Review

Which are the main fluorophores in skin and oral mucosa? A review with emphasis on clinical applications of tissue autofluorescence

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ABSTRACT

Objectives: The present review provides information about which molecules appear to be the main fluorophores in skin and oral mucosa, together with their clinical applications.

Design: The MEDLINE database was searched, using "oral mucosa AND fluorophores", "skin AND fluorophores", "epidermal AND fluorophores", "dermal AND fluorophores" and "cutaneous AND fluorophores" as entry terms.

We searched the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The level of evidence in the studies was assessed using the Classification of the Oxford Centre for Evidence-based Medicine (CEBM) Levels for Diagnosis.

Results: Five papers and 17 were primarily focused on description of fluorophores in oral mucosa and skin Evidence exists that fluorophores of oral mucosa and skin are mainly proteins such as collagen, elastin, keratin and tryptophan. Other possible fluorophores identified are: porphyrins, advanced glycation end products, flavins, lipopigment, nicotinamide adenine dinucleotide, flavin adenine dinucleotide, pheomelanin, eumelanin and components of lipofuscin.

Clinical applications of oral mucosal autofluorescence (AF) are related to management of malignant and potentially malignant lesions. In the skin, AF has been used for acne assessment, diagnosis of sweat-gland pathologies, glycemic control and management of malignant lesions and as a marker for skin aging.

Conclusion: Fluorophores stimulated through AF devices are implied in different physiologic and pathologic processes. AF seems to be useful for several clinical applications, especially in skin department.

Because most of the studies show a low level of evidence, further studies are necessary in such a promising and fascinating field.

1. Introduction

Fluorophores are molecules that can absorb and re-emit specific wavelengths of light and are therefore responsible for the phenomenon of fluorescence.

Fluorophores can be classified into endogenous, when produced within the body, and exogenous, if they originate from outside the organism. Endogenous fluorophores are either intracellular or extracellular, both the cause of biological tissue autofluorescence (AF). AF is defined as a peculiar visual property of some tissues directly associated with the concentration and distribution of specific fluorophores. A diagram illustrating the excitation of molecules and the following phenomenon of fluorescence (Jablonski diagram) is shown in Fig. 1.

Cells and extracellular compartments within dysplastic and malignant lesions may display modifications of the amount, distribution and physicochemical properties of some endogenous fluorophores (Monici, 2005). Malignant and potentially malignant changes may therefore result in AF variations, potentially useful for diagnostic purposes (Fig. 2).

AF evaluation might improve the sensitivity and specificity of cancer diagnosis, having been tested on several organs, such as colon, lung, cervix and oesophagus (Schantz, Savage, Sacks, & Alfano, 1997). Evidence has recently emerged that supports the usefulness of AF for head and neck cancer diagnosis, at both the mucosal and cutaneous levels (Monici, 2005; Schantz et al., 1997).

However, even though some fluorophores have been identified and

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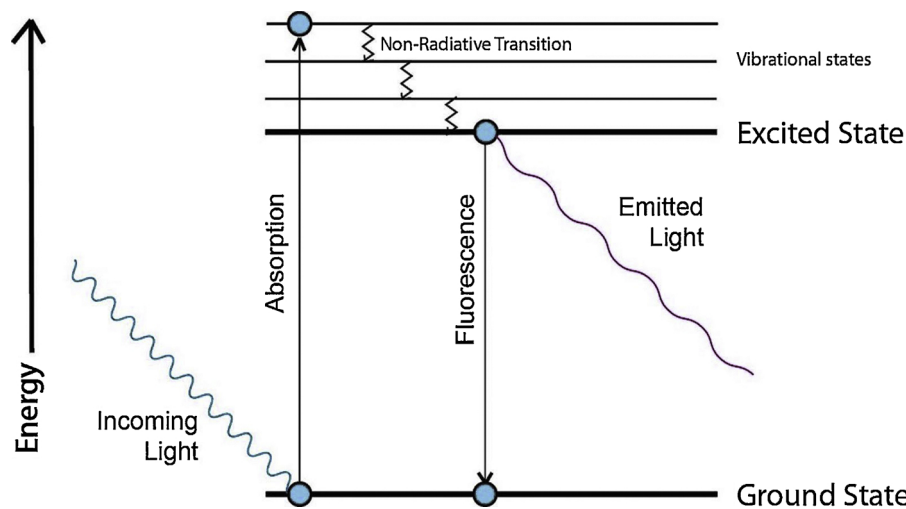*E-mail address:* ilaria.giovannacci@gmail.com (I. Giovannacci).

Fig. 1. Jablonski diagram, showing the molecular mechanism underlying the phenomenon of autofluorescence.
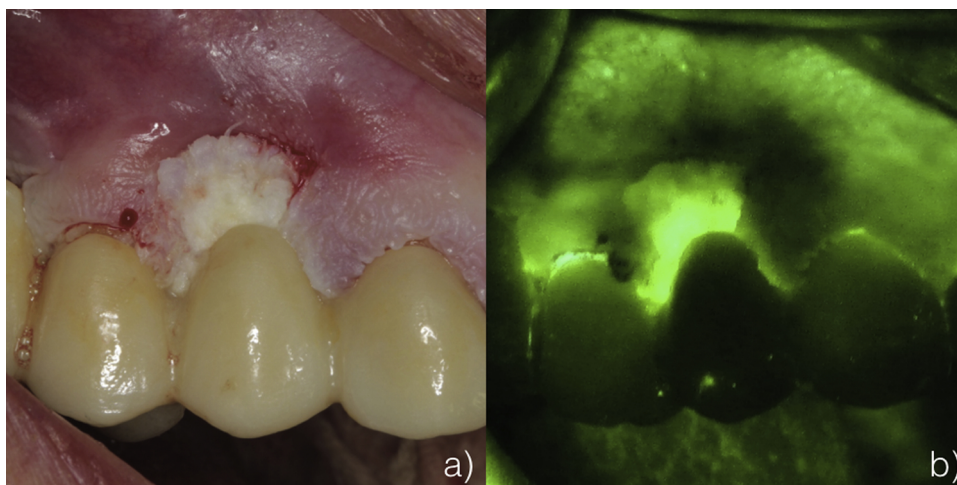

Fig. 2. Oral verrucous carcinoma surrounded by dysplastic tissue in a 65-year-old female patient. (a) clinical view; (b) fluorescence view.

described in detail, there is currently a gap of knowledge about other specific molecules responsible for tissue AF as well as about the mechanisms through which they cease to emit fluorescence in cases of malignant or pre-malignant lesions (Schantz et al., 1997).

The present review provides information about which molecules show evidence of being the main fluorophores in skin and oral mucosa, together with their excitation and emission wavelengths and the devices used for inducing fluorescence, as well as a discussion of clinical applications of AF.

1.1. Methods for the literature search

The MEDLINE database was searched, using "oral mucosa AND fluorophores", "skin AND fluorophores", "epidermal AND fluorophores", "dermal AND fluorophores" and "cutaneous AND fluorophores" as entry terms.

To avoid accidental loss of relevant studies, we searched the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement published in 2009 (Moher, Liberati, & Tetzlaff, 2009).

Reference lists of reviews were screened to identify papers potentially not identified in the database search.

