

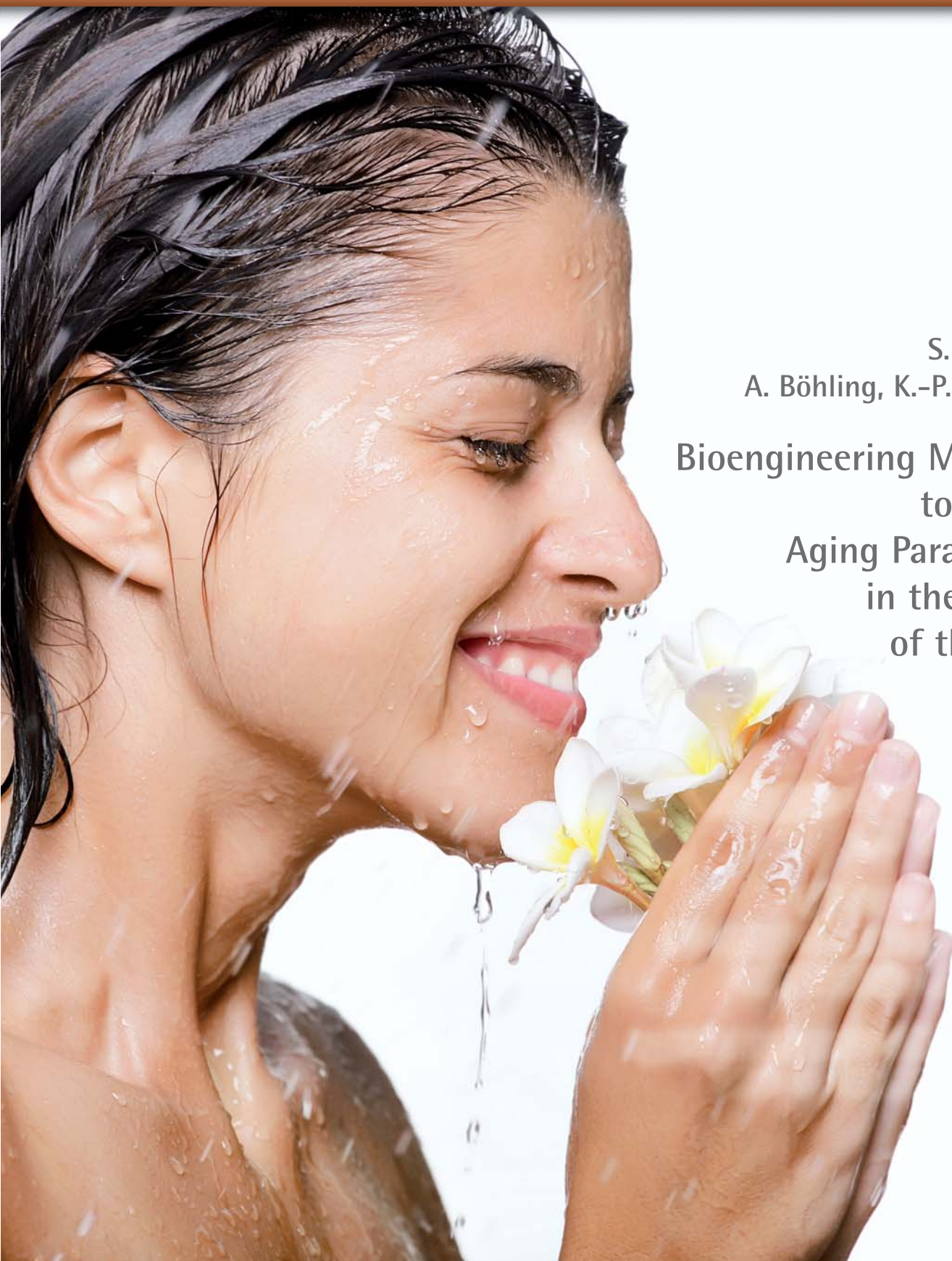
SOFW

JOURNAL

3-2011

English Edition

International Journal for Applied Science
• Personal Care • Detergents • Specialties



