SKIN CANCER BIOMARKER DETECTION BY INFRARED SPECTROSCOPY

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

This invention pertains in general to the field of identification of a molecular cancer biomarker in human skin epidermal cancerous tissue from a biopsy through IR (infrared) spectral assignment of the multiplet at around 1055 cm^{-1}. More specifically, this involves the detection method, IR spectroscopy, to identify the presence of DNA/RNA ratio peak in human epidermis, diagnostic for squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and malignant melanoma (MM). The specificity and sensitivity of skin cancer biomarker band is correlated with skin cancer type and its progression, towards determination of prognosis and prediction of the treatment in the patient. Qualitative and quantitative assessment of a molecular cancer biomarker in human skin epidermal cancerous tissue all together indicate prognosis, diagnosis and prediction of epidermal skin cancers and precancers. Detection of the multiplet together with patterned appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1} additionally indicates relation to certain types of tumour and malignancy, also indicating progression of malignancy and progression towards malignancy.

Although skin tissue is used as the representative tissue, the detection method for a biomarker is not limited to measuring skin epidermal tissue and can be exploited with other tissues, as will be apparent to the skilled person.

2. PRIOR ART

The specificity and sensitivity of clinical diagnosis of epidermal skin cancers varies from approximately 40 to 80 %. The diagnostic accuracy depends largely on the length of training and experience of the clinician: reported to be 80 % for trained dermatologists, 62 % for senior registars, 56 % for registars and approximately 40 % for non-dermatologists. So, clinical diagnostic accuracy of basal cell carcinoma (BCC), the most common cancer of the skin, is only 65 % for practising dermatologists. Another skin cancer, derived from the middle part of the epidermis, squamous cell carcinoma (SCC), is easily diagnosed in typical clinical cases, but may sometimes difficult to diagnose when located on the genitalia and lips, leading to misdiagnosis and rapid metastasizing, with death resulting in about 75 % of the patients. Precancerous dermatoses, such as Bowen's disease (intraepidermal carcinoma *in situ*) and many others, are often difficult to diagnose in a

dermatology clinic, because many other skin lesions mimic them. Clinical diagnosis of malignant melanoma (MM) can be often confused with benign pigmented lesions, resulting in up to 80% of unnecessary biopsies. Importantly, patients with diagnosis of MM should be monitored for the rest of their lives and examined for local recurrence, metastasis and fresh malignant lesions.

Dermoscopy has been reported to improve the diagnostic accuracy of epidermal skin cancers and precancers, based on numerous features for establishing morphological pattern recognition. Nevertheless, recognition of these patterns requires a long training in dermatology, and still interpretation of numerous cases vary largely between the experts.

Confocal microscopy might be useful in the diagnosis of epidermal skin cancers and precancers, based on interpretion of cellular images of keratinocytes in the epidermis, based again on cellular morphological recognition of pathology-related patterns in conjunction with correlation to standard histology. Although this technique increases the diagnostic accuracy of epidermal skin cancers and precancers, there have been described limitions for certain skin lesions.

Conventional histopathological identification of cancerous tissues from a biopsy by morphological pattern recognition remains gold standard for clinical diagnosis of skin tumours, though the major drawbacks of the histopathological assessment are delays in diagnosis and interpretational variations among the pathologists.

In order to reduce false-negative results, an increasing requirement has been felt for a new and complimentary diagnostic technique to improve the diagnostic potential of "difficult to determine cases" in a shorter time-frame. Thus, there is an urgent need in clinical dermatology today for novel, rapid, non-invasive diagnostic techniques, that can be applied for real-time primary and follow-up screening to compliment a clinical diagnosis of skin tumours, ideally replacing human interpretation.

Infrared (IR) microspectroscopy is a tool for spectroscopic evaluation of unstained tissues from biopsies at any anatomical location. Allowing easy visualization of cellular components based on their intrinsic properties and chemical composition without a requirement of external contrast-inducing agents, this technique provides sample-specific molecular information, unique spectral fingerprints for molecules of nucleic acids, proteins, lipids, water, thus providing a potential route to obtain diagnostic markers for diseases.

Spectroscopic analysis of cancerous tissues has received considerable attention to be a diagnostic technique due to its sensitivity to biochemical variations in the samples from a variety of human cancers.