1.1.1. Study selection and data extraction
1.1.1.1. Inclusion criteria

- Papers focused on identification and characterisation of endogenous fluorophores, reporting studies performed in *in vivo* (humans, animals), *ex vivo* and *in vitro* models
- No time limits

1.1.1.2. Exclusion criteria

- Papers with unavailable abstract
- Papers not in English language
- Congress proceedings
- Review papers
- Papers not clearly detailing the fluorophores investigated
- Papers not indicating excitation wavelength used for inducing fluorescence
- Studies evaluating fluorescence induced by exogenous fluorophores

A PRISMA flow diagram was used to summarise the study selection process. The flowchart followed for selection of studies of oral mucosa is shown in Fig. 3. The flowchart for selection of studies on fluorophores in skin is shown in Fig. 4.

The level of evidence in the studies was assessed using the Classification of the Oxford Centre for Evidence-based Medicine (CEBM) Levels for Diagnosis, updated in March 2009.
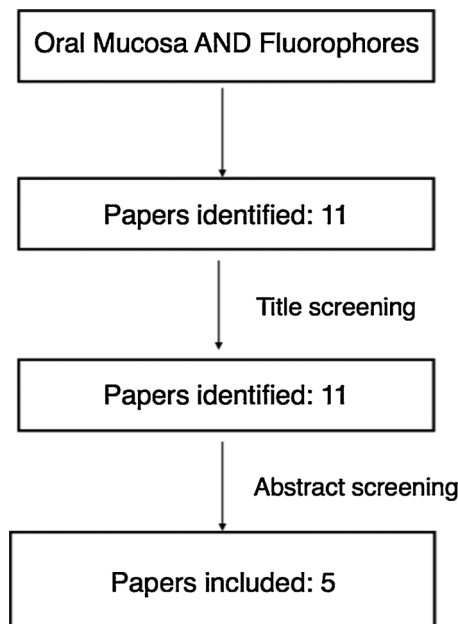
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│   Oral Mucosa AND Fluorophores       │
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                  │
                  ▼
┌─────────────────────────────────────┐
│       Papers identified: 11          │
└─────────────────────────────────────┘
                  │  Title screening
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┌─────────────────────────────────────┐
│       Papers identified: 11          │
└─────────────────────────────────────┘
                  │  Abstract screening
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┌─────────────────────────────────────┐
│        Papers included: 5            │
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Fig. 3. Flowchart reporting the selection procedures for papers on oral mucosa.

2. Fluorophores of oral mucosa

Five papers evaluating fluorophores in oral mucosa were identified (Fig. 3) (Gurushankar, Nazeer, Jayasree, & Krishnakumar, 2015; Jayanthi et al., 2009; Lane et al., 2006; Nazeer, Asish, Venugopal, Anita, & Gupta, 2014; Vedeswari, Jayachandran, & Ganesan, 2009).

Molecules identified as fluorophores of the oral mucosa are: collagen, the reduced form of nicotinamide, adenine dinucleotide (NADH), flavin, adenine dinucleotide (FAD), porphyrins, elastin and keratin. Specific data extracted from each study is shown in Table 1.

Collagen, NADH and FAD were indicated as fluorophores in four studies, porphyrins in two studies and elastin and keratin in one study.

Excitation wavelengths used for the identification of the presumptive fluorophores ranged from 320 to 460 nm (including ultraviolet [UV] radiations). Instead, the emission spectra ranged from 350 to 750 nm.

2.1. Reported clinical applications

Data on clinical applications was available in all five of the studies selected for the present review.

In two studies AF was investigated as a potential aid for improving cancer diagnostic accuracy (excitation wavelength used: 400–460 nm) (Jayanthi et al., 2009; Lane et al., 2006). In one study, AF spectroscopy was used for monitoring the therapeutic response and for characterising tissue reaction after treatment of oral submucous fibrosis (excitation wavelength used: 330 nm) (Vedeswari et al., 2009). Another study demonstrated a variation in AF spectra between the oral mucosa of habitual tobacco users and the oral mucosa of a group of volunteers without any habits (excitation wavelength used: 410 nm) (Nazeer et al., 2014).

In the only animal study identified, fluorescence spectroscopy was proposed for monitoring or potentially predicting response to cancer therapy (excitation wavelength used: 320 nm) (Gurushankar et al., 2015).

2.2. Level of evidence

Four out of the five studies on oral mucosa and fluorophores were *in vivo* studies, performed on humans. One study was a randomised trial in an animal model (Gurushankar et al., 2014). The Oxford CEBM and National Institutes of Health (NIH) Quality Assessment classifications were therefore applicable for four studies (Jayanthi et al., 2009; Lane et al., 2006; Nazeer et al., 2014; Vedeswari et al., 2009).

Level of evidence was 2b for all studies evaluated.

3. Fluorophores of skin

Fifteen papers were identified by using "Skin AND Fluorophores" as entry terms, five papers by using "Epidermal AND Fluorophores" and one paper by using "Dermal AND Fluorophores". Four papers were repetitions. The database search using "Cutaneous AND Fluorophores" as entry terms did not identify papers dealing with the topic of the present review. Seventeen papers were eventually included (Fig. 4) (Breunig, Studier, & König, 2010; Dimitrow, Riemann et al., 2009, 2009b; Eny et al., 2015; Gillies, Zonios, Anderson, & Kollias, 2000; Krasieva et al., 2013; Laiho, Pelet, Hancewicz, Kaplan, & So, 2005; Leupold et al., 2011; Miyamoto & Kudoh, 2013; McMullen, Chen, & Moore, 2012; Na, Stender, Henriksen, & Wulf, 2011; Patalay et al., 2011; Richter, Trojahn, Dobos, Blume-Peytavi, & Kottner, 2016; Stirban, Nandrean, Negrean, Koschinsky, & Tschoepe, 2008; Sandby-Møller, Thieden, Philipsen, Heydenreich, & Wulf, 2004; Yu et al., 2012; Zhao et al., 2016).

Molecules identified as possible fluorophores in the skin were porphyrins, advanced glycation end products (AGEs), flavins, lipopigment, the reduced form of nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), pheomelanin, eumelanin, collagen, elastin, tryptophan, components of lipofuscin and keratin. Details of studies and molecules are shown in Table 2.

Excitation wavelengths used for inducing AF ranged from 260 to 1000 nm (including UV). Wavelengths used with two-photon tomography and femtosecond laser ranged from 720 to 1000 nm. Wavelengths used with other devices ranged from 260 to 488 nm.

3.1. Reported clinical applications

Clinical applications of skin AF displayed a great heterogeneity.

In four of the selected studies, skin AF was proposed as a possible new diagnostic tool for malignant melanoma. In all these papers, a two-photon tomography instrument was the device used for investigation (Dimitrow, Riemann et al., 2009, 2009b; Krasieva et al., 2013; Miyamoto & Kudoh, 2013).

In two out of 17 papers, skin AF was investigated as a marker for photo-aging, using UV wavelengths (330 and 370 nm) (Na et al., 2011; Sandby-Møller et al., 2004).

Other applications reported for skin AF were measurement of acne severity (excitation wavelength: 400 nm) (Richter et al., 2016); diagnosis and therapy of eccrine sweat gland disease (excitation wavelength: 488 nm) (Zhao et al., 2016); screening for patients with undetected diabetes or cardiovascular risk (excitation wavelength used: 300–420 nm) and assessment of the effectiveness of anti-proliferative agents (excitation wavelength used: 260–480 nm) (Gillies et al., 2000; Stirban et al., 2008).