S. Bielfeldt

A. Böhling, K.-P. Wilhelm

**Bioengineering Methods
to Assess
Aging Parameters
in the Depth
of the Skin**

S. Bielfeldt, A. Böhling, K.-P. Wilhelm*

Bioengineering Methods to Assess Aging Parameters in the Depth of the Skin

Abstract

Non invasive *in vivo* methods are presented to assess age dependent changes in the different layers of the skin. Measurement of epidermal thickness parameters and papillary index by use of confocal reflectance microscopy are explained as well as the count of capillary loops, a parameter to assess age dependent skin nutrition. To assess photo aging of the upper dermis, 22 MHz ultrasound measurement is described. Thickness and intensity of the sub epidermal low echogenic band is a suitable parameter for that. It can be measured in ultrasound B-images by help of image analysis. With increasing age the chemical composition of the skin barrier changes. The depletion of NMF components and the reduced repair capacity of the barrier are well documented. *In vivo* confocal Raman spectroscopy can be used to quantify these age related processes. The described endpoints are all well established in literature and therefore suitable for the claim support of anti aging cosmetics.

■ Introduction

Anti aging efficacy is one of the most important claims for leave-on cosmetic products. Reduction of the traces of aging is one of the most wanted demands of cosmetic customers in a society that becomes older and older. Scientifically sound claim substantiation is required by authorities and there is a growing need for suitable, reliable and well documented skin aging parameters for cosmetic claim support. Most favorite are parameters derived from non invasive *in vivo* methods because they are not harmful to the volunteers and represent most closely the situation of the customer.

Already for decades a wide range of non invasive skin surface methods have been established. These are skin capacitance measurement to assess moisture, wrinkle measurements by the use of replicas or direct surface scanning, measurement of transepidermal water loss, skin pigmentation assessment by colorimetric methods and blood flow measurement by colorimetry or laser Doppler imaging. For the assessment of deeper skin layers, skin ultrasound methods are well established for a long time but their resolution is not high enough to discriminate structures in the epidermis.

High resolution information from the depth of the skin was mainly limited to more or less invasive *ex vivo* methods as sampling of Stratum corneum cells and layers or histological methods on full thickness skin biopsies. Mainly in the last decade the situation has changed when *in vivo* confocal technologies for skin research were established. The typical lateral resolution of these technologies is

one micron and the depth resolution is in the range of four to five microns. The high energy of focused near infrared lasers deeply penetrate the skin. Non invasive *in vivo* investigation of all epidermal layers and the reticular dermis can be obtained. Not only imaging parameters are assessable as by the use of the confocal reflectance microscope, but also the chemical composition of the skin can be analyzed, as by *in vivo* confocal Raman spectroscopy.

■ Epidermal Parameters Assessed by Confocal Reflectance Microscopy

High resolution confocal reflectance microscopy can be used to assess several age related parameters in the epidermis which are well documented in literature. In 1990 *Prenchat* (1) observed a clear age dependent decrease of epidermal thickness in the range of 6% per decade of aging, starting at an age of 30. These results were obtained by morphometric analysis of punch biopsies of the inner upper arm. In 2002 *Butis et al.* (2) found results in line with these findings by use of confocal microscopy. On a first view these results seem to be contradictory to the findings of *Guérif-Ferreira* (3). They report increased epidermal thickness with age, which was highly significant ($p < 0.001$, $r = 0.74$). The reason for this contradictory finding is that *Guérif-Ferreira* measured the distance between skin surface and the beginning of the dermal papillae, while the other authors included the complete dermal papillae into their measurement of epidermal thickness. *Guerie* and *Ferreira* also es-

tablished a measurement of the height of the dermal papillae and found a highly significant negative correlation of it with age ($p < 0.001$, $r = -0.854$). These findings make clear how the epidermis changes over age. In youth the dermal epidermal junction is dominated by large dermal papillae enabling optimal nourishment of the epidermis by diffusion from the blood vessels of the dermis. Diffusion processes are strictly related to the surface of the junction. The larger the surface, the better is the diffusion into epidermis. During aging the papillae become flattened and diminished (4). As a result the nourishment of the epidermis is impaired. The non papillary part of the epidermis becomes thicker because of larger granular layer cells resulting in a thickened basal layer in aged skin. This was demonstrated by *Sauer-mann* (4) by use of confocal reflectance microscopy. The consequences of the impaired nutrition situation of the aged epidermis was demonstrated by *Gadial-ly et al.*, 1995 (5). While in young adults the recovery time after experimental barrier impairment with organic solvents, detergents or tape stripping took about 72 hours in aged humans this recovery period was found to last a whole week.

