameter, were observed in the area between the basal layer to the stratum corneum. The Fontana-Masson staining identified the granules as melanin. The new technique was then applied for *in-vivo* observation of melanin in the human arm skin. An accumulation of the granules was visualized around the nuclei as melanin caps on the image derived from the ratio of a1/a2.
Conclusion: Our findings confirmed that FLIM can non-invasively provide us data of melanin distribution with almost the same quality as the conventional method, and also that FLIM is applicable for *in-vivo* observation of melanin granules in human skin.
Keywords: Non-invasive imaging, Twophoton microscope, FLIM, Fluorescence decay curve, Life time, Melanin, Skin

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Quantitative assessment of dermal extracellular matrix components by high resolution multiphoton tomography: *in vivo* double blind study of a cosmetical product

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Purpose: To study the advantages of a new *in-vivo* non invasive method, multiphotonic tomography for quantifying the effects of a skincare product on the components of the superficial dermis.
Methods: Multiphoton tomography of human skin with subcellular resolution using the CE-marked imaging system *DermaInspect* (JenLab GmbH, Jena, Germany) was performed in 24 women aged between 45 and 65 years The product was tested in a randomized double blind test against vehicle, with application twice daily for 3 months on the volar forearm. High-resolution multiphoton images of all epidermal skin layers as well as of the dermal collagen and elastin network were obtained by two photons auto fluorescence and second harmonic generation. The measurements were carried out

at baseline and, after 1 and 3 months of treatment. A quantification procedure was implemented to quantify the ratio of the two signals SHG and AF between 88.5µm and 180µm.

Results: Statistical analyses reveal an increase of this ratio after application of the cream compared to vehicle indicating an increase of extracellular matrix proteins.

Conclusions: The multiphoton tomography DermaInspect is a promising tool to assess non-invasively the effect of drugs and cosmetic products on the different skin layers.

Keywords: Skin, Multiphoton, Collagen, Elastin, Cosmetic product

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Infrared spectroscopic imaging of molecular composition and structure during human corneocyte maturation

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Background: The outermost region of the epidermis, the stratum corneum (SC), provides an essential barrier to water loss and protects against exogenous substances. The functional integrity of the SC depends on a complex maturation and exfoliation process, which is often perturbed in skin diseases and when the skin is damaged by environmental stresses.

Purpose: The current study was designed to development new biophysical measurements that provide insights into the structural and compositional changes accompanying SC, and particularly corneocyte, maturation.

Methods: Infrared (IR) spectroscopic imaging was employed to evaluate the molecular changes occurring with the maturation of corneocytes isolated from different depths in healthy human SC.

Results: The quality of spectra from the IR measurements of individual corneocytes was high and revealed depth dependent variations in molecular composition. Among the spectral changes identified were several arising from alterations in the concentration of the major constituents of natural moisturizing factor (NMF), a SC component critical in maintaining SC hydration. A significant and progressive decrease in the concentration of NMF was observed for corneocytes isolated from superficial compared to deeper SC layers (layer 3 *vs*. layer 11, respectively).

Conclusion: A generally applicable IR parameter that measures the relative NMF concentration in isolated corneocytes was successfully developed. This IR parameter identifies corneocytes at different stages of maturation by directly measuring the NMF chemical composition within individual corneocytes. The potential biomedical applications of vibrational imaging in evaluating corneocyte composition and molecular structure in the treatment of NMF-related diseases will be discussed.

Keywords: NMF, Corneocytes, IR Imaging, Maturation, Stratum corneum

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Identification of skin cancers using confocal raman microscopy

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Background: Skin cancer has a cure rate of 100% if it is early detected. Unfortunately, early detection is very difficult with current medical diagnosis because diagnosis is still based on morphological inspection by a pathologist. In the present study, the confocal Raman microscopic technique was applied to the early dermatological diagnosis of BCC.

Purpose: To confirm the potential applicability of confocal Raman microscopy as a direct diagnostic tool for precancerous and noncancerous lesions.

Materials/Methods: BCC tissues used for confocal Raman measurements were obtained from 10 patients using a routine biopsy. Cross-sections 20 µm thick were cut with a microtome at –20 ^{o}C, and frozen sections were stored in liquid nitrogen before use. Two thin sections were cut for the experiments. One section was used for confocal Raman profiling experiments and the other section was stained with H&E and sent to an expert pathologist for a routine cancer diagnosis. The H&E section was also used as a Raman reference to locate the boundaries between the different skin-layers in the unstained section. Confocal Raman measurements were performed using a Renishaw 2000 Raman microscope system with an argon ion laser operating at $\lambda = 514.5$.

Results: Direct Rama signal differences between benign and BCC tissues could be observed. In particular, the enhancement of the amide III band and the disappearance of the PO_2^- stretching band in BCC tissue could be used for the direct dermatological diagnosis of a skin cancer. In addition, confocal Raman depth profiling could be used to accurately determine the BCC area from healthy surrounding tissue, making it possible to precisely differentiate a cancerous area from surrounding non-cancerous tissues. Mixed amide I bands of benign and BCC tissues around the borderline, as well as irregular patterns of PO_2^- bands of benign tissue caused by inhomogeneous metastasis inside the BCC area, could also be detected.

Conclusions: A fast and accurate BCC diagnostic tool for the screening and selection of biopsy is important in surgical operations. Our results show that by applying the confocal Raman depth profiling technique to human skin tissue, it is possible to precisely determine cancerous tissue from surrounding non-cancerous tissue. We predict that this confocal Raman profiling technique has strong feasibility as a dermatological diagnostic tool.

Keywords: BCC, Confocal raman microscopy, Skin

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Clinical Studies

6(*Invited Lecture*)
Asian skin: characteristic of microrelief of photo-protected / photo-exposed sites and the crow's feet wrinkle

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Background: Clinic epidemiological survey for skin wrinkles is necessary and important for better understanding the skin aging. However, it is non-quantitative and impossible to observe fines changes.
Purpose: To evaluate the microtopography of forehead as photo-exposed sites, and abdomen as relative photo-protected sites by silicone replicas, meantime the differences of the wrinkle between Caucasian and Asian women were observed.
Methods: A totally 120 Chinese women from 3 to 90 were recruited for the study. The silicone mould that held skin microtopography was scanned by the Profilometry of interference fringe projection (LIBC and Optical Laboratory, PM DUFFIEUX, CNRS UMR 6603, Besançon, France) and the three-dimensional image of the silicone mould was recorded. The image was dealt with Microtopography Three-dimensional Digital Image Processing Software (ProjectFringes, Besançon, France). With the same method, we also compared the wrinkles volume and depth of the crow's feet between the 50 Chinese women and 50 France women. They were age-paired and distributed by age decade.
Results: All the roughness (Sa) and the height / depth parameters (Sq, Spm, Svm and Stm) were increased with age. Their correlation coefficients with age were between 0.63 and 0.78 with high significance. the forehead's microtopography increased in the puberty sharply whereas the roughness of abdomen increased later during life. The wrinkles volume and depth of Chinese women were fewer than those of French women; there were nearly 10 years delay for the same wrinkle intensity in the Chinese women before aged 50.
Conclusion：There were significant differences for the microtopography between the photo-exposed and the photo-protected sites and comparing with the Caucasian women, the wrinkle in Asian women appeared late.
Keywords: Asian skin, Microtopography, Wrinkle, Site, Ethnic, Profilometry

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OCT imaging of the dermal epidermal junction of perioral skin and lips from French caucasian and Japanese women

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Purpose: Compared to the skin, there are very few studies aiming at acquiring structural information of the lip *in vivo* with non invasive cross-sectional imaging methods. The objective of this study was to measure with OCT imaging the thickness of the epidermis and visualize the dermal epidermal junction (DEJ) of perioral skin and lips from French Caucasian and Japanese women and to assess:
1) the age effects on both perioral skin and lips,
2) the ethnic differences between French Caucasian and Japanese lips and perioral skin,
3) the differences between the perioral skin and lips.
Methods: 174 French Caucasians and 200 Japanese participated in this study during the winter season. The thickness of the epidermis and the DEJ mean height of the perioral skin and lower lip were imaged and quantified using a homemade Optical Coherence Tomography (OCT) system.
Results: Main results are:
1) The thickness of the epidermis and the DEJ mean height of the perilabial skin decrease significantly with age whereas the epidermal thickness of the lower lip is unchanged.
2) Compared to the Japanese women, the epidermis of the French Caucasian lower lips is significantly thicker, whereas the DEJ mean height is not different.
3) The thickness of the epidermis and the DEJ mean height of the lower lip are higher that the perioral skin ones.
Conclusion: OCT is a valuable non invasive method to gain new information on lip anatomy *in vivo*. Such measurements emphasize the request to formulate tailored-made products based on lips specificities
Keywords: *In Vivo*, Non-invasive imaging, Optical coherence tomography, Skin, Lip, Ethnicity

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Sweat activity measured by skin impedance

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Background: Measurement of sweat activity is a useful clinical tool for diagnosis and treatment evaluation of diseases afflicting the sweating physiology such as hyperhidrosis. Current methods of measurement are limited to controlled laboratory research due to a lack of portability.

Purpose: The purpose of this project was to develop a new method of measuring sweat activity which enables long-term measurements at several skin sites with a pocket-size portable system.
Methods: We present a solution based on electrical measurements in the stratum corneum enabling 24h monitoring and logging of sweat activity in four channels using a pocket-size portable device. The device uses skin-surface electrodes for each channel, and wireless communication between the device and a monitoring PC for real-time measurements or downloading of logged data.
Measurements have been done on 30 healthy volunteers during relaxation, acoustic stimuli, heat exposure and physical exercise.
Results: The new method enables measurements of quick changes in sweat activity at four different skin sites simultaneously. The portability and robustness of the system enables long-term recording of sweat activity under circumstances such as daily errands, exercise and sleep.
Some typical measurements on the healthy volunteers are presented. Our measurements show large interindividual differences in the sweat activity of the healthy volunteers, and intraindividually that the sweat activity was very dependent on skin site.
Conclusions: The skin impedance measurement method is well suited for portable multichannel measurements of sweat activity.
Keywords: Skin, Sweat, Conductance, Bioimpedance

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Imaging of hair cuticle under traction test

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Background/Purpose: Nowadays, hair is an important part of our appearance and brightness the main evaluation criteria of goodness. Unfortunately, daily washing, combing or drying induce cracks and breaks on the outer part of hair, the cuticle, decreasing in the same time the inner part, the cortex, mainly on a mechanical point of view. and sensitively, these changes provoke a loose of brightness. These daily modifications are often studied after the treatment comparing it to the initial state. But, for a better comprehension, it seems to be interesting to develop a method that can observe these modifications during the treatment. Thus, we develop a method to observe the cuticle deformation during a tensile test.
Methods: A traction machine has been specially developed to perform tensile test on hair. It uses the ability of the interferometer to make a topographic image with high resolution and in short time, less than one minute. Samples are 20 mm long Caucasian hair and undergo thermal and moisture treatments. The whole device is positioned under an optical device design to measure the topography of surfaces. The interferometer Veeco Instruments, Wyko, Cambridge, UK), is used in the Vertical Scanning Interferometry mode with a magnification of 73.4 and the image obtained measure 80 µm lengths by 60 µm height. An image of each area is taken before the pull test. During the test, images are taken on the areas located, and the corresponding force and strain is recorded. Thus, images of the same area can be compared. From the image, it is possible to extract a profile and observe the elongation of the cuticle.
Results: This study allows to distinguish two step in the scale deformation: an elongation that causes an decrease of the roughness followed by an wrench and an increase of roughness. The stress strain curves extracted from each samples shows significant differences with the evolution of the treatment temperature and the humidity rate. The wrench beginning also varies with the different treatments. Finally, the ability of cuticle recovery with the increase of humidity is shown for elongation less than 15%.
Conclusion: This method shows significant results for the study of cuticle modification caused by traction and gives information on the behaviour of hair under daily combing solicitations. It could be extended to the study of hair photo-damage and shampoo or conditioner to evaluate there ability to reinforced hair structure.
Keywords: Traction test, Hair cuticle, Interferometry

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Imaging of cellulite: a review

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There is little coherency or consistency within the scientific literature as to the manifestation of so-called cellulite which is neither a skin disorder, disease, nor a tissue dystrophy. It is an inevitable reality of the genetic makeup of the female human species interlinked with steroid hormones and external influences. Cellulite is a condition that needs to be managed since it cannot be cured inside the strict confines of cosmetic definition.
There are a number of clinical methods available to evaluate cellulite, though the limitations of each method alone are such that more than one method is required in order to correlate clinical findings with any laboratory findings. From a scientific viewpoint, a clearer understanding of the influences on adipose tissue metabolism, and connective tissue structure, is required for the development of new test methods in order to advance a more rational approach to understanding cellulite. Furthermore the development of clinical methods, which can correlate in vitro studies, will finally bring logic and understanding to the scientific literature, the condition, and ultimately manage the continued expectations and unmet needs of the consumer.
Without a coherent understanding of the appearance of cellulite, much of the clinical aspects regarding evaluation have relied on subjective methods which do not provide information indicating whether treatments have improved the condition. They only indicate a

change in adipose thickness, since there is no categorical demonstration of changes in the fibrosclerotic tissue which would lead to a physical change in preventing the protrusion and/or development of papillae adipose. Although histopathology studies have provided a clear insight into the physical manifestation of cellulite, a real need remains for useful and reproducible methods in order to visualize cellulite at cellular and structural levels. This is necessary to verify the true effectiveness of so-called anti-cellulite products/ingredients, and to provide coherency to literature findings and current methods.
There are a number of clinical methods available to evaluate the cellulite condition though the limitations of each alone are such that more than one is required in order to correlate clinical findings with any laboratory findings. This review deals with currently available imagining methods and their opportunities and limitations in the understanding and evaluation of cellulite.
Keywords: Cellulite, Imaging, Review

Cross-sectional imaging (confocal microscopy, OCT and MRI)

11(*Invited lecture*)
High resolution cross-sectional imaging of the skin: sonography, optical coherence tomography and magnetic resonance imaging modalities

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This lecture gives a state-of-the-art overview on high resolution sonography, magnetic resonance imaging and optical coherence tomography to study healthy skin, inflammatory skin diseases and skin tumors.
Using ***high resolution sonography*** (50-100 MHz), stratum corneum and living epidermis can be differentiated on palmar and plantar skin and some sites of glabrous skin. Stripping and swelling experiments show that the skin entry echo originates from the water (coupling medium) - stratum corneum border. On the palms and the soles, where the stratum corneum is thick, a second echorich line becomes visible beneath the skin entry echo, corresponding to the stratum corneum - stratum Malpighii interface. The upper and lower echopoor zone between these echorich lines correlate to the str. corneum and Malpighii histologically. The stratum papillare of the dermis, which has a fine fibrillary structure, is also echopoor and is part of the lower echopoor zone. Inflammatory diseases and skin tumors are visible as echopoor region within the dermis. When they extend beyond the dermis, the in-depth demarcation can become difficult. As in 20 MHz sonography, with higher frequencies tissue differentiation is not possible [1].
Magnetic resonance imaging enables us to visualize the detailed architecture of different skin layers, skin appendages, skin tumors and of the inflammatory infiltrate. 10 years ago, we used a 9.6 Tesla MR-spectroscope with an 3D imaging unit. This enabled us to get an excellent resolution, however we had to accept a serious disadvantage: due to the small probe volume we had to do our experiments ex-vivo. Determination of the proton relaxation times T1 and T2 provided a highly significant differentiation of the various tissues (normal skin structures, skin tumors) [2], showing that this method has great potential.In the past years, new dedicated surface coils combined with “normal” MRI-equipment have been developed. The image quality has been greatly improved and new modalities, such as “functional imaging” give us new insights on the skin and its appendages, skin tumors and inflammatory diseases.
Optical Coherence Tomography visualises the upper dermis and epidermis at very high resolution [3]. Changes of the Stratum corneum and of the viable epidermis can be visualized under experimental conditions and in inflammatory dermatoses. This method is particularly useful in follow-up studies when focusing on the up-most skin layers. In the past years, “functional imaging” has also been developed for OCT. Recently very promising studies on UV-radiation, inflammatory diseases and differentiation of very superficial skin tumors have made this new method popular.

[1] El Gammal S et al.: J Invest Dermatol 113 (1999): 821-829 [2] El Gammal S et al.: J Invest Dermatol (1996): 1287-1292 [3] El Gammal S et al.: Akt. Dermatol. 26 (2000): 183-196.

Keywords: *In Vivo*, Non-invasive imaging, Sonography, OCT, MRI, Skin

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New visualization and quantification of the internal skin elasticity by ultrasound imaging

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Background/Purpose: In the world of cosmetic dermatology, skin elasticity has been assessed only in surface layers due to the technical difficulty of measuring multiple heterogeneous layers of skin. Recently, we have developed a new method that uses tissue strain imaging (TSI) technology, which is a widely employed ultrasound diagnostic procedure that can determine the composition and contours of nearly all body tissues. The aim of this study was to test the new method in assessing elasticity in dermis, subcutaneous and fat layers of human cheek skin.
Methods: Using a pressure device with a 14-MHz ultrasound transducer (1.2 mm in diameter), constant and

linear compressions was given to the cheek skin of 35 volunteers (aged: 20-60 years). The elasticity of each layer was measured and then analyzed by the TSI application software incorporated into the Ultrasound system (Aplio™ XV Toshiba medical Systems Corp). A skin tissue-equivalent phantom, which is a block of material with the acoustic velocity (1530m/s) of human skin, was collaboratively developed with OST inc. (Japan). This phantom was placed between skin and the transducer as a reference material of pressure intensity received on the skin.

Results: Skin elasticity was clearly visualized and quantified in each layer of skin. An age-dependent reduction of elasticity was determined in all layers. In comparison among three internal skin layers (dermis, subcutaneous, fat layers), the highest elasticity was determined in the subcutaneous layer followed by the fat layer.

Conclusion: These findings support the validity and sensitivity of this method in assessing the elasticity of multiple layers of skin.

Keywords: Non-invasive imaging, Elastograpy, Elasticity, Diagnosis, Ultrasound, Skin

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B-spline based algorithm for the automatic detection of the dermal-epidermal junction on OCT images

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Purpose: Optical Coherence Tomography (OCT) performs cross-sectional imaging of the internal structures of the skin *in vivo* with high resolution (~ 10 µm). Therefore, it allows to characterize the junction between the dermis and the epidermis: Dermal-Epidermal Junction (DEJ). OCT images present however a poor contrast, so that the detection of the DEJ is not easy, whether manually or automatically. The objective of this work was to apply a new dedicated image processing algorithm to detect automatically the DEJ.

Methods: The method we developed contains two main stages. First of all, having detected the surface of the skin, we smooth the OCT image with 2D B-Splines in order to reduce the optical speckle (considered as noise) while preserving the morphological information contained in the image (set of two smoothing parameters: p and q, one for each dimension). In a second stage, we detect from this smoothed image the border between the dermis and the epidermis. Detected border can present discontinuities that are smoothed by 1D robust B-Splines. This algorithm thus allows to quantify automatically the DEJ morphology on the 2D image: epidermis (average thickness, etc.) or the rete ridge of the DEJ (interdigitation index, mean height, etc.). It was tested on a skin image data base (skin of the face) containing more than 200 subjects (18-70 years) distributed in 5 age groups.

Results: The results obtained with the automatic method are in good agreement with the standard and interactive method based on 1D-profile analysis. The detected border on the image corresponds to what we guess on the images. With age, the results show differences between the different age groups: we observe a decrease of the average thickness of the epidermis (65 µm to 50 µm) and a decrease of the nomalized developed length of the Dermal-Epidermal Junction (1.4 to 1.1). Besides, the extracted measures either concerning the epidermis or the DEJ are coherent with the literature.

