BMJ Open

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (FLIMMA STUDY)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-012730
Article Type:	Protocol
Date Submitted by the Author:	19-May-2016
Complete List of Authors:	Fink, Christine; University Hospital Heidelberg, Department of Dermatology Hofmann, Maja; Charité Berlin, Department of Dermatology Jagoda, Agata; Charité Berlin, Department of Dermatology Spaenkuch, Iris; University of Tübingen, Department of Dermatology Forschner, Andrea; University of Tübingen, Department of Dermatology Tampouri, Ioanna; University of Tübingen, Department of Dermatology Lomberg, Diana; University of Tübingen, Department of Dermatology Leupold, Dieter; Magnosco GmbH Garbe, Claus; University of Tübingen Haenssle, Holger; University Hospital Heidelberg, Department of Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Diagnostics
Keywords:	Melanoma, Diagnostic, Two step photon excitation, Melanin fluorescence, Magnosco dermatofluoroscope

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (*FLIMMA STUDY*)

Authors:

Christine Fink[1], Maja Hofmann[2], Agata Jagoda[2], Iris Spaenkuch[3], Andrea Forschner[3], Ioanna Tampouri [3], Diana Lomberg[3], Dieter Leupold[4], Claus Garbe [3], Holger A Haenssle[1]

Departments/Institutions:

[1]Department of Dermatology, University of Heidelberg, Germany

[2] Charité Berlin, Department of Dermatology, Berlin, Germany

[3] University of Tübingen, Department of Dermatology, Tübingen, Germany

[4] Magnosco GmbH, Deuben, Germany

Keywords: Melanoma, Diagnostic, Two step photon excitation, melanin fluorescence, Magnosco dermatofluoroscope

Word count: 2291

Corresponding Author:

PD Dr. med. Holger Haenssle

Department of Dermatology, University of Heidelberg,

Im Neuenheimer Feld 440, 69120 Heidelberg

Holger.Haenssle@med.uni-heidelberg.de

Fon: +49-6221-56-39555

Fax: +49-6221-56-4996

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

For peer review only

ABSTRACT

Introduction Non-invasive, nanosecond, stepwise two-photon laser excitation of skin tissue was shown to induce melanin fluorescence spectra that allow for the differentiation of melanocytic nevi from cutaneous melanoma.

Methods and analysis This prospective, non-controlled, multicenter clinical study is performed to evaluate the diagnostic performance of the stepwise two-photon excited melanin fluorescence in the detection of cutaneous melanoma. The comparator will be the histopathological diagnosis. A total of 620 pigmented skin lesions suspicious for melanoma and intended for excision will be enrolled.

Ethics and dissemination Ethics approval was provided by the local ethic committees of the medical faculties of the University of Tuebingen, Heidelberg and Berlin.

Study registration: The FLIMMA study is registered at ClinicalTrials.gov (Identifier: NCT02425475).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time the diagnostic performance of non-invasive, nanosecond, stepwise two-photon laser excitation in the detection of melanoma will be evaluated.
- The University of Tuebingen acts as the sponsor of the study, thus reducing the influence of commercial interests and bias.
- The FLIMMA study is designed as a prospective, multicenter, observational study. Histopathologists are blinded to the results of the test device and a central review board of histopathologists will review all false negative results of the test device. While randomized controlled trials generate the most reliable evidence, the protocol described here is a necessary preliminary step in this challenging area of research and was closely adapted from FDA-approved protocols in the field of medicinal products for the diagnosis of melanoma.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

1 INTRODUCTION

1.1 Background

Melanoma is a malignant tumor, which develops from the pigment producing melanocytes by neoplastic transformation. Malignant melanoma is identified as one form of cancer of increasing incidence, which is now among the 10 most frequent human malignancies. In Europe the regional incidence rates reside between 10 and 25 per 100.000 per year and increase, especially in elderly people.[1-6] After diagnosis of a malignant melanoma the prognosis depends strongly on the thickness of the tumor and lymphatic or hematogenous metastases.[7] Once melanomas cause metastases, the prognosis of survival worsens dramatically, with a 5-year survival of patients with stage IV disease between 6.7% and 18.8%.[8-9] This underlines the urgent need to diagnose melanoma as early as possible. The diagnostic accuracy for the clinical melanoma diagnosis does not exceed 75% and may be increased to up to 90% by the use of dermoscopy in the hands of experts; however, approximately 10% of melanomas will be missed despite all these aforementioned diagnostic efforts. The term "featureless melanoma" has been coined for this phenomenon.[10] Particularly in these difficult to diagnose and "featureless" melanomas additional strategies for melanoma diagnosis would be extremely helpful.

1.2 Two step photon excitation of melanin fluorescence

Autofluorescence spectra of human skin tissue are usually excited by one-photon absorption in the UV-A region.[11] However, by this form of excitation the ultra-weak fluorescence of melanin is undetectably hidden by the emission from the main endogenous fluorophores NAD(P)H and flavins.[12-13] A more specific excitation of the melanin fluorescence may be useful for gaining information about the potential malignancy of pigmented skin lesions. A first step to overcome this lack of specific melanin fluorescence was the application of more targeted fluorescence excitation technique based on two photon absorption from an 800nm-femtosecond laser. All endogeneous skin tissue fluorophores except melanin do not absorb at 800nm; nevertheless, upon irradiation with 800nm-laser pulses they may show their well known fluorescence in the spectrum of visible light. This is caused by a special

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

non-linear optical effect called simultaneous two photon absorption. The intensity of the excited fluorescence is comparably weak. In contrast, melanin shows absorption upon irradiation with 800nm-laser pulses and absorbs two photons in a stepwise process via an intermediate excited electronic state.[12] At physiologically acceptable laser intensities, the latter mechanism is much more effective.[14] By this procedure the main autofluorescence of skin will be partly suppressed and the melanin fluorescence becomes measurable. Investigations in a variety of pigmented skin lesions gave first hints on the differences between the fluorescence from common nevi as compared to malignant melanoma.[15] Also in other melanin-containing fluorophore compositions this fluorescence discrimination in favour of melanin can be observed, e.g. in the choroidea and the retinal pigment epithelium of the eye.[16] A further essential improvement in measuring the melanin fluorescence from skin tissue as selectively as possible could be achieved only recently by using nanosecond pulses instead of femtosecond pulses.[17]

1.3 The dermatofluoroscope Magnosco DFC 1 for in-vivo diagnostics of melanoma

The investigational device in this study is the dermatofluoroscope Magnosco DFC 1 by Magnosco GmbH, Berlin, Germany, with a two-photon excitation with 800 nm/nanosecond pulses from a dye laser, equipped with a spectrometer and a sensitive photon detector. It is designed for use by dermatologists and trained medical personnel and should be applied to patients with skin type I, II, III and IV, who show atypical melanocytic lesions. For the investigation with the Magnosco DFC 1 the patient has to be at physical rest. The overview CCD camera is applied to take a macroscopic image for documentation reasons. After cleaning and shaving the location of interest, the lesion is covered with a specific cover shield with mask. The latter helps to place the scanning head onto the intact skin. After fixing it by an adhesive pad, a dermoscopic image of the lesion is taken. The spectral data are gathered automatically, while the lesion is raster-scanned. The results of the data analysis are presented on the computer screen: 1. Dermoscopic image overlaid with the scanning raster: fluorescence spectra indicating malignancy are visualized as red spots. 2. A score given on a green/red bar indicates the result of data analysis: a) presence of malignant melanoma, b) no indication of malignant melanoma, c) no valid result. The analysis of the spectral data in conjunction with images

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

and patient master data are documented as one file in the dermatofluoroscope Magnosco DFC 1 data base. All files can be transferred with the customized USB stick or printed for patient information.