Based on the reported findings we recommend the following non invasive epidermal parameters for anti aging studies with cosmetic products:

- A) Measurement of the epidermis thickness excluding the papillae
- B) Measurement of the heights of dermal papillae
- C) Measurement of the number of dermal papillae per cm^2
- D) Measurement of the number of capillary loops per cm^2

Confocal Reflectance Microscopy Technology

The general advantage of a confocal microscope compared to a standard light microscope is that it is not limited to the surface of partly transparent objects but enables the observation of a target structure in a defined layer at a chosen depth of such an object.

For example the VivaScope® (Mavic, Munich, Germany) is a confocal laser scanning microscope designed for *in vivo* examination (6). With this technique skin can be imaged *in vivo* in its native state. The human skin is an optically inhomogeneous tissue which is mostly impervious to light. Nevertheless, wavelengths between 600 nm and 1300 nm penetrate the skin (optical window). The light reflexion of the tissue is depending on wavelength and skin type and can be increased up to 60% by use of a laser of about 800 nm.

To generate the confocal image, a laser beam in the near infrared range (gallium-arsenide laser; 830 nm) is directed through an interconnected lens system with beam splitter onto the skin area to be examined. The part of the laser beam reflected by the skin returns through the beam splitter, passes a pinhole and is captured by the detector. Compared to a standard light microscope the pinhole prevents light reflected outside the focal plane to reach the detector. This leads to images of only the focal plane with a vertical resolution of about 4–5 μm . The light source, the illuminated spot on the skin, and the aperture opening of the detector are on optically conjugated focal planes – they are confocally interconnected.

In vivo mapping of the skin is possible down to a depth of approximately 350 μm depending on the skin type. Different microstructures within the skin cause natural variations of the refraction index and therefore provide the contrast in the image. For example, cytoplasm with a refraction index coming close to that of water (refraction index 1.33) is depicted with a very low contrast. Melanin and keratin (refraction index 1.7), however, have a relatively high refraction index and thus act as natural contrasting agents. Confocal laser scanning microscopy uses a water-immersible objective lens which is optically corrected for an aqueous fluid between the objective lens and the adhesive window, such as ultrasound gel. To avoid a discontinuous transition of the refraction indices (which might reflect the laser light) between the adhesive windows and the skin, the optical transition must be corrected by an index matching fluid. This fluid might be a specific oil or a water based gel.



Fig. 1 *In vivo* confocal microscopy of the skin

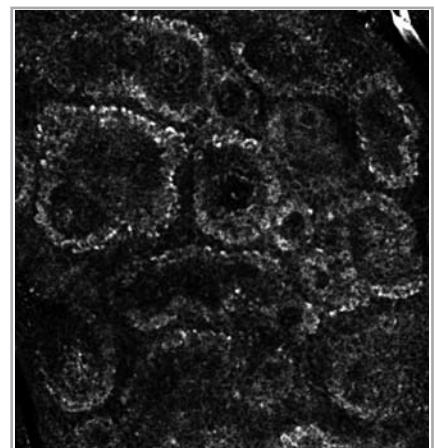


Fig. 2 *In vivo* confocal microscopic image of papillary structures

Confocal reflectance microscopy can produce images of *in vivo* skin sections with an optical section thickness less than 5 micrometers and therefore is comparable with histological skin sections. An integrated dermatoscope takes an image of the skin surface to document the lateral localisation of the confocal image. This allows for precise repositioning and repeated assessments on exactly the same test area (Fig. 1 and 2).

