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Original Paper

Detection of Melanomas by Digital Imaging of Spectrally Resolved Ultraviolet Light-induced Autofluorescence of Human Skin

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Digital images of autofluorescence excited with 366 nm Hg line were recorded in a narrow 475 nm band for 408 pigmented lesions of the skin (90 melanomas, 205 common melanocytic and dysplastic naevi, 113 lesions of different kinds) and analysed photometrically with respect to spatial distribution of intensity to differentiate between melanomas and other melanocytic lesions. Earlier reports describing patterns of intensity distributions characteristic for melanomas have not been confirmed in this study. However, our evaluations showed that an algorithm based on ratios of maximum intensity recorded outside the lesions and minimum intensity found within them, allows melanomas to be detected with a sensitivity of 82.5%, a specificity of 78.6% and a positive predictive value of 58.9% (melanomas versus common and dysplastic naevi) or 76.7% (melanomas versus other pigmented lesions). The method is now being tested in a multicentre study involving three groups in three different cities in Poland. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: melanoma, naevi, *in situ* detection, autofluorescence, digital imaging, spectrally resolved imaging
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INTRODUCTION

THE INCIDENCE of melanomas has been increasing worldwide, and according to statistical data, the number of diagnosed melanomas doubles every 10–15 years. At the same time, the most important prognostic determinant is the early diagnosis of melanoma. Unfortunately, in most cases, the clinical diagnosis of pigmented tumours is relatively difficult. Diagnosis is mainly based on a set of morphological criteria. According to recent studies, up to 50% of early melanomas may escape detection during routine clinical examinations, while experts achieve accuracy of approximately 80–90% [1]. In general, an improvement can be achieved by using epiluminescence microscopy, but even with this, the interpretation depends on the experience of the examiner. An exact diagnosis can be obtained only after histological examination of the removed tissues, although histopathological interpretation of melanocytic lesions of the skin also remains one of the most difficult areas in pathology [2].

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Melanomas typically arise in skin tissues. Such a location makes it possible to use a new approach for diagnosis based on methods of so-called tissue spectroscopy. The first attempt at using a fluorescence method for *in situ* detection of melanoma was made in 1988 by Lohmann and Paul [3]. The authors excited *in vivo* autofluorescence of skin tissues with ultraviolet (UV) light and recorded spectra of light emitted by healthy tissues, naevi and melanomas. They found that the spectra were similar in all cases and had a simple structure, with one maximum at approximately 475 nm. The authors noticed, however, that melanomas generated specific patterns of variations in the intensity of the fluorescence. Usually, the intensity was very low for a melanoma and there was a local increase in intensity in a transition zone between the melanoma and areas of healthy skin, followed by a drop in the intensity measured for the latter. Such local maxima of intensity were not found for naevi, and the authors concluded that the effect may be of importance for the differentiation between melanomas and naevi. The hypothesis was tested in a study involving a large sample of patients [4]. Those early results were not confirmed by an independent study of

Sterenborg and colleagues [5], who later continued their experiments on an animal model [6]. Recently Bono and associates [7] reported a new diagnostic algorithm based on imaging a reflectance of the skin and pigmented lesions in infrared. The preliminary results reported by Bono and associates [7] were very promising, with 77% of lesions correctly diagnosed against 81% of correct clinical diagnoses.

The aim of the current study was to elaborate an optical fluorescence method for *in situ* detection of melanomas of the skin. Our approach consisted of using a digital imaging technique for quantitative measurements of spatial distributions of the intensity of the autofluorescence emitted in the selected band of the spectrum by the cells in the skin areas of interest. Such a methodology enabled us to measure the autofluorescence intensities of different regions simultaneously at exactly the same conditions of excitation and, thus, was much more objective than the point-by-point scanning approach of Lohmann and associates [3, 4] who measured the whole emission spectrum in each point and evaluated the intensity from such data.

