Thesis for the Degree of Doctor of Philosophy

Aspects on in vivo imaging techniques for diagnostics of pigmented skin lesions

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Cover:
The cover picture illustrates a melanoma, with the dermoscopic image on the left side and the SIAscopic images of dermal melanin, blood and collagen, on the right side.

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Abstract

Problem*:* Non-invasive diagnostic techniques to facilitate diagnosis of pigmented skin lesions (PSL) are being developed. Dermoscopy and SIAscopy are two such techniques, and they are evaluated in this thesis.
Aims*:* Pp I: To investigate if primary care physicians (PCPs) improve their ability to diagnose melanoma using dermoscopy after a short education intervention. Pp II: To describe relevant morphological features of pigmented basal cell carcinomas (BCCs) using dermoscopy and to create a diagnostic method based on these findings. Pp III: To evaluate if SIAscopy could be used to diagnose pigmented BCCs. Pp IV: i) To find out if SIAscopic findings topographically correlated with histopathological findings of melanoma; ii) if SIAscopy could give a topographic indication of the localisation of maximum tumour thickness, iii) provide a guide for appropriate sectioning of the specimen for histopathological evaluation.
Methods*:* Pp I: The diagnostic accuracy for melanoma and non-melanoma PSLs were tested among 74 PCPs, divided into an education intervention group and a control group. Both groups were re-tested after the education intervention. Pp II: 426 dermoscopic images of pigmented BCCs, melanomas and benign PSLs were scored for dermoscopic features. Based on the results an algorithm was derived. Pp III: 21 pigmented BCCs were analysed regarding dermoscopic and SIAscopic findings. Pp IV: 60 PSLs, i.e. 29 invasive melanomas, 13 melanoma *in situ* and 18 benign PSLs, showing positive SIAscopic findings were included. Topographic comparisons were made between SIAscopic findings and histopahology.
Results: Pp I: There was a significant improvement in sensitivity for melanoma diagnosis among PCPs who were educated in dermoscopy. Pp II: A dermoscopic algorithm for diagnosing pigmented BCCs was created. The algorithm had a sensitivity of 93% for the diagnosis of pigmented BCCs, a specificity of 89% for invasive melanoma and 92% for benign pigmented skin lesions. Pp III: The same SIAscopic features that had previously been shown to be frequent in melanomas, were seen in pigmented BCCs. Using dermoscopy 90% of the pigmented BCCs were correctly diagnosed. Pp IV: In only 11 of 29 invasive melanomas the SIAgraphs topographically matched the area of invasion on histology. A high concentration of dermal melanin was the SIAscopic signal with best correlation to melanoma invasion, although it also proved to have low specificity.
Conclusions*:* Pp I: Dermoscopy significantly improves sensitivity for melanoma when used by primary care physicians, after a short education intervention on dermoscopy. Pp II: A robust dermoscopy algorithm that allows the diagnosis of pigmented BCCs from invasive melanoma and benign pigmented skin lesions has been developed. Pp III: SIAscopy has no advantage over dermoscopy when diagnosing pigmented basal cell carcinoma, and can be misleading if the examiner has little or no knowledge of dermoscopy. Pp IV: Information regarding microscopic structure and architecture given by the SIAscope does not represent reliable diagnostic information related to the lesions internal structure, when compared to histopathology. Therefore SIAscopy cannot be used as a guide for localising the maximum tumour thickness when performing histopathological examination.

Key words: Melanoma, pigmented nevi, basal cell carcinoma, differential diagnosis, dermoscopy, spectrophotometric intracutaneous analysis, pathology

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LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. K Westerhoff, W H McCarthy and S W Menzies. Increase in the sensitivity for melanoma diagnosis by primary care physicians using skin surface microscopy. *British Journal of Dermatology 2000; 143: 1016-1020.*

II. S W Menzies, K Westerhoff, H Rabinovitz, A W Kopf, W H McCarthy and B Katz. Surface microscopy of pigmented basal cell carcinoma. *Archives of Dermatology 2000; 136: 1012-1016.*

III. K Terstappen, O Larkö and A-M Wennberg. Pigmented basal cell carcinoma – Comparing the diagnostic methods of SIAscopy and dermoscopy. *Acta Dermato-Venereologica 2007; 87: 238-242.*

IV. K Terstappen, M Suurküla, H Hallberg, M Ericson and A-M Wennberg. Poor correlation between spectrophotometric intracutaneous analysis and histopathology in melanoma and non-melanoma lesions. *Submitted for publication.*

ABBREVIATIONS

ABCD	asymmetry, border, colour, dermoscopic structures
BCC	basal cell carcinoma
DNA	deoxyribonucleic acid
IDS	International Dermoscopy Society
ISO	International organization for standardization
LED	light emitting diodes
LMM	lentigo malignant melanoma
NM	nodular melanoma
PCP	primary care physician
SIA	spectrophotometric intracutaneous analysis
SCC	squamous cell carcinoma
SSM	superficially spreading melanoma
UV	ultraviolet
UVA	ultraviolet A radiation
UVB	ultraviolet B radiation
UVC	ultraviolet C radiation
UVR	ultraviolet radiation

‘O-o-o-gh! What a…! It’s all black.’
‘It’s been black ever since I was born. I had a big birthmark here, but you can see it’s degenerated.’
‘What’s that there?’
‘They’re three fistulas that remained after each of the three times it discharged. You see, Dyoma, my tumour’s quite different from yours. Mine’s a melanoblastoma, a real merciless bastard. As a rule, it’s eight months and you’ve had it.’
‘How do you know all this?’
‘I read a book about it before I came. It was only after I read it that I faced up to what I’d got. But the point is that even if I’d come earlier they still wouldn’t have been able to operate. A melanoblastoma is such a swine, you only have to touch it with a knife and it produces secondaries. You see, it wants to live too, in its way. Then, because I waited those months, this suddenly appeared in my groin.’…
…‘No, Dyoma, it’s far too late to cure me. Nobody’s cured of a melanomblastoma. There just aren’t any instances of recovery. In my case, cutting of a leg wouldn’t be enough, and where could they cut higher up…’

From *Cancer Ward* by A. Solzhenitsyn, 1968

INTRODUCTION

Skin cancer incidence is rising in the Western World and subsequently the number of patients seeking dermatologic consultation is steadily increasing. Today patients with skin tumours, benign or malignant, represent 40-50% of the total patient amount in Dermatology departments in Sweden.

The incidence of melanoma is increasing among Caucasians and in Sweden the rate has quadrupled from 1970 to 2006 (The Swedish Cancer Registry, The National Board of Health and Welfare, Sweden). Early detection of melanoma is critical for patient survival prognosis, which for patients with advanced melanoma remains poor. It has been shown that the 10 years survival probability for patients with early thin melanomas, i.e. thickness less than 0.75 mm, is as high as 97% compared with only 32% for patients with thick melanomas, i.e. thickness more than 4.0 mm [1]. Melanomas are responsible for 90% of the mortality due to skin cancer in Sweden (Cause of Death Statistics 2005, The National Board of Health and Welfare, Sweden).

Facts on melanoma

- Invasive melanomas constitute 4.2% of all malignant tumours registered in Sweden.
- It is the 7th most common malignancy among men and the 6th most common malignancy among women in Sweden.
- The age-adjusted incidence rate in 2005 was 25.5/100 000 for men and 21.3/100 000 for women in Sweden.
- The median age for melanoma diagnosis in Sweden is 63 years of age for men and 58 years of age for women.

Data from: Cancer Incidence in Sweden 2005. National Board of Health and Welfare, Stockholm, 2007 and National guidelines for melanoma 2007, Sweden

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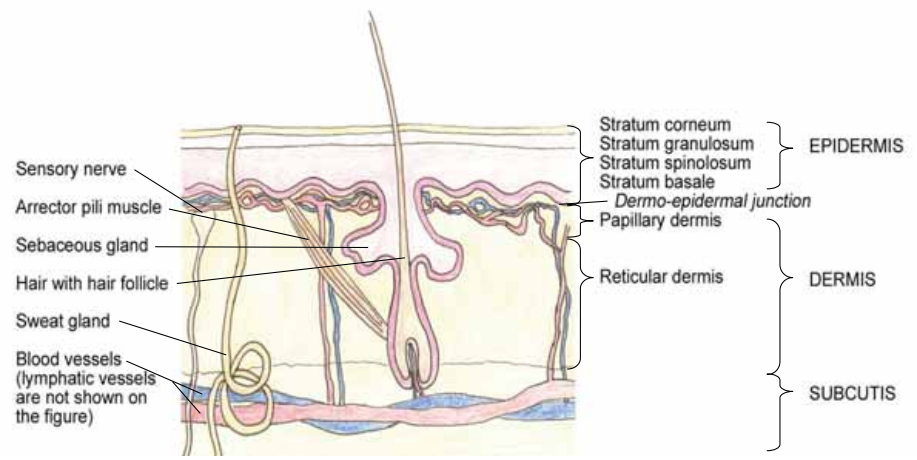
THE HUMAN SKIN

The human skin consists of three layers; the epidermis, the dermis and the subcutis (fig. 1). The epidermis is divided into four morphological layers; the outermost layer being stratum corneum and then follows stratum granulosum, stratum spinosum and stratum basale. The epidermis mainly consists of keratinocytes. Other cells that can be found in the epidermis are melanocytes, Langerhans cells and Merkel cells. Below the epidermis is the dermis which largely consists of connective tissue. In this layer, contrary to the epidermis, blood- and lymphatic vessels are present. Also the dermis is devided into morphological layers (the papillary dermis, which is a thin superficial layer underneath the epidermis, and the reticular dermis). In between the epidermis and the dermis the dermo-epidermal junction is situated. The subcutis is a layer consisting mainly of loose connective tissue and fat cells.