■ **Measurement of the Degree of Photo Aging of the Skin**

Total skin thickness does not vary significantly between the first and seventh decade of live and is only reduced after

the eighth decade (7). As a consequence total skin thickness as can be measured with ultrasound is not a good parameter of intrinsic aging. However, extrinsic aging due to chronic sun exposure can be detected easily in the dermis with ultrasound. Already 1989 Deregull et al. (7) reported a low echogenic band in the upper dermis that increased linearly in thickness with increasing age. This result was confirmed by other authors (8-10). The age related low echogenic band corresponds to areas which were histologically diagnosed as solar elastosis. This photodamage of elastic and collagen fibers in the dermis is caused by chronic sun exposure (9). We routinely measure the low echogenic band with a 22MHz ultrasound device (Taberna Pro Medicum, Lueneburg, Germany). The images are obtained from dorsal forearm, ventral forearm or cheek.

To obtain unbiased results in measurement of the low echogenic band (Fig. 3) it is important to take into account changes of the total echogenicity of the skin due to transparency effects of test products remaining on the skin. Further a diurnal rhythm of the low echogenic band has to be considered. A clear diurnal variation was seen in 74% of the volunteers (11).

■ Age Related Changes of Chemical Composition and Regeneration Properties of the Skin Barrier

The most important barrier of the skin is its uppermost layer, the Stratum corneum. Though its thickness is only 12 - 20 microns in most regions of the human body, it protects the body from water loss and environmental stress as UV-radiation or mechanical and chemical hazards. Two components of the Stratum corneum are well known to impair with increasing age. One is the Natural Moisturization Factor (NMF) the other the barrier lipid system (ceramides, cholesterol and other lipids in a highly organized structure). In elderly individuals amino acids of the Stratum corneum which are part of the NMF are known to be reduced. For these subjects a significant correlation exists between skin xerosis and amino acid content (12). This

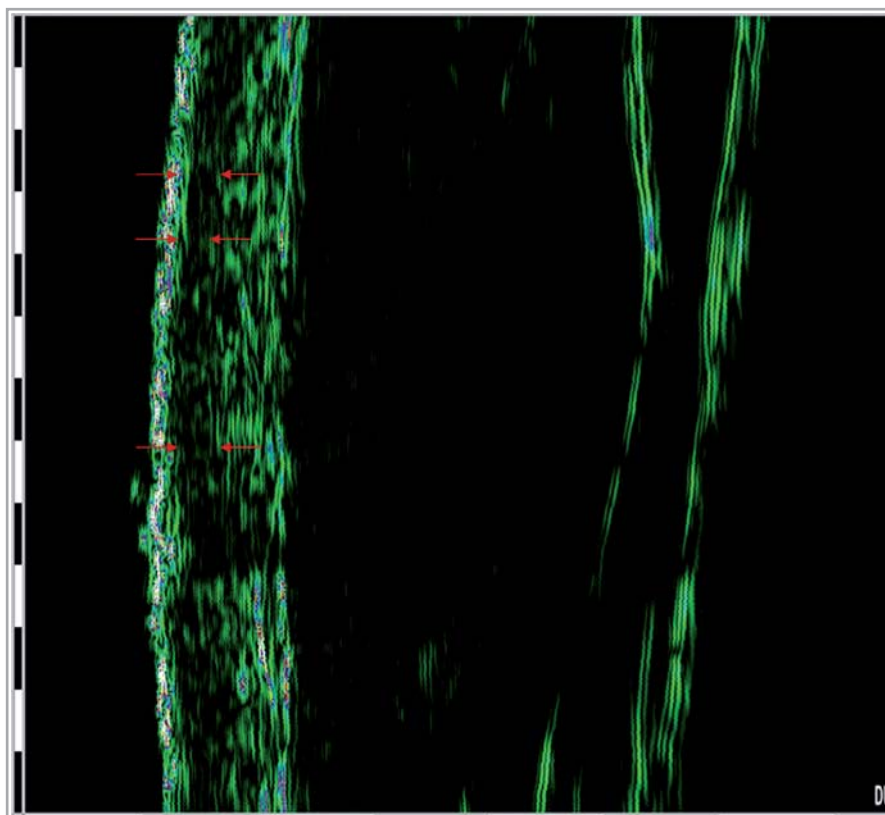


Fig. 3 Low echogenic band (between arrows) in the upper dermis indicates photoaging of the skin

means that age related skin dryness is at least partly due to reduced NMF levels in the Stratum corneum.

