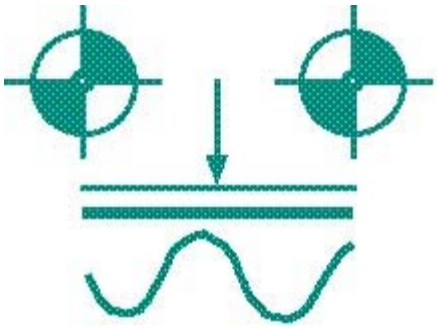

PROGRAM

U.S. Technical Symposium of
The International Society for Biophysics
and Imaging of the Skin

Westin Hotel Dallas Galleria, *Dallas, TX*
March 18-21, 2009

Scientific Committee

- **Ernie Braue, Ph.D. (Chairman)**
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- **Martha Tate, Ph.D.**
- **Stacy Hawkins, Ph.D.**
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Welcome to the USA 2009 symposium of the International Society for Biophysics and Imaging of the Skin.

As the local hosts of the symposium Barry Reece and I want to welcome you to Dallas Texas! Whether this is your first encounter with a meeting of the Society or like some of us you've been attending meetings since the Society was formed in the '70's, you are bound to learn something new that can inspire your work when you return home. This is a "small" meeting, so we have planned plenty of opportunity to discuss shop informally. Please take advantage of this opportunity and meet some colleagues you didn't know you had.

All podium sessions will be held in Dallas Ballroom 1. The other activities will take place in the Dallas Foyer and Ballroom 2.

David Miller

Barry Reece

Things to do:

SHOP- The Westin Hotel is directly connected to the Galleria Dallas. More than 200 of the most celebrated shops from around the world along with delectable restaurants and eateries surround an impressive ice rink, a genuine Dallas experience - world-class shopping at its best!

CHECK OUT DALLAS CULTURE- Downtown Dallas is just a 40 minute bus ride from a stop directly across from the hotel on the parkway: walk to JFK Memorial, 6th Floor Museum, Dallas Museum of Art, Nasher Sculpture Center, Meyerson Symphony Center.

GETTING AROUND- DART Bus 183 runs between the Hotel area and downtown Dallas, $1.50 one way. Taxi fare to down town (non rush hour) is about $35. Airport transfers by Taxi (~$40) and Super Shuttle shared ride ($28) .

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U.S. Technical Symposium of
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U.S. Technical Symposium of
The International Society for Biophysics and Imaging of the Skin

Westin Hotel Dallas Galleria, Dallas, Texas March 18-21, 2009

SCHEDULE OF EVENTS

Wednesday, March 18, 2009

12:00-3:00 **Technical Showcase setup** in Dallas Ballroom 2

10:00-5:00 **Registration in** Dallas Foyer 1

1:00-5:00 **Pre-Symposium Workshop** in Dallas Ballroom 1
Moderator: Ernie Braue

1:00-1:30 Photography and 3-D Imaging by **Stacy Hawkins**

1:30-2:00 Electrical Properties by **Randy Wickett**

2:00-2:30 Evaporative Water Loss by **Gary Grove**

2:30-3:00 Mechanical Properties by **Randy Wickett**

3:00-3:30 Confocal Raman Spectroscopy by **Joachim Fluhr**

3:30-5:00 **Hands-on Demonstrations** in Dallas Ballroom 2

5:00-7:00 **Symposium Opening Wine and Cheese Reception** in Dallas Ballroom 2 (sponsored by **BioNet, Inc**.)

Thursday, March 19, 2009

7:00-5:00 **Registration** in Dallas Ballroom pre-function area

7:00-8:30 **Poster Setup** and **Continental Breakfast** in Dallas Ballroom 2 **(**Sponsored by an anonymous donor) Posters to be displayed until 12:00, March 21; must be down by 1:00

8:00-4:00 **Technical Showcase in** Dallas Ballroom 2

8:30-4:05 **Podium Session 1 and 2** in Dallas Ballroom 1

8:30-8:45 **Opening Remarks**

8:45-9:45 Symposium **keynote** address by **Andrzej Slominski**

- **(001) Neuroendocrine functions of the skin**

Podium 1: **Molecular Imaging**
Moderator: **Joachim Fluhr**

9:45-10:30 Session **keynote** by **Joachim Fluhr**

- **(002)** Molecular Imaging

10:30-10:50 **Break and Exhibition** in Foyer and Dallas Ballroom 1 **(**Sponsored by **Hilltop Laboratories)**

10:50-11:10 **Sachin V. Patwardhan**, Dan DiGregorio, Ramazan Demirli, Bill Halas, & Doug Canfield
- **(003)** Multi-spectral Fluorescence Imaging for Classification & Evaluation of Acne

11:10-11:30 **Paul Wighton,** Tim K. Lee, M. Stella Atkins, Harvey Lui, David I. McLean
- **(004)** Calibrating an Inexpensive Skin Lesion Imaging System

11:30-11:50 **Frank Joa**
- **(005)** Technical Comparison of Facial Wrinkle Image Systems

12:00-1:00 **Lunch in** Dallas Ballroom 2

1:00-2:00 **Poster Session A in** Dallas Ballroom 2**,** odd numbered posters manned

Podium 2: **Biophysics of the Skin**
Moderator: **Stacy Hawkins**

2:00-2:45 Session **keynote** by **Reinhold Dauskardt**
(006) Biomechanics of Human Skin: Predicting Skin Damage and the Effects of Treatments

2:45-3:05 **Break and Exhibition** in Foyer and Dallas Ballroom 2 **(**Sponsored by **Stephens & Associates)**

3:05-3:25 **Betsy Hughes-Formella** and Joachim Fluhr
- **(007)** Proof-of-concept: The use of biophysical measurement methods to establish efficacy

3:25-3:45 **Natalja Skrebova Eikje**, Takayuki Sota, and Katsuo Aizawa
- **(008)** Non-invasive characterization and monitoring of glucose in the skin of healthy, prediabetes and diabetes subjects by ATR-FTIR spectroscopy

3:45-4:05 **Moditha Nawinne,** Hamid Tehrani, Mark Pitt, Bianca de Gama-Rose, and Milind Dalal
- **(009)** The role of the SIAscope in the treatment for Basal Cell Carcinoma

6:00-9:00 **Grand Reception in** Dallas Ballroom 2

Friday, March 20, 2009

7:00-5:00 **Registration** in Dallas Foyer 2

7:00-8:30 **Continental Breakfast** in Dallas Ballroom 2 **(**Sponsored by **Mary Kay)**

8:00-5:00 **Technical Showcase in** Dallas Ballroom 2

8:30-4:25 **Podium Sessions 3 and 4** in Dallas Ballroom 1

Podium 3: **Skin Protection and Raman Spectroscopy (**Sponsored by **Unilever)**
Moderator: **Martha Tate**

8:30-9:15 Session **keynote** by **Gopi Menon**
- **(011) Skin Protection: A Biological Perspective**

9:15-9:35 **Marty Visscher**, Radhika Utturkar, Angela LaRuffa, Marisa Robinson, William Pickens, Randy Wickett, and Steven Hoath
- **(012)** Neonatal Skin Maturation – Vernix Caseosa and Natural Moisturizing Factor

9:35-9:55 **Jeffrey E. Berg**, James P. Bowman, Rick Zepp, David W. Koenig, and Scott W. Wenzel
- **(025)** Instrumental Evaluation of Uninvolved and Involved Skin Sites in Adults with Atopic Dermatitis

9:55-10:15 **Jennifer A. Davis,** Marty O. Visscher, and R. Randall Wickett
- **(013)** Investigation of a cytokine polymorphism and neurosensory irritation in hand dermatitis among health care workers

10:15-10:45 **Break and Exhibition in** Foyer Dallas Ballroom 2

10:45-11:05 **Johanna de Sterke**, André van der Pol, Peter Caspers, and John Battista
- **(014)** Validating In Vivo Skin Composition Analysis by Raman Spectroscopy

11:05-11:25 David Koenig, **Andrea Smiltneek**, Douglas Hoffman, Andrew Basehoar, Lisa Stabe, Tina Nussbaum, Barry Reece, and Corey Cunningham
- **(015)** Confocal Raman Studies of Stratum Corneum Water Profiles Following Treatment with Moisturizers

11:25-11:45 M. E. Darvin, **J. W. Fluhr**, P. Caspars, J. A. van der Pool, H. Richter, A. Patzelt, W. Sterry, and J. Lademann
- **(016)** In vivo distribution of carotenoids in different anatomical locations of human skin: comparative assessment with two different Raman spectroscopy methods

12:00-1:00 **Lunch** in Dallas Ballroom 2

1:00-2:00 **Poster Session B** in Dallas Ballroom 2**,** even numbered posters manned

Podium 4: **Claim Support**
Moderator: **Randy Wickett**

2:00-2:45 Session **keynote** by **Tony Johnson**
- **(017)** The Role of the Skin Bioengineer in the Challenging World of Product Claims

2:45-3:05 **Gary Grove**, Jonn Damia, Mary Jo Grove, and Timothy Houser
- **(018)** Guideline for performing adhesive peel tests from human skin

3:05-3:25 **Break and Exhibition** in Foyer and Dallas Ballroom 2 **(**Sponsored by **CPT Laboratories)**

3:25-3:45 **Stacy Hawkins**
- **(019)** Case Studies: How Consumer Perception Relates to Objective Claims

3:45-4:05 **Judy Woodford**
- **(020)** Facial Shine – By Which Perspective?

4:05-4:25 **Martha Tate**
- **(021)** Overview of Skin Product Claims Substantiation

6:00-10:30 **Gala Dinner** in Dallas Ballroom 2 ("Hard Nights Day" sponsored in part by **RCTS and CyberDerm**)

Saturday, March 21, 2009

7:30-9:00 **Registration and Continental Breakfast** in Foyer and Dallas Ballroom 2 **(**Sponsored by **AMA Labs)**

8:00-11:00 **Technical Showcase** in Dallas Ballroom 2

8:30-11:45 Podium Session 5 in Dallas Ballroom 1

Podium 5: **General Session**
Moderator **Stephan El Gammal**

8:30-9:15 Session keynote by **Stephan El Gammal**
- **(022)** UpDATE ON Cross-Sectional Imaging of the Skin: High Resolution Sonography, Optical Coherence Tomography and MagnetiC Resonance Imaging

9:15-9:45 **Jean Luc Lévêque** and Lucien Aubert
- **(023)** Upper dermis echogenicity: Which meaning?