3.2. Level of evidence

Seven out of 17 studies on skin/epidermal fluorophores were *in vivo* studies performed on humans; four out of 17 studies selected were *ex vivo* and *in vivo* studies, and six out of 17 studies were *ex vivo* or *in vitro* studies (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Eny et al., 2015; Gillies et al., 2000; Krasieva et al., 2013; Leopold et al., 2011; McMullen et al., 2012; Na et al., 2011; Patalay et al., 2011; Richter et al., 2016; Stirban et al., 2008; Laiho et al., 2005; Sandby-Møller et al., 2004; Yu et al., 2012; Zhao et al., 2016).

The Oxford CEBM guidelines were applicable in 11 studies (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Eny et al., 2015;
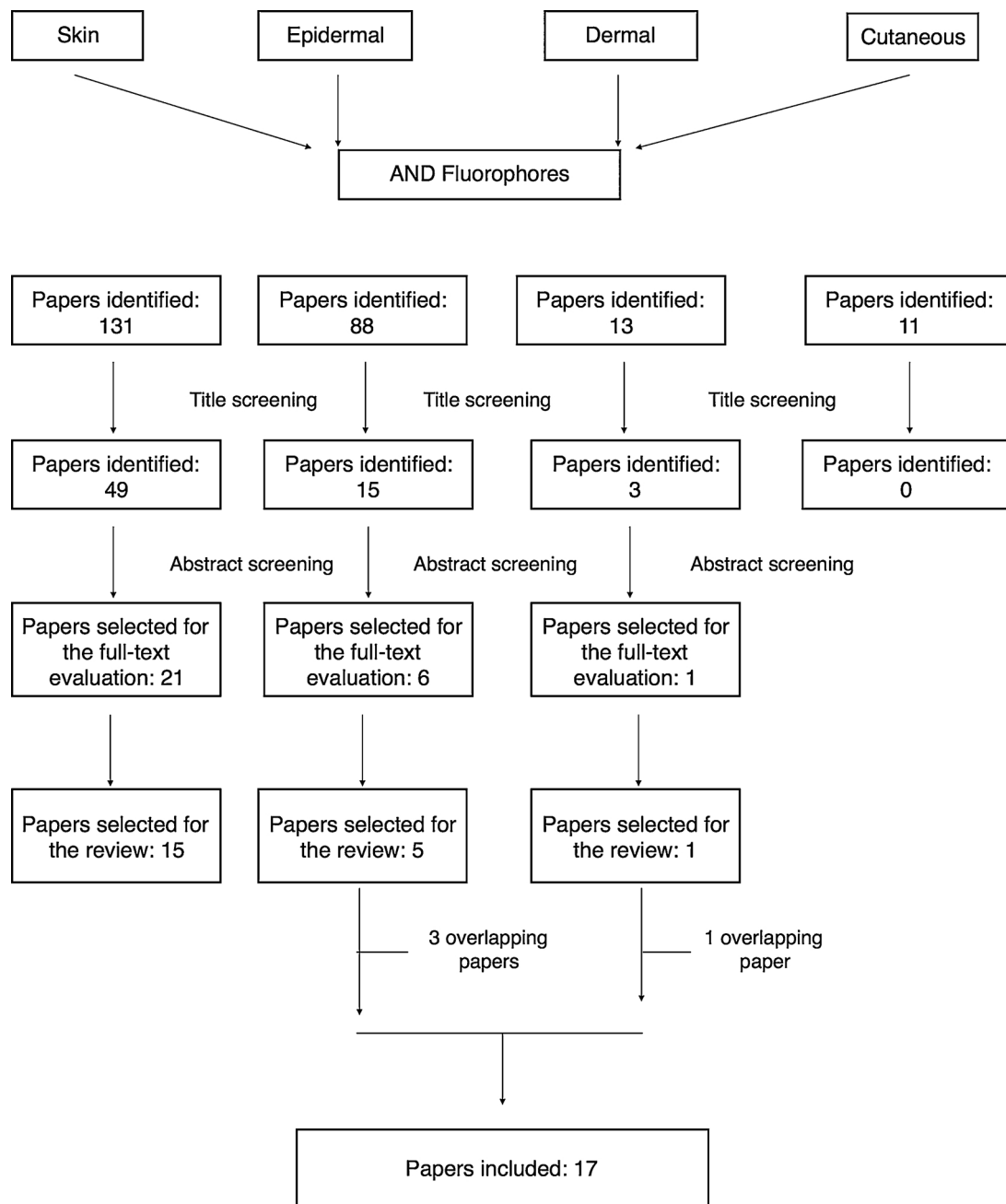
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┌──────────┐   ┌──────────────┐   ┌──────────┐   ┌──────────────┐
│   Skin   │   │  Epidermal   │   │  Dermal  │   │  Cutaneous   │
└──────────┘   └──────────────┘   └──────────┘   └──────────────┘
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        \             │                │               /
         ┌────────────────────────────────────────┐
         │            AND Fluorophores              │
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Papers identified: 131	Papers identified: 88	Papers identified: 13	Papers identified: 11

Title screening · Title screening · Title screening

Papers identified: 49	Papers identified: 15	Papers identified: 3	Papers identified: 0

Abstract screening · Abstract screening · Abstract screening

Papers selected for the full-text evaluation: 21	Papers selected for the full-text evaluation: 6	Papers selected for the full-text evaluation: 1

Papers selected for the review: 15	Papers selected for the review: 5	Papers selected for the review: 1

3 overlapping papers · 1 overlapping paper

Papers included: 17

Fig. 4. Flowchart reporting the selection procedures for papers on skin.

Gillies et al., 2000; Miyamoto & Kudoh, 2013; McMullen et al., 2012; Na et al., 2011; Richter et al., 2016; Stirban et al., 2008; Sandby-Møller et al., 2004).

The level of evidence was 1b for one study, 2b for six studies and 3b for four studies.

4. Commercial devices used to induce and visualise fluorescence

Commercial devices reported in the literature are usually based on simple setups, (e.g., peculiar excitation and emission wavelength bands, obtained by using excitation light sources [light editing diode LED]). Such devices have been optimised for specific applications, (e.g., Visiopor© (Courage + Khazaka Electronic GmbH, Germany), including the quantitative measurement of the AF emitted by bacterial porphyrins in patients with acne (Richter et al., 2016).

A qualitative analysis of tissue AF is possible through devices similar to those described. The VELscope^{TM} system (LED Medical Diagnostics Inc., Barnaby, Canada) has been widely employed for diagnostic purposes, particularly for oral mucosa.

Other devices are based on multi-photon tomography (e.g., DermaInspec© [JenLab GmbH, Germany]). The technique, used for *in vivo* non-invasive tomography of human skin (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Miyamoto & Kudoh, 2013; Patalay et al., 2011), is based on the near-infrared femto laser beam scanning technology. Devices employing multi-photon tomography allow optical biopsies with subcellular spatial resolution. The technology generates an image based on the AF intensity and time decay of fluorophores, including NADH, flavins, porphyrins and elastin at different tissue depths.

For more specific *ex vivo* or *in vitro* AF measurements, particular microscopes (e.g., Olympus IX71©) are used. Depending on their setup, such microscopes allow high-resolution imaging of reflected or

Table 1
Outline of papers investigating fluorophores in oral mucosa.