Barrier lipids mainly ceramides are not clearly reduced in elderly people but as already reported the barrier recovery after experimental barrier destruction by organic solvents, detergents or tape stripping is clearly prolonged compared to younger people. This means that age related skin dryness can also be caused by reduced barrier recovery properties after environmental stress of the skin. NMF levels and composition as well as the concentration of ceramides can be measured in one step by *in vivo* confocal Raman spectroscopy.

Confocal Raman Spectroscopy of the Skin

Atoms in molecules are not rigidly bound. They move around their equilibrium position in so called vibrational modes. When light interacts with a molecule by inelastic collision, energy can be trans-

ferred into the vibrational modes or to the photon. In both cases the energy and the wavelength of the scattered light changes. This effect was discovered by Sir C.V. Raman in 1928 and was awarded by the Nobel Prize in Physics in 1930. The Raman Effect can be measured with a high resolution spectrometer which measures the spectra of the remitted light. Besides the wavelength of the incident light this spectra illustrates peaks at wavelengths below and above the incident wavelength. The difference between the wavelength below and that of the incident light, called the Raman-Shift, is analyzed and expressed as relative wavenumber in cm^{-1} .

The electronic structure of the molecule determines to a very high degree the energy and Raman activity of the vibrational modes: the Raman spectrum is therefore truly a molecular fingerprint. The Raman spectrum of skin is in essence a superposition of the Raman spectra of every substance present. If the spectra of the investigated molecules are known,

then by detailed analysis of the skin spectrum its concentration (relative to the amount of keratin) can be measured. Hydration of the Stratum corneum, which is exposed to the relatively dry external environment, is maintained by NMF since it is a highly hygroscopic and water-soluble mixture of amino acids and specific salts, e.g. urea.

Separate determinations of the NMF components in the fingerprint region (400 bis 1800 wave numbers) can be performed *in vivo* with confocal Raman spectroscopy. The NMF concentration is obtained by adding up the contributions of the NMF components.

The Raman spectra can be analyzed automatically which leads to an effective and fast analysis process. Each spectrum is described as a linear combination of carefully measured spectra of pure skin components stored in the software. The validity of this approach has been demonstrated by comparison to classical *in vitro* methods (Fig. 4 and 5).

From the same spectra the main components of the barrier lipids (total ceramides and cholesterol) can be quantified.

Based on the reported findings we analyze the following Raman parameters for anti aging studies:

- A) Measurement of total NMF and of the single components of NMF as urea, lactate, PCA
- B) Measurement of the different amino acids which contribute to the NMF
- C) Measurement of the total content of Ceramides
- D) Measurement of Cholesterol

Since the regeneration of all of these components is retarded in aged skin, test models with artificially induced barrier impairment by tape stripping, or treatment with a detergent as Sodium Dodecylsulfate can be used to investigate the efficacy of anti aging cosmetic products. The measurement of the recovery speed of barrier relevant molecules by confocal Raman spectroscopy leads to very useful and specific efficacy endpoints.



Fig. 4 *In vivo* measurement of skin molecules with Confocal Raman spectroscopy

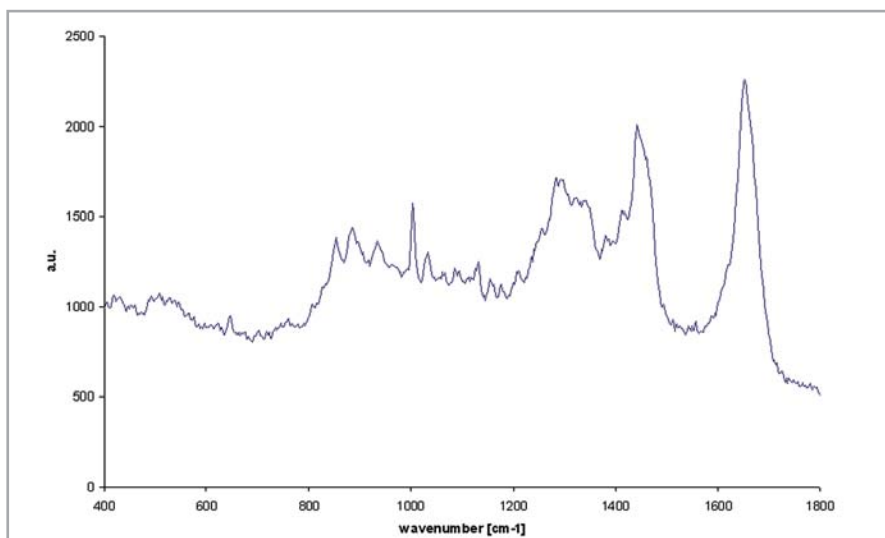


Fig. 5 Raman spectrum of skin in a defined depth captured by confocal Raman spectroscopy

