Malignant Melanoma Diagnosis by Raman Spectroscopy and Neural Network: Structure Alterations in Proteins and Lipids in Intact Cancer Tissue

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ABSTRACT

Malignant melanoma (MM) is the most aggressive skin cancer. The specificity and sensitivity of clinical diagnosis varies from around 40% to 80%. Here, we investigated whether the chemical changes in the melanoma tissue detected by Raman spectroscopy and neural networks can be used for diagnostic purposes. Near-infrared Fourier transform (NIR-FT) Raman spectra were obtained from the samples of malignant melanoma (MM, n=22) and other skin tumors that can be clinically confused with MM: pigmented nevi (PN, n=41), basal cell carcinoma (BCC, n=48), seborrhoeic keratoses (SK, n=23) and normal skin (NOR, n=89). A sensitivity analysis of spectral frequencies used by a neural network was performed to determine the importance of the individual components in the Raman spectra.

Visual inspection of the Raman spectra suggested that MM could be differentiated from PN, BCC, SK and normal skin due to the decrease in the intensity of the amide I protein band around 1660 cm⁻¹. Moreover, MM and BCC showed an increase of the intensity of the lipid specific bands peak around 1310 cm⁻¹ and 1330 cm⁻¹, respectively. Band alterations used in the visual inspection were also independently identified by neural network for melanoma diagnosis.

The sensitivity and specificity for diagnosis of malignant melanoma achieved by neural network analysis of Raman spectra were 85% and 99%, respectively.

We propose that neural network analysis of NIR-FT Raman spectra could provide a novel method for rapid, automated skin cancer diagnosis on unstained skin samples.

Key words: skin tumor/ basal cell carcinoma/ nevi/ seborhoeic keratosis

INTRODUCTION

Malignant melanoma (MM) is the most aggressive of skin cancers and is invariably fatal if left untreated (MacKie, 2000; Marks, 2000; Martinez et al., 2001). MM removal at early stages is almost always curative and therefore early detection is essential. Clinical diagnostic sensitivity differs greatly depending on the length of the training of the clinician: 80% for trained dermatologist, 62% for senior registrar, 56% for registrars and approximately 40% for non-dermatologists (Cassileth *et al*, 1986; Morton and Mackie 1998; Chen *et al*, 2001).

Difficulties in diagnosis of cutaneous MM arise because benign lesions like pigmented nevi, seborrhoeic keratosis and other types of skin cancer such as basal cell carcinoma may resemble malignant melanoma (MacKie, 2000; Kanzler and Mraz-Genrnhard, 2001). Histopathological examination of the excised suspicious element is considered to be the golden standard (Slater, 2000). However, removal of every pigmented lesion is unacceptable for the patient, especially in case of multiple skin lesions or lesions localized in cosmetically important parts of the body such as the face because of risk of scarring. Eighty percent of biopsies taken by non-dermatologists of suspected malignant skin lesions have been reported to be benign (Jones *et al*, 1997) and thus inappropriate surgery is frequent (Cox *et al*, 1992).

Development of non-invasive diagnostic methods is therefore crucial for malignant melanoma detection. Despite many attempts to implement various instrumental techniques (total body

photography, dermatoscopy, high-frequency skin ultrasonography, fluorescence spectroscopy, positron emission tomography, etc.) a non-invasive, reliable method for malignant melanoma diagnosis has not yet been established (Bafounta *et al*, 2001; Harland *et al*, 2000; Kittler *et al*, 2002; Prichard *et al*, 2002).

Raman spectroscopy is a technique that provides information about the molecular structure of the investigated sample and has been widely used for the past seventy years for non-destructive chemical analysis (Raman and Krishanan, 1928; Edwards *et al*, 1995; Hanlon *et al*, 2000; Choo-Smith *et al*, 2001). In Raman spectroscopy a sample is irradiated with laser light, which results in light scattering. The majority of scattered light has unchanged frequency (so-called Rayleigh line) whereas the rest is shifted in frequency (Raman effect). Those frequency shifts can be analyzed and presented as a Raman spectrum. Raman effect is caused by molecular vibrations in the irradiated sample and thus gives information about the structure of the molecules. Molecular vibrations can be divided into deformational vibrations (δ) where the angles between the bonds change, and stretching vibrations (v) where the lengths of the bonds change but the angle between them remains constant.

