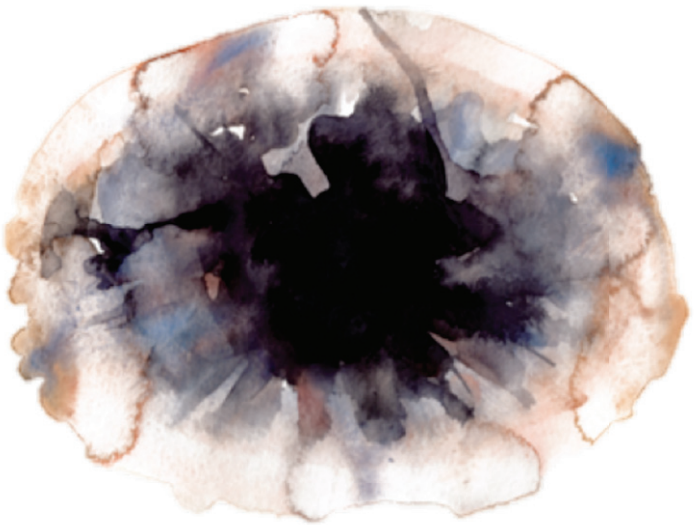
EX VIVO DERMOSCOPY WITH DERM DOTTING

A new method for lesion specific and targeted cutting

Marc Haspeslagh



Thesis submitted as fulfilment of the requirements for the degree of
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“They didn’t know it was impossible, so they did it”

- Marc Twain -

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List of abbreviations

BCC	basal cell carcinoma
DD	derm dotting
EVD	ex vivo dermoscopy
EVD-DD	ex vivo dermoscopy with derm dotting
HE	hematoxylin-eosin
IVD	in vivo dermoscopy
NOS	not otherwise specified
NPD	non-polarised dermoscopy
PD	polarised dermoscopy
SCC	squamous cell carcinoma
TAT	turnaround time

Introduction

1 History of dermoscopy

Dermoscopy is a relatively young and dynamic diagnostic field in dermatology. It is a simple non-invasive technique that bridges the gap between clinical dermatology and histopathology. Dermoscopy uses a hand-held instrument which incorporates a magnification zoom lens and a light source. It allows the clinician to see skin structures that are not visible to the naked eye, situated at the level of the stratum corneum, the epidermis and the (superficial) dermis.

Dermoscopy has its origin in the 17th century, when in 1655 first Pierre Borel (Petrus Borrelius, 1620-1689) and few years later, in 1663, Kolhaus examined nail fold capillaries under the microscope. A liquid interface to reduce refraction and increase the resolution of microscopy was first used by Ernst Karl Abbe in 1878. In 1879, Hueter performed surface microscopy on lip capillaries [1]. In 1893, Unna used the term 'diascopy' to examine lupus and lichen planus with immersion oil and a glass spatula [2]. Saphier introduced the term dermatoscopy in 1920 [3-6]. In the mid-20th century, Zeiss produced the first binocular dermoscope. For many years, 'epiluminescence microscopy', 'dermatoscopy' and eventually 'dermoscopy' were used to describe surface microscopy. Despite the use of dermoscopy as a diagnostic tool on patients with Raynaud's disease, lupus, systemic sclerosis and dermatomyositis since 1920, dermoscopy for evaluation of skin tumours was only used in the late 20th century. In 1951, Goldman [7] developed the first portable dermoscope. He was the first to use this method for evaluating pigmented skin lesions. In 1971, Rona MacKie described the use of dermoscopy for the pre-operative evaluation of pigmented lesions and for the differential diagnosis between benign and malignant lesions. Fritsch and Pechlaner described pattern analysis in dermoscopy in 1981 and Soyer correlated the dermoscopic findings with their morphologic substrate in 1989, the year in which the first consensus conference meeting on skin surface microscopy was held in Hamburg. In 1990, the first dermoscopic atlas was published. In 1994, Stolz introduced the ABCD rule of dermoscopy [8]. In 2001, the first virtual consensus Net Meeting on Dermoscopy was held in Rome. From that moment on, dermoscopy became more and more known throughout the world, which led to the foundation of the International Dermoscopy Society in 2003. Stolz, Argenziano, Menzies, Soyer and others gradually developed criteria to distinguish naevi from melanomas [9-11]. Over the last few years, many different diagnostic algorithms have been suggested and dermoscopy on non-melanocytic tumoural lesions and other dermatological conditions such as inflammatory skin disease, hair disorders, nail fold capillaries was also introduced [12].

2 Principles of polarised and non-polarised dermoscopy

Most dermoscopes are hand-held instruments, usually with a magnification of X10 to X20. They contain a light-emitting diode to provide illumination. Three types of light can be identified: surface glare, superficial light and penetrating light (*Figure 1)*.

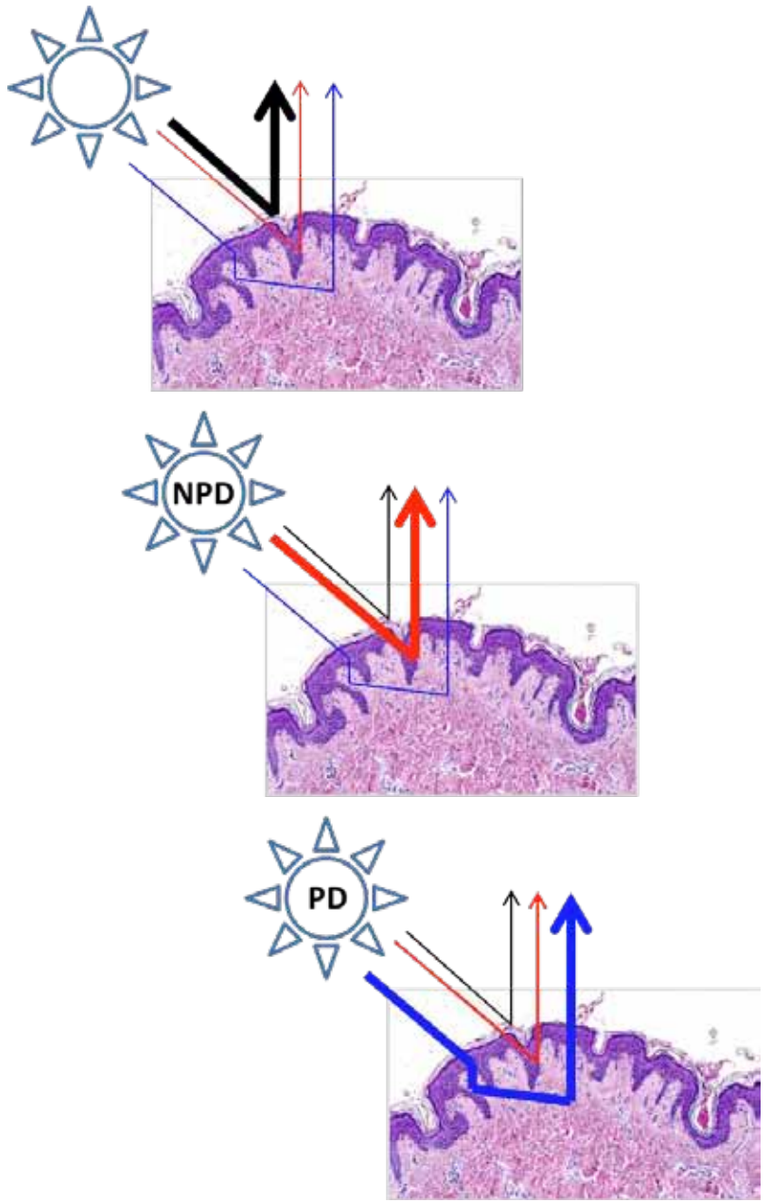


Figure 1 Optical properties of light detected with the naked eye include mostly surface glare (black arrow), with non-polarised dermoscopy (NPD) mostly superficial light (red arrow) and with polarised dermoscopy (PD) mostly penetrating light (blue arrow).

Most light that is detected by the naked eye is superficial reflected light. Due to the reflective property of the stratum corneum (surface glare) most light is scattered. Therefore, we mainly observe the characteristics of the surface layer and can only minimally evaluate the colours and structures of deeper layers of the epidermis and dermis.

A non-polarised dermoscope (NPD) must be in direct contact with the skin and a liquid interphase (oil, alcohol or ultrasound gel) is needed. The replacement of the skin air interface by a skin liquid interface matches closer the refraction index of the horny layer decreasing the refraction of the light, allowing us to identify superficial epidermal and dermal structures that are not visible to the naked eye.

In 2000, dermoscopes with polarised light were introduced (PD). In contrast to NPD, most light in PD is penetrating, thus visualising deeper structures. Another advantage of PD is that no direct skin contact or a liquid interphase is needed. With PD however, a superficial area with a thickness of 0.06 to 0.1 mm becomes invisible. Superficial structures, like orthokeratosis and milia-like cysts, become less conspicuous than with NPD, while blood vessels, deeper pigmentation and white shiny structures are more conspicuous with PD.

NPD and PD provide complementary information and therefore, the use of both provides the clinician the best information for diagnosis [13]. Since most current dermoscopes are provided with both NPD and PD, users can easily switch between both techniques.

3 Histopathologic tissue correlate of dermoscopic structures

Contrary to histopathologic examination, dermoscopy shows colours and structures beneath the surface of the skin in a horizontal plane view. These colours and structures have specific histological correlates and are not perceived by the naked eye.

The main chromophore in the skin is melanin. Other chromophores are hemoglobin and collagen fibers. The melanin in pigmented lesions is perceived as a range from black to dark or light brown, grey, and blue, depending on the depth of the localization of the pigment (Tyndall effect). Pink and red are produced by hemoglobin, white by collagen fibers in fibrosis or sclerosis, respectively (Figure 2). Other chromophores are keratin or exogenous pigmentation. Colours in dermoscopy can also be produced by a combination of different chromophores. The Tyndall effect cannot explain every colour. Black can be induced by coagulated blood in an angiokeratoma without melanin in the upper epidermis. White can also be produced by scales on the surface of a tumour.

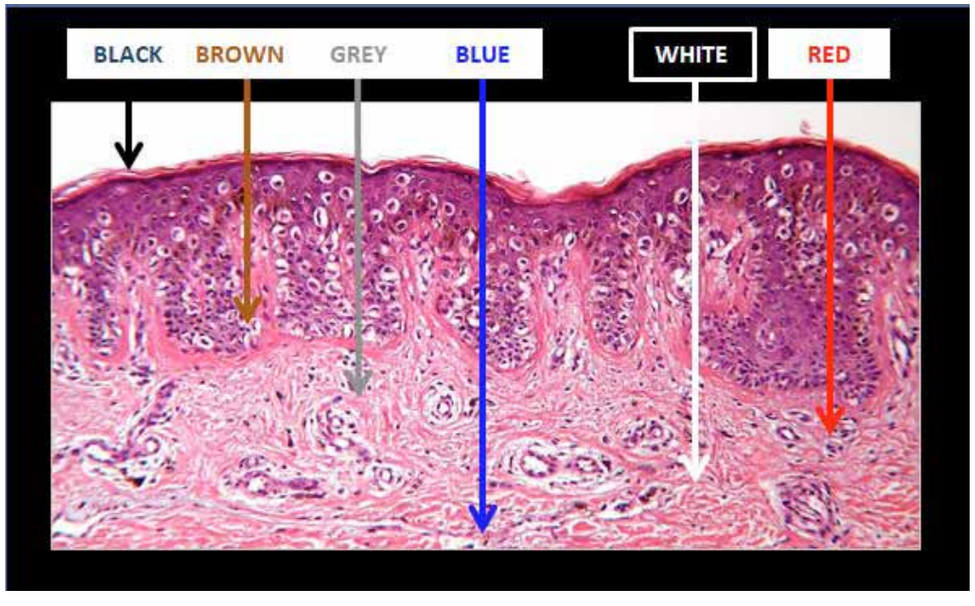


Figure 2 Colours in dermoscopy depending on the quantity and localisation of melanin, blood vessels, keratinocytes and collagen

Dermoscopic structures are two-dimensional surface projections of 3D tissue structures at different depths of the skin. The depth at which structures are visible is limited to the papillary dermis. The most important dermoscopic structure in melanocytic lesions is a network formed by melanin in basal keratinocytes or melanocytes along the dermo-epidermal junction. This network represents the rete ridges pattern in a horizontal way. The non-pigmented “holes” correspond to the tips of the papillae. In dysplastic or melanomatous lesions, the regularity of this network becomes atypical.

Other important diagnostic structures are streaks, globules, structureless areas, dots, pseudo-network, crystalline structures, and vascular structures. Many other structures and new patterns continue to be described. Most of them correspond to well-defined histopathologic correlates. There are different diagnostic algorithms like the ABCD rule, the Menzies method, the 7-point checklist, the 3-point checklist, chaos and clues, and CASH (colour, architecture, symmetry, and homogeneity) to diagnose pigmented skin lesions [12]. Experienced dermoscopists use pattern analysis as preferred method to diagnose lesions. Dermoscopy findings in other skin conditions, such as inflammatory skin conditions, hairs and nails, … are also being published regularly.

