In vivo determination of the skinís molecular composition offers many advantages over ex vivo methods. However, the number of methods to study the molecular composition of the skin in vivo is limited. Raman spectroscopy is a light scattering technique that provides detailed information about molecular composition and has excellent possibilities for in vivo application on the human skin. We have shown in vitro that Raman spectroscopy can distinguish basal cell carcinoma (BCC) from its surrounding tissue, based on subtle but clearly detectable differences in the composition of the tissues. *In vivo* application of this methodology has great potential for assisting, guiding and accelerating onco-surgical procedures such as the time-consuming Mohsí micrographic surgery. *In vivo* confocal Raman microspectroscopy, applied directly on the living skin, provides information about the composition of the skin with high spatial resolution. This enables objective assessment of a personís skin condition, monitoring of the penetration and effects of topically applied products.

Skin characterization with *in vivo* Raman spectroscopy

Peter Caspers, Annieke Nijssen, Gerwin Puppels

The authors gratefully acknowledge the financial support from and collaboration with Philips Research.

Introduction

Basal cell carcinoma (BCC) is the most common cancer of the skin and its incidence is ever increasing. Although slowly growing and rarely metastatic, BCC can cause significant local destruction. Histopathological examination is the gold standard for confirming the diagnosis. For most types of BCC, surgery is recommended as first-line treatment. A problem is presented by the fact that the tumor borders cannot be detected visually with 100% accuracy. This results in five-year recurrence rates for primary BCC ís of 10.1% after simple surgical excision, due to irradical resection. Mohs' micrographic surgery is a technique in which resected material is inspected histopathologically during the course of the surgical procedure. With this approach tumor margins can be detected with approximately 100% certainty: five-year recurrence rates are only 1%. However, Mohs' micrographic surgery is a time-consuming method, both for the pathologist and the surgeon, which prevents its widespread use. Real-time intra-operative ***in vivo*** determination of the tumor border would be a solution to this problem.

The SC is a physical barrier that protects the viable cells against dehydration and chemical and microbial assault from the environment. The barrier function depends on proper moisturization, lipid balance and the presence of humectants, especially constituents

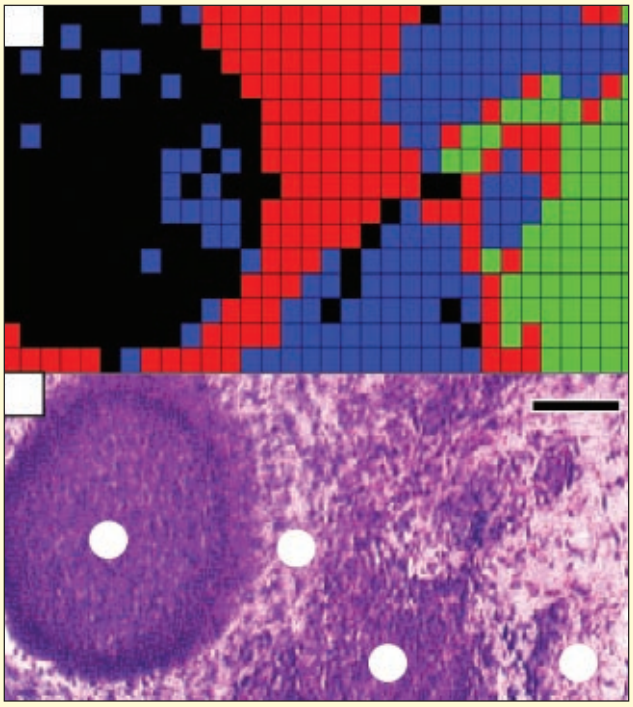

Figure 1. *Comparison of pseudo-color Raman map with image of H&E stained adjacent section. BCC and surrounding dermis: KCA distinguishes BCC (1), dermis directly surrounding the tumor (2) and dermis at a greater distance from the tumor (3). Furthermore a dense inflammatory infiltrate is present (4) and detected by Raman spectroscopy. Colors: black=BCC, red=dermis close to tumor, green=dermis at a greater distance from tumor, blue=dense chronic inflammatory infiltrate. (Scale bar: 100 µm.)*