Previous studies of the samples obtained from the genital tract, brain, breast and laryngx revealed that the transition from normal to cancer tissue is associated with significant differences in chemical structure which are reflected in the Raman spectra (Liu *et al*, 1991; Mizuno *et al*, 1994; Hanlon *et al*, 2000; Stone *et al*, 2000). Benign and malignant changes in the gastrointestinal tract have recently been studied intraoperatively *in vivo* with the aid of Raman spectroscopy (Shim *et al*, 2000; Dacosta *et al*, 2002). Our earlier studies have shown that samples of various benign skin lesions and non-melanoma skin cancer have characteristic Raman spectra (Gniadecka *et al*, 1997a; Gniadecka *et al*, 1997b; Gniadecka *et al*, 1998a) and that this technique is highly reproducible (Gniadecka *et al*, 1998b). Recent studies on basal cell carcinoma by Raman spectroscopy showed that it is feasible to differentiate malignant tissue from the healthy surrounding tissue (Nijssen *et al*, 2002).

However, Raman spectra are complex and the traditional interpretation of the spectra by their visual inspection is subjective and time consuming. Artificial neural network is a pattern recognition tool for modeling non-linear functional relationships in data. Neural networks may be applied to problems where the relationship is complex or unknown. Automated computer methods like principal component analysis and neural networks have in the last decades been developed for many different applications in biomedical research (Hanlon *et al*, 2000; 18,19). Recent studies have shown that neural networks can be successfully used for analysis of dermatoscopic images (Rubegni *et al*, 2002a; Rubegni *et al*, 2002b; Picollo *et al*, 2001).

Here we have developed a neural network system for the automated classification of Raman spectra, allowing to differentiate malignant melanoma from other clinically similar skin tumors. Firstly, all Raman spectra were preprocessed (see Patients and Methods). Then, examples of Raman spectra were used to train the neural network for learning the spectral patterns. The performance of the neural network was tested on an independent set of spectra, by prediction of lesion type and comparing it to the true class. Moreover, the importance of specific spectral bands for the classification of individual

classes could be evaluated, thus making it possible to extract the most meaningful parts of spectra important for the neural network classification.

PATIENTS AND METHODS

Skin samples

Three millimeter punch biopsies of malignant melanoma (n=22; including in situ, superficial spreading and nodular malignant melanoma), pigmented naevi (n=41), and punch biopsies or curettage specimens of basal cell carcinoma (n=48; including nodular and superficial spreading types), seborrhoeic keratosis (n=23) and normal skin (n=89, biopsies of the skin in the vicinity of the lesions from various body regions) were collected for Raman spectroscopy. The samples were kept at +4°C in closed vessels during the short period before analysis. The majority of the samples were analyzed immediately by Raman spectroscopy. A part of the samples were snap frozen in liquid nitrogen and stored at -80°C. The frozen samples were transferred to closed vessels and allowed to thaw at +4°C for 3-5 minutes. The Ethics Committee of Copenhagen City, Denmark accepted the protocol.

Raman spectroscopy

A Bruker IFS 66 interferometer equipped with a FRA 106 module or FRA 100 (Karlsruhe, Germany) were used as previously described (Gniadecka *et al*, 1998bc). The laser beam (1064 nm line at 300 mW from a continuous wave Nd: YAG laser) was focused to a spot of approximately 100 μ m in diameter on the epidermal site of the biopsy or curetted specimens, which during the procedure was placed in a stainless - steel cup. Typically 250 scans at a 4 cm⁻¹ spectral resolution were collected from each sample over approximately 7 min. In preliminary experiments we found no significant overheating or thermal injury of the samples at these conditions (unpublished). Zero-filling was used for all spectra. OPUS software (Bruker) was used to evaluate spectral characteristics in the region from 200cm⁻¹ to

3500 cm⁻¹. Before the visual evaluation, the spectra were normalized for the 1450 cm⁻¹ band intensity in the 800 to 1800 cm⁻¹ region and for the 2940 cm⁻¹ band intensity in the 2500 cm⁻¹ to 3500 cm⁻¹ region. The intensity of 1450 cm⁻¹ and 2940 cm⁻¹ bands remain stable under a variety of conditions and have therefore been routinely chosen for spectra normalization by us and numerous other researchers. For neural network analysis spectral region from 200cm⁻¹ to 3500 cm⁻¹ was used.