Figure 1. Schematic structure of the skin



Melanocytes

Melanocytes are the pigment producing cells in the skin. They are evenly distributed in the skin along the basal layer at the dermo-epidermal junction. Melanocytes produce melanosomes, which are melanin-laden organelles. The melanosomes can be transferred to the surrounding keratinocytes. The melanin is the major pigment factor in the skin and ethnic differences in skin colour are due to variation in number, size and distribution of melanosomes. If stimulated by UV-radiation, or hormones, the pigmentation increases.

PIGMENTED SKIN LESIONS

The purpose of this chapter is not to give a complete overview of all types of pigmented skin lesions, but to provide a short presentation of the most common lesions that are of importance for understanding the following chapters in this thesis.

Skin lesions having melanocytic origin

Benign melanocytic lesions

Freckles and lentigo

A number of benign pigmented lesions can arise in the skin. In freckles there is a temporary overproduction of melanin in skin due to exposure to UV-radiation, while in lentigo there are an increased number of melanocytes in the dermo-epidermal junction.

Melanocytic nevi

Nevi are lesions which are the result of proliferation of melanocytes at the dermo-epidermal junction. These clusters of melanocytes can either stay at this position or migrate into the dermis. Nevi can be either congenital or acquired. There are variants of nevi with different growth patterns which renders them different clinical appearance and different names; junctional nevi, compound nevi, dermal nevi (also Unna´s nevi or Miescher´s nevi), Spitz nevi, Reed nevi, blue nevi and more. The majority are totally benign with no, or very limited, malignant potential. Although, it

has been shown that multiple common nevi is a strong risk factor for melanoma [2, 3].

Atypical or dysplastic nevi

A subgroup of nevi have, on histological examination, architectural and cytological atypia, and they are called atypical or dysplastic nevi (also Clark nevi). Clinical characteristics of atypical nevi are several; they tend to be larger in size (> 5 mm in diameter) but small nevi can also be atypical. They are often asymmetrical and have an ill-defined border. Colour variation often occurs with different shades of brown and pink in an irregular fashion. The clinical importance of atypical nevi lies in their association with increased melanoma risk [4-9]. An individual with multiple atypical nevi and a family history of multiple atypical nevi have an increased risk of developing melanoma. This risk increases substantially if there is also a personal or family history of melanoma.

Malignant melanocytic lesions

Melanoma

The most malignant cutaneous skin tumour, and subsequently the most important tumour to identify for the dermatologist, is melanoma. Melanomas arise from malignant melanocytes. They can have their origin in melanocytes in a nevus or from melanocytes in normal skin, arising *de novo.* As long as the malignant clone is only growing in the epidermis the lesion is called a melanoma *in situ.* When the malignant melanocytes invade the dermis they have metastatic potential and the lesion has become an invasive melanoma. The level of invasion in the dermis, through to the subcutaneous fat, is measured during histopathologic examination of the tumour after excision. Two measurements are made; the invasion depth according to Breslow is the thickness, in mm, from the stratum granulosum in the epidermis to the deepest invasive melanoma cell [10]. The Clark measurement are invasion levels numbered I-V, where level I represents intraepidermal growth, i.e. *in situ*, level II a few cells in the papillary dermis, level III occupation and expansion of the papillary dermis, level IV invasion of the reticular dermis and level V invasion into subcutaneous fat [11, 12]. Melanomas are divided into subsets due to clinical and pathological features;

superficially spreading melanoma (SSM), nodular melanoma (NM), lentigo malignant melanoma (LMM) and acral lentigious melanoma.

Skin lesions having non-melanocytic origin

An abundance of skin lesions; benign, premalignant and malignant, can arise from the epidermis and the skin appendages (hair follicles, sweat glands, sebaceous glands). Here only those which are more common malignancies or which are benign but are important differential diagnoses to melanoma are presented.

Benign non-melanocytic lesions

Seborrhoeic keratosis

Seborrhoeic keratosis is a benign, often pigmented, tumour composed of epidermal keratinocytes. They are very common, especially among the elderly. They can be flat but are more commonly verrucous in their appearance.

Malignant non-melanocytic lesions

Basal cell carcinoma

Basal cell carcinomas (BCC) are the most common skin malignancy among Caucasians. They are thought to originate from immature cells of the epidermis [13]. Basal cell carcinomas rarely metastasise but if left untreated they can grow in an invasive manner and cause great tissue damage and be surgically difficult to remove. A proportion of BCCs contain pigment. In the largest histological series studied the incidence of pigmented BCC ranges from 6.7% to 8.5%, but a racial predilection probably exists [14, 15].

Squamous cell carcinoma

Squamous cell carcinomas (SCC) arise from the keratinocytes of the epidermis. SCCs begin when the atypical keratinocytes grow through the basement membrane and invade the dermis. When growing only in the epidermis they are considered precancerous, and this condition is called actinic keratosis. More advanced changes, with full epidermal thickness dysplasia, but no dermal invasion is called squamous

cell carcinoma *in situ,* or Bowen´s disease. Once an invasive SCC has developed it has metastatic potential and can be fatal. Within the English literature pigmented squamous cell carcinomas is rarely reported, with an incidence that ranges from 0.01% to 7% of all squamous cell carcinomas [16, 17].

DIAGNOSING SKIN CANCER

It is of great importance that malignant skin tumours are diagnosed at an early stage, especially melanoma. The differential diagnosis between malignant and benign skin tumour can sometimes be difficult, particularly if they are pigmented. Specialized pigmented lesion clinics are held at many Dermatology departments since dysplastic nevi have been identified as a strong risk factor for melanoma [4-9]. Because atypical/dysplastic nevi present colour variation, asymmetry and border irregularity they are often difficult to differentiate from early melanomas. Another group of lesions, that can be clinically difficult to diagnose as melanoma, are small lesions. In Sweden 22% of melanomas are less than 5mm in diameter at the time for detection [18]. Therefore there is a need for techniques which allows for non-invasive diagnostics of tumours, to facilitate early diagnosis. Since many of these techniques are dependent on the optical properties of the skin, a section about this subject precede the presentation of different non-invasive diagnostic techniques.

Optical properties of the skin

The optical pathways in the skin are described by Anderson & Parrish [19]. A schematic figure of the optics of normal skin can be seen in figure 2. See also figure 3 for explanation of terminology. Due to the change in refractive index, when light travels from air to stratum corneum of the skin, a portion of the light is reflected. Because the surface of the stratum corneum is not smooth and planar, the reflectance from the skin is not specular, i.e. does not maintain an image. The incoming light that is not reflected may be absorbed or scattered. This can occur in any layer of the skin. Scattering is the result of inhomogeneities in a medium's refractive index, and depend on the wavelength. Scattering is low in the epidermis, but of major importance in the dermis, especially at shorter wavelengths (UV). Longer wavelengths, in the visible-near infrared spectrum, penetrate the skin to a greater extent than do shorter wavelengths. This is called the optical window of tissue.

Absorption is the major optical barrier in the stratum corneum and epidermis[19]. Absorption is dependent of the chromophores present. For wavelengths below 300 nm, aromatic amino acids, nucleic acids, urocanic acid and melanin are the major epidermal absorbers. In the range 350-1200 nm, melanin is the major absorber, especially at shorter wavelengths. Absorption in the dermis is mainly due to blood-borne pigments; haemoglobin, beta-carotene and bilirubin [19, 20].

Fig. 2. Schematic diagram of optical pathways in the skin, redrawn from Anderson & Parrish [19]

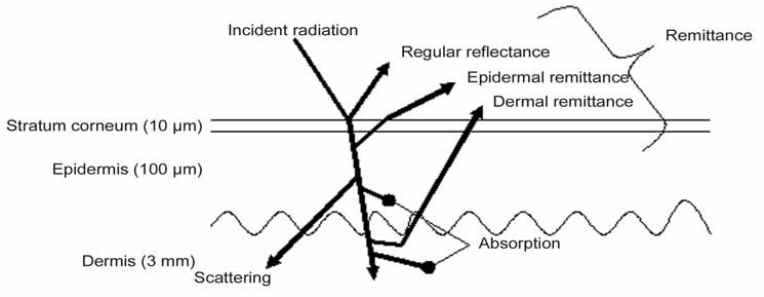
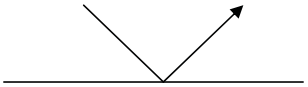
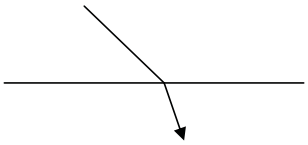


Fig. 3.

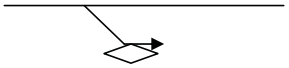
- ***Reflection:*** the light ”bounces” off the skin surface due to changes in refractive index



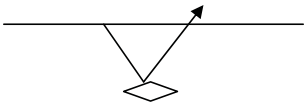
- ***Refraction:*** the light deviates due to changes in refractive index



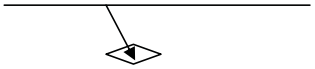
- ***Scattering:*** the light is reflected due to changes in refractive index



- ***Remittance:*** the light is returned back to the surface due to scattering events or reflection



- ***Absorption****:* various chromophores present in the skin absorb light



Interactions between radiation and skin

In this context, in a thesis about diagnosing skin cancers, it is important to also mention the effects of light, especially UV radiation, on the skin. UV radiation (UVR) is divided into three regions: UVC, 100-280nm; UVB; 280-315nm; and UVA, 315-400nm (ISO 21348 Process for Determining Solar Irradiances). In natural sunlight, UVC is absorbed by the earth's ozone layer, UVB is partly absorbed by the ozone layer and UVA not absorbed at all. UVR is electromagnetic radiation in the form of high energy photons. When these photons are absorbed by biological molecules the molecules enters an excited state. For the molecule to get back to the ground state it has to lose the energy, and can do so by, for example, losing energy as heat, emit fluorescence or form photoproducts. In the skin the chromophores discussed earlier are the UVR absorbing molecules. Melanin absorbs UVR and transform this energy into heat [21]. It also scavengers hydroxyl radicals or molecular oxygen and protects the DNA from being damaged by these free radicals. Still, UVR, especially UVB, may induce DNA damage by inducing pyrimidine dimers, and incorrect repair of these products may lead to mutations. Other UVR induced photoproducts can also lead to DNA damage and produce mutations. When these mutations affect the function of oncogenes, tumour-suppressive genes or genes involved in DNA repair mechanisms this can eventually lead to the development of cancer.