4 History of tissue processing

Antoni van Leeuwenhoek developed the first microscope in 1673. The first microtome was introduced in 1848. During the 19th century, paraffin was introduced as embedding material and the hematoxylin-eosin (HE) stain was described in 1875. During the 20th century, this method of processing has been nearly unchanged. During the last two decades, molecular biology and genetics became important tools to add diagnostic, predictive and prognostic information in many tumours. However, in daily practice a technically well cut paraffin block and a HE slide remain a primary key stone for an adequate diagnosis.

5 Standard method of dermatopathology processing

Skin biopsies are fixed in 10% formalin, which fixes the tissue by forming cross links between amino-acid residues in proteins. The formalin is buffered at pH 7 and slowly penetrates into the tissues without altering the structure of the proteins. This 10%-buffered formalin penetrates about 1 mm/hour. Depending on the thickness, at least 12-24 hours are needed to fix the specimen properly. Guidelines for gross dissection practice differ, and are influenced by the specimen type and the clinical context. In most dermatopathology textbooks, the bread-loaf transverse-method with processing of tips is advocated. With this method, 2-3 mm-thick sections are made and paraffin-embedded. There are three types of skin biopsies to be processed: excision biopsies, punches, and shavings or curettages. After proper fixation, the excision biopsy is measured and visually inspected. In most laboratories, the subcutaneous section plane of the specimen is marked with Indian ink or other marking inks. Punches are mostly used in case of an inflammatory skin disease. Some punches are diagnostic biopsies from focal suspicious areas in a larger tumoral lesion. Punches of 2-3 mm are embedded intact; larger punches (4-6 mm) are bisected. Shavings are embedded intact or bisected. Excision specimens are usually transversely sectioned in 3-mm-slides (bread-loaf sections). In most laboratories, the tips are also routinely processed. *(Figure 9 p 32*).

All tissue sections are then placed into a plastic cassette to hold the tissue while being processed. Later on, the cassette will also serve as the backing for the final paraffin block. In most laboratories, the tissue sections in the cassettes are processed overnight in a tissue processor. The first step in a tissue processor, is a final formalin fixation step. Then, the tissue passes into ethanol to remove the water from the tissue. The ethanol is cleared by xylene. Finally, the tissue passes into the paraffin bath.

In the morning, a metal mould is used to make a paraffin block that includes the processed tissue. During this step, the tissue has to be oriented properly in order to make transversely oriented sections. Good orientation is made possible by inking the lower side of the specimen and providing a schematic drawing of the sectioning.

The paraffin is cooled on a cool plate, which hardens the paraffin block. The paraffin block can then be cut on a microtome which moves the block over a very sharp knife to make 4-µm-thin sections. There is a marked variation in the extent of leveling performed by different laboratories. The paraffin cuts are “caught” in a water bath, stretched out on a glass slide and then dried on a hot plate.

An automatic stainer deparaffinizes the sections with sequential immersion in xylene, alcohol and water. The slides are then routinely stained with hematoxylin and eosin. The stained slides are dried and automatically transferred into a mounting station where a coverslip is placed over the section.

The slides are now ready for the pathologist, who examines them and reports the microscopic description, the diagnosis and possible comments. In some cases, special stains or immunostains are required to make a final diagnosis.

6 Flaws of the standard method

With the introduction of dermoscopy in dermatology, ever smaller suspicious lesions are detected and more focal changes in lesions are now a reason to excise. In most laboratories, this **morphologic information is not at the disposal of the pathologist** while grossing the specimen and examining the slide. This is in contrast with the actual quality requirements according to which dermatopathologists have to fully integrate the clinical context of each case. As such, the diagnostic standard requires access and use of this dermoscopic information. The actual standard method does not fulfill these requirements, because the tissue sectioning is performed without clinicopathologic correlation. With the “blind” bread- loaf method it is not uncommon that embedded tissue sections from excised small lesions do not contain the lesion. In addition, focal diagnostic areas risk to be transected. Since some of these blocks only contain a small part of the lesion, the transected focal area risks to get lost during the initial trimming and not be included when cut at the microtome (*Figure 9*), which results in slides without lesional tissue. **Processing of non-diagnostic tissue** results in needless embedding, cutting, staining and interpretation of skin sections.

The bloc is cut “blindly” and tissue ribbons are not conserved while cutting at different levels. Most laboratories use a water bath to collect the paraffin cuts on a glass slide. The tissue that is trimmed before the diagnostic cut (that is mounted on glass slide) is thrown away. Since the cutting is done blindly, targeting the most diagnostic tissue depth by the technician is not possible. If the technician is too careful and does not cut deep enough, the pathologist risks to examine a non-representative slide, in which case deeper cuts will be necessary to make a diagnosis. However, performing additional levels results in a higher workload, and extra technical and material expenses. Exhaustion of the complete paraffin block is both impractical and not economically feasible. When at the initial trimming the technician passes trough the area of interest, the **diagnostic area will be lost** forever and no proper diagnosis, despite cutting of the complete paraffin block, can be made.

Usually less than 2% of tissue is examined and with this approach [14], focal lesions are easily missed. Moreover, the **margin status is also only incompletely evaluated** on such a small amount of tissue. Schnebelen [15] found that about one quarter of positive margins of shavings of non-melanoma skin cancer may be missed. Chang [16] found a false negative margin in one third of punch excisions of melanocytic lesions upon thorough block sectioning. Since Barr [17] found that in more than one third of atypical naevi heterogeneity of atypia is present, one can assume that grading of these atypical naevi in blind sections of less than 2% of the lesion is a risky and questionable way of processing.

When deeper cuts or special stains are demanded, the archived block has to be retrieved and recut on the microtome. The new cuts are always deeper cuts and not necessarily located next to the tissue of the lesion on the first HE slides. The **focal lesion identified on the initial slide risks to be absent** in these deeper cuts.

Especially when no diagnostic findings are seen in the slide, these negative findings are **not correlated adequately with the dermoscopic and clinical aspect** of the lesion. The pathologist has **no internal control** on whether what he examines is representative for the submitted lesion or not.

Chapter I Rationale and specific aims

1 Rationale

In most general pathology laboratories skin biopsies are processed in a routine manner. Macroscopy of skin lesions is usually limited to a quick visual inspection and by measuring the resection specimen and the lesion. A schematic drawing of the lesion is often made. This is followed by inking of the bottom side and transverse sectioning of the lesion every 3 mm, with horizontal processing of the tips. Per microscopy slide, three different levels of microtome cuts are generally assembled. With this standard method only 2% or less of the excised lesion is examined, potentially leading to inadequate evaluation of section margins of neoplastic skin lesions, misclassification especially of melanocytic lesions, and misdiagnosis of small lesions not visible by the naked eye.

In 2007, Scope and co-workers described the added value of ex vivo dermoscopy (EVD) for the evaluation of skin lesions by guiding macroscopy. The advantage of EVD in the histopathological diagnosis of skin lesions was confirmed by others, but the need remained for a practical way of marking and targeting suspicious margins and focal alterations in lesions seen on the EVD. For this purpose, a marking method was searched and found with the application of nail varnish to dot focal lesions. The use of this new method of combining EVD with derm dotting (EVD with DD) was introduced in our laboratory in 2011.

The general aims of this study are to describe the advantages of EVD with DD, to prove that EVD with DD can improve the reliability of pathological margin evaluation and accuracy of diagnosis, and to evaluate the impact of this new method on the need of additional cuts and turnaround times.

2 Specific aims

1) Comparison of EVD and in Vivo Dermoscopy (IVD) in the dermatopathologic evaluation of skin tumours (chapter III).

EVD has been reported as a potential valuable tool in routine dermatopathology. To compare IVD and EVD and to provide guidance for routine dermatopathological evaluations, 101 consecutive IVD and EVD images of skin tumours from a private practice laboratory were collected from March 1st to September 30th, 2013. Colours, structures, and vessels of EVD images were scored and compared with those of the corresponding IVD images by four observers. The concordance between the EVD and IVD images, and gain or loss of colours, structures, and vessels on EVD relative to IVD images were examined.

2) To compare the performance of pathologic evaluation of skin tumours by EVD with derm dotting (DD), with the standard method of skin biopsy processing (chapter IV).

Derm dotting is a simple and easy method to mark focal areas or suspected section planes observed on EVD. To compare the diagnostic performance of the standard method of skin biopsy processing with the practice of EVD with DD, the diagnostic performance in 6.526 skin biopsy specimens examined from 2008 to 2010 with a standard method of processing was compared with 8.584 biopsy specimens examined in 2015 with EVD with DD. Biopsy specimens from both periods were diagnosed by the same dermatopathologist. In this retrospective study, the main outcome measures were clinicopathological characteristics, usefulness of EVD with DD, and turnaround times.

3) To evaluate the application of DD in oral and maxillofacial surgery (chapter V).
The nail varnish used for DD is resistant to different forms of tissue processing, discernible while cutting the paraffin block, and easily recognizable under the microscope. To determine the effectiveness of DD in intraoral tissue specimens, this procedure was used on the intraoral tissues of nine patients who underwent resection of squamous cell carcinoma. The DD method was also tested on frozen sections and, in particular, its usefulness to orient the resection specimen, and to mark suspect borders or areas of interest were examined in this prospective study.

4) To find a histological correlate and an optical explication for rosettes and other white shiny structures observed in polarised dermoscopy (chapter VI).
Rosettes are a specific form of white shiny structures that can be observed with polarised dermoscopy. The precise morphological correlate and optical explanation of this dermoscopic feature are not known. To find an explanation and histopathological correlate, a series of 6.108 consecutive skin biopsies was examined with EVD and when rosettes were present, serial transverse sections were examined with polarisation.

Chapter II Description of Ex Vivo Dermoscopy with Derm Dotting

A new method of processing in dermatopathology should -in theory- meet the following requirements:
‘While processing skin tumours, the dermoscopic information is at disposal and used to orient the macroscopic cutting process in order to make targeted microtome sections. Correlation of the dermoscopic information is made during the microscopic examination. The method has to be implementable in a repetitive and economically justifiable way with acceptable TATs.

Others before us realised that a more guided approach is needed to make sure that the sections in skin pathology are more representative. Different new targeting methods have been proposed. Braun [18] described a micro punch technique. However, this marking system, with a 1-mm micro punch, creates an artefact in the biopsy. Others used stitches to mark and orient the excision specimen [19]. These stitches have to be removed before microtome cutting, also leaving a tissue artifact. Coloured gelatines, acrylic pigments and even Tipp-Ex were tried to mark margins or focal alterations (“hot spots”) in specimens. Yet, none of these methods were practical and none of them met the quality requirements for lesion specific and targeted dermatopathology.
The quality and detailed information of nowadays dermoscopy images could form the informative bridge between the clinician and pathologist and could be the key to a different lesion specific approach of diagnosing skin biopsies. In 2001, Bauer [20] already noticed that dermoscopy could turn the histopathologist’s attention to the suspicious areas in melanocytic lesions. In 2007, Scope [21] was the first to use polarised non-contact dermoscopy as an ancillary method of gross pathology. In a study of seven cases he concluded that the findings of EVD were comparable to IVD and that it was about time for pathologists to learn dermoscopy. Since 2012, Amin [22] used ex vivo contact dermoscopy as a routine part in the examination of pigmented lesions. In a retrospective study of 517 cases, he recommended EVD as a valuable adjunct to histopathology and a surrogate for less than optimal clinical information. In 2013, Malvehy described that the use of ex vivo dermoscopy on fresh tissue can help in selecting the most representative tissue for molecular analysis without compromising the routine diagnostic work on the remaining tissue [23].
Impressed by the potential of this method, we realized that EVD could be an important step towards finding a new lesion specific diagnostic approach of skin tumours. The same year, we routinely started making an ex vivo dermoscopic picture of skin tumours with a Dermlite III dermoscope, connected to a Nikon 1-J1 camera. Technicians were trained in a beginner and later on, an advanced course of in vivo dermoscopy and as such became part of the diagnostic process. The availability of this detailed ex vivo dermoscopic information was a first important step, but secondly a way of marking and targeting these hot spots detected by the ex vivo dermoscopy was needed. After many trials with different dyes and punction methods, simple

nail varnish was tried out as a marking system for suspicious margins and focal lesions seen on the EVD. Nail varnish was found to be an ideal tissue marker for different reasons: it dries fast, it is resistant to processing, and is also visible and traceable in the paraffin block when cut on the microtome (*Figure 3*).