Spectra evaluation

To analyze alterations in protein and lipid structure, the positions, shape and intensity of spectral bands for proteins, lipids and water were studied in the region 1200 cm⁻¹-1750 cm⁻¹. This area comprises the major protein and lipid vibrations. Among seven important protein vibrations, the amide I and amide III vibrations are clearly represented in Raman spectra of the skin (Tu, 1986; Edwards et al., 1995). The amide I (around 1650 cm⁻¹) reflects mainly the C=O stretching vibrations, whereas the amide III band (around 1270 cm⁻¹) is a more complex mode involving several chemical bonds. Combined maximum intensity frequencies of the amide I and III reflect protein secondary structure (α -helix conformation, β sheet, random coil). Moreover, a strong C-C stretch band at approximately 935 cm⁻¹ is typical for an α helix. Lipid specific vibrations are found around 1300 cm⁻¹ (twisting and wagging vibrations) and in the region extending to 1340 cm⁻¹ (Edwards *et al*, 1995).

The intensities of the described spectral bands were calculated using Matlab software, The MathWorks Inc. USA. To demonstrate spectral changes for proteins, the ratio between the amide I band and δ (CH)₂(CH)₃ in proteins and lipids (I_{1650 cm-1}/I_{1450 cm-1}) and the ratio between the amide III and lipids at around 1320 cm-1 were calculated (I_{1270 cm-1}/I_{1320 cm-1}) (Tables 1 and 2).

Neural network analysis

The raw Raman spectra were fed to the system where they were preprocessed. The preprocessing stage included background suppression¹ and dimension reduction² of the spectrum. Based on the preprocessed spectrum, the neural network predicted the probability that a spectrum belonged to the lesion types³. The spectra were assigned to the class that had the highest predicted class probability. Before the system could be used for classification, the weights (parameters) of the neural network were trained (optimized) from a set of spectra with class labels⁴. The training needed careful control to avoid over-fitting⁵ the training data, as the neural network architecture used here was extremely flexible and able to fit the data with arbitrary accuracy. The neural network classifier software was written in Matlab and is freely available at http://mole.imm.dtu.dk/toolbox/ann/index.html.

¹ The background suppression was obtained by numerically computing the local first derivative of each spectrum. ² The number of dimensions is reduced with principal component analysis (PCA) of the data and by removing the least significant principal components. The number of retained principal components was determined with the Bayesian information criterion (BIC), using the maximum likelihood formulation of the PCA (Hansen and Larsen, 1996).

³ The neural network architecture was based on the feed-forward architecture with two layers of weights and hyperbolic tangent function as activation function of the hidden units. To interpret the output of the neural network as probabilities, the outputs were extended with an exponential transformation called softmax.

⁴ The weight optimization was done by a quasi-Newton scheme, described in detail in (Nielsen, 2000).

⁵ The approach adapted here used regularization and outlier detection to prevent over-fitting. A Bayesian ML-II approximation was used to estimate a regularization parameter and outlier probability (Sigurdsson *et al.*, 2002).

The importance of specific spectral bands for the neural network classification was evaluated with sensitivity analysis⁶. The results are visualized with sensitivity maps, where high sensitivity of a specific spectral band was interpreted as highly important for the neural network classification. The NPAIRS framework⁷ was used to determine the reproducibility of the sensitivity maps. By using this framework, confidence intervals were determined for the sensitivity maps to reflect their reproducibility. Sensitivity maps that are highly reproducible have corresponding high confidence.