9:45-10:05 **Marty Visscher**, M. Victoria deCastro, Lisa Combs, Lori Perkins, Jill Winer, Nancy Schwegman, Claire Burkhart, and Pattie Bondurant
- **(024)** Neonatal Skin Integrity at PICC Line Sites: Effect of Chlorhexidine Gluconate

10:05-10:35 **Break and Exhibition** in Foyer and Dallas Ballroom 2

10:35-10:55 **Randy Wickett**
- **(010)** Influence of Dressings on Barrier Repair: Effects on Biophysical Measurements and Natural Moisturizing Factors

10:55-11:15 **Di Qu,** James R. Mayne, Richard B. Bylsma, and G. Paul Seehra
- **(027)** Skin Color Measurement from Digital Photograph Using Novel Color Correction and Analysis Algorithms

11:15-11:35 J.S. Graham, R.S. Stevenson, R.R. Deckert, R.F. Railer, L.W. Mitcheltree, T.A. Hamilton, RB Lee, and **E.H. Braue Jr**
- **(026)** Medical Management of Cutaneous Sulfur Mustard Injuries

11:35-11:45 **Silvia Perez Damonte**
- Update on the next International ISBS Symposium in Buenos Aires, Argentina, September 24-26, 2010

11:45-12:00 **Best Poster Award (**Sponsored by **Academ, Inc)** and **Closing Remarks**

U.S. Technical Symposium of
The International Society for Biophysics and Imaging of the Skin

Technical Showcase

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ACADERM, Inc.
3723 Haven Avenue #104
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Phone: 650-980-3322
Fax: 650-980-3323
Email: acaderm@aol.com

Attendees: Todd Maibach

Canfield Imaging Systems
253 Passaic Avenue
Fairfield, NJ 07004-2524 USA

Phone: 973-276-0336
800-815-4330
Fax: 973-276-0339
Email: info@CanfieldSci.com
Web: www.canfieldsci.com

Attendees: Dennis DaSilva
Sachin Patwardhan
Scott Kastner

cyberDERM inc.
275 New Darlington Road
Media, PA 19063-5607

Phone: 610-325-0112
Fax: 610-325-0881
Email: cyberDERM@comcast.net
Web: www.cyberderm-inc.com

Attendees: Gary & Mary Jo Grove
Jonn Damia
Tim Houser

CuDerm Corporation
2929 Carlisle, Ste. 380
Dallas, TX 75204

Phone: 972-248-8095
800-690-1933
Fax 972-248-1094
Email sales@cuderm.com
Web: www.cuderm.com

Attendees: Sheila Dauth
Allison Gray

Courage + Khazaka GmbH Mathias-Bruggen-Str.91 50829 Koln Germany Phone: ++49 221 956 4990 Fax: ++49 221 956 4991 Email: info@courage-khazaka.de Web: www.courage-khazaka.de	**Delfin Technologies, Inc.** 62 Southfield Avenue, Suite 201 Stamford, CT 06902 USA Phone: 203-554-2707 Fax: 203-357-9955 Email: info@delfintech.com Web: www.delfintech.com Attendees: Aki Immonen
River Diagnostics 32 Sea Spray Drive, Suite B Biddeford ME 04005 Phone: 678-595-8774 Fax: 207-284-8141 Email: america@riverd.com Web: www.riverd.com	**STE, Inc.** George Kramer 8209 Rider Avenue Towson, MD 21204-1946 Phone: 410-821-8441 Fax: 410-821-8429 Email: gkramer@SkinTestEquip.com Web: www.SkinTestEquip.com Attendees: George Kramer Bob Imhof

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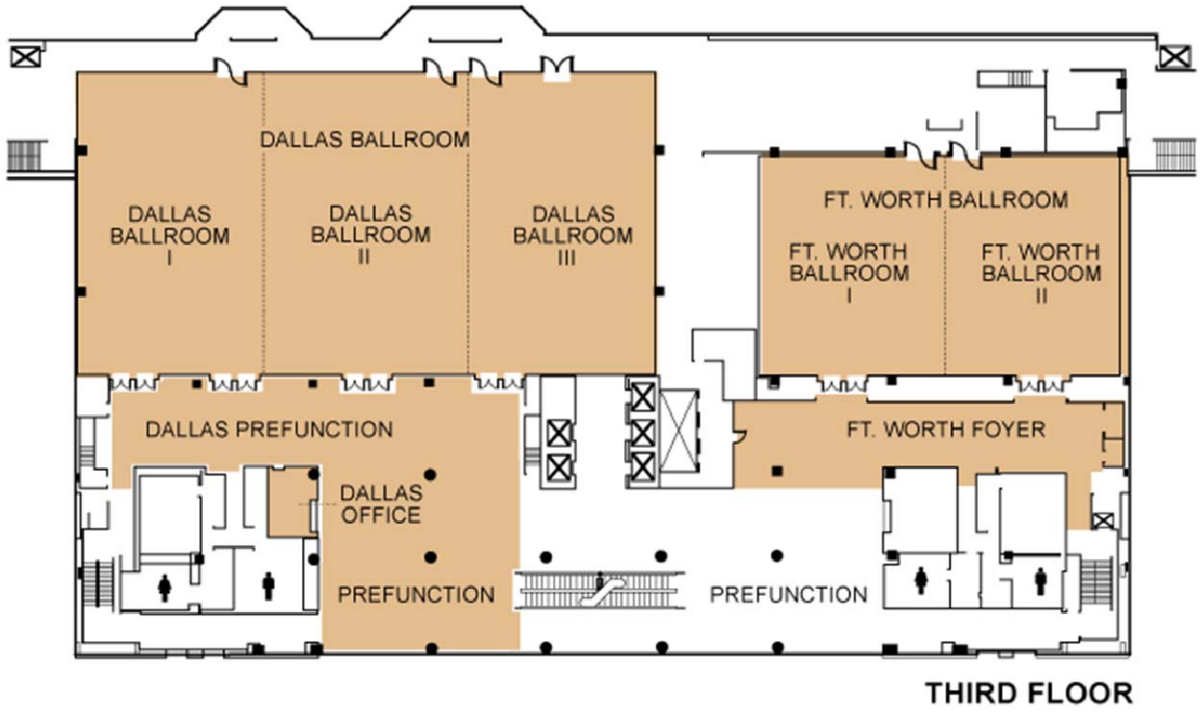

ABSTRACTS

001
Neuroendocrine functions of the skin

Andrzej T. Slominski, MD, PhD; Department of Pathology and Laboratory Medicine; University of Tennessee Health Center; Memphis, TN, USA.

The skin is strategically located at the interface with the external environment where it has evolved to detect, integrate and respond to a diverse range of stressors including solar radiation. Recent findings have established the skin as an important peripheral neuro-endocrine organ that is tightly networked to central stress axes. These neuroendocrine functions of the skin not only contribute to the maintenance of skin homeostasis but also have systemic effects. Specifically, significant experimental evidence has gone on to show that epidermal cells produce and respond to classical stress neurotransmitters, neuropeptides and hormones, and that this production is stimulated by ultraviolet radiation (UVR), biological factors and other agents (Endocrine Rev 21, 457-487, 2000; Physiol Rev 80, 979-1020, 2000; Physiol Rev 84, 1155-1228, 2004; FASEB J 15, 1678-1693, 2001; FASEB J 19, 176-194, 2005; Mol Cell Endocrinol 265-266, 143-149, 2007; J Clin Invest 117, 3166-3169, 2007; Trend Endocrinol Metab 19, 17-24, 2008). Examples of potent epidermal products include biogenic amines (catecholamines, serotonin and N-acetyl-serotonin) and melatonin, proopiomelanocortin-derived ACTH, □-endorphin or MSH peptides, corticotropin-releasing factor and related urocortins, thyroid-related hormones and corticosteroids (e.g., cortisol/corticosterone as well as their precursor molecules). Importantly, the production of these molecules is hierarchical and it follows the algorithms of classical neuroendocrine axes (e.g. hypothalamic pituitary adrenal axis (HPA), hypothalamic-thyroid axis, serotoninergic /melatoninergic, catecholaminergic and cholinergic systems). Moreover, dysregulation of these axes may lead to skin diseases. These endocrine factors represent an exquisite regulatory layer addressed at restricting maximally the effect of noxious agents in the skin to preserve local and consequently global homeostasis, they can also enter circulation leading to the systemic effects (endocrine effects). Furthermore, the skin-derived factors may also activate cutaneous sensory nerve endings to alert the brain to changes in the epidermal environment, or alternatively to activate other coordinating centers by direct spinal cord neurotransmission without brain involvement. Thus, the skin cells can coordinate not only cutaneous but also global homeostasis.

002
Molecular Imaging

Joachim W. Fluhr, MD; bioskin, Berlin, Germany and Department of Dermatology, Charité University Clinic, Berlin, Germany

Skin or more precisely the epidermis are the outermost part of the human body and represents the interface to the potentially harmful environment. As an external organ skin is approachable for visual assessment which is to date still the gold standard in clinical diagnostic and research. Molecular mechanisms and their modulation are classically studied *in vitro* (e.g. in cell culture models) or using invasive biopsy techniques and subsequent immune-histochemistry coupled with different light microscopy techniques (e.g. confocal microscopy or fluorescent life time imaging) or molecular biology techniques (e.g. RT-PCR).
Recently non- or minimal-invasive methods are used in different cutaneous research areas derived from other indications. The resolution approaches the nanoscale-range. These methods include multidimensional imaging and image analysis of digital pictures, *in vivo* multiphoton spectroscopy, optical coherence tomography, atomic force microscopy, near-infrared spectroscopy (NIR) and *in vivo* Raman Spectroscopy. Furthermore protein-protein interactions can now be visualized e.g. using fluorescent proteins. Additionally optical reporter genes were coupled to microPET and microCAT e.g. in cancer gene therapies.
The goal of these new technologies is to optain multidimensional imaging for quantitative and qualitative studies in humans *in vivo*. The drawback of some of the presented methods is the use of fluorophores that are not yet approved for use in humans *in vivo*. Furthermore the required financial resources for many of the technologies are quiet high and might be an obstacle for standard use.
The lecture will give an overview on new molecular imaging technologies with some examples where skin was already a target. Furthermore potential future indications for cutaneous studies will be discussed.