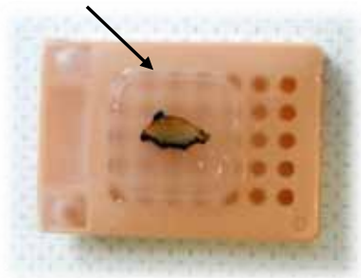


Figure 3 Tissue bloc with blue dot

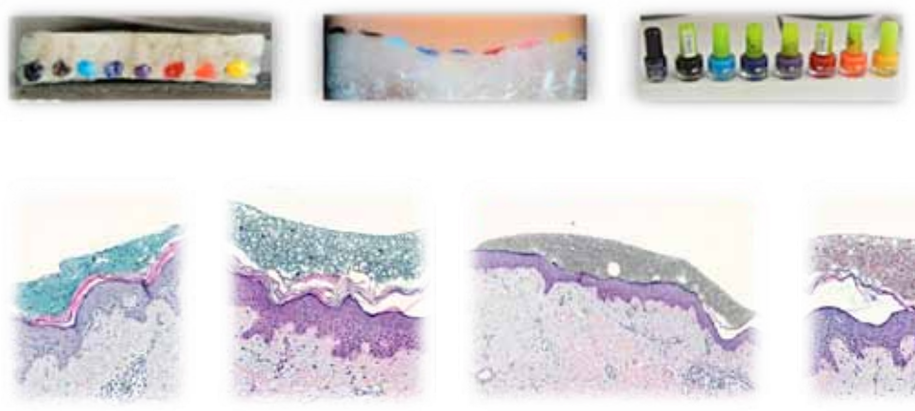


Figure 4 Different colours of nail varnish on skin, in paraffin bloc and in microscopic pictures.

Dots on focal diagnostic lesions are made with a varnish brush or, for very small dots, with a toothpick. Marking lines are made with a special varnish liner brush.

Figure 5 A varnish brush is used for larger dots, varnish liner for marking one lateral border, and a toothpick to mark very small lesions.

'EVD with Derm Dotting' was born and the author gradually realized that this method could meet most of the requirements for lesion specific and targeted examination of skin tumours. After more than 4 years of experience, the author wanted to describe and examine 'EVD with DD' in a scientific controlled way to find out to what extent this new method was implementable on a daily routine basis and to what extent it met the requirements of lesion specific and targeted processing in the diagnosis of skin biopsies.

After 4 years of experience with this method, the following protocol was established: (*Figure 9).*

1) After proper fixation, the specimen is inspected and measured by a technician with experience in dermoscopy. The technician decides whether an EVD image should be made (in about 40-50% of excision specimens) or not. Advice can be asked to the supervising dermatopathologist. A digital photo is taken with a Dermlite III dermoscope connected to a Nikon1-J1 camera (*Figure 6*). As polarised light is used, contact with the specimen is not obligatory.

Figure 6 Dermlite III dermoscope connected to camera

The EVD image is analyzed to identify the most diagnostic areas and critical margins. This dermoscopic information is used to decide how to section and mark the lesion in order to include and not to transect the diagnostic areas, and to ensure the suspicious margins are contained in the sections. The "photograph" is attached to the specimen file in the laboratory informatic system.

2) General orientation of excisions is obtained by inking half of the bottom green and black, respectively. A horizontal border line is also made on the skin rim with black nail varnish. Focal lesions seen on dermoscopy are marked with a nail varnish dot. Different colours can be used. We use three different colours: orange, black and blue. Orange is used when focal lesions have to be traced in the block at microtome sectioning; in this case, fluorescent orange is popping out when approaching the level of the dot with the microtome knife. Blue and black

can easily be noticed under the microscope as a darker blue or black grey granular layer (*Figure 4*).
Punch biopsies, taken to investigate inflammatory diseases, are dotted in the center to guide the technician into the most active part of the lesional skin. When, focal lesions, like colour changes or crusts are seen, they are marked and traced.

The inking and marking procedure based on dermoscopic information permits lesion-specific sectioning in order to centrally include and not transect the diagnostic areas or suspicious margins. Most clinically benign melanocytic lesions are bread-loaf transverse-sectioned without processing of tips. In non-melanoma skin cancer lesions, the tips are always processed. In atypical melanocytic lesions and melanomas, the transverse sections are oriented in order to include the different structures, suspicious margins or focal lesions identified on EVD. Elongated lesions with clear transverse margins on EVD are sectioned horizontally or tangentially. Acral naevi are cut perpendicular to the epidermal ridge pattern.

Orientation, inking, nail varnish and section planes are indicated on a drawing to help orienting the tissue for the pathologist.

3) Controlled microtome cutting is performed and paraffin ribbons are made from one or two depths (depending on the size of the biopsy) before reaching the dotted area of interest. Specimens without dot are cut at 2 or 3 levels.The ribbons are not dropped in a water bath, but stretched in rows on a wooden plate. From every depth, two to three cuts (depending on the size of the fragments) are stretched on a glass slide and dried on a hot plate (*Figure 7*). This results in one slide with two (big biopsy) or three (small biopsy) depths on it, with the deepest depth being the one in the dot.
The residual ribbons are identified and conserved at +4°C in the fridge, in case additional stainings are needed.

Figure 7 Plate with paraffin ribbons and slide with three cuts from three depths.

In the latter case, the pathologist can ask for a specific cut from the ribbons to further examine the lesion. Only when the case has been signed out, the remaining cutted sections are thrown away.

4) Under the microscope, the pathologist can easily orient the fragments permitting a fast and lesion specific examination. Furthermore, the markings on slides prove that cuts are representative of the areas identified at EVD. The ex vivo image is correlated with the histological findings. Only when all these findings correspond and fully explain the dermoscopic findings, a final diagnosis can be made *(Figure 8).*

Figure 8 A pathologist correlates histologic aspect with the dermoscopic image and the clinical information

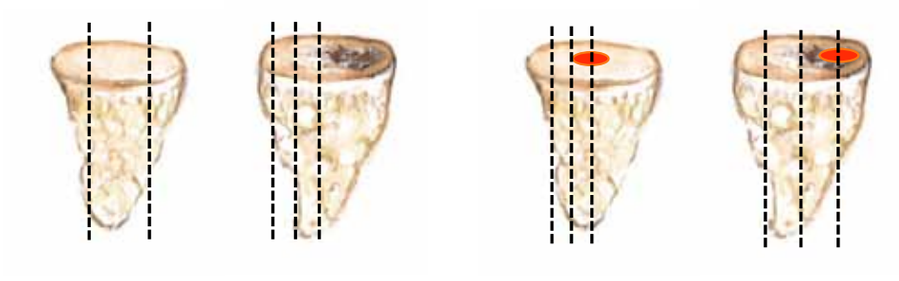
Standard method

EVD with DD

Punch biopsy

Random cuts give no central information, focal lesions may be missed. Block can be exhausted for eventual additional stains.

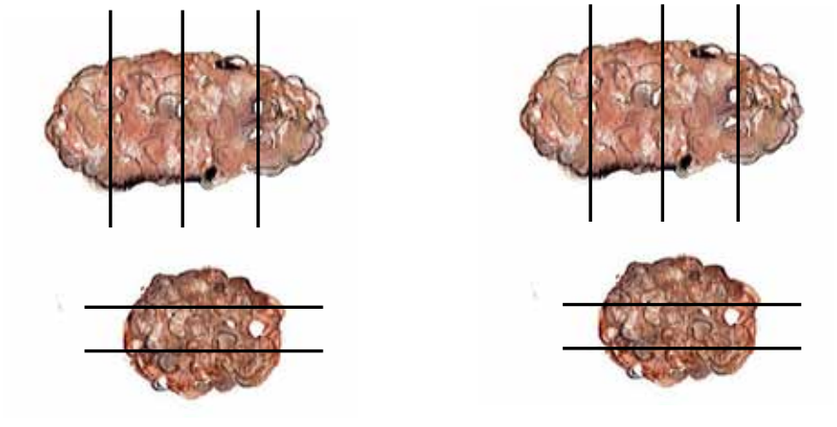
Three depths with marked central section and residual tissue for deeper sections. Marked section of focal lesions.



Shaving / curettage

Random longitudinal or transverse sections at three levels result in representative slides of the lesion.

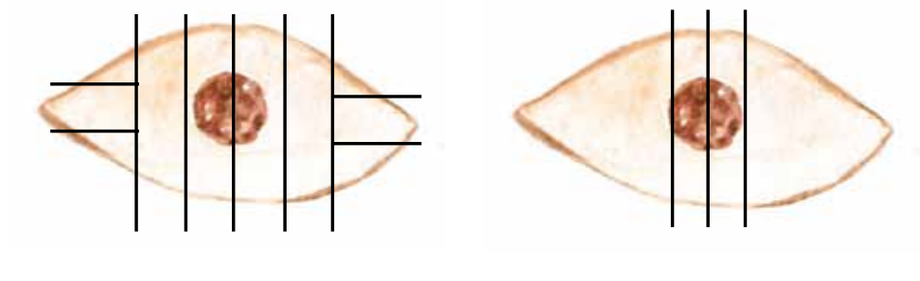
Random longitudinal or transverse sections at three levels result in representative slides of the lesions.



Naevi

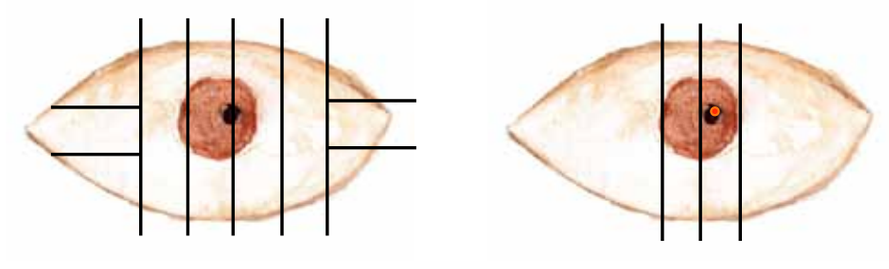
Blind transverse bread-loaf sections with processing of tips results in many slides without lesional tissue.

Oriented sectioning without processing tips results in slides with only lesional tissue.



Small, focal lesions can be transected and risk to get lost in slides.

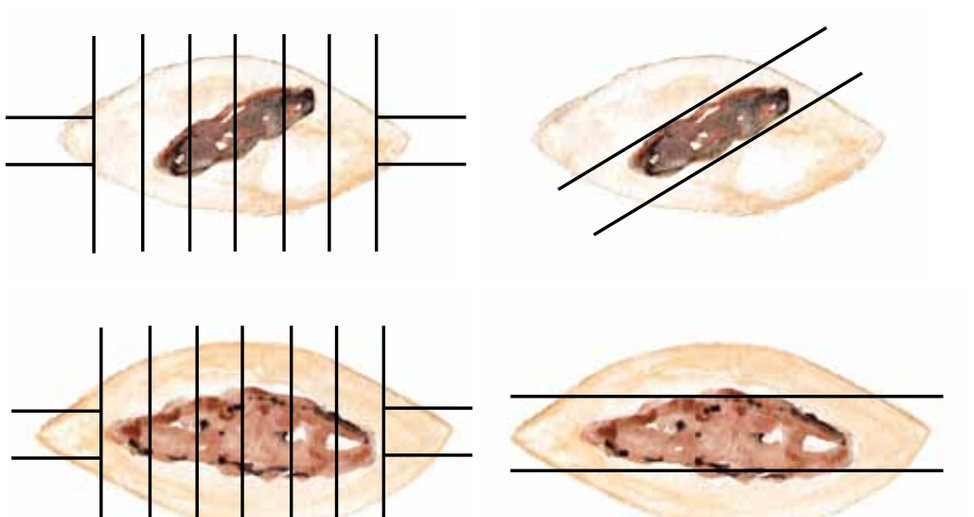
Focal lesion is dotted and traced in the slide.



Elongated / perpendicular lesions

Blind transverse bread-loaf sections result in slides with short transverse cuts of the lesional tissue.

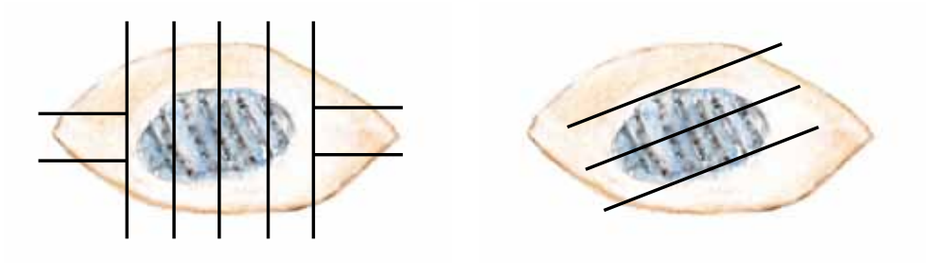
Longitudinal or perpendicular sections result in less slides and longitudinal lesional slides.



Acral naevi

Blind transverse bread-loaf section results in slides without parallel furrow pattern information.

Oriented sectioning results in slides with parallel furrow pattern information.