Author	Year	Type of paper	Fluorophores	Excitation wavelength	Emission wavelength	Device (excitation source)	Application fields or conclusions
K. Gurushankar et al	2015	Animal scientific study (hamster)	- Collagen - nicotinamide adenine dinucleotide (NADH) - flavin adenine dinucleotide (FAD)	320 nm	ranging from 350 to 550 nm	Datamax software (Datamax, Round Rock, Texas) and the inbuilt double-grating monochromator.	Fluorescence spectroscopy for monitoring or potentially predicting response to cancer therapy.
SS. Nazeer et al	2014	Clinical cohort study	- FAD - Porphyrins - Hemoglobin (chromophore)	410 nm	ranging from 450 to 750 nm	Instruments consists of a 450-W Xenon arc lamp, a double excitation monochromator, a double emission monochromator, and a photomultiplier tube.	The study demonstrated a variation in auto-fluorescence spectra from the oral mucosa of habitual tobacco users with that of a group of volunteers without any habits in a clinical setup.
CP. Vedeswari et al	2009	Clinical cohort study	- Collagen - NADH	330 nm	ranging from 350 to 600 nm * emission spectra in normal mucosa different from oral submucous fibrosis and different after treatment	A monochromator with a 150 W ozone-free Xenon lamp	Autofluorescence spectroscopy for monitoring the therapeutic response and characterize the tissue transformation after treatment of oral submucous fibrosis.
JL. Jayanthi et al	2009	Clinical cohort study	- Collagen - NADH - Elastin - Keratin - FAD - Porphyrins	404 nm	- peak in healthy patient: 500 nm - peaks in malignant lesions: 635, 685 and 705 nm	A 404 nm, 50 mW diode laser (Stocker Yale, Montreal, Canada)	Autofluorescence can improve diagnostic accuracy.
PM. Lane et al	2006	Clinical pilot study	- Collagen - NADH - FAD	400-460 nm	> 475 nm	The light source X-Cite 120, EXFO used a 120-W metal-halide arc lamp with integral elliptical reflector optimized for near- UV/blue reflection.	Direct fluorescence visualization device has potential as a simple, cost-effective screening device for the early detection of oral premalignant lesions.

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Table 2
Outline of papers investigating fluorophores in skin.

Author	Year	Type of paper	Fluorophores	Excitation wavelength	Emission wavelength	Device (excitation source)	Application fields or conclusions
Richter C et al	2016	Clinical study	Porphyrins	UVA light (about 400 nm)	Red fluorescence	Visiopor PP 34 (Courage + Khazaka Electronic GmbH)	Fluorescence quantity for measuring acne severity
Eny KM et al	2015	Clinical study	Advanced glycation end products (AGEs)	SIF 1: 375 nm; SIF 14: 456 nm	SIF 1: 435-655 nm; SIF 14: 491-655 nm	SCOUT DS SF spectrometer (VeraLight, Inc., Albuquerque, NM)	Skin intrinsic fluorescence is associated with caffeine consumption
Zhao HL et al	2015	Ex vivo study	Flavin; Lipopigment	488 nm	2 peaks: 530 nm and 590 nm	Olympus Microscope IX71, OLYMPUS CORPORATION, Tokyo, Japan	Autofluorescence for diagnosis and therapy of eccrine sweat glands disease
Miyamoto K et al	2013	Clinical study	NADPH	near-infrared (760 nm)	–	Two-photon tomography (DermalInspect/MPTflex, JenLab GmbH, Jena, Germany)	NADPH visualization and quantification in living keratinocyte for evaluating skin aging and its progression
Krasieva TB et al	2013	In vitro scientific study	Pheomelanin; Eumelanin	900-1000 nm	Pheomelanin: 615-625 nm Eumelanin: 640-680 nm	Meta detector of the Zeiss LSM 510 Meta NLO microscopy system; Chameleon-Ultra femtosecond pulsed tunable laser (Coherent Inc.)	Two-photon excited fluorescence (TPF) spectral index and phasor fluorescence lifetime imaging microscopy (FILM) used for rapid melanin ratio characterization boh in vivo and in vitro.
Yu Y et al	2012	In vitro + ex vivo scientific study	Keratin; NADH; FAD; Melanin; Collagen; Elastin	ranging from 730 to 920 nm	Keratin: 485-525 nm NADH: 460 nm FAD: 550-543 nm Melanin: 579 nm Collagen: 458-475-510 nm Elastin: 454-472-508 nm	720-950 nm femto-second laser (Chameleon, Coherent Inc., Santa Clara, CA)	Imaging guided TPF excitation-emission-matrix (EEM) spectroscopy provides useful information for developing clinical devices for skin disease diagnosis
McMullen RL et al	2012	Ex vivo animal study + in vivo human study	(human skin) - Collagen - Tryptophan	ranging from 270 to 450 nm	(human skin) - Collagen: 466 nm - Trp: 350 nm	UVA lamp	Collagen fluorescence has different characteristics depending on type of skin tissue investigated.
Patalay R et al	2011	Ex vivo scientific study	Collagen; Elastin; Melanin; Keratin; Porphyrins; NADPH; Flavins	760 nm	NADPH: 450-460 nm (main fluorophore in the blue channel) Melanin: 550 nm Flavins: 525 nm (main fluorophores in the green channel)	(modified) Two-photon tomography (DermalInspect/MPTflex, JenLab GmbH, Jena, Germany)	Fluorescence for differential diagnosis from dysplastic nevi and malignant nodular basal cell carcinoma.
Leupold D et al	2010	Ex vivo scientific study	Pheomelanin; Eumelanin	810 nm	Common melanocytic nevi: band peaking 590 nm Melanoma: band peaking 640 nm	2.5 ns-pulsed via 810 nm wo-photon	Two-photon excited fluorescence is a possible new diagnostic tool on the basis of the red-shift of melanin fluorescence in melanoma.
Breunig HG et al	2010	Clinical study	Keratin; NADH; FAD; Melanin; Collagen; Elastin	ranging from 710 to 920 nm	–	Sapphire laser (Mai Tai XF, Spectra Physics)	–
Dimitrow E et al.	2009	In vivo + ex vivo scientific study	- FAD - NAD(P)H - metal-free porphyrins - components of lipofuscin - melanin - elastin - collagen - keratin	760 nm and 800 nm	–	Two-photon tomography (DermalInspect/MPTflex, JenLab GmbH, Jena, Germany)	Early detection of black skin cancer.

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Table 2 (*continued*)

Author	Year	Type of paper	Fluorophores	Excitation wavelength	Emission wavelength	Device (excitation source)	Application fields or conclusions
Dimitrow E et al.	2009	In vivo + ex vivo scientific study	- NAD(P)H - metal-free porphyrins - components of lipofuscin - melanin - elastin - collagen - keratin	750 nm - 850 nm	–	Two-photon tomography (DermaInspect/ MPTflex, JenLab GmbH, Jena, Germany)	Multiphoton laser tomography for malignant melanoma diagnosis
Stirban A et al	2008	Clinical study	AGEs	ranging from 300 to 420 nm	–	AGE Reader (Diagnoptics BV, Groningen, The Netherlands)	Skin Autofluorescence as a non-invasive tool for screening people for undetected diabetes or cardiovascular risk.
Laiho LH et al	2005	ex vivo study	Tryptophan; NADPH; Melanin; Elastin; Collagen.	ranging from 375 to 600 nm	Collagen: peak 400-425 nm Tryptophan: peak 425 nm NADPH: peak 475 nm Elastin: peak475 nm Melanin: peak 550nm	Ti:sapphire laser (Tsunami,Spectra Physics, Palo Alto, California))	Basis for future in vivo skin spectroscopy studies and clinical examinations.
Sandby-Møller J et al	2004	Clinical study	Collagen	EX1 370 nm EX 2: 330nm	EM 1: 455 nm EM 2: 455 / 370nm	Quarz Y-fiber (wavelenght allowance range: 120-1600 n. Volpi AG, Zurich, Switzerland)	Collagen-linked autofluorescence decreased with UVR exposure. F ratio might be the best measure of individual photodamage and a marker of individual cumulative UVR dose.
Na R et al	2001	Clinical study	–	EX1 330 nm EX 2: 370nm	EM 1: 375 nm; EM 2: 455 nm	Quarz Y-fiber (wavelenght allowance range: 120-1600 n. Volpi AG, Zurich, Switzerland)	375 nm skin autofluorescence as a biologic marker of skin aging in vivo.
Gillies R et al	2000	In vivo + ex vivo scientific study	Tyrosine; Tryptophan; Collagen; Elastin	ranging from 260 to 480 nm	Tyrosine: EX 270 EM: 320 nm Tryptophan: EX:295 EM: 345 nm Pepsin digestible collagen cross-link: EX: 335 EM: 390 nm Collagenase digestible collagen cross-link: EX: 370 EM: 460 nm Elastin: EX: 440 EM: 520 nm	–	Tryptophan fluorescence is potentially associated with proliferation and may be a useful marker in assessing the effectiveness of anti-proliferative agents or treatment.