003
Multi-spectral Fluorescence Imaging for Classification & Evaluation of Acne

Sachin V. Patwardhan[1], Dan DiGregorio, Ramazan Demirli, Bill Halas, & Doug Canfield
[1]Senior Scientist, Canfield Scientific Inc., Fairfield, NJ 07004

Background: Acne severity is assessed based upon the number & type of lesions, & area of involvement using the Investigator's Global Assessment (IGA). Due to lack of a proven imaging technique, it relies on tedious & unreliable manual examination.
Purpose: A multi-modal, & multi-spectral facial imaging system is designed for capturing acne related fluorescence, absorption, & topological information in a calibrated, consistent, & efficient lighting environment. Image analysis techniques are then used for lesion classification & assigning an IGA grade to the subject. Quantitative evaluation of lesion progression, erythema, effectiveness of treatment, & post acne pigmentary changes is also possible.
Methods: The imaging system captures a set of images of the subject's face from 3 angles using calibrated sources (xenon flashes) & a digital color camera. Captured images include: 1. white light image for visual inspection, 2. white flat-lit image for surface topology, 3. cross-polarized image for skin pigment analysis, 4. porphyrin fluorescence image for bacteria -analysis, 5. horns fluorescence image for localizing active lesions, 6. collagen fluorescence image for subsurface topology, & 7. excitation light image for normalization. Captured images are analyzed using feature detection algorithms for identification of porphyrin, horns, hemoglobin, melanin, & pus distributions. These when combined with topological skin features allow fast, automatic, & reliable acne lesion classification & assignment of an IGA grade. Lesions are classified into open/close comedones, papules, pustules, nodules, cysts, burnt-out & excreted types.
Results: Maps of detected lesions, lesion counts, & area- /severity-based scores are produced after analyzing the images. Results indicate that the proposed imaging system is at least 5 times more sensitive in identifying acne lesions; in particular, the non-inflammatory lesions. Further, it is more accurate in distinguishing papules from pustules, burnt-out from active lesions, calculating areas of inflammation, & assigning an IGA grade when compared with manual evaluation.
Conclusions: Proposed imaging system provides accurate & reliable evaluation of acne lesions as compared to the tedious & unreliable manual lesion counting. The IGA grade assigned by the algorithm is more consistent than manual grading. We expect the use of this technology to set new standards in acne treatment & aid physician with various treatment options based on the severity scores weighted by the detected lesion types. Further, the use of this imaging system will benefit drug development efforts & to establish efficacy.

Key Words: Multi-spectral imaging, Acne Classification, Porphyrin fluorescence, Hemoglobin absorption.

004
Calibrating an Inexpensive Skin Lesion Imaging System

Paul Wighton[1,2,3], Tim K. Lee[1,2,3], M. Stella Atkins[1], Harvey Lui[2,3], David I. McLean[2]
[1]School of Computing Science, Simon Fraser University, Burnaby, BC, Canada, [2]Photomedicine Institute, Department of Dermatology and Skin Science, University of British Columbia and Vancouver Coastal Health Research Institute, [3]Cancer Control Research Program and Cancer Imaging Department, BC Cancer Research Centre, Vancouver, BC, Canada

Background: An inexpensive, easy to use skin lesion imaging system is being designed using a conventional dermoscope and a consumer level digital camera. It is designed to have broad appeal to physicians and skin scientists due to its low cost and ease of use. Such a system could lead to improved and more efficient patient care through the use of teledermatology or automated methods. In addition, it could be used to generate a large database of various skin conditions to further future research. However, the inexpensive optics, combined with the limitations of the consumer level camera pose several challenges including 1) inaccurate color representation; 2) inconsistent illumination across the image; 3) inconsistent spatial resolution across images and cameras; and 4) image distortions (both geometric and chromatic).
Purpose: The purpose of this research is to design a fast and simple method of dermoscope calibration to mitigate the shortcomings listed above.

Methods: The dermoscope is a Dermlite II Pro attached to a Canon Powershot G9 digital camera. This camera allows the user access to the 'RAW' sensor data, before it has been compressed or processed for optimized JPEG photographs. We have developed software to generate images from the raw data capture using calibration information obtained a priori. Calibration is achieved by acquiring two reference images: a grey card and a checkered pattern. The grey card is used for color balancing and vignetting correction while the checkered pattern is used to correct for geometric and chromatic lens distortions. A first-order radial symmetric distortion model is applied for chromatic aberration correction. The magnification level can be also inferred.
Results: After calibration, illumination (which is originally 2.5 times brighter in the center than the periphery) is rendered constant and distortions due to chromatic aberration are reduced by approximately 74%.
Conclusions: It is possible to mitigate the shortcomings of inexpensive dermoscope components using a fast and simple calibration procedure.

Key Words: Digital Dermoscopy; Calibration; Color Balancing; Vignetting; Chromatic Aberration; Geometric Distortion.

005
Technical Comparison of Facial Wrinkle Image Systems

Frank Joa, Miami Valley Innovation Lab, Cincinnati, OH 45252

Method for comparing facial imaging systems is explored utilizing image standards and charts to measure the technical response of these systems in their ability to image wrinkles. These evaluations provide objective measures with which each systems can be evaluated and contrasted with the performance of the other imaging systems for the detection of wrinkles. These measures are also compared to some perceptual measures of the systems and the differences in the objective and the perceptive measures are explored.

006
Biomechanics of Human Skin: Predicting Skin Damage and the Effects of Treatments

Reinhold H. Dauskardt, Department of Materials Science and Engineering, Stanford University

We describe a thin-film mechanics approach to characterize and model the biomechanical function of human skin with a particular focus on the outermost stratum corneum (SC) layer. The SC provides both mechanical protection and a controlled permeable barrier to the external environment while subject to highly variable conditions including changing temperature, humidity, mechanical and abrasive contact. The biomechanical properties of the SC are crucial in understanding the mechanical and biophysical function of skin, its cosmetic "feel" and appearance, and play a central role in skin damage processes of skin chapping and cracking.

Techniques involving both in-plane and out-of-plane mechanical and intercellular delamination characterization are described. We demonstrate how environmental, enzymatic, moisturizing and chemical treatments that influence components of the SC tissue can dramatically affect resulting mechanical properties and biomechanical function. Treatments were selected to systematically manipulate intercellular lipids, corneodesmosomes and intracellular keratin. In addition to stress-strain and viscoelastic properties, we describe novel thin-film methods to probe the resistance to time dependent intercellular delamination and the stresses that arise naturally in SC as a result of treatment or environmental conditions.

We finally demonstrate how damage processes in human skin can be quantitatively modeled and predicted based on thin-film biomechanics and cracking processes. We believe that this represents a new approach to characterize and model the fundamental biomechanics of human skin.

007
Proof-of-concept: The use of biophysical measurement methods to establish efficacy

Betsy Hughes-Formella and Joachim Fluhr, bioskin GmbH, Hamburg and Berlin Germany

Whereas biophysical measurement methods are routinely used for the investigation of skin function and cosmetic claim support, these methods are rarely used in drug development. However, these methods lend themselves as surrogate endpoints for objective assessment of clinical effects. Appropriate designs are often better suited than subjective clinical assessments for Proof-of-Concept (POC).
Since early efficacy and POC studies lead to go/no go decisions in clinical development, it is crucial to critically evaluate every facet of study design. Important design considerations (e.g. sample size, choice of endpoints, duration and manner of treatment, choice of comparators) for POC studies using biophysical measurements as primary endpoints is important e.g. in a Psoriasis Plaque Test (PPT).
Patients (n=12-20) with stable psoriatic plaques are suitable for inclusion in the PPT. Small test fields located on one or two comparable plaques are treated in parallel over a 12-28 day period, preferably under occlusion. Test chambers are seated into holes punched in a hydrocolloid bandage. Treatments are renewed once daily 6x per week. The primary variable is the extent of the psoriatic infiltrate measured in sonographic images (20 MHz) at defined intervals during the treatment period. Optionally, the intensity of erythema can be measured in the test fields using chromametric a*-values. Clinical assessment of improvement or worsening is included as secondary criteria.
The PPT has been used to investigate efficacy of corticosteroids, vitamin D analogs, oligonucleotides, diverse inflammatory inhibitors, and immunmodulators, among others. It is well suited for evaluation of new chemical entities, alternative formulations or initial studies of dose response.
By using innovative test designs or models such as the PPT with parallel "intra-individual" comparison in "symptomatic" volunteers or healthy subjects and objective measurement of skin condition it is often possible to reduce the number of subjects needed in early trials.

Key words: Drug development, Proof-of-Concept, Psoriasis Plaque Test

008
Non-invasive characterization and monitoring of glucose in the skin of healthy, prediabetes and diabetes subjects by ATR-FTIR spectroscopy

Natalja Skrebova Eikje, Takayuki Sota, Katsuo Aizawa, Department of Electrical Engineering and Bioscience, Waseda University, 3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169-8555, Japan

ATR-FTIR spectroscopy has been widely introduced as a fast and non-invasive method for molecular gross investigations of the surface of the skin in the spectral range between 5000 and 1000 cm^{-1}. However, *in vivo* detailed description of glucose-specific signals in the 1160 -1000 cm^{-1} region, that might be important for understanding biochemical, metabolic and (patho-)physiological processes of glucose in the skin has not been reported in the literature until recently. Here we represent HATR-FTIR (horizontal attenuated total reflectance Fourier transform infrared) spectroscopy technique for fast real-time monitoring of D-α- and β-glucose biomolecules in the skin tissue, for their *in vivo* comparative qualitative and quantitative molecular characterization among healthy, prediabetes and diabetes subjects. Based on calculated mean values of determined *in vivo* 5 glucose-specific peaks at about 1153, 1118, 1080, 1041 and 1030 cm^{-1} intra- and inter-subject glucose activity levels at different times and doses of OGTT (oral glucose tolerance test) were assessed. Our study has successfully demonstrated potential of HATR-FTIR spectroscopy technique to intra- and inter-individually analyze and compare behaviour of D-α- and β-glucose biomolecules in monitored skin before and after OGTT in healthy, prediabetes and diabetes subjects.

009
Title: The role of the SIAscope in the treatment for Basal Cell Carcinoma

Dr Moditha Nawinne,[*] Dr Hamid Tehrani,[*] Dr Mark Pitt,[‡] Dr Bianca de Gama – Rose,[‡] and Dr Milind Dalal.[*]
* Department of Plastic Surgery, Royal Preston Hospital, Preston, Lancashire, UK.
‡ Department of Pathology, Royal Preston Hospital, Preston, Lancashire, UK

Background/Purpose: A major treatment dilemma faced by clinicians in the management of BCCs is the accurate assessment of lesion thickness. Thin BCCs may be successfully treated by non-surgical methods, however clinical examination is not always reliable in identifying such lesions. The SIAscope (Spectrophotometric Intracutaneous Analysis) which uses a light source with four discrete wave bands to illuminate the skin is a proven diagnostic aid in the management of BCCs. This study evaluates the role of the SIAscope in assessing the thickness of BCCs, using previously described SIAscopic features: flare, paleness, branched-vessels and ulceration.
Material /Methods: 42 lesions, clinically diagnosed as thin BCCs, were imaged using the SIAscope prior to surgical excision. The lesions were then step-sectioned and the thicknesses measured at each SIAscopic feature.
Results: ANOVA analysis revealed a significant difference in mean thicknesses of all four features. The mean thicknesses for each feature are; flare 0.45mm, branched vessels 0.96mm, paleness 1.33mm and ulceration 1.8mm ($p<0.0001$ at 95% CI).
Conclusions: Since available literature suggests a thickness of ≤1.5mm is easily penetrable by non-surgical treatment agents, the study concludes that clinically diagnosed thin lesions which display only 'flare' and/or 'branched-vessels' on a SIAscopic image may successfully be treated by non-surgical methods.