Skin tissue IR absorbance spectrum fits all the characteristics for a development of a clinically useful biomarker. Proteins, the most abundant species in cells, dominate in the observed spectra of cells and tissues, including skin, because their consistence in those is about 60 %. Nucleic acids in IR spectra from benign, premalignant and malignant skin cancers have been always observed in the

presence of proteins. The nucleic acids DNA and RNA are especially important, because they carry within their structure hereditary information that determines the identity and structure of proteins. The mid-IR absorbance spectrum is one of the most information-rich and concise way to represent the whole proteomics of a cell, because of its high sensitivity to the conformation of nucleic acids and their conformational transitions, identification of base composition, its sensitivity to the effect of base-pairing interactions and base-stacking effects, etc. This makes IR spectroscopy technique more advantageous over another vibrational spectroscopy technique, Raman spectroscopy, that only provides information related to individual nucleotides.

Recent reports demonstrate FT (Fourier transform) IR spectroscopy as a potentially powerful analytical method with a high sensitivity, based on the results to identify the spectral changes of biological activity in cancerous cells in culture before appeared morphological changes, to commonly and specifically characterize skin carcinogenesis in human skin tumours based on analysis of intra- and inter-molecular interactions for nucleic acids and proteins expressed in IR spectra from benign, premalignant and malignant skin lesions.

Due to these methodological advantages, it is a purpose of this invention to determine and validate consistent and significant spectral parameters (biomarkers), which can evidently discriminate between normal (healthy, unchanged) and cancerous cells in human skin tissues, useful for prognosis, diagnosis and prediction of epidermal skin cancers and precancers.

Verification and validation of skin cancer biomarker band in IR spectra from human skin tissue might have further impact on determination of the most effective therapy in each patient and even determination of the most effective dosage of drug, aiding the field of personalized medicine.

SUMMARY OF THE INVENTION

The invention presents methods of using FTIR microspectroscopy for detecting the multiplet at about 1055 cm^{-1}, whose presence and increased expression in epidermal skin tissue is indicative for diagnosis and follow-up of epidermal skin cancers and precancers. The invented method is based on identification of the ratio of intensity of two bands of nucleic acids, DNA and RNA, in IR spectra of biopsied cancerous skin tissue samples. More particularly, provided detection method of a biomarker is a fast and reagent-free method for qualitative and quantitative analysis of identified multiplet at about 1055 cm^{-1} in epidermal cancerous skin tissues, that is not detected in normal epidermal skin tissue. Detection of the multiplet together with patterned appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1} additionally indicates relation to certain types of tumour and malignancy, also indicating progression of malignancy and progression towards malignancy.

The method can be carried out manually and comprises of the following steps:

- obtaining FTIR spectra from a multitude of histopathologically proven pathological sites in epidermis on a sample, ideally from a whole pathology area
- possibly, obtaining control measurements throughout unchanged, healthy epidermis, for spectral comparison with pathological sites within each sample of the individual
- normalizing each of the spectrum to amide I peak (1650 cm^{-1})
- averaging all epidermal measurements to obtain an average for the sample
- if identifiable, an assignment of the multiplet at about 1055 cm^{-1} in the specified region for nucleic acids between 900 and 1300 cm^{-1}

In the above method, the biopsied tissue samples with established histopathological diagnosis are prepared for measurements by using FTIR microspectroscopy by the following steps:

- strictly sequential 2 sample cuts having a thickness of 6 micrometers
- staining 1 sample cut with hematoxilin and eosin for histopathological evaluation
- air-drying 1 sample cut on CaF_2 slide glass for collection of FTIR spectra

All the above and other characteristics and advantages of the invention will be further described.

DETAILED DESCRIPTION OF THE INVENTION

The method of the invention will now be described in more detail.

FTIR microspectrometer by JEOL Co. Ltd. (Tokyo, Japan), a model IR-MAU200, has been used for IR spectral acquisition from skin tissue samples. Before each measurement, a calibration was

performed using a sample provided with the instrument by the manufacturer, and proper operating conditions of FTIR microspectrometer was confirmed.

The tissue samples used for FTIR microspectroscopy measurements were in the dry state, while the corresponding slides observed by light microscope were stained with hematoxilin and eosin for the identification of pathology-related tissue structure.

Initially, the background spectrum was collected. After the measurement site was chosen by using the visible light, the microscope was changed to IR mode. The number of co-added scans were increased to 127 in order to achieve high signal-to-noise ratio. The aperture size used in the measurements was 25 x 25 micrometers. The measured spectra at a resolution of 4 cm^{-1} covered the wavenumber range between 800 and 4000 cm^{-1}.