PATIENTS AND METHODS

The study was continued for 4 years, from 1993. More than 700 patients were examined. In 408 cases, the results of the fluorescence measurements could be compared with the results of histopathological examination of the tissues removed during surgical operation. Such complete pairs of data were obtained for 90 patients with melanomas of different forms and stages (Clark I, 3; Clark II, 28; Clark III, 34; Clark IV, 18; Clark V, 7), 169 patients with 205 pigmented naevi and 105 patients with 113 skin lesions of different types, hereafter referred to as others (verruca seborhoica, 40; carcinoma basocellulare, 17; haemangioma, 13; dermatofibroma, 10; keratosis seborhoica, 9; other lesions of different kind, 24). Some of the lesions under study had features disqualifying them from fluorescence investigations, such as bleeding, crust formation, necrosis, visible inflammatory reactions, etc. Such lesions (10 melanomas, 10 other cases) were not included in the analyses of the diagnostic parameters of the method. All patients gave their informed consent and the study was approved by the District Committee of Ethics of Scientific Research at the School of Medicine, Bydgoszcz, Poland.

The patients from several hospitals and clinics located in different cities (mostly Toruń, Łódź, Bydgoszcz, Gdańsk) were examined. The results of histological evaluations performed in those units were used as a reference for the fluorescence diagnosis. As a rule, the histopathologists did not know the results of the fluorescence examination. The main element of the apparatus, comprising the light source and the camera, was suspended on a holder, with many degrees of freedom, allowing for easy illumination and imaging of any part of a patient's body. The light source for the excitation of fluorescence was a high-pressure Xe–Hg lamp (Hamamatsu, Japan), with a custom-made water filter, lenses and an interference filter transmitting the 366 nm line of Hg. The digital imaging part of the apparatus was based on a CCD camera (SpectraSource, U.S.A.) designed for low light applications. The images were corrected for dark counts, bias voltages of the pixels and also for variations in the sensitivity of the pixels of the CCD matrix, according to procedures suggested by the manufacturer. The contrast, grey values, gain, etc. were always the same as required for photometric measurements. The optical system of the camera included a suitable interference filter with maximum transmission at 475 nm and an objective lens of a photographic camera (wide angle, f 37 mm). An AT486 computer was sufficient to control the measurements and to perform digital analyses of the images of the autofluorescence.

Images of the autofluorescence were recorded in a dark room, although no extraordinary precautions were needed to reduce the level of background light produced by the exciting lamp and the computer monitor display to zero. The autofluorescence was typically excited in areas of the skin with a diameter of 3–5 cm. The distance between the region subject to examination and the camera was approximately 30 cm. Such an arrangement reduced errors in the determination of fluorescence intensity due to changes in the distance between the often curved regions of the skin and the objective lens.

In our method, the exciting light did not need to be focused and its intensity at skin level was less than 0.1 mW/cm^2. Typical exposure times ranged from seconds to minutes, depending on the pigmentation of the skin and lesions and on the distance from the source of excitation light. At such conditions, doses of UVA received by the patients (from 1 to 30 mJ/cm^2) were always well below the level of the minimal erythema dose (1 MED = 60 J/cm^2 at 366 nm for type 1 skin, i.e. skin of an individual who never suntans and always burns [8]).

RESULTS

Typical images of the skin lesions taken at white light illumination and of the autofluorescence of the same lesions are shown in Figures 1–3. The autofluorescence was excited with 366 nm line and observed in the 475 nm band. The images of the autofluorescence showed typically characteristic patterns of fluorescence intensity distributions, reflecting to some extent the outlines of the lesions. The zones of equal intensity became diffused with increased distance from the lesions. Healthy skin was characterised by a relatively homogenous distribution of fluorescence intensity within the regions under study (diameters of 3–5 cm) and average values of a ratio of the maximum and minimum intensity measured within such areas were approximately 2.0 for all skin types and all locations on the body (the average value of that ratio obtained from all measurements was 1.9 ± 0.2). At the same time, however, the existence of such variations of autofluorescence intensity within relatively small areas indicated that the locally measured fluorescence intensities should not be considered a proper indicator of changes of the skin's characteristics. Leffell and colleagues [9], for instance, reported an increase in fluorescence intensity of approximately 10% with age. The present data, similar to the results of Sterenborg and associates [5], show that the diagnostic value of such a correlation may not be significant, because of the natural variability masking the effect in individuals.