1.4 Preliminary data with the Magnosco DFC 1 device

The preclinical data available for assessing the suitability of the dermatofluoroscope Magnosco DFC 1 for melanoma diagnostics are based on ex vivo and histological specimen examination. The specimens examined so far were freshly excised pigmented nevoid lesions and their corresponding paraffin-embedded histological samples. In 167 freshly excised tissue specimens from clinically suspicious pigmented lesions (suspected malignant melanoma/dysplastic nevi), the diagnosis was first made based on the new fluorescence-spectroscopic diagnostic method before the histopathological diagnosis was available. In relation to the histopathological diagnosis as the current gold standard of melanoma diagnostics, the new diagnostic method showed a sensitivity of melanoma detection of 93.5%, a specificity of 80.0% and a diagnostic accuracy of 82.6% on freshly excised pigmented lesions.[18] In a study on 125 paraffin embedded specimens of melanocytic melanomas (n = 60) and melanocytic nevi (n = 65), a sensitivity of melanoma detection of 82.5% and a specificity of 72.5% were detected.[19]

2 DESIGN/METHODS

2.1 Study design

The FLIMMA study is designed as a prospective, non-controlled, multicenter clinical study in patients with suspected malignant melanoma.

2.1 Objectives

The primary objective of this study is to determine sensitivity and specificity of the algorithm for the fluorescence diagnostics of melanoma. The comparator and gold standard for the diagnosis will be the histopathological diagnosis of the pigmented lesions. Secondary objectives are to collect data for training and optimization of the diagnostic algorithm, to assess the safety of the device, and to assess the incidence of adverse events.

2.2 Endpoints

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

The primary endpoint is to determine sensitivity and specificity of this fully-automated, non-invasive, in-vivo method. Secondary endpoints include the assessment of the safety of the device as well as the collection of data for training and optimization of the computerized diagnostic algorithm.

2.3 Recruitment and status of the study

Approval of the local Ethics Committees was granted 2014/07/02 for all three participating centers: University Hospital of Tuebingen, University Hospital of Heidelberg, and Charité Berlin). Date of first enrollment was 2014/09/17. The recruitment of patients is in progress. The estimated total time frame for recruitment of 620 patients is 20 months. The total duration of the study is expected to be 26 months, including analysis.

2.4 Study population

A total of 620 patients, who show pigmented lesions with suspicion of dysplastic nevus or melanoma and in whom an excision is indicated, will be recruited.

2.5 Criteria for Inclusion-/ Exclusion

Patients having pigmented lesions with suspicion of dysplastic nevus or melanoma, in whom an excision is indicated in order to exclude or diagnose malignant melanoma, who are equal to or greater than 18 years of age, and who have given written informed consent will be eligible. Patients with skin type V and VI according to Fitzpatrick's scale; patients, where there is a risk that the scanning head is detached because the patient cannot be placed at rest, patients who cannot understand the patient information and who cannot provide informed consent, patients with deep dermal lesions ≥ 5 mm beneath the stratum corneum, clinically or dermoscopically obviously nonmelanocytic lesions, peri- and subungual lesions, mucosal lesions, lesions with trauma, erosion, excoriation, or ulceration on more than 50% of the lesion area, tattooed lesions, patients suffering from albinism, pregnant or breast feeding women, lesions with dominant (>50%) regression, and lesions which are not suitable to fix the scanning head will be excluded from the FLIMMA study.

2.6 Methods

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

A total of 620 pigmented skin lesions intended for excision to either confirm or rule out melanoma will be enrolled after written informed consent at the participating centers. Three centers participate within Germany: University Hospital of Tuebingen as the lead-center, University Hospital of Heidelberg and Charité Berlin. Clinical and dermoscopic images will be recorded for all cases. Then, as a second diagnostic procedure, fluorescence diagnostics based on the two photon excitation from a dye-laser will be performed. The classification as non-melanoma or malignant melanoma by the medical dermatofluoroscope Magnosco DFC 1 will be documented prior to the excision. The in-vivo melanin fluorescence assessment will be performed no longer than 14 days prior to excision. Histopathologists on the study sites will be blinded to the diagnoses attained by the analyses of fluorescence spectra. Moreover, all false negative cases with a disagreement in the diagnosis by the test method and the histopathological examination on site will be submitted to a blinded central pathology review board. The histopathologic diagnosis will serve as gold standard for subsequent evaluations of the diagnostic accuracy. The FLIMMA study was registered at ClinicalTrials.gov (Identifier: NCT02425475).

2.7 Statistical considerations

In this study 560 evaluable lesions will be recruited, including 80 evaluable melanomas. In order to compensate for any drop outs, a total of 620 specimens are examined. It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35%. With 70 evaluable melanoma specimens and an observed sensitivity of 90%, the two-sided 95% confidence interval is 0.80 - 0.96. The specificity is assumed to be in the order of 35%, which is evaluated in 420 specimens, according to negative gold standard specimen results in a confidence interval of 0.30-0.40. This accuracy is considered sufficient for the method to be evaluated. The study data and the cohort under evaluation will be analysed by means of descriptive statistics. Furthermore, the main endpoint of the present study is the diagnostic accuracy. Sensitivity and specificity of the diagnostic method of fluorescence based pigment analysis will be determined. The comparator and goal standard for this analysis is the histopathological diagnosis.

3 ETHICS AND DISSEMINATION

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

3.1 Declarations and ethic aspects

The study is conducted in accordance with the Declaration of Helsinki principles (2013),[20] requirements and guidance provided in ISO 14155 (2012)[21] and applicable local government regulations and Independent Ethics Committee policies and procedures. In the context of the approved standard operating procedures (SOPs) which are based on ICH-GCP guidelines (E6) and the German implementation of Good clinical practice (GCP) for the clinical work, the patients will be informed orally and in written form about aim, character and consequences of the procedure. Before initiation of the study the protocol, the patient information sheet and the consent form were presented to the independent ethic committee. The names of patients and all confidential data are subject to professional discretion and the "Bundesdatenschutzgesetz (BDSG)". Processing of medical data will only take place in pseudonymous form. In case of withdrawal from the study, the data that has already been collected will be destroyed. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The investigator will explain to each participant the nature of the study, its purpose, the procedure involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Additionally, all participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure. The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator and it will be retained as part of the study records.

3.2 Risk-Benefit relationship

The decision for excision is based on the clinical and dermoscopic diagnosis of the pigmented lesion, and will not be biased by the diagnosis of the device under investigation (dermatofluoroscope Magnosco DFC 1). The melanin fluorescence measurements of the FLIMMA study will not influence

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

the clinical procedures. Therefore, there is no risk for the patient that his participation may deteriorate the management rate of his pigmented lesions. The information of the patient and the measurement procedure itself will generally take less than 15 minutes. In case of a false negative diagnosis by the dermatofluoroscope Magnosco DFC a second independent histopathological review will be performed. This will be in favor of a higher diagnostic accuracy and may be beneficial for the patient.