of Natural Moisturizing Factor (NMF). Several skin problems have been described, ranging from dry skin to severe forms of psoriasis where these parameters were perturbed. Obviously, there is a need for techniques that provide detailed knowledge about these parameters of the skin. Only noninvasive methods allow studying the skin in its natural state without affecting its integrity, morphology or molecular composition. Measurements can be repeated on the same skin area, and can thus be used to monitor changes in the skin over time. Noninvasive characterization of the thin (~12 µm) superficial skin layer, the stratum corneum (SC), requires a technique with good spatial resolution and molecular specificity, which is offered by ***in vivo*** confocal Raman microspectroscopy.

Technical solutions

The laboratories of the CODT have developed unique expertise and instrumentation for tissue classification based on Raman spectroscopy. It was shown that BCC can be distinguished from non-cancerous tissue. 15 excision specimens of histologically proven BCCís were collected. Biopsies were taken from the specimens and cryo-sectioned on CaF_2 substrates. Adjacent sections were fixed in formalin and H&E-stained for histopathologic inspection. The unstained sections were placed under a Raman microscope, which was developed in the CODT laboratories. The sections were scanned on a grid of 10x10µm^2 pixels, each of which produced a Raman spectrum. K-means clustering analysis (KCA) was then used to group the spectra in clusters, based on spectral similarity. This resulted in a pseudo-color Raman map, where different colors represent clusters based on differences in spectral characteristics between the grid elements. Figure 1 shows a comparison between a pseudo-color Raman image and the H&E stained tissue section. Both images are highly similar, demonstrating that Raman spectroscopy clearly distinguishes between BCC, (altered) dermis close to the tumor, (normal) dermis distant from the tumor and a region of dense inflammatory infiltrate. The method applied to the set of 15 BCC specimens showed a result of 100% sensitivity and 93% selectivity for basal cell carcinoma.
River Diagnostics in collaboration with the laboratories of the CODT are world leaders in instrumentation and methodology for ***in vivo*** confocal Raman microspectroscopy on the human skin. River Diagnostics' Model 3510 Skin Analyzer (see figures 2 and 3) combines high spatial resolution (~5 µm) with molecular specificity, which enables rapid quantitative and depth-resolved determination of the molecular composition of the SC.

Skin hydration

The Raman signal of skin in the 2500-4000 cm^{-1} spectral region is primarily due to CH_2- and CH_3 stretching vibrations in proteins and lipids (~ 2800-3000 cm^{-1}) and to the OH-stretching vibrations of water (~ 3100-3700 cm^{-1}). The water concentration, in mass-%, is determined from the intensity ratio of two well chosen spectral intervals within these CH-stretching and OH-stretching regions. Figure 4 shows the in vivo water concentration profile across the SC of the arm. The results are shown in triplicate, measured on three spots close to one another on the inner forearm.

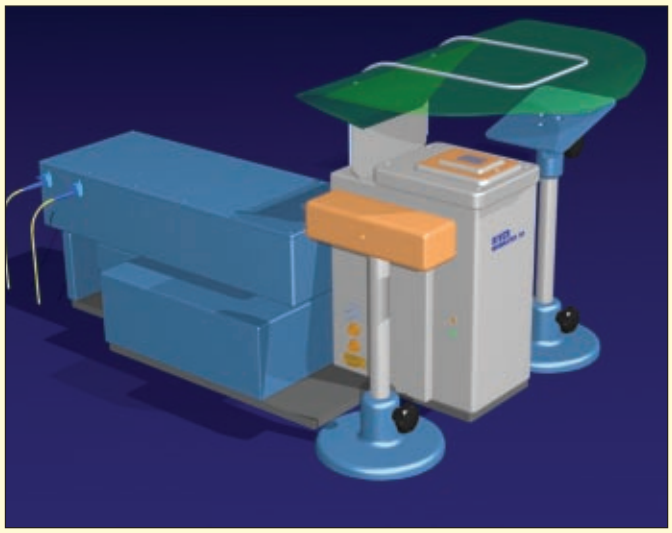

Figure 2. *Model 3510 Skin Analyzer for in vivo confocal Raman measurements on the skin*