transmitted fluorescence, by selecting the chosen excitation and emission bands, followed by digital image processing (Ericson et al., 2008).

Scout DS© (Veralight) performs a non-invasive automatic quantitative fluorescence spectroscopy to detect the levels of advanced glycation end products (AGEs) in the skin (Eny et al., 2015) of patients with diabetes.

Among the optical components more frequently used in clinical settings, the monochromator is a tunable optical filter that enables selection and transmission of wavelengths of only a specific, narrow band of the incident light (Gurushankar et al., 2015; Na et al., 2011; Nazeer et al., 2014; Vedeswari et al., 2009). Such a device can be used to filter narrow-band light, starting from a wide-band source to elicit tissue AF (Breunig et al., 2010; Drakaki, Dessinioti, Stratigos, Salavastru, & Antoniou, 2014; Gurushankar et al., 2015; Lane et al., 2006; Nazeer et al., 2014; Na et al., 2011; Sandby-Møller et al., 2004; Vedeswari et al., 2009; Yaroslavsky, Neel, & Anderson, 2004). A second monochromator can be used to select the wanted emission narrow-band light by removing the unwanted reflected excitation light. In some cases, photomultiplier tubes, which are extremely sensitive detectors of light signals, can be used (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Gurushankar et al., 2015; Krasieva et al., 2013; Leopold et al., 2011; Nazeer et al., 2014; Vedeswari et al., 2009; Yu et al., 2012).

5. Discussion

Innovative, non-invasive visual tools, possibly improving diagnostic accuracy, have recently gained interest. Among these, devices evaluating tissues' auto-fluorescence (AF) are emerging (Drakaki et al., 2014; Giovannacci, Vescovi, Manfredi, & Meleti, 2016).

It seems likely that malignant or potentially malignant changes in soft tissues may induce variations of the tissue AF spectra, which can be visualised in real time by non-invasive methods.

On the basis of the present literature review, some evidence exists that endogenous fluorophores of skin, stimulated by ultraviolet or blue excitation wavelengths, are proteins such as collagen, elastin, keratin and the amino acid tryptophan. On the other hand, the very limited number of papers (5) investigated for fluorophores in the oral mucosa does not allow drawing any robust conclusion on such an anatomical compartment. Data on AF molecules in oral mucosa should, therefore, be considered preliminary and presumptive, and they should be confirmed by further research.

Even though skin and oral mucosa share some similarities in terms of overall histological architecture and molecule distribution, it should be borne in mind that specific features (e.g., width of the keratin layer, presence of peculiar glands, *etc.*) may be associated with different behaviours in terms of AF (light absorption and emission). Nevertheless, it would seem that both in skin and oral mucosa, dysplastic and neoplastic processes induce some alteration of fluorophores' structure and/or concentration, thus producing a variation of normal AF (Croce & Bottiroli, 2014; Jayanthi et al., 2009).

Among fluorophores identified in the present review, localised either in the oral mucosa or skin, there are molecules involved in tissue metabolism such as the reduced forms of NAD (NADH) and NAD phosphate (NADPH) and FAD. Metabolic activity of the relative amounts of NADH, NADPH and FAD and the microenvironment of these metabolic electron carriers can apparently be used to monitor changes in metabolism non-invasively, which is one of the hallmarks of carcinogenesis (Gurushankar et al., 2015; Skala et al., 2007). Such coenzymes are the main ones responsible for AF rising from cell cytoplasm, with their amount, redox and bound-free state in close relationship with their engagement in energetic metabolism, cell oxidative defence, reductive biosynthesis, and signal transduction (Croce & Bottiroli, 2014).

Particularly, NADH is more fluorescent than the oxidised form, NAD^+ (Miyamoto & Kudoh, 2013). Depending on the metabolic status of cells (e.g., prevalence of reactions of oxidation or reduction), the oxidised or reduced form of the coenzyme will be prevalent in the

cytoplasm, thus leading to a higher or lower degree of fluorescence (Croce & Bottiroli, 2014). It has been widely demonstrated that neoplastic cells display an increase in oxidative processes, and it is therefore reasonable to hypothesise a shift in the NAD^+/NADH equilibrium toward a predominance of NAD^+ (Drakaki et al., 2014; Gào & Schöttker, 2017).

In addition, Drakaki et al., in their review on laser-induced fluorescence for diagnosis of basal cell carcinoma, confirmed that AF variation in malignant tissues was caused by decreased NADH levels and by a shifted equilibrium between the highly fluorescent NADH and the less-fluorescent oxidised-form $NAD+$ (Drakaki et al., 2014).

Similar to NAD, NADP is fluorescent in its reduced form (NADPH); the AF emission properties were strictly dependent on the bound/free condition (Miyamoto & Kudoh, 2013). The typical energetic anaerobic metabolism was observed in most neoplastic cells results, in general, in lower values in the NADPH bound/free ratio with respect to non-neoplastic cells (Croce & Bottiroli, 2014; Salmon et al., 1982). Such a low value of the bound/free ratio is, most probably, one of the biological features associated to the loss of AF observed in tumour cells.

As part of the group of flavin *free derivatives*, FAD exhibits absorption/excitation and emission properties at 440–450 nm and 525 nm (Penzer, 1980; Wolfbeiss, 1985). The fluorescence emission from FAD has been reported to be strongly affected by the nature of the protein to which the prosthetic group is bound (Kunz & Kunz, 1985; Kunz, 1986). Interestingly, the first description of FAD fluorescence was performed in 1962, in intracellular granules of eosinophils (Grossi & Zaccheo, 1963). It seems worthy to mention here that further studies demonstrated an increase in AF amplitude and a shift from the blue to the yellow region when the circulating cells settled in the tissues (Barnes, Aggarwal, Thomsen, Fitzmaurice, & Richards-Kortum, 1992; Mayeno, Hamann, & Gleich, 1992; Weil &, 1981).