4 SAFETY

This is a non-invasive diagnostic procedure based on low-intensity visible light exposure, which has no capacity to injure tissues. Therefore, no adverse reactions related to the optical procedures are expected. All adverse events (AEs) will be recorded and documented. SAE will be reported in accordance with the Medizinprodukte-Sicherheitsplanverordnung (MPSV) ordinance.

5 FOOTNOTES

5.1 Funding statement

This research received a grant from a commercial sector.

5.2 Study registration

The FLIMMA study was registered at ClinicalTrials.gov with the Identifier: NCT02425475.

5.3 Conflicts of interest

The authors declared that they have no competing interests.

5.4 Authors' contribution

H. Haenssle, C. Garbe, and M. Hofmann participated in the development and the implementation of the study (sample size calculations, writing of the protocol, submission to ethics committee, data management). I. Spaenkuch, D. Leupold, and C. Garbe performed the data handling and statistical analysis. C. Fink, A. Forschner, I. Tampouri, A. Jagoda and D. Lomberg helped to draft and to review the paper. All authors read and approved the final manuscript.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

6 REFERENCES

1. Stang A, Stang K, Stegmaier C et al. Skin melanoma in Saarland: incidence, survival and mortality 1970-1996. *Eur J Cancer Prev* 2001; 10: 407-15.

2. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clin Dermatol* 2009; 27: 3-9.

3. Lasithiotakis KG, Leiter U, Gorkievicz R et al. The incidence and mortality of cutaneous melanoma in Southern Germany: trends by anatomic site and pathologic characteristics, 1976 to 2003. *Cancer* 2006; 107: 1331-9.

4. Lasithiotakis KG, Petrakis IE, Garbe C. Cutaneous melanoma in the elderly: epidemiology, prognosis and treatment. *Melanoma Res* 2010; 20: 163-70.

5. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. *Adv Exp Med Biol* 2008; 624: 89-103.

6. Bosetti C, La VC, Naldi L et al. Mortality from cutaneous malignant melanoma in Europe. Has the epidemic levelled off? *Melanoma Res* 2004; 14: 301-9.

7. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. *N Engl J Med* 2004; 351: 998-1012.

8. Balch CM, Soong SJ, Gershenwald JE et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001; 19: 3622-34.

9. Weide B, Elsasser M, Buttner P et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. *Br J Cancer* 2012; 107: 422-8.

10. Menzies SW, Ingvar C, Crotty KA et al. Frequency and morphologic characteristics of invasive melanomas lacking specific surface microscopic features. *Arch Dermatol* 1996; 132: 1178-82.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

For peer review only

11. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. *Annu Rev Phys Chem* 1996; 47: 555-606.

12. Teuchner K, Freyer W, Leupold D et al. Femtosecond two-photon excited fluorescence of melanin. *Photochem Photobiol* 1999; 70: 146-51.

13. Zeng H, MacAulay C, McLean DI et al. Spectroscopic and microscopic characteristics of human skin autofluorescence emission. *Photochem Photobiol* 1995; 61: 639-45.

14. Teuchner K, Ehlert J, Freyer W et al. Fluorescence studies of melanin by stepwise twophoton femtosecond laser excitation. *J Fluoresc* 2000; 20: 275-81.

15. Hoffmann K, Stucker M, Altmeyer P et al. Selective femtosecond pulse-excitation of melanin fluorescence in tissue. *J Invest Dermatol* 2001; 116: 629-30.

16. Schneider M, Teuchner K, Leupold D. [Two-photon fluorescence of ocular melanomas. Studies on a new diagnostic method]. *Ophthalmologe* 2005; 102: 703-7.

17. Eichhorn R, Wessler G, Scholz M et al. Early diagnosis of melanotic melanoma based on laser-induced melanin fluorescence. *J Biomed Opt* 2009; 14: 034033.

18. Scholz M, Stankovic G, Scholz C, Leupold D et al. En route to a new in vivo diagnostic of malignant pigmented melanoma. *PCMR* 2012 Mar;25(2):281-3.

19. Freudenberger S, [Informationsgehalt der Melanin-dominierten Fluoreszenz pigmentierter kutaner Läsionen in Paraffin]. Dissertation Universitätsklinik Tübingen 2014.

20. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053.

21. Clinical investigation of medical devices for human subjects - Good clinical practice (ISO 14155:2011 + Cor. 1:2011); German version EN ISO 14155:2011 + AC:2011

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

BMJ Open

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (FLIMMA STUDY)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-012730.R1
Article Type:	Protocol
Date Submitted by the Author:	15-Jul-2016
Complete List of Authors:	Fink, Christine; University Hospital Heidelberg, Department of Dermatology Hofmann, Maja; Charité Berlin, Department of Dermatology Jagoda, Agata; Charité Berlin, Department of Dermatology Spaenkuch, Iris; University of Tübingen, Department of Dermatology Forschner, Andrea; University of Tübingen, Department of Dermatology Tampouri, Ioanna; University of Tübingen, Department of Dermatology Lomberg, Diana; University of Tübingen, Department of Dermatology Leupold, Dieter; Magnosco GmbH Garbe, Claus; University of Tübingen Haenssle, Holger; University Hospital Heidelberg, Department of Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Diagnostics
Keywords:	Melanoma, Diagnostic, Two step photon excitation, Melanin fluorescence, Magnosco dermatofluoroscope

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (*FLIMMA STUDY*)

Authors:

Christine Fink[1], Maja Hofmann[2], Agata Jagoda[2], Iris Spaenkuch[3], Andrea Forschner[3], Ioanna Tampouri [3], Diana Lomberg[3], Dieter Leupold[4], Claus Garbe [3], Holger A Haenssle[1]

Departments/Institutions:

[1]Department of Dermatology, University of Heidelberg, Germany

[2] Charité Berlin, Department of Dermatology, Berlin, Germany

[3] University of Tübingen, Department of Dermatology, Tübingen, Germany

[4] Magnosco GmbH, Deuben, Germany

Keywords: Melanoma, Diagnostic, Two step photon excitation, melanin fluorescence, Magnosco dermatofluoroscope

Word count: 2291

Corresponding Author:

PD Dr. med. Holger Haenssle

Department of Dermatology, University of Heidelberg,

Im Neuenheimer Feld 440, 69120 Heidelberg

Holger.Haenssle@med.uni-heidelberg.de

Fon: +49-6221-56-39555

Fax: +49-6221-56-4996

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

ABSTRACT

Introduction Non-invasive, nanosecond, stepwise two-photon laser excitation of skin tissue was shown to induce melanin fluorescence spectra that allow for the differentiation of melanocytic nevi from cutaneous melanoma.

Methods and analysis This prospective, non-controlled, multicenter clinical study is performed to evaluate the diagnostic performance of the stepwise two-photon excited melanin fluorescence in the detection of cutaneous melanoma. The comparator will be the histopathological diagnosis. A total of 620 pigmented skin lesions suspicious for melanoma and intended for excision will be enrolled.

Ethics and dissemination Ethics approval was provided by the local ethic committees of the medical faculties of the University of Tuebingen, Heidelberg and Berlin.