Conclusion: B-Spline based algorithm seems a very attractive method to detect automatically the 2-dimensional DEJ morphology on OCT images. It will allow us to use OCT imaging in clinical studies on large cohorts of subjects. We can intend to spread this method (by fitting if needed some parameters) to OCT images from other anatomical zones as the lip (slightly different contrast) or images acquired with the new generation of frequency-domain OCT systems.

Keywords: Non-invasive imaging, Optical Coherence Tomography, Skin, Image processing

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In vivo and *in vitro* 4.7T MR imaging using lenti ef-1α hfth transfection in skin tumor model

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Background: Ferritin, the iron storage protein, was recently promise useful detection of reporter gene expression by the 4.7T magnetic resonance imaging (MRI). Non-invasive molecular images are providing researchers with new opportunities to develop and to study various animal models.

Purpose: Our purpose of this study is that non invasive MR image comparison between Feridex® labeled cell and Human havy chain Ferritin transfected stable cell implanted animal model. And Tumor development post implantation Feridex® labeled or transfected tumor cells.

Methods: We transfected mouse melanoma (CRL-6323™, ATCC, USA), Human Basal Cell Carcinoma (CRL-7762™, ATCC, USA), for MRI image of tumor development, All cells were stably expressing of EF-1α hFTH by clonal selection using puromycin. 30 mice per each skin cancer were implantaed transfected skin cancer via lumbar subcutaneous. Feridex® labeled skin cancer cell were injected thoracic vertebra subcutaneous region to compare the MR images.

Results: We demonstrated that using feridex® labeled cell were high intensity than transfected cell in short term but after time dependent study were dramatically higher expression of hFTH in transfected cell line. Also histological evaluation was done with Prussian blue staining.
Conclusions: The Lenti EF-1α hFTH transfected stable cell line implanted animal model provides tumor development and non invasive molecular imaging of reporter gene expression *in vivo* by MRI. Using Ferritin transfected cell lines give a promise for pre-clinical study.
Keywords: Melanoma, Basal cell carcinoma, Ferritin, Lenti EF-1α hFTH

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Development of multi-modal *in-vivo* confocal microscopy capable of reflectance, fluorescence and raman imaging

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Background: Confocal scanning microscopy is a versatile tool for investigating various specimens in bio-science. Its non-invasive optical sectioning ability is very useful to inspect inner-structure of living biological tissues or cells. Confocal scanning microscopy can be classified by imaging mode into two types of reflectance and fluorescence. Each imaging modes has unique functions and characteristics. Reflectance confocal microscopy shows tissue architecture and cellular morphology without any preprocessing of samples. Fluorescence confocal microscopy visualizes molecular properties of tissue with high-contrast. The integration of these two modes in one system will create many advantages, since the morphology and molecular properties can be showed simultaneously in the same field of view.
Purpose: The objective of our research is a development of multi-modal *in-vivo* confocal microscopy that has the capability of reflectance, fluorescence and Raman imaging.
Materials/Methods: In order to increase penetration depth, our implementation uses a near infrared diode laser of 810nm wavelength as the light source for reflectance mode. The reflectance mode uses the intensity of reflected light by refractive index mismatching in the specimen as the source of contrast. Because this intensity generally very weak, high power diode laser should be used. The light source for fluorescence mode is Ar-ion laser of 488nm wavelength. This green laser is good for various fluorophores. The detectors for both of fluorescence and reflectance mode are PMTs. Due to the high-sensitivity and high-bandwidth of the PMT, this point detector is widely used in various applications which need high speed and contrast. Resonant mirror which has resonant frequency of 4kHz is used for fast scanner. And galvo-mirror is used for slow scanner. With these two scanners, we can obtain two dimensional images at the speed of 8~32 frames/s. In the case of Raman imaging, Ar-ion laser and line CCD is used as the light source and detector. In conclusion, our implementation can obtain fluorescence and reflectance image simultaneously and also can obtain Raman image.
Results: The reflectance and fluorescence images for various tissues biopsied from mouse are obtained to verify the imaging performance of our system. In the images obtained and displayed in real-time, sub-cellular structures can be clearly distinguished.
Conclusions: We developed multi-modal *in-vivo* confocal scanning microscopy capable of reflectance, fluorescence and Raman imaging. The resolution and imaging speed performances are verified by imaging various mouse tissues.
Keywords: *In vivo*, Non-invasive, Confocal laser scanning microscopy, Raman spectroscopy, Reflectance, Fluorescence

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Skin color, blood flow and vascular functions

16(*Invited lecture*)
Image-based control of skin appearance

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We introduce a practical skin color, texture and translucency analysis/synthesis technique for E-cosmetic function in digital images based on physics and physiologically-based image processing. In a general comparison of the use of facial cosmetics, it seems that the application of lipstick and makeup with some color is the general preference in Europe and America. In Asia, however, the application of a cosmetic for skin care, such as whitening essence, and the natural change of skin color is generally preferred. In this work, the "E-cosmetic" function focuses on Asian people. The inverse lighting technique is the key technology for E-cosmetic, because it is necessary to obtain a unique reflectance on the skin by discounting illuminants. E-cosmetic appropriately applies image-processing techniques based on the original skin color.

In this work, the skin color image is analyzed by extracting hemoglobin, melanin and shading components by two steps. In the first step, shading on the face is removed by a simple color vector analysis in the optical density domain as an inverse lighting technique. The image without shading is analyzed by independent component analysis to extracts hemoglobin and melanin components. Experimental results using UV-B irradiation and the application of methyl nicotinate on the arms support the physiological validity of the analysis and the effectiveness of the proposed shading removal.

By controlling the amount of hemoglobin, melanin components, and inversely process the components into the skin color as the synthesis process, we can realistically synthesized the facial color images. We also proposed a technique to synthesize the change of texture in pigment due to aging. The pyramid-based texture analysis/synthesis technique was used for the spatial processing of texture. Using the proposed technique, we could realistically change the skin color and texture of a 50 year-old woman to that of a 20 year-old woman.

A useful tool is also introduced for controlling the skin melanin texture continuously. The melanin texture can be continuously and physiologically controlled based on the analysis of 123 skin textures in our database. The physiological validity for the change of the melanin texture is confirmed by comparing the synthesized image with an ultraviolet image, which can predict the change of melanin texture due to aging. The control processes are implemented on programmable graphics hardware, and real-time processing is achieved for a facial video stream.

Controlling the skin translucency is also one of the important tasks in the reproduction of preferred skin appearance. The extracted shading component is controlled to change the translucency of the skin by simple kernel operations for the component. The physical validity for the change of translucency is confirmed by using the images of optical skin phantoms.

Keywords: Skin color, Skin texture, Skin translucency, Melanin, Hemoglobin, Inverse lighting, Independent component analysis, Pyramid-based texture analysis and synthesis, Physiologically-based rendering, Real-time processing

17
Obtain skin pigment concentration map from single color image

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Purpose: Melanin and hemoglobin play dominant roles in skin appearance. The objective of the study was to automatically extract melanin and hemoglobin distribution within the skin a single digital image.

Methods: Firstly, the relationship between color of digital skin image and skin pigment concentration are mathematically built by analyzing light interaction with skin. A new algorithm is then proposed to automatically obtain pigment concentration without the need of skin image database by finding the most reasonable separation vectors from all candidates. Separation vector is calculated by applying Independent Component Analysis in every sub-region of the whole skin image. The candidate vectors are then chosen according to our proposed set of rules.

Results: Melanin and hemoglobin concentration map is directly shown by images with the same size as the input single color image. Visually, the map is evaluated by observing the different features (like freckles, pimples etc.) appear in different pigment distribution map. Furthermore, the algorithm was validated by comparing pigment concentration maps with melanin and hemoglobin indexes calculated from spectrocolorimetric data on the same regions of interest acquired on about 50 volunteers. The algorithm was also tested on different skin phototypes and gave good results except on very dark skin subjects. Both the visual appearance and scientific experiences strongly prove the proposed algorithm is robust and effective.

Conclusion: A robust algorithm is proposed to automatically decompose skin color into melanin and hemoglobin maps. It is expected that the method will be useful in applications like cosmetic product evaluation, and dermatological follow-up of pigmented or inflammatory lesions.

Keywords: Digital imaging, Skin color, Melanin, Hemoglobin, Image processing

18
Study for matching method based on independent component analysis of skin using stereo images

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Background/Purpose: Solving Matching problems are important in seeking the fundamental matrix that includes epipolar geometric information. We can find 3D information of stereo skin images from the fundamental matrix. Up to now the feature point extraction which uses scale invariant feature transform (SIFT) is the most accurate among the feature point extraction algorithms. However, it is difficult to find corresponding points in stereo skin images because the stereo skin images have very similar patterns. Therefore, we need a more intelligent matching algorithm for the stereo skin images. The researching fields that use independent component analysis (ICA) in neuroinformatics are developing rapidly. The main idea of this paper is to find a more

reliable corresponding point and fundamental matrix by using SIFT and computational neuroscientific method on ICA in stereo skin images.

Methods: Our proposed method consists of eight major stages: (1) estimate the ICA transformation matrix, (2) determine the parameters of the corresponding stabilizing shrinkage function, (3) compute the projections on the sparsifying basis by using the ICA transformation matrix, (4) apply the corresponding shrinkage nonlinearity, (5) transform the estimated stabilized sparse code back by inverse the ICA transformation matrix, (6) extract the feature points by applying SIFT, (7) solve the matching problem with feature points, (8) estimate the fundamental matrix with corresponding points. Computational neuroscientific method and modified sparse code shrinkage (SCS) are used for the first five stages. RANSAC (RANdom SAmple Consensus) algorithm is used to estimate the fundamental matrix in the seventh and eighth stage.

Results: The position of the SIFT feature points is accurate within half a pixel. We use geometric error to evaluate the performance of our method. Experimental results show that geometric error is even smaller than half a pixel.

Conclusions: Our proposed method can extract a more reliable and accurate feature points that satisfy epipolar geometry from stereo skin images. Our future work is to study independent subspace analysis (ISA) and topographic independent component analysis (TICA) instead of ICA for a more intelligent image processing method.

Keywords: Stereo skin images, Matching, Independent Component Analysis(ICA)

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Measurement of skin ashiness

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Background/Purpose: Skin ashiness makes the skin appear dull and unhealthy. It is usually due to skin being dry. Topical applications of skin moisturizers are the routine treatment to restore skin hydration and the healthy appearance. Measurement of skin ashiness is traditionally conducted by clinical visual evaluation. An objective, quantitative and instrumental measurement of skin ashiness should be useful to evaluate the skin condition as well as the immediate and long lasting benefit of skin moisturizers.

Methods: Skin ashiness is the result of increased light scattering at skin surface. Light scattering at skin surface is wavelength dependent with more at blue wavelength and less at red wavelength. This change of skin surface scattering from skin ashiness or the application of skin moisturization can be measured as the change of diffuse reflectance. We demonstrate the use a handheld spectrophotometer to evaluate skin ashiness and the effect of skin hydration.

Results/Conclusions: We find that skin spectral reflectance and scattering coefficient are high for ashy skin and low for moisturized skin. This is consistent with the expected change of skin surface scattering. The experiment results show that skin moisturization decreases surface scattering of light by stratum corneum. The change of skin spectral reflectance from the ashy skin to well moisturized skin is a good measure of skin ashiness.

Keywords: Skin surface scattering, Diffuse reflection, Spectral reflectance, Skin ashiness, Skin hydration

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Use of digital photography and image analysis techniques to quantify erythema in health care workers

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Background/Aims: The goal of this study was to quantify erythema on the hands of health care workers (HCWs) using image analysis of digital photographs (DIA) to evaluate the entire hand surface and to compare erythema measurements derived from photographs graded both live (LSG) and using a Visual Perception System (VPS) that allows simultaneous viewing of before and after images on a high resolution monitor.

Methods: Hands HCWs were evaluated prior to the initial scrub and at the end of multiple 2-3 day work cycles during spring (n = 54) and winter (n = 60) trials. Skin condition was measured with LSG, DIA and VPS.

Results: HCWs had significantly higher values of erythema compared to a non-HCW control group. Knuckle erythema increased over the cycle in both seasons. It decreased between cycles in spring but continued to increase between cycles in winter. For DIA area of excess redness, the quantitative measure of erythema, the decrease over the cycle in spring was significantly different than the increase over the cycle in winter. Minimal changes in area of excess redness occurred during recovery in both seasons. VPS comparisons showed a decrease in erythema during recovery in spring and an increase during recovery in winter, indicating significant differences for spring versus winter ($p < 0.05$). Correlations were observed for (1) results for the VPS versus the LSG method and (2) between excess erythema ($\mu+\sigma$) from digital image analysis and the VPS erythema scores.

Conclusions: Significant work cycle effects for spring versus winter were observed with DIA, while significant effects were found during the recovery period with VPS.

DIA produced an objective quantitative measure of erythema that was not limited or influenced by other aspects of skin irritation (e.g., dryness, scaling) or texture encountered in the visual methods of LSG and VPS. The DIA method minimizes the difficulty in differentiating erythema severity. Standardization of image capture and processing allows assessment of skin condition across clinical locations. The VPS is a more reliable way to compare skin condition at different times, i.e. beginning versus the end of a treatment cycle, because images are viewed simultaneously and can be carefully examined for differences.

Keywords: Irritant dermatitis, Erythema, Skin imaging, Digital image analysis, Visual perception system

Molecular imaging of skin II

21(*Invited lecture*)
Confocal microscopy of skin cancers

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Confocal microscopy is a well-known modality for "optical sectioning" of tissue: thin planes or slices within tissue are noninvasively imaged with high resolution and high contrast without physically cutting the tissue. Real-time confocal scanning laser microscopes have been designed for imaging human skin, oral mucosa and other tissues. Tissues are imaged both *in vivo* and in fresh biopsies or excisions *ex vivo*. Early laboratory prototypes were developed into commercial microscopes. Images are produced in real-time at 10-30 frames/second, with water immersion objective lenses of magnification 30X-100X and numerical apertures 0.7-1.2, near-infrared wavelengths 800-1064 nm and safe illumination power levels of 5-20 milliwatts. Epithelial and connective tissue layers are imaged to depths of 200-500 μm. The lateral resolution is 0.5-1.0 μm and optical section thickness is 2-5 μm. The optical sectioning compares very well to that of standard pathology for which 5 μm-thin slices of tissue are typically prepared. Nuclear and cellular morphology in the superficial epidermis, and collagen and blood flow in the deeper dermis is imaged in real-time *in vivo*. Correlation of confocal images *in vivo* to the corresponding pathology is excellent. Recent clinical studies have shown that melanocytic lesions including melanomas are detected with sensitivity of 95-100% and specificity of 70-90%. Basal cell carcinomas are detected with 97% specificity and 92% sensitivity. Confocal imaging-guided surgery of lentigo maligna melanomas has proven successful on patients, with the post-surgical cancer-free margins correlating well with the post-surgical pathology. Current instrumentation research is focused on investigating one-dimensional line-scanning (instead of the standard two-dimensional point-scanning) toward creating simpler, smaller and less expensive confocal microscopes. Confocal mosaicing microscopy is another instrument that is being developed for rapid detection of non-melanoma skin cancers in large excisions from Mohs surgery. Using acridine orange to stain nuclei in fluorescence, basal cell carcinomas are detectable in confocal mosaics with excellent correlation to pathology. Creating a mosaic requires only 5-9 minutes, compared to 20-45 minutes for preparation of frozen pathology. Mosaicing may enable rapid pathology-at-the-bedside in large areas of excised tissue to potentially guide surgery. Confocal microscopy is a promising imaging technology for noninvasive real-time clinical screening and diagnosis and surgical guidance applications in dermatology.

Keyword: Confocal microscopy, Mosaicing, Human skin, Skin cancer, Melanoma, Mohs surgery

22
In vivo three-dimensional skin imaging for early cancer detection with a handheld dual-axes confocal fluorescence microscope

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More than 1 million new cases of skin cancer are diagnosed each year in the US resulting in more than 10,000 U.S. deaths annually - the majority of which is caused by melanoma. Invasive biopsies, in which suspicious skin lesions are removed and examined, are currently the primary means of skin cancer diagnosis. Unfortunately, many clinically suspicious but histologically benign lesions are removed as well.

A noninvasive technique that minimizes this error will significantly improve clinical diagnosis, reduce unnecessary biopsies, and potentially capture melanomas in the most curable stage as well as non-melanoma skin cancers which will reduce morbidity. It may also be used for margin control during skin cancer surgery.

We demonstrate a Dual-Axes Confocal (DAC) fluorescence microscope capable of three-dimensional (3-D) *in vivo* real-time imaging in a handheld package (10-mm diameter), weighing less than 12 ounces, that can fundamentally change the way clinical dermatological diagnosis has been performed. Genecreme® (TransDerm, Inc.) containing fluorescent infrared dye can penetrate the stratum corneum layer of skin and in combination with DAC microscopy technology to reveal the intricate structures of epidermal and dermal skin layers. The key technologies enabling miniaturization of the microscope are Microelectromechanical-Systems (MEMS) and miniature-optics.

The imaging demonstrations with sub-cellular resolution from the handheld microscope are demonstrated on both *in vivo/ex vivo* human and mice skin. The microscope achieves a maximum imaging frame rate of 15 frames/second. The field of view (FOV) as large as 750 × 300 μm^2 is demonstrated. The microscope achieves

full-width-half-maximum (FWHM) transverse and axial resolutions of 4 μm and 6 μm, respectively. Currently, the maximum imaging depth of the microscope can go as deep as 200 μm in mouse skin. A larger FOV (> 2 mm^2) of skin can be acquired by real-time "mosaicing" software integrated with our imaging acquisition system. We have demonstrated a handheld DAC microscope (785-nm-wavelength) for real-time 3-D *in vivo* skin imaging. The microscope and the acquisition systems are mounted on a clinical cart to enable a broad set of skin imaging applications.

Keywords: *In vivo*, Non-invasive imaging, Confocal microscopy, Skin mosaicing, Dual-axes confocal

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In vivo molecular imaging of skin cancer with thymidine kinase-transfected cell in mice using nuclear medicine imaging system

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Background: The most diseases bring on a cellular activity changes before anatomical changes. A good way to find cellular activity is by Nuclear Medicine Imaging System (NMIS). Depending on physical characteristics of the substance used, a short-wave X-ray radiation (gamma radiation) or a positron radiation is emitted. These substances are basis of NMIS. Gamma camera and Positron Emission Tomography (PET) is NMIS. NMIS is capacity for biochemical and functional activities of diseases. The cellular activity means biochemical and functional activities. An advantage of detecting tumor in NMIS is that longitudinal study from single animal. This study has sought to proof the suitable for monitoring transplanted cell in living mice using Nuclear medicine molecular imaging system.

Purpose: In this study, the skin cancer with Thymidine Kinase (TK) transfected Cell made molecular images using Nuclear Medicine Imaging System.

Methods: All experiments were performed using C57BL/6 and BALB/c nude inbred mice in accordance to Korea university medical center IRB standards and procedure protocols. Induced skin cancer by Melanoma and Basal Cell Carcinoma cell lines. Each cell line was transfected with Thymidine Kinase. The C57BL/6 Mice and BALB/C nude mice were used for skin tumor models. These studies were performed using NMIS. Each skin cancer mouse model was injected intravenously with [^{18}F]FIAU before NMIS scanning. For a static [^{18}F]FIAU scan one should wait sufficient uptake time after the injection to scan the mouse.

Results: Nuclear medicine imaging allows the biodistribution, magnitude and time variation of Thymidine Kinase gene expression to be analyzed within the whole body of a living small animal which is also quicker and may reduce the number of animals required for a study. In this study, we were able to obtain molecular images of transfected cell with Thymidine Kinase in living small mice using NMIS.

Conlclusions: Molecular images of transfected cell with Thymidine Kinase were obtained noninvasively. With further development, *in vivo* molecular imaging studies should change the transfection vector.