Key words – Basal Cell Carcinoma, SIAscope.

010
Influence of Dressings on Barrier Repair: Effects on Biophysical Measurements and Natural Moisturizing Factors

R. Randall Wickett[1], Marisa Robinson[1], and Marty Visscher[2], [1]College of Pharmacy, University of Cincinnati, Cincinnati, OH; [2]The Skin Sciences Institute, Cincinnati Children's Hospital Medical Center

We have investigated the effects of common treatments on the amino acid component of natural moisturizing factor measured by HPLC from D-Squames(r) and biophysical properties of the stratum corneum. A mild acetone/ether extraction that did not significantly increase in TEWL did significantly reduce the ability of the skin to retain NMF amino acids during a subsequent water soak. In another study the skin's barrier function was damaged by tape stripping with Scotch Tape(r) to four times the baseline TEWL. The effect of three treatments, semi-permeable membrane, a wet dressing and no occlusion on the rate of SC barrier repair relative to untreated control was determined by biophysical measurements and visual grading. At day 5 of recovery, D-Squames(r) were collected from all test sites in order to measure NMF levels and determine the influence of hydration on NMF generation from filaggrin during barrier repair. In agreement with our previous work, the semi-permeable membrane produced the fastest barrier repair, however NMF regeneration was not different from no occlusion. The wet dressing impeded both barrier repair and regeneration of NMF.

011
SKIN PROTECTION : A BIOLOGICAL PERSPECTIVE

Gopinathan K. Menon, PhD, Global R & D, ISP Corporation, Wayne, NJ 07470.

Skin protection is a complex subject, as its definition can be dependent on the context : whether Industrial, medical, occupational, recreational or personal care. Hence skin protectants can be clothing, devices, drugs, sunscreens, insect repellents or bioactive molecules and phytochemicals. As the interface of the body with our environment, skin is an organ with the most protective functions, or barriers to various environmental stressors, and keeping this defensive organ in optimal health is a concern for almost all . From a Biological perspective, skin protection can include preventing UV damage (to the DNA) , protect from the deleterious effects of Free radicals, maintain innate immunity , shore up the barrier repair responses to various types of chemical and biological assaults, and in essence

" stress-proof" the skin. This talk will focus on the innate ability of skin to respond to various deleterious effects of environmental stressors, as well as the rationale for boosting cell/ tissue functions of skin via topically active agents . Some of the recent trends in developing such actives, in vitro and ex vivo studies to validate them, and potential benefits in terms of skin protection would also be discussed.

012
Neonatal Skin Maturation – Vernix Caseosa and Natural Moisturizing Factor

Marty Visscher[1], Radhika Utturkar[2], Angela LaRuffa[1], Marisa Robinson[2], William Pickens[1], Randy Wickett[2], and Steven Hoath[1]. [1]The Skin Sciences Institute, Cincinnati Children's Hospital Medical Center and [2]College of Pharmacy, University of Cincinnati, Cincinnati, OH

Background: Full term neonatal skin hydration decreases rapidly and then increases during the first two postnatal weeks, indicating adaptive changes in the water handling properties of the upper stratum corneum (SC). Transition from high to low humidity at birth may initiate epidermal changes such as the proteolysis of filaggrin to natural moisturizing factor (NMF). Newborn skin with vernix caseosa left intact at birth is more hydrated, less scaly, and undergoes a more rapid decrease in pH than with skin with vernix removed. Vernix retention facilitates postnatal hydration and influences acid mantle formation.
Purpose: To examine the potential roles of vernix caseosa (VC) and SC natural moisturizing factor (NMF) in postnatal adaptation and determine the implications for preterm SC maturation.
Methods: NMF was quantified from the upper SC at 24 hours in (1) parallel cohorts of full term infants randomly assigned to have VC intact or removed at birth and (2) from full term infants at birth and one month. They were compared to NMF in similar samples from neonatal foreskin, vernix and adult volar SC. Samples collected 24 hrs post birth with D'squame disks were analyzed using reverse-phase high performance liquid chromatography and fluorescence detection. Statistical evaluations were made with Students t-test and ANOVA.
Results: Retention of VC resulted in significantly higher NMF levels at 24 hours post birth compared to VC removed subjects. NMF levels paralleled the higher SC hydration and lower skin pH observed for VC retention. NMF levels were very low at birth, significantly higher at one month but lower than adult values. Relative to infant and adult SC, vernix had higher levels of glutamic acid and histidine. NMF increased as a function of SC depth in adult SC and decreased in neonatal foreskin.
Conclusions: Retention of NMF containing VC appears to facilitate increased hydration and reduction in pH at birth. Production of NMF may be initiated with the transition from high to low humidity in the neonatal period. The findings are relevant for the skin care of premature infants for whom the postnatal SC acidification continues for as long as nine weeks after birth.

Key Words: stratum corneum, neonatal skin, vernix caseosa, adaptation, stratum corneum hydration, natural moisturizing factor, skin pH.

013
Investigation of a cytokine polymorphism and neurosensory irritation in hand dermatitis among health care workers

Jennifer A. Davis, BS, [1, 2] Marty O. Visscher, PhD, [2] R. Randall Wickett, PhD[1]
[1] College of Pharmacy, University of Cincinnati [2] The Skin Sciences Institute, Cincinnati Children's Hospital Medical Center

Background: Performance of repetitive hand hygiene procedures is required for health care workers (HCWs) to minimize the spread of infection in health care settings. However, compliance with required hand hygiene procedures is low (approximately 30%) due to the irritant contact dermatitis (ICD) that results. Susceptibility to ICD cannot currently be predicted, in part, because the biological basis of the inter-subject variability is not well understood.
Purpose: The purpose of this study was to evaluate the relationship between two biological skin parameters, TNF-α polymorphism -308 and neurosensory irritation (NSI), and the irritant response to repetitive hand hygiene procedures in HCWs.
Methods: NSI was evaluated with the lactic acid sting test (LAST) and quantified with a labeled magnitude scale (LMS). DNA genotyping was done by quantitative polymerase chain reaction (Q-PCR) on blood samples to

determine presence of a TNF-α polymorphism at locus -308. ICD was evaluated by expert visual grading of erythema and dryness and with digital imaging to quantify excess erythema.
Results: Subjects with high knuckle dryness had a higher number of A alleles than expected and those with low knuckle dryness had a lower number than expected (chi-squared test) though the differences were not significant (P = 0.11). No correlation was found between TNF-α polymorphism -308 and visual erythema or excess erythema from image analysis. Subjects with low visual dryness had higher, but not significant, LAST scores as compared to those with high visual dryness (P = 0.11). Individuals with the AA/GA genotypes had higher LAST scores than those with the GG genotype but differences were not significant (P = 0.39).
Conclusions: The findings suggest that TNF-α polymorphism -308 and NSI may be associated with ICD severity and may be useful in predicting susceptibility to ICD. However, further work is needed to clarify these relationships, particularly in the context of chronic irritant dermatitis from repetitive exposure to water, surfactants, and alcohol based hand sanitizers.

Key words: health care workers (HCWs), irritant contact dermatitis (ICD), neurosensory irritation (NSI), TNF-α polymorphism -308.

014
Validating In Vivo Skin Composition Analysis by Raman Spectroscopy

Johanna de Sterke, André van der Pol, Peter Caspers, John Battista, River Diagnostics, Rotterdam, The Netherlands

Background: In vivo confocal Raman micro-spectroscopy of skin was introduced ten years ago as a novel research tool. Its use lies in claim substantiation of product efficacy and fundamental skin science. The technology is now routinely used in human panelist studies in the skin sciences. There is no alternative methodology for direct measurement of concentrations of substances in vivo, at defined depths below the surface of the skin. Therefore, direct validation of the measured concentrations is not possible. Recently, researchers have started to compare in a systematic manner the concentrations reported by confocal Raman spectroscopy to concentrations obtained by in vitro methods.
Purpose: This lecture discusses the technical details of ways to validate the compositional analysis by confocal Raman spectroscopy. The approach is illustrated by several applications.
Methods: Raman depth profiles are recorded in vivo on the arms of volunteers, reflecting the spatially-resolved molecular composition of the stratum corneum before and after treatment with topically applied products. Novel developments now allow for detailed quantification of the endogenous and exogenous compounds in the skin. The obtained profiles of depth versus composition can be recast in other formats, for example into a flux. In this way, direct comparison to concentrations as measured by other technologies in vitro becomes possible.
Results: After a review of the limited validation studies up to now, new data will be presented. Among the applications are water measurements, NMF components, retinol, caffeine and UV absorbers such as octyl methoxy-cinnamate.
Conclusions: In vivo Raman spectroscopy is increasingly being accepted as a quantitative method to investigate the penetration and effects of topically applied products. New developments have enabled better quantification of molecular components in the skin and validation studies are increasingly supporting the establishment of Raman spectroscopy as a rapid, non-invasive tool in the skin sciences.

Key words: confocal Raman spectroscopy, in vivo skin composition, validation.

015

Confocal Raman Studies of Stratum Corneum Water Profiles Following Treatment with Moisturizers

David Koenig[1], **Andrea Smiltneek[1]**, Douglas Hoffman[1], Andrew Basehoar[1], Lisa Stabe[1], Tina Nussbaum[1], Barry Reece[2], and Corey Cunningham[1]. [1]Kimberly-Clark Corporation, Neenah, WI 54956 and [2]Reliance Clinical Testing Services Irving, TX 75062 USA.