All spectra were examined at frequencies from 200 cm⁻¹ to 3500 cm⁻¹ at spectral resolution of around 2 cm⁻¹ (zero-padded original spectrum). By using the preprocessing method, the dimension of the spectra was reduced from the original 1711 frequency components to 7 inputs to the neural network. The 7 inputs matched the first 7 principal components.

The neural network had initially 7 input units, 20 hidden units and 5 output units, one for each class. The generalization capability of the network was tested with leave-one-out (LOO) cross-validation, where a single example is used for testing and all others for training. This was done for all examples. The dimension reduction was calculated on the training set and then applied on the test set, so that the test data was truly independent of the training data. After training, the optimal neural network was

⁶ Sensitivity was evaluated by computing the derivative of an output with respect to an input, using the absolute value average sensitivities (Zurada *et al.*, 1994). The sensitivity measurements were mapped back through the PCA basis, to form the so-called sensitivity map of the individual classes.

⁷ The data set was randomly divided in two equal halves (split half resampling). Then, two independent networks were trained on each half of the data and the corresponding sensitivity maps were computed. The similarity of the sensitivity maps determined the reproducibility. For details see (Strother *et al.*, 2002).

evaluated using the test set and the classification errors were calculated. The reported specificity and sensitivity for each of the classes was determined based on the average of the 5 LOO cross-validations.

A sensitivity analysis was performed in order to obtain information about the relevant frequency bands in the Raman spectra for each class. The reported average sensitivity map with confidence interval for the malignant melanoma was obtained from the average of 100 pairs of sensitivity maps using the NPAIRS framework.

Statistical analysis

Data were not normally distributed and therefore non-parametric Wilcoxon test was used to compare the values. Confidence intervals were calculated as described in Altman (1991). $P \le 0.05$ was considered significant.

RESULTS

Spectra of normal skin

The spectra of normal skin were similar to those previously reported (Barry *et al*, 1992; Edwards *et al*, 1995; Gniadecka *et al*, 1998bc). Peaks originating from vibrations within protein molecules and lipids were clearly resolved (Fig. 1 and Table 1). In normal skin the positions of the amide I and III bands (1650 cm⁻¹ and 1270 cm⁻¹) and the presence of a well developed band around 935 cm⁻¹ suggested helical protein structure. Pigmentation increased the background level of the spectra which was most pronounced in the region 1800-2500 cm⁻¹ as previously described for normal skin (Knudsen *et al.,* 2002) (Fig. 2). This region does not contain protein and lipid bands.

Skin tumor spectra reveal protein and lipid alterations

Spectra revealed clear cut changes between the investigated types of tumors allowing differentiation of malignant melanoma from other investigated lesions (Fig. 1, Table 1 and 2). MM presented with a decrease in the amide I band intensity resulting in flattening of the spectral area between 1500 cm⁻¹ and 1800 cm⁻¹ (Fig. 1A and Table 2). This suggested a change in molecular composition. Moreover, an increase in intensity of the band at 1300 cm⁻¹ of the CH twisting and wagging in lipids and the band around 1310-1330 cm⁻¹ was seen with relative decrease in the amide III regions of proteins (Fig. 1B and Table 2).

BCC showed similar spectral changes in the region 1300-1340 cm⁻¹ (Fig. 1B and Table 2). The emerging bands in this region were found around 1300 cm⁻¹ and 1330 cm⁻¹.

Both MM and BCC spectra showed a decrease in protein band intensity around 940 cm⁻¹ (Fig. 1C). In contrast to MM, BCC spectra did not show any decrease in intensity of the amide I band (Fig. 1A).

SK presented with a prominent increase of intensity in the 1300 cm⁻¹ making the discrimination from BCC possible (Fig. 1B). The band around 1450 cm⁻¹ reflecting (CH)₂ (CH)₃ in lipids and proteins was shifted in MM, BCC and SK (Fig. 1D and Table 1).