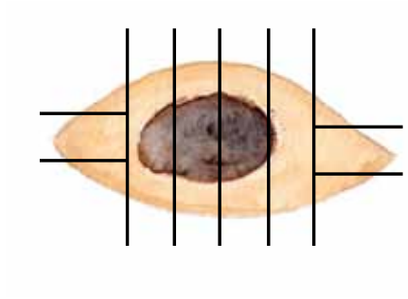
Atypical melanocytic lesions, melanomas and non-melanoma skin cancer

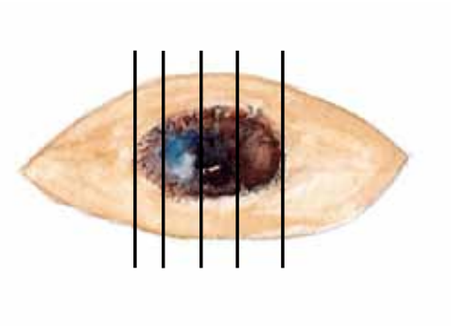
Excision is inked at dermal and subcutaneous side. It is not possible to correctly orientate the lesion.

Excision is inked with green and black and one lateral border is lined with black varnish, in order to correctly orient specimen.

With naked eye inspection, no structures and only one colour is seen. Dermal information is missing. With bread-loaf sectioning there is no control that all diagnostic areas are present in the slides. Deeper cuts may be necessary to correctly diagnose the lesion.

EVD discloses colours and structures. Blue, grey or crystalline structures can lead to areas of regression, fibrosis or the deepest melanin deposition. Sectioning can be oriented to include all diagnostic areas of the lesion.





Naked eye inspection misses small areas or ulceration or suspicious section margins.

EVD discloses small areas of ulceration and suspicious section margins. They can be dotted and traced in the slide.

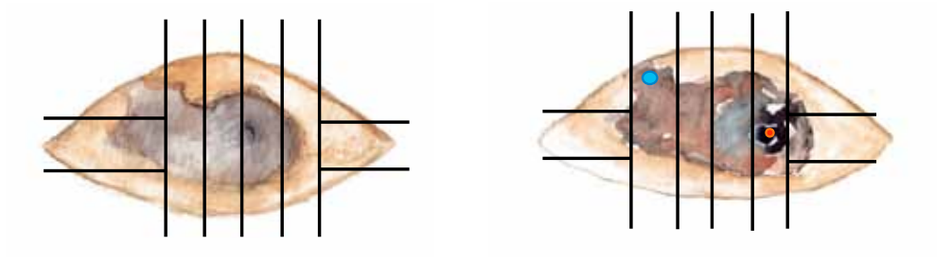


Figure 9 Comparison between standard method and EVD with DD

Routine Use of Ex Vivo Dermoscopy With "Derm Dotting" in Dermatopathology

To the Editors:

Recently, Amin and Fraga[1] presented in this journal their experience with ex vivo dermoscopy of cutaneous biopsies for melanocytic lesions. They reexamined 517 melanocytic neoplasms and were able to correlate in most cases the dermoscopic patterns with the microscopic findings. Reevaluation of 25 remaining ambiguous lesions with concurrent ex vivo dermoscopy images (EVDI) lead to a definite diagnosis in 16 cases, only 7 remained inconclusive. They recommend EVDI as a valuable adjunct to histopathology and a surrogate for less than optimal clinical information.

Scope et al[2] already described the use of this method in dermatopathology and correlated the ex vivo pictures with the in vivo pictures of the same lesions. They advocate the diagnostic aid of this technique in the macroscopy of the skin biopsies and its usefulness as first step to guide tissue sectioning in gross pathology.

Since 2012, we also routinely take an ex vivo dermoscopic image of most pigment lesions and other tumoral lesions we receive at our dermatopathology laboratory. We use a dermlite II dermoscope that is connected to a Nikon 1-J1 camera. As described by Amin and Fraga,[1] the disposition of this extra information during the microscopic examination is very helpful especially in ambiguous lesions and the evaluation of section margins of the lesion.

As mentioned by Scope et al,[2] with this tool, technicians who handle the tissue can better orient and decide how to process and cut the lesion. In our laboratory, the 2 technicians responsible for the processing of skin biopsies have learned the basic dermoscopic principles and use this extra information to bring

The authors declare no funding and conflicts of interest.

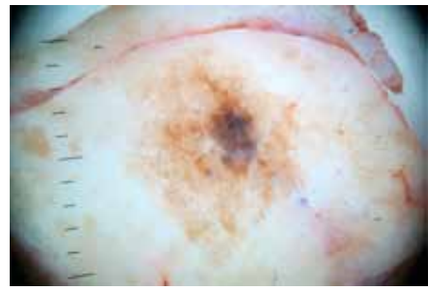


FIGURE 1. Ex vivo dermoscopic picture of an irregular nevus with a new black dot.

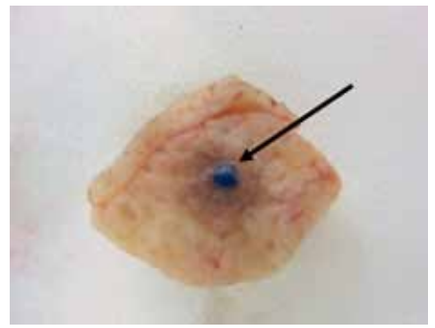


FIGURE 2. Blue varnish marking of the black dot.

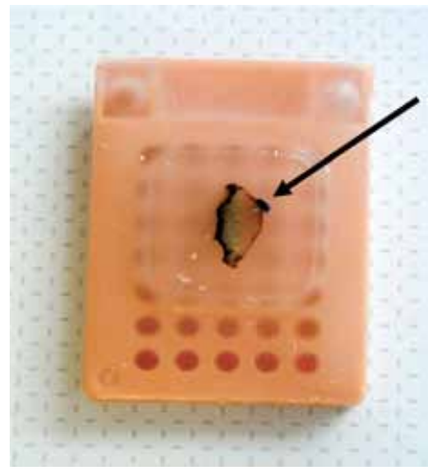


FIGURE 3. Tissue block cut at the level of the dotted area.

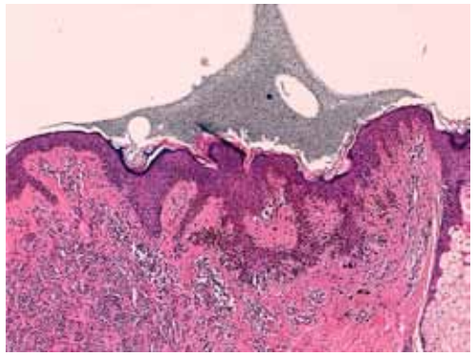


FIGURE 4. Dotted area corresponds to the area of focal hyperpigmentation with lentiginous epithelial reaction.

the most diagnostic tissue cuts on the microscope slide. Especially in lesions where the dermatologist excises a lesion because of a focal change or new lesion in a skin tumor, the EVDI enables the technician to include the diagnostic zones in the chosen tissue block.

To optimize the use of this extra information, we developed a simple and cheap marking system that makes it possible to recognize and trace this diagnostic areas revealed by the dermoscopy. Black dots, areas of depigmentation, structureless zones, ulcerated areas, and suspicious section margins are marked with a simple color dot immediately after their detection with the dermoscope (Figs. 1, 2). For this "derm dotting", we use standard nail varnish. This type of varnish dries very fast and enables us to make marks from 1 to 3 mm. The product is applied with a brush for larger marks and with a toothpick for smaller dots. The color dot is resistant to the different tissue processing phases and permits the technician to cut safely and immediately into the center of the diagnostic zone. The dot area is easily discernable while cutting the block (Fig. 3). Under the microscope, the marked zone is recognized as a thick black gray granular plaque on the horny layer with a thickness of 2–3 mm (Fig. 4). We do not only use this derm dotting for pigment lesions but also for other tumoral lesions where dermoscopy shows diagnostically interesting areas. Narrow margins seen on the EVDI can be selectively marked, selected for imbedding, and easily traced under the microscope. Ulceration, important in staging of pigment lesions, is often inadequately assessed with current standard techniques. With this marking system, we can cut right in the center of the ulcerative part and stage the lesion in a correct way. Also basal cell carcinomas often penetrate deepest in their ulcerated part, and examination of this part leads to a more correct assessment of thickness and more secure margin evaluation. Finally, in the smaller whole-mounted lesions, the center of the lesion can be marked to lead the technician safely when cutting the block.

We are very enthusiast about this technical "trick" and have happier technicians who have to make less numerous deeper cuts. Besides this important economical time-saving advantage, it also upgrades the work of the processing technicians who become part of the diagnostic process. The technique is simpler than the micropunch technique developed by Braun et al.[3] Furthermore, our technique does not interfere with the quality of the histology of the underlying lesion. Their marking system with a 1-mm micropunch creates an artifact in the biopsy that interrupts the histological picture and that can create problems in the handling of the

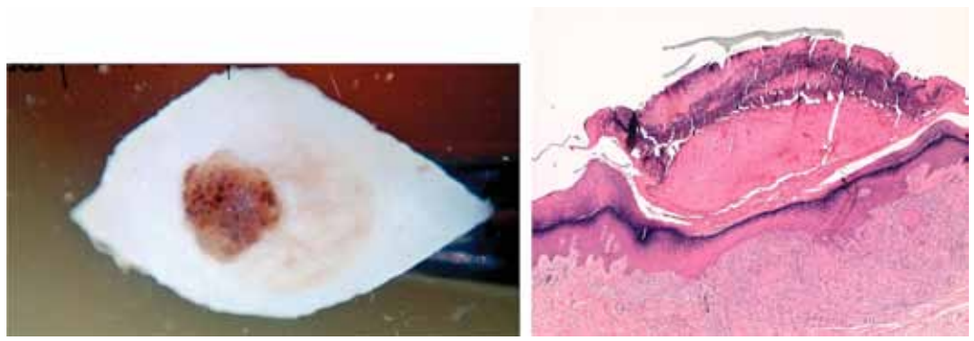


FIGURE 5. Traumatized nevus with marked fibrino pustulous crust formation.

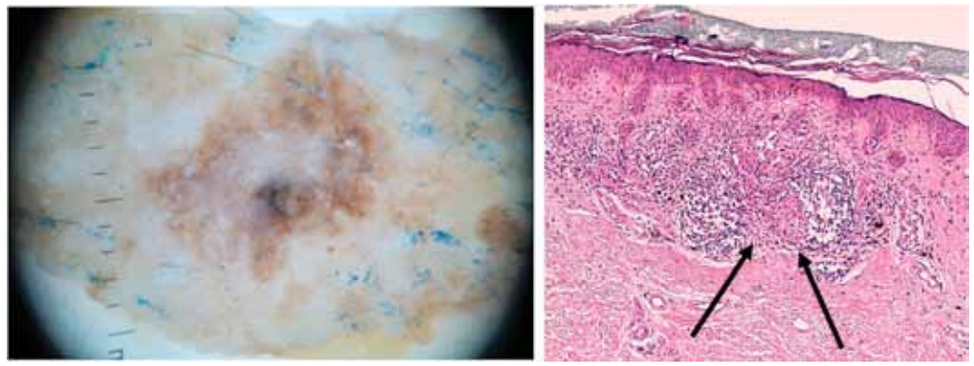


FIGURE 6. Melanoma in situ with central structureless pale zone, after derm dotting corresponding to the zone of maximal vertical invasion with a depth of 0.39 mm.

tissue section. The derm dotting can also be, as in their study, primarily done by the referring dermatologist just before the resection of the lesion. In this setting, however, a part of the dermoscopic information is hidden for the dermatopathologist. We prefer to have the intact dermoscopic picture and decide ourselves which areas will be marked.

Finally, it enables the dermatopathologist to better understand the lesion and make a mirror correlation with the dermoscopic information. Black dots, blue zones, depigmentation zones, and areas of hemorrhage are easily traceable and find their histological explanation (Figs. 5, 6). This individualized skin biopsy approach, however, demands a continuous communication and education of the dermatopathologist and his technical staff. We believe that this derm dotting technique of dermoscopically selected areas makes diagnosing a lesion by the dermatopathologist more fun, more accurate, and at the end makes him more confident when signing out the protocol he was interpreting the right spot of the lesion and was not making a false-negative or understaged diagnosis. It can be an interesting, economizing, cheap, and easily implementable technique for all dermatopathology laboratories.

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Chapter III Comparison of ex vivo and in vivo dermoscopy in dermatopathologic evaluation of skin tumours

Original Investigation

Comparison of Ex Vivo and In Vivo Dermoscopy in Dermatopathologic Evaluation of Skin Tumors

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IMPORTANCE Ex vivo dermoscopy (EVD) can be a valuable tool in routine diagnostic dermatopathologic evaluation.

OBJECTIVES To compare in vivo dermoscopy (IVD) and EVD and to provide guidance for routine dermatopathologic evaluations.