Study registration: The FLIMMA study is registered at ClinicalTrials.gov (Identifier: NCT02425475).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time the diagnostic performance of non-invasive, nanosecond, stepwise two-photon laser excitation in the detection of melanoma will be evaluated.
- The University of Tuebingen acts as the sponsor of the study, thus reducing the influence of commercial interests and bias.
- The FLIMMA study is designed as a prospective, multicenter, observational study. Histopathologists are blinded to the results of the test device and a central review board of histopathologists will review all false negative results of the test device. While randomized controlled trials generate the most reliable evidence, the protocol described here is a necessary preliminary step in this challenging area of research and was closely adapted from FDA-approved protocols in the field of medicinal products for the diagnosis of melanoma.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

1 INTRODUCTION

1.1 Background

Melanoma is a malignant tumor, which develops from the pigment producing melanocytes by neoplastic transformation. Malignant melanoma is identified as one form of cancer of increasing incidence, which is now among the 10 most frequent human malignancies. In Europe the regional incidence rates reside between 10 and 25 per 100.000 per year and increase, especially in elderly people.[1-6] After diagnosis of a malignant melanoma the prognosis depends strongly on the thickness of the tumor and lymphatic or hematogenous metastases.[7] Once melanomas cause metastases, the prognosis of survival worsens dramatically, with a 5-year survival of patients with stage IV disease between 6.7% and 18.8%.[8-9] This underlines the urgent need to diagnose melanoma as early as possible. The diagnostic accuracy for the clinical melanoma diagnosis does not exceed 75% and may be increased to up to 90% by the use of dermoscopy in the hands of experts; however, approximately 10% of melanomas will be missed despite all these aforementioned diagnostic efforts. The term "featureless melanoma" has been coined for this phenomenon.[10] Particularly in these difficult to diagnose and "featureless" melanomas additional strategies for melanoma diagnosis would be extremely helpful.

1.2 Two step photon excitation of melanin fluorescence

Autofluorescence spectra of human skin tissue are usually excited by one-photon absorption in the UV-A region.[11] However, by this form of excitation the ultra-weak fluorescence of melanin is undetectably hidden by the emission from the main endogenous fluorophores NAD(P)H and flavins.[12-13] A more specific excitation of the melanin fluorescence may be useful for gaining information about the potential malignancy of pigmented skin lesions. A first step to overcome this lack of specific melanin fluorescence was the application of more targeted fluorescence excitation technique based on two photon absorption from an 800nm-femtosecond laser. All endogeneous skin tissue fluorophores except melanin do not absorb at 800nm; nevertheless, upon irradiation with 800nm-laser pulses they may show their well known fluorescence in the spectrum of visible light. This is caused by a special

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

non-linear optical effect called simultaneous two photon absorption. The intensity of the excited fluorescence is comparably weak. In contrast, melanin shows absorption upon irradiation with 800nm-laser pulses and absorbs two photons in a stepwise process via an intermediate excited electronic state.[12] At physiologically acceptable laser intensities, the latter mechanism is much more effective.[14] By this procedure the main autofluorescence of skin will be partly suppressed and the melanin fluorescence becomes measurable. Investigations in a variety of pigmented skin lesions gave first hints on the differences between the fluorescence from common nevi as compared to malignant melanoma.[15] Also in other melanin-containing fluorophore compositions this fluorescence discrimination in favour of melanin can be observed, e.g. in the choroidea and the retinal pigment epithelium of the eye.[16] A further essential improvement in measuring the melanin fluorescence from skin tissue as selectively as possible could be achieved only recently by using nanosecond pulses instead of femtosecond pulses.[17]

1.3 The dermatofluoroscope Magnosco DFC 1 for in-vivo diagnostics of melanoma

The investigational device in this study is the dermatofluoroscope Magnosco DFC 1 by Magnosco GmbH, Berlin, Germany, with a two-photon excitation with 800 nm/nanosecond pulses from a dye laser, equipped with a spectrometer and a sensitive photon detector. It is designed for use by dermatologists and trained medical personnel and should be applied to patients with skin type I, II, III and IV, who show atypical melanocytic lesions. For the investigation with the Magnosco DFC 1 the patient has to be at physical rest. The overview CCD camera is applied to take a macroscopic image for documentation reasons. After cleaning and shaving the location of interest, the lesion is covered with a specific cover shield with mask. The latter helps to place the scanning head onto the intact skin. After fixing it by an adhesive pad, a dermoscopic image of the lesion is taken. The spectral data are gathered automatically, while the lesion is raster-scanned. The results of the data analysis are presented on the computer screen: 1. Dermoscopic image overlaid with the scanning raster: fluorescence spectra indicating malignancy are visualized as red spots. 2. A score given on a green/red bar indicates the result of data analysis: a) presence of malignant melanoma, b) no indication of malignant melanoma, c) no valid result. The analysis of the spectral data in conjunction with images

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

and patient master data are documented as one file in the dermatofluoroscope Magnosco DFC 1 data base. All files can be transferred with the customized USB stick or printed for patient information.

1.4 Preliminary data with the Magnosco DFC 1 device

The preclinical data available for assessing the suitability of the dermatofluoroscope Magnosco DFC 1 for melanoma diagnostics are based on ex vivo and histological specimen examination. The specimens examined so far were freshly excised pigmented nevoid lesions and their corresponding paraffin-embedded histological samples. In 167 freshly excised tissue specimens from clinically suspicious pigmented lesions (suspected malignant melanoma/dysplastic nevi), the diagnosis was first made based on the new fluorescence-spectroscopic diagnostic method before the histopathological diagnosis was available. In relation to the histopathological diagnosis as the current gold standard of melanoma diagnostics, the new diagnostic method showed a sensitivity of melanoma detection of 93.5%, a specificity of 80.0% and a diagnostic accuracy of 82.6% on freshly excised pigmented lesions.[18] In a study on 125 paraffin embedded specimens of melanocytic melanomas (n = 60) and melanocytic nevi (n = 65), a sensitivity of melanoma detection of 82.5% and a specificity of 72.5% were detected.[19]

2 DESIGN/METHODS

2.1 Study design

The FLIMMA study is designed as a prospective, non-controlled, multicenter clinical study in patients with suspected malignant melanoma.

2.1 Objectives

The primary objective of this study is to determine sensitivity and specificity of the algorithm for the fluorescence diagnostics of melanoma. The comparator and gold standard for the diagnosis will be the histopathological diagnosis of the pigmented lesions. Secondary objectives are to collect data for training and optimization of the diagnostic algorithm, to assess the safety of the device, and to assess the incidence of adverse events.

2.2 Endpoints

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

The primary endpoint is to determine sensitivity and specificity of this fully-automated, non-invasive, in-vivo method. Secondary endpoints include the assessment of the safety of the device as well as the collection of data for training and optimization of the computerized diagnostic algorithm.

2.3 Recruitment and status of the study

Approval of the local Ethics Committees was granted 2014/07/02 for all three participating centers: University Hospital of Tuebingen, University Hospital of Heidelberg, and Charité Berlin). Date of first enrollment was 2014/09/17. The recruitment of patients is in progress. The estimated total time frame for recruitment of 620 patients is 20 months. The total duration of the study is expected to be 26 months, including analysis.