Porphyrins are indicated as fluorophores both in oral mucosa and in skin tissues, with an excitation wavelength of about 400 nm and emission wavelength around 630–690 nm. Variation in porphyrin concentration is considered an important marker for differentiating malignant disorders from healthy tissues (Jayanthi et al., 2009). According to Bagri-Manjrekar et al., the mechanism responsible for such a phenomenon seems to depend on one or more of the following possibilities: (1) porphyrins of host origin and excreted from the circulating blood by the tumour; (2) the tumour itself produces porphyrins and (3) porphyrins are the product of microbial activity restricted to the ulcerated surface of the tumour (Bagri-Manjrekar et al., 2018).

In particular, Nazeer et al. demonstrated a more elevated level of porphyrins in the tissues of patients with oral leukoplakia and among tobacco users compared to non-tobacco users. Jayanthi et al. reported that the level of porphyrins increases with the increase in pathological grading, and Croce et al. reported that an excess of protoporphyrin IX (PpIX) occurs naturally in cancers and their metastases (Croce et al., 2011; Jayanthi, Subhash, Stephen, Philip, & Beena, 2011).

Interestingly, Inaguma et al. demonstrated that when exposed to 410 nm wavelength light, 85% of oral carcinomas returned porphyrin-like fluorescence spectra, whereas the normal mucosa of the oral cavity did not display such a feature (Inaguma & Hashimoto, 1999).

Moreover, the Mallia et al. study highlighted that the peak observed at 685 nm, especially in neoplastic and dysplastic tissues, could depend on the accumulation of the endogenous fluorophore coproporphyrin III, which is a precursor of PpIX in the heme synthesis. This peak was absent in tissues from healthy volunteers, which confirmed such findings (Mallia et al., 2008).

Among less-reported fluorophores, the present review identified flavin, lipopigment, advanced glycation end products (AGEs), pheomelanin and eumelanin.

Zhao et al. reported flavin and lipopigment as endogenous fluorophores of eccrine sweat glands. Following irradiation with a 488 nm wavelength, a single eccrine sweat gland isolated from normal skin tissue under a microscope showed AF with two peaks: 530 and 590 nm,

corresponding to flavin and lipopigment peaks. Such molecules are coenzymes in many metabolic pathways. AF measurement could therefore be performed to monitor cells and tissue energy metabolism (Zhao et al., 2016).

AGEs are a heterogeneous group of molecules resulting from non-enzymatic glycation of proteins, lipids, and nucleic acids. The measure of AGE residues provides information on glycemic control in diabetic patients (Eny et al., 2015; Stirban et al., 2008). It would seem that their concentration can be indirectly measured by evaluating skin AF. Stirban et al., using an excitation light source between 300 and 420 nm, demonstrated that skin AF increases after lunch in both healthy and diabetic patients (Stirban et al., 2008).

Light absorption behaviour of melanin is completely different from the absorption of other organic fluorophores (Hoffmann, Stucker, Altmeyer, Teuchner, & Leupold, 2001). In fact, melanin AF in the spectral region of 450–650 nm is extremely weak in human skin tissue and is completely hidden from the main endogenous fluorophores (e.g., NADPH e flavins) (Teuchner et al., 1999; Zeng, MacAulay, McLean, & Palcic, 1995). Such a disadvantage has been overcome only recently, by using special fluorescence techniques such as one-photon excitation and two-photon absorption (Weinberger et al., 2006; Teuchner et al., 1999). Upon irradiation with 800-nm laser pulses, melanin absorbs two photons in a stepwise process through an intermediate excited electronic state (Teuchner et al., 1999).

The multiphoton laser tomography (MLT), developed on the basis of the aforementioned technologies, enables a three-dimensional optical sectioning with a subcellular spatial resolution, thus allowing selective melanin imaging (Koenig & Riemann, 2003).

Investigation of melanin fluorescence, within a different set of pigmented lesions of the skin, showed a marked difference in AF behaviour of benign pigmentations (e.g., melanocytic nevi) with respect to melanoma (Hoffmann et al., 2001). Leupold et al. confirmed that through the use of a two-photon excitation technology with 2.5-ns pulses, melanin fluorescence peak is 590 nm in all types of benign melanocytic nevi, although it shifts to red light with 640 nm in melanoma (Leupold et al., 2011). Such observation led to the hypothesis that malignant transformation of melanocytes is associated with changes in melanogenesis and in the amounts of cellular eumelanin and pheomelanin (Leupold et al., 2011).

The reason for the red-shift of melanin fluorescence in melanoma is the modification of the eumelanin/pheomelanin ratio with an higher concentration of pheomelanin in malignant lesions (Leupold et al., 2011; Simon, Peles, Wakamatsu, & Ito, 2009; Yaroslavsky et al., 2004).

Dimitrow et al. further demonstrated that MLT can differentiate between benign and malignant melanocytic lesions both *in vivo* and *ex vivo*. In fact, among 83 pigmented lesions of the skin, the authors highlighted distinct morphological differences in melanoma compared with melanocytic nevi. In particular, six characteristic features of AF malignant melanoma were specified (architectural disarray, pleomorphic cells, poorly defined keratinocyte cell borders, large intercellular distance, ascending melanocytes and dendritic cells). The technique showed an overall sensitivity of 75% *in vivo* and 93% *ex vivo* (Dimitrow, Ziemer et al., 2009).

All clinical applications of oral mucosal AF reported in the papers included in the present review were related to the diagnosis and follow-up of malignant and potentially malignant lesions (Gurushankar et al., 2015; Jayanthi et al., 2009; Lane et al., 2006; Vedeswari et al., 2009).

It should be borne in mind that a potential risk of malignant transformation associated with the use of UV-emitting devices exists (D'Orazio, Jarrett, Amaro-Ortiz, & Scott, 2013). Therefore, caution should be used in clinical settings, when fluorescence is excited through UV wavelengths. It would, however, seem improbable that the limited time of exposure necessary in the reported techniques is sufficient to generate some damage at the cellular and tissue levels.

AF detection in skin seems to cover a wider range of applications than in oral mucosa. Several papers are focused on the diagnosis and

follow-up of malignant and potentially malignant lesions, both non-melanoma skin cancers and melanoma. Skin AF has also been proposed for assessing the severity of acne, for the diagnosis of sweat gland pathologies, for monitoring glycemic control in diabetic patients and as a marker for skin aging.

6. Conclusion

Some evidence exists for the usefulness of AF in the management of potentially malignant and malignant lesions of the skin. On the other hand, the very limited number of papers (5) investigating fluorophores in oral mucosa, and their behaviour in physiologic and pathologic conditions, does not allow any robust conclusions about such anatomical compartmentalisation. Further to diagnosis of malignant lesions, potential fields of application of skin and mucosal AF have been explored. However, on the basis of the weak level of evidence, more well-designed studies are needed to confirm such potentialities.

The heterogeneity of AF devices as well as their applications makes it difficult to compare the results of the studies included in the present review.

References

Bagri-Manjrekar, K., Chaudhary, M., Sridharan, G., Tekade, S. R., Gadbail, A. R., & Khot, K. (2018). *In vivo* autofluorescence of oral squamous cell carcinoma correlated to cell proliferation rate. *Journal of Cancer Research and Therapeutics, 14*(3), 553–558. https://doi.org/10.4103/0973-1482.172710.

Barnes, D., Aggarwal, S., Thomsen, S., Fitzmaurice, M., & Richards-Kortum, R. (1992). A characterization of the fluorescent properties of circulating human eosinophils. *Photochemistry and Photobiology, 58*, 297–303. https://doi.org/10.1111/j.1751-1097.1993.tb09565.x.