Spectra of pigmented nevi were almost identical to those of normal skin but consistently showed a decrease in intensity in the right wing of the amide I band (Fig. 1A and Table 2). This suggested minor changes in composition or conformational alterations in the proteins. Moreover, in the region from 1800 cm⁻¹ to 2500 cm⁻¹, an increase in intensity of the otherwise flat spectral region was observed (Fig. 2). This phenomenon occurred most probably due to an increase in pigmentation of the lesional skin, similar to that of highly pigmented skin and in MM spectra.

Neural network analysis classifies MM with 85 % sensitivity and 99 % specificity

Neural network analysis of Raman spectra achieved the diagnostic sensitivity of 85% and specificity of 99% for the diagnosis of malignant melanoma (Table 3). BCC diagnosis was 97% and 98 % respectively. The confusion map, established by neural network, presenting wrongly classified skin lesions is presented in Table 4. MM had tendency to be misdiagnosed as BCC and PN, while PN were confused with normal skin. Seborhoeic keratosis was diagnosed with 96 % sensitivity and was most

frequently confused with BCC. Pigmented nevi were diagnosed with 78 % sensitivity.

The sensitivity map for MM in Fig. 3 showed important spectral intensities at 1620-1670 cm⁻¹, 1230-1300 cm⁻¹ and 1430-1450 cm⁻¹, which matched the spectral differentiation marked A, B and D in Fig. 1. Moreover, the sensitivity map pointed out new frequencies not used for visual inspection of the spectra, 2840-3000 cm⁻¹, 2000-2350 cm⁻¹ and at 1000 cm⁻¹, marked E, F and G in fig. 2, respectively. Vibrations reflected at this spectral regions are most probably characteristic for (E) CH stretching in proteins and lipids (Gniadecka *et al.*, 1998), (F) skin fluorescence (Knudsen et al., 2002) and (G) ring vibrations in aromatic aminoacids residues (Gniadecka *et al.*, 1997).

DISCUSSION

We have demonstrated that it is possible to differentiate malignant melanoma from pigmented nevi, basal cell carcinoma and seborrhoeic keratosis by the neural network analysis of the NIR-FT Raman spectra.

The neural network analysis used spectral alterations reflecting changes in composition and structure of proteins and lipids. Malignant melanoma and basal cell carcinoma spectra showed similar patterns but were distinctive. Intensities in the amide III regions of proteins decreased relatively to the lipid-specific bands. An emergence of the lipid component of the $\delta(CH_{2})(CH_{3})$ accounting for shifting of the 1450 cm ⁻¹ band occurred both in BCC and MM spectra. For SK, a major increase in intensity of the 1300 cm⁻¹ band of twisting and wagging vibrations of lipids reflected lipid accumulation in the horny cysts of SK.

The first Raman spectroscopy study on cancer recognition was performed on uterus, ovaries and cervix cancer tissue where the decrease if intensity of the amide III region was reported (Liu *et al*, 1992). This phenomenon has also been observed for laryngeal cancer, where additionally increase in the lipids bands around 1310-1340 cm⁻¹ was found (Stone *et al*, 2000). The peak around 1320 cm⁻¹ can be attributed to both collagen and nucleic acids (DNA), more specifically to the purine bases guanine and adenine (Perno *et al.*, 1989; Stone *et al.*, 2000). Raman spectra obtained from malignant melanoma and basal cell carcinoma show similar alterations in the 1310-1340 cm⁻¹ region. Therefore it is most probable that malignant transformation triggers similar molecular changes independently of the tissue

involved. Alterations of the amide bands in Raman spectra are attributed to the conformational changes of proteins. Collagen contributes predominantly to Raman spectra of the skin (Johansson *et al*, 2000) and therefore changes in its structure or its degradation could explain the decrease in the amide band intensities. Collagen degradation is influenced by matrix metaloproteinases (Yoshizaki et al, 2002). Upregulation of metaloproteinases were found in invasive skin tumors like MM and BCC (Hofmann *et al.*, 2000; Varani et al, 2000) but also in the endometrial and laryngeal carcinoma (Magary et al, 2000; Di Nezza LA et al, 2002). Cancer - stroma interactions (Ruiter *et al*, 2002), where ground substance and fibrobalasts are affected are most probably reflected in the alterations of the protein, lipid and water structure detected here by Raman spectroscopy.