Dermoscopy *(dermatoscopy, skin surface microscopy, in vivo cutaneous surface microscopy, epiluminescence microscopy, magnified oil immersion microscopy)*
Skin surface microscopy has been used by dermatologist for centuries to facilitate diagnosis of different skin conditions. The instruments used have been colposcopes, mono- and binocular microscopes [22, 23]. In the 1950s Goldman was the first to publish findings when using skin surface microscopy to study pigmented skin lesions [24].

Several magnification instruments are available with magnification ranging from x6 to x100. Most commonly used are hand held devices, called dermatoscopes, which have x10 magnification. They were introduced on the market in the early 1990s. The light source in these are non-polarized. In the last few years dermatoscopes with LED light with polarization has been introduced.

To prevent surface reflection, immersion liquids, usually mineral oils or alcohol, and direct contact between the dermatoscope and the skin is needed when using dermatoscopes with non-polarized light. In this way the change in refractive index normally occurring between air and skin is minimised, eliminating surface scattering. In more recent instruments using polarized light, immersion liquid is no longer necessary, and some of these instruments do not need direct skin contact. Here the incident light is polarized and a polarizer (filter put in front of the lens) blocks the reflected light, allowing only the light that has been scattered within the skin to be viewed for better visualization of pigmentation [25]. Dermoscopy allows the epidermis to become translucent, and therefore permits a detailed examination of the pigmented structures of the epidermis, dermo-epidermal junction and, to a lesser extent also the dermis. The result is the visualisation of a multitude of morphological features, not visible with the naked eye. Figure 4 show dermoscopic images of melanomas and figure 5 show dermoscopic images of pigmented BCCs, with significant features marked in the images.

Non-polarized versus polarized light and contact versus non-contact dermocopy gives somewhat different appearance of the examined lesions in regards to colour and visualization of vessels. In a study by Benvenuto-Andrade et al. they report

excellent agreement for most dermoscopic colours, with the exception of blue-white veil and pink (red) colour when comparing non-polarized and polarized light [26]. They also conclude that most dermoscopic structures had fair to perfect agreement, with the exception of milialike cysts and comedolike openings, which seem to be better visualized with non-polarized light [26]. Polarized light improves visualizing of red areas and vessels, especially the latter with non-contact dermoscopy [26].

All research done on dermoscopy, up until the last years, has been done using non-polarized contact dermoscopy, and all textbooks and atlases on the subject are based on this modality. From the late 1980s and forward multiple articles have been published on contact dermoscopy and pigmented skin lesions. Most of the early work from the late 1980s and 1990s are regarding diagnosing melanoma. Different diagnostic algorithms for melanoma have been presented by several research groups [27-32]. They all present results on sensitivity and specificity that is better than when diagnosing with the naked eye. A review of previous publications to compare the accuracy of melanoma diagnosis with and without dermoscopy was published in 2002 by Kittler et al [33]. They identified 27 studies, published between 1987 and 2000, eligible for meta-analysis. In conclusion they found that the diagnostic accuracy for melanoma was significantly higher with dermoscopy than without this technique. The diagnostic accuracy significantly depended on the degree of experience of the examiner. Almost all of the studies (26 of 27) on accuracy of melanoma diagnosis included in the meta-analysis were carried out among dermatologists. The only study among non-dermatologists is the study that is presented in this thesis as paper I, which will be discussed in detail further on.

In regards to diagnosis of non-melanoma skin cancer and non-malignant pigmented skin lesions using dermoscopy several studies have been published in resent years, showing that dermoscopy enhance the clinical diagnosis of nearly all pigmented skin lesions [28, 32, 34-44]. In this thesis, as paper II, a simple diagnostic algorithm to diagnose pigmented basal cell carcinoma is presented, which was the result of a study done in collaboration with Scott Menzies and co-authors.

A consensus net-meeting on dermoscopy was held 2000, over the internet, to refine the dermoscopic terminology, and to investigate the interobserver and intraobserver

reproducibility and validity of the various dermoscopic criteria and diagnostic algorithms [45, 46]. A two step procedure to differentiate melanocytic from non-melanocytic lesions in the first step and then, in the second step, differentiate melanoma from benign melanocytic lesions, were validated [45, 46]. This two step procedure is presented in table 1 and figure 6. The four diagnostic algorithms for diagnosing melanoma, which have gained the largest interest among demoscopy users, are presented in table 2-5 [27-30, 32, 45-47]. Pattern analysis was the first dermoscopic method presented for diagnostics of pigmented skin lesions [27]. This method has further been modified and refined through work by the International Dermoscopy Sociaty (IDS) and the modified version is presented in table 2. Pattern analysis based diagnosis demands a critical assessment of the dermoscopic features seen in a pigmented skin lesion. Semi quantitative/quantitative dermoscopic algorithms have been presented as more simplified diagnostic methods for differential diagnosis between malignant and benign melanocytic lesions. The three algorithms with largest impact; ABCD rule, Menzies method and the 7-point checklist, are presented in table 3-5 [28-30]. Pattern analysis, ABCD rule, Menzies method and the 7-point checklist were all evaluated in the consensus net meeting, showing similar results on sensitivity for melanoma, but specificity differed slightly in favour of pattern analysis [45].

Digital dermoscopy offers a possibility to monitor melanocytic lesions over time. Several digital systems are available on the market. The advantage of monitoring lesions on a long term basis is that unnecessary excisions can be minimized without the risk of overlooking an early or featureless melanoma [48]. Digital dermoscopy thus represent a valuable tool in screening patients with multiple atypical melanocytic nevi, and it has been used in pigmented lesions clinics over the last decade. Digital dermoscopy also enables teledermoscopy, where images of lesions can be evaluated by remote consultants, offering the possibility of second expert opinion, if necessary [49-51].

Table 1. The dermoscopic features for the first and second step in the 2-step diagnostic procedure as presented in fig 6. (For description of globular pattern and cobblestone pattern; patterns used in the first step to identify melanocytic lesions, see table 2)

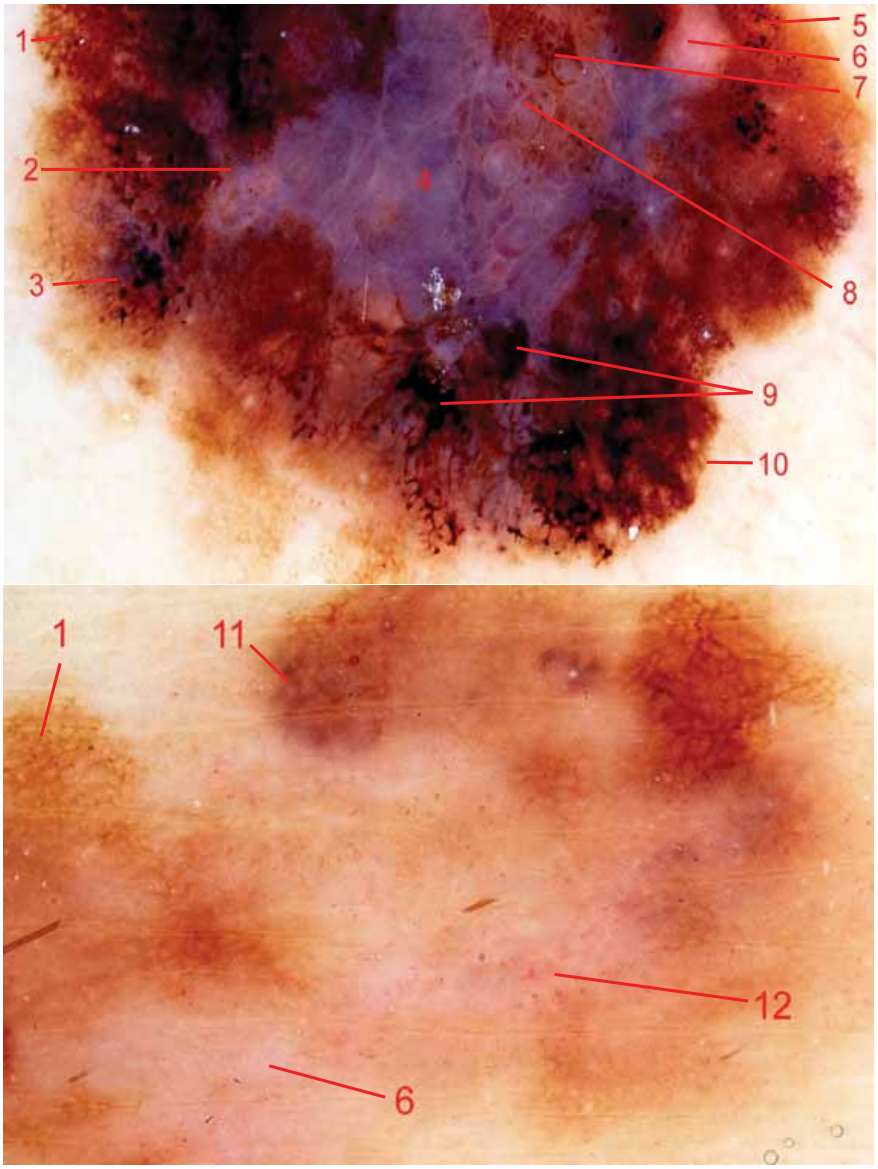
Dermoscopic feature	Definition	Histopathological correlate (if described)	Lesions
Pigment network (fig 4, feature 1)	Brown lines which build up net-like structure with light "holes" in between the grids.	Melanocytes and keratinocytes containing melanin at the basal layer. The vertical projection of these cells produce areas of dense pigment at the rete ridges (=grids/lines) and lighter colour at the dermal papilla (=holes) [52-54]	Melanocytic lesions (freckles, lentigo, junctional and compound nevi, melanoma)
Pseudonetwork	Network-like pattern in the face due to epidermal and/or dermal pigmentation (grids/lines) around closely situated follicular openings (holes).		Melanocytic lesions in the face (nevi, lentigo, lentigo maligna, lentigo maligna melanoma) Non-melanocytic lesions in the face, for example, seborrhoeic keratosis [55].
Parallel pattern	Pigmentation in lesions on palms/soles and mucosa following surface sulci or cristae.	Benign nevi: Nests of nevus cells, mainly located in the crista profunda limitans; epidermal rete ridges underlying the sulci superficiales [56]. Melanoma: Increased number of atypical melanocytes arranged as solitary units in the crista profunda intermedia; epidermal rete ridges underlying the cristae superficiales [57]	Melanocytic lesions (acral nevi and melanoma)
Homogeneous blue pigmentation	Homogeneous structureless blue pigmentation throughout the entire lesion	Heavily pigmented melanocytes with long dendritic processes located among thick collagen bundles in mid/lower dermis [58]	Blue nevi
Globules, brown to black (fig 4, feature 3 and 7)	Accumulation of pigmentation in round or oval structures. They can be brown to black in colour, regular or irregular in size and distribution.	Brown globules represent pigmented nests (melanocytes) in the lower epidermis or papillary dermis [54, 59]. Black globules correlate with melanin in the stratum corneum (as cluster of melanocytes or as "free" melanin) [54]	Melanocytic lesions (benign or dysplastic nevi, melanoma) -in benign nevi usually regular in size and distribution, brown -in melanoma irregular in size and distribution, often black
Dots, brown to black (fig 4, feature 2 and 5)	See globules, smaller in size.	Brown dots: Melanocytes/neoplastic cells arranged predominantly at the dermo-epidermal junction and in the epidermis [60]. Black dots: see black globules	See globules