DESIGN, SETTING, AND PARTICIPANTS This observational study collected 101 consecutive IVD and EVD images of skin tumors from a private dermatology practice from March 1 to September 30, 2013. Four observers (3 dermatologists and 1 dermatopathologist) blinded to the histopathologic diagnoses independently scored and compared the colors, structures, and vessels of EVD images with those of the corresponding IVD images. Data were analyzed from January 1 to March 31, 2014.

MAIN OUTCOMES AND MEASURES Concordance between the EVD and IVD images and gain or loss of colors, structures, and vessels on EVD relative to IVD images.

RESULTS The final analysis included 404 observations of 101 images. The EVD image was generally similar to the corresponding IVD image but clearly darker, with new areas of blue in 130 of 404 observations (32.2%) and white in 100 of 404 observations (24.8%) and loss of red in 283 of 404 observations (70.0%). Most structures were well preserved. New structureless areas were found in 78 of 404 observations of EVD images (19.3%), and new crystalline structures were detected in 68 of 404 observations of EVD images (16.8%). On EVD images, squames and crusts were lost in 56 of 404 observations (13.9%) and 43 of 404 observations (10.6%), respectively. Blood vessels were lost in 142 of 404 observations of EVD images (35.1%).

CONCLUSIONS AND RELEVANCE The EVD image is an important new tool in dermatopathology and may give direction to targeted tissue processing and examination of skin tumors.

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In daily practice, most pathology laboratories process skin biopsy specimens without access to the clinical and/or dermoscopic images. In pigmented skin tumors, this information can be crucial to process and diagnose the lesion correctly. Ex vivo dermoscopy (EVD) was first introduced as a valuable tool in dermatopathology by Scope et al,[1] who compared EVD and in vivo dermoscopy (IVD) of 7 lesions and found that EVD reduced errors by aiding selection of areas in which to perform step sectioning. Amin and Fraga[2] reviewed 517 biopsy specimens with EVD and histopathologic correlation. In their study, most features observed in IVD images were also present in EVD images. In 72% of the ambiguous lesions, a more definite diagnosis was assigned after review with the EVD image. In 7.7% of cases, the section margins were reclassified after reviewing the EVD image. Thus, support for the use of EVD for diagnosis and clinicopathologic correlation exists.

With increasingly smaller diameter lesions undergoing biopsy, the focal changes are only visible with dermoscopy; therefore, communication of this dermoscopic information to the pathologist is important. In many dermatopathology laboratories, this communication is often insufficient or totally absent, and one can presume that these suspect areas are often missed with the standard random sectioning technique that examines less than 2% of the tissue.[3] Some surgeons mark areas of interest for the pathologist with a 1-mm punch or use suture marking to direct the pathologist to these suspect areas.[4] However, in our experience, these methods are not satisfactory because they create a disturbance artifact in the tissue

specimen. In combination with marking specific and suspected lesions on the biopsy specimen with nail varnish (derm dotting),[5] EVD is a simple and easy method that brings this crucial information to the pathologist and in the slides to be examined. The practical method and advantages of this new clinicopathologic approach of dermatopathology have been described elsewhere.[5] Using this method, the exact morphologic correlation of dermoscopic characteristics, such as rosettes, can be identified.[6] In a recent study,[7] derm dotting has been used successfully as a marking system on mucosal tissue, namely, in maxillofacial surgery.

Although several studies have compared IVD and EVD,[1,8,9] no systematic comparison by multiple observers in a real-world community setting has been performed, to our knowledge. Therefore, 101 EVD images of consecutive tumoral skin lesions excised in a single dermatology practice were scored independently for different dermoscopic colors, structures, and vessels by 4 independent observers. We compared the results of both techniques to define similarities and relevant differences.

Methods

We included 101 consecutive skin tumors obtained from a private dermatology practice of 2 of us (K.V. and S.L.) from March 1 to September 30, 2013 (**Table 1**). All lesions were documented with in vivo contact polarized dermoscopy (Dermlite 3; 3Gen, Inc) connected to a camera (PowerShot G11; Canon). After fixation in 10% buffered formalin for 12 to 24 hours, we (M.H., I.D.W., and N.D.) obtained contact ex vivo images at the dermatopathology laboratory with a digital camera (Nikon 1 series; Nikon) connected to a polarized dermoscope (Dermlite 3). The ethics committee of University Hospital Ghent approved this study. Because deidentified images and histopathologic diagnoses were examined, the ethics committee waived the need for informed consent.

The IVD and EVD images (JPEG) were scored separately by 3 dermoscopy-experienced dermatologists (K.V., S.L., and L.B.) and 1 dermatopathologist with experience in EVD (M.H.). The 4 observers were blinded to the histopathologic diagnosis (Table 1). The quality of each image was scored as good, moderate, or bad by each observer. After elimination of 1 case (scored as bad quality by all 4 observers), the scores of the remaining 101 IVD and EVD images were compared for significant characteristics, with each observer blinded to the reading of the others (McNemar test). All observations per characteristic (all added scores by item by 4 observers) were subtyped as concordant (no difference between the IVD and EVD images) or discordant. The latter images were subtyped as loss in EVD (ie, the presence of an item on the IVD image but not on the EVD image) and gain in EVD (ie, the presence of an item on the EVD image but not on the IVD image).

Data were analyzed from January 1 to March 31, 2014. We calculated a κ value per item. A significant difference between IVD and EVD was defined as a significant McNemar test result in at least 2 observers and preferentially in 3 or even 4 observers. In a second phase, a similar analysis was performed in the diagnostic subcategories of basal cell carcinomas (BCCs) (n = 31) and invasive melanomas (n = 12). All statistical tests were 2 tailed, and $P < .05$ was considered statistically significant. The analyses were conducted in SPSS software (version 22; IBM).

Table 1. Histopathologic Diagnosis of the 101 Included Skin Tumor Lesions

Type of Tumor	No. of Cases
Melanocytic lesions	47
Congenital nevi	1
Clark or flat nevi	17
Light to moderate dysplasia	16
Severe dysplasia	1
Unna nevi	2
Miescher nevi	1
Acral nevi	1
Blue nevi	5
Spitz or Reed nevi	6
Lentigo maligna	1
Melanoma in situ	1
Invasive melanoma	12
Nonmelanocytic lesions	54
Dermatofibroma	6
Seborrheic keratosis	4
Solar lentigo	3
Angioma	2
Bowen disease	1
Invasive squamous cell carcinoma	3
Basal cell carcinoma	31
Superficial	7
Nodular	21
Infiltrative	3
Pilomatricoma	1
Molluscum contagiosum	1

Results

In total, we included 47 melanocytic and 54 nonmelanocytic lesions (Table 1). For each characteristic, 404 observations were registered. We found concordance in the presence or absence of a feature in 10 549 of 13 736 scored observations (76.8%) and discordance in the remaining 3187 scored observations (23.3%) (**Table 2**).

Colors

In general, colors are well preserved in EVD compared with IVD images. Brown showed concordance between EVD and IVD images in 348 of 404 observations (86.1%). In EVD images, we found a gain of dark brown in 72 of 404 observations (17.8%) (2 observers). A gain of blue on EVD images was seen in 130 of 404 observations (32.2%) and a gain of white in 100 of 404 observations (24.7%) (3 observers for both). In contrast, red disappeared in 283 of 404 observations of EVD images (70.0%) (4 observers).

Table 2. Results of Scored Characteristics in 101 Tumoral Skin Lesions

Characteristic	No. (%) of Observations (N = 404)			κ Value	No. of Observers With Significant McNemar Test[a]
	Concordant Observations	Loss on EVD	Gain on EVD		
Black	350 (86.6)	23 (5.7)	31 (7.7)	0.65	1
Brown	348 (86.1)	14 (3.5)	42 (10.4)	0.21	2
Dark brown	310 (76.7)	22 (5.4)	72 (17.8)	0.49	2
Light brown	300 (74.3)	59 (14.6)	45 (11.1)	0.10	1
Blue	261 (64.6)	13 (3.2)	130 (32.2)	0.27	4
Red	118 (29.2)	283 (70.0)	3 (0.7)	0.01	4
White	277 (68.6)	27 (6.7)	100 (24.8)	0.35	3
Comedolike openings or milialike cysts	389 (96.3)	7 (1.7)	8 (2.0)	0.59	0
Blue ovoid nests	369 (91.3)	21 (5.2)	14 (3.5)	0.50	0
Red-blue lagoons	395 (97.8)	8 (2.0)	1 (0.2)	0.51	0
Network	352 (87.1)	35 (8.7)	17 (4.2)	0.66	1
Branched streaks	351 (86.9)	34 (8.4)	19 (4.7)	0.64	1
Globules and dots	328 (81.2)	31 (7.7)	45 (11.1)	0.57	1
Structureless areas	304 (75.2)	22 (5.4)	78 (19.3)	0.25	3
Crystalline structures	299 (74.0)	37 (9.2)	68 (16.8)	0.46	2
Yellowish globular structures	403 (99.8)	1 (0.2)	0	0.75	0
Strawberry pattern	390 (96.5)	14 (3.5)	0	0.35	1
White circles	390 (96.5)	9 (2.2)	5 (1.2)	0.24	0
Ulceration	383 (94.8)	16 (4.0)	5 (1.2)	0.35	0
Squames or keratin	318 (78.7)	56 (13.9)	30 (7.4)	0.32	0
Crusts	345 (85.4)	43 (10.6)	16 (4.0)	0.42	2
Pseudonetwork	388 (96.0)	13 (3.2)	3 (0.7)	0.56	0
Hyperpigmented follicular openings	395 (97.8)	8 (2.0)	1 (0.2)	0.71	0
Annular granular pattern	399 (98.8)	5 (1.2)	0	0.58	0
Rhomboidal structures	398 (98.5)	5 (1.2)	1 (0.2)	0.77	0
Peppering	380 (94.1)	7 (1.7)	17 (4.2)	0.63	0
Brainlike or fingerprint appearance	395 (97.8)	7 (1.7)	2 (0.5)	0.36	0
Vessels	252 (62.4)	142 (35.1)	10 (2.5)	0.25	4
Commalike	394 (97.5)	8 (2.0)	2 (0.5)	0.31	0
Dotted	353 (87.4)	43 (10.6)	8 (2.0)	0.02	2
Hairpin	389 (96.3)	10 (2.5)	5 (1.2)	0.36	0
Branched	312 (77.2)	85 (21.0)	7 (1.7)	0.26	4
Linear irregular	337 (83.4)	53 (13.1)	14 (3.5)	0.32	2
Glomerular	397 (98.3)	6 (1.5)	1 (0.2)	0.23	0

Abbreviation: EVD, ex vivo dermoscopy.

[a] All observations per characteristic (all added scores by item by 4 observers) were subtyped as concordant or discordant. A significant difference between in vivo dermoscopy and EVD was defined as a significant McNemar test result in at least 2 observers and preferentially in 3 or even 4 observers.

Similar color differences existed for diagnostic subgroups, especially in BCCs. A gain of brown in BCCs was seen in 22 of 124 observations (17.7%) and a gain of light brown in 24 of 124 observations (19.4%) (2 observers) (eTable 1 in the Supplement). A gain of blue was present in 51 of 124 observations (41.1%) (3 observers) and a gain of white in 41 of 124 observations (33.1%). We found a loss of red in 109 of 124 observations (87.9%) (4 observers). Among invasive melanoma lesions, a gain of white was seen in 17 of 48 observations (35.4%) (2 observers). Loss of red was seen in 35 of 48 observations of melanoma cases (72.9%) (3 observers) (eTable 2 in the Supplement).

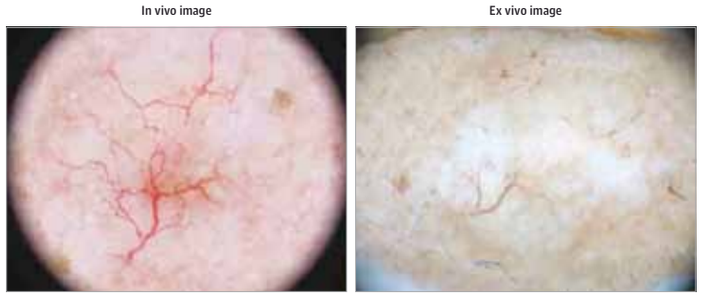
Structures

Most structures were well preserved, and the concordance between EVD and IVD images was good, with a 95% CI of 0.50 to 0.77. Structureless areas and crystalline structures were observed more frequently on the EVD images, with a gain in 78 of 404 observations (19.3%) (4 observers) and in 68 of 404 observations (16.8%) (3 observers), respectively. In BCCs, crystalline structures were found on EVD images in 67 of 124 observations (54.0%). Crusts were lost in 13 of 124 observations (10.5%) (3 observers).

Vascular

Vessels were lost on EVD in 142 of 404 observations (35.1%), which means that in 142 of 212 vessel observations on IVD (67.0%), the vessels disappeared on EVD images (4 observers). In 70 of 212 IVD vessel observations (33.0%), some vascularization was preserved on EVD as light brown, interrupted, branched or irregular linear structures (4 observers).