2.4 Study population

A total of 620 patients, who show pigmented lesions with suspicion of dysplastic nevus or melanoma and in whom an excision is indicated, will be recruited.

2.5 Criteria for Inclusion-/ Exclusion

Patients having pigmented lesions with suspicion of dysplastic nevus or melanoma, in whom an excision is indicated in order to exclude or diagnose malignant melanoma, who are equal to or greater than 18 years of age, and who have given written informed consent will be eligible. Patients with skin type V and VI according to Fitzpatrick's scale; patients, where there is a risk that the scanning head is detached because the patient cannot be placed at rest, patients who cannot understand the patient information and who cannot provide informed consent, patients with deep dermal lesions ≥ 5 mm beneath the stratum corneum, clinically or dermoscopically obviously nonmelanocytic lesions, peri- and subungual lesions, mucosal lesions, lesions with trauma, erosion, excoriation, or ulceration on more than 50% of the lesion area, tattooed lesions, patients suffering from albinism, pregnant or breast feeding women, lesions with dominant (>50%) regression, and lesions which are not suitable to fix the scanning head will be excluded from the FLIMMA study.

2.6 Methods

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

A total of 620 pigmented skin lesions intended for excision to either confirm or rule out melanoma will be enrolled after written informed consent at the participating centers. Three centers participate within Germany: University Hospital of Tuebingen as the lead-center, University Hospital of Heidelberg and Charité Berlin. Clinical and dermoscopic images will be recorded for all cases. Then, as a second diagnostic procedure, fluorescence diagnostics based on the two photon excitation from a dye-laser will be performed. The classification as non-melanoma or malignant melanoma by the medical dermatofluoroscope Magnosco DFC 1 will be documented prior to the excision. The in-vivo melanin fluorescence assessment will be performed no longer than 14 days prior to excision. Histopathologists on the study sites will be blinded to the diagnoses attained by the analyses of fluorescence spectra. Moreover, all false negative cases with a disagreement in the diagnosis by the test method and the histopathological examination on site will be submitted to a blinded central pathology review board. The histopathologic diagnosis will serve as gold standard for subsequent evaluations of the diagnostic accuracy. The FLIMMA study was registered at ClinicalTrials.gov (Identifier: NCT02425475).

2.7 Statistical considerations

In this study 560 evaluable lesions will be recruited, including 80 evaluable melanomas. In order to compensate for any drop outs, a total of 620 specimens are examined. It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35%. With 70 evaluable melanoma specimens and an observed sensitivity of 90%, the two-sided 95% confidence interval is 0.80 - 0.96. The specificity is assumed to be in the order of 35%, which is evaluated in 420 specimens, according to negative gold standard specimen results in a confidence interval of 0.30-0.40. This accuracy is considered sufficient for the method to be evaluated. The study data and the cohort under evaluation will be analysed by means of descriptive statistics. Furthermore, the main endpoint of the present study is the diagnostic accuracy. Sensitivity and specificity of the diagnostic method of fluorescence based pigment analysis will be determined. The comparator and goal standard for this analysis is the histopathological diagnosis.

3 ETHICS AND DISSEMINATION

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

3.1 Declarations and ethic aspects

The study is conducted in accordance with the Declaration of Helsinki principles (2013),[20] requirements and guidance provided in ISO 14155 (2012)[21] and applicable local government regulations and Independent Ethics Committee policies and procedures. In the context of the approved standard operating procedures (SOPs) which are based on ICH-GCP guidelines (E6) and the German implementation of Good clinical practice (GCP) for the clinical work, the patients will be informed orally and in written form about aim, character and consequences of the procedure. Before initiation of the study the protocol, the patient information sheet and the consent form were presented to the independent ethic committee. The names of patients and all confidential data are subject to professional discretion and the "Bundesdatenschutzgesetz (BDSG)". Processing of medical data will only take place in pseudonymous form. In case of withdrawal from the study, the data that has already been collected will be destroyed. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The investigator will explain to each participant the nature of the study, its purpose, the procedure involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Additionally, all participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure. The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator and it will be retained as part of the study records. All records relating to this study are stored in an external archive and must be retained for at least ten years after completion of the research.

3.2 Risk-Benefit relationship

The decision for excision is based on the clinical and dermoscopic diagnosis of the pigmented lesion, and will not be biased by the diagnosis of the device under investigation (dermatofluoroscope

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

Magnosco DFC 1). The melanin fluorescence measurements of the FLIMMA study will not influence the clinical procedures. Therefore, there is no risk for the patient that his participation may deteriorate the management rate of his pigmented lesions. The information of the patient and the measurement procedure itself will generally take less than 15 minutes. In case of a false negative diagnosis by the dermatofluoroscope Magnosco DFC a second independent histopathological review will be performed. This will be in favor of a higher diagnostic accuracy and may be beneficial for the patient.

4 SAFETY

This is a non-invasive diagnostic procedure based on low-intensity visible light exposure, which has no capacity to injure tissues. Therefore, no adverse reactions related to the optical procedures are expected. All adverse events (AEs) will be recorded and documented. SAE will be reported in accordance with the Medizinprodukte-Sicherheitsplanverordnung (MPSV) ordinance.

5 FOOTNOTES

5.1 Funding statement

The University of Tuebingen acts as the sponsor of the study. This research received no grant from a commercial sector.

5.2 Study registration

The FLIMMA study was registered at ClinicalTrials.gov with the Identifier: NCT02425475.

5.3 Conflicts of interest

The authors declared that they have no competing interests.

5.4 Authors' contribution

H. Haenssle, C. Garbe, and M. Hofmann participated in the development and the implementation of the study (sample size calculations, writing of the protocol, submission to ethics committee, data management). I. Spaenkuch, D. Leupold, and C. Garbe performed the data handling and statistical

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

analysis. C. Fink, A. Forschner, I. Tampouri, A. Jagoda and D. Lomberg helped to draft and to review the paper. All authors read and approved the final manuscript.

6 REFERENCES

1. Stang A, Stang K, Stegmaier C et al. Skin melanoma in Saarland: incidence, survival and mortality 1970-1996. *Eur J Cancer Prev* 2001; 10: 407-15.

2. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clin Dermatol* 2009; 27: 3-9.

3. Lasithiotakis KG, Leiter U, Gorkievicz R et al. The incidence and mortality of cutaneous melanoma in Southern Germany: trends by anatomic site and pathologic characteristics, 1976 to 2003. *Cancer* 2006; 107: 1331-9.

4. Lasithiotakis KG, Petrakis IE, Garbe C. Cutaneous melanoma in the elderly: epidemiology, prognosis and treatment. *Melanoma Res* 2010; 20: 163-70.

5. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. *Adv Exp Med Biol* 2008; 624: 89-103.

6. Bosetti C, La VC, Naldi L et al. Mortality from cutaneous malignant melanoma in Europe. Has the epidemic levelled off? *Melanoma Res* 2004; 14: 301-9.

7. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. *N Engl J Med* 2004; 351: 998-1012.

8. Balch CM, Soong SJ, Gershenwald JE et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001; 19: 3622-34.

9. Weide B, Elsasser M, Buttner P et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. *Br J Cancer* 2012; 107: 422-8.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

10. Menzies SW, Ingvar C, Crotty KA et al. Frequency and morphologic characteristics of invasive melanomas lacking specific surface microscopic features. *Arch Dermatol* 1996; 132: 1178-82.

11. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. *Annu Rev Phys Chem* 1996; 47: 555-606.

12. Teuchner K, Freyer W, Leupold D et al. Femtosecond two-photon excited fluorescence of melanin. *Photochem Photobiol* 1999; 70: 146-51.

13. Zeng H, MacAulay C, McLean DI et al. Spectroscopic and microscopic characteristics of human skin autofluorescence emission. *Photochem Photobiol* 1995; 61: 639-45.

14. Teuchner K, Ehlert J, Freyer W et al. Fluorescence studies of melanin by stepwise twophoton femtosecond laser excitation. *J Fluoresc* 2000; 20: 275-81.

15. Hoffmann K, Stucker M, Altmeyer P et al. Selective femtosecond pulse-excitation of melanin fluorescence in tissue. *J Invest Dermatol* 2001; 116: 629-30.

16. Schneider M, Teuchner K, Leupold D. [Two-photon fluorescence of ocular melanomas. Studies on a new diagnostic method]. *Ophthalmologe* 2005; 102: 703-7.

17. Eichhorn R, Wessler G, Scholz M et al. Early diagnosis of melanotic melanoma based on laser-induced melanin fluorescence. *J Biomed Opt* 2009; 14: 034033.

18. Scholz M, Stankovic G, Scholz C, Leupold D et al. En route to a new in vivo diagnostic of malignant pigmented melanoma. *PCMR* 2012 Mar;25(2):281-3.

19. Freudenberger S, [Informationsgehalt der Melanin-dominierten Fluoreszenz pigmentierter kutaner Läsionen in Paraffin]. Dissertation Universitätsklinik Tübingen 2014.

20. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

For peer review only

21. Clinical investigation of medical devices for human subjects - Good clinical practice (ISO 14155:2011 + Cor. 1:2011); German version EN ISO 14155:2011 + AC:2011

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

BMJ Open

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (FLIMMA STUDY)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-012730.R2
Article Type:	Protocol
Date Submitted by the Author:	21-Sep-2016
Complete List of Authors:	Fink, Christine; University Hospital Heidelberg, Department of Dermatology Hofmann, Maja; Charité Berlin, Department of Dermatology Jagoda, Agata; Charité Berlin, Department of Dermatology Spaenkuch, Iris; University of Tübingen, Department of Dermatology Forschner, Andrea; University of Tübingen, Department of Dermatology Tampouri, Ioanna; University of Tübingen, Department of Dermatology Lomberg, Diana; University of Tübingen, Department of Dermatology Leupold, Dieter; Magnosco GmbH Garbe, Claus; University of Tübingen Haenssle, Holger; University Hospital Heidelberg, Department of Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Diagnostics
Keywords:	Melanoma, Diagnostic, Two step photon excitation, Melanin fluorescence, Magnosco dermatofluoroscope

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

STUDY PROTOCOL FOR A PROSPECTIVE, NON-CONTROLLED, MULTICENTER CLINICAL STUDY TO EVALUATE THE DIAGNOSTIC ACCURACY OF A STEPWISE TWO-PHOTON EXCITED MELANIN FLUORESCENCE IN PIGMENTED LESIONS SUSPICIOUS FOR MELANOMA (*FLIMMA STUDY*)

Authors:

Christine Fink[1], Maja Hofmann[2], Agata Jagoda[2], Iris Spaenkuch[3], Andrea Forschner[3], Ioanna Tampouri [3], Diana Lomberg[3], Dieter Leupold[4], Claus Garbe [3], Holger A Haenssle[1]

Departments/Institutions:

[1]Department of Dermatology, University of Heidelberg, Germany

[2] Charité Berlin, Department of Dermatology, Berlin, Germany

[3] University of Tübingen, Department of Dermatology, Tübingen, Germany

[4] Magnosco GmbH, Deuben, Germany

Keywords: Melanoma, Diagnostic, Two step photon excitation, melanin fluorescence, Magnosco dermatofluoroscope

Word count: 2291

Corresponding Author:

PD Dr. med. Holger Haenssle

Department of Dermatology, University of Heidelberg,

Im Neuenheimer Feld 440, 69120 Heidelberg

Holger.Haenssle@med.uni-heidelberg.de

Fon: +49-6221-56-39555

Fax: +49-6221-56-4996

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

ABSTRACT

Introduction Non-invasive, nanosecond, stepwise two-photon laser excitation of skin tissue was shown to induce melanin fluorescence spectra that allow for the differentiation of melanocytic nevi from cutaneous melanoma.

Methods and analysis This prospective, non-controlled, multicenter clinical study is performed to evaluate the diagnostic performance of the stepwise two-photon excited melanin fluorescence in the detection of cutaneous melanoma. The comparator will be the histopathological diagnosis. A total of 620 pigmented skin lesions suspicious for melanoma and intended for excision will be enrolled.

Ethics and dissemination Ethics approval was provided by the local ethic committees of the medical faculties of the University of Tuebingen, Heidelberg and Berlin.

Study registration: The FLIMMA study is registered at ClinicalTrials.gov (Identifier: NCT02425475).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time the diagnostic performance of non-invasive, nanosecond, stepwise two-photon laser excitation in the detection of melanoma will be evaluated.
- The University of Tuebingen acts as the sponsor of the study, thus reducing the influence of commercial interests and bias.
- The FLIMMA study is designed as a prospective, multicenter, observational study. Histopathologists are blinded to the results of the test device and a central review board of histopathologists will review all false negative results of the test device. While randomized controlled trials generate the most reliable evidence, the protocol described here is a necessary preliminary step in this challenging area of research and was closely adapted from FDA-approved protocols in the field of medicinal products for the diagnosis of melanoma.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

1 INTRODUCTION

1.1 Background

Melanoma is a malignant tumor, which develops from the pigment producing melanocytes by neoplastic transformation. Malignant melanoma is identified as one form of cancer of increasing incidence, which is now among the 10 most frequent human malignancies. In Europe the regional incidence rates reside between 10 and 25 per 100.000 per year and increase, especially in elderly people.[1-6] After diagnosis of a malignant melanoma the prognosis depends strongly on the thickness of the tumor and lymphatic or hematogenous metastases.[7] Once melanomas cause metastases, the prognosis of survival worsens dramatically, with a 5-year survival of patients with stage IV disease between 6.7% and 18.8%.[8-9] This underlines the urgent need to diagnose melanoma as early as possible. The diagnostic accuracy for the clinical melanoma diagnosis does not exceed 75% and may be increased to up to 90% by the use of dermoscopy in the hands of experts; however, approximately 10% of melanomas will be missed despite all these aforementioned diagnostic efforts. The term "featureless melanoma" has been coined for this phenomenon.[10] Particularly in these difficult to diagnose and "featureless" melanomas additional strategies for melanoma diagnosis would be extremely helpful.