Streaks (=radial streaming and pseudopods) (fig 4, feature 10)	Linear structures (=radial streaming), sometimes with a bulb at the distal end (=pseudopodes), brown to black, in the periphery of a lesion	Melanoma: Neoplastic cells, confined to the epidermis, distributed in confluent nests accentuated toward the periphery of the lesion [60]. Spitz/Reed nevus: Presumably spindle and/or epitheloid melanocytes closely packed along the dermoepidermal junction [61]	If regularly distributed around the edge of the lesion (starburst pattern); Spitz/Reed nevus If irregularly distributed around the edge of a lesion; melanoma
Blotches (fig 4, feature 9)	Diffuse area of pigmentation, dark brown to grey-black, without any other dermoscopic features.	Melanin at all levels of the epidermis and/or large area of the dermis containing melanin [54]	If regular; usually benign melanocytic nevi If irregular; high risk for melanoma
Blue-white veil (fig 4, feature 4)	Irregular, structureless area of confluent blue pigmentation with an overlying "ground-glass" film.	Compact orthokeratosis [54] with more or less pronounced hypergranulosis [59] usually overlying a large melanin-containing area in the dermis.	Melanoma Spitz/Reed nevi
Hypopigmentation	Areas of decreased pigmentation within a pigmented lesion	Hypopigmented epidermis with or without poorly developed rete ridges [54]	Dysplastic nevi, melanoma
Regression areas (fig 4, feature 6 and 11)	1.White, sometimes scar-like, areas 2. Blue-grey areas 3. Blue-grey dots	1. Thickened papillary dermis with fibrosis [60] 2. Melanosis with a variable amount of melanophages within a thickened fibrotic papillary dermis [60] 3. Melanophages, surrounding the superficial vascular plexus [60].	Melanoma Blue-grey dots can also be seen in regressing nevi and regressing seborrhoeic keratosis
Milia-like cysts	White-yellow round structures	Intraepidermal horn globules underneath the surface [59]	Seborrhoeic keratosis Less frequently in papillomatous dermal nevi and very rarely in melanoma.
Commedo-like openings	Brown-black oval/irregular structures, sharply circumscribed	Intraepidermal horn globules reaching the surface [59]	Seborrhoeic keratosis Less frequently in papillomatous dermal nevi.
Light brown fingerprint-like structures	Delicate light brown linear pattern		Seborrhoeic keratosis
Cerebriform pattern (brain-like appearance)	Brown furrows and ridges in a pattern resembling the gyri and sulci at the cerebral surface		Seborrhoeic keratosis
Maple leaf-like structures (fig 5, feature 1)	Brown to grey-blue discrete bulbous extensions forming a maple leaf-like pattern	Pigmented epithelial nodules of BCC in the papillary dermis [59]. Pigment also in the tumour stroma [62]	Pigmented basal cell carcinoma

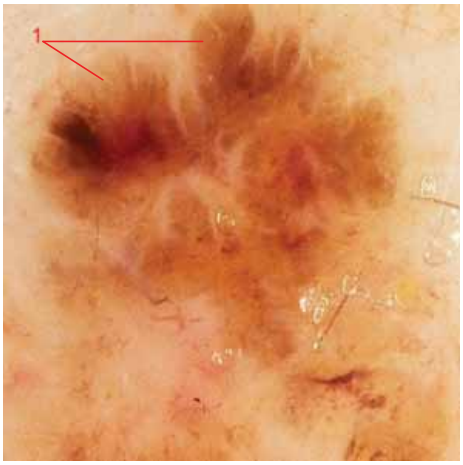
Spoke-wheel areas (fig 5, feature 4)	Well-circumscribed radial projections, usually tan but sometimes blue or grey, meeting at an often darker (dark brown, black or blue) central axis.	Pigmented basal cell carcinoma
Large blue-grey ovoid nests (fig 5, feature 2)	Well-circumscribed, confluent or near confluent pigmented ovoid or elongated areas, larger than globules, and not intimately connected to a tumour body	Pigmented basal cell carcinoma
Multiple blue-grey globules	Multiple grey-blue round to oval structures, should be differentiated from grey-blue dots	Pigmented basal cell carcinoma
Ulceration	Absence of the epidermis, often with congealed blood	Basal cell carcinoma Melanoma
Blood vessels [63, 64]	1. Comma vessels 2. Wreath vessels 3. Arborizing vessels (fig 5, feature 3) 4. Hairpin vessels 5. Dotted vessels 6. Linear irregular vessels (fig 4, feature 8) 7. Vessels within regression structures (fig 4, feature 12)	1. melanocytic nevi, rarely melanoma 2. sebaceous hyperplasias 3. commonly in basal cell carcinomas, rarely in melanomas, nevi and seborrhoeic keratosis 4. commonly in melanomas and seborrhoeic keratoses, sometimes also in basal cell carcinomas, keratoacanthomas and in melanocytic nevi 5. melanocytic tumours. Sometimes also seen in seborrhoeic keratoses, rarely in basal cell carcinomas 6. Relatively common in melanomas, especially when thicker than 0.75 mm 7. Regressive melanomas

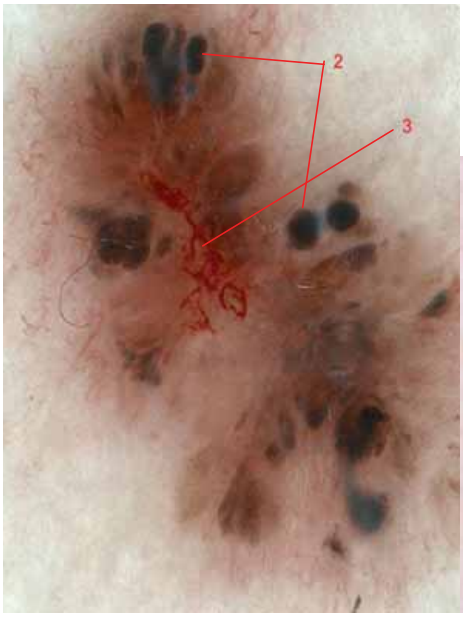
Red-blue lacunas	Round or oval red to red-blue, sometimes red-black, areas	Dilated vascular structures in the upper dermis [59]	Hemangiomas Angiokeratomas
Red-blue to red-black homogeneous area	Sharply circumscribed homogeneous red-blue to red-black maculae	Subcorneal aggregation of red blood cells	Subcorneal hemorrhage

Fig. 4 a-b. Two melanomas illustrating some of the most important dermoscopic features in melanoma lesions. Some of the features can also be present in benign nevi.



1. atypical network
2. dots, brown
3. globules, black
4. blue-white veil
5. dots, black
6. regression area, white
7. globules, brown
8. linear irregular vessel
9. blotch
10. streaks
11. regression area, blue
12. blood vessels within regression area





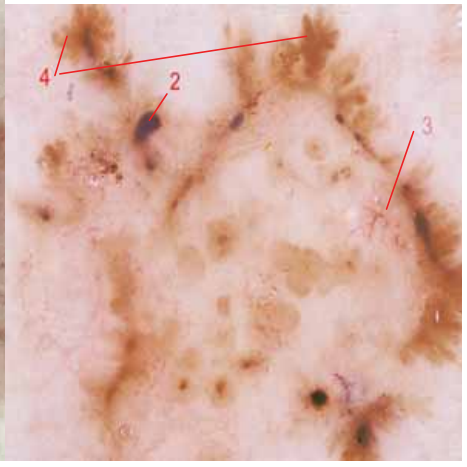


Fig. 5 a-c. Some features of pigmented basal cell carcinoma illustrated.