Keywords: PET, Gamma camera, NMIS, Thymidine kinase, Molecular imaging, Skin disease

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Cosmetics applications of confocal raman microscopy

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Background: One of the major fields of investigation of L'ORÉAL Research is to improve the understanding of the chemical composition and structure of skin and hair. To enable a better design of cosmetic products, a thorough understanding of the mechanism and the nature of the interactions of cosmetic ingredients with these substrates is necessary.

Purpose/Method: Raman is a key analytical technique in cosmetics and dermatology, as it enables a non destructive and non invasive molecular characterization of the different substrates (skin, hair, nail) before and after treatment with cosmetic products. In the confocal mode, Raman spectroscopy has the additional advantage of providing 3D information as it enables in depth measurements. The 3D information is crucial to improve our understanding of the skin and hair by in situ analysis of the chemical composition of water, lipids, proteins, sulphur, and amino-acids. The fantastic advantage of this technique is the possibility to carry out experiments under *in vitro* as well as *in vivo* conditions.

Results: L'ORÉAL Research has demonstrated the relevance of this instrument for the *in vivo* investigation of skin. The Raman probe provides the high spatial resolution required to study the different layers of the skin and to detect variations in the structure of skin between different anatomical sites and volunteers. The confocal Raman probe also enables the determination of *in vivo* water content from the surface to several microns below the skin surface to assess its barrier properties and water-holding capacity. The monitoring of applied cosmetic products, such as moisturizing cream, is also possible allowing a better understanding of their interaction with skin components and the determination of their penetration in the skin layers as related to depth

and time.
Conclusions: The aim of this presentation is to present an iconography, based upon confocal Raman microspectroscopy, of various fields of investigation involved cosmetic research and routinely used by us to access cosmetic treatments.
Keywords: Spectroscopy raman, Confocal, Skin, Hair, Cosmetic

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Confocal raman studies of stratum corneum biochemistry as a function of age

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Introduction: Changes in the skin due to aging are related to both chronological aging and environmental factors. Raman spectroscopy of skin allows for a more complete assessment of aging on skin biochemistry.
Methods: The study consisted of 58 subjects of differing race and skin type. The subject pool contained 11 children (<3 years), 31 adults (25-35 years), and 16 elderly adults (>65 years). Following a 15 minute equilibration in an environmentally controlled, transepidermal water loss, skin capacitance, and water and fingerprint profiles using a Raman spectrometer were taken.
Three anatomical test sites were evaluated on adult and elderly subjects (volar forearm, cheek, and buttock) and 2 test sites were evaluated on children (volar forearm and buttock). Measurements were repeated at a minimum of 7 different locations for both water and fingerprint profiles within each site.
Results: Children's arms tended to have higher water content and significantly lower total natural moisturizing factor as compared to adult or elderly skin. There was evidence that adults had higher water levels in buttock skin than elderly. It also appeared that the water content of the elderly cheek was higher than adults. The water to depth relationship was parallel for all age groups, suggesting no difference in average skin thickness.
There were few statistically significant differences for ceramide and cholesterol between locations and age groups. The exception was the higher ceramide level near the surface of the cheek skin. Additionally, there was evidence to suggest adults have higher cholesterol levels in their cheeks than elderly participants.
Conculsions: Raman spectroscopy allowed for assessment of stratum corneum characteristics associated with aging. Skin aging appears to alter stratum corneum biochemistry. The most pronounced change with age appears to be associated with stratum corneum urea levels.
Keywords: Raman spectroscopy, Stratum corneum, Skin aging, Urea

Claims support in cosmetology and pharmacology

26
The innovator's dilemma: biophysics and imaging of the skin. The CRO perspective

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Background: The life in skin biophysics and skin imaging research has been greatly enriched in the last decade by the continuous improvement of existing techniques like greater resolution of digital imaging devices and even more by new non-disruptive techniques as for instance MRI, Confocal imaging and Raman spectroscopy. While in the early days of our society the founding members knew the operating principles of all available instruments and many were able to operate them, this has dramatically changed by the year 2008.
Purpose: To examine the challenges and dilemma of driving innovation in skin imaging within the CRO setting.
Methods: The investigator in a CRO environment faces a number of additional challenges as compared with their colleagues in basic research either in industry or academia. Providing high level research at a competitive cost, training of staff, GCP-compliance, proof of reliability and validity of methods and last but not least validation of hard- and software according to pertinent standards or guidelines, e.g. GAMP 4 or FDA 21 CFR part 11. Examples of those challenges especially in the area of skin imaging and image analysis will be presented along with strategies how to address them.
Results: The choice of skin measuring devices has had a great influence on contract research organizations (CRO's) conducting studies in the skin field. No single institution can own or operate all instruments. Cost is one reason, in depth knowledge and intensive training requirements are indeed the even more limiting reasons. Choices have to be made.
Conclusions: The modern advent of skin imaging provides the researcher and clinician alike with powerful new tools to record and measure many skin aspects. Access to state of the art technology in the clinical setting is no guarantee of quality, and hence validity, reproducibility and standardization are essential prerequisites to successful GCP-compliant studies.
Keywords: *In vivo*, Non-invasive imaging, Confocal laser scanning microscopy, Image analysis claims support studies

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Non invasive strategies for claim substantiation of various antiaging skin care products

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Background: Photoaging refers to chronic changes in skin due to repeated sun exposure. Free radicals are implicated and cause the skin to age rapidly with appearance of wrinkles, fine lines and loss of firmness, radiance, elasticity & skin tone. There is growing concern about aging in areas of the face and around the eyes. More women are demanding products to prevent aging but are unwilling to pursue aggressive, invasive and expensive measures like lifts for face, eyes and lips. Antioxidants are being used to combat harmful effects of UV radiation and play an important role in antiaging skin care. As more antiaging products are entering the market place, today's techno savvy consumer expects and demands proof of what exactly to expect from a product before accepting brand reliability. Thus, claim substantiation trials assume prime importance. These trials help collect information from multiple disciplines like sensory evaluations by experts, consumer evaluation / self assessment questionnaires, clinical assessments and instrument evaluations.
Purpose: Our primary objective was to capture characteristics of photoaging and provide non invasive testing strategies for claim substantiation of antiaging facial regimens by checking for improvement and consumer acceptance of skin care products.
Methods: Two trials, one involving the whole face and the other involving the area around the eyes will be presented. Both trials involved enrollment of healthy female subjects (35-60 years) who gave signed informed consent. Subjects for both trials were screened for extent of photodamage and those with mild to moderate overall photodamage meeting study criteria were enrolled in clinical trials ranging from 4-8 weeks. Subjects were to use the antioxidant rich antiaging regimens twice daily as per instructions provided for the entire duration of the studies. Measurements included clinical visual assessments by a blinded expert evaluator, subjective assessments, silicon replicas and digital photographs at baseline, midpoint and at the end of the clinical trials.
Results: Our results allow us to clinically demonstrate and capture photoaging relevant information. Further, they provide strategies to better appreciate differences in skin aging parameters for claim substantiation of product efficacy safety and consumer acceptance.
Conclusions: Testing strategies tend to establish not only product efficacy but also provide claim substantiation and information regarding consumer acceptance. In addition, this can be useful for assessing comparative performances from competitors.
Keywords: Photoaging, Antioxidants, Claim substantiation, Non-invasive strategies

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Set up of a sensoskin™ reference frame for describing the evolution of the cutaneous relief

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Starting from the realisation of accurate double replicas (negative/positive) of the cutaneous relief realised on subjects from a few days to 80 years old, a reference frame called SENSOSKIN™, designed to describe the evolution of the cutaneous topography, has been set up.
The SENSOSKIN™ reference frame includes plates, obtained by techniques of injection of elastomeric materials, and is built from the planar adjacent combination of the realised replicas.
These plates, the hardness of which is approximately between 20 and 30 Shore A, can be provided in different colours characterising the most common typologies.
Thus, we obtain a series of samples which form groups according to relevant one-dimensional sensorial descriptors (braking, slippery, roughness) corresponding to a gradual evolution of sensations.
Three-dimensional images, obtained by Scanning Mechanical Microscopy, show more precisely the most characteristic morphological differences.
Keywords: Reference frame, Cutaneous relief, Sensorial descriptor, Scanning mechanical microscopy

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Sensory perception pattern is altered in sensitive skin

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Background: Sensitive skin is characterized by subjective symptoms that are hard to quantify. Most symptoms were originated from individual experiences regarding sensory recognition toward irritation response.
Purpose: We aimed to see whether a neurobiological approach could improve our understanding of the nature of skin sensitivity.
Methods: In this study, we measured the sensory perception of well-controlled electric currents on the skin that stimulated sensory nerve fibers such as the myelinated A fiber, A-delta fiber and unmyelinated c-fiber. The sensory perception thresholds were obtained quantitatively from subjects with sensitive-prone skin and controls. Application of 0.075% capsaicin, known to stimulate the nociceptor c-fiber, was topically given, then the sensory perception thresholds were measured to determine whether the prior exposure of nociceptive stimulation can affect the subsequent sensory perception.
Result: The results showed that the perception thresholds of skin sensitive-prone subjects were low for the c-fiber measurements at 5Hz electric current stimulation. Furthermore, a wide variation in sensory perception was noted in sensitive-prone skin following topical application of capsaicin.

Conclusion: In conclusion, the abnormal sensory perception in sensitive skin appears to be related to neurological instability, where c-fiber nociception plays a role. Thus, quantitative sensory perception threshold measurement could be a useful method to discriminate the skin sensitive-prone subjects.
Keywords: Sensitive skin, Sensory perception, Non-invasive measurement, Electric current, C-fiber nerve

Mechanical properties (friction and elasticity)

30(Invited lecture)
Biomechanics and tribology of the skin from nanoscale to macroscale

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In objective to develop a non invasive metrology to characterise the mechanical and tribological properties of skin *in vivo,* a specific indentation/friction device has been designed. The bio-tribological equipment is an original system based on the measurement of static and sliding contact forces, friction noise and acoustic emission.
The indentation test consists in monitoring the penetration of the indenter in the surface while displacement δ and the normal force FN are continuously measured with a high sensitivity. The results obtained by this approach show identical values of the Young modulus E* and the shear modulus G* of six dermal equivalents obtained from a 62 years old subject (E* = 8.5 ± 1.74 KPa and G* = 3.3 ± 0.46 KPa) and *in vivo* total skin of 20 subjects aged from 55 to 70 years. (E* = 8.3 ± 2.1 KPa, G* = 2.8 ± 0.8 Kpa). The comparison between the Young modulus of the skin *in vivo* and the dermal equivalents (DEs), shows a high similarity, what lets think that the characterization of the skin *in vivo* by compressive test in the range of normal load (0.5 to 2g) and contact pressure (0.5 to 2 KPa), is more sensitive to the answer of the collagen and elastin fibers of the demis, which assure the skin elasticity.
When the probe is extremely close to the surface, attractive forces between skin surface film and the indentor appear. This interaction is detected by the presence of negative forces. During the unloading procedure, the adhesive forces and adhesive energy are measured as a separation resistance between the probe and the surface film. The attractive forces were measured in different situations: normal skin: the attractive force F_a = 0.2 mN at a distance of 60 µm, the effect of hydration with water, five minutes after: F_a = 0.35 mN at a distance of 210 µm, ten minutes after: F_a = 0.28 mN at a distance of 80 µm. The effect of artificial sebum application: F_a = 1.2 mN at a distance of 320 µm. The adhesive forces were measured at the end on the unloading procedure: normal skin F_a = 0.9 mN and the adhesive energy W_{ad} = 30 mJ/m² which is in accordance with the literature concerning wettability. The effect of artificial sebum gives an adhesive force of 3.88 mN.
When the probe is moved along the surface over a certain length with sliding velocity in the range of 100 µm/s to 1000 µm/s, the friction force F_T of a surface can be characterized in two distinct stages: in the static phase the lateral force increases linearly to reach a maximum which corresponds to the static limit friction force. The slope of the evolution of static friction froce allows to quantify the lateral stiffness K_T (N/m) and the shear modulus G*. In the sliding stage, the simultaneous measurement of the normal and friction force F_N, F_T , can be used to determine the dynamic friction coefficients, μ_d.
A discontinuous phenomenon called stick-slip, due to compression and distortion of the skin, occur during the permanent sliding and generate friction noise. These characteristics of friction dissipation are therefore the key phenomenon for the feeling of smoothness. To assess this signature of friction, a specific tribo-acoustical probe has been developed. This is the sort of artificial finger whose load is controlled and equipped with a microphone to measure the sound pressure level. When rubbing the probe on various state of skin, it is possible to compare the acoustical level and therefore to assess the relative smoothness of the surfaces. The stratum corneum (SC), or cornea layer, is the micro-scale of skin surface, which is permanently requested during a friction test, and constitute the sources of bet in vibration of the probe.
The effect of the density of the keranocytes was highlighted by the technique of wrenching by an adhesive (tape stripping). Acoustic acquisitions were made before and after five and ten tape-stripping. (extraction of the stratum corneum layers).The study of the two areas, showed a significant decrease of the average sound level (ASL) after the first five tape-stripping (-4 dBA) and a weaker one after the five following tape-stripping (-5 dBA, cumulated value), with the change of frequencies bands. This closed evolution on the two sites, which differed by their dryness and surface roughness suggested that the main acoustic information was provided by the upper keratinized layer of the skin, i.e. the stiffness and microscopic roughness of the stratum corneum. However, higher ASL with specific frequencies was observed on the calf than on the forearm in each condition studied.
The results show that the acoustic emission is correlated to the stiffness and roughness of the stratum coreneum. The application to the analysis of ageing, shows clearly the increase of acoustic emission during ageing of human skin.

31
In-vivo exploration of the mechanical properties of human dermis with 2D high resolution elastography at 50 MHz

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Background: Skin mechanical behavior under stress can be finely explored *in vivo* using 2D high frequency elastography. We described and validated in this context in previous works [1][2] an elastographic imaging system based on the combination of two devices: an extensiometer that submitted skin to an uni-axial stress-cycle and a high resolution echographic system at 20 MHz.

Purpose: The purpose was to observe *in vivo* mechanical behavior of the skin (i.e. the dermis) under stress at a higher resolution by using a 50 MHz ultrasound device. This device allowed describing strain and displacements under biological tissue at a few hundreds of µm-scale.

Methods: The echographic system at 50 MHz had an axial resolution of 60 µm and a large field probe giving images of 16 mm width and 3.2 mm depth.

The stretching stress was applied by means of a new extensiometer. This device was used *in-vivo* to assess the mechanical behavior of the skin under uniaxial stretching. This system was multi skin area and was used to perform axial strain and lateral displacement kinetics during a stress-cycle of 20 seconds: stretching, holding and releasing. The uniaxial stretching values were between 20 and 30 %. Each kinetic was associated to a homogeneous region of interest set in the dermis corresponding to an explored area of about 1 mm².

Results: Results *in vivo* at 20 and 50 MHz were compared. We confirmed two mechanical behaviors of the dermis on the forearm : a thinning down of the dermis obtained for low stress (< 0.3 N) values and corresponding to a negative strain, and a thickening of the dermis obtained for higher stress values (>0.6 N) and corresponding to positive strain.

Conclusion: High resolution elastograms performed between 20 and 50 MHz allowed to describe the inhomogeneous mechanical behavior of dermis according to depth and to differentiate this behavior for several skin areas.

[1] S.Gahagnon, et al., « *In-vivo* exploration of the mechanical properties of healthy and pathological human dermis with 2D high resolution elastography», IEEE Ultr. Symp., Vancouver, 2006.

[2] Mofid Y, et al., « High frequency elastography for *in-vivo* study of the mechanical behaviour of skin», IEEE Trans. on Ultr., Ferr. and Freq. Contr., Vol. 53, N° 5, 925-936, 2006..

Keywords: Skin, Ultrasound, *In vivo*, Elastography, High resolution

32
Measurement of mechanical skin parameter by an air-flow device

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Background: Mechanical properties of the skin have been studied noninvasively by a number of methods, using uneasily stretching, torsion, suction, ballistometric techniques, indentation and shearwave propagation. Each one of the mentioned methods can be used to extracted mechanical parameters of skin but the information and correlation among the results can differ depending of the tested side and the calculated parameters.

Purpose: In this paper we developed an air-flow device that extracts mechanical parameters of skin without any physical contact with the skin.

Methods: A laser displacement sensor with accuracy of ± 5 microns and a computer controlled Digital Flow Meter are used to create skin deformation and measure its displacement. Different air profiles were applied on skin to extract mechanical parameters.

Results: Comparative measurement with Cutometer® from different parts of the body and different skin treatments are presented in this paper showing significant changes in skin parameters before and after treatment.

Conclusions: New mechanical parameters as maximum displacement and yield pressure are defined to characterize skin firmness.

Keywords: Air-flow, Skin, Mechanical properties

33
In vivo viscoelasticity measurement of skin using dynamic indentation

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Background: Human skin is one of the most important organ of the human body. Measurement of its mechanical properties can provide very important informations, for example for clinical or cosmetic research. Thus, it is essential to have objective and quantitative measurements to compare studies made by different experimenters in different centers. Dynamic indentation method is often used on polymer materials and has been specially developed for skin application to provide informations on its viscoelastic properties. This study shows the ability of this method to measure the skin stiffness and damping in a wide frequency range.

Methods: A complete device has been developed. A cylindrical indenter with a radius of 2 mm is used. As the mechanical properties are mainly due to the superficial layers (the epidermis and the dermis), it is very important to stress only this part. The displacement amplitude varies from 1 to 10 µm and the indenter penetration is about 200µm. The stress frequency ranges from 10 to 60 Hz. In order to access any area of the body, a complete

mobile adjustable frame is used. Tests on inert materials have been done to validate the device. A study on 46 subjects aged from 18 to 70 years divided in 3 groups has been performed. Measurements included dynamic indentation, hydration measurement and topographic analysis (plates area measurement).

Results: Results show that the mechanical behaviour of the skin can be described by a Kelvin Voight model under dynamic indentation. As a whole, the stiffness found decreases with ageing. A good correlation between the plates area of skin topography with the stiffness and damping has been found. These plates area correspond to the tension state of the collagen and elastin network. It shows that dynamic indentation measurements correspond mainly to the natural tension state of the skin on the body. The complex modulus measured by dynamic indentation at 10 Hz frequency stress ranges from 7.2±2.1 kPa for the oldest group to 10.7±2.6 kPa for the youngest group.

Conclusion: This device gives convincing results. Advantages of this method are the small contact area, the absence of adhesive and the possibility to make several measurements without changing the skin properties. The stiffness and damping obtained are important to study ageing and cosmetic effects.

Keywords: *In vivo*, Viscoelasticity, Dynamic indentation

34
Skin friction and elasticity in young and aged persons

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Background/Purpose: The mechanical properties of human skin are known to change with ageing, rendering skin more vulnerable as well as less resistant to friction and shear forces, which are considered major clinical risk factors for wounds or decubitus in bedridden persons. Until now, there are only few and contradictory results on the age-dependent frictional properties of skin. This study investigated in detail the influence of age and age-related skin changes on the friction of human skin against textiles.

Methods: *In vivo* skin friction measurements on a force plate were combined with skin analyses concerning elasticity, hydration, pH value and sebum content. 32 young (29.2±9.2 years) and 28 aged, hospitalised persons (81.8±6.3 years) rubbed their volar forearm in a reciprocating motion against various textiles (cotton-polyester, PTFE, viscose) on the force plate, using defined normal loads and sliding velocities representing clinically relevant contact conditions.

Results: Mean friction coefficients ranged from 0.30±0.04 (PTFE) to 0.43±0.04 (cotton-polyester). Interestingly, no significant differences in the friction properties of skin were found between both age-groups despite skin elasticity was significantly lower in the aged persons. Contrary to expectations, skin hydration was significantly higher in the institutionalised patients, whereas no significant differences were observed in skin pH and sebum content. Within each age-group, men and women showed consistent results in all parameters studied.