The initial idea of this work was to use digital imaging to apply the diagnostic algorithm of Lohmann and Paul [3] in a more objective manner than their scanning technique. However, it was clear that despite using the same excitation wavelength and fluorescence detection band as Lohmann and Paul [3], we could never observe the characteristic increases in fluorescence intensity around the edges of melanomatic lesions described by Lohmann and colleagues [3, 4]. In contrast, the images of the autofluorescence showed inhomogeneous spatial distributions of intensity, both within the lesions and around them. As a rule, one can find in the areas

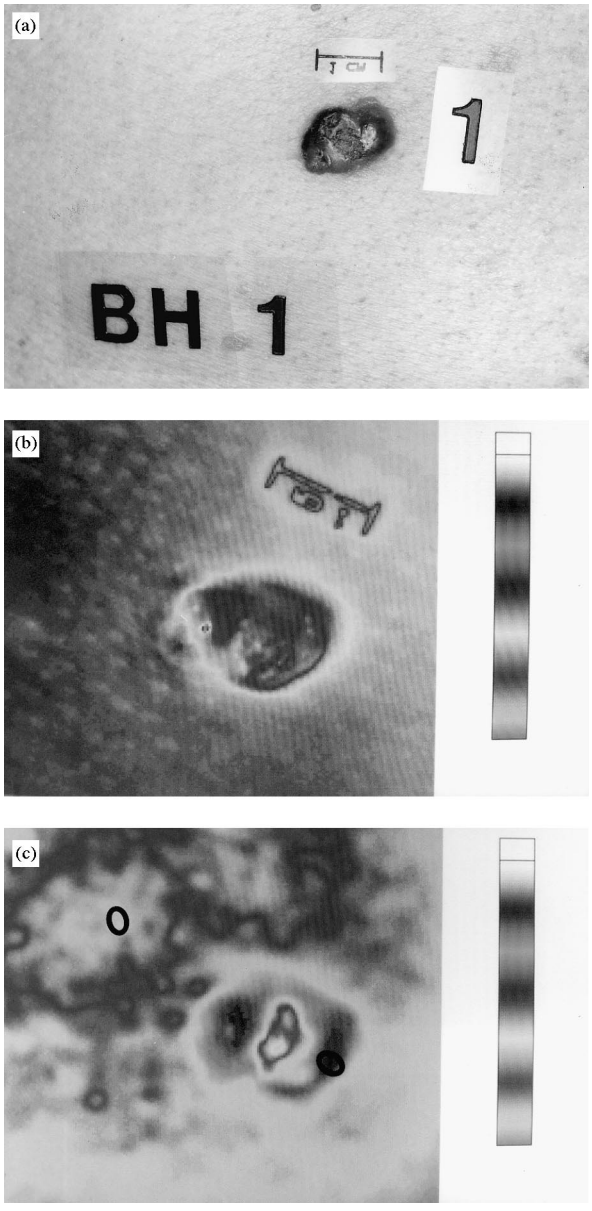

Figure 1. Malignant melanoma (Clark III): (a) classical photograph, (b) image recorded at white light illumination and (c) fluorescence image. False colours of (b) and (c) represent fluorescence intensities (maximum = white). The areas of minimum and maximum intensity used to calculate the diagnostic parameter *R* are marked by circles in (c).