Breunig, H. G., Studier, H., & König, K. (2010). Multiphoton excitation characteristics of cellular fluorophores of human skin in vivo. *Optics Express Journal, 18*(8), 7857–7871. https://doi.org/10.1364/OE.18.007857.

Croce, A. C., & Bottiroli, G. (2014). Autofluorescence spectroscopy and imaging: A tool for biomedical research and diagnosis. *European Journal of Histochemistry, 58*(4), https://doi.org/10.4081/ejh.2014.2461.

Croce, A. C., Santamaria, G., De Simone, U., Lucchini, F., Freitas, I., & Bottiroli, G. (2011). Naturally-occurring porphyrins in a spontaneous-tumour bearing mouse model. *Photochemical & Photobiological Sciences, 10*(7), 1189–1195. https://doi.org/10.1039/c0pp00375a.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. *International Journal of Molecular Sciences, 14*(6), 12222–12248. https://doi.org/10.3390/ijms140612222.

Dimitrow, E., Riemann, I., Ehlers, A., Koehler, M. J., Norgauer, J., Elsner, P., et al. (2009). Spectral fluorescence lifetime detection and selective melanin imaging by multi-photon laser tomography for melanoma diagnosis. *Experimental Dermatology, 18*(6), 509–515. https://doi.org/10.1111/j.1600-0625.2008.00815.x.

Dimitrow, E., Ziemer, M., Koehler, M. J., Norgauer, J., König, K., Elsner, P., et al. (2009). Sensitivity and specificity of multiphoton laser tomography for in vivo and ex vivo diagnosis of malignant melanoma. *The Journal of Investigative Dermatology, 129*(7), 1752–1758. https://doi.org/10.1038/jid.2008.439.

Drakaki, E., Dessinioti, C., Stratigos, A. J., Salavastru, C., & Antoniou, C. (2014). Laser-induced fluorescence made simple: Implications for the diagnosis and follow-up monitoring of basal cell carcinoma. *Journal of Biomedical Opticst, 19*(3), 30901. https://doi.org/10.1117/1.JBO.19.3.030901.

Eny, K. M., Orchard, T. J., Miller, R. G., Maynard, J., Grant, D. M., Costacou, T., et al. (2015). Caffeine consumption contributes to skin intrinsic fluorescence in type 1 diabetes. *Diabetes Technology & Therapeutics, 17*(10), 726–734. https://doi.org/10.1089/dia.2015.0017.

Ericson, M. B., Simonsson, C., Guldbrand, S., Ljungblad, C., Paoli, J., & Smedh, M. (2008). Two-photon laser-scanning fluorescence microscopy applied for studies of human skin. *Journal of Biophotonics, 1*(4), 320–330. https://doi.org/10.1002/jbio.200810022.

Gào, X., & Schöttker, B. (2017). Reduction–oxidation pathways involved in cancer development: A systematic review of literature reviews. *Oncotarget, 8*(31), 51888–51906. https://doi.org/10.18632/oncotarget.17128.

Gillies, R., Zonios, G., Anderson, R. R., & Kollias, N. (2000). Fluorescence excitation spectroscopy provides information about human skin in vivo. *The Journal of Investigative Dermatology, 115*(4), 704–707. https://doi.org/10.1046/j.1523-1747.2000.00091.x.

Giovannacci, I., Vescovi, P., Manfredi, M., & Meleti, M. (2016). Non-invasive visual tools for diagnosis of oral cancer and dysplasia: A systematic review. *Medicina Oral, Patología Oral y Cirugía Bucal, 21*(3), e305–15. https://doi.org/10.4317/medoral.20996.

Grossi, C. E., & Zaccheo, D. (1963). Sulla fluorescenza delle granulazioni specifiche dei leucociti eosinofili. *Bollettino Società Italiana Biologia Sperimentale, 39*, 421–423.

Gurushankar, K., Nazeer, S. S., Jayasree, R. S., & Krishnakumar, N. (2015). Evaluation of antitumor activity of hesperetin-loaded nanoparticles against DMBA-induced oral

carcinogenesis based on tissue autofluorescence spectroscopy and multivariate analysis. *Journal of Fluorescence, 25*(4), 931–939. https://doi.org/10.1007/s10895-015-1575-4.

Hoffmann, K., Stucker, M., Altmeyer, P., Teuchner, K., & Leupold, D. (2001). Selective femtosecond pulse-excitation of melanin fluorescence in tissue. *The Journal of Investigative Dermatology, 116*, 629–630. https://doi.org/10.1046/j.1523-1747.2001.01288.x.

Inaguma, M., & Hashimoto, K. (1999). Porphyrin-like fluorescence in oral cancer: In vivo fluorescence spectral characterization of lesions by use of a near-ultraviolet excited autofluorescence diagnosis system and separation of fluorescent extracts by capillary electrophoresis. *Cancer, 86*(11), 2201–2211. https://doi.org/10.1002/(SICI)1097-0142(19991201)86:11<2201::AID-CNCR5>3.0.CO;2-9.

Jayanthi, J. L., Mallia, R. J., Shiny, S. T., Baiju, K. V., Mathews, A., Kumar, R., et al. (2009). Discriminant analysis of autofluorescence spectra for classification of oral lesions in vivo. *Lasers in Surgery and Medicine, 41*(5), 345–352. https://doi.org/10.1002/lsm.20771.

Jayanthi, J. L., Subhash, N., Stephen, M., Philip, E. K., & Beena, V. T. (2011). Comparative evaluation of the diagnostic performance of autofluorescence and diffuse reflectance in oral cancer detection: A clinical study. *Journal of Biophotonics, 4*(10), 696–706. https://doi.org/10.1002/jbio.201100037.

Koenig, K., & Riemann, I. (2003). High-resolution multiphoton tomography of human skin with subcellular spatial resolution and picosecond time resolution. *Journal of Biomedical Optics, 8*, 432–439. https://doi.org/10.1117/1.1577349.

Krasieva, T. B., Stringari, C., Liu, F., Sun, C. H., Kong, Y., Balu, M., et al. (2013). Two-photon excited fluorescence lifetime imaging and spectroscopy of melanins in vitro and in vivo. *Journal of Biomedical Opticst, 18*(3), 31107. https://doi.org/10.1117/1.JBO.18.3.031107.

Kunz, W. S. (1986). Spectral properties of fluorescent flavoproteins of isolated rat liver mitochondria. *FEBS Letters, 195*, 92–96. https://doi.org/10.1016/0014-5793(86)80137-5.

Kunz, W. S., & Kunz, W. (1985). Contribution of different enzymes to flavoproteins fluorescence of isolated rat liver mitochondria. *Biochimica et Biophysica Acta, 841*, 237–246. https://doi.org/10.1016/0304-4165(85)90064-9.

Laiho, L. H., Pelet, S., Hancewicz, T. M., Kaplan, P. D., & So, P. T. (2005). Two-photon 3-D mapping of ex vivo human skin endogenous fluorescence species based on fluorescence emission spectra. *Journal of Biomedical Opticst, 10*(2), 024016. https://doi.org/10.1117/1.1891370.

Lane, P. M., Gilhuly, T., Whitehead, P., Zeng, H., Poh, C. F., Ng, S., et al. (2006). Simple device for the direct visualization of oral-cavity tissue fluorescence. *Journal of Biomedical Opticst, 11*(2), 024006. https://doi.org/10.1117/1.2193157.