Conclusion: The friction of volar forearm skin against textiles was found to be independent of gender and age, although elastic and surface properties considerably change with ageing. In the elderly, the reduction in skin elasticity (resilience) and skin turgor leads to more pronounced skin wrinkling and skin tissue displacements during friction contacts. In contrast, young skin shows a smoother surface and only slight deformations. According to the literature, adhesion is assumed to be the dominant factor for skin friction, but our observations imply that deformation is an important factor in the friction of aged skin. Age-independent skin friction indicates that contributions from adhesion and deformation add up to relatively constant values. Clinically, low friction medical textiles could improve the quality of life in the growing number of high-maintenance persons.

Keywords: Skin, Friction, Elasticity, Hydration, Age, Textiles

Skin function (barrier and hydration)

35
In-vivo stratum corneum thickness and water diffusion coefficient measurements using opto-thermal radiometry and condenser-chamber tewl method

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Background: Stratum corneum (SC) thickness and its water diffusion coefficient are two key parameters that would affect the trans-dermal drug delivery. In this paper, we report the development of a new method for measuring these two parameters of *in-vivo* stratum corneum by using opto-thermal transient emission radiometry (OTTER) and condenser-chamber TEWL (trans-epidermal water loss) method.

Purpose: The purpose in this study is to develop a new method for SC thickness and water diffusion coefficient measurements.

Methods: OTTER is an infrared remote sensing technology that can be used to measure SC surface water concentration and water concentration gradient, from which we can calculate the SC thickness. The newly improved mobile measurement head makes it easy to access any skin sites of the body.

Results: Fig. 1 shows the *In-vivo* SC thickness results of nine different skin sites, namely finger back, finger front, palm, hand, volar forearm low, volar forearm high, neck, cheek, and forehead. Condenser-chamber TEWL method is a new closed chamber technology that can be used for accurate TEWL measurements. Combination of the OTTER measurements and TEWL measurements[3], allow us for the first time to study the *in-vivo* SC water diffusion coefficients and its dependency on water concentration. Fig. 2 shows how SC water diffusion coefficients changes against SC hydration level.

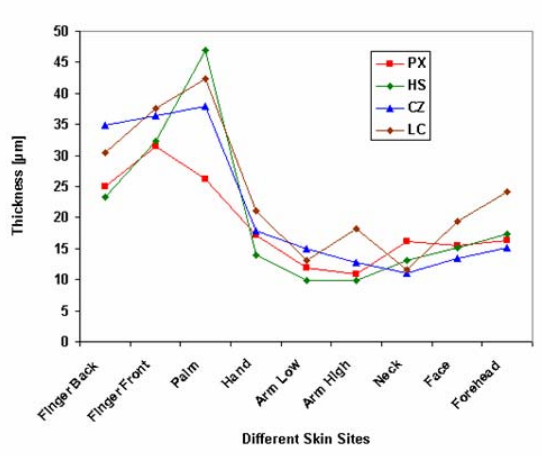

Fig. 1 SC thickness of nine different skin sites from four different volunteers.

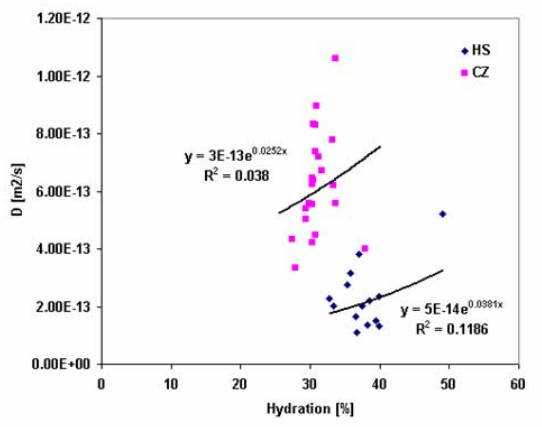

Fig. 2 SC water diffusion coefficient at different water concentration level during the recovery of immersive hydration.

Conclusions: Our study shows that combining opto-thermal measurements and condenser-chamber TEWL measurements, can provide a new information, and can be used to study the thickness and water diffusion coefficients of *in-vivo* SC of different skin sites, of different volunteers, and at different conditions.

Keywords: *In Vivo*, Skin, SC thickness, Diffusion coefficient, Opto-thermal, Condensor-TEWL method

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Functional characteristics of the skin surface of children approaching puberty: age and seasonal influences

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Background: There is amazingly few information about the skin surface properties of children nearing puberty despite the great differences in them between children and adults.
Purpose: To fill the lack of the information about the skin surface properties of children nearing puberty, we conducted bioengineering evaluation of the skin surface stratum of the cheeks and flexor forearms in comparison with their mothers.
Method: We conducted various biophysical measurements of the skin surface of the cheek and forearms of 32 healthy Japanese children aged from 10 to 14 years and their mothers (average age 40years) in summer and in the following winter.
Results: Corresponding to their clinical findings of xerotic changes observed in the children in winter, they showed markedly lower values of skin surface hydration than their mother. SC barrier function evaluated in terms of transepidermal water loss was also poorer on the forearm in the children regardless of the season. In contrast, the SC barrier of the cheek, which was better in the children, tended to become poorer when they reached puberty. The corneocyte size was significantly smaller in the children. Skin surface lipid amount measurable on the cheek remained low until age 13, but at age 14 it increased remarkably, approaching the adult level.
Conclusion: The obtained findings indicate that most of the functional characteristics of the skin in children remain distinct from those of adults until puberty.
Keywords: Barrier, Child, Hydration, Puberty, Sebum, Stratum corneum

37
Kinetics of barrier reformation in stratum corneum following thermal perturbation: IR studies of lipid structural reorganization

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Background: Stratum corneum, the outermost layer of the epidermis, constitutes the primary barrier to permeability in skin. As such, it has been the target of many approaches for dermal and transdermal drug delivery based on methods involving transient modifications of the barrier, particularly focusing on the extracellular lamellar lipids of the SC.

Purpose: The purpose of this study was to develop an infrared (IR) spectroscopic method to monitor the kinetics of barrier restoration following an external perturbation. In the current case, temperature perturbation was selected as a convenient means to induce structural changes in the barrier.
Method: The IR spectroscopic method is based on the observation that the ordered lipid phases of the barrier in isolated human stratum corneum exist, in part, in orthorhombically packed lipid lamellar subcells. Such phases display a characteristic splitting of the CH_2 rocking vibrations with component frequencies at 720 and 729 cm^{-1}. The latter is reliably diagnostic for orthorhombic phases and is markedly reduced in intensity following a thermal perturbation to 55° C.
Results: The kinetics of barrier recovery following quenching from 55 °C (where all orthorhombic organization is gone) to either 25 °C or 30 °C were monitored by tracking the restoration of the 729 cm^{-1} band intensity. In the initial stages of structural reorganization, the kinetic processes were dominated by exponential growth, followed by linear increases at longer times. The half lives for exponential growth regimes were 52.4 hours for the 25 °C quench and 13.8 hours for the 30 °C quench.
Conclusion: These values obtained for restoration of lipid organization are in reasonable accord with those in the literature that were determined with more phenomenological approaches, typically based on restoration of some barrier function. This novel IR spectroscopic method for monitoring structural reorganization kinetics in intact stratum corneum can readily be extended to evaluate barrier recovery following a variety of barrier perturbation approaches used to enhance drug delivery.
Keywords: Stratum corneum, Lipids, Barrier, Permeation, FTIR spectroscopy

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A comparative study on the efficacy and safety of 308-nm excimer laser and targeted UVA/UVB phototherapy (dualight®) in the treatment of vitiligo

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Background: Despite the high medical cost, 308-nm excimer laser became a useful modality in the treatment of limited number of vitiligo lesions especially when they are located in hard-to-reach sites. Targeted phototherapy system delivering high-intensity UVA/UVB light has been introduced to attain comparable efficacy at reduced cost with excimer laser.
Objective: We compared the efficacy and safety of 308-nm excimer laser and targeted UVA/UVB phototherapy system (Dualight®) in the treatment of localized vitiligo.
Methods: Twice weekly treatment with 308-nm excimer laser or targeted UVA/UVB phototherapy system was given to selected vitiligo lesions for a maximum of 10 weeks. Efficacy of treatment was evaluated 8 weeks after initiation with global assessment or mechanical analysis where possible using computer-assisted image analysis of area of repigmentation or using reflectance spectrometer (DermaSpectrometer®) for the level of repigmentation.
Results: 14 patients were treated with 308-nm excimer laser, 13 patients with UVB of targeted UVA/UVB phototherapy system, and 12 patients with PUVA using UVA of targeted UVA/UVB phototherapy system. After 8 weeks of treatment, the number of patients showing treatment response was 13(92.9%), 12(92.3%), 11(91.7%) and that of treatment success was 5(35.7%), 2(15.4%), 0(0.0%) respectively. Side effects were mild and transient in all treatment groups.
Conclusion: Both 308-nm excimer laser and targeted broadband UVA/UVB phototherapy system seemed to offer comparable effects and safety for vitiligo patients with limited number of lesions.
Keywords: 308-nm excimer laser, Targeted phototherapy, Vitiligo

Poster session
Molecular imaging of skin

P39
In vivo optical molecular imaging for skin disease using QDs

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Background: Molecular imaging is guide a very important role in the biomedical sciences. Optical imaging is a rising star in the field of molecular imaging. Molecular optical imaging using fluorescent semiconductor nano crystals probes, also known as quantum dots or QDs, is rapidly being implemented not only in many clinical fields but also in various fields from tracking gene expression, protein-protein interaction or migrating cells to molecular diagnosis and treatment.
Purpose: The purpose of this study was observation to process skin cancer therapy and wound healing using QDs. We then analyze multi-spectral imaging using engineering methods.
Methods: All experiments were performed using C57BL/6 and BALB/c nude inbred mice in accordance to Korea university medical center IRB standards and procedure protocols. We induce C57BL/6J mice and

BALB/c nude mice skin diseases such as melanoma cancer and wound. Using both subcutaneous injection of QDs-tagged cells, such as melanoma cancer cells and mixed cells of fibroblast and keratinocyte, and systemic injection of multifunctional QD probes. We have achieved multi-spectral imaging of melanoma cancer and wound animal model under *in vivo* conditions.

Results: *In vivo* studies of melanoma cancer decrease in mice indicate that the QD probes accumulate at cancer both by the enhanced permeability and retention of tumor sites and by antibody binding to cancer-specific cell surface biomarkers. And we were able to see wound healing processes as same methods.

Conclusions: And the detection of QDs-tagged cells, such as melanoma cancer cells and mixed cells of fibroblast and keratinocyte, are possible to separate accurately more than the past *in vivo* optical molecular image. The new generations of QDs have far reaching potential for the study of intracellular processes at the single-molecule level, high-resolution cellular imaging *in vivo* observation of cell tracking, tumor targeting, and diagnosis.

Keywords: Molecular imaging, Q-dots, Melanoma, Fibroblast

Acknowledgements

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P40

Observation of melanoma and fibroblast cell in phase contrast x-ray image using gold nano particle label technology

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Background: Molecular imaging, such as Optical fluorescent imaging, Optical luminescence imaging and MR imaging, PET imaging, are extensively used for basic research. Also, molecular imaging by using nano particles label technique has been extensively interested to biomedical field. Recently, scattering-based phase contrast x-ray imaging technique is emerging as a new method for biomedical imaging.

Purpose: The purpose of this study was observation to morphological feature gold nano paticles labeled melanoma and fibroblast cell using phase contrast hard x-ray technique method.

Methods: We were preparation cells for attachment and culture on the silicon nitride membrane. To attach the gold nano particles selectively onto biomarker antibodies in cells, anti-tubulin was conjugated to gold nano particles surface. We then used to phase contrast hard x-ray microscope, 1B2 beam line, with sub-micrometer spatial resolution a Pohang Accelerator Laboratory (PAL).

Results: Ability to image molecular biomarkers associated with cancer or other cells is limited. Here, we observe a new method of molecular specific contrast agents for phase x-ray imaging based on gold nano particles attached to probe molecules with high affinity for specific cellular biomarkers. We were able to obtaine high resolution image such as phase contrast x-ray image less than 40 μm × 40 μm size. In this image, we were able to show very clear gold nano particles labeled cell image.

Conclusions: In this article, we discuss first of all. According to our results, phase contrast x-ray is very excellent molecular imaging method. This technique provides high resolution imaging method compared with other imaging modality. To better improve spatial resolution in phase contrast x-ray imaging, gold nano particles (size; 70nm) are used as contrast agents. And gold nano particles labeled cell are distinguished very easy than other cell.

Keywords: Molecular imaging, Gold nono particle, Phase contrast x-ray, Melanoma, Fibroblast

Acknowledgements

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Noninasive image of firefly luciferase gene expression in different animal cancer model

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Background: Luciferase has been widely used as reporter gene study over decade. Bioluminescence provides tracking tumor cells, stem cells, tool for rapid identification in real time analysis.

Purpose: Our goal of this study is using Lenti- CMV Luciferase transfected skin cancer graft mouse model in real-time analysis and evaluating our lab developed new optic instrument for Bioluminescence image.

Methods: We transfected mouse melanoma (CRL-6323™, ATCC, USA), Human Basal Cell Carcinoma (CRL-7762™, ATCC, USA), for optic image of tumor development, All cells were stably expressing of CMV–

Luciferase by clonal selection using puromycin and real-time PCR. After implantation to mouse subcutaneous, mouse were anesthetized and immediately administrated luciferin substrate via I.P. on post implantation day 1, 3, 5, 7, 14, 21and 28.

Results: We demonstrated that using luciferase transfected skin cancer transplantation animal models shows long-term stable expression of luciferase expression for up to mouse death of tumor metastasis resulting cachexia. Also metastasis region were detected after administration of luciferin substrates via I.P injection. New designed optic instrument by RISI were success to get fluorescence *in vivo* and *in vitro* images compared conventional optic instrument.

Conclusions: The Lenti CMV-firefly Luciferase transfected stable cell line implanted animal model provides non-invasive imaging of reporter gene expression *in vivo* by Optic instrument. Using Luciferase transfected cell lines give a easy and fast real-time detection bioluminescence image with cheap prices also our new developed optic instruments demonstrates high sensitivity of fluorescence. But we found degradation of luciferase intensity though skin or animal muscle layer depth. Optical imaging of reporter gene expression study has many potential applications.

Keywords: Melanoma, Basal cell carcinoma, Luciferase, Lenti CMV luciferase, Optical study

Acknowledgements

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P42

A cationic lamellar lipid technology that provides clinically effective skin moisturization from an in-shower rinse-off conditioning lotion

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Background: Daily cleansing can result in skin barrier damage often leading to painful dry skin and subsequent skin irritation, conditions often exasperated in low-humidity environments. A new personal care product form (in-shower skin conditioners) was recently introduced to the skincare market and a clinical protocol was recently developed and described ("Controlled Exposure Protocol to Assess the Dry Skin Improvement Potential of In-Shower Body Lotions", Ertel et al., American Academy of Dermatology 2006) for evaluating the clinical efficacy of such topical formulations.

Purpose: This presentation will describe human *in vivo* clinical studies, and *in vitro* biophysical studies, evaluating a new cationic lamellar lipid formulation technology (hydroxyethyl cetearylamidopropyldimonium chloride in a behenyl, stearyl, and cetyl alcohol matrix) formulationed into an in-shower skin conditioner. The purpose of this was to determine the efficacy of this systems in provided skin moisturization.

Methods: Microscopy and infrared spectroscopy data will be presented demonstrating the ordered lamellar structure of this technology and the highly ordered phases present on the skin. These lipid structures provide the material properties that restore barrier function to the skin resulting in improved skin moisturization. In addition to *in vitro* biophysical studies, this presentation will include clinical data from a recent study following the above mentioned clinical protocol. In brief, seventeen panelists were dosed on their forearms (randomized marked sites) with a cationic lamellar lipid in-shower lotion once a day for five days, after which photographs and skin hydration measurements were collected. In addition to the cationic lipid treated skin, each panelist had an untreated site, and a site treated with a benchmark in-shower lotion previously reported to be an effective product for this application.

Results: The sites treated with the cationic lamellar lipid technology were statistically equivalent to the previously reported effective benchmark formulation, and both these sites were significantly ($p<0.05$) more hydrated than the untreated sites. The clinical results for the cationic lamellar lipid technology formulation are consistent with the biophysical studies showing the highly ordered and substantive nature of this lamellar cationic lipid technology.

Conclusion: These results demonstrate that this cationic lamellar lipid technology can prevent and help mediate the occurrence of cleansing induced dry skin, and thus significantly improve consumers' skin health through effective skin moisturization.

Keywords: Hydration, Stratum corneum, Skin condition, Cationic lamellar lipid

P43

Intravital imaging in zebrafish using quantum dots

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Background/Purpose: The purpose is to investigate noninvasive intravital imaging of transparent zebrafish embryos, using molecular imagings with quantum dots(QDs). Semiconductor quantum dots are nanometer-sized crystals with unique photochemical and photophysical properties. Luminescent quantum-dots is a promising alternative to organic dyes for fluorescence-based applications. In comparison with organic dyes and fluorescent proteins, quantum dots are brighter, more stable against photobleaching, and can be excited for multicolor emission with a single light source.