1.2 Two step photon excitation of melanin fluorescence

Autofluorescence spectra of human skin tissue are usually excited by one-photon absorption in the UV-A region.[11] However, by this form of excitation the ultra-weak fluorescence of melanin is undetectably hidden by the emission from the main endogenous fluorophores NAD(P)H and flavins.[12-13] A more specific excitation of the melanin fluorescence may be useful for gaining information about the potential malignancy of pigmented skin lesions. A first step to overcome this lack of specific melanin fluorescence was the application of more targeted fluorescence excitation technique based on two photon absorption from an 800nm-femtosecond laser. All endogeneous skin tissue fluorophores except melanin do not absorb at 800nm; nevertheless, upon irradiation with 800nm-laser pulses they may show their well known fluorescence in the spectrum of visible light. This is caused by a special

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

non-linear optical effect called simultaneous two photon absorption. The intensity of the excited fluorescence is comparably weak. In contrast, melanin shows absorption upon irradiation with 800nm-laser pulses and absorbs two photons in a stepwise process via an intermediate excited electronic state.[12] At physiologically acceptable laser intensities, the latter mechanism is much more effective.[14] By this procedure the main autofluorescence of skin will be partly suppressed and the melanin fluorescence becomes measurable. Investigations in a variety of pigmented skin lesions gave first hints on the differences between the fluorescence from common nevi as compared to malignant melanoma.[15] Also in other melanin-containing fluorophore compositions this fluorescence discrimination in favour of melanin can be observed, e.g. in the choroidea and the retinal pigment epithelium of the eye.[16] A further essential improvement in measuring the melanin fluorescence from skin tissue as selectively as possible could be achieved only recently by using nanosecond pulses instead of femtosecond pulses.[17]

1.3 The dermatofluoroscope Magnosco DFC 1 for in-vivo diagnostics of melanoma

The investigational device in this study is the dermatofluoroscope Magnosco DFC 1 by Magnosco GmbH, Berlin, Germany, with a two-photon excitation with 800 nm/nanosecond pulses from a dye laser, equipped with a spectrometer and a sensitive photon detector. It is designed for use by dermatologists and trained medical personnel and should be applied to patients with skin type I, II, III and IV, who show atypical melanocytic lesions. For the investigation with the Magnosco DFC 1 the patient has to be at physical rest. The overview CCD camera is applied to take a macroscopic image for documentation reasons. After cleaning and shaving the location of interest, the lesion is covered with a specific cover shield with mask. The latter helps to place the scanning head onto the intact skin. After fixing it by an adhesive pad, a dermoscopic image of the lesion is taken. The spectral data are gathered automatically, while the lesion is raster-scanned. The results of the data analysis are presented on the computer screen: 1. Dermoscopic image overlaid with the scanning raster: fluorescence spectra indicating malignancy are visualized as red spots. 2. A score given on a green/red bar indicates the result of data analysis: a) presence of malignant melanoma, b) no indication of malignant melanoma, c) no valid result. The analysis of the spectral data in conjunction with images

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

and patient master data are documented as one file in the dermatofluoroscope Magnosco DFC 1 data base. All files can be transferred with the customized USB stick or printed for patient information.

1.4 Preliminary data with the Magnosco DFC 1 device

The preclinical data available for assessing the suitability of the dermatofluoroscope Magnosco DFC 1 for melanoma diagnostics are based on ex vivo and histological specimen examination. The specimens examined so far were freshly excised pigmented nevoid lesions and their corresponding paraffin-embedded histological samples. In 167 freshly excised tissue specimens from clinically suspicious pigmented lesions (suspected malignant melanoma/dysplastic nevi), the diagnosis was first made based on the new fluorescence-spectroscopic diagnostic method before the histopathological diagnosis was available. In relation to the histopathological diagnosis as the current gold standard of melanoma diagnostics, the new diagnostic method showed a sensitivity of melanoma detection of 93.5%, a specificity of 80.0% and a diagnostic accuracy of 82.6% on freshly excised pigmented lesions.[18] In a study on 125 paraffin embedded specimens of melanocytic melanomas (n = 60) and melanocytic nevi (n = 65), a sensitivity of melanoma detection of 82.5% and a specificity of 72.5% were detected.[19]

2 DESIGN/METHODS

2.1 Study design

The FLIMMA study is designed as a prospective, non-controlled, multicenter clinical study in patients with suspected malignant melanoma.

2.1 Objectives

The primary objective of this study is to determine sensitivity and specificity of the algorithm for the fluorescence diagnostics of melanoma. The comparator and gold standard for the diagnosis will be the histopathological diagnosis of the pigmented lesions. Secondary objectives are to collect data for training and optimization of the diagnostic algorithm, to assess the safety of the device, and to assess the incidence of adverse events.

2.2 Endpoints

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

The primary endpoint is to determine sensitivity and specificity of this fully-automated, non-invasive, in-vivo method. Secondary endpoints include the assessment of the safety of the device as well as the collection of data for training and optimization of the computerized diagnostic algorithm.

2.3 Recruitment and status of the study

Approval of the local Ethics Committees was granted 2014/07/02 for all three participating centers: University Hospital of Tuebingen, University Hospital of Heidelberg, and Charité Berlin). Date of first enrollment was 2014/09/17. The recruitment of patients is in progress. The estimated total time frame for recruitment of 620 patients is 20 months. The total duration of the study is expected to be 26 months, including analysis.

2.4 Study population

A total of 620 patients, who show pigmented lesions with suspicion of dysplastic nevus or melanoma and in whom an excision is indicated, will be recruited.

2.5 Criteria for Inclusion-/ Exclusion

Patients having pigmented lesions with suspicion of dysplastic nevus or melanoma, in whom an excision is indicated in order to exclude or diagnose malignant melanoma, who are equal to or greater than 18 years of age, and who have given written informed consent will be eligible. Patients with skin type V and VI according to Fitzpatrick's scale; patients, where there is a risk that the scanning head is detached because the patient cannot be placed at rest, patients who cannot understand the patient information and who cannot provide informed consent, patients with deep dermal lesions ≥ 5 mm beneath the stratum corneum, clinically or dermoscopically obviously nonmelanocytic lesions, peri- and subungual lesions, mucosal lesions, lesions with trauma, erosion, excoriation, or ulceration on more than 50% of the lesion area, tattooed lesions, patients suffering from albinism, pregnant or breast feeding women, lesions with dominant (>50%) regression, and lesions which are not suitable to fix the scanning head will be excluded from the FLIMMA study.

2.6 Methods

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

A total of 620 pigmented skin lesions intended for excision to either confirm or rule out melanoma will be enrolled after written informed consent at the participating centers. Three centers participate within Germany: University Hospital of Tuebingen as the lead-center, University Hospital of Heidelberg and Charité Berlin. Clinical and dermoscopic images will be recorded for all cases. Then, as a second diagnostic procedure, fluorescence diagnostics based on the two photon excitation from a dye-laser will be performed. The classification as non-melanoma or malignant melanoma by the medical dermatofluoroscope Magnosco DFC 1 will be documented prior to the excision. The in-vivo melanin fluorescence assessment will be performed no longer than 14 days prior to excision. Histopathologists on the study sites will be blinded to the diagnoses attained by the analyses of fluorescence spectra. Moreover, all false negative cases with a disagreement in the diagnosis by the test method and the histopathological examination on site will be submitted to a blinded central pathology review board. The histopathologic diagnosis will serve as gold standard for subsequent evaluations of the diagnostic accuracy. The FLIMMA study was registered at ClinicalTrials.gov (Identifier: NCT02425475).