Spectral preprocessing methods applied to all measured FTIR spectra have included:

- if necessary, spike removal and smoothing, in order to reduce the high frequency instrumental noise
- baseline correction, in order to eliminate the dissimilarities between spectra due to shifts in baseline
- the amide I normalization at about 1650 cm^{-1}, due to its high sensitivity to small changes in molecular conformation and hydrogen bonding of peptide groups
- pick peaks assignment in the 900-1700 cm^{-1} region

The invention method included the following steps for detecting the ratio of intensity of two bands for nucleic acids, DNA and RNA, in the multiplet around 1055 cm^{-1} in epidermal skin cancerous tissues:

- assignment of the multiplet at around 1055 cm^{-1}
- calculation of mean value of assigned multiplet at around 1055 cm^{-1}
- calculation of the intensity ratio I_{1055}/I_{1245}

Data used in the examples below derive from histopathologically confirmed tissue specimens from the biopsy archive of the Department of Dermatology, Tokushima University, Tokushima, Japan.

Together, the data employed in the skin cancerous tissue samples were extracted from 24 subjects.

A database of having measured 87 spectra from 13 patients with epidermal skin cancers, including 6 patients with BCC, 4 patients with SCC and 3 patients with MM, in comparison to a database of 21 measured spectra from normal epidermis in 4 healthy subjects.

In examples there were also used a database of having measured 80 spectra from 3 patients with Bowen's disease, a precancerous dermatosis, and a database of having measured 80 spectra from 4 patients with melanocytic nevi, benign compound type.

EXAMPLES

Tables 1-7 is a summary of data employed from the patients with BCC, SCC, MM, Bowen's disease and melanocytic nevus.

DETECTION OF THE MULTIPLET IN EPIDERMAL SKIN CANCERS

Skin cancer biomarker, the multiplet expressed by the level of the peak at about 1055 cm^{-1}, has been significant in all measured epidermal skin cancers - BCC, SCC and MM, since there was no appearance of this peak in normal skin tissue epidermis. Mean intensities of the peak at about 1055 cm^{-1} has varied from 0.09 to 0.47 in BCC; from 0.13 to 0.66 in SCC; from 0.14 to 0.26 in MM patients, thus differing as low, medium and high within their variation ranges.

Example 1

BASAL CELL CARCINOMA (BCC)

Table 1 summarizes IR spectral detection of a biomarker band by means of analysis of mean values

of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in 6 patients with BCC.

Patients with BCC	No. of spectra	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
patient 1	10 spectra	0.47 (0.40-0.59)	0.96 (0.93-0.98)	1060 cm^{-1} (1059-1061)
patient 2	10 spectra	0.43 (0.31-0.45)	0.95 (0.94-0.96)	1060 cm^{-1} (1055-1067)
patient 3	5 spectra	0.44 (0.27-0.59)	0.94 (0.84-0.98)	1058 cm^{-1} (1052-1064)
patient 4	10 spectra	0.19 (0.13-0.26)	0.87 (0.50-1.00)	1055 cm^{-1} (1051-1061)
patient 5	9 spectra	0.14 (0.12-0.18)	0.67 (0.63-0.86)	1053 cm^{-1} (1047-1062)
patient 6	8 spectra	0.09 (0.07-0.13)	0.59 (0.54-0.63)	1061 cm^{-1} (1057-1063)

In the presented 52 spectra from 6 patients with BCC the intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} has varied between 0.59 and 0.96.

Right-shifted mean peak position of the multiplet has been observed in 4 patients independently from the level of intensity of a biomarker. Nevertheless, right-shifted variations for mean peak position of a biomarker has been seen in all patients with BCC.

Example 2

SQUAMOUS CELL CARCINOMA (SCC)

Table 1 summarizes IR spectral detection of a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in 4 patients with SCC.

Patients with SCC	No. of spectra	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)

patient 1	5 spectra	0.66	1.00	1060 cm^{-1}
patient 2	3 spectra	0.30 (0.26-0.34)	0.81 (0.74-0.94)	1054 cm^{-1} (1049-1058)
patient 3	9 spectra	0.13 (0.07-0.25)	0.77 (0.50-1.04)	1059 cm^{-1} (1056-1064)
patient 4	3 spectra	x	x	x

In the presented 20 spectra from 4 patients with SCC the intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} has been presented only in 3 patients with SCC and varied between 0.77 and 1.00.

Right-shifted variations for mean peak position of the multiplet at about 1055 cm^{-1} has been seen in 3 patients with SCC, but right-shifted mean peak position has been detected in 2 patients with SCC with different intensity levels.

Example 3

MALIGNANT MELANOMA (MM)

Table 3 summarizes IR spectral detection of a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in 3 patients with MM.