Figure 1. Example of Basal Cell Carcinoma (BCC)



The nodular type BCC was located on the back of a man in his 40s. The vessels were partly preserved on ex vivo dermoscopy as light brown curved and branched lines. The ex vivo dermoscopic image showed a new white crystalline structureless area that created a sharper delineation of the tumor compared with the in vivo dermoscopic image.

Hairpin vessels, dotted vessels, and glomeruloid vessels more often were lost completely. In BCCs, in vivo vessels were seen in 115 of 124 observations (92.7%), and the vascularization disappeared ex vivo in 76 of 115 observations (66.1%), but partial preservation was seen in 45 of 115 vessel observations (39.1%) (3 observers). Most preservation occurred among the thicker branching vessels.

Discussion

In general, most structures and colors observed on IVD can also be recognized on EVD, confirming earlier findings.[1,2] In our study, we found a general concordance of 76.8% between EVD and IVD images. The most important differences between IVD and EVD images concern the colors. In this comparative study, red disappeared completely on EVD in 70.0% of the cases. In BCCs and melanomas, these percentages were even higher at 87.9% and 72.9%, respectively. Malvehy et al[9] also showed that colors and structures were comparable between IVD and EVD performed on freshly excised tissue. On the fresh tissue examined with EVD, they found loss of vessels, red, pink, and vascular blush. In the present comparative study, EVD was performed on fixed biopsy specimens.

Loss of red was reported earlier by Scope et al.[1] Red disappears mainly by collapse of the vascular structures. However, red also transforms into brown by the fixation process with formaldehyde. In the skin, melanin and hemoglobin are the most important chromophores that selectively absorb different wavelengths. Owing to cross-linking on the molecular level during the formalin fixation process, oxyhemoglobin gradually oxidizes into methemoglobin.[10] The spectrum of light absorption hereby changes, and the red changes into brown.

In accordance with these findings, most vessel structures were no longer discernible on EVD or visible as light brown straight, curved, or clothed lines (**Figure 1**). This change was observed mainly for branched vessels and linear irregular vessels. The visualization of blood vessel remnants as these light brown lines or clods seems to depend on the blood vessel diameter; the larger the vessels, the greater chance that some remnants will be observed on EVD images (eg, dotted, comalike, hairpinlike, and glomerular vessels are more difficult to recognize on EVD images). However, with the shift to brown after fixation, smaller vessel structures are easily overlooked and mistaken for dots, small crusts, or focal erosive areas. This vessel color change may explain in part the observed shift in general from light brown to dark brown with a gain of dark brown in 72 of 404 observations (17.8%) and a loss of light brown in 59 of 404 observations (14.6%).

Another factor that seems to influence the observation of vessel structures with EVD is the fixation time. With shorter fixation times, the chance of observing even smaller vessel remnants on EVD increases. In insufficiently fixed biopsy specimens, some vascular red can still be observed.

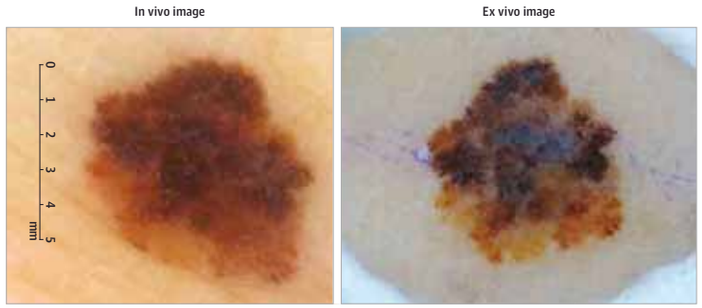
In contrast to the red that almost completely disappeared on EVD, the blue and white were observed more frequently, with an increase in 32.2% and 24.8% of the cases, respectively (for BCC, 41.1% and 33.1%, respectively). Blue and white probably become visible or are accentuated by the loss of red during the fixation process. Optical clearing of the tissue by methylene glycol, the hydrated metabolite of formaldehyde, during the fixation process, with reduced scattering of light in collagenous skin tissue; therefore, deeper penetration may also explain the appearance or accentuation of blue and white.[11] Moreover, when this newly discernable blue or white on the EVD image is traced with derm dotting,[5] microscopic examination may reveal important diagnostic areas, such as the foci of invasion in a melanoma lesion that otherwise would be diagnosed as a purely in situ lesion (**Figure 2**).

In addition to an increased number of cases in which blue could be seen on EVD images, the blue present on IVD images was also more prominent on EVD images. This phenomenon was seen through accentuation or appearance of a blue veil (**Figure 3**) and in lesions with a significant presence of deeper dermal melanin pigment (Figure 2). Especially in blue nevi, the diagnostic steel blue color was well preserved, and the image was almost identical to the IVD image.

This gain in white and structureless areas with EVD was accentuated in BCCs, for which 106 of 124 observations (85.5%) showed a white structureless area. In this way, EVD revealed the underlying fibrotic stromal reaction and demonstrated a white structureless area in nearly one-third of the EVD images. Furthermore, the fixation process led to more frequent presence and clearer visualization of crystalline structures in 21 of 124 observations (16.9%). This finding is typical in the case of dermatofibromas and BCCs. The ex vivo image of the dermatofibromas with the stellar white center and crystalline structures hereby becomes very diagnostic. In BCCs, crystalline structures were seen in 54.0% of the EVD images, an increase of 16.1% compared with the IVD images. In some cases of mainly superficial BCCs, these changes created a very different image with a zone of new white, crystalline structures and sharper demarcation (Figure 1). This change could be very useful for proper orientation and evaluation of free margins in BCCs. Other structural elements that can show variation include crusts that tend to disappear on EVD images, especially in BCCs. This tendency is probably owing to detachment of these structures during the fixation and manipulation of the biopsy specimen.

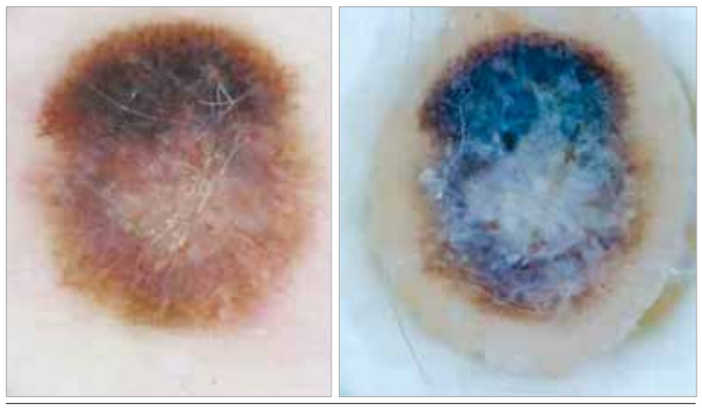
A limitation of this study was the use of 2 different camera systems (Canon vs Nikon) to obtain the IVD and EVD images. In daily practice of IVD, however, different systems are used. Although this finding may have accounted for subtle differences, the observers had the experience that the reported differences between IVD and EVD could not be allocated to the different camera systems. As described in the introduction, our conclusions are in line with the results of other reports. Although the present study pointed out the benefits of EVD for the diagnostic process of dermatopathology, this study did not assess the implications of adding EVD to the potentially improved pathologic diagnostic accuracy. Future research will examine the implications for the diagnostic process.

Figure 2. Example of Melanoma



The lesion (Breslow thickness, 0.5 mm) was located on the abdomen of a woman in her 60s. A new blue area on the ex vivo dermoscopic image disclosed the only invasive area in this melanoma.

Figure 3. Example of Spitz Nevus



The lesion was located on the right upper arm of a girl in her teens. A large new blue veil was seen on the ex vivo image.

Conclusions

Ex vivo dermoscopy images are broadly similar to IVD images but also have clear differences and the potential to improve the pathologic diagnosis of skin tumors by provid-

ing direction to target tissue for processing and examination. Training in dermoscopy is required for the pathologist and the technical staff. More research is needed to determine the exact effect of this new method on margin evaluation and on the accuracy of diagnosis and appropriate staging of melanoma.

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Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Haspeslagh, Noë, Degryse, Brochez.
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Supplementary Online Content

Haspeslagh M, Vossaert K, Lanssens S, et al. Comparision of ex vivo and in vivo dermoscopy in dermatopathologic evaluation of skin tumors. *JAMA Psychiatry*. Published online January 6, 2016. doi:10.1001/jamadermatol.2015.4766.

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Results of Scored Characteristics for Colors and Vessels in Basal Cell Carcinomas

Characteristic	Concordant observations	Loss on EVD	Gain on EVD	Kappa value	Number of observers with significancy (Mc Nemar)
Black	115 (92.8%)	5 (4.0%)	4 (3.2%)	0.49	0
Brown	97 (78.2%)	5 (4.0%)	22 (17.8%)	0.02	2*
Dark brown	90 (72.6%)	7 (5.6%)	27 (21.8%)	0.36	0
Light brown	84 (67.7%)	16 (12.9%)	24 (19.4%)	-0.03	2*
Blue	67 (54.1%)	6 (4.8%)	51 (41.1%)	0.17	3**
Red	15 (12.1%)	109 (87.9%)	0 (0.0%)	0.05	4***
White	77 (62.1%)	6 (4.8%)	41 (33.1%)	0.24	3**
Vessels	45 (36.3%)	76 (61.3%)	3 (2.4%)	-0.08	3**
Comma like	123 (99.2%)	0 (0.0%)	1 (0.8%)	0.75	0
Dotted	115 (92.7%)	5 (4.1%)	4 (3.2%)	0.21	0
Hairpin	119 (96.0%)	2 (1.6%)	3 (2.4%)	0.23	1
Branched	46 (37.1%)	76 (61.3%)	2 (1.6%)	0.06	3**
Linear irregular	85 (68.5%)	27 (21.8%)	12 (9.7%)	-0.03	1
Glomerular	123 (99.2%)	0 (0.0%)	1 (0.8%)	0.75	1

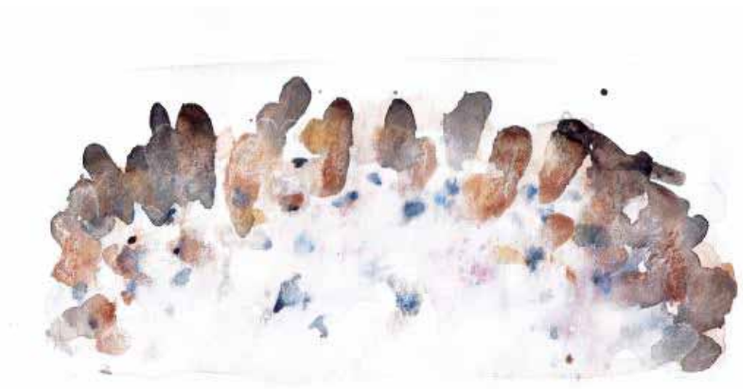
N=4×31 (124).

eTable 2. Results of Scored Characteristics for Colors and Vessels in Melanomas

Characteristic	Concordant observations	Loss on EVD	Gain on EVD	Kappa value	Number of observers with significancy (Mc Nemar)
Black	35 (72.9%)	5 (10.4%)	8 (16.7%)	0.26	0
Brown	42 (87.5%)	0 (0.0%)	6 (12.5%)	0.25	0
Dark brown	37 (77.1%)	0 (0.0%)	11 (22.9%)	0.22	0
Light brown	37 (77.1%)	5 (10.4%)	6 (12.5%)	0.13	0
Blue	36 (75.0%)	0 (0.0%)	12 (25.0%)	0.6	0
Red	12 (25.0%)	35 (72.9%)	1 (2.1%)	0.01	3**
White	27 (56.3%)	4 (8.3%)	17 (35.4%)	0.19	2*
Vessels	40 (83.3%)	7 (14.6%)	1 (2.1%)	0.17	0
Comma like	46 (95.8%)	2 (4.2%)	0 (0.0%)	0.04	0
Dotted	45 (93.8%)	3 (6.2%)	0 (0.0%)	0.06	0
Hairpin	47 (97.9%)	1 (2.1%)	0 (0.0%)	0.02	0
Branched	45 (93.7%)	1 (2.1%)	2 (4.2%)	0.06	0
Linear irregular	40 (83.3%)	8 (16.7%)	0 (0.0%)	0.17	0
Glomerular	48 (100.0%)	0 (0.0%)	0 (0.0%)	0.00	1

N=4×12 (48).

Chapter IV Pathologic evaluation of skin tumours with ex vivo dermoscopy with derm dotting



JAMA Dermatology | Original Investigation

Pathologic Evaluation of Skin Tumors With Ex Vivo Dermoscopy With Derm Dotting

Marc Haspeslagh, MD; Isabelle Hoorens, MD; Nele Degryse, BA; Ine De Wispelaere, BA; Annemarie Degroote, MD; Sarah Van Belle, BA; Jan Verboven, IR; Katrien Vossaert, MD; Fabio Facchetti, MD, PhD; Jo Van Dorpe, MD, PhD; Sofie De Schepper, MD, PhD; Lieve Brochez, MD, PhD

IMPORTANCE Ex vivo dermoscopy (EVD) with derm dotting (DD) improves clinicopathologic correlation and the quality of diagnosis in skin tumors.