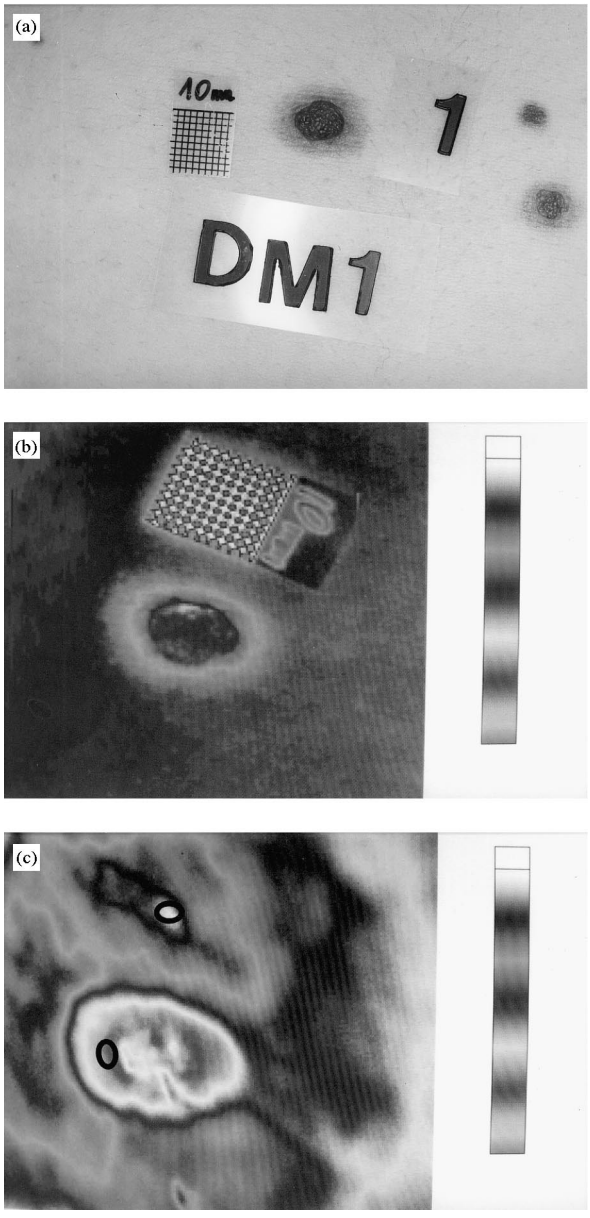

Figure 2. Pigmented naevus: (a) classical photograph, (b) image recorded at white light illumination and (c) fluorescence image.

surrounding the lesions relatively well-defined regions of increased intensity of autofluorescence, but such regions can be found for all types of lesions under study, and they do not occur symmetrically around lesions and can be localised at distances from 2–40 mm from their edges. Similarly, relatively well-localised regions of minimum and maximum intensities could be found within the lesions.

In such a situation, we decided to determine if ratios of a maximum intensity of fluorescence measured for regions located up to 40 mm from the lesions to a minimum intensity determined for the lesions, might be useful for *in situ* detection of melanomas. The appropriate areas of maximum and minimum intensity were located in the digital images of fluorescence. Typically, those regions had highly irregular shapes and areas of several mm^2 (see Figures 1c, 2c, 3c). The so-called hot spots, with areas of less than 1 mm^2, were not taken into account. Average intensities normalised to a unit surface (1 pixel) were then determined for the selected regions using numerical procedures. Since the diagnostic parameter of choice was a ratio $R = I_{max}/I_{min}$, it was not important that areas represented by single pixels differed slightly from one case to another. Histograms of R values obtained in the current study are shown in Figure 4. The R values vary in a relatively wide range for each of the three groups of lesions (melanoma, naevus, other). Average values of R (and their standard deviations) are: for melanomas $(R) = 14.3$ (16.3), for naevi $(R) = 5.7$ (4.3) and for other skin lesions $(R) = 6.1$ (6.0). Large scatter of the measured values of the diagnostic parameter seems to suggest that, despite the difference in average values found for melanomas and for the