1. maple leaf-like pattern
2. large grey-blue ovoid nests
3. arborizing telangiectasia
4. spoke wheel

Fig. 6. Schematic illustration of the two-step algorithm for differentiation between melanocytic and pigmented non-melanocytic lesions (first step), and between melanoma and benign melanocytic lesion (second step) [46]. In the first step more features could be included for non-melanocytic lesions, as the examiner gets more experienced in dermoscopy [65-67].

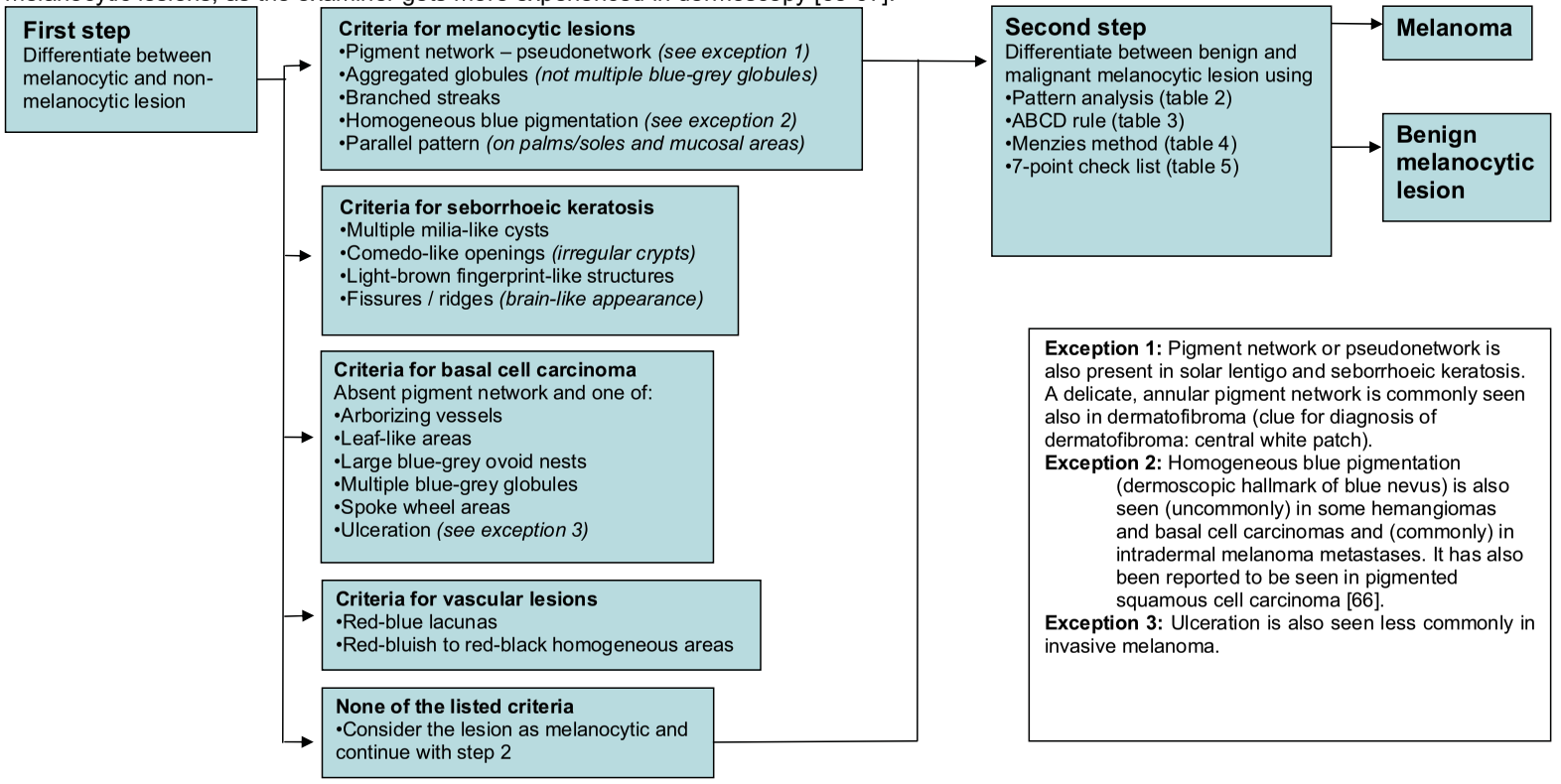


Table 2. Modified pattern analysis criteria for the dermoscopic differentiation between benign melanocytic lesions and melanoma as presented by Malvehy et al. on behalf of the International Dermoscopy Society (IDS) Board members [47], with addition of site-related features [46]. This modified pattern analysis is based on previous work by Pehamberger et al and the IDS [27, 32, 45, 46].

DERMOSCOPIC FEATURE	DEFINITION	DIAGNOSTIC SIGNIFICANCE
Global pattern		
Reticular pattern	Pigment network covering most parts of the lesion	Melanocytic lesion
Globular pattern	Numerous, variously sized, round to oval structures with various shades of brown and grey-black	Melanocytic lesion
Cobblestone pattern	Large, closely aggregated, somewhat angulated globule-like structures resembling a cobblestone	Dermal nevus
Homogeneous pattern	Diffuse, brown, grey-blue to grey-black pigmentation in the absence of other distinctive local features	Melanocytic (blue) nevus
Starburst pattern	Pigmented streaks in a radial arrangement at the edge of the lesion	Spitz/Reed nevus
Parallel pattern	Pigmentation on palms/soles that follow the sulci or the cristae superficiales, occasionally arranged at the right angles to these structures	Acral nevus/melanoma
Multicomponent pattern	Combination of three or more above patterns	Melanoma
Nonspecific pattern	Pigmented lesions lacking above patterns	Possible melanoma

Local features		
Pigment network	Typical pigment network: light- to dark-brown network with small, uniformly spaced network holes and thin network lines distributed more or less regularly throughout the lesion and usually thinning out at the periphery.	Benign melanocytic lesion
	Atypical pigment network: black, brown or grey network with irregular holes and thick lines	Melanoma
Dots/globules	Black, brown, round to oval, variously sized structures regularly or irregularly distributed within the lesion	If regular; benign melanocytic lesion If irregular; melanoma
Streaks	These have previously been described separately as pseudopods and radial streaming. Streaks are bulbous and often kinked or finger-like projections seen at the edge of a lesion. They may arise from network structures but more commonly do not. They range in colour from tan to black.	If regular; benign melanocytic lesion (Spitz/Reed nevus) If irregular; melanoma
Blue-white veil	Irregular, structureless area of confluent blue pigmentation with an overlying “ground-glass” film. The pigmentation cannot occupy the entire lesion and usually corresponds to a clinically elevated part of the lesion.	Melanoma
Regression structures	White scar-like depigmentation and/or blue pepper-like granules usually corresponding to a clinically flat part of the lesion	Melanoma
Hypopigmentation	Areas with less pigmentation than the overall pigmentation of the lesion	Nonspecific
Blotches	Black, brown, and/or grey structureless areas with symmetrical or asymmetric distribution within the lesion.	If symmetrical; benign melanocytic lesion If asymmetrical; melanoma

Vascular structures	Comma-like vessels	Dermal nevus
	Hair-pin vessels	If uniformly distributed; seborrheic keratosis. If irregularly distributed; melanoma
	Dotted vessels	Melanoma
	Linear irregular vessels	Melanoma
	Vessels and/or erythema within regression structures	Melanoma
Site-related features		
Face	Typical pseudonetwork (round, equally sized network holes corresponding to the pre-existing follicular ostia)	Benign melanocytic lesion
	Annular-granular structures (multiple blue-grey dots surrounding the follicular ostia with an annular-granular appearance)	Melanoma
	Grey pseudonetwork (grey pigmentation surrounding the follicular ostia, formed by the confluence of annular-granular structures)	Melanoma
	Rhomboidal structures (grey-brown pigmentation surrounding the follicular ostia with a rhomboidal appearance)	Melanoma
	Asymmetric pigmented follicles (eccrine annular pigmentation around follicular ostia)	Melanoma
Palms/soles	Parallel-furrow pattern (pigmentation following the sulci superficiales)	Acral nevus
	Lattice-like pattern (pigmentation following and crossing the sulci superficiales)	Acral nevus
	Fibrillar pattern (numerous, finely pigmented filaments perpendicular to the sulci et cristae superficiales)	Acral nevus
	Parallel-ridge pattern (pigmentation aligned along the cristae superficiales)	Melanoma

Table 3. ABCD rule for the dermoscopic differentiation between benign melanocytic lesions and melanoma [28].

Formula for calculating total score: [(A score x 1.3)+(B score x 0.1) +(C score x 0.5)+(D score x 0.5)]. Interpretation of total score: <4.75, benign melanocytic lesion; 4.75-5.45, suspicious lesion (close follow-up or excision recommended); >5.45, lesion highly suspicious for melanoma.