Methods: We prepared transgenic fish line of olig2 EGFP, nk2.2 mGFP, and GFAP EGFP. Qdot 605 antibody conjugation was performed according to manufacturer's recommendation (*In vitro* gen). Qdot 605 and antibody labeled Qdot 605 were injected into one- to two-cell-stage zebrafish embryos. Embryos were anesthetized in 0.01% tricaine and mounted in 1.5% methylcellulose. Images of QD605-injected embryos were recorded with a digital camera (AxioImager, Zeiss).
Results: We could image nerve networks of olig2 and angiogenesis at once by using olig2-Dsred transgenic zebrafish and Qdot.
Conclusions: The results indicate that QDs can be very effective in imaging for the study of nerve network and cardiovascular development with zebrafish.
Keywords: Zebrafish, Quantum dot, Molecular imaging

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Clinical studies

P44
Increased carbonyl protein level in the stratum corneum of inflamatory skin disorders

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Background/Purpose: Stratum corneum (SC) is the interface of body and environment, and is continuously exposed to oxidative stress, resulting in carbonyl modification of proteins. We have developed a simple and non-invasive method to assess carbonyl protein (CP) level in the SC, applied it to various kinds of skin, and revealed a link between the stratum corneum carbonylated protein (SCCP) level and water content in the SC. The purpose of the present study is to examine the SCCP level in inflammatory skin disorders associated with xerosis. Psoriasis vulgaris (PV) and atopic dermatitis (AD) are typical inflammatory skin disorders, of which the stratum corneum shows markedly low water content.
Methods: SC samples were non-invasively collected from the lesional and non-lesional areas of PV and AD by adhesive tape stripping, and their carbonyl groups were determined by reaction with fluorescein-5-thiosemicarbazide. The average fluorescence intensity of the SC was calculated as SCCP level.
Results: Higher SCCP level was observed in lesional area of PV as compared with non-lesional area or healthy control. Lesional area of AD also exhibited higher SCCP level than corresponding non-lesional area, of which SCCP level was slightly higher than the healthy control.
Conclusion: These data suggest the involvement of oxidative modification of the SC protein, at least in part, in generation of xerotic skin in inflammatory skin disorders as well as dry skin in healthy subjects.
Keywords: Atopic dermatitis, Carbonyl protein, Oxidation, Psoriasis vulgaris, Stratum corneum

P45
Detection method of the skin tumor and wound healing models using stereo image system

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Background/Purpose: It is very difficult to make the non-convergence stereo system for the stereo matching problem. In spite of convergence model system, we can solve this problem by using the epipolar geometry of image rectification. The main idea of this paper is to find object contour on skin by segmenting the disparity map. This image has the least information which can recognize objects. So we will think disparity map as segment image in this paper. Because the edge means a sudden change in image intensity, the general edge may not guaranty the object contours. For the reason, it is very important process to get the segment image by using disparity map.
Methods: The edge may not guaranty the object contour on skin. So our main idea is to apply the edge detector for finding object boundary from the segment image. Given an image of disparity map, we can combine the edge detector for detecting object boundary or contours on skin.
Results: There are many edge detector to perform edge detection such as Roberts, Prewitt and Sobel based on the concept of gradient. Among them we use the Canny edge detector known as the optimal detector around the world which is based on the gradient magnitude of a smoothed image by Gaussian.
Conclusions: In this paper we have proposed that combination of edge detector and Dynamic Programming is useful algorithm for detecting object contour like skin tumor and wound healing models. However it is mismatched a bit if compared with an accurate object boundary. For improving this problem our further work is to use the snake algorithm of active contour mode and Graph cut.
Keywords: Disparity, Stereo image, Canny edge detector, Skin tumor, Wound healing

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P46
Quantitative analysis for malignant melanoma diagnosis using dermascopy

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Background: Malignant melanoma (M.M.) is increasing fast nowadays through all over the world. It has good prognosis if it treated in the curable early stage. Dermoscopy, also known as dermatoscopy or epiluminescence microscopy (ELM), is a non-invasive, *in vivo* technique, which permits visualization of features of malignant melanomas and many melanocytic lesions that are not discernable by examination with the naked eyes. Specially, skin lesion color and the shape are important features for diagnosing M.M.. However, it is not easy to use these features for diagnosis of skin lesion because of a lot of information.
Purpose: The purpose in this study is to evaluate difference between melanoma and other skin lesions.
Methods: In this study, we evaluated the color features and the shape features of M.M.. Each feature is detected through using the difference of MM and other skin lesions. Color features are evaluated using R-ratio (Red ratio) and intensity based on RGB color space. R-ratio is the red distribution of RGB color component which is the most important component of skin color. R-ratio is not the color and even though there are same R-ratios, the colors can be different. It means that it is possible not to be matched between the R-ratios and the colors. Shape features are evaluated using the circularity, the symmetry and the mid-point assessment. First, the circularity is a grade of the round shape of a skin lesion. Second, the symmetry is matching rate of each 4 areas separated into two parts. Finally, the mid-point assessment is deviation of the middle points.
Results: In this study, we found features of M.M.. M.M. has more R-ratios and intensity ranges and more shape variations than other skin lesions. Each feature we used was able to detect the M.M. respectively.
Conclusions: We detected color and shape features using the difference of M.M. and other skin lesions from this study. Although these features are able to detect the M.M. Respectively, using these features together is able to detect the M.M. more accurately.
Keywords: Malignant melanoma, Dermascopy, Skin color, Skin image analysis

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P47
In vitro measurement of the effects of skin occlusion on the growth of candida albicans

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Background: The growth of the pathogenic yeast, *Candida albicans*, has been associated with numerous aliments such as thrush in infants, diaper rash, and urogenital infections in adult females. It is also well know that water activity (a_w) is a primary factor in the regulation of microbial growth. Therefore, the occlusivity caused by a material placed over the skin should directly impact a_w of the skin's surface and the growth of associated yeast. Investigations of these interactions *in vivo* are confounded by safety concerns for the addition of live pathogens to skin as well as the complexity of recovering live microbes from human skin quantitatively. It would therefore be advantageous to investigate the impact of skin biophysical conditions on pathogen control and survival *in vitro*.
Methods: An *in vitro* skin apparatus was designed to simulate the skin biophysical conditions for perianal skin. This model was used to measure the effect of material breathability on *C. albicans* growth. Briefly, the apparatus consisted of stainless steel cups filled with semisolid growth medium over-laid with Vitro-Skin™ and placed in a dry heating bath at 37 °C to simulate the temperature and a_w of the skin surface. All experiments were done in a temperature and humidity controlled environmental chamber. Water loss across the Vitro-Skin™ and surface temperatures were found to closely match *in vivo* observations of transepidermal water loss (TEWL) and temperature measurements for infant and adult perianal skin. The growth of the yeast was monitored using plate counts.
Results: Using this apparatus it was observed that breathability, as opposed to the use of a ZnO containing ointment on the material, was the major factor controlling yeast growth on occluded skin. The most breathable material reduced yeast survival by more then 70%. Mirroring *in vivo* observations, the more occlusive the material the more the yeast growth.
Conclusions: The *in vitro* skin apparatus will allow for evaluation of materials that would not be appropriate for human clinical testing. Other parameters related to microbial growth on the skin can be simulated using this apparatus, including modulation of CO_2 levels through incorporation of carbonates or entrapped gases, pH gradients by incorporation of biological buffers, ion gradients through the use of various salts, as well as the inclusion of immunochemicals and defensins. The

apparatus and method described here has the potential to be an excellent biophysical and biochemical representation of *in vivo* skin.

Keywords: Candida albicans, Yeast Infection, Occlusion, *In vitro* skin model

P48
Improvement of microenvironment for wound healing in diabetic mice

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Background: In diabetic mellitus, the skin wound healing is difficult because of complication of elevated blood glucose level. EPCs(endothelial progenitor cells) are essential in vasculogenesis and wound healing, but circulating and wound level numbers are decreased in diabetes. Diabetic mice showed impaired phosphorylation of BM eNOS, decreased circulating EPCs, and diminished SDF-1a expression in cutaneous wounds (JCI 2007). The diabetic wound healing required cell therapy products from outside and more fundamentally, systematic treatment that can improve microenvirunment. In this study we used Substance-P and GM-CSF which improve inflow of cells to wound site that make blood vessel and make better cure condition.

Purpose: In this study, we evaluated effects of treatment of wound healing using Substance-P, GM-CSF in diabetic mice which were induced with Streptozotocin.

Methods: Briefly, Diabetic mice were received an intraperitoneally injections of 100 nM Substance-P or (5 μg/kg) GM-CSF or mixture Substance-P and GM-CSF. Mice were treated with five consecutive daily injections. After 14days, mice were sacrificed and tested macroscopic wound closure effect, formation ability of granulation tissue, and composition ability of collagen. To proof these products capacity of mobilization of EPC & MSC to wound site, CD29 (integrin b1) & CD117 (SCF R/c-kit) Immune staining was performed using paraffin section staining methods.

Results: Rapidly wound closure and high formation ability of granulation tissue improved in Substance-P /GM-CSF treated mice compared with that of untreated mice,. We could identify the cells which look like EPC in Substance-P /GM-CSF group by Immune staining with famous EPC marker of CD117.

Conlclusions: We observed that Substance-P and GM-CSF improve inflow of cells to wound site that make blood vessel and make better cure condition. We are expecting improvement of microenvironment for wound healing on Substance-P and GM-CSF.

Keywords: Wound healing, Diabetic mice, Substance-P, GM-CSF

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Cross sectional imaging (confocal microscope and OCT)

P49
In vivo confocal laser scanning microscopy for non-invasive diagnosis of pemphigus foliaceus

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Background: *In vivo* confocal laser scanning microscopy (CLSM) is a modern non-invasive method for investigation of the skin that allows real-time visualization of individual cells and sub-cellular structures at resolution similar to the one provided by routine histopathology. Numerous applications of CLSM for both diagnostic and research purposes have been described. However, data on the application of the method for the non-invasive investigation of autoimmune bullous diseases are limited so far.

Purpose: Our aim was to test the potential of CLSM for real-time *in vivo* diagnosis of pemphigus foliaceus (PF).

Methods: Pre-existing and mechanically induced lesions of PF were examined by the means of CLSM, parallel to routine histology, direct immunofluorescence microscopy and enzyme linked immunosorbent assay (ELISA) performed in the same patients.

Results: The morphological features characteristic for PF namely a subcorneal intraepidermal blister with acantholytic cells in the blister cavity, were readily detectable by CLSM. The findings were consistent across the patients and the investigated lesions. The confocal images were in agreement with the routine histology of the pre-existing lesions. No differences in the confocal images of pre-existing lesions compared to mechanically induced ones were observed.

Conclusions: Our findings provide evidence for the potential of CLSM as a non-invasive tool for the diagnosis of pemphigus and the differentiation of its subtypes. The method may be successfully used as real time *in vivo* screening tool to facilitate the diagnosis and point to the need for further investigation of the patient.

Keywords: Confocal laser scanning microscopy, Non-invasive skin imaging, Pemphigus, Autoimmune bullous disease

P50
Semiology of actinic lentigos intrinsic heterogeneity: *in vivo* confocal microscopy versus dermatoscopy

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Background/Purpose: *In vivo* confocal microscopy has revealed different stages of Actinic Lentigo (AL) development [1]. The aim of this study was to establish the confocal semiology of AL in comparison to dermatoscopy of the lesions using confocal images from a large field of view.

Methods: Volunteers with several AL on the dorsum of hand were included, 80 AL were assessed. Clinical investigation and dermatoscopy (Fotofinder dermoscope®, Teachscreen software GmbH; Bad Birnbach, Germany) were combined with *in vivo* confocal investigation (Vivascope 1000, Lucid Inc, Henrietta, NY, USA) on the lesion and on the adjacent skin (control area). Confocal images were obtained from a large field of view (3x4.5 mm).

Results: The Dermal Epidermal Junction (DEJ) appears in hypersignal with a modified appearance of the dermal papillae sections. This distribution reflects the known histological structure of AL, with the presence of crowed, long and short, thick and thin budding configurations of the epidermis [1]. These confocal structures have been confirmed by comparison and superimposition with dermatoscopy pictures (x70 magnification).

Observed structures of confocal images were not the same for all of the AL included in the study. Moreover, confocal images from a large field of view or high magnification of dermatoscopic pictures have shown that the observed structures can be very different within a given AL, suggesting that different areas of an AL could be in different grades of severity.

Conclusion: High magnifications of an AL have shown that the observed structures are very different within the lesion which proves the heterogeneity of Actinic Lentigo architecture, which has not been described before. It enhances our knowledge on this pigmented disorder genesis and could be of significant help for evaluating the effects of whitening agents on actinic lentigos, by measuring early changes in the DEJ organisation and its hypersignal variations.

Keywords: *In vivo* confocal microscopy, Dermatoscopy

Reference

1. Nouveau-Richard S, Monot M, Passeron T, Ostovari N, Zakaria W, Ortonne JP, de Lacharrière O. Semiology of actinic lentigos with *In Vivo* confocal microscopy. 21st WCD, Buenos Aires, Argentina 2007

P51
High frequency ultrasound image

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Background: During the past 15 years, ultrasound image of skin has gained increasing importance as non-invasive imaging method in dermatology. Structures of the epidermis cannot be differentiated due to the lack of resolution using commercially available transducer with frequency of 20 to 25 MHz

Purpose: To investigate the epidermis, resolution must be improved. By the rising the frequency of ultrasound transducer, resolution increase, but the signal penetration depth into the skin is reduced. We modified the 50 to 100 MHz transducer technology in such a way the skin structure can be visualized.

Materials/Methods: An experimental ultrasound imaging unit was developed the can be operated with different transducers in a frequency range of 50 to 200 MHz. To study the epidermis and eye ball, we used a 100 MHz ceramic transducer. For image acquisition, high speed signal processing for ultrasound were developed by Prof. Yoon's group. Sonogram were taken volunteers with health skin in dorsal forearm, pig skin and pig eye ball.

Results: In 100MHz ultrasound image of forearm of human skin, and pig skin, an echo rich entry echo is seen at the upper border. Below the entry echo, there is an echo poor band, which we call EPB1(Echo Poor Band 1). It is follow by echo rich liver, which runs parallel to the entry echo but has intense below EPB1, a second echo poor band is seen which we will call EPB2 (Echo Poor Band 2) is defined by the scattered reflects of the dermis. In pig eye ball, we can see the cornea, anterior chamber and lens structure in 100 MHz ultrasound image.

Conclusions: Our high frequency ultrasound equipment allows a more detailed visualization of the upper skin layer. The significant intensity difference between stratum corneum and stratum malpighi - visible as echo rich line – can be explained by the hydration state of this layer.

Keywords: High frequency ultrasound, Epidermis, Eye ball

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P52
A study on improvement of efficiency to enhance the contrast for confocal microendoscope with grin probe

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Background: An endoscopic version of a confocal microscope could be especially helpful for an early cancer diagnosis during a minimally invasive operation. In many types of endoscope systems, fiber-bundle confocal microendoscope system with GRIN probe can be easy to miniaturize, because of no distal scanning mechanism and small size of GRIN lenses with relatively high numerical aperture.
Purpose: The high coupling efficiency of fiber-bundle confocal microendoscope leads to a higher contrast. But the numerical aperture of the coupling lens of GRIN probe is smaller than that of the fiber-bundle in previous researches, coupling efficiency between the fiber-bundle and GRIN probe is too low. So we introduce the method to make desired NA of coupling lens for improving the coupling efficiency between coherent fiber-bundle and GRIN probe.
Materials/Methods: New method to make desired NA of coupling lens is proposed to improve the coupling efficiency between coherent fiber-bundle and GRIN probe. In previous research, the coupling lens was built with a only GRIN lens of nominal NA of 0.2. But the coupling lens designed by new method for improving the coupling efficiency is composed of GRIN lenses of nominal NA of 0.2 and 0.6.
Results: Experimental results had verified the improvement of the coupling efficiency by increasing NA of the coupling lens. But spherical aberration caused by new method reduces the improvement of coupling efficiency. So new method needs to correct the spherical aberration by an optimized adaptation of the refractive index profile.
Conclusions: Coupling efficiency can be improved by NA of coupling lens of GRIN probe by new method and optimized index profile. It is expected to improve contrast for confocal microendoscope with a coherent fiber-bundle.
Keywords: Confocal reflectance microscopy, Endoscopy, Gradient-index(GRIN) optics, Optical biopsy, Image fiber bundle

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Skin color, blood flow and vascular functions

P53
The effect of custom white balance on color assessment using digital camera

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Background: The light determines the color we perceive, and is of prime importance in terms of color appraisal. Tungsten lamp is an excellent daylight simulator in visible light range, and is thought to be a candidate for ideal light source for color appraisal. However, in general, they have low color temperatures. Filter for light source is a way to overcome different color temperatures. The various filters for color temperature conversion are designed for modifying color temperatures of light sources. White balance (WB) of digital camera is another way of acquiring correct colors in circumstances of lights of different color temperatures. In this regards, it is needed to define the effect of WB function on photographic images.
Purpose: The aim of this study was to define the effect of custom WB function of digital camera in terms of color assessment using digital camera or colorimetric photography.
Methods: We used tungsten lamp and four blue filters to get light sources of various color temperatures. Two different color charts were used, one for color measurement method or statistical calibration and the other for assessment of color measurement accuracy. All colored squares on a color chart were photographed using digital camera using each combination of lamp and filters, analyzed and converted into mean CIELAB values. These values were compared statistically with reference CIELAB values obtained previously with spectrophotometer, and produced calibration method for color measurement for given light sources. The accuracy of the color measurement was compared according to different light sources by measuring the colored squares of the other color chart using above method. These processes are repeated twice using fixed predetermined WB and custom WB using gray card.
Results: As expected, deltaE or color difference from reference CIELAB coordinates were decreased by some amount using custom WB only. However, as for individual light sources statistically calibrated results with custom WB are not always better than calibrated results with fixed WB. The efficiency of statistical calibration decreased with custom WB. The smallest deltaE was obtained in unexpected light source in method using custom WB.
Conclusions: As for colorimetric photography, custom WB makes the choice of optimal light source difficult. The most accurate measurement of color could be obtained in very particular circumstances using both custom WB and statistical calibration. However, those can be generalized because of unexpectedness of custom

WB. It seems rational to optimize the light sources rather than customizing WB of digital camera.
Keywords: Photography, White balance, Color

P54
A study about the efficacy and safety of TCA-based blue peel in oriental melasma patients

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Background: Melasma is a common skin problem. Multiple therapeutic modalities have been used, such as bleaching agents and chemical peelings. However, the optimal, safe agent has not been discovered. The conventional trichloroacetic acid (TCA) peel or phenol peel may cause prolonged erythema or permanent scar if uneven penetration happen or too high concentration is used. Especially in oriental melasma patients, the possibility of postinflammatory hyperpigmentation or depigmentation is relatively high. But TCA-based blue peel is used with relatively low concentration of TCA with blue base. Therefore, it is easy to perform even application and safe procedure of medium to deep depth peeling.
Purpose: Our purpose of study was to analyze the efficacy and safety of TCA-based blue peel and to find the optimal method of TCA-based blue peel in treating Oriental melasma patients.
Methods: This study was conducted at Modelo Clinic(Seoul, Korea) in the period between June and December 2007. Twenty eight patients with melasma were enrolled in this study. Before and after the peeling, topical program constitute of 0.1% tretinoin, 4% hydroquinone, AHAs(6% glycolic acid, 4% lactic acid) was applied. We spiltted the face randomly. On one side, we performed TCA-based blue peel into papillary dermis level one time. On the other side, we did into basal layer level three times at three weeks interval. To assess the severity of melasma and treatment efficacy, Mexameter® and modified Melasma Area Severity Index (MASI) score were used. The Objective clinical evaluation score and subjective satisfaction score were also graded (0 to 4). Adverse reactions were described as Visual Analogue Scale (VAS). Follow-up was carried out for six months after the beginning.
Results: The mean age of the patients was 45±10 years and the skin type was 3 or 4. (12 and 16 patients, respectively). During the pre-peel conditioning for six weeks, the mean Melanin Index (MI) was decreased from 193.5±10.4 to 148.1±9.5 ($p<0.05$). Modified MASI score was also decreased from 7.95±2.29 to 5.26±0.78 ($p<0.05$) during that time. After the peeling, all the patients showed the statistically significant decrease of MI. On the papillary dermis depth one-time peeling sides, the mean MI decreased from 147.4±7.8 to 127.6±8.1 after three weeks and sustained for six months. On the basal layer depth peeling sides, the mean MI decreased from 148.5±8.1 to 139.3±8.4 with one-time peeling, and changed to 133.5±5.6 with three-time peeling. The objective clinical evaluation score (3.9 versus 3.5) and subjective satisfaction score (3.85 versus 3.5) also showed more excellent results on papillary dermis depth peeling sides. The VAS of adverse reactions were not statistically different on both sides except crust. The mean healing (decrusting) time was 7.5 days on papillary dermis depth peeling sides versus 5.2 days on basal layer depth peeling sides.
Conclusion: TCA-based blue peel showed good results in treating Oriental melasma patients. The one-time papillay dermis depth peeling was more efficient and showed more excellent clinical score than multiple basal layer depth peeling. The safety profiles were not statistically different between two other peeling depths except crust.
Keywords: TCA-based blue peel**,** Optimal method**,** Melasma

P55
Biophysical changes after application of topical anesthetic cream

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Background/Purpose: EMLA cream is a mixture of lidocaine and prilocaine, which provide analgesic effect during laser treatments. We often see the blanching after application of EMLA, but no study has been ever tried to evaluate the extent or mechanism of blanching. Thus we would like to investigate the biophysical changes after application of EMLA cream.
Methods: 10 volunteers (22-36 years old) were included in this study. 0.5g of EMLA and placebo were applied on arm with occlusive dressing. Erythema index and Melanin Index were measured with mexameter after 15 min, 30 min, 60 min application.
Results: Within 15 min, EMLA and placebo both have blanching effect, but after 30 min only EMLA applied site shows reduction of Erythema index.($P<0.05$) Maximum reduction of Erythema index was 40%, 60 min after application of EMLA. Melanin index and hydration were similar between two groups.
Conclusion: Even though hydration itself has a vasoconstrictive effect, but EMLA is more potent up to 40% reduction of Erythema Index. But at least 30 min to get such EMLA itself induced blanching effect.
Keywords: EMLA, Erythema index, Mexameter