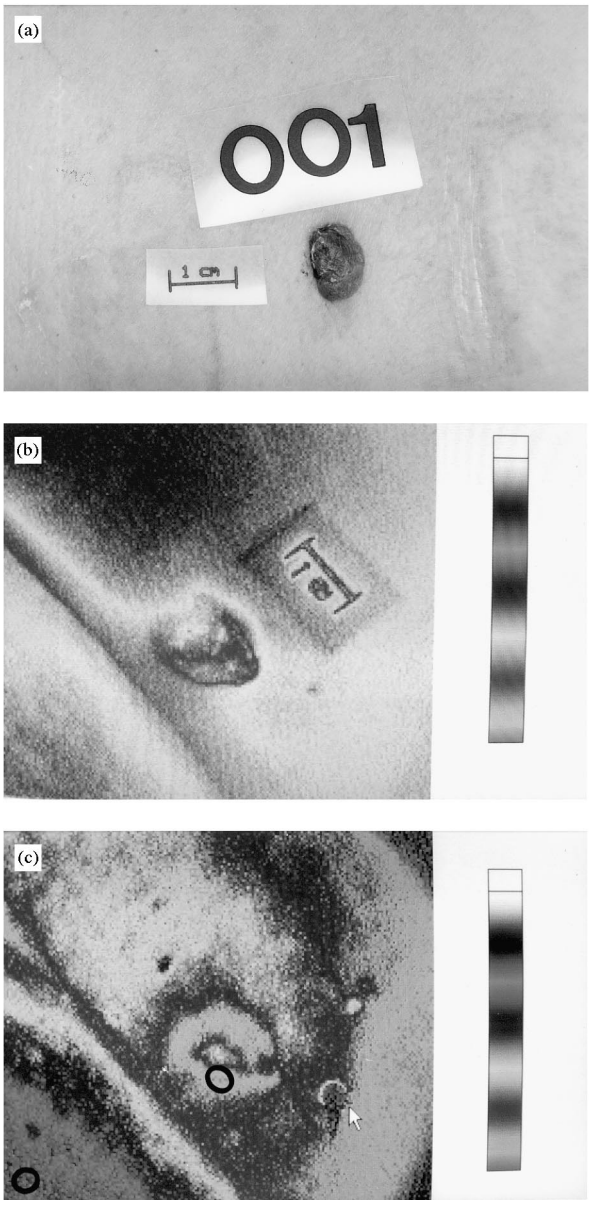

Figure 3. Verruca seborrhoica: (a) classical photograph, (b) image recorded at white light illumination and (c) fluorescence image. The arrow in (c) points to a 'hot spot' which has not been taken into account in the analysis of the intensities.

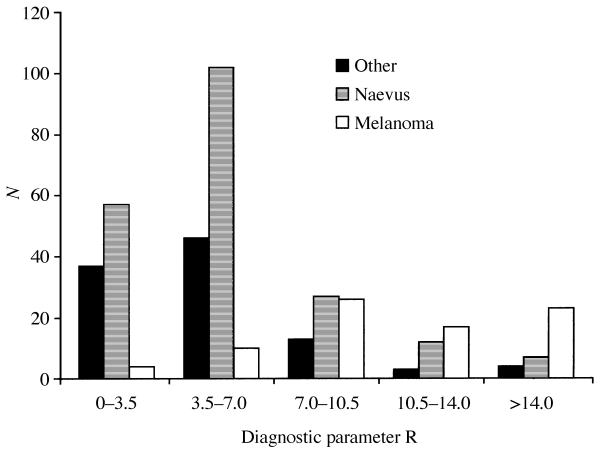

Figure 4. Histogram for values of the parameter *R* measured for lesions histologically classified as melanoma, naevus or pigmented lesion of other kind.

other two groups of skin lesions, the parameter as such might have only a very limited diagnostic potential. It should be noted that the distributions of R (Figure 2) were highly asymmetric and, thus, the average values of R and their standard deviations did not characterise properly differences in frequencies of the occurrence of different R values in the three groups. Such differences were better shown by median values, which for melanomas, pigmented naevi and other lesions, were, respectively, 10.4, 4.6 and 4.5. No correlation was found between the stage of melanoma (both in Clark and Breslow classification) and the value of R. A more detailed analysis of the data showed that, from the point of view of the diagnostic characteristics of the method, the optimum threshold value for detecting melanomatic lesions was $R = 7.0$, i.e. any result of $R \geq 7.0$ is now considered an indicator that the lesion of concern should be carefully examined by a specialist. The threshold value of $R = 7.0$ was selected as a compromise between the sensitivity and the specificity of the algorithm. Three typical parameters were calculated to estimate the diagnostic potential of such an approach [10]: C_M, sensitivity towards melanomas; S_{NM}, specificity of classifying the lesions as different from melanoma; PPV; positive predictive value for detecting melanomas. 66 of 80 melanomas yielded $R \geq 7.0$. Thus, the sensitivity of detecting melanomas was $C_M = 82.5\%$. For benign naevi, a positive result of $R \geq 7.0$ was obtained for 46 lesions, while for 159 naevi, the result was negative. In the group of other pigmented lesions of different types, 20 yielded $R \geq 7.0$ and 83 were classified correctly as negative cases. The specificity of the method calculated for the two classes of pigmented lesions was $S_{NM} = 78.6\%$. The PPV estimated for all the cases examined in this study was 50.0%. However, among the pigmented entities were also those that could be easily differentiated from melanomas on the basis of their visual characteristics. Therefore, it is interesting to calculate the PPV for a limited sample of cases including only benign naevi and melanomas. For the two groups the PPV was 58.9%.