BACKGROUND: Humectants, emollients, and skin protectants are commonly used in cosmetic products to provide skin moisturization benefits. While these cosmetics appear to moisturize the skin indirectly as measured by impedance, their impact on direct observations of stratum corneum (SC) water profiles is poorly understood.
PURPOSE: To investigate the impact of moisturizers on SC biochemistry, normal and exaggerated use of these cosmetics was studied using *in vivo* confocal Raman spectroscopy and confocal microscopy.
METHODS: Several studies were performed to investigate the impact of single or multiple (4) application of moisturizers over a 24 hr period. Studies using *in vivo* Raman spectroscopy and biophysical measurements were conducted in Irving, TX, using female subjects, aged 18-65. Studies using *in vivo* confocal microscopy and biophysical measurements were conducted in Neenah, WI, with both male and female subjects, aged 18-65. Three types of moisturizers (50% Cetiol HE, 50% glycerin, or petrolatum) were applied to the volar forearm. Sites were analyzed for changes in impedance (NOVA DPM 9003), TEWL (Tewameter® TM 300), biochemical profiles (*in vivo* confocal Raman spectroscopy, Model 3510 Skin Analyzer®), and SC thickness (i*n vivo* confocal microscopy, Vivascope® 1500), dependent on the specific study design. Measurements were taken before addition of the moisturizers and at either 4 and/or 24 hrs after treatment.
RESULTS: An increase in impedance was observed after an application of each moisturizer with glycerin producing the largest change. However, glycerin and petrolatum had no impact on SC water profiles. Interestingly, Cetiol HE produced a significant drop in water concentration post treatment. Exposure of the volar forearm to a single application of petrolatum did not change SC thickness as measured by confocal imaging. Repeated application of each moisturizer increased impedance. In contrast to a single application, confocal imaging indicated that repeated application of each moisturizer increased the apparent SC thickness by approximately 5 μm.
CONCULSIONS: Single application of moisturizers to the skin appears to not change the water content or thickness of the SC. In contrast, exaggerated moisturization appears to increase SC thickness. These contrary findings implicate the need for divergent analytical techniques to fully understand complex interactions of water and the SC.

KEYWORDS: Raman spectroscopy, *in vivo* confocal imaging, stratum corneum thickness, moisturizers.

016

In vivo Distribution of Carotenoids in Different Anatomical Locations of Human Skin: Comparative Assessment with Two Different Raman Spectroscopy Methods

M. E. Darvin[1], **J. W. Fluhr**[1,2], P. Caspars[3], J. A. van der Pool[2], H. Richter[1], A. Patzelt[1], W. Sterry[1], J. Lademann[1]
[1] Center of Experimental Applied Cutaneous Physiology, Department of Dermatology and Allergology, Charité-Universitätsmedizin Berlin, Germany; [2] Bioskin GmbH Berlin, Germany; [3] River Diagnostic B.V. Rotterdam, Netherlands

Background: The cutaneous antioxidants form an efficient protection system against the destructive potential of free radicals, produced by environmental factors, such as UV-sun irradiation, hazardous substances and life style habits. Most of the antioxidants cannot be produced by the human organism. Thus they have to be incorporated by food and beverages.
Material and Methods: In the present manuscript, the distribution of carotenoids as a marker for antioxidative potential in human skin was investigated with two different in vivo Raman spectroscopy methods with an excitation wavelength of 785 nm (Skin analyzer) and at 488nm (resonance Raman spectroscopy). The carotenoid profile was assessed at three different anatomical locations (palm, forehead and volar forearm) in 12 healthy volunteers.
Results: In untreated skin, the major fraction of the carotenoids is located in the upper part of the SC. The amount of carotenoid is lower in the upper part of the SC on the forearm compared to forehead and palm shown with both methods. Both methods detect similar distinction patterns of carotenoid levels for the three anatomical locations.
Conclusion: The present study supports the hypothesis that antioxidative substances; here carotenoids, are secreted via eccrine sweat glands and/or sebaceous glands to the skin surface. Raman spectroscopic methods are an efficient

tool to analyze the distribution of carotenoids in the human skin over time and with the Skin Analyzer over different layers of the epidermis. Resonance Raman spectroscopy is suited to analyze deeper parts of the skin.

Key words: In vivo Raman Spectroscopy, Carotenoids, Anatomical Location.

017
The Role of the Skin Bioengineer in the Challenging World of Product Claims

Anthony Johnson, Unilever R&D, Trumbull, CT 06611

In a broad sense, claims are pervasive in our every day lives. From the campaign promises of a new president to the guarantee of weight loss from a new diet pill, we are bombarded with all types of claims everyday. Much of what we do and think about is in fact driven by the claims we hear and see, accept or reject, from the daily exposure to communications from multiple sources. Much of the input is received subconsciously or subliminally, but nevertheless shapes the beliefs that drive our everyday actions and activities.
With regard to personal product claims of the type we deal with, there are many facets to consider before products are launched in the market place. What do we want to communicate to the consumer and how do we do this effectively and what are the legal and regulatory constraints? These are important questions but addressing them with a check list of dos and don'ts is not inspiring. For more interest and a degree of humor one could look at the plethora of product claims in the marketplace and consider how the more outrageous are made without it seems any serious risk of imprisonment for deception and fraud. Dubious claims are not the province of major manufacturers so will not be a main feature of my presentation.
I have watched the development of product claims over many years and have a sense that there are fashions and phases. Today we are in an age of great marketing sophistication and advanced communication technologies. Claims are made, supported and delivered quite differently than in the past. It seems that manufacturers often invest more in claims and communication than in making a product in the first place. More claims fail than succeed if judged by impact on product sales. And yet some simple claims become icons. Effective claims are clearly a most important part of the personal care product marketing mix.
In recent years we have had an explosion in the availability and use of highly sensitive bioengineering instruments and techniques. These tools often require considerable expertise and insight to use them to maximum advantage for the products they examine and support. It is easy to over-claim and this is ultimately to the detriment of the product. Consumers are often gullible but seldom stupid. Skin bioengineering scientists are no longer simply custodians of sophisticated instruments but pivotal for achieving the right interpretation and use of results from increasingly complex studies. They have a responsibility for helping their marketing colleagues to maximize performance insights without going over the top. Developing and supporting truly differentiating products claims is becoming increasing difficult and more dependent on technical understanding and insight.
I trust that the issues I present will encourage bioengineering scientists to see their role in a new light and resolve to ensure that their science is used well.

018
Guideline for performing adhesive peel tests from human skin

Gary Grove, Jonn Damia, Mary Jo Grove, and Timothy Houser
cyberDERM, inc., Media, PA, USA

Pressure-sensitive adhesives are widely used in the medical field to secure surgical dressings, tapes, bandages, transdermal drug delivery patches, cutaneous electrodes and many other devices to the skin. It is generally recognized that most adhesives are not "skin friendly" and will cause substantial discomfort, therefore damaging the underlying skin when removed; especially in those cases where this is done frequently. Although standard testing methods have been developed for evaluating the aggressiveness of pressure sensitive adhesives from rigid substrates, it has been our experience that this type of peel test does not predict how these adhesives behave when stuck to human skin. Thus, to fully understand the performance properties of medical adhesives, one must explore the relationship between skin damage and peel test measurements using human volunteers. We have found by using computerized evaporimetery, increased TEWL rates result from the mechanical disruption of the Stratum Corneum barrier. This method proved to be a very reliable measure of the degree of damage associated with adhesive tape trauma. Learning how to properly conduct peel tests in vivo has been much more difficult. We have developed a

special tensile testing apparatus for these types of studies. Factors such as peel rate, peel angle, peel direction, dwell time, body site and preexisting skin conditions, all need to be carefully considered to get truly meaningful results. It is extremely important that the chosen testing conditions be relevant to real world usage.

Key words: TEWL, peel test, tape trauma.

020
Facial Shine – By Which Perspective?

Judith K. Woodford, Ph.D., Desiree Butcher, Barry Reece, M.S.* Kao Brands Company Cincinnati OH, *RCTS, Inc Irving TX

Facial shine is a common concern of young adults and many products have promised to reduce or control this issue. Shine can be measured by a multitude of methodologies. For a particular project, the best-suited method will depend upon the question to be answered. Studies will be presented illustrating these techniques.

021
Overview of Skin Product Claims Substantiation

Martha Tate, Kimberly-Clark Corporation, Roswell, GA USA

Examples of skin and hair product claims are abundant in fashion magazines every month. In this presentation, we will look at some fashion magazine advertisements. We will review categories of claims and how studies are set up. Study and statistical design are important in the substantiation of product claims. Skin product benefits can be addressed through clinical studies, consumer perception, and a variety of instrumental evaluations. Many of the instruments are available in the ISBS meeting's Technical Showcase. Finally, we will review classic examples of claims from advertisements clipped from the newsstand.

Key Words: Claims, instrumental evaluations, consumer perception

022
UpDATE ON Cross-Sectional Imaging of the Skin: High Resolution Sonography, Optical Coherence Tomographyand MagnetiC Resonance Imaging

Stephan El Gammal[1]; Alexander Knüttel[2], Michael Vogt[3]
[1]Dermatological Clinic, Hospital Bethesda, Freudenberg, Germany, [2]ISIS Optronics GmbH, Mannheim, Germany, [3]Institute for High Frequency Engineering of the Ruhr-University, Bochum, Germany

This lecture gives a state-of-the-art overview on sonography, magnetic resonance microscopy and opticalcoherence 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.

Magnetic Resonance Microscopy 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, 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. 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.

Keywords: Cross-sectional Imaging, High Resolution Sonography, Magnetic Resonance Imaging (MRI), Optical Coherence Tomography, Update

023
Upper dermis echogenicity : Which meaning ?

Jean Luc Lévêque[1] and Lucien Aubert,[2] [1]Consultant, Paris, [2]L'Oréal Recherche, Monaco

Aims: Since the pioneering work by de Rigal et al in 1989, most of the scientists involved in skin research using ultrasound imaging agree that a subepidermal low echogenic band (SLEB) exists and reflects ageing and/or photoageing of the skin. Numerous methods have been proposed for measuring SLEB but there is no agreement on an objective and standardized methodology of evaluation. Moreover the interpretation of this band is still controversial. There is also some disagreement about the influence of age on the forearm skin thickness.
Method: Ultrasonic images of the skin forearm (ventral and dorsal) were recorded by means of a Dermascan C(Cortex Technology, Monaderm, Monaco) on 304 women (20-102 years). The severity of skin photoageing on the forearm was also recorded. A second study was carried out by 100 women: US images were recorded on three sites around the forearm.
Results: The first study confirms the progressive increase of the echogenicity ratio of the low echogenic pixels between upper and lower dermis (LEP(u/l)) versus age, independently of the forearm side and independently of the photoageing grade. There is also no correlation between this parameter and the thickness of the SLEB. The second study demonstrates that a relatively UV-protected zone exists between the ventral and dorsal areas. Concerning skin thickness, the study confirms that skin is thicker on the dorsal side compared to the ventral one and that atrophy only begins beyond the seventh decade.
Conclusion: SLEB thickness and LEP(u/l) are not equivalent. These parameters are not related to visually assessed photoaging but are probably related to the mechanical alteration of the dermis. Ventral face of the forearm must not be considered as a UV protected area.