This is the first report where neural network of Raman spectra has been employed for MM diagnosis. We have previously used this method for BCC diagnosis (Gniadecka *et al*, 1997a). Neural networks and other computer methods like principal component analysis have been successfully used as aid in the detection of gastrointestinal tract malignancies and breast cancer (Hanlon *et al*, 2000; Christoyianni *et al*, 2002; Dacosta *et al*, 2002). In all cancer studies including ours, neural network becomes subjective as it solely relies on the expertise of the pathological diagnosis, recognized as a golden standard. It is possible that Raman spectroscopy possessing the strength of the specificity of the biochemical information may reveal early signs of the cancer transformation before any morphological structure analysis is seen in histopathology.

Neural network analysis based only on the spectral information allowed to diagnose MM with sensitivity of 85 % and the specificity was 99 %. This is comparable to the diagnostic accuracy for MM achieved by trained specialists in dermatology (Cassileth *et al*, 1986; Morton and Mackie 1998; Chen *et al*, 2001). A recent study, using a similar neural network architecture trained on features extracted from digitized dermatoscopic images, obtained 75 % sensitivity for malignant melanoma (Hintz-Madsen *et al.*, 2001). Other recent studies (Rubegni *et al*, 2002a; Rubegni *et al*, 2002b; Piccolo *et al*, 2002), also using dermatoscopic features and neural networks, have shown an extremely good performance. The sensitivity was 92-94 % for malignant melanoma, but these studies do not appear to apply a fully independent test data.

The neural network presented here has the advantage that the parameter optimization does not rely on cross-validation or tuning of parameters. Moreover, the important input features (Raman frequencies) can be estimated directly from the optimized neural network. Interestingly, neural network sensitivity map showed that the computer system had used identical spectral bands that are usually used for the visual spectra differentiation. Alterations in bands not previously perceived on visual inspection were 2840-3000 cm⁻¹ and at 1000 cm⁻¹, suggesting molecular changes in CH stretching in proteins and in aromatic side chains.

At the present stage of technical development it is possible to obtain Raman spectra directly from skin surface in vivo, but the quality and reproducible of spectra is lower that that yielded in skin biopsies in vitro. For this reason we included here the in vitro data only. Obviously, in vivo Raman spectroscopy is going to play an increasingly important role, as exemplified by a recent study on gastrointestinal tract tumors (Shim *et al*, 2000). Preliminary Raman spectroscopic studies revealed that *in vivo* spectra of MM and BCC did not differ from those of *in vitro* (Gniadecka *et al*, 1998a). Development of Raman spectroscopy *in vivo* should ease tracking skin malignancy in clinical practice and further detection of alterations in protein and lipid structure may add to the understanding of carcinogenic processes.

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LEGENDS TO FIGURES

Fig. 1. NIR-FT Raman spectra of: **NOR**, normal skin; **PN**, pigmented naevus; **MM**, malignant melanoma; **BCC**, basal cell carcinoma; **SK**, seborrhoeic keratosis. Spectral alterations of following major spectral bands are shown: **A**, major decrease of the intensity of the amide I band of proteins in MM, slight decrease of the right wing of the band in PN; **B**, the amide III band region around 1270 cm⁻¹ and increase in the lipid specific region 1300-1340 cm⁻¹ in MM, BCC and SK; **C** decrease of the v(C-C) band around 940 cm⁻¹ in proteins in MM, BCC and SK; **D**, widening of the δ CH₂) (CH₃) in proteins and lipids in MM, BCC and SK. For intensity comparison all spectra were normalized to 1450 cm⁻¹ band.

Fig. 2. NIR-FT Raman spectra of: **NOR**, normal skin; **PN**, pigmented naevus; **MM**, malignant melanoma; **BCC**, basal cell carcinoma; **SK**, seborrhoeic keratosis. Note the influence of the pigmentation on the 1800 cm⁻¹ to 2500 cm⁻¹ region.