DISCUSSION

The origin of the observed autofluorescence has not been fully understood. Leffell and colleagues [9] suggested that endogenous fluorophores responsible for the autofluorescence of the skin are collagen, elastin and desmosine. Lohmann and associates [3, 4] related this fluorescence mostly to NADH (nicotinamide adenine dinucleotide), while Sterenborg and colleagues [5] strongly opposed such an opinion and thought that the main skin fluorophores are collagen and keratin. Also, the origin of the observed patterns of spatial distribution of skin autofluorescence is not clear and so extensive studies are required to explain the molecular basis of the present fluorescence method of detecting melanomas *in situ*. The spatial distributions of autofluorescence intensity obtained in the current study do not show the increased level of emission in the border areas of melanomas described by Lohmann and associates [3, 4]. Similar data contradicting the observations of Lohmann and associates [3, 4] have already been published by Sterenborg and colleagues [5]. In the current study, however, excitation and detection bands

similar, if not the same, as those described by Lohmann and associates [3, 4] were used. One possible explanation for this discrepancy may be that the geometry of the excitation and of the detection, as well as the intensity of the exciting light were, in our experiment, different from those used by Lohmann and associates [3, 4]. In our method, the exciting light was defocused to illuminate an area with a diameter of up to 5 cm, while Lohmann and associates [3, 4] focused their exciting beam. Thus, in the experiments reported by Lohmann and associates [3, 4] the exciting light penetrated deeper than in the current study and more cellular layers contributed to the detected fluorescence signal.

In conclusion, the results obtained indicate that the fluorescence method of *in situ* detection of melanoma presented in this work has a potential for becoming a useful tool for screening large populations of patients. It is objective, simple and does not require the involvement of a dermatologist or other specialists. Fluorescence examination of a single lesion requires only a few minutes and is cheap. Moreover it allows for systematic long-term studies of suspected lesions. The images can be stored and used for future quantitative comparisons of fluorescent and morphological characteristics of the lesions of interest. The diagnostic characteristics of the method, i.e. its sensitivity, specificity and PPV show that when used in screening programmes, the method will allow for selection, with reasonable sensitivity, of a group of patients who should be carefully examined by a specialist. In the future, both the image analysis and calculations of the diagnostic parameter R can be performed automatically. The method does not require any exogenous fluorescent agents and the dose of UVA radiation received by a patient during the examination is much lower than 1 MED.

We have now started a second phase of this project in which the method will be tested independently by three groups in Poland.

1. Grin CM, Kopf AW, Welkovich B, Bart RS, Levenstein MJ. Accuracy of clinical diagnosis of malignant melanoma. *Arch Dermatol* 1990, **126**, 763–766.
2. Barnhill RL, Busam KJ. *Pathology of Melanocytic Nevi and Malignant Melanoma*. Newton, Butterworth-Heinemann, 1995.
3. Lohmann W, Paul E. *In situ* detection of melanomas by fluorescence measurements. *Naturwissenschaften* 1988, **75**, 201–202.
4. Lohmann W, Nilles M, Boedeker RH. *In situ* differentiation between nevi and malignant melanomas by fluorescence measurements. *Naturwissenschaften* 1991, **78**, 456–457.
5. Sterenborg HJCM, Motamedi M, Wagner RF, Duvic M, Thomsen S, Jacques SL. *In vivo* fluorescence spectroscopy and imaging of human skin tumours. *Lasers Med Sci* 1994, **9**, 191–201.
6. Sterenborg HJCM, Thomsen S, Jacques SL, Motamedi M. *In vivo* autofluorescence of an unpigmented melanoma in mice. Correlation of spectroscopic properties to microscopic nature. *Melanoma Res* 1995, **5**, 211–216.
7. Bono A, Tomatis S, Bartoli C, *et al*. The invisible colours of melanoma. A telespectrophotometric diagnostic approach on pigmented skin lesions. *Eur J Cancer* 1996, **32A**, 727–729.
8. Kohen E, Santus R, Hirschberg JG. *Photobiology*. San Diego, Academic Press, 1995.
9. Leffell DJ, Stetz ML, Milstone LM, Deckelbaum LI. *In vivo* fluorescence of human skin. *Arch Dermatol* 1988, **124**, 1514–1518.
10. Choi SC. *Introductory Applied Statistics in Science*. New Jersey, Englewood Cliffs, Prentice-Hall Inc., 1978.

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