OBJECTIVE To compare the diagnostic performance of the standard method of skin biopsy processing with the practice of EVD with DD.

DESIGN, SETTING, AND PARTICIPANTS This retrospective study compares the diagnostic performance in 6526 skin biopsy specimens examined from 2008 to 2010 with a standard method of processing with 8584 biopsy specimens examined in 2015 with EVD and DD. Data were analyzed from January 1 to March 31, 2016. A total of 15 110 skin biopsy specimens were included. The biopsy specimens from 2008 to 2010 were processed in a hospital-based general pathology laboratory; the biopsy specimens from 2015 were processed in a private dermatopathology laboratory. Biopsy specimens from both periods were diagnosed by the same dermatopathologist.

MAIN OUTCOMES AND MEASURES The primary outcome measures were clinicopathological characteristics, usefulness of EVD with DD, and turnaround times (TATs).

RESULTS Use of EVD with DD increased the detection of positive section margins in nonmelanoma skin cancer from 8.4% to 12.8%. The most significant increase was seen in Bowen disease, invasive squamous cell carcinoma, and a superficial type of basal cell carcinoma (BCC). With EVD and DD, a specific clinicopathologic diagnosis was made in 27.7% of nevi compared with only 10.3% using the standard method. The incidence of moderately and severely dysplastic nevi increased from 1.0% to 7.2% and from 0.6% to 1.4%, respectively. The detection of ulceration in melanomas with thicker than 1 mm increased from 24.0% to 31.3%. The number of nevi-associated melanomas increased from 15.5% to 33.3%. The number of collision lesions from 0.07% to 1.07%. The TAT for nevi decreased from 2 days to 1 day, for melanomas from 5 days to 2 days, and for BCC from 2 days to 1 day.

CONCLUSIONS AND RELEVANCE Ex vivo dermoscopy and DD with adapted sectioning in a dermatopathology setting allows a more accurate and less time consuming histopathologic diagnosis of skin tumors. These findings suggest that pathologists involved in skin tumor evaluation should be encouraged to learn dermoscopy and replace random transverse cutting with lesion-specific and DD-guided cutting.

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Supplemental content

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Most pathology laboratories process skin biopsy specimens without access to clinical and/or dermoscopic images. Macroscopy of skin tumors is usually limited to a quick visual inspection, measurement of the lesion, standard transverse sectioning, and random cutting at 2 to 3 different levels.

Since the introduction of dermoscopy in the follow-up of pigmented skin lesions, focal areas that are visible only with dermoscopy may be the reason for diagnostic excision of lesions. Communication of this information to the pathologist may be of importance for a correct evaluation of these lesions.[1] A routine diagnostic procedure submits only 2% or less of the excised tissue for examination.[2] This is a worrisome fact, especially for melanocytic tumors , as one can imagine that small specific areas of concern for the clinician can be missed by the pathologist if these are not discernable by the naked eye. Merkel et al[3] applied a micropunch during in vivo dermoscopy to mark these areas of interest, which can, however, create an artifact and result in detaching of tissue.

In 2007, Scope et al[4] described the added value that ex vivo dermoscopy (EVD) can give to the pathologist by guiding tissue sectioning. Cabete et al[5] found that the combined use of EVD and histopathology improves clinicopathological correlation and allows selection of representative areas for sectioning. In 13.1% of their cases, a different final diagnosis was rendered after dermoscopy-guided specimen sectioning. Since 2011 we have systematically used EVD with derm dotting (DD) in our dermatopathology laboratory. With this simple and easy method, focal areas or suspected section planes observed on EVD are marked with nail varnish. This method of derm dotting was described by our team in 2013.[6] Others have afterward implemented this method in the examination of pigmented lesions.[7] The dotting method was also successfully used during oral surgery.[8] In a systematic comparison of 101 tumoral skin lesions, we demonstrated that EVD is broadly similar to in vivo dermoscopy but also has clear differences, providing possible direction to target tissue for processing and examination.[9]

In the present study we evaluated the impact of EVD with DD on the reliability of pathological margin evaluation and accuracy of diagnosis. We also looked at the impact on time of this method by comparing additional cuts and turnaround time (TAT) (time from tissue cutting to pathological protocol).

Key Points

Question What is the diagnostic performance of processing skin tumors with ex vivo dermoscopy (EVD) and derm dotting (DD) compared with the standard method?

Findings We observed that EVD and DD significantly increase the detection of positive section margins in nonmelanoma cancers and result in a better recognition of specific clinicopathological subtypes of nevi; a higher incidence of moderately and severely dysplastic nevi, nevi-associated melanomas, and collision tumors; and a decrease in the median turnaround time for melanocytic tumors.

Meaning Ex vivo dermoscopy and DD permit a more accurate and less time-consuming histologic diagnosis of skin tumors.

Methods

Skin Biopsy Specimens and Histological Processing

We compared 6526 skin biopsy specimens processed from January 1, 2008, to December 31, 2010, in a general pathology laboratory with 8584 skin biopsy specimens examined in a dermatopathology setting from January 1 to December 31, 2015. The skin biopsy specimens processed in this new dermatopathology setting can be considered comparable with those in the general hospital in terms of referred cases and patient population, since all the dermatologists sending to the general hospital pathology laboratory switched to the new dermatopathology laboratory. The number of skin biopsy specimens increased through growth of the existing dermatology practices and by referral of cases from dermatologists at new private dermatology practices in the same region.

Because the submitting dermatology practices were all located outside the hospital, samples from both periods were considered comparable. The same dermatopathologist (M.H.) diagnosed the lesions from both periods.

The lesion specimens from the first time period were processed using the standard transverse method for skin biopsy specimen processing. In first instance, skin tumors were inspected by a technician by the naked eye. Measurements were taken, and a schematic drawing of lesion was made. The bottom side was inked. The lesion was standard bread-loaf cut every 3 mm, with horizontal processing of tips. On 1 microscopic slide, 3 different levels of microtome cuts were assembled. Paraffin ribbons were conserved for possible additional staining.

Ex vivo dermoscopy and DD were used during the second time period. In the dermatopathology laboratory setting, skin tumors are inspected by a technician by the naked eye and measured. The technicians are trained in dermoscopy and decide if a dermoscopic image needs to be taken. A dermoscopic image (EVD, taken with a PowerShot G11, Canon; Dermlite 3, 3Gen Inc) was made of all flat melanocytic lesions, all lesions suspicious for dysplasia or skin cancer (both melanoma and nonmelanoma), and when specific dermoscopic information was provided by the clinician. Also, when a focal area of interest or suspect margin was noticed, a dermoscopic picture was made. When a technician was in doubt, the advice of the pathologist was asked. The images were only connected to the final histopathological diagnosis and no other personal data from patients were included. Moreover, according to Belgian law , no written informed consent was required. We therefore waived submission to the local human subjects ethics committee.

The following handling protocol was used (eFigure 1 in the Supplement): a horizontal border line was made on the skin rim with black nail varnish to orient the specimen (**Figure 1**). One half of the bottom side was inked green, the other half black. Focal lesions seen on dermoscopy were marked with a nail varnish dot. Different colors could be used. The varnish dot was discernable under the microscope as a granular brown-gray plaque on top of the horny layer (**Figure 2**). Punch biopsy specimens, taken to invest inflammatory diseases, were dotted in the center. Controlled microtome cutting was done until the area of interest (dotted) was reached. In addition, 2 previous depths were mounted on the same slide. Orientation, inking, nail varnish, and section planes were indicated on a drawing to help orient the tissue by the pathologist. Paraffin ribbons were conserved for possible additional staining.

Most benign melanocytic lesions were bread-loaf transverse-sectioned without processing of tips. In nonmelanoma skin cancer lesions, the tips were processed. In atypical melanocytic lesions and melanomas, the transverse sections were oriented to include the different structures, suspect margins, or focal lesions seen on EVD. In elongated lesions with clear transverse margins on EVD, horizontal or tangential sections were made. Acral nevi were cut perpendicular to the parallel ridge pattern.

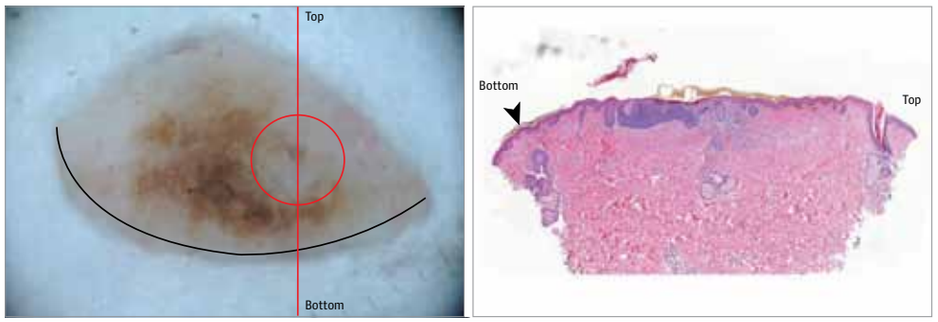
Outcome

To evaluate the quality of the diagnostic process, the following features were systematically scored and compared for both periods: (1) the proportion of positive section margins in nonmelanoma skin cancer lesions; (2) the proportion of nevi subtypes; (3) the proportion of moderately and severely dysplastic nevi, melanoma in situ, and invasive melanomas; (4) the proportion of nevi-associated melanomas; (5) the incidence of ulceration in melanomas thicker than 1 mm; and (6) the proportion of collision lesions.

To evaluate the economical and practical consequences of this extra macroscopic work, the number of deeper cuts and TATs from gross sectioning until the electronic validation of the definitive protocol was compared for both periods.

All categorical variables were compared using Pearson χ^2 test. Median TAT was compared using Mann-Whitney test. All statistical tests were 2-tailed, and $P < .05$ was considered statistically significant. Statistical analysis was done using SPSS software (version 21.0; IBM).

Figure 1. Example of Collision of a Basal Cell Carcinoma in a Flat Nevus



A, Dotting of the pale area in this pigmented lesion (circle) on the chest of a woman in her 40s identified a superficial basal cell carcinoma in collision with a flat nevus. The vertical line indicates the section plane. A horizontal border line of black nail varnish was applied to orient the specimen (arrowhead). B, Histopathologic image, hematoxylin-eosin. A and B, "Top" and "bottom" indicate the top and bottom of the section plane.

Results

Positive Margins in Nonmelanoma Skin Cancer

With EVD and DD, the number of nonmelanoma skin cancer lesions with positive section margins increased significantly from 8.4% to 12.8% ($P < .001$) (**Table 1**). In squamous cell carcinoma (SCC), the prevalence of positive margins increased from 8.7% to 16.9% ($P < .001$). For Bowen disease and in situ squamous carcinoma (ISCC), this number increased from 9.3% 19.1% ($P = .02$) and from 8.6% to 15.7% ($P < .001$), respectively. In the group of BCC, a positive margin was found in 11.1% compared with 8.3% without EVD and DD ($P = .02$). In the superficial type of BCC, the incidence of positive margins increased from 1.8% to 4.8% ($P = .046$).

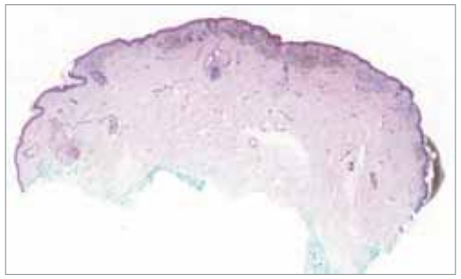
Melanocytic Lesions

Of 6526 biopsy specimens seen from 2008 to 2010, 1946 were diagnosed as nevi (29.8%) compared with 2808 of 8584 (32.7%) in 2015 (**Table 2**). For both periods, 2% of biopsy specimens were melanomas. For Miescher, flat, blue, and spitzoid types of nevi, a similar incidence was found in the 2 periods (respectively, 15.6% vs 14.6%, 14.2% vs 14.8%, 2.5% vs 2.0%, and 3.8% vs 3.2%). Only for Unna type nevi was a significant increase found (from 23% to 31.4%). In addition to these common variants, with EVD and DD, 27.7% of nevi were diagnosed as nevi with special clinicopathological characteristics compared with only 10.3% before using EVD and DD ($P < .001$). Table 2 lists the incidence of these subtypes for both methods. The number of nevi with moderate dysplasia increased from 1.0% to

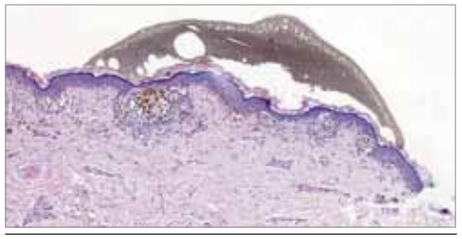
Figure 2. Example of a Melanoma

A Melanoma

B Original magnification × 12.5



C Original magnification × 50



A, This superficial spreading melanoma with a thickness of 0.6 mm on the clavicula of a woman in her 60s shows on ex vivo dermoscopy a focal suspicious margin (circle), confirmed to be positive after dotting and guided cutting. The line indicates the section plane. B and C, Histopathologic images, hematoxylin-eosin.