Fig. 3. The sensitivity map of neural network weighting of spectral frequencies, used for malignant melanoma classification. The dashed line indicates 99 % confidence interval. Spectral bands marked **A**, **B**, **D** correspond to the description from Fig. 1 showing differences detected on visual classification of the spectra. $\mathbf{E} - CH_3$ stretching vibrations in proteins and lipids (around 2940cm⁻¹), **F** - vibration caused by skin fluorescence (2000-2350 cm⁻¹), **G** - ring vibrations in aminoacids (around 1000 cm⁻¹).

Table 1. Major protein and lipid band positions of NIR-FT Raman spectra of normal skin (NOR),pigmented nevi (PN), malignant melanoma (MM), basal cell carcinoma (BCC) and seborrhoeickeratosis (SK). Values represent means with 95% confidence intervals.

	Amide I	Amide III	δ (CH) ₂ (CH) ₃	
	in proteins	in proteins	in proteins and lipids	
NOR	1662 (1656-1668)	1264 (1241-1287)	1451 (1449-1453)	
PN	1660 (1652-1668)*	1264 (1242-1286)	1451 (1448-1454)	
MM	1655 (1647-1663)*	1272 (1258-1286)*¶	1449 (1443-1455)*	
BCC	1655 (1651-1659)*	1268 (1253-1283)	1449 (1445-1453)*	
SK	1653 (1649-1657)*	1276 (1266-1286)*	1446 (1441-1451)*	

*significant as compared to NOR

¶significant as compared to BCC

Table 2. Major band ratios in NIR-FT Raman spectra of normal skin (NOR), pigmented nevi (PN), malignant melanoma (MM), basal cell carcinoma (BCC) and seborrhoeic keratosis (SK). Values represent means with 95% confidence intervals.

	Amide I	Amide III	C – C	
	in proteins	in proteins	in proteins	
	$I_{1650 \text{ cm}-1}/I_{1450 \text{ cm}-1}$	I _{1270 cm-1} /I _{1320cm-1}	$I_{930 \text{ cm-}1}/I_{1000 \text{ cm-}1}$	
NOR	0.69 (0.54-0.84)	1.15 (0.96 -1.34)	2.49 (1.24-3.74)	
PN	0.62 (0.39-0.85)*	1.05 (0.98 -1.12)*	2.24 (0.64-3.84)*	
MM	0.29 (-0.27-0.85)*¶	0.99 (0.93 -1.05)*¶	0.77 (-0.29-1.83)*	
BCC	0.86 (0.51-1.21)*	0.94 (0.86 -1.02)*	0.82 (0.10-1.54)*	
SK	0.81 (0.33-1.29)*	0.95 (0.82 -1.08)*	0.85 (0.14-1.56)*	

*significant as compared to NOR

¶significant as compared to BCC

Table 3. Specificity and sensitivity of neural network analysis of Raman spectra for classification of normal skin (NOR), pigmented nevi (PN), malignant melanoma (MM), basal cell carcinoma (BCC) and seborrhoeic keratoses (SK). Sensitivity and specificity are defined as in Altman (1991) and shown \pm two times the SD of the approximated normal distribution to the binomial distribution.

	Sensitivity	Specificity
NOR	96 % ± 3	94 % ± 3
PN	78 % ± 6	97 % ± 2
MM	85 % ± 5	99 % ± 1
BCC	98 % ± 2	98 % ± 2
SK	96 % ± 3	$100\% \pm 0$

Table 4. Confusion map of the neural network predictions of normal skin (NOR), pigmented nevi (PN), malignant melanoma (MM), basal cell carcinoma (BCC) and seborrhoeic keratoses (SK). The top row indicates correct labels, while the far left column (marked with stars) indicates network predictions. The confusion map presents the probability of different classes estimated by the neural network given the true class label.

	NOR	PN	MM	BCC	SK
NOR*	96 %	20 %	0 %	0 %	0 %
PN*	3 %	78 %	10 %	2 %	0 %
MM*	0 %	2 %	85 %	0 %	0 %
BCC*	1 %	0 %	5 %	98 %	4 %
SK*	0 %	0 %	0 %	0 %	96%