024
Neonatal Skin Integrity at PICC Line Sites: Effect of Chlorhexidine Gluconate

Marty Visscher, PhD[1], M. Victoria deCastro, MSN, RNC[2], Lisa Combs,RN[2], Lori Perkins, BSN, RN[2], Jill Winer, RN[2], Nancy Schwegman, RNC[2], Claire Burkhart, BSN, RN[2], and Pattie Bondurant, MN, RN, CNS[2].
Skin Sciences Institute[1], Regional Center for Newborn Intensive Care[2], Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: To reduce PICC line associated infections, the skin is treated with chlorhexidine gluconate (ChloraPrep®, 2% CHG, 70% alcohol, water) before insertion and application of tapes (steri-strips) and dressings (semi-permeable, e.g., Tegaderm™). While CHG is commonly used with central lines, there is no published information regarding the effects on skin, i.e., irritation, inflammation, and barrier integrity. CHG (0.5%) was more

effective than 10% povidone-iodine against colonization, but effects on skin integrity were not reported. Severe contact dermatitis occurred in preterm infants (5.7%) treated with a CHG dressing (Biopatch). Barrier compromise increases infection risk.
Purpose: To test the hypothesis that the condition of skin treated with CHG and a semipermeable dressing (Tegaderm™) will not differ from skin with the dressing only.
Methods: NICU patients with arm or leg PICCs were eligible (n=40, GA 32.1 ± 4.7). Measures of stratum corneum barrier integrity (TEWL), erythema, rash, and dryness/scaling were made at the PICC site (CHG + dressing, P), a contralateral site (Tegaderm™, D) and an untreated control (C) at insertion and dressing changes. Statistical evaluations were made using ANOVA and linear mixed models repeated measures procedures.
Results: At week 1, the PICC site had the highest erythema score (P 1.6 ± 0.2, D 0.8 ± 0.2, C 0.0 ± 0.0, $p \leq 0.05$, ANOVA). Dryness was higher for the PICC site (2.0 ± 0.2) than D (1.2 ± 0.2) and C (0.7 ± 0.2), as was TEWL (P 21.8 ± 4.6, D 15.9 ± 1.7, C 13.9 ± 1.6). At week 2, the PICC and Tegaderm™ sites were each significantly higher than the control for erythema (P 1.4 ± 0.2, D 0.8 ± 0.2, C 0.0 ± 0.0) and dryness (P 1.8 ± 0.3, D 1.7 ± 0.3, C 0.8 ± 0.2) but not different from each other. TEWL was higher for P (P 29.0 ± 10.5, D 15.8 ± 2.1, C 12.7 ± 1.6). Similar results were found for infants ≤ 29 weeks gestation.
Conclusions: The dressings applied to PICC sites, rather than CHG, contribute to the observed skin breakdown and thereby alter the normal stratum corneum barrier development in neonates. Consideration of alternative strategies and/or products for securing PICC lines is warranted.

Key Words: stratum corneum, barrier compromise, erythema, neonate, chlorhexidine gluconate, dryness, TEWL, PICC.

025

INSTRUMENTAL EVALUATION OF UNINVOLVED AND INVOLVED SKIN SITES IN ADULTS WITH ATOPIC DERMATITIS

Jeffrey E. Berg[1], James P. Bowman[1], Rick Zepp[2], David W. Koenig[2], Scott W. Wenzel[2]
[1]Hill Top Research Corporation, Miamiville, Ohio, USA; [2]Kimberly Clark Corporation, Neenah, Wisconsin, USA

BACKGROUND / PURPOSE: The objective of this research was to determine whether or not values from various skin biophysical measurements (TEWL, D-Squame, Sebumeter, Sebutape® Skin Indicators, Nova DPM 9003 and silicone skin replicas) would indicate a relationship between skin sites involved with atopic dermatitis and uninvolved skin sites on the same subjects as well as on skin sites from matched paired controls.
METHODS: Trans-epidermal water loss (TEWL) measurements, skin impedance measurements, Sebumeter measurements, Sebutape® Skin Indicators, silicone skin replicas and D-Squames were taken to quantify the overall condition of the skin sites being evaluated. Ten subjects in each group (1 male, 9 female) completed all phases of the study. No test articles were used in the study.
RESULTS: There were no adverse events reported or observed during the course of the study. Overall, the results indicated a few significant differences between involved and uninvolved test sites for the parameters tested. The results of the correlation analyses indicated no significant relationship between TEWL, impedance and D-Squame data. Data from Sebumeter measurements indicated a significant correlation between involved and uninvolved skin sites from the atopic subjects. The D-squame image analysis results suggest a significant scaling difference between atopic subjects and healthy subjects, but not within the atopic subjects themselves. The replica results indicated the texture of the atopic subject's involved sites to be somewhat rougher than their contra-lateral uninvolved sites. An even stronger difference in roughness was found when comparing the atopic sites with the matched healthy subjects.
CONCLUSION: Although not all of the measures employed in this small study yielded statistically significant results, a majority of the data did show anticipated directionality, indicating that the skin of the atopic group can be objectively and subjectively quantified as being in poorer condition than that of similar subjects without dry, compromised skin. The lack of significance observed in this study was likely attributed to small sample sizes.

KEY WORDS: D-squame, Sebumeter, silicone replicas, atopic dermatitis, skin health.

026
Medical Management of Cutaneous Sulfur Mustard Injuries

J.S. Graham, R.S. Stevenson, R.R. Deckert, R.F. Railer, L.W. Mitcheltree, T.A. Hamilton, RB Lee, and **E.H. Braue Jr.,** U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400

Background: Sulfur mustard (2,2′-dichlorodiethyl sulfide; SM) is a potent vesicating chemical warfare agent that poses a continuing threat to both military and civilian populations. Significant cutaneous SM injuries can take several months to heal, necessitate lengthy hospitalizations, and result in long-term complications. There are currently no standardized or optimized methods of casualty management. New strategies are needed to provide for optimal and rapid wound healing.
Objective: The primary aim of this research was to develop improved clinical strategies (treatment guidelines) for optimal treatment of superficial dermal (second degree) cutaneous SM injuries, with the goal of returning damaged skin to optimal appearance and normal function in the shortest period of time.
Methods: Superficial dermal SM injuries were created on the ventral abdominal surface of weanling pigs. At 48 hours post-exposure, lesions were laser debrided and a treatment adjunct applied. Cultured epithelial allografts and eleven commercial off-the-shelf (COTS) products were examined for their efficacy in improving wound healing of these injuries. Clinical evaluations and a variety of non-invasive bioengineering methods were used at 7 and 14 days post-surgery to follow the progress of wound healing and evaluate various cosmetic and functional properties of the wounds. Measurements included reflectance colorimetry to measure erythema; evaporimetry to examine transepidermal water loss as a method of evaluating barrier function; torsional ballistometry to evaluate the mechanical properties of skin firmness and elasticity; and two-dimensional high frequency ultrasonography (HFU) to monitor skin thickness (e.g., edema, scar tissue). Digital images were used to assess wound contraction by image analysis. Histopathology and immunohistochemistry were performed 14 days following surgery to examine structural integrity and quality of healing. Logical Decisions® for Windows was used to rank the twelve treatment adjuncts that were studied.
Results: The most efficacious treatment adjuncts included (1) Vacuum Assisted Closure™, V.A.C., involving application of topical negative pressure, (2) Amino-Plex Spray (biO2 Cosmeceuticals International, Inc., Beverly Hills, CA), a nutritive cosmeceutical product that is designed to increase oxygen in cells, stimulate ATP synthesis, improve glucose transportation, stimulate collagen formation, and promote angiogenesis, and (3) ReCell Autologous Cell Harvesting Device (Clinical Cell Culture Americas LLC, Coral Springs, Florida), an innovative medical device that was developed to allow rapid harvesting of autologous cells from a thin split-thickness biopsy followed by spray application of a population of skin cells onto wounds within 30 minutes of collecting the biopsy, without the need of culturing the keratinocytes in a clinical laboratory.
Conclusions: Complete re-epithelialization of debrided SM injuries in 7 days is possible. In general, shallow laser debridement through the basement membrane zone (100 um) appears to provide better results than deeper debridement (400 um) with respect to early re-epithelialization, cosmetic appearance, functional restoration, and structural integrity. Of the twelve treatment adjuncts examined, the most promising included Vacuum Assisted Closure™, Amino-Plex Spray, and ReCell Autologous Cell Harvesting Device.

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army or the Department of Defense.

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027
Skin Color Measurement from Digital Photograph Using Novel Color Correction and Analysis Algorithms

Di Qu, Ph. D., James R. Mayne, Richard B. Bylsma, Ph. D., and G. Paul Seehra, Ph. D. ARTISTRY™ Center for Skin Health Research, Amway Corporation, Ada, MI, USA

Background: Clinical skin color measurement can be assessed using methods of clinical grading, colorimetry, and image analysis of photographs. However, variability associated with each method impacts its reproducibility and sensitivity. For clinical grading, subjectivity is a known cause of variability. With the colorimeter, skin contact has been a major drawback which changes skin color and results in inaccurate data. Image analysis of photographs

provides an objective way for skin color measurement. Its accuracy, however, is limited by the inconsistence in photographing techniques, which causes variability.
Purpose: In this study, we attempted to improve the accuracy of the image color analysis method. Color correction was identified as a key step toward minimizing picture-to-picture variation, and mathematical algorithms combined with use of color standards were developed to reduce measurement variability.
Methods: Color measurements and digital photographs were collected using Minolta Chromameter and Nikon D200 digital camera in the following studies: 1) repeated measurements on an inert surface, 2) repeated measurements on a single site of human skin, and 3) measurements on skin of multiple human volunteers in a clinical study. Custom-designed color chips were used as standards in the image analysis technique for color correction. Average intensity values of the individual RGB channels were analyzed, and mathematical algorithms were developed to correct color mapping caused by changes in light intensity during photographing. The measured color properties were compared between the two methods to assess accuracy and variability.
Results: From the repeated tests on a single skin site, an 88% reduction in variability of measured L*a*b* values was achieved using the color correction method over a Minolta Chromameter. From 26 clinical photographs, the variation of measured L*a*b* values reduced by 52% after color correction and analysis.
Conclusions: Variability of skin color measurement is introduced by subjectivity, skin contact, or photographing technique in various conventional methods. Data in this study suggest digital photography paired with novel image correction may provide a more accurate and less variable method of measuring skin color for application in ethnic skin research and product efficacy studies.