DERMOSCOPIC FEATURE	DEFINITION	SCORE	WEIGHT FACTOR
Asymmetry	In 0, 1, or 2 perpendicular axes; assess not only contour, but also colours and structures	0-2	X1.3
Border	Abrupt ending of pigment pattern at the periphery in 0-8 segments	0-8	X0.1
Colour	Presence of up to six colours (white, red, light-brown, dark-brown, blue-grey, black)	1-6	X0.5
Dermoscopic structures	Presence of network, structureless (homogeneous) areas, branched streaks, dots, and globules	1-5	X0.5

Table 4. Menzies method for the dermoscopic differentiation between benign melanocytic lesions and melanoma [29]. For melanoma to be diagnosed a lesion must have neither of both negative features and 1 or more of the 9 positive features.

DERMOSCOPIC FEATURE	DEFINITION
Negative features	
Symmetry of pattern	Symmetry of pattern is required across all axes through the lesion's center of gravity (center of the lesion). Symmetry of pattern does not require shape symmetry.
Presence of a single colour	The colours scored are black, grey, blue, dark brown, tan and red. White is not scored as a colour.
Positive features	
Blue-white veil	An area of irregular, structureless confluent blue pigmentation with an overlying white "ground-glass" haze. It cannot occupy the entire lesion and cannot be associated with red-blue lacunas.
Multiple brown dots	Focal areas of multiple brown (usually dark brown) dots (not globules).
Pseudopods	Bulbous and often kinked projections that are found at the edge of a lesion either directly connected to the tumour body or pigmented network. They can never be seen distributed regularly or symmetrically around the lesion. When connected directly to the tumour body, they must have an acute angle to the tumour edge or arise from linear or curvilinear extensions. When connected to the network, the width of the bulbous ending must be greater than the width of any part of the surrounding network and at least double that of its directly connected network projection.
Radial steaming	Finger-like extensions at the edge of a lesion which are never distributed regularly or symmetrically around the lesion.
Scar-like depigmentation	Areas of white distinct irregular extensions (true scarring), which should not be confused with hypo- or depigmentation due to simple loss of melanin.
Peripheral black dots/globules	Black dots/globules found at or near the edge of the lesion.
Multiple (5-6) colours	The colours scored are black, grey, blue, dark brown, tan and red. White is not scored as a colour.

Multiple blue/grey dots	Foci of multiple blue or grey dots (not globules) often described as “pepper-like” granules in pattern
Broadened network	A network made up of irregular thicker “cords” of the net, often seen focally thicker

Table 5. 7-point checklist for the dermoscopic differentiation between benign melanocytic lesions and melanoma [30]. The individual scores are added. A minimum total score of 3 is required for the diagnosis of melanoma, whereas a total score of less than 3 is indicative of non-melanoma.

DERMOSCOPIC FEATURE	DEFINITION	SCORE
Atypical pigment network	Black, brown or grey network with irregular holes and thick lines	2
Blue-whitish veil	Irregular, structureless area of confluent blue pigmentation with an overlying white “ground-glass” film. The pigmentation cannot occupy the entire lesion and usually corresponds to a clinically elevated part of the lesion	2
Atypical vascular pattern	Linear-irregular or dotted vessels not clearly combined with regression structures	2
Irregular streaks	Brown to black, bulbous or finger-like projections irregularly distributed at the edge of a lesion. They may arise from network structures but more commonly do not.	1
Irregular dots/globules	Black, brown, round to oval, variously sized structures irregularly distributed within the lesion	1
Irregular blotches	Black, brown, and/or grey structureless areas asymmetrically distributed within the lesion	1
Regression structures	White scar-like depigmentation and/or blue pepper-like granules usually corresponding to a clinically flat part of the lesion	1

Spectrophotometric intracutanous analysis

Spectrophotometric intracutaneous analysis (SIA) is a technique based on an optical image formation model of human skin first presented by S Cotton in 1998 [68]. The model is based on mathematical theories (Kubelka-Munk theory, Bouger´s law) on how light interacts with the skin as it is transmitted through the different layers of the skin [68]. The different interaction processes are remittance, scattering and absorption, as schematically presented in figure 3. The model was implemented to analyse colouration of human skin using spectrophotometric analysis of remitted light in the visible and infrared spectra [68]. This technique generates images showing melanin, and its position in relation to the dermo-epidermal junction. The analysis does not only indicate detection of dermal melanin but also other histological and structural features, such as dermal blood and thickness of papillary dermis [68]. Continued development of this technology, resulted in a commercial instrument called SIAscope (Astron Clinica Ltd., United Kingdom). The SIAscope is equipped with a handheld unit that operates by probing the skin spectrally in the range from visible light, 400-700 nm, to near-infrared radiation, 700-1000 nm. The remitted light from the skin is digitally recorded, and the input is analysed spectrophotometrically by computer algorithms based on the optical model presented by Cotton [68]. Whether any changes have been made in the implementation of the algorithm to the commercial instrument have not been made public. The analysis is reported to give information regarding total melanin content of the epidermis and the papillary dermis, collagen and haemoglobin content, as well as the presence of melanin in the papillary dermis (fig 7). SIAscopy was presented by Moncrieff et al. in 2002 as a tool to facilitate diagnosis of melanoma [69]. Significant findings in invasive melanoma were the presence of blood displacement with erythematous blush, collagen holes and presence of dermal melanin within the lesion (Table 6).

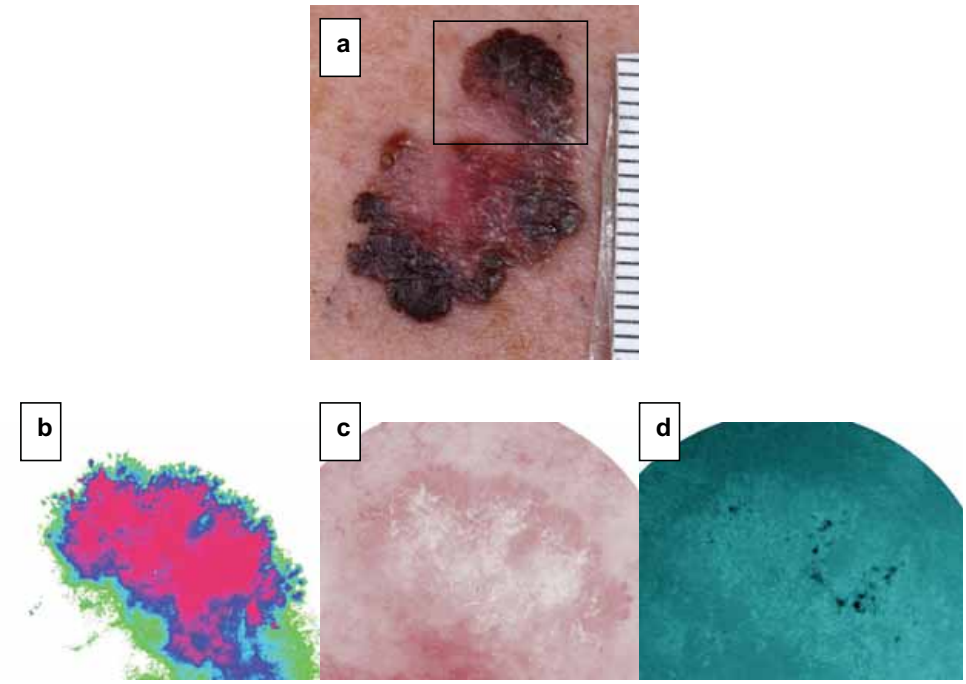
Table 6. Definition of SIAscopic features [69]

Dermal melanin	The presence of dermal melanin within the lesion not due to hairs
Erythematous blush	A peripheral increase in blood within the lesion compared with the surrounding normal skin for ¾ of the circumference of the lesion.
Blood displacement	A confluent, non-pixelated area demonstrating an absence of blood within the lesion.
Collagen holes	An area within the lesion coinciding with melanin (dermal or total) demonstrating an absence of collagen not due to hair follicles or sebaceous / sweat ducts.

In the Moncreiff study SIAscopy had a sensitivity of 82.7% and a specificity of 80.1% when diagnosing invasive melanoma. Only one additional study has been published in a scientific medical journal regarding melanoma diagnosis, comparing clinical diagnosis of melanoma by experienced dermatologist and SIAscopy, showing comparable diagnostic accuracy between clinician and SIAscopy [70]. Thus, there is a lack of studies regarding this diagnostic tool and its clinical use when diagnosing melanoma. In this thesis, in paper IV, is put forward a study that compares the correlation between SIAscopy and histopathology on melanoma.

Regarding the use of SIAscopy for diagnosing non-melanoma skin cancer a few more studies have been published [71-73]. They suggest that SIAscopy can be of diagnostic use when diagnosing non-melanoma skin cancer. In regards to pigmented non-melanoma skin cancer one study is published and it is presented in this thesis as paper III.

Fig 7 Melanoma with the SIAscopically examined area marked on the clinical image (a). Below the clinical image the SIAgraphs showing (b) dermal melanin image with high concentration of dermal melanin, (c) blood image showing blood displacement and erythematous blush, and (d) collagen image with collagen holes.



Other *in vivo* diagnostic techniques

Confocal microscopy, multiphoton microscopy, optical coherence tomography and fluorescence diagnostics are some of the techniques that are being used in research projects to evaluate their possible use in *in vivo* diagnosis of melanoma and non-melanoma skin cancer [74-79]. Of these *in vivo* diagnostic techniques, most clinical research work is done on confocal microscopy and skin cancer, particularly melanoma. Confocal reflectance microscopy works by focusing a low-power laser beam on a specific point in the skin, detecting only the reflected light from that point through a spatial filter. This beam is the scanned horizontally over a 2-dimensional grid to obtain a horizontal microscopic section [75]. The main advantage of this technique is that it enables imaging of cellular components of intact skin with detail approaching that of histology [75, 76]. The limitation is that assessment can only be done to a depth of 350 μm, which corresponds to the papillary dermis [76].