2.7 Statistical considerations

In this study 560 evaluable lesions will be recruited, including 80 evaluable melanomas. In order to compensate for any drop outs, a total of 620 specimens are examined. It is assumed that the true sensitivity is in the order of 90%, and the true specificity is in the order of 35% based on the available preclinical data described in more detail in the introduction section. With 70 evaluable melanoma specimens and an observed sensitivity of 90%, the two-sided 95% confidence interval is 0.80 - 0.96. The specificity is assumed to be in the order of 35%, which is evaluated in 420 specimens, according to negative gold standard specimen results in a confidence interval of 0.30-0.40. This accuracy is considered sufficient for the method to be evaluated. The study data and the cohort under evaluation will be analysed by means of descriptive statistics. Furthermore, the main endpoint of the present study is the diagnostic accuracy. Sensitivity and specificity of the diagnostic method of fluorescence based pigment analysis will be determined. The comparator and goal standard for this analysis is the histopathological diagnosis.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

3 ETHICS AND DISSEMINATION

3.1 Declarations and ethic aspects

The study is conducted in accordance with the Declaration of Helsinki principles (2013),[20] requirements and guidance provided in ISO 14155 (2012)[21] and applicable local government regulations and Independent Ethics Committee policies and procedures. In the context of the approved standard operating procedures (SOPs) which are based on ICH-GCP guidelines (E6) and the German implementation of Good clinical practice (GCP) for the clinical work, the patients will be informed orally and in written form about aim, character and consequences of the procedure. Before initiation of the study the protocol, the patient information sheet and the consent form were presented to the independent ethic committee. The names of patients and all confidential data are subject to professional discretion and the "Bundesdatenschutzgesetz (BDSG)". Processing of medical data will only take place in pseudonymous form. In case of withdrawal from the study, the data that has already been collected will be destroyed. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The investigator will explain to each participant the nature of the study, its purpose, the procedure involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Additionally, all participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure. The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator and it will be retained as part of the study records. All records relating to this study are stored in an external archive and must be retained for at least ten years after completion of the research.

3.2 Risk-Benefit relationship

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

The decision for excision is based on the clinical and dermoscopic diagnosis of the pigmented lesion, and will not be biased by the diagnosis of the device under investigation (dermatofluoroscope Magnosco DFC 1). The melanin fluorescence measurements of the FLIMMA study will not influence the clinical procedures. Therefore, there is no risk for the patient that his participation may deteriorate the management rate of his pigmented lesions. The information of the patient and the measurement procedure itself will generally take less than 15 minutes. In case of a false negative diagnosis by the dermatofluoroscope Magnosco DFC a second independent histopathological review will be performed. This will be in favor of a higher diagnostic accuracy and may be beneficial for the patient.

4 SAFETY

This is a non-invasive diagnostic procedure based on low-intensity visible light exposure, which has no capacity to injure tissues. Therefore, no adverse reactions related to the optical procedures are expected. All adverse events (AEs) will be recorded and documented. SAE will be reported in accordance with the Medizinprodukte-Sicherheitsplanverordnung (MPSV) ordinance.

5 FOOTNOTES

5.1 Funding statement

The University of Tuebingen acts as the sponsor of the study. This research received no grant from a commercial sector.

5.2 Study registration

The FLIMMA study was registered at ClinicalTrials.gov with the Identifier: NCT02425475.

5.3 Conflicts of interest

The authors declared that they have no competing interests.

5.4 Authors' contribution

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

H. Haenssle, C. Garbe, and M. Hofmann participated in the development and the implementation of the study (sample size calculations, writing of the protocol, submission to ethics committee, data management). I. Spaenkuch, D. Leupold, and C. Garbe performed the data handling and statistical analysis. C. Fink, A. Forschner, I. Tampouri, A. Jagoda and D. Lomberg helped to draft and to review the paper. All authors read and approved the final manuscript.

6 REFERENCES

1. Stang A, Stang K, Stegmaier C et al. Skin melanoma in Saarland: incidence, survival and mortality 1970-1996. *Eur J Cancer Prev* 2001; 10: 407-15.

2. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clin Dermatol* 2009; 27: 3-9.

3. Lasithiotakis KG, Leiter U, Gorkievicz R et al. The incidence and mortality of cutaneous melanoma in Southern Germany: trends by anatomic site and pathologic characteristics, 1976 to 2003. *Cancer* 2006; 107: 1331-9.

4. Lasithiotakis KG, Petrakis IE, Garbe C. Cutaneous melanoma in the elderly: epidemiology, prognosis and treatment. *Melanoma Res* 2010; 20: 163-70.

5. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. *Adv Exp Med Biol* 2008; 624: 89-103.

6. Bosetti C, La VC, Naldi L et al. Mortality from cutaneous malignant melanoma in Europe. Has the epidemic levelled off? *Melanoma Res* 2004; 14: 301-9.

7. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. *N Engl J Med* 2004; 351: 998-1012.

8. Balch CM, Soong SJ, Gershenwald JE et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001; 19: 3622-34.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

9. Weide B, Elsasser M, Buttner P et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. *Br J Cancer* 2012; 107: 422-8.

10. Menzies SW, Ingvar C, Crotty KA et al. Frequency and morphologic characteristics of invasive melanomas lacking specific surface microscopic features. *Arch Dermatol* 1996; 132: 1178-82.

11. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. *Annu Rev Phys Chem* 1996; 47: 555-606.

12. Teuchner K, Freyer W, Leupold D et al. Femtosecond two-photon excited fluorescence of melanin. *Photochem Photobiol* 1999; 70: 146-51.

13. Zeng H, MacAulay C, McLean DI et al. Spectroscopic and microscopic characteristics of human skin autofluorescence emission. *Photochem Photobiol* 1995; 61: 639-45.

14. Teuchner K, Ehlert J, Freyer W et al. Fluorescence studies of melanin by stepwise twophoton femtosecond laser excitation. *J Fluoresc* 2000; 20: 275-81.

15. Hoffmann K, Stucker M, Altmeyer P et al. Selective femtosecond pulse-excitation of melanin fluorescence in tissue. *J Invest Dermatol* 2001; 116: 629-30.

16. Schneider M, Teuchner K, Leupold D. [Two-photon fluorescence of ocular melanomas. Studies on a new diagnostic method]. *Ophthalmologe* 2005; 102: 703-7.

17. Eichhorn R, Wessler G, Scholz M et al. Early diagnosis of melanotic melanoma based on laser-

18. Leupold D, Scholz M, Stankovic G et al. The stepwise two-photon excited melanin fluorescence is a unique diagnostic tool for the detection of malignant transformation in melanocytes. Pigment Cell Melanoma Res. 2011 Jun;24(3):438-45.

19. Freudenberger S, [Informationsgehalt der Melanin-dominierten Fluoreszenz pigmentierter kutaner Läsionen in Paraffin]. Dissertation Universitätsklinik Tübingen 2014.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

20. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053.

21. Clinical investigation of medical devices for human subjects - Good clinical practice (ISO 14155:2011 + Cor. 1:2011); German version EN ISO 14155:2011 + AC:2011

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2016-012730 on 19 December 2016. Downloaded from http://bmjopen.bmj.com/ on April 24, 2024 by guest. Protected by copyright.