Leupold, D., Scholz, M., Stankovic, G., Reda, J., Buder, S., Eichhorn, R., et al. (2011). The stepwise two-photon excited melanin fluorescence is a unique diagnostic tool for the detection of malignant transformation in melanocytes. *Pigment Cell & Melanoma Research, 24*(3), 438–445. https://doi.org/10.1111/j.1755-148X.2011.00853.

Mallia, R. J., Thomas, S. S., Mathews, A., Kumar, R. R., Sebastian, P., Madhavan, J., et al. (2008). Laser-induced autofluorescence spectral ratio reference standard for early discrimination of oral cancer. *Cancer, 112*, 1503–1512. https://doi.org/10.1002/cncr.23324.

Mayeno, A. N., Hamann, K. J., & Gleich, G. J. (1992). Granule-associated flavin adenine dinucleotide (FAD) is responsible for eosinophil autofluorescence. *Journal of Leukocyte Biology, 51*, 172–175. https://doi.org/10.1002/jlb.51.2.172.

McMullen, R. L., Chen, S., & Moore, D. J. (2012). Spectrofluorescence of skin and hair. *International Journal of Cosmetic Science, 34*(3), 246–256. https://doi.org/10.1111/j.1468-2494.2012.00709.x.

Miyamoto, K., & Kudoh, H. (2013). Quantification and visualization of cellular NAD(P)H in young and aged female facial skin with in vivo two-photon tomography. *The British Journal of Dermatology, 169*(Suppl 2), 25–31. https://doi.org/10.1111/bjd.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. *PLoS Medicine, 6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097.

Monici, M. (2005). Cell and tissue autofluorescence research and diagnostic applications. *Biotechnology Annual Review, 11*, 227–256. https://doi.org/10.1016/S1387-2656(05)11007-2.

Na, R., Stender, I. M., Henriksen, M., & Wulf, H. C. (2011). Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *The Journal of Investigative Dermatology, 116*(4), 536–540. https://doi.org/10.1046/j.1523-1747.2001.01285.x.

Nazeer, S. S., Asish, R., Venugopal, C., Anita, B., & Gupta, A. K. (2014). Noninvasive assessment of the risk of tobacco abuse in oral mucosa using fluorescence spectroscopy: A clinical approach. *Journal of Biomedical Opticst, 19*(5), 057013. https://doi.org/10.1117/1.JBO.19.5.057013.

Patalay, R., Talbot, C., Alexandrov, Y., Munro, I., Neil, M. A., König, K., et al. (2011). Quantification of cellular autofluorescence of human skin using multiphoton tomography and fluorescence lifetime imaging in two spectral detection channels. *Biomedical Optics Express Journal, 2*(12), 3295–3308. https://doi.org/10.1364/BOE.2.003295.

Penzer, G. R. (1980). Molecular emission spectroscopy (fluorescence and phosphorescence). In S. B. Brown (Ed.). *An introduction to spectroscopy for biochemists* (pp. 70–114). London: Academic Press.

Richter, C., Trojahn, C., Dobos, G., Blume-Peytavi, U., & Kottner, J. (2016). Follicular fluorescence quantity to characterize acne severity: A validation study. *Skin Research and Technology, 22*(4), 451–459. https://doi.org/10.1111/srt.12286.

Salmon, J. M., Kohen, E., Viallet, P., Hirschberg, J. G., Wouters, A. W., & Kohen, C. (1982). Microspectrofluorometric approach to the study of free/bound NAD(P)H ratio as metabolic indicator in various cell types. *Photochemistry and Photobiology, 36*, 585–593. https://doi.org/10.1111/j.1751-1097.1982.tb04420.x.

Sandby-Møller, J., Thieden, E., Philipsen, P. A., Heydenreich, J., & Wulf, H. C. (2004). Skin autofluorescence as a biological UVR dosimeter. *Photodermatology, Photoimmunology & Photomedicine, 20*(1), 33–40. https://doi.org/10.1111/j.1600-0781.2004.00059.x.

Schantz, S. P., Savage, H. E., Sacks, P., & Alfano, R. R. (1997). Native cellular fluorescence and its application to cancer prevention. *Environmental Health Perspectives, 105*(Suppl 4), 941–944. https://doi.org/10.1289/ehp.97105s4941.

Simon, J. D., Peles, D., Wakamatsu, K., & Ito, S. (2009). Current challenges in understanding melanogenesis: Bridging chemistry, biological control, morphology, and function. *Pigment Cell & Melanoma Research, 22*(5), 563–579. https://doi.org/10.1111/j.1755-148X.2009.00610.x.

Skala, M. C., Riching, K. M., Bird, D. K., Fitzpatrick, A. G., Eickhoff, J., Eliceiri, K. W., et al. (2007). In vivo multiphoton fluorescence lifetime imaging of protein-bound and free nicotin- amide adenine dinucleotide in normal and precancerous epithelia. *Journal of Biomedical Opticst, 12*, 024014. https://doi.org/10.1073/pnas.0708425104.

Stirban, A., Nandrean, S., Negrean, M., Koschinsky, T., & Tschoepe, D. (2008). Skin autofluorescence increases postprandially in human subjects. *Diabetes Technology & Therapeutics, 10*(3), 200–205. https://doi.org/10.1089/dia.2007.0275.

Teuchner, K., Freyer, W., Leupold, D., Volkmer, A., Birch, D. J., Altmeyer, P., et al. (1999). Femtosecond two-photon excited fluo- rescence of melanin. *Photochemistry and Photobiology, 70*, 146–151. https://doi.org/10.1111/j.1751-1097.1999.tb07982.x.

Vedeswari, C. P., Jayachandran, S., & Ganesan, S. (2009). In vivo autofluorescence characteristics of pre- and post-treated oral submucous fibrosis: A pilot study. *Indian Journal of Dental Research, 20*(3), 261–267. https://doi.org/10.4103/0970-9290.57354.

Weinberger, A. W., Lappas, A., Kirschkamp, T., Mazinani, B. A., Huth, J. K., Mohammadi, B., et al. (2006). Fundus near infrared fluorescence correlates with fundus near infrared reflectance. *Investigative Ophthalmology & Visual Science, 47*(7), 3098–3108. https://doi.org/10.1167/iovs.05-1104.

Wolfbeiss, O. S. (1985). The fluorescence of organic natural products. In G. S. Schulman (Ed.). *Molecular luminescence spectrometry: Methods and applications — Part 1* (pp. 168–370). New York: Wiley.

Yaroslavsky, A. N., Neel, V., & Anderson, R. R. (2004). Fluorescence polarization imaging for delineating nonmelanoma skin cancers. *Optics Letters, 29*(17), 2010–2012. https://doi.org/10.1364/OL.29.002010.

Yu, Y., Lee, A. M., Wang, H., Tang, S., Zhao, J., Lui, H., et al. (2012). Imaging-guided two-photon excitation-emission-matrix measurements of human skin tissues. *Journal of Biomedical Opticst, 17*(7), 077004. https://doi.org/10.1117/1.JBO.17.7.077004.

Zeng, H., MacAulay, C., McLean, D., & Palcic, B. (1995). Spectroscopic and microscopic characteristics of human skin autofluo- rescence emission. *Photochemistry and Photobiology, 61*, 39–645. https://doi.org/10.1111/j.1751-1097.1995.tb09881.x.

Zhao, H. L., Chen, Y., Zhao, H. J., Tan, Z. J., Zhang, C. P., Fu, X. B., et al. (2016). Autofluorescence of eccrine sweat glands. *Skin Research and Technology, 22*(1), 98–103. https://doi.org/10.1111/srt.12234.