Keywords: skin, image analysis, Chromameter, color correction algorithms

POSTERS

P001
Skin Color Measurements with the DSMII colorimeter

Jonn Damia,[1] Timothy Houser,[1] Mary Jo Grove,[1] Gary Grove,[1] Carol Wellington[2], Sarah Joseph,[2] Logan Kennedy, [2] and Phil Diffenderfer,[2] [1]cyberDERM, inc. Media, PA USA, [2]Dept. of Computer Science, Shippensburg University, Shippensburg, PA, USA

Variations in human hair and skin color are the most striking visible aspect of human phenotype, yet very little is known about the genetics that underlie this diversity. Recent developments in a number of fields have provided the tools that will allow us to better understand the diversity of human pigmentation. This includes the recently introduced DSMII Colorimeter that was developed by our group at cyberDERM and now manufactured by Cortex Technology (Hadsund, Denmark). The DSMII is a portable optoelectronic instrument that allows objective measurements of human skin and hair using the RGB color space model. We have compared the capabilities of this instrument to that of the Minolta Chromameter which uses the CIE L*a*b* color system. Comparative color measurements were carried out first in vitro on standardized color charts, and subsequently in vivo on populations of various ethnic origins. Skin color changes induced by various physico-chemical treatments were also quantitatively evaluated with these instruments. Moderate to highly significant linear correlations could be established between the CIE L*a*b* color parameters and those derived using the RGB color space model. We found that in the in vivo studies the repeatability and sensitivity of the DSMII was better than that of the Minolta Chromameter. This is due largely in part to the operator being allowed to view the actual area being measured in real time (which is possible thanks to a small, transparent probe head). What is most exciting is that the DSMII provides a platform for developing more sophisticated types of analysis with little to no modification to the basic device.

Key words: chromameter, skin color.

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P002
Guidelines for determining the mechanical properties of the skin with the DermaLab Elasticity Module

Timothy Houser, Jonn Damia, and Gary Grove, cyberDERM, inc., Media, PA, USA

The DermaLab Elasticity Module is a commercially available device for measuring skin deformation and recovery that is made by Cortex Technology (Hadsund, Denmark). The probe consists of a lightweight plastic chambered head that is attached to the skin with double-sided adhesive tape. Within the suction chamber, there are 2 light beams that serve as level detectors. As the skin is lifted into the chamber by increased suction, it will sequentially block these light beams and the negative pressure to do so at each level can be measured. Since these data provide the stress and strain at 2 fixed points, the deformation characteristics can be determine using Hooke's Law. It is also possible to assess the recovery properties by timing how long it takes for the deformed skin to drop from the upper to lower light beams. We have undertaken a number of studies using physical standards, such as latex sheets, with different elastic properties to validate this approach. These results were quite consistent with that predicted, and even showed the non-ideal behavior of latex when excessively stretched. We found that, due to the non-linearity of the suction pump pressure profile, the deformation measurements based on time, such as dT, were not appropriate. We have also performed a number of clinical studies using normal healthy volunteers to study how skin deformability and recovery vary according to anatomical site, gender and age. We found that this simple and non-invasive device was very useful in the study of the mechanical aspects of the skin, especially for detecting age-associated changes. Of the various parameters examined, the pressure required to lift the skin to the higher level (Upper pressure) and the retraction time for it to fall back (Retraction) were found to be the most informative. In contrast to the manufacturer's claims, we were not able to confirm that VE, which combines both aspects in a single value, to be a meaningful measure.

Key words: skin elasticity, suction cup.

P003
SkinGlossMeter – a novel device for the measurement of skin gloss

[1]**Aki Immonen**, [2]Kari Myller, [1]Jouni Nuutinen, [2]Tapani Lahtinen; [1]Delfin Technologies Ltd, Kuopio; [2]MGM-Devices Ltd, Joensuu; [2]Department of Physics, University of Kuopio; Finland

Background: Easily applicable instrument to measure gloss of skin or lip, nail and hair has not been available.
Purpose: To develop a hand-held device for easy determination of local gloss of biological materials, especially gloss of skin.
Methods: A small hand-held and battery-operated device with a patented diffractive optical element and laser light source (635 nm) was constructed. The specular reflectance later called gloss was defined as part of the incident light reflecting back at a small angle (≤ 5 degrees). The specular reflectance was measured from a gloss standard. Furthermore, the gloss of volar and dorsal forearm, cheek and forehead was measured with six healthy Caucasian volunteers.
Results: There was a significant correlation between the SkinGlossMeter reading and gloss standard. The coefficient of variation for ten repeated measurements varied between 2 and 5 % depending on the standard gloss site. Marked differences between glosses of anatomical sites existed with highest gloss values on the forehead. The time for single measurement was 2 sec.
Conclusions: A novel glossmeter for easy assessment of gloss was developed. Preliminary measurements indicate that in addition to skin the instrument can be applicable also with lip, nail and hair. The SkinGlossMeter allows rapid and accurate measurement of gloss with applicants and formulations affecting human skin appearance.

Key words: Skin gloss, diffractive optics, hair, lip, nail

P004

Quantitative analysis of bioluminescence imaging of Malignant melanoma with luciferase in mouse models using optical imaging system

Jaeyoung Kim[1], Seunghan Ha[1], Gunwoo Lee[1], Onseok Lee[1], Sunghee Moon[1], Gyuman Park[1], Kyungho Kang[3] and Chilhwan Oh[1,2]; [1]Research Institute for Skin Image, [2]Dept. of Dermatology, [3]Dept. of Internal Medicine, Korea University College of Medicine, Seoul, Korea

Background/Purpose: Annually, incidence of malignant melanoma in Asian has been increased as western people had been done. This cancer needs a lot of researches for early diagnosis and treatment. The purpose of this study is to analyze quantitatively in mouse melanoma model of subcutaneous melanoma for using preclinical evaluation of new management methods. Nowadays, non-invasive evaluation of small animal tumor models could be used with Micro Magnetic Resonance Imaging (MRI), Micro Computed Tomography (CT), Single Photon Emission Computed Tomography (SPECT), Micro Positron Emission Tomography (PET). These devices are very expensive to be used and it takes long time to obtain the image of cancer model using these devices. However, the bioluminescence and fluorescence imaging system is much cheaper than others. Also it takes short time to acquire image. The bioluminescence and fluorescence imaging is a widely used non-invasive method for sensing gene expression, protein functions and other biological processes in animal models.
Methods: All experiments were performed using BALB/c nude inbred mice in accordance to Institutional Review Board (IRB) standards and procedure protocols of Korea University Medical Center (KUMC). Subcutaneous xenografts were established by injection of 5×10^6 B16-F1 cell line that had been transduced lentiviral vector containing luciferase. In vitro and in vivo bioluminescence images were obtained using homemade system. In our imaging system, the cooling Charge-Coupled Device (CCD) camera (Princeton Instruments PIXIS, Roper Scientific, Trenton, NJ) was used for data acquisition. Acquired images were analyzed using Matlab™ software (The mathworks, Inc., MA, U.S.A.).
Results/Conclusions: Bioluminescence imaging could be used for evaluation of degree of subcutaneous xenograft tumor models (melanoma B16-F1 cell line that tranceduced lentiviral vector containing firefly luciferase). Bioluminescent signal had increased according to tumor size. Bioluminescence imaging is supposed to be a powerful tools for *in vivo* evaluation of mouse model of xenograft melanoma. This technology is a cost effective and has high sensitivity. So, our bioluminescence imaging system can be use for preclinical study for new therapeutic agent against to malignant melanoma.

Key words: bioluminescence, melanoma, xenograft model, imaging, luciferase.

P006

3D Image Analysis of the Aging Face using the derma*TOP*-blue System

Matthew Liskowycz [1,2], **Gary Grove** [1], Mary Jo Grove [1], William Mongon [3], Greg Czepiel [3] and Jean-Jacques Servant [4]; [1.] cyberDERM, inc. Media, PA, [2.] Penn State University-Brandywine, Media, PA, [3]. Accurex Dimensional Measurement Systems, Swarthmore, PA , [4.] EOTech SA, Marcoussis, France.

It is generally known that the human face undergoes very characteristic changes with aging that are readily appreciated visually. These include crow's feet wrinkles in the periorbital area, furrows on the forehead, purser's lines around the lips and other more subtle changes in the skin's microrelief. Our group at cyberDERM has considerable experience in objectively evaluating anti-aging and wrinkle erasing products by using optical profilometry to measure changes in silicon rubber impressions taken from the skin surface. Our previous attempts to do *in vivo* assessments directly on the skin using various scanning systems have been somewhat disappointing. The biggest problem is that several seconds are required to complete a scan and the surface features are often blurred due to the panelist moving ever so slightly during this time. In the present study, we have utilized the dermaTOP-blue system which has an acquisition time of 1 second with the critical period for non-movement being less than 260 msec. This is a non-contact method that projects a series of miniature patterns of blue fringes that is optimized for precise measurement of the skin surface. To facilitate repositioning of the face for multiple measurements at different times, we utilized a custom designed support bench and chair with an integrated head holding device. A built in laser line projected onto the panelist's face verifies that they are properly positioned. Different sensor configurations are available depending upon the field of view desired. It is quite possible to measure lip volume, sagging jowls and baggy eyes but in this study our focus was on crow's feet wrinkles. With the OptoCat software

and a group of application macros, one can produce a 3D pseudocolor, topographic map of the skin surface which allows product effects to be demonstrated by superimposing pre and post treatment images that have been properly aligned. Additional parameters such as Ra, Rz, LR and other roughness statistics can be obtained by profile analysis of the wrinkles. We have found that this approach allows for a very powerful analysis by which the effectiveness of various dermatological and cosmetic treatments for the aging face can be evaluated.

Key Words: 3D Imaging, wrinkles, microrelief.

P007
Perception of Sensorial Irritation by Different Ethnic Groups

O'Leary J,[1] Foy V,[1] McGlone F,[2] and Meldrum H;[1] [1]Unilever R&D 40 Merritt Boulevard, Trumbull CT06611 USA; [2]Port Sunlight, Wirral CH63 3JW UK.

Sensorial irritation is any unpleasant response (sting, itch, burn, tingling etc.) that is perceived from the topical application of skin care products, and has a profound effect on the preference of such products by consumers. Understanding skin sensorial irritation of different ethnicities is a key priority for the development of non-irritating, efficacious skin care products suitable for consumers globally. Traditional sting testing has been done by the evaluation of lactic acid on the cheek (naso-labial fold) area of the face. The purpose of this investigation was to explore the sensorial irritation to glycolic acid of 3 different ethnic groups: Japanese, Chinese and Caucasian subjects living in the United States.