P56
Comparative study for an objective measurement of meds using spectrophotometer between psoriasis and vitiligo patients according to skin phototypes

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Backgrounds: The assessment of cutaneous sensitivity of UV radiation is important in setting the phototherapy protocol. Traditionally, minimal erythema dose(MED) have been regarded as a key factor in determining irradiation dose. But, MED evaluation is a quite subjective process because the measured values merely reflect the doses from light sources and there are few data concerning the objective measurement of depth of erythema responses, especially in MED, according to skin phototypes.
Objectives: The objective of our study is to compare various parameters of spectrophotometer at the observed MEDs between psoriasis and vitiligo patients according to skin phototypes.
Methods: A total of 29 psoriasis and 24 vitiligo patients were selected before receiving NBUVB phototherapy. To perform phototesting, 10 sites on back skin were vertically exposed to NBUVB in a series of 10 among 14 doses between 340 and 1400mJ/cm2. Three color attributes of the skin; hue, lightness, and saturation, were measured by spectrophotometer(CM-2600d, Konica Minolta Holdings, Inc., Tokyo, Japan) at each erythema spots and control skin. This spectrophotometer employs a xenon arc lamp emitting wavebands from 460 to 760nm at 10-nm intervals. Reflected light is detected by a silicon photodiode array housed in a dual 40 glass element structure. Also, we evaluated the relationship between a*b* values and skin phototype in psoriasis and vitiligo patients. The L*a*b* color space, devised by Commission Internationale de l'Eclairage in 1976, was adopted to measure object color. This color space has been shown to visual differences and is now used worldwide for color communication. L* indicates lightness and color directions are indicated as a*(red-green) and b*(yellow-blue). As the a* and b* values increases, the saturation of the color increases. The pressure on the skin during measurement was kept constant to prevent changes in the skin color due to physical contact.
Result: In all subjects, MED were measured in the 400-1000mJ/cm2 range. The average of colorimetric values at MEDs in overall patients with skin type III and IV were a* 11.60±2.16, L*61.53±3.44 and a*11.98±1.93, L*60.29±4.49, respectively and, in psoriasis patients with skin type III(n=24) and IV(n=5), a*12.08±2.04, L*61.26±3.72 and a*11.24±1.06, L*60.34±4.00, respectively, and in vitiligo patients with skin type III (n=18) and IV(n=6), a*11.06±2.17, L*61.91±3.03 and a*12.87±2.46, L*60.23±5.52. Skin type IV patients showed a steeper inverse correlation slope than that of skin type III series in a*L*curve, irrespective of psoriasis or vitiligo. Especially in type III patients, vitiligo patients series showed a steeper slope than psoriatics. However, another index, b* values, did not show a significant correlation with a* values.
In summary, L* value was inversely correlated with a representative value for erythema(a*) in overall patients. Especially among type III patients, the vitiligo patients series(n=18) showed a more prominent inverse correlation than that of psoriatics(n=24) in a*L*curve.
Conclusions: Spectrophtometer enables UV erythema to assess objectively on quantitative basis. And it can make up for the disadvantages of arbitrary values in MED determination in aspects of irradiation doses from the light sources.
Our study results indicate that L* value is generally inversely correlated with a* value, but it is unreasonable that the fact is applied in each skin phototypes. By the result of a a* L* curve concerning skin type III and IV persons, the skin type IV series reflected a*L* correlation better than those of skin type III. Therfore, objectively measured skin color may be useful for predicting an individual's sensitivity to NBUVB radiation. Moreover, we propose very cautiously L* value may be used as another index for tanning level instead of b* value.
Keyword: Spectrophotometer, Skin phototype, L* value, A*value

P57
Efficacy assessment of moisturizers by mathematical modeling of the vasodilatory response to nicotinates

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Background: Xerosis, or dry skin, is a common disorder that causes abnormal epidermal proliferation and differentiation, which then results in impairment of skin barrier function. The assessment of alterations on skin permeability is, therefore, relevant to the study of the therapeutic efficacy of moisturizers designed to improve xerotic skin. Laser Doppler Flowmetry is a valuable tool to study the vasodilatory effect of nicotinates on the cutaneous microcirculation and, indirectly, investigate the penetration enhancing/retarding potential of substances and formulations. The correlation of these two variables is complex, because of the multiple factors involved in the response to nicotinates, but valid extrapolations have previously been established. Most authors analyse the blood flow curves by determining lag time between application and initial response (t_{onset}) and time for maximum response (t_{max}), but these are usually difficult to pinpoint with precision. The main objective of this investigation is to improve the methodology employing nicotinates to investigate skin barrier function by application of mathematical modelling to characterize more accurately the response to ethyl nicotinate and, in turn, assess the efficacy of a moisturizer.
Methods: A total of fifteen female volunteers with dry skin participated in the study. Volunteers were instructed to apply for two weeks to an assigned area in the leg a

formulation designed for ichthyosis-prone skin containing 15% urea (Kératosane 15™). The entire study was conducted in the frontal aspect of the lower leg of the volunteer, where dry skin is usually more evident. Two sites were marked in this area: one where the skin was treated with cream and another in an untreated control. A 0.2M aqueous solution of ethyl nicotinate was applied to each site for 60 seconds using a saturated filter paper disc. As soon as the paper disc was removed, alterations in the blood flow in both sites were measured for 60 minutes using a two-probe laser Doppler perfusion monitor. The classic variables, i.e. time for onset (t_{onset}) and time for maximum response (t_{max}) were determined. Additionally, a sigmoid emax mathematical model was fitted to the data using a nonlinear regression routine. The calculated parameters considered significant to the study were lag time until initial response (t_{lag}), time for maximum response (t_m) and perfusion slope.

Results: Results obtained in this study indicate minor differences between the permeation of ethyl nicotinate in the treated and control sites. The molecule permeated slightly faster through the skin that was treated with the cream and provided a slower vascular response in the xerotic (untreated) skin. This effect could not be demonstrated when t_{onset} and t_{max} obtained in the treated and control sites were compared. It could only be shown more evidently when the parameters resulting from the mathematical modeling were submitted to statistical analysis.

Conclusion: The study indicates that the moisturizer has not significantly improved the barrier function of dry skin after treatment for two weeks. It seems that the moisturizer is merely improving the water content of the stratum corneum and has besen unable to influence the causes that lead to poor barrier function- the alterations in epidermal proliferation and differentiation. Additionally, results show that the application of the product can facilitate the permeation of mildly lipophilic substances (such as ethyl nicotinate), which may expose individuals to irritant and allergen substances in the environment.

Keywords: Efficacy, Moisturisers, Mathematical modelling, Nicotinates

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Claims support in cosmetology and pharmacology

P58

A study of cutaneous physiological parameters before and after oral isotretinoin treatment in acne patients

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Background: Oral isotretinoin is widely used for the treatment of severe acne. Although isotretinoin therapy usually produces desquamation and tight feeling of the skin, there have been few investigations as to whether long-term isotretinoin use is associated with barrier disruption, leading to dryness of the skin.

Purpose: We have conducted a research to investigate whether long-term isotretinoin therapy can cause significant physiological derangement of the skin including TEWL, pH, hydration and sebum secretion.

Methods: We have measured TEWL, pH, hydration level and sebum secretion of a total of 12 acne patients before and after long-term oral isotretinoin. Isotretinoin was administered with the goal of cumulative dose of 120mg/kg.

Results: The results showed that all the physiological parameters changed significantly after treatment. Sebum secretion decreased significantly that is compatible with pharmacological action of isotretinoin on the sebaceous glands. Skin hydration level slightly increased after the treatment and barrier disruption indicated by TEWL decreased significantly after the treatment. Skin pH decreased slightly after long-term isotretinoin use.

Conclusion: In contrast to the long-held belief that isotretinoin can impair skin barrier function, leading to dehydration of the skin this study demonstrated that, after long-term use of isotretinoin in acne patients, barrier function was not impaired and skin hydration level was not decreased. Dry feeling in isotretinoin use may be related to increased desquamation from isotretinoin rather than actual barrier disruption.

Keywords: Acne, Corneometer, Isotretinoin, pHmeter, Sebumeter, TEWL

P59

A study for the therapeutic efficacy of pedunculagin for atopic dermatitis

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Background/Purpose: Atopic Dermatitis (AD) is a chronic relapsing inflammatory skin disorder with increasing prevalence. Experimental animal model are indispensable tools to study the pathogenic mechanisms and to test novel therapeutic approaches *in vivo*. AD-like lesions can be induced experimentally in NC/Nga mice. Pedunculagin, an ellagitannin purified from *Alnus hirsuta* var. *microphylla, Betulaceas*, is a novel immunomodulator. To evaluate the therapeutic efficacy of pedunculagin for AD-like dermatits of NC/Nga mice

by clinical method and noninvasive methods.

Methods: AD-like lesions can be induced experimentally in NC/Nga mice using 2, 4, 6-Trinirtochlorobenzene (TNCB). Left upper back remained untreated and right upper back was administered by vehicle. Left lower back was administered by 0.1% pedunculagin and right lower back was administered by 0.5% pedunculagin as daily application for 4 weeks. Clinical severity scores of the dermatitis and ear thickness, transepidermal water loss (TEWL), water holding capacity (WHC), pH, skin roughness were measured noninvasively at baseline and at 1day, 3day, 1weak, 2weak, 4weak after pedunculagin and vehicle application respectively.

Results: AD has been induced using TNCB successfully. We evaluated the therapeutic efficacy of pedunculagin for atopic dermatitis using evaluation methods of the clinical severity score and noninvasive biomedical engineering tools. The therapeutic efficacy of pedunculagin for atopic dermatitis was confirmed using Corneometer®, Evaporimeter® and SOT especially.

Conclusions: These results suggest that pedunculagin might improve the disruption of permeability barrier with improvement of the water holding capacity of the stratum corneum.

Keywords: Atopic dermatitis, Pedunculagin, SOT, Therapeutic efficacy

Acknowledgements

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P60
Quantitative sensory methods: investigation of skin sensations in response to a chemical stress

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Background: Self-perception of sensitive skin has been previously defined by immediate skin sensations or transient visible reactions in response to various stimuli ranging from environmental causes to contact causes. Skin sensations are investigated by the analysis of perception thresholds of cutaneous nerve fibers in response to a stress.

Purpose: In this context, our objective was to investigate the effect of a topical application of a non-irritant dose of menthol on skin nerve fibers using two Quantative Sensory Testing (QST) methods.

Methods: Twenty nine Caucasian women aged 25-35 years, with an healthy skin, were recruited according to well defined inclusion criteria. Thermal Perception Thresholds (TPT) and Current Perception Thresholds (CPT) were measured using a thermal sensory analyzer (TSA 2001, Medoc Inc) and a current perception sensory device (Neurometer®, Neurotron), respectively. Left and right cheeks were investigated separately. TPT were measured before and after 2% menthol application on the left cheek, followed by CPT measured before and after 2% menthol application on the right cheek.

Results/Discussion: We have demonstrated that a non-irritant dose of menthol increased CPT of A nerve fibers in human skin by altering their depolarization thresholds. Results are in agreement with earlier findings suggesting that cold and menthol could lead to membrane depolarization on a subpopulation of primary afferent neurons.

However, no significant effect of menthol on cold perception thresholds or on warm perception thresholds was observed. These results are in line with previous studies showing that menthol had no effect on thermal detection excepted on inhibition of the perception of warmth on the lips.

Conclusions: Our results suggest that a topical application of 2% menthol increased A fibers perception thresholds without changing cold or warm detection.

Two hypotheses could lead to explain our results: the method used did not allow to measure thermal perception changes induced by topical application of menthol on the cheek, or menthol does not affect thermal sensations in the range of temperature studied.

Keywords: Current perception thresholds, Thermal perception thresholds, Menthol

P61
Reduced potency after refrigerated storage of botulitum toxin a: human extensor digitorum brevis muscle study

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Objective: To determine whether the potency of botulinum toxin A (BTA) decreases after being reconstituted with normal saline and stored in refrigerator

Methods: We injected one side of the extensor digitorum brevis muscle with 2.5 units of botulinum toxin A that had been immediately reconstituted with saline, and the contralateral side with identical material that had been reconstituted and stored in a refrigerator for preselected periods (1, 2, and 4 weeks) in 32 healthy volunteers.

Results: Mean compound muscle action potential amplitudes expressed as a percentage of the baseline amplitude were more reduced in sides injected with immediately reconstituted BTA than in sides injected with BTA stored for 1 week or more ($P < 0.05$). No

bacterial growth was observed in any stored BTA samples.

Conclusion: Storage of reconstituted BTA at low temperatures may affect the potency of the toxin. Therefore, the use of BTA after refrigerated storage is not recommended.

Keywords: Botulinum toxin A, Potency, Refrigerated storage

P62

Comparing *in vivo* skin surface roughness measurement using laser speckle imaging with red and blue wavelengths

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Background: Certain biomedical clinical applications require measuring the roughness of a biological surface *in vivo* accurately. However, fast, non-invasive, *in vivo* measuring techniques are not yet widely available. We have been studying the technique of measuring skin roughness by laser speckle imaging. Laser speckle, a stochastic interference pattern, is formed when a laser beam is reflected by a rough surface. We have investigated the theory and the optimal parameters that allow us to relate the contrast of a laser speckle pattern directly to the surface roughness.

Purpose: The aim of this study is to construct a laser speckle imaging device that can measure *in vivo* skin roughness. In addition, we compare the effectiveness of red versus blue laser illumination for the construction.

Methods: A prototype laser speckle imaging device was designed by employing an open geometry scheme and a CCD camera without an imaging lens. In order to remove the contribution of intracutaneous volume scattering, we utilized polarization filtering on two registered channels with co- and cross-linear orientations. Skin roughness was then computed directly from the contrast of the registered speckle patterns. In the prototype, we incorporated a blue (408 nm) and a red diode laser (663 nm) for testing their effectiveness in detecting surface roughness; the imaging procedure was performed separately for each laser. In addition, we obtained a skin replica of the same skin patch immediately after the imaging procedure. The replica roughness was measured by a laser profilometer, and was compared with the roughness obtained by our speckle imaging prototype.

Results: We constructed a portable prototype, which was applied to the hand of a group of human volunteers. Co- and cross- polarized speckle patterns were obtained for each laser, and skin roughness was computed for the red and blue lasers separately. The roughness derived from the blue laser was much closer than the red laser to the roughness measured from the replicas and to the values published in literature. The reason may be that the overwhelming volume scattering in the red wavelength range was not rejected effectively, while the volume scattering in the blue wavelength is much smaller and had less interference with the surface scattering.

Conclusions: We demonstrated the technique of measuring skin roughness using the contrast of laser speckle images. In particular, preliminary results show that the blue laser may be more suitable for skin surface roughness detection than the red laser.

Keywords: Skin roughness, Laser speckle imaging, Contrast, *In vivo*, Non-invasive

P63

Predicting a person's age from his face image

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Background/Objective: The shape, texture, and color of the face are modified by age, involving a combination of changes in the bone, fat, muscles, and skin tissues. Human beings have developed a great ability to interpret all these changes and to guess people's age based on their facial appearance. Our objective was to build an algorithm, which could predict someone's age based on his front face pictures.

Subjects/Methods: 173 subjects, uniformly distributed between 18 and 74 years of age were enrolled for the study. A front face picture was taken under controlled lighting and positioning environment. A learning by sample classifier based on Partial Least Square (PLS) regression was built in order to predict the subjects' age from the shape, color, and texture of their faces.

Firstly, landmarks where manually placed around the main facial features (Jaw line, nose, eyes, lips). A PLS model was built to predict the age from the relative position of the landmarks leading to a shape model of face aging. Then textural PLS models were built from the values of the pixels of the face (one model for each RGB Channel). Combination of the textural models enables to capture color changes associated with age. Finally, a full regression model of age was built, by merging the shape and the textural models into another PLS model. Age prediction models were validated using the leave-one-out strategy.

Results: The full model enables to predict someone's age with an average absolute error of 4.8 years for the validation set. The shape model captures information such as the reduction of lips thickness and eye size; the transformation of the jaw line shape from oval to square. The color and textural models capture information such as the aggravation of the nasolabial fold, the corner of the month wrinkles and the bags under the eyes. They also put forward the yellowish of the skin tone and the progressive atrophy of the cheeks.

Conclusion: The proposed age prediction model shows performances that matches human accuracy. This model gives an interesting insight to the cosmetic dermatologist since it enables to build a picture summary of the changes that occur in facial appearance. While using this model, it clearly appears that facial aging is a multi-dimensional problem. Therefore efficient face rejuvenation should simultaneously address several signs of ageing in order to produce an "aesthetically" consistent whole.

Keywords: Clinical photography, Facial aging, Face analysis, Skin color, Wrinkles, Sagging, Apparent age

P64

Non-invasive imaging to quantify the grade of cellulite severity

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Cellulite is a condition reflected in adipose biochemistry and altered connective tissue structure, and is a gender-related condition with clinical expression of conformational changes taking place in the fibrous strands partitioning the hypodermis. Objective methods to measure the efficacy of cosmetic products are of growing importance. Many studies have relied on subjective methods which do not provide information as to whether applications of cosmetic products have improved the condition. Even investigations using more objective methods have yet to lead to a proper understanding of cellulite and its possible reduction by cosmetic treatment. Consequently, the need for objective methods to investigate dimpling skin remains to be compared with subjective methods.

The aim of our work was to investigate ranking of macrophotos; ultrasound measurements; skin surface texture measurements; *in vivo* confocal microscopy; and thermography measurements for their ability to characterize the dimpling of skin.

Standardization of macrophotography minimized differences in image features between evaluation times, therefore enabling follow-up rating assessments of the images. Volunteers and experts scored significant improvement of skin appearance over the course of a three month cosmetic treatment. Image analysis of ultrasound imaging was automated, and a modification of the commonly known roughness parameter Ra was implemented to characterize cellulite severity. Profilometry measurements that are standardized and easy-to-handle methods, also give further aspects of the status of cellulite skin. Profilometry measurements were performed with a DermaTOP instrument, providing a measurement area of 60 * 80 mm. Using the principle of fringe projection, the instrument determines the surface of the skin. Roughness and volume parameters are calculated from these measurements to obtain data evaluable for statistical analysis.

Very good differentiation of cellulite profiles were obtained and provided a greater sensitivity than visual assessments.

Macrophotography and ultrasound imaging can be regarded as important tools for determining and quantifying the aspects of cellulite. Profilometry also gives further aspects of the status of cellulite skin. Despite being one of the more complex methods, confocal microscopy provides useful information about the grade of cellulite on the cellular level. The combination of the methods mentioned is well able to define cellulite-reducing efficacy from the cosmetic point of view.

Keywords: Cellulite, Imaging, Skin surface texture, Profilometery, Macrophotography

P65

Assessment by fringe projection of the immediate smoothing effect of a wrinkle filler after single application

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Introduction: The product « Touche Précieuse » is a wrinkle filler designed to attenuate the skin relief immediately. It combines a «silicon gum» texture, hyaluronic filling spheres and a soft focus effect generated by powders with a high refractive index. The objective of this work was to quantify its smoothing effect on the wrinkles.

Method: Assessment were performed before and after application of the product in 10 healthy women with wrinkles of light to moderate intensity on the forehead and crow's foot. To assess the smoothing effect, 3-dimension images of these wrinkles were taken with profilometry by fringe projection *in vivo* then their volume, maximal depth, mean depth and surface were characterized. Facial and hemifacial standardized photographs were taken with and without (cross, parallel) polarization filters.