■ Summary and Conclusion

Advanced non invasive *in vivo* technology is available to measure age related effects in the depth of the skin. The use of confocal reflectance microscopy, *in vivo* confocal Raman spectroscopy and skin ultrasound technology enables a measurement of highly specific and sensitive endpoints of skin aging and skin nourishment. These methods provide good chances of a scientifically sound, claim support for cosmetic anti aging products.

References

- (1) Branchet MC, Boissac S, Frances C, Robert AM, Skin thickness changes in normal aging skin. *Gerontology* 1990 36 (1), 28-35
- (2) Batisse D, Bazin R, Baldeweck T, Querleux B, Leveque JL, Influence of age on the wrinkling capacities of skin. *Skin Res Technol* 2002 8 (3), 148-154
- (3) Guérif-Ferreira Y, Oberto G, Berghi A, Cucumel K, dal Farra C, Domloge N, *In Vivo* study of Age-Related Skin Change Through *In Vivo* Confocal Microscopy. 70th Annual Meeting of the Society for Investigative Dermatology 2010, Atlanta, Georgia

- (4) *Sauermann K, Clemann S, Jaspers S, Gambichler T, Altmeyer P, Hoffmann K, Ennen J*, Age related changes of human skin investigated with histometric measurements by confocal laser scanning microscopy *in vivo*. *Skin Res Technol* 2002 8 (1), 52-56
- (5) *Elias PM*, Stratum Corneum Defensive Functions: An Integrated View. *Journal of Investigative Dermatology* 2005 125 (1), 183-200
- (6) *Sauermann K, Clemann S, Jaspers S, Gambichler T, Altmeyer P, Hoffmann K, Ennen J*, Age related changes of human skin investigated with histometric measurements by confocal laser scanning microscopy *in vivo*. *Skin Research And Technology* 2002 8 (1), 52-56
- (7) *de Rigal J, Escoffier C, Querleux B, Faivre B, Agache P, Leveque JL*, Assessment of aging of the human skin by *in vivo* ultrasonic imaging. *J Invest Dermatol* 1989 93 (5), 621-625
- (8) *Gnaidecka M, Jemec GB*, Quantitative evaluation of chronological aging and photoaging *in vivo*: studies on skin echogenicity and thickness. *Br J Dermatol* 1998 139, 815-821
- (9) *Richard S, de Rigal J, de Lacharriere O, Berardesca E, Leveque JL*, Noninvasive measurement of the effect of lifetime exposure to the sun on the aged skin. *Photodermatol Photoimmunol Photomed* 1994 10 (4), 164-169
- (10) *Tsukahara K, Takema Y, Moriwaki S, Fujimura T, Kitahara T, Imokawa G*, Age-related alterations of echogenicity in Japanese skin. *Dermatology* 2000 200 (4), 303-307
- (11) *Gnaidecka M, Gniadecki R, Serup J, Sondergaard J*, Ultrasound structure and digital image analysis of the subepidermal low echogenic band in aged human skin: diurnal changes and interindividual variability. *J Invest Dermatol* 1994 102 (3), 362-365
- (12) *Denda M, Hori J, Koyama J, Yoshida S, Namba R, Takahashi M, Horii I, Yamamoto A*, Stratum corneum sphingolipids and free amino acids in experimentally-induced scaly skin. *Arch Dermatol Res* 1992 284 (6), 363-367

* *Authors' address:*

Stephan Bielfeldt
Arne Böhling
Klaus-Peter Wilhelm
proDERM Institute for
Applied Dermatological Research
Kiebitzweg 2
22869 Schenefeld / Hamburg
Germany
Email: SBielfeldt@proDERM.de