In one study, Caucasian subjects' responses were compared to Japanese subjects using a half face design. In a separate study, Caucasian subjects were compared with Chinese subjects using a full face study design. These designs were used to capture the typical magnitude of sensorial irritation by subjects and how it changes over time. Subjects' self-perceived unpleasant responses to 4% and 8 % glycolic acid in water at pH 3.8 were recorded over a 7.5 minute trial. Over 90% of both the Chinese and Caucasians reported sensorial irritation to 4% and 8% Glycolic acid in water when applied to the entire face. Both the Chinese and Caucasian populations initially reported higher sting versus itch response to 4% Glycolic Acid in water. Over the course of the 7.5 minute trial these responses reversed for both populations (Chinese: 11.4% sting, 36.4% itch, Caucasian: 14.6% sting, 41.7% itch). The 8% Glycolic acid responses mimicked the 4% Glycolic acid response pattern for both the Caucasian and Chinese populations. In the ½ face design with 4% glycolic acid in water, a similar trend was observed in responses for the Caucasian and the Japanese subjects, which matched the Chinese subjects' trends. Sensorial irritation breakdown (sting, itch, burn other) to glycolic acid was similar for both Asian populations (Japanese and Chinese) at all time points. The Japanese and Chinese populations showed higher baseline trans-epidermal water loss compared to Caucasians in both studies. These studies indicated the sensorial response to glycolic acid sting was similar for Japanese and Chinese women, all living in the USA.

Key Words: Sting, ethnic differences.

P009
Comparison of Stratum Corneum Thickness Calculated from *in vivo* Raman Spectroscopy and Confocal Imaging

Andrea Smiltneek[1], Douglas Hoffman[1], Andrew Basehoar[1], Lisa Stabe[1], Tina Nussbaum[1], Barry Reece[2], Corey Cunningham[1], and David Koenig[1], [1]Kimberly-Clark Corporation, Neenah, WI 54956 and [2]Reliance Clinical Testing Services Irving, TX 75062 USA

BACKGROUND: *In vivo* Raman spectroscopy and confocal imaging have been reported to provide a non-invasive means to determine stratum corneum (SC) thickness. The latter is a direct method while Raman spectroscopy is an indirect measure. It is anticipated that the two methods will complement each other with regards to defining SC thickness. Raman spectroscopy utilizes depth-resolved water profiles while confocal imaging utilizes the densely-packed keratin found in SC to calculate thickness.
PURPOSE: To compare SC thickness measurements obtained from Raman spectroscopy and confocal imaging at baseline and following a cosmetic treatment.

METHODS: Two studies were utilized to compare SC thickness values obtained from Raman spectroscopy and confocal imaging. One study employed *in vivo* Raman spectroscopy and was conducted in Irving, TX, using female subjects, aged 18-65. The second study employed *in vivo* confocal imaging and was conducted in Neenah, WI, with male and female subjects, aged 18-65. Measurements were taken from the volar forearm. Water profiles from Raman spectroscopy were obtained using a Model 3510 Skin Analyzer® (River® Diagnostics) and confocal images were obtained using a Vivascope® 1500 (Lucid®, Inc). Measurements were taken just prior to petrolatum application and 24 hrs after application. SC thickness values were calculated using methods provided by River® Diagnostics and Lucid, Inc., respectively.
RESULTS: *In vivo* Raman spectroscopy and confocal imaging yielded significantly different measurements for mean SC thickness at baseline (95% confidence) though the magnitude of the difference was relatively small (~2 μm). Specifically, a mean thickness of 19.8 ± 3.2 μm was measured using Raman spectroscopy while confocal imaging indicated a thickness of 17.8 ± 1.5 μm. Neither method was able to detect a significant increase in SC thickness following a 24 hour application of petrolatum.
CONCULSIONS: SC thickness measurements obtained from *in vivo* Raman spectroscopy and confocal imaging appear to have a small but significant difference. Calculated thickness measurements from *in vivo* confocal imaging had less variability between replicates than those obtained from Raman spectroscopy. This would indicate *in vivo* confocal imaging is better able to detect a change in SC thickness resulting from a treatment. These findings help demonstrate the complement of *in vivo* confocal imaging and Raman spectroscopy for detecting relatively small changes in SC thickness that may occur as a result of a cosmetic treatment.

KEY WORDS: Raman spectroscopy, *in vivo* confocal imaging, petrolatum, stratum corneum thickness.

P010
Treating Cosmetic Intolerance Syndrome and Inflammatory Skin Reactions

Walter Smith Ph.D., Future Beauty Laboratory, Michael Bishop, Active Organics

Skin sensitivity can arise from impaired barrier function or exaggerated response topical ingredients. As we age, skin sensitivity generally increases due in part to a decreased production of key barrier lipids, allowing for an increased penetration into the skin. Reactivity can also change due to altered expression of key genes involved in regulation of the inflammatory response. Together these changes are responsible for an increase in "Cosmetic Intolerance Syndrome" (CIS). CIS is an idiopathic response to the use or overuse of multiple cosmetic products. CIS reactions range from simple stinging and burning to an all out allergic contact dermatitis.
We have defined a strategy to reduce CIS and increase the skin's resistance to inflammatory reactions in general. Actilipid Ultra, a wheat extract containing ceramides and peptides, improves barrier function, and up regulates the production of ceramides, filaggrin and aquaporins. Actisoothe contains partially hydrolysed peptides from Coriolus and Cordyceps and down regulates COX 2 production. COX 2 is the rate limiting enzyme in the production of prostaglandins a key mediator of the inflammatory response. Actisoothe also immediately reduces redness and irritation from sun, mechanical or chemical irritants. ActImmune is a broccoli sprout extract with glutathione conjugates, suforaphane and DIM and has immune regulatory activity as well as sun protection attributes.
Subjects with CIS and sensitive skin were recruited and via clinical evaluations, only those with impaired barrier function (I), skin hyper-reactive and sensitive to the application of chemical irritants (2), and those with both fragile and reactive skin (3) participated in the study. Subjects were examined via a battery of tests prior to the study start and after one and three months, during which subjects used a placebo or products containing individual or combinations of the test materials described above.
After and three months significant improvements in skin condition were noted with daily application of the individual or a combination of the test products:

- Sensitivity to chemical irritants was dramatically reduced
- Skin dryness and itching due to poor barrier function was virtually eliminated
- The occurrence of adult onset inflammatory acne was decreased by more than 40%
- CIS reactions was reduced by more than 80% over the three month test period
- Photo-sensitivity was reduced by more than 40%

Sensitive skin can arise from a number of etiologies. Combining ingredients to improve barrier function and one to reduce skin reactivity and another to address skin immunological behavior is an excellent strategy to control consumer adverse reactions and assure acceptance of cosmetic products.

P011
Treatment of Cutaneous Sulfur Mustard (SM) Injury Using Mechanical Wound Debridement Methods and Amino-Plex® Spray Therapy

R.S. Stevenson, J.S. Graham, R.R. Deckert, R.F. Railer, B.F. Doxzon, H.L. Lumpkin, J.L. Devorak, L.W. Mitcheltree, T.A. Hamilton, J.I. Azeke and E.H. Braue; U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400

Background: Sulfur mustard [bis(2-chloroethyl)sulfide; SM] is a potent chemical warfare agent that induces blister and lesion formation. The resulting injuries, depending on severity, take weeks or months to heal. Amino-Plex® (biO_2 Cosmeceuticals International, Inc., Beverly Hills, CA) is a nutritive product designed to stimulate ATP synthesis, improve glucose transportation, stimulate collagen formation, promote angiogenesis and improve wound healing.
Purpose: The goal of this study is to examine the efficacy of Amino-Plex® treatment following mechanical debridement of SM injuries.
Methods: Partial-thickness sulfur mustard injuries were generated in the weanling pig model. At 48 hours, lesions were debrided using an Er:YAG laser or nitrogen-driven dermatome and treated with daily application of Amino-Plex® for seven days. A variety of non-invasive bioengineering methods were used to assess wound healing and evaluate the functional and cosmetic properties of the skin during a 14-day healing period: digital photographs (for visual documentation and gross observation), ballistometry (to assess skin hardness and elasticity), reflectance colorimetry (to evaluate changes in skin hue, chroma, and lightness), 2D high-frequency ultrasound (to examine changes in skin thickness), and evaporimetry (to measure transepidermal water loss as a way to evaluate barrier function). Cutaneous tissues were collected for histopathology and immunohistochemistry at 14 days post-surgery.
Results: Improved aesthetic and biomechanical results were obtained with 7-day Amino-Plex® treatment following debridement. Laser debridement, at any depth, proved more efficacious than dermatome debridement or no debridement at both the macro- and microscopic levels. Deeper debridement (200 □m) gave the most consistent and desirable cosmetic results, and showed the highest percentage of normal localization of basement membrane zone proteins associated with healthy tissue.
Conclusions: Amino-Plex® in conjunction with debridement is an effective treatment of cutaneous sulfur mustard injuries. Partial-thickness wounds, treated within two days of exposure, have shown to fully heal within two weeks.

Key Words: sulfur, mustard, chemical, vesicant, burn, Amino-Plex, wound, healing, laser, dermatome, debridement, non-invasive, bioengineering

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army or the Department of Defense.

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P012
In vivo confocal Raman spectroscopic measurements of ingredient penetration and water profiles in the skin

Chunhong Xiao, Jaime O'Leary, Melody Edmunds, Thomas Hancewicz, Shuliang Zhang and Manoj Misra; Unilever R & D, Trumbull, CT, USA

Vibrational spectroscopy has been used for structural analysis in biophysics and biochemistry for many decades. The technique provides important information about sample composition, protein structure, lipid conformational order, phase behavior, and intermolecular interactions. More recently, it has been increasingly used for in vivo studies of the skin for pharmaceutical and cosmetic applications. It provides both quantitative and structural information pertaining to the skin as well as exogenous materials delivered

from skin products, without the use of probes or dyes. Skin moisturization is essential for maintaining skin health and appearance. In particular, the stratum corneum (SC) provides a critical barrier against environmental insults and undergoes constant stress with changes in ambient humidity. Although there are many commercially available noninvasive techniques for measuring bulk hydration levels in skin, confocal Raman spectroscopy has the added advantage of measuring the water gradient in skin, and therefore provides depth-resolved information. In addition, Raman spectroscopy can be used for measuring natural moisturizing factors (NMFs) and SC thickness, and for studying ingredient penetration. With this technique, differences in SC hydration were detected on untreated skin at different body sites. The effects of moisturizers with various properties on skin hydration were detected and compared.