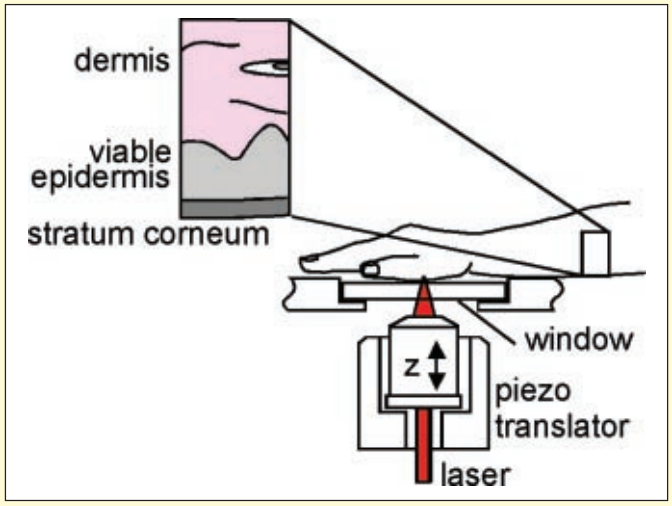

Figure 3. *Schematic of the measurement layout for in vivo confocal Raman measurements on the skin. Laser light (NIR, low power) is focused to a small spot at a selected depth below the skin surface, which can be varied. The Raman signal generated at the laser focus is collected and recorded with a CCD-based spectrometer. The inset shows a magnification of the various skin layers.*

Skin NMF

The Raman spectrum of skin in the 400-2000 cm^{-1} spectral region contains a variety of bands that are specific for the various molecular species in the skin. Mathematical modeling (multiple least squares fitting) with in vitro spectra of NMF constituents enables identification and quantification of skin these constituents. Figure 5 shows the ***in vivo*** concentration profile of NMF across the stratum corneum of the arm. The results are shown in triplicate, measured on three spots close to one another on the inner forearm.

Conclusions

In vivo Raman spectroscopic skin characterization has great potential for assisting, guiding and accelerating onco-surgical procedures such as the time-consuming Mohs' micrographic surgery.
Applications of ***in vivo*** measurements of skin hydration and NMF can be found in fundamental skin research, clinical monitoring of medical skin treatment (e.g. psoriasis), skin typing and development and clinical testing of moisturizing personal care products.

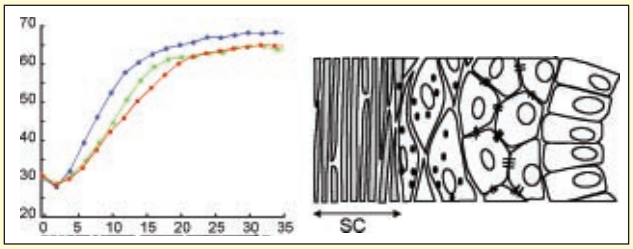

Figure 4. *In vivo water concentration profiles of the human skin, measured in triplicate on the inner forearm. Laser wavelength: 671 nm; laser power on the skin: ~15 mW; measurement time: 1 s per point*

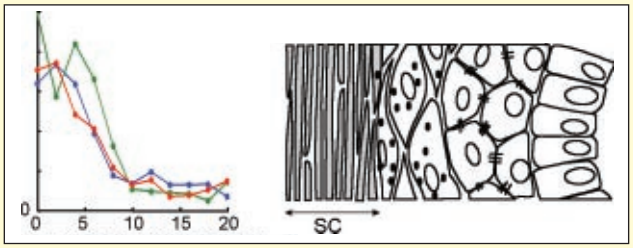

Figure 5. *In vivo NMF concentration profiles of the human skin, measured in triplicate on the inner forearm. Laser wavelength: 785 nm; laser power on the skin: ~20 mW; measurement time: 5 s per point.*

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