AIMS OF THE INVESTIGATION

Paper I

The aim was to investigate if primary care physicians could improve their ability to diagnose melanoma using dermoscopy after a short education intervention.

Paper II

The aim was to describe relevant morphologic features of pigmented basal cell carcinomas using dermoscopy and to create a simple diagnostic method based on these findings.

Paper III

The aim was to evaluate if SIAscopy could be used to diagnose pigmented basal cell carcinomas.

Paper IV

The aims were: i) to find out if SIAscopic findings, e.g. presence of dermal melanin, blood displacement and collagen holes, topographically correlated with histopathological findings of melanoma; ii) if SIAscopy could give a topographic indication of the localization of maximum tumour thickness, and iii) provide a guide for appropriate sectioning of the surgical specimen for histopathological evaluation.

MATERIAL AND METHODS

Paper I

Seventy-four practicing primary care physicians (PCPs) completed a pre-test, containing matched clinical and dermoscopic photographs, of 50 melanomas and 50 atypical pigmented non-melanomas. PCPs were randomized between a group undergoing dermoscopy education intervention and a control group. The ability to diagnose melanoma was assessed by an identical post-test.

Paper II

In vivo dermoscopic images of 142 pigmented basal cell carcinomas, 142 invasive melanomas and 142 benign pigmented skin lesions were included in the study. The images were randomly divided into 2 equally sized training and test sets. All the included pigmented skin lesions had been excised and reviewed for histological diagnosis. Images from the training set were scored for 45 dermoscopy features. Based on the results an algorithm was derived and tested on the test set.

Paper III

Twenty-one pigmented basal cell carcinomas were analysed, comparing dermoscopic and SIAscopic findings. The lesions were analysed using the dermoscopy method described by Menzies et al. [80] and the SIAscopic technique described by Moncrieff et al. [69] to measure melanin content (total and dermal) and the influence of the tumour on collagen and blood content.

Paper IV

Sixty lesions; i.e. 29 invasive melanomas, 13 melanomas *in situ* and 18 pigmented non-melanoma lesions, were included. The lesions were, at inclusion, all clinically suspicious for melanoma and all displayed positive SIAscopic findings indicating melanoma [69]. After excision the specimens went through non-routine histopathological investigation to compare the SIAscopic findings with the histopathological findings.

Statistical methods

Paper I

Sensitivity and specificity for the diagnosis of melanoma were calculated. Differences between the scores in the education intervention group and the non-intervention group, and clinical versus surface microscopic scores, were analyzed using two-tailed t-tests. When comparing the results within each group of pre- versus post-test and clinical versus dermoscopy results, paired t-tests were used. Unpaired t-tests were done to compare the differences in scores between the education intervention group and the non-intervention group. Melanoma and non-melanoma scores were examined separately. A power analysis prior to the study (power=0.95, one-sided α=0.05) showed that a 15% improvement between intervention and non-intervention scores and dermoscopic versus clinical scores could be detected with our sample size.

Paper II

In the training set the difference between the proportions of pigmented BCCs and non-BCCs identified with each feature was analyzed in a series of χ^2 tests of independence. A test-wise error rate of $\alpha<0.05$ was used to determine the statistical significance of each test. Because multiple tests were performed, the experiment-wise error rate was not controlled, and the type I error rate (i.e. accepting a chance difference) was clearly inflated. This strategy was justified by the potential clinical benefit of identifying new predictors of BCC. Rejecting a real effect (type II error) was of greater concern than accepting a chance difference (type I error).

The sensitivity for diagnosis of pigmented BCCs is defined as the number of scored positive BCCs divided by the total number of BCCs (expressed as a percentage). The specificity for the diagnosis is defined as the number of scored negative non-BCCs divided by the number of non-BCCs (expressed as a percentage).

From the training set data a simple algorithm for diagnosing pigmented BCCs were constructed. The algorithm was based on the principle that individual features were selected for low sensitivity (negative features) and high specificity for invasive melanoma and benign pigmented skin lesion (PLS) sets (positive features). Models were constructed to achieve adequate sensitivity for diagnosis while achieving high

specificity using the benign PLS set and moderate to high specificity using the invasive melanoma set. In the second phase of the study the single optimal algorithm derived from the training set was scored on the independent test set, without prior knowledge of the diagnosis of each lesion.

Paper III

The dermoscopic and SIAscopic findings, in each lesion, were accounted for in a table. No statistics were made due to the small number of included lesions.

Paper IV

Sensitivity and specificity for each SIAscopic feature were calculated. Also the sensitivity and specificity for the combined features (presence of blood displacement with erythematous blush, collagen holes and presence of dermal melanin) were calculated. Positive and negative predictive values for the combined features were calculated. 95% confidence intervals for sensitivity, specificity, positive and negative predictive value were shown. As the traditional method for calculating confidence interval based on normality approximation is not adequate for very low observed proportions (or very large) we used another method that is recommended in such situations [81].

Kappa-statistics were applied to compare the agreement between SIAcopy and histopathology on the features of dermal melanin, blood displacement and collagen holes.

Ethics

Papers III and IV

The regional ethical review board in Göteborg approved the studies.

RESULTS

Paper I

There was a significant improvement between the post- versus pre-test for the education intervention group, with respect to both clinical melanoma diagnosis (63% vs 55% $p<0.01$) and dermoscopy melanoma diagnosis (76% vs 58% $p<0.001$). No significant differences were found in the control group between the post- versus pre-test, neither for clinical melanoma diagnosis (54% vs 51% $p=0.2$) nor the dermoscopy melanoma diagnosis (55% vs 53% $p=0.6$). Following training the ability to diagnose melanoma was significantly higher using dermoscopy compared to clinical diagnosis (76% vs 63% $p<0.001$). This difference was absent in the control group (55% vs 54% $p=0.6$). No significant difference was found in clinical versus dermoscopy post-test results for non-melanoma PSL in either the intervention group or control group. Improvement in the sensitivity for the diagnosis of melanoma with dermoscopy was observed without a decrease in specificity.

Paper II

The following algorithm was created. For a pigmented BCC to be diagnosed it must not have the negative feature of pigment network and must have 1 or more of the following 6 positive features: large grey-blue ovoid nests, multiple grey-blue globules, maple leaf-like areas, spoke wheel areas, ulceration, and arborizing “treelike” telangiectasia. On an independent test set the model had a sensitivity of 97% for the diagnosis of pigmented basal cell carcinoma, a specificity of 93% for the invasive melanoma set and 92% for the benign pigmented skin lesion set. Combining test and training sets resulted in a sensitivity of 93% for the diagnosis of pigmented BCCs, a specificity of 89% for invasive melanoma and 92% for benign pigmented skin lesions.

Paper III

SIAscopic signal for dermal melanin was observed in all of the pigmented BCCs included in the study. Erythematous blush was also common (43%) and blood displacement as well as collagen holes occurred (19% and 14% respectively). Thus, pigmented BCCs display the same SIAscopic features that previously have been

presented for melanoma [69], although not as frequently as for melanoma. Dermoscopy, using the algorithm by Menzies et al for pigmented BCCs (see paper II, [80]), correctly diagnosed 90% (19 of 21) of the pigmented BCCs. Two nodular BCCs were not correctly diagnosed using dermoscopy. Thus, the study implies that SIAscopy is not suitable as the sole diagnostic tool for diagnosing pigmented basal cell carcinomas.

Paper IV

In only 11 of 29 invasive melanomas the SIAgraphs topographically matched the area of invasion on histology. A high concentration of dermal melanin was the SIAscopic signal with best correlation to melanoma invasion, although it also proved to have low specificity. It is concluded that SIAscopy-based diagnosis has a low diagnostic accuracy for melanoma and does not provide reliable diagnostic information relating to the lesions internal structure on histopathology examination and therefore cannot be used as a guide for localizing the maximum tumor thickness when performing the histopathological examination

DISCUSSION

Methodological considerations

In paper I and II all evaluations were made on photographic images of the lesions, in paper I clinical and dermoscopic images (paper copies) and in paper II dermoscopic images (Dia-slides). Photographic images of lesions are not identical to the images received during clinical examination in regards to exact colour reproduction and "clinical feeling", but great effort was put in to making the photographic images as authentic as possible, with manual colouration of paper copies of images in study I.

In paper III the study material was small, only 21 pigmented basal cell carcinomas, which was a limitation to the study. Even so, the results were fairly clear-cut and conclusions could be drawn from it.

In paper IV, due to stepwise development of the histopathological procedure during the early inclusion period, precise adherence to all the details in the study protocol, as described in "Materials and methods" (see appendix IV), did not occur in 30 of the 60 study cases. Mostly however this was deemed not to significantly impair the validity of the observations made, although 9 lesions had to be excluded due to technical problems or due to failure in the histopathological processing.

General discussion

Differential diagnosis between benign and malignant pigmented skin lesion is an important but sometimes difficult task for both dermatologists and primary care physicians (PCP). Consequently several diagnostic tools for *in vivo* diagnosis have been developed. Some of them have been used for a few decades, and are validated by numerous independent studies; others are new and have not so far encompassed the same evaluation. A well established diagnostic tool is dermoscopy. When performing a search on the Medline database for dermoscopy, more than seven hundred hits regarding this subject are obtained. Another diagnostic tool is SIAscopy which has been commercially available since 2000, and a similar search on the Medline database, on the term spectrophotometric intracutaneous analysis, yields 13 hits. Dermoscopy and SIAscopy are the methods which are focused on in this thesis,

specifically in regards to their diagnostic use in melanoma and pigmented basal cell carcinoma.