Patients with MM	No. of spectra	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
patient 1	4 spectra	0.26 (0.26-0.27)	0.69 (0.68-0.71)	1054 cm^{-1} (1052-1056)
patient 2	7 spectra	0.14 (0.13-0.15)	0.47 (0.43-0.50)	1050 cm^{-1} (1043-1064)
patient 3	4 spectra	x	x	x

In 11 spectra obtained in the epidermis of 2 patients with MM the intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} has varied between 0.47 and 0.69.

Left-shifted mean peak position of the multiplet detectable in 2 patients with MM, have showed large variations between 1043 and 1064 cm^{-1}.

Example 4

BCC vs. SCC vs. MM vs. HEALTHY EPIDERMIS

Table 4 summarizes IR spectral detection of a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in all measured patients with epidermal skin cancers (BCC, SCC. MM) vs. healthy subjects.

Subjects	No. of patients	No. of spectra	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
Healthy	4	21 spectra	x	x	x
BCC	6	52 spectra	**0.30** (0.09-0.44)	**0.83** (0.59-0.96)	**1058 cm^{-1}** (1053-1062)
SCC	3	17 spectra	**0.36** (0.13-0.66)	**0.86** (0.77-1.00)	**1057 cm^{-1}** (1054-1060)
MM	2	11 spectra	**0.20** (0.14-0.26)	**0.58** (0.47-0.69)	**1052 cm^{-1}** (1043-1064)

Significant mean values of a biomarker band at about 1055 cm^{-1} has been calculated as 0.20 for MM, 0.30 for BCC and 0.36 for SCC, in comparison with no peak appearance in healthy epidermis.

The intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} was 0.58 for MM, with similar to each other, 0.83 for BCC and 0.86 for SCC.

Right-shifted mean peak position of a biomarker band was similar in 6 patients with BCC and in 3 patients with SCC, accordingly at 1057 cm^{-1} and 1058 cm^{-1}. Largely variated left-shifted mean peak position of a biomarker band in 2 patients with MM was detected at 1052 cm^{-1}.

DETECTION OF THE MULTIPLET IN EPIDERMAL PRECANCERS

A precancerous dermatosis, Bowen's disease, is the earliest form of skin cancer, so-called carcinoma *in situ*. Untreated cases are reported to progress into invasive carcinoma.

Example 1

BOWEN'S DISEASE

Table 1 summarizes IR spectral detection a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in 3 patients with Bowen's disease.

Patients with BD	Total No. of spectra	No. of spectra with the multiplet	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
patient 1	10 spectra	x	x	x	x
patient 2	24 spectra	**3 spectra**	0.15 (0.13-0.16)	0.66 (0.63-0.69)	1053 cm^{-1} (1051-1056)
patient 3	36 spectra	**10 spectra**	0.26 (0.22-0.29)	0.77 (0.63-1.00)	1052 cm^{-1} (1050-1057)

In epidermally measured 70 spectra in 3 patients with Bowen's disease, only 13 spectra in 2 patients selectively detected the appearance of a biomarker band, the multiplet at about 1055 cm^{-1}.

So, in one patient with histopathologically proven Bowen's disease, mean value of a biomarker band calculated on the basis of three IR spectra was 0.15, with small variations between 0.13 and 0.16. The intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} was 0.66, varying between 0.63 and 0.69. Left-positioned mean peak at 1053 cm^{-1} has varied between 1051 and 1056 cm^{-1}.

In another histopathologically proven patient with Bowen's disease, mean value of a biomarker band calculated on the basis of ten IR spectra was 0.26 with the range between 0.22 and 0.29. The intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} was 0.77, varying between 0.63 and 1.00. Similar to other measured patient, left-positioned mean peak at 1052 cm^{-1} has varied between 1050 and 1057 cm^{-1}.

Example 2

BOWEN'S DISEASE vs. SCC vs. HEALTHY EPIDERMIS

Table 2 summarizes IR spectral detection of a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in all measured patients with SCC, Bowen's disease vs. healthy subjects.

All subjects	Total No. of spectra	No. of spectra with 1055 cm^{-1}	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
4 healthy	21	21 spectra in 4 subjects	x	x	x
3 **BD**	70	**13 spectra** in 2 patients	**0.21** (0.15-0.26)	**0.72** (0.66-0.81)	**1053 cm^{-1}** (1052-1053)
3 **SCC**	20	**17 spectra** in 2 patients	**0.36** (0.13-0.66)	**0.86** (0.77-1.00)	**1057 cm^{-1}** (1054-1060)

Detected biomarker band was not constant and prominent at the appearance in IR spectra measured in the patients with Bowen's disease (only in 13 spectra of 2 patients out of 70 spectra of 3 patients), in comparison to SCC (in all spectra measured in 2 patients among 3 patients with SCC). Mean value of a biomarker for Bowen's disease was 0.21 with a variation between 0.15 and 0.26, but mean value of a biomarker for SCC was 0.36 with a variation between 0.13 and 0.66.

The intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} was lower in Bowen's disease, mean calculated as 0.72 with a variation between 0.66 and 0.81, in comparison to SCC mean calculated intensity of a biomarker as 0.86, with a variation between 0.77 and 1.00.

It is obvious detection of a left-shifted position of mean peak at 1053 cm^{-1} in the patients with Bowen's disease, compared to a right-shifted position of mean peak at 1057 cm^{-1} in the patients with SCC.

DETECTION OF THE MULTIPLET IN MELANOCYTIC NEVUS

Histopathologically, nevus cells (melanocytes) are normally localized in the basal layer of the epidermis. Their proliferation may appear in the epidermis (junctional nevus), in the epidermis and dermis (compound nevus) or only in the dermis (intradermal nevus). All types of nevi are melanocytic (nevocellular, melanocellular), benign nevi.

Therefore, there were used histopathologically confirmed samples of compound nevi for comparative epidermal acquisition of IR spectra of benign melanocytic nevi in 4 patients

Example 1

Table 1 summarizes IR spectral detection of a biomarker band by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) and mean peak position at about 1055 cm^{-1} with variations in 4 patients with benign melanocytic nevi, compound nevus type.

Patients with MN	Total No. of measured spectra	No. of spectra with 1055 cm^{-1}	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
patient 1	12	5	0.22 (0.15-0.31)	0.71	1055 cm^{-1} (1051-1055)
patient 2	10	10	0.19 (0.08-0.33)	0.59	1054 cm^{-1} (1052-1058)
patient 3	8	2	0.24	0.75	1055 cm^{-1}
patient 4	18	9	0.17 (0.09-0.25)	0.68	1053 cm^{-1} (1051-1058)

A biomarker band at about 1055 cm^{-1} has been detected in all measured patients with melanocytic nevus, a compound type nevus, but not constantly. In the epidermis, a biomarker band has been detected in 26 spectra out of totally obtained 48 spectra in 4 patients with melanocytic nevus: in 5 out of 12 measured spectra in Patient 1, in all 10 measured spectra in Patient 2, in 2 spectra out of 8 measured spectra in Patient 3, and in 9 spectra out of 18 measured spectra in Patient 4.

Mean values for detected biomarker have varied between 0.17 and 0.24 in 4 patients with melanocytic nevus.

Mean intensities of detected biomarker band by the intensity band ratio I_{1055}/I_{1245} ranged within 0.59 - 0.75 among patients with melanocytic nevi.

In the patients with melanocellular nevi mean peak position for a biomarker has been determined between 1053 and 1055 cm^{-1}, thus with left-shifted position.

Example 2

MM vs. MN vs. HEALTHY EPIDERMIS

Table 2 summarizes IR spectral detection of a biomarker by means of analysis of mean values of the multiplet at about 1055 cm^{-1} with variations, mean intensity ratio (I_{1055}/I_{1245}) with variations and mean peak position at about 1055 cm^{-1} with variations in all measured patients with MM, melanocytic nevus (MN) (benign compound nevus) vs. healthy subjects.

Subjects	Total No. of spectra	No. of spectra with multiplet	Mean value (variation)	I_{1055}/I_{1245} (variation)	Mean peak position (variation)
4 healthy	21	21 spectra in 4 subjects	x	x	x
4 MN	48	26 spectra in 4 patients	**0.20** (0.17-0.24)	**0.68** (0.59-0.75)	**1054 cm^{-1}** (1053-1055)

3MM	15	11 spectra in 2 patients	**0.20** (0.14-0.26)	**0.58** (0.47-0.69)	**1052 cm^{-1}** (1043-1064)

Detected biomarker band was not constant and prominent at the appearance in IR spectra measured in the patients with melanocytic nevi (only in 26 spectra out of totally measured 48 spectra in 4 patients), in comparison to SCC (in all spectra measured in 2 patients among 3 patients with MM).

Mean value of a biomarker for melanocytic nevus was 0.20 with a variation between 0.17 and 0.24, similar to mean value of 0.20 for MM, with a variation between 0.14 and 0.26.

The intensity of a biomarker band by the intensity ratio I_{1055}/I_{1245} was similar in the patients with melanocytic nevus and MM.