Results: The smoothing effect of the product was evidenced by fringe projection: on the forehead a significant decrease of the volume (by about 17% (-0.7 mm^3) p=0.03), the maximal depth and the mean depth of the wrinkles (p=0.0261) was observed. The wrinkle is not only less deep but its extent is also reduced (decrease of the surface of 3 mm^2). On the crow's foot, the volume tends to decrease by 23% (-0.6 mm^3) and the surface decreases significantly (p=0.0098) by almost 24% (-4mm^2). The photographs confirm the « filling » or « blurring » effect of the product. The « soft focus » effect is particularly visible on those taken in parallel polarized light, where the reflection is much less marked after application of the product.

All these results are confirmed by the subjects

declaration (100 % confirm an attenuation of their crow's feet wrinkles, 90 % of those of their forehead). 90% of the volunteers are satisfied with the smoothing and the matifying effects, 80 % declare that the product clears away the shadowed areas from their face.
Conclusion: The smoothing effect of this wrinkle filler could be demonstrated on both sites. This effect is immediate but also sufficiently intense to be detected by only 10 volunteers. The association of fringe projection with digital photography provides an accurate and coherent analysis of the skin relief and guarantees quality illustrations.
Keywords: Fringe projection, Wrinkle filler, Soft focus effect, Smoothing effect, Standardized photographs, Polarization filters

P66
3D measurement of lip volume and relief
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Background: Quantification on the lip surface and volume is of great interest in the cosmetic and aesthetic fields. Due to the important curvature and the mobility of this area, analysis of replica could be deceiving to characterise the lip relief. Other methodologies give a 2D image of the lips useful for relief quantification but insufficient for volume measurement.
Purpose: We studied the 3D image acquisition by fringe projection to quantify the volume and the roughness parameters of the lips. Two clinical studies were performed in order to validate this method for product evaluation.
Methods: The head and the lips of the subjects were kept in a standardised position. 3D image were acquired with a Dermatop system (Breukmann – Eotech) with a 50 mm field of view. Rectilinear equidistant parallel lines were projected on the subject's lip. The inferior lip was numerically extracted for further volume and roughness analysis.
Repeatability and reproducibility were previously assessed.
Short term efficacy testing was performed on 15 women 30 and 60 minutes after two applications of a lip product (LP1) separated by 90 minutes. For long term efficacy evaluation, a lipstick (LP2) containing a combination of active ingredients was tested on 27 women vs. a placebo group during 1 month.
Results: After the first application of LP1, the rugosity parameters Ra and Rz were significantly lower. After the second application, rugosity parameters decreased and volume increased significantly.
Long term application of LP2 showed a significant decrease of every roughness parameters and a significant increase of volume vs. placebo after 1 month of application.
Conclusions: Thanks to its speed and precision, 3D acquisition of the lip volume by fringe projection was shown to be useful tool for quantification of lip product efficacy. This methodology is adapted to the evaluation of short term improvement due to, for instance, to emollient or smoothing effect of the lip product. It could also detect improvements linked to long term effect of lip care.
Keywords: Lip, 3D, Fringe projection

P67
Noninvasive techniques for the evaluation of the facial volume
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Background: Due to the technological advance in science, many kinds of the instruments regarding noninvasive skin evaluation methods have been developed and used. For instance, colorimeter and mexameter which are used to compare skin color, TEWAmeter which is used to analyze epidermal damage and tonometer which is used to evaluate elasticity. These have been widely considered as a reliable and objective method for evaluating the effect of cosmetics and dermatologic procedure before and after. However, for the volume change, it difficult to assess the effect unless you are the well trained evaluators since there is no other way except simple photography compare method.
Purpose/Methods: We have difficulty in the objective assessing for the volume. We would like to introduce several various applications using Vetra 3D (Canfield Scientific Inc., USA) which is recently developed system for the facial volume evaluation method.
Results/Conclusions: We were able to detect the facial volume changes in several different conditions. So this system could be a useful and objective system to assess volume change.
Keywords: Vectra, Non-invasive imaging, Volume

Mechanical properties (friction and elasticity)

P68
Effect of the subcutaneous tissues on the skin mechanical properties
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Purpose: Knowledge about the human skin mechanical properties is essential in several domains, particularly for dermatology, cosmetic, to detect some cutaneous pathology or to correctly protect the skin against external mechanical aggressions, which is ensured by a reversible deformation of its structure. This study proposes a new

method to determine the human skin mechanical properties *in vivo* using the indentation test. Usually, the skin mechanical parameters obtained with this method are influenced by the mechanical properties of the subcutaneous layers, like muscles.

Methods: In this study, different mechanical models were used to evaluate the effect of the subcutaneous layers on the measurements and to extract the skin elastic properties from the global mechanical response. To measure the skin mechanical properties *in vivo*, a specific indentation device has been developed.

Results: The obtained results demonstrate that it is necessary to take into account the effect of the subcutaneous layers to correctly estimate the skin Young's modulus. Moreover, the results illustrate that the variation of the measured Young's modulus at low penetration depth cannot be correctly described with usual one-layer mechanical models. Thus a two-layer elastic model was proposed, which highly improved the measurement of the skin mechanical properties.

Conclusions: This study shows that for high penetration depth the one-layer mechanical model is sufficient to correctly estimate the skin mechanical properties, but for low penetration depth a refinement of the model, which consists by considering a two-layers mechanical model, is necessary. It permitted to correctly describe the elastic behaviour of the skin until a penetration depth equal to its thickness.

Keywords: Biomechanic, Mechanical model, Young's modulus, Indentation

P69
Evaluation of skin mechanical properties by determining of resonant frequency and loss resistance with tactile sensor

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Purpose: Was to clarify the characteristics of resonance frequency change (⊿f) and loss resistance (Zr) by determining the mechanical properties of skin with a tactile sensor (Venustron® Axiom Inc., Japan), which is a device used to elucidate the mechanical characteristics of skin based on implementation of a resonance circuit and piezoelectric oscillator.

Materials/Methods: Two different experiments were performed with 30 healthy Japanese males as subjects. In Experiment 1, ⊿f and Zr were measured at different body sites, including the cheek, forehead, medial upper arm, lateral forearm, and heel. In Experiment 2, those parameters were determined at the cheek and medial upper arm both before and after lotion application. In both experiments, we also measured other parameters of viscoelasticity, including Uf and Ur/Uf with a Cutometer®, and resonance running time (RRT) with a Reviscometer®.

Results: Results of skin viscoelasticity skin shown by ⊿f, Uf, Ur/Uf, and RRT vales showed similar tendencies for the different body sites, while Zr was different from the other parameters. In addition, ⊿f changed significantly after application of lotion, in contrast to the other parameters.

Conclusion: The results obtained with a tactile sensor showed that ⊿f and Zr differ from other generally used parameters of viscoelasticity determined with a Cutometer® and Reviscometer®. Based on our results and the characteristics of the device, we consider that these two parameters are able to separately reveal the viscous and elastic characteristics of skin.

Keywords: Skin, Resonance frequency change, Loss resistance, Elasticity, Viscosity

P70
Skin softness and visco-elasticity measurements with frequency shift technique

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Background: Skin mechanical properties are very important parameters of healthy skin, and good markers of skin aging. They also affect the appearance and the perception of skin. Skin mechanical properties are determined by the quality and quantity of the different layers: epidermis, dermis and subcutis that affected by the aging, body site, external conditions and diseases. They are also strongly affected by the skin hydration condition. Quantitative measurement of skin mechanical properties has been used effectively in the investigation of physiological changes in skin tissue structure and function to determine the skin condition, the treatment efficacy.

Purpose: Traditional devices of skin mechanical property measurement have been designed to measure the stress-strain relationship that induced by a suction, a stretching, a pushing or a torsion. Recently there is a technique (Venustron) that not only measures the stress-strain of skin, it also utilizes a vibration sensor to measure the acoustic frequency shifts due to differences in acoustic impedance of the skin. In this report, we will discuss the *in-vivo* application of this technique.

Methods: Measure the skin softness and visco-elasticity on the arm, leg, cheek and chest of subjects in a young group and an old group with Venustron.

Results/Conclusion: We found out that there are the significant differences of mechanical properties between different body sites and there are also some differences in body sites between the age groups.

Keywords: Skin softness, Visco-elasticity, Acoustic frequency shift, Body site, Age

P71
Dermis structures change under skin

compression: age effect

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Purpose: During the course of ageing, the skin is transformed in terms of composition, structural organization, size and naturally, its properties. Regarding biomechanical skin change, several studies have revealed that during the course of ageing, the skin becomes less extensible, and less able to return to its initial shape after being subjected to deformation.
The purpose of the present study was to investigate the effect of a horizontal wrinkling on skin structures using ultrasound imaging. The influence of age on skin structures change after application of the stress was also studied.
Materials/Methods: 48 women were enrolled in this study and distributed in 2 age groups of 24 women as follows:

- Younger group [18-30y]
- Older group [45-60y]

In order to apply a horizontal wrinkling on the skin, a new device, the Densiscore© , was used on the ventral forearm of the subjects. This system developed by ourselves, allows standard compression of 42% in the plane of kin. It comprises of 2 blocks affixed to the skin by means of double-sided tape. The 2 blocks can be moved towards each other or away from each other, encompassing an area of skin to be examined using an ultrasound imaging. This device equipped with a high frequency broadband transducer working at 25MHz and providing axial resolution of 75µm at -6dB. The ultrasound characterization in the dermis was obtained from 3 separate rectangular regions of interest (ROI) of equal thickness (33% of the whole skin thickness) coming from the surface echo of the skin to the dermis-hypodermis junction. Within each of these regions of interest, the mean grey values within the images were determined before and during the skin compression.
Results: We have revealed significant change of the skin thickness, as well as structural echogenicity of the dermis during the skin compression. The skin thickness increased and the echogenicity of the dermis decreased with variable amplitude according to the 3 regions of dermis interest selected.
Regarding the age effect, differences were also revealed between the 2 age groups especially in the region of interest located under the surface echo of the skin, corresponded to the upper dermis.
These results will be presented in details and related to the composition of the skin, in order to explain the change observed, in terms of fibers network and water content.
Conclusion: This study has also pointed out that this system combining a skin compression device with an ultrasound imaging technique seems to be a convenient and fast way to investigate both the mechanical behavior of the dermis and skin age effect, and in this way will be very useful to evaluate anti-ageing products efficacy.

Keywords: Skin compression, Ultrasound imaging, Age effect

P72
Dermis water mobility investigated under skin indentation: age effect

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Purpose: Aging process induces changes in skin structures and water mobility within the dermis leading less skin elasticity with age. The purpose of this study was to investigate the effect of an indentation on the structures of the skin using an ultrasound imaging system. The influence of age on skin structures change after application of the indentation stress was also studied.
Matrial/Methods: 48 women were enrolled in this study and distributed in 2 age groups of 24 women as follows:

- Younger group [18-30y]
- Older group [45-60y]

The skin indentation was done by using a new device called the DermoTrace©. A grid, made of seven grooves of 2mm width, punched out from a disk of 30mm in diameter, was pressed on to the skin under a controlled 1.5kg pressure for 45 seconds.
Then investigation of the skin structures during the indentation was performed in real time by an ultrasound investigation system on the compressed site.
More precisely, before and 2, 5, 10, 15, 20, 25 and 30 minutes after removing of the grid of the DermoTrace©, echographic image of the dermis tissue was recorded and analyzed. This was done using an homemade imaging ultrasound system equipped with a high frequency broadband transducer working at 25 MHz and providing axial resolution of 75µm at -6dB.
The ultrasound characterization of the dermis was obtained by analyzing the echogenicity of the dermis on different regions of interest from the surface echo of the skin to the dermis-hypodermis junction, as well as the determination of an ultrasound pseudo-attenuation index..
Results: Regarding the skin surface, we revealed that the indentation of the grid into the skin was significantly higher and lasted longer for the older group than the younger one

- No change of the echogenicity of the superficial dermis has been revealed for both age groups after the indentation of the grid
- Significant change of the echogenicity of the deep dermis has been revealed for the older group after the indentation, while no change was observed for the younger group. Thus, for the older group a drastic decrease of the echogenicity was observed 2mn after removing of the grid with a return to the original state 30mn later.

Conclusion: These results will be presented in details

and discussed in terms of dermis water mobility versus age.

Keywords: Skin indentation, Ultrasound imaging, Age effect

Skin function (barrier and hydration)

P73
Skin homeostasis assessment *in vivo* by tcpO2 measurements

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Background: Experimental dermatology has tried to find one objective indicator of normal skin homeostasis. This indicator would be a measure of the tissue "vitality" (breathability, metabolic capacity, or other) and help to define "healthy skin". The immediate interest (clinical, aesthetical, technological) of this indicator is obvious but, the lack of complementary technology as practically reduced the assessment to tcp O_2 measurements obtained at skin surface in normal breathing conditions. However, breathing in a 100% O_2 saturated atmosphere, may offer new insights allowing not only to assess the maximum O_2 reaching the tissue but, by mathematical modeling, to know about the O_2 distribution from the blood to the tissue.

Purpose: To design a mathematical model allowing to quantitatively characterize the two components of the local microcirculation: flow and oxygen (bio)availability.

Methods: Healthy volunteers, both gender (n=60, mean: 31,5 + 14,5 y.o.) were included, after informed written consent. The protocol involved a basal measurement followed by a dynamical provocation by breathing an atmosphere of 100% Oxygen during 10 min and recovery (normal air breathing). All the evaluation was performed in the most peripheral territory (foot). Chosen variables were Trans Epidermal Water Loss (TEWL Tewameter TM300), blood flow (LDF, Periflux PF500) and transcutaneous gases (tcpO2 and tcpCO2, Periflux PF5000). A bicompartmental model was applied to the tcpO2 data allowing to obtain the most relevant parameters (t1/2 distribution). Mean values and statistical analysis were obtained with MS Excel and SPSS. Non linear regression was calculated using Winnonlin. The significance level was set to 95%.

Results: Following provocation, a tcpO2 increase was observed, saturating after 10 min. During this period LDF values decreased by a local vasoconstrictor effect. Data modelling suggests a fast distribution of the oxygen from the blood to the tissue (low t1/2 distribution) that is clearly slower in the older volunteers. This fact, suggests a decrease in the metabolic capacity of the tissue, which may be further explored as a predictor of skin homeostasis or its alteration (e.g. pathophysiological process such as ageing)

Conclusions: The proposed model fits remarkably well to data, allowing to obtain dynamical parameters, such as t1/2 distribution, that can be used as a quantitative indicators of skin health.

Keywords: Homeostasis, tcpO2, Bioavailability

P74
Skin capacitance imaging for surface profiles and dynamic water concentration measurements

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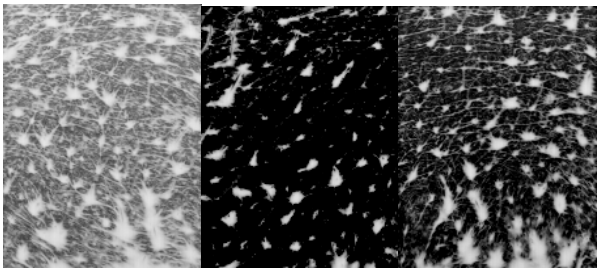
Background: Previous studies show that capacitance based Fingerprint card sensors, originally designed for fingerprint imaging, can be used for skin hydration imaging, surface analysis, and skin micro relief measurements. In this paper, we present our latest work on stratum corneum (SC) 3D surface profiles and SC dynamic water concentration measurements by using Fingerprint card sensors.

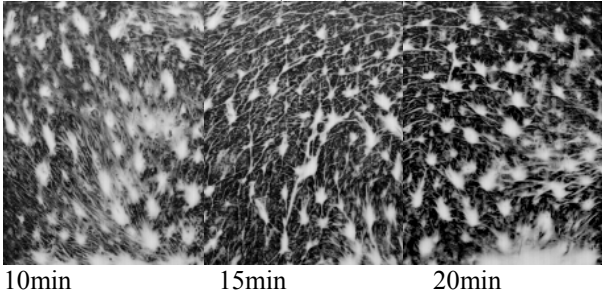
Purpose: The purpose in this study is to develop a new method for SC 3D surface profiles and SC dynamic water concentration measurements.

Methods: SC dynamic water distribution is achieved by immersing test skin sites in room temperature water for 20 minutes, three skin sites (face, thumb and volar forearm) are studied, and measurements are performed both before and periodically thereafter.

Results: Fig. 1 shows the skin images of a face skin test site, before and after the immersive hydration. By analysing the image grayscale values, we can also show the 3D skin surface profiles, and how they change with the water content. The results show that thumb skin site has the most significant hydration increase during the immersive hydration, it is also the quickest to recover to its normal hydration level. While face and volar forearm skin sites have also hydration increases, the face skin site is the slowest to recover.



before after (0 min) 5min



10min 15min 20min

Fig. 1 Skin images of a face skin test site before and after a 20-minute immersive hydration.

Conclusions: Fingerprint card sensors can be used for SC surface profile and SC dynamic water concentration measurements. The different dynamic water distributions reflect different skin sites' different characteristics, such as SC water holding/binding capabilities and its barrier functions.

Keywords: *In vivo*, Skin surface profiles, SC water content, Fingerprint card, Opto-thermal, Condensor-TEWL method

P75
Changes in the depth profile of water in the stratum corneum treated with water

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Background/Purpose: Dermatologists and cosmetic scientists are increasingly interested in the stratum corneum (SC) hydration, because SC plays an important role in keeping the skin surface soft and smooth. Information regarding the molecular composition of skin can be obtained non-invasively by a latest developed confocal Raman spectrometer. In this study, we investigate changes in the depth profile of water in SC treated with water by Raman spectroscopy. We also unveil a relationship between the depth profile patterns and the cutaneous sensation.

Methods: Depth profiles of Raman spectra in the region 2,600 - 4,000 cm^{-1} were obtained using a 671-nm laser at 2 μm intervals from the skin surface towards the interior with a confocal Raman spectrometer (Model 3510, River Diagnostics BV, The Netherlands). Water content (mass%), expressed in grams of water per 100 g of wet tissue, was calculated from the water-to-protein ratio of the Raman band (Caspers et al., 2000). Skin surface temperature was measured by an infrared irradiation thermometer (IT-340S, HORIBA, Japan). Changes in the depth profile of water after water treatment by cotton mask for various time periods and the skin temperature were measured at 1 and 10 min after the water application to the skin surface. In addition, questionnaires regarding cutaneous sensation were obtained in various water-depth profile patterns.

Results: Water content in the middle to lower part of SC increased with the increase of the water-application time. Warming skin during the water application increased the water penetration amount, the depth, and the holding time in SC. In addition, the increasing water content in the upper part of SC was associated with cutaneous sensations, "hydrate" and "refresh" feelings. On the other hand, the increasing water content in the middle and lower part of SC was associated with "refresh to deeper area" and "firm" feelings.

Conclusions: The water content in the upper part of the SC was mainly changed in daily environmental conditions (Egawa et al., 2008). However, when water was externally applied, water content both in the upper part of the SC and under middle part of the SC was increased with the increase of the water-application time. In addition, the temperature of applying water affected the penetration depth, the amount, and the holding time of water. Thus, we could control the depth profile of water by externally applying water. The location of water also affected cutaneous sensations.

Keywords: Raman, Skin, Temperature, Water

P76
Biophysical functions of the stratum corneum of the areola skin

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Background: The areola skin is often to be involved lesion in atopic dermatitis (AD) patients and it is considered as one of minor clinical features of AD.

Purpose: To elucidate the functional properties of the stratum corneum (SC) of the areola skin with non-invasive methods.