Several studies have shown that the diagnostic accuracy for melanoma increases with the use of dermoscopy [33]. Only few of these studies have been done among non-dermatologists, e.g. PCPs. The PCP is often the physician with whom the patient comes in first contact. In paper I, we show that PCPs improve their ability to correctly diagnose invasive melanoma with more than 20% (from “pre-education intervention” sensitivity for clinical melanoma diagnosis of 55% to “post-education intervention” sensitivity for dermoscopic melanoma diagnosis of 76%) with only a short education intervention on dermoscopy. This marked improvement in sensitivity with dermoscopy, was observed without a decrease in specificity, indicating that the effect should occur without increasing the number of needless excisions. Why specificity did not increase by the use of dermoscopy is unclear. However, it has been shown previously, as experience with the dermoscopy technique increases, the diagnostic accuracy (which includes specificity) should also increase [82]. Thus, in countries were melanoma leads to significant mortality PCPs should be formally trained in dermoscopy. In Sweden today only dermatologists and dermatology registrars have formal education opportunities in dermoscopy, but in my opinion PCPs should be educated as well. Dermoscopy is not more difficult than ophthalmoscopy, which is something every PCP is supposed to master. If PCPs could learn to use the two-step procedure (fig. 6) when diagnosing pigmented skin lesions, their ability to select the appropriate cases for referral to dermatologists or surgeons would increase dramatically. It is my belief though, that it is important to use dermoscopy regularly and frequently, on both benign and malignant pigmented skin lesions, to uphold the skill and continue to perform with a high diagnostic accuracy. Thus, only physicians with a larger number of patients with pigmented skin tumours have the real opportunity to practice their dermoscopy skills.

Basal cell carcinoma (BCC) is a very common skin malignancy. The pigmented form of BCC can be an important differential diagnosis to melanoma. In dermoscopy pigmented BCCs show some characteristic features and in paper II we presented a simple diagnostic algorithm for pigmented BCCs. The algorithm is based on the negative feature of pigmented network, which can not be found in pigmented BCCs,

and six positive features, i.e. large grey-blue ovoid nests, multiple grey-blue globules, maple leaf-like areas, spoke wheel areas, ulceration and arborizing "treelike" telangiectasia, where at least one must be found. The algorithm has a sensitivity of 93% for the diagnosis of pigmented BCC and a specificity of 89% for invasive melanoma and 92% for benign pigmented skin lesions (when combining the results from the test- and training-set, see paper II). This was the first study, to our knowledge, that statistically analyzed the dermoscopic features of pigmented BCCs in a larger series of tumours, including pigmented BCCs as well as melanomas and benign pigmented skin lesions. This algorithm for diagnosing pigmented BCC has gained acceptance among dermoscopy users and is part of step one in the two step procedure to diagnose melanoma (see fig 6) presented by the International Dermoscopy Society in an atlas, based on the results of the consensus net meeting on dermoscopy held in 2000.

SIAscopy is a different *in vivo* diagnostic technique based on spectrophotometric analysis of remitted light from the skin when probing a skin lesion with lengths in the light spectrum from short wavelength visible light to long wave near-infrared spectrum. With mathematical algorithms the remitted light is interpreted based on a mathematical optical skin model. The resulting images give information regarding total melanin content of the epidermis and the papillary dermis, collagen and haemoglobin content, as well as the presence of melanin in the papillary dermis. This diagnostic tool is more recent than dermoscopy and has not been so extensively evaluated in a clinical research setting as dermoscopy. In a majority of the few studies that have been carried out, up to date, by other research groups SIAscopy is presented as a valuable tool to diagnose melanoma as well as non-melanoma skin cancer. [68, 69, 71-73]. We have, in paper III and IV evaluated the use of SIAscopy in diagnosing pigmented BCC and melanoma. In regards to pigmented BCCs we show (paper III) that pigmented BCCs display the same SIAscopic features that previously have been presented for melanoma (table VI) [69], although not as frequently as for melanoma. The finding of dermal melanin is the most common SIAscopic feature in pigmented BCCs, occurring in all of the pigmented BCCs included in the study presented in paper III. Also the features erythematous blush, blood displacement and collagen holes are commonly occurring. We therefore conclude that the sole use of SIAscopy, as diagnostic tool, is not reliable when

diagnosing pigmented BCCs or when differentiating pigmented BCCs from melanoma.

Furthermore SIAscopy is reported to give information pertaining to the microscopic structure and architecture of a skin lesion by images showing the distribution, position and quantity of melanin, blood and collagen. If this information is valid the technique could be used not only to facilitate the clinical diagnosis of melanoma but also the histopathological examination. In paper IV we compare the SIAscopic findings in melanoma and pigmented non-melanoma lesions with histopathology, using SIAgraphs as a guide when performing tumour sectioning and microscopic examination. We conclude that the information regarding microscopic structure and architecture given by the SIAscope does not represent reliable diagnostic information relating to the lesions internal structure and therefore cannot be used as a guide for localizing the maximum tumour thickness when performing the histopathological examination. Among the study cases topographical correspondence between the SIAscopic signal of dermal melanin and presence of melanophages and/or heavy epidermal pigmentation was observed. Topographical correlation between the histologically confirmed presence of high amounts of melanin, both epidermal and dermal, and SIAscopic indications of blood displacement and collagen holes were also observed. These false SIAscopic indications, we believe, are due to disturbances in the colorimetric analysis which, in turn, are based on some fault in the algorithms in the optical image formation model of human skin which are used in the software of the SIAscope.
In paper IV we also show a low sensitivity for melanoma using the SIAscopic algorithm presented by Moncreiff et al [69].

The idea that *in vivo* diagnostic techniques can be used as a guide when sectioning tumour specimens for histopathologic examination, to possibly improve the diagnostic accuracy of routine histopathologic investigation of melanoma, is appealing. Paper IV presents a specially developed preparatory and documentary procedure for histopathology which enables the accurate topographical comparison between the SIAscopy or dermoscopy image and the histopathologic specimen. If this type of collaboration between clinician and pathologist could be used also in routine cases it

is my belief that it could be rewarding for all parties, including the patients, most importantly!

In summary; of the two *in vivo* diagnostic techniques investigated in this thesis, the well established dermoscopy seems to be the most reliable diagnostic tool to help differentiating melanocytic from non-melanocytic lesions and to help diagnosing melanoma and pigmented basal cell carcinoma. Further research in this field will probably lead to development of other diagnostic tools, but to gain wide acceptance among dermatologists and other physicians they will have to be easy to use and cost effective.

CONCLUSIONS

- Dermoscopy significantly improves sensitivity for melanoma when used by primary care physicians, after a short education intervention on dermoscopy. All primary care physicians in countries where melanoma leads to significant mortality should be trained in dermoscopy (paper I).
- A robust dermoscopy algorithm that allows the diagnosis of pigmented BCCs from invasive melanoma and benign pigmented skin lesions has been developed. The algorithm is based on the negative feature of pigment network, which can not be found in pigmented BCCs, and six positive features; large grey-blue ovoid nests, multiple grey-blue globules, maple leaf-like areas, spoke wheel areas, ulceration and arborizing “treelike” telangiectasia, where at least one must be found (paper II).
- SIAscopy has no advantage over dermoscopy when diagnosing pigmented basal cell carcinoma, and can be misleading if the examiner has little or no knowledge of dermoscopy (paper III).
- Information regarding microscopic structure and architecture given by the SIAscope does not represent reliable diagnostic information related to the lesions internal structure, when compared to histopathology. Therefore SIAscopy cannot, in its present form, be used as a guide for localizing the maximum tumour thickness when performing the histopathological examination (paper IV).

FUTURE PROSPECTS

This thesis is not by far a concluding work on this research topic but only covers a part of *in vivo* imaging techniques for diagnostics of pigmented skin tumours. Dermoscopy has been, and continues to be, a field of extensive research in research groups and international research collaborations all over the world. SIAscopy is a method that has not yet been so extensively investigated but will probably be further developed. In addition other techniques such as confocal microscopy, multiphoton microscopy, optical coherence tomography and fluorescence diagnostics will continue to be investigated as methods of diagnosing skin cancer; both melanoma and non-melanoma skin cancer.

In regards to the two techniques that are investigated in this thesis; dermoscopy and spectrophotometric intracutaneous analysis, possible future research prospects include:

- Further investigation on dermoscopic features in dysplastic nevi to evaluate if there are specific features that can predict an increased risk of melanoma development.
- Further investigations on dermoscopic features seen in pigmented mucosal lesions.
- Further work on form-, colour- and texture analysis on digital dermoscopy to identify features that topographically correlate with histopathological findings of i) early signs of melanoma development, ii) invasive tumour growth at different levels of invasion.
- Further technical development of spectrophotometric intracutaneous analysis to overcome the problem with over-diagnosing presence of dermal melanin in lesions with high levels of epidermal melanin, as well as miss-diagnosing blood displacement and collagen holes in lesions with high epidermal and/or dermal melanin. If this can be achieved spectophotometric intracutaneous analysis could be a very useful *in vivo* diagnostic tool for pigmented skin lesions.

- Texture analysis of the SIAgraphs to define the textural properties of a signal that topographically correlates with histopathological findings of invasive skin tumour growth, versus a SIAgraphic signal that not topographically correlate with verified invasive tumour.

The challenging work to correlate digital images (from any *in vivo* imaging technique), with histopathology will have to continue, since closer collaboration between clinician and pathologist will need to become more frequent, as the diagnostic techniques gets more advanced and detailed. Additional work will have to be done on this subject, to further develop exact matching procedures for digital images and histopathology images, usable not only in research settings but also in everyday clinical work.

In addition to further research in the fields of *in vivo* diagnostic techniques, future prospects should also involve extending education opportunities in dermoscopy to include primary care physicians. The number of people seeking medical consultation for pigmented skin lesions is increasing with increased public awareness of the rising melanoma incidences and it is not possible for all to be examined by dermatologists.

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