There was detected a similar left-shifted position of mean peak at 1053 cm^{-1} in the patients with melanocytic nevus and at 1052 cm^{-1} in the patients with MM, with the only difference that mean peak in the patients with melanocytic nevus variated between 1053 cm^{-1} and 1055 cm^{-1}, but in the patients with MM mean peak has had large variation between 1043 cm^{-1} and 1064 cm^{-1}.

TUMOUR-RELATED DETECTION OF THE MULTIPLET (1055 cm^{-1}) WITH DNA/RNA TRIAD PEAK (1071, 1084, 1095 cm^{-1}) PATTERNS

In IR absorption spectra from benign, premalignant and malignant skin cancerous tissues the multiplet at about 1055 cm^{-1} is always bounded with DNA/RNA triad peaks at about 1071, 1084 and 1095 cm^{-1} at different shapes and intensities, related to pathology type of skin tumours.

Moreover, the activity of the multiplet has been reported to interact with the activity of the most prominent peak in DNA/RNA triad peak in the patients with different epidermal skin cancers [PCT/EE2013/000001].

Example 1

BASAL CELL CARCINOMA (BCC)

Table 1 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 6 patients with BCC.

BCC	1055 cm^{-1}	1071 cm^{-1}	1084/1085 cm^{-1}	1095 cm^{-1}
1 pt.	0.44	x	0.45	x
2 pt.	0.43	0.36	0.48	0.36
3 pt.	0.42	x	0.40	x
4 pt.	0.19	x	0.23	0.08
5 pt.	0.14	x	0.18	x
6 pt.	0.09	x	0.16	x

In all patients with BCC detected biomarker band at about 1055 cm^{-1} is clearly tied with the appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1}.

Results displayed in Table 1 identify 3 patterns:

- 1055 cm^{-1} with 1084/1085 cm^{-1} (in 4 patients)
- 1055 cm^{-1} with 1071, 1084/1085, 1095 cm^{-1} (in 1 patient)
- 1055 cm^{-1} with 1084/1085, 1095 cm^{-1} (in 1 patient)

Example 2

SQUAMOUS CELL CARCINOMA (SCC)

Table 2 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 4 patients with SCC.

SCC	1055 cm^{-1}	1071 cm^{-1}	1084/1085 cm^{-1}	1095 cm^{-1}
1 pt.	0.66	x	0.65	x
2 pt.	0.30	0.35	0.29	0.34
3 pt.	0.13	x	0.16	0.14

4 pt.	x	0.11	0.12	0.09

In all patients with SCC detected biomarker band at about 1055 cm^{-1} is clearly tied with the appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1}.

Results displayed in Table 1 identify 3 patterns:

- 1055 cm^{-1} with 1084/1085 cm^{-1} (in 1 patient)
- 1055 cm^{-1} with 1071, 1084/1085, 1095 cm^{-1} (in 2 patients)
- 1055 cm^{-1} with 1084/1085, 1095 cm^{-1} (in 1 patient)

Example 3

MALIGNANT MELANOMA (MM)

Table 3 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 3 patients with MM.

MM	**1055 cm^{-1}**	**1071 cm^{-1}**	**1084/1085 cm^{-1}**	**1095 cm^{-1}**
1 pt.	0.26	0.27	0.29	0.30
2 pt.	0.14	x	0.27	0.18
3 pt.	x	x	0.35	x

In all patients with MM detected biomarker band at about 1055 cm^{-1} is clearly tied with the appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1}.

Results displayed in Table 1 identify 3 patterns:

- 1055 cm^{-1} with 1071, 1084/1085, 1095 cm^{-1} (in 1 patient)
- 1055 cm^{-1} with 1084/1085, 1095 cm^{-1} (in 1 patient)

- no detection of 1055 cm^{-1} with detected 1084/1085 cm^{-1} (in 1 patient)

Example 4

BOWEN'S DISEASE

Table 4 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 3 patients with Bowen's disease.

Bowen's disease	**1055 cm^{-1}**	**1071 cm^{-1}**	**1084/1085 cm^{-1}**	**1095 cm^{-1}**
1 pt.	x	x	0.07	x
2 pt.	0.15	x	0.18	x
3 pt.	0.26	x	0.29	x

In all patients with Bowen's disease detected biomarker band at about 1055 cm^{-1} is clearly tied with the appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1}.

Results displayed in Table 1 identify 2 patterns:

- 1055 cm^{-1} with 1084/1085 cm^{-1} (in 2 patients)
- no detection of 1055 cm^{-1} with detected 1084/1085 cm^{-1} (in 1 patient)

Example 5

MELANOCYTIC NEVUS

Table 5 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 4 patients with melanocytic nevi.