Methods: Nine male aged 20-28 years with AD (AM; atopic males), 9 female aged 20-24 years with AD (AF; atopic females), 9 normal males aged 20-33 years (NM; normal males) and 9 normal females aged 21-34 years (NF; normal females) were included. High frequency conductance, a parameter for skin surface hydration, transepidermal water loss (TEWL), skin surface lipid level were measured on the areola skin and the adjacent skin with Skicon 200 EX, DermaLab and Sebumeter SM 825, respectively. Size of the corneocytes obtained with adhesive tape from the skin surface of the areola and adjacent skin were measured. None of AD patients had inflammatory skin lesion on the test sites.

Results: In all groups, TEWL was significantly higher on the areola together with smaller corneocytes of the skin surface than on the adjacent skin especially in AM. High frequency conductance was significantly lower in AM and higher in NF on the areola than on the adjacent skin. There was no significant difference in conductance between the areola and the adjacent skin in AF and NM. Skin surface lipid level was significantly higher on the areola than on the adjacent skin in females both atopics and normal controls.

Conclusions: Biophysical functions of the SC on the areola skin were proved to be impaired as compared with the adjacent skin. Due to the impaired function of the SC, the areola skin is often affected in AD.

Keywords: Stratum corneum, Areola, Hydration, TEWL, Skin surface lipid, Atopic dermatitis

P77
A novel method to evaluate liquid soap performance in cleansing soiled hands

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Background: Liquid hand cleansers are designed to quickly and easily remove dirt and discoloration. A standardized method is needed to assess the cleansing efficacy of a variety of cleansers.
Purpose: The primary objective of this study evaluated the effect of washing time on hand cleansing using a standard soil. A secondary objective compared the cleansing efficacy of three liquid soap products.
Methods: This was an evaluator blinded, three-way crossover design conducted in 15 subjects. Subjects were randomly assigned to one of three treatment groups on each of three test days. A test day consisted of three supervised washing cycles where the subject was randomly assigned a washing time sequence using one test article. The standardized soil consisted of a 21 gram mixture of silicone dioxide (black chalk) and a marketed hand lotion. Soil was uniformly applied to a 2.25 in^2 area of the right or left palm prior to each supervised wash. The mixture was allowed to dry for approximately 15 minutes prior to washing. Hand cleanliness was assessed at baseline, following soil application and following the supervised wash. Subjects washed for 5, 12 or 20 seconds according to a randomization scheme. Cleansing efficacy was assessed by skin reflectance measurements (Minolta Chroma Meter®) and subjectively by technician and subjects.
Results: Longer wash times improved the overall cleanliness of the hands regardless of the soap used. Chromameter readings detected statistically significant differences in efficacy between wash times and treatments. The standardized soil, along with objective and subjective measures of efficacy, allowed for discrimination between products in terms of overall efficacy.
Conclusions: Skin reflectance measurements showed that wash time is a significant factor in hand cleansing. Subjective assessments of efficacy, both subjects and technician, correlated well with objective measurements.
Keywords: Minolta chroma meter, Liquid hand soap, Cleansing efficacy

P78
Analysis of the skin hydration states using high resolution MR microscope

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Background: Magnetic Resonance (MR) technique has been rapidly developed, and Magnetic Resonance Image (MRI) is now the most versatile non-invasive diagnostic tool with a much higher resolution than other imaging modalities. The technique which analyses stratum corneum plays a highly important role in determining skin hydration effects, new methodology was developed for measuring the skin hydration effects directly and objectively using 3T high resolution MR microscope. Modifications of high sensitivity RF coil and gradient coil in the high resolution MR microscope are necessary to evaluate skin hydration states. A planar RF and a gradient coil system were modified to acquire high resolution MR images.
Purpose: In this study, a high resolution MR microscope was used to evaluate quantitatively the efficacy of moisturizers with rapid test in 'before and after' moisturizer.
Materials/Methods: Because the technique which analyses stratum corneum plays a highly important role in determining skin hydration effects, new methodology was developed for measuring the skin hydration effects directly and objectively using 3T high resolution MR microscope. Modifications of high sensitivity RF coil and gradient coil in the high resolution MR microscope are necessary to evaluate skin hydration states. A planar RF and a gradient coil system were modified to acquire high resolution MR images. In this study, a high resolution MR microscope was used to evaluate quantitatively the efficacy of moisturizers with rapid test in 'before and after' moisturization. It has been compared between the results of high resolution MR and conventional bioengineering devices.
Results: It has been compared between the results of high resolution MR and conventional bioengineering devices. The results by the classical bioengineering methods were similar to those obtained by MR imaging analysis and confirmed the reliability and validity of high resolution MR spectroscopy.
Conclusions: The results by the classical bioengineering methods were similar to those obtained by MR imaging analysis and confirmed the reliability and validity of high resolution MR spectroscopy. Additionally High resolution MR microscopy may be very useful tool to evaluate the hydration states of stratum corneum.
Keywords: MRI, Human Skin, Stratum corneum, Hydration

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P79
Barrier integrity testing with a condenser-chamber TEWL instrument

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The aim of this study was to assess the performance of a condenser-chamber TEWL instrument for barrier integrity testing. According to Netzlaff *et al* [1] open-chamber TEWL measurements appears to be of limited use for such tests, being able to detect only severe damage in the samples they examined. Problems identified included topically adhering water and the permeation of condensed water via capillary action through deliberately made pinholes in artificial membranes.

Topically adhering water is less of a problem with the condenser-chamber TEWL method, because the chamber microclimate provides consistent conditions for rapid evaporation, irrespective of ambient humidity. Topical water shows up as a transient peak in the recorded water vapour flux time-series curve, whereas the water diffusing through the membrane generally settles to a lower level. Such flux curves give detailed information about the properties of the membranes and the validity of the tests.

We will report measurements using both artificial membranes (Sil-Tec and PTFE) and bio-membranes (excised human stratum corneum and snake sheddings) to illustrate the capabilities of this approach.

Keywords: Membrane, Barrier integrity, TEWL, Condenser-chamber, Open-chamber

Reference

1. Netzlaff F, Kostka KH, Lehr CM, Schaefer UF. TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz type diffusion cells *in vitro*. Limitations shown by transport data testing. European Journal of Pharmaceutics and Biopharmaceutics 2006; 63: 44-50.

P80
Is TEWL a flow related variable?

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Background: Several transcutaneous variables obtained by non invasive methods are known to be flow related. Recently an inverse relationship between TEWL and Flow, obtained by Laser Doppler Flowmetry under controlled conditions was described, suggesting that the local haemodinamical perfusion conditions do influence this indicator of the cutaneous barrier. The complex mechanisms ruling these factors are not entirely identified but, the influence of thermoregulation over the skin physiology is known.

Purpose: The present study was designed to dynamically obtain local flow and TEWL *in vivo* signals during local heat provocation, in order to further understand the nature of that TEWL-Flow inverse relationship.

Methods: Healthy female (n=37, 18-35 years) were tested after informed written consent, and submitted to two experimental protocol groups: I (n=27) basal posture in the seated position evaluation, followed by passive leg elevation at 90º (10 min), then returning to the basal position (10 min); II (n=10) basal skin temperature evaluation (10 min), local provocation at 44ºC (40 min), return to basal temperature (10 min). Trans Epidermal Water Loss (TEWL Tewameter TM300) and microcirculation blood flow (LDF, Periflux PF500) were measured. Mean values and Pearson correlations between basal and provocations were calculated by MS Excel and SPSS. The significance level was set to 95%.

Results: The passive postural change revealed an inverse significant relationship between TEWL and LDF as described before, probably due to a local adaptative regulation involving the Starling forces ruling the fluid homeostasis at the measuring area. After heat provocation a direct relation between TEWL and LDF values was obtained. This fact suggests that local vasodilation induced by heat, alters the local homeostasis, definitely involving the Starling forces in the process, and overriding the inverse relationship between TEWL and LDF.

Conclusions: The local flow conditions influences TEWL depending on the hemodynamic modification involved. Nevertheless these relationships may find other expression in some special conditions such as aging or peripheral vascular disease.

Keywords: TEWL, FLOW, TEWL/LDF relation, Temperature effect

P81
Dynamical LDF/tcpO2 relationships as a quantitative indicator of peripheral vascular function *in vivo*

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Background: Studying Peripheral Vascular Disease has involved LDF and tcO2 (static) measurements. The immediate application of this information is mostly clinical, regarding the stage of the pathology or an eventual intervention (e.g. amputation decision). However, many other information maybe extracted from the analysis of flow variables relationships (LDF and tcpO2) especially if obtained from dynamical assessments *in vivo*.

Purpose: To test a non-invasive experimental model based in dynamical provocations that can be used to obtain predictors of the microcirculation functional state.

Methods: Healthy volunteers, both gender (n=60, mean: 31,5 + 14,5 y.o.) were included, after informed written consent, fulfilling 2 experimental protocols: (I) testing the effect of a passive postural change, from decubitus, passive elevation of the leg at 45º; (II) testing

the effect of a suprasystolic pressure occlusion of the ankle, followed by reactive hyperemia.
All the measurements were taken continuously, in the foot, and data obtained in 3 moments: basal, after dynamical provocation and recover to basal condition. Chosen variables were Trans Epidermal Water Loss (TEWL Tewameter TM300), local blood flow (LDF, Periflux PF500) and transcutaneous gases (tcpO2 and tcpCO2, Periflux PF5000). Mean values and statistical analysis were obtained with MS Excel and SPSS. The significance level was set to 95%.
Results: Both protocols reveal the same profile variation, with a significant decrease of the LDF and tcpO2 values, followed by a reactive hyperemia. However, the magnitude of the suprasystolic occlusion is clearly different. In normal conditions, LDF and tcpO2 values recover to the initial levels, suggesting a normal vascular function. Decay rates of tcpO2 and LDF during the hyperemic responses shows slower decays for the older volunteers when compared with the younger ones. This maybe explained by the pathophysiological process (aging) effects since other clinical evidences were absent and the volunteers classified as healthy.
Conclusion: The experimental model is rather easy and fast to apply, allowing to assess a physiological adaptation (dynamical) process *in vivo*. It maybe useful to characterize the peripheral vascular condition of the individual in very early stages of impairment, simply by studying the LDF-tcpO2 relationships.
Keywords: Flow, tcpO2, LDF/tcpO2 relation, Vascular function

P82
The GlaSbox® registration chamber allowing measurement of contractile forces generated by fibroblasts

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Beside their primarily role in extracellular matrix production, fibroblasts exhibit some features of smooth muscle cells such as alpha-smooth muscle actin (αSM actin) filament bundles, suggested that they might produce the force of wound contraction. An in vitro model that is considered to be an equivalent of the process of wound contraction has been established. Bell et al. reported that the incorporation of fibroblasts in a collagen gel induces a progressive contraction of the gel, resulting in the formation of a dense collagen disc, called retracted lattice.
We have developed a new device, the GlaSbox®, which can measure directly the forces exerted by fibroblasts on the collagen gel. The culture model used is a tense lattice in which cells are seeded within a collagen matrix immobilized at its extremities. Contraction is inhibited and lattice formation is associated with the production of internal tension. Such tense lattices are more closely related to skin than retracted lattices in terms of both internal structure and mechanical behaviour, because skin is also permanently under tension. The GlaSbox® cell chamber is composed with 8 rectangular culture wells in which lattices developed. Two opposite silicon beams hang down into each well at a distance of 27 mm apart. The lattice is attached to this sensor through a grid directly etched on the lower part of the beams. A strain gauge is deposited at the beam surface and connected to form a Wheastone bridge. The strain gauges signal output is amplified then converted and collected by a computer which included an acquisition card and a specific program for giving directly the forces in real time.
Different cell sources have been studied to test the idea that the ability of fibroblasts to generate contractile forces varies between populations. Fibroblasts from early striae distensae generated strong contractile forces contrary to fibroblasts from wrinkles which exhibit significant reduced contractile capacities.
Keywords: GlaSbox®, Fibroblasts, Contractile forces

P83
An apparatus to control environmental parameters influencing xenobiotics permeation through human skin

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Molecules penetration through human skin depends on their physical-chemical proprieties and the skin conditions. In the literature, it is confirmed that polarity of molecules is an essential factor which affects their penetration and permeation. Furthermore, some authors demonstrated that increase in temperature and humidity enhances molecules skin permeation. A number of apparatus have been employed for measuring *ex-vivo* chemicals percutaneous absorption. Most of experiments did not take into consideration the variability of environmental parameters during skin exposition. The change of exposure conditions from a laboratory to another gives disparate results. Consequently, an *ex-vivo* technique with a control of the surrounding conditions, could allow replicating the microclimate above the skin during its exposition to chemicals. The aim of our work is to present an original apparatus to assess human skin permeation and retention of compounds under controlled environmental conditions (temperature, humidity, concentration). With this apparatus we demonstrated that butoxyethanol vapours crossing the skin epidermis-dermis layers change in respect of different environmental conditions (Temperature 23.5 °C, Relative humidity 20%, 55% and 88%) : The cumulative quantity in receptor fluid in Franz cells after 8 hours of exposure to butoxyethanol vapours was 20.2 ± 0.51

(μg/Cm^2/8hours) at 20% relative humidity, 22.4 ± 0.7 (μg/Cm^2/8hours) at 55 % relative humidity and 25.2 ± 0.45 (μg/Cm^2/8hours) at 88% relative humidity. Such a device is very useful to create many situations encountered at different scenarios of skin exposure to chemicals, including drugs and cosmetic preparations.

Keywords: Measuring device, Environmental parameters, Human skin permeation

P84
Wettability of Vernix Caseosa: *ex vivo* studied

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Introduction: Well before birth, the fetal skin is protected from the external environment (amniotic liquid) by a specific layer called Vernix Caseosa (VC). This protection begins towards the 24th gestation week and is made of a layer containing up of 80% water, 10% lipid and 10% protein.
The water contact angle θ_w is a indicator of the surface hydrophobic/hydrophilic tendency.
Objective: The objective of this work was to quantify the hydrophilic/hydrophobic tendency of VC in order to compare it with in vivo human skin wettability.
Materials/Methods: The water contact angle θ_w was measured on the fleshly VC by a tool specially conceived for *in vivo* and/or *in vitro* wettability studies.
Results: The water contact angle θ_w was about 88°. This value classed VC within hydrophobic tendency surfaces.
Discussion: In this work, our results were closed to those in the literature. The values classed VC as a surface of hydrophobic tendency compared to that measured on the volar forearm (poor in sebum). In the forehead (rich in sebum) the θ_w = 55°.
Conclusion/Perspectives: The physicochemical parameters of VC are very recent (2001) and less published.
However, in order to best understand the exact nature, role and for future exploitation of this material, both faces not yet studied till today, should be tested.
The bibliographic data show that VC material may be used *in vivo* for dry skin hydration and could help in anti microbial and innate immunity defense.

Keywords: Vernix Caseosa, Water contact angle, Wettability, Human skin, Hydrophobic/hydrophilic balance

Skin histo-morphological correlation with optical

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Claim support for cosmetic products should be based on scientific evidence. Optical Coherence Tomography (OCT) is a promising non-contact, non-destructive diagnostic technique for skin imaging in vivo. The purpose of this study is to endorse OCT as a skin morphological analysis technique with the potential to substitute invasive procedures such as punch biopsies with similar accuracy as histological analysis. For this purpose, ear pigskin were sewed to copper rings and a surgical suture was used as a fixed marker. Our OCT device has a Ti:Al2O3 laser with a central wavelength of 800nm, transverse resolution of 10μm and longitudinal resolution of 10μm. We collected series of OCT images oriented at right angles to the course of the suture 1mm after the entrance of the suture. Samples were embedded in paraffin to obtain histological slices with 6μm thick and stained with hematoxylin and eosin for comparison purposes. We were able to positively identify the epidermal layers such as stratum corneum, deeper epidermal structures, dermal papillae and dermal-epidermal junctions both in OCT and in histology. The thickness of the stratum corneum and epidermis measured by OCT and histology were not statistically different, confirming thus the viability of OCT for skin analysis. Our method may provide quantitative and histological information the potential of efficacy of cosmetic and or cosmeceutical treatments on different skin layers. Thus, OCT imaging appears to be a reliable, quick and non invasive technique to analyze several skin treatments. OCT could represent a new innovative way to diagnosis and detect benefits of topical treatments.

How to assess anti-cellulite treatment?

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Background: Cellulite is a common condition affecting over 80% postpubertal women. It is characterized by a dimpled or "orange-peel" appearance of the skin. It is frequently found on the thigh and buttock regions ("culotte de cheval") and is associated to a bad lymphatic drainage.
Purpose: The aim of the paper is to present assessing complementary solutions to evaluate cellulite treatments effectiveness through symptoms quantification.
Methods: -Reminder: clinical evaluation
The grade of cellulite can be evaluated on a scale of listing 4 points, none (no cellulite), mild (pad only to pinch), moderate (pad standing), high (pad standing and lying).
-Thigh circumference measurement
This technique enables to follow the thigh circumference evolution. The measurement is always taken at a specific

distance from the soil (on an anatomical landmark) with a meter of seamstress.
-Fringes projection
The roughness of the padding as well as the volume or surface of the thigh in relation to a reference height can be calculated from an interferometry technique, the fringe projections. This in vivo system (DermaTop, Eotech, France) projects fringes on the test surface. The surface irregularities distort the fringes proportionally to the relief. Computerized algorithm reconstitutes then the skin relief by calculating the height of each spot of the surface.
-Contact thermography
Thermographic images (Thermo-cell-test® system) allow to split up volunteers into four fundamental groups (none (no cellulite), mild (pad only to pinch), moderate (pad standing), high (pad standing and lying)). The method -easy to apply- consists in placing upon the skin surface special plates containing cholesterol microcrystals (encapsulated) whose colors change according to the temperature; different stage of cellulite are thus observed.
-Photography
The macrophotography of the buttocks muscles relaxed and contracted can be achieved to follow and compare cellulite state at different times; this necessitates that the attitude of the subject reveals the cellulite structure, and requires rigorous conditions standardization (clothes, light and distance…).
Conclusion: In associating these different techniques, it is possible to quantify and illustrate the effects of an anti-cellulite treatment.
Keywords: Cellulite, Thermography, Fringes projection

Non invasive methods of assessing wheals and other edematous lesions of skin

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Background: The assessment of wheal and other edematous lesions on human skin has traditionally been done clinically and by measuring the size, transepidermal water loss and erythema. The advancements in biophysical and medical technology have made it possible to study this phenomenon in more detail. The edematous lesions invariably have accumulation of fluid at various levels of skin and are generally associated with inflammatory processes. Some of these lesions are prominently raised above the skin surface e.g. the sub-papillary dermal wheal whereas some of these lesions, e.g. those associated with irritation, are more subtle. In these lesions, there is a marked change in skin structure and optical properties. Generally these lesions occur as a result of a disease or contact with an allergen or a toxic chemical. Mechanical trauma and exposure to certain radiation, e.g. ultraviolet light, can also induce these lesions.
Purpose: To explore new and advanced technology to study the wheal and other edematous lesions on skin.
Methods: In this study the edematous lesions were produced experimentally and then evaluated using a variety of non-invasive biophysical techniques: Diffuse Reflectance Spectrophotometry, fringe-projection device, ultrasonography, optical coherence tomography, confocal microscopy, ballistrometry, laser Doppler blood flow imaging etc.
Results: Using a spectrophotometric technique, the start, maturation and the resolution of a wheal were followed. The surface structure and shape of the wheal was studied using a fringe projection device. A variety of imaging techniques were used to visualize the sub-surface structural changes in skin. The Ultrasound device showed the changes in the echo-density in the dermis. Optical Coherence Tomography showed cross-sectional structure with evidence of fluid accumulation. Horizontal sections, using Confocal Microscopy also revealed the structure of the lesions. Changes in visco-elastic properties were measured using a ballistometer. Laser-Doppler blood flow imager documented the spatial dimensions of the inflammatory reaction.
Conclusion: The use of advanced biophysical measurements enhances our ability to study edematous lesions.