MN	**1055 cm^{-1}**	**1071 cm^{-1}**	**1084/1085 cm^{-1}**	**1095 cm^{-1}**

1 pt.	0.22	0.22	0.24	x
2 pt.	0.19	0.20	0.23	x
3 pt.	0.24	x	0.27	x
4 pt.	0.17	0.16	0.19	x

In all patients with melanocytic nevi detected biomarker band at about 1055 cm^{-1} is clearly tied with the appearance of DNA/RNA triad peaks at about 1071, 1084/1085 and 1095 cm^{-1}.

Results displayed in Table 1 identify 2 patterns:

- 1055 cm^{-1} with 1084/1085 cm^{-1} (in 1 patient)
- 1055 cm^{-1} with 1071, 1084/1085 cm^{-1} (in 3 patients)

Example 6

HEALTHY EPIDERMIS vs. BD vs. MN vs. MM vs. BCC vs. SCC

Table 6 summarizes epidermally detected mean values of a biomarker at about 1055 cm^{-1} and mean values of the peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1} in 4 healthy subjects, in 3 patients with Bowen's disease, in 4 patients with melanocytic nevi, in 3 patients with MM, in 4 patients with SCC and 6 patients with BCC.

PATIENTS	**1055 cm^{-1}**	**1071 cm^{-1}**	**1084/1085 cm^{-1}**	**1095 cm^{-1}**
healthy epidermis	x	0.50	0.30	0.50
Bowen's disease	0.21	x	0.18	x
melanocytic nevus	0.21	0.19	0.23	x
MM	0.20	0.27	0.31	0.24
SCC	0.36	0.23	0.31	0.19
BCC	0.29	0.36	0.32	0.22

Results displayed in Table 6 detect biomarker band at about 1055 cm^{-1} in all measured pathologies in the epidermis (BCC, SCC, MM, Bowen's disease, melanocellular nevus), but with no detection of a biomarker band in healthy, non-pathological epidermis.

Mean values of detected biomarker band at about 1055 cm^{-1} for benign at 0.21, premalignant at 0.21 and malignant skin tumours at 0.20 for MM, at 0.29 for BCC, and at 0.36 for SCC are significant.

However, alone, mean values of a biomarker in 6 patients with BCC and 2 patients with SCC are much more higher than mean values of a biomarker in 2 patients with MM, 4 patients with MN and 3 patients with Bowen's disease.

Importantly, epidermally detected biomarker band in 2 patients with MM is strongly tied with the appearance of all peaks in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1}, that is not observed in the patients with melanocytic nevus (benign compound nevus) and Bowen's disease (precancerous dermatosis). The same pattern of a biomarker band detection together with the appearance of all peaks in DNA/RNA triad is observed in SCC and BCC patients.

So, in epidermally measured patients with melanocytic nevus (benign compound nevus) detection of a biomarker band at about 1055 cm^{-1} is tied with the appearance of 2 peaks in DNA/RNA triad, at about 1071 cm^{-1} and 1084/1085 cm^{-1}, but never at about 1095 cm^{-1}.

In 2 patients with Bowen's disease, mean value of detected biomarker band at 0.20 is tied with the appearance of one peak in DNA/RNA triad, at about 1084/1085 cm^{-1}.

The invention method claimed is:

1. A method of detecting whether a molecular biomarker band is presented in IR spectra from pathological epidermis in the samples related to skin cancerous tissue of benign, premalignant and malignant skin tumours comprising of:

(i) IR spectra normalization to amide I (at about 1650 cm-1) in the 700-4000 cm^{-1} region

(ii) baseline correction in the fingerprint region, in order to eliminate the dissimilarities between spectra

(iii) peak assignment of the multiplet at around 1055 cm^{-1} together with assignment of all peaks detected in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1}

(iv) determination of left-shifted or right-shifted mean peak position of the multiplet at around 1055 cm^{-1}, with additional determination of right-shifted or left-shifted variations

(v) calculation of mean value of assigned multiplet at around 1055 cm^{-1}

(vi) calculation of mean value(s) of assigned peaks in DNA/RNA triad at about 1071 cm^{-1}, and/or 1084/1085 cm^{-1} and/or 1095 cm^{-1}

(vii) calculation of the intensity ratio, I_{1055}/I_{1245}, where the peak at about 1245 cm^{-1} is a known peak of a combination of phosphodiester vibration (DNA) and the amide III in IR spectra

2. The method of Claim 1, wherein said individual is a human subject.

3. The method of Claim 1 of epidermal detection of a biomarker band, with additional recognition of related to certain types of tumour and malignancy patterns of:

(i) detection of the peak at about 1055 cm^{-1} with strong appearance of the peak at about 1084/1085 cm^{-1} in DNA/RNA triad peak, thus supporting diagnosis of BCC, SCC, MM, Bowen's disease and melanocytic nevus

(ii) detection of the peak at about 1055 cm^{-1} with strong appearance of 3 peaks in DNA/RNA triad peak at about 1071, 1084/1085 cm^{-1} and 1095 cm^{-1}, thus supporting diagnosis of BCC, SCC and MM

(iii) detection of the peak at about 1055 cm^{-1} with strong appearance of 2 peaks in DNA/RNA triad peak at about 1071 and 1084/1085 cm^{-1}, thus supporting diagnosis of benign melanocytic nevus, a compound type

4. A method according to Claims 1-3, additionally recognising:

(i) left-shifted position of a biomarker band (below 1055 cm^{-1}), thus supporting diagnosis of benign melanocytic nevus (a compound type), Bowen's disease and MM

(ii) right-shifted position of a biomarker band (higher 1055 cm^{-1}), thus supporting diagnosis of SCC and BCC

(iii) low, medium and high values and intensities for detected biomarker band, thus indicative for prognosis, diagnosis and prediction of epidermal skin cancers and precancers

(iv) constant or non-constant spectral appearance of a biomarker band in epidermally measured IR spectra, thus indicating certain types of tumour and malignancy

5. A method according to Claims 1-4, additionally recognising:

(i) progression of malignancy

(ii) progression towards malignancy

CLAIMS

The invention method claimed is:

1. A method of detecting whether a molecular biomarker band is presented in IR spectra from pathological epidermis in the samples related to skin cancerous tissue of benign, premalignant and malignant skin tumours comprising of:

(i) IR spectra normalization to amide I (at about 1650 cm-1) in the 700-4000 cm^{-1} region

(ii) baseline correction in the fingerprint region, in order to eliminate the dissimilarities between spectra

(iii) peak assignment of the multiplet at around 1055 cm^{-1} together with assignment of all peaks detected in DNA/RNA triad at about 1071, 1084/1085 and 1095 cm^{-1}

(iv) determination of left-shifted or right-shifted mean peak position of the multiplet at around 1055 cm^{-1}, with additional determination of right-shifted or left-shifted variations

(v) calculation of mean value of assigned multiplet at around 1055 cm^{-1}

(vi) calculation of mean value(s) of assigned peaks in DNA/RNA triad at about 1071 cm^{-1}, and/or 1084/1085 cm^{-1} and/or 1095 cm^{-1}

(vii) calculation of the intensity ratio, I_{1055}/I_{1245}, where the peak at about 1245 cm^{-1} is a known peak of a combination of phosphodiester vibration (DNA) and the amide III in IR spectra

2. The method of Claim 1, wherein said individual is a human subject.

3. The method of Claim 1 of epidermal detection of a biomarker band, with additional recognition of related to certain types of tumour and malignancy patterns of:

(i) detection of the peak at about 1055 cm^{-1} with strong appearance of the peak at about 1084/1085 cm^{-1} in DNA/RNA triad peak, thus supporting diagnosis of BCC, SCC, MM, Bowen's disease and melanocytic nevus

(ii) detection of the peak at about 1055 cm^{-1} with strong appearance of 3 peaks in DNA/RNA triad peak at about 1071, 1084/1085 cm^{-1} and 1095 cm^{-1}, thus supporting diagnosis of BCC, SCC and MM

(iii) detection of the peak at about 1055 cm^{-1} with strong appearance of 2 peaks in DNA/RNA triad peak at about 1071 and 1084/1085 cm^{-1}, thus supporting diagnosis of benign melanocytic nevus, a compound type

4. A method according to Claims 1-3, additionally recognising:

(i) left-shifted position of a biomarker band (below 1055 cm^{-1}), thus supporting diagnosis of benign melanocytic nevus (a compound type), Bowen's disease and MM

(ii) right-shifted position of a biomarker band (higher 1055 cm^{-1}), thus supporting diagnosis of SCC and BCC

(iii) low, medium and high values and intensities for detected biomarker band, thus indicative for prognosis, diagnosis and prediction of epidermal skin cancers and precancers

(iv) constant or non-constant spectral appearance of a biomarker band in epidermally measured IR spectra, thus indicating certain types of tumour and malignancy

5. A method according to Claims 1-4, additionally recognising:

(i) progression of malignancy

(ii) progression towards malignancy