7.2% ($P < .001$), whereas the number of nevi with severe dysplasia increased from 0.6% to 1.4% ($P = .005$).

In the periods 2008 to 2010 and 2015, similar numbers of melanoma in situ were observed (respectively, 0.6% and 0.7%). Also, the incidence of invasive melanomas was similar for both periods (1.3% and 1.4%). There was an increase in the diagnosis of residual nevi in melanomas from 15.2% to 33.3% ($P = .06$). A trend toward an increase was found for ulceration in melanomas thicker than 1 mm (from 24.0% to 31.3%; $P = .54$).

Collision Lesions

Ex vivo dermoscopy and DD revealed a collision lesion in 1.1% of the cases, compared with only 0.07% of cases diagnosed between 2008 and 2010 ($P < .001$).

Deeper Cuts

Comparing both periods, a slight, nonsignificant, increase in demand for deeper cuts was found using EVD and DD (6.4% vs 5.3% using the standard method). For nonmelanoma skin cancer, a decrease was found (from 3.8% to 2.9%). For nevi, deeper cuts were asked in 5.3% of cases, compared with 5.1% with the standard method. For melanomas, there was an increase from 25.5% to 57.9%.

Turnaround Time

The median TAT significantly decreased during the second time period from 2 days to 1 day for nevi, from 5 to 2 days for melanoma, and from 2 to 1 day for BCC ($P < .001$).

Discussion

The significant increase in positive section margins in nonmelanoma skin cancer lesions (from 8.4% to 12.8%) indicates a higher sensitivity of the EVD and DD method. In ISCC, the visualization of a yellow-brown keratotic or crusty surface on EVD permits delineation of the lesion and can guide us to suspicious margins. Many Bowenoid lesions show skip lesions. They may be covered with a keratotic crust or show brown dots corresponding to residual glomerular vessels.[9] These characteristics on EVD can disclose a positive margin and guide the tissue sectioning. In BCCs, EVD with DD proved to increase the number of positive margins significantly (from 8.3% to 11.1%). This was mainly due to superficial BCCs that may grow multifocal and may not be well delineated clinically.

As we described in a previous report,[9] in some BCCs, the loss of red and intensification of the white structureless areas on EVD (reflecting a fibrosing stromal reaction) permits a better margin evaluation.[6] In other cases of superficial BCCs, small brown crusts or blue-gray ovoid nests (corresponding to pigmented basal nests) may give a hint to the most marginal tumoral nests. Nodular BCCs are mostly well delineated clinically, and surgery often results in clean margins. Margin positivity in these cases are mostly found in the deeper section plane or deeper lateral sides, and this information cannot be captured by EVD.

As demonstrated in a previous study,[9] EVD of pigmented lesions provides a sharp and detailed picture, allowing an excellent orientation and guided cutting of the lesions. Suspicious superficial borders can easily be identified (Figure 2).

During both investigated periods, a similar percentage of common variants of nevi was found. Only for Unna-type nevi was a significant increase in the amount of nevi found (from 23% to 31.4%). This probably reflects more attention for sub-

Table 1. Positive Section Planes in Nonmelanoma Skin Cancer

Type of Tumor	General Hospital Period, 2008-2010, No./Total No. (%)	Private Dermatopathology Period, 2015, No./Total No. (%)	*P* Value
Nonmelanoma skin cancer	130/1540 (8.4)	239/1874 (12.8)	<.001[a]
Squamous cell carcinoma	41/471 (8.7)	89/524 (16.9)	<.001[a]
Bowen disease	12/129 (9.3)	37/194 (19.1)	.02[a]
In situ NOS	1/10 (10.0)	3/9 (33.4)	.21
Keratoacanthoma type SCC	3/48 (6.3)	5/41 (12.1)	.33
Invasive SCC, other	25/284 (8.6)	44/280 (15.7)	<.001[a]
Basal cell carcinoma	89/1069 (8.3)	150/1350 (11.1)	.02[a]
Superficial	5/276 (1.8)	16/336 (4.8)	.046[a]
Superficial/nodular	3/11 (27.3)	12/103 (11.7)	.15
Nodular/noduloulcerative/nodulocystic	62/710 (8.7)	82/725 (11.3)	.10
Nodular/infiltrative	2/8 (25.0)	2/13 (15.4)	.59
Infiltrative/morpheiform	4/8 (50.0)	28/102 (27.5)	.18
Basosquamous	4/21 (19.0)	1/6 (16.7)	.89
Others or NOS	9/63 (14.3)	9/65 (13.8)	.94

Abbreviations: NOS, not otherwise specified; SCC, squamous cell carcinoma.

[a] Statistically significant.

typing and a lower threshold for diagnosing a nevus as papillomatous since the use of EVD.

In 27.7% of nevi, many of which were removed because of clinically atypical aspects, EVD with DD led to a more specific clinicopathologic subtyping (27.7% vs 10.3% without EVD and DD) (Table 2). The only nevus subtype that could be easily identified without EVD and DD were hypermelanotic flat and/or lentiginous nevi (8.4% vs 8.1% in 2015). Diagnoses of large nested nevus increased from 1.2% to 2.2%. Ex vivo dermoscopy and adapted cutting may have contributed to a bet-

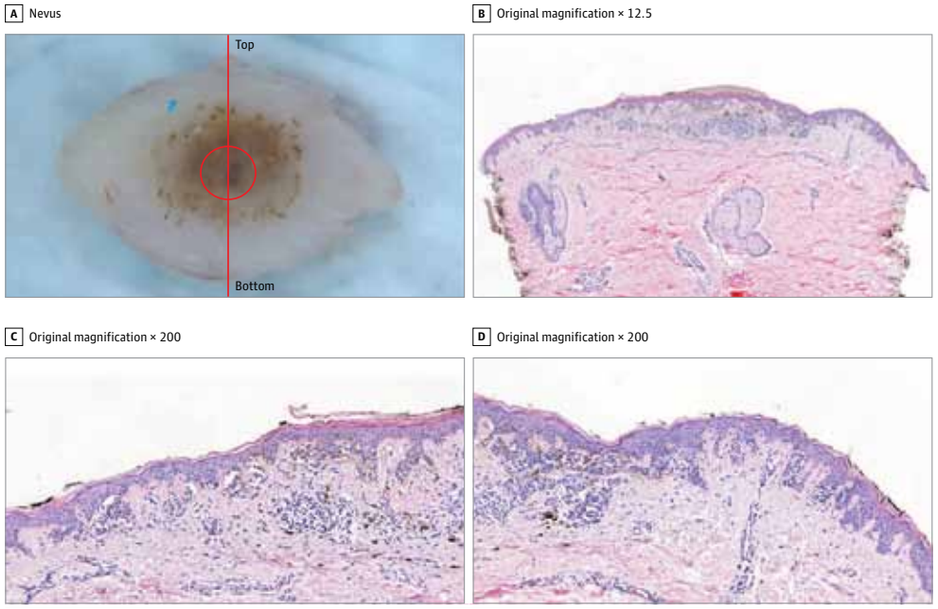
Table 2. Pigmented Lesions

Type of Nevus	General Hospital Period, 2008-2010, No. (%)	Private Dermatopathology Period, 2015, No. (%)	*P* Value
Total biopsies	6526	8584	
Nevi	1946 of 6526 (29.8)	2808 of 8584 (32.7)	<.001[a]
Unna	449 (23.0)	884 (31.4)	<.001[a]
Miescher	303 (15.6)	409 (14.6)	.34
Flat	277 (14.2)	416 (14.8)	.58
Blue	49 (2.5)	55 (2.0)	.20
Spitz/Reed	74 (3.8)	91 (3.2)	.30
Other	418 (21.5)	420 (15.0)	<.001[a]
NOS	376 (19.3)	533 (19.0)	.77
Clinicopathologic subtypes	200 (10.3)	777 (27.7)	<.001[a]
Flat hyperpigmented/lentiginous	158 (8.1)	236 (8.4)	.21
Large nested	23 (1.2)	63 (2.2)	<.001[a]
Actively growing	0	35 (1.2)	<.001[a]
Asymmetric shoulder	0	97 (3.4)	<.001[a]
Fibrosing/sclerosing	6 (0.3)	168 (6.0)	<.001[a]
Sclerosing	6 (0.3)	104 (3.7)	<.001[a]
Superficial fibrosing	0	37 (1.3)	<.001[a]
Perifollicular fibrosing	0	27 (1.0)	<.001[a]
Nevus with dot	0	26 (0.9)	<.001[a]
Irritative/traumatized	1 (0.1)	118 (4.2)	<.001[a]
Eczematized	5 (0.3)	17 (0.6)	.08
Recurrent	7 (0.4)	17 (0.6)	.24
Nevi with moderate dysplasia	20 (1.0)	201 (7.2)	<.001[a]
Nevi with severe dysplasia	11 (0.6)	40 (1.4)	.008[a]
Melanoma in situ	42 (0.6)	59 (0.79)	.74
Invasive melanomas	87 (1.3)	116 (1.4)	.80
Ulceration in melanomas >1 mm	6 of 25 (24.0)	10 of 32 (31.3)	.54
Nevi-associated melanomas	5 of 33 (15.2)	18 of 54 (33.3)	.06[a]

Abbreviation: NOS, not otherwise specified.

[a] Statistically significant.

Figure 3. Example of Actively Growing Nevus



A, This pigmented lesion on the chest of a man in his 20s with a history of growth in the past 6 months shows a homogeneous center and a separated peripheral rim of brown globules corresponding to a flat compound nevus. The dotted blue-gray center (circle) corresponds to an area of chronic inflammation and melanophagia. The line indicates the section plane. B-D, Histopathologic images, hematoxylin-eosin. C and D, The peripheral rim of globules corresponds to larger or fused junctional nests.

ter understanding of the specific architecture of melanocytic lesions. After correlation with EVD, 1.2% of nevi were diagnosed as actively growing nevi with peripheral globules, corresponding to another specific nevus with large nests (**Figure 3**). This diagnosis is almost impossible to make without dermoscopic information.

Focal areas of fibrosis and/or sclerosis in nevi may cause concern for the clinician and often prompt resection, but these areas may be difficult to trace without EVD and DD. The diagnosis of a fibrosing or sclerosing nevus by use of EVD was made in 6% of nevi compared with only in 0.3% without EVD. The latter were all examples of fully sclerosing nevi. Many fibrosing, sclerosing nevi however, show only focal sclerosis, often starting in a perifollicular way. Without EVD and DD these areas can be missed. A correct interpretation of fibrosing/sclerosing nevi is important because the differential diagnosis of these cases with regressive melanoma can be very difficult, even with the aid of EVD and DD information.

Nevi that were resected as consequence of the appearance of a new black, blue, or brown dot were marked at this dot and diagnosed readily through EVD without need for deeper cuts or risk of transection of the dot.

Irritated, traumatized nevi were also significantly more frequently diagnosed than previously. There was a also an increase in the diagnosis of recurrent and eczematized nevi. This increase, probably owing to the low number, was not significant. Ex vivo dermoscopy often revealed focal changes in these lesions (crusts, erosions, pigment changes), permitting marking and guided cutting.

Ex vivo dermoscopy with DD resulted in a significant increase of moderately and severely dysplastic nevi, from 1.0% to 7.2% and from 0.6% to 1.4%, while the incidence of melanoma in situ, an invasive melanoma, remained the same. This observation suggests a higher sensitivity of EVD with DD specifically for the identification of areas of significant dysplasia. This is in line with the retrospective study of Merkel et al[9] on the use of dermoscopy-guided histologic sectioning for the diagnosis of melanocytic lesions. This has clinical consequences because in severely dysplastic nevi a broader resection to 0.5 cm is advised.

Besides an adequate control of section planes, EVD with DD may also help to identify a preexisting nevus rest in melanomas or a focus of melanoma in a benign nevus. The observation of small areas with a residual regular network or conserved globules in a dermoscopically malignant lesion can disclose a preexisting nevus. Focal areas with a disturbed network or a blue-gray aspect on EVD can guide the pathologist to a small focus of melanoma in a benign nevus. By dotting and

thereby facilitating microscopic traceability of these areas, there was an increase in the diagnosis of residual nevi in melanomas (from 15.2% to 33.3%). The diagnosis of ulceration in melanomas thicker than 1 mm increased from 24.0% compared with 31.3%. However, this increase was not significant, probably owing to the low number of cases included in this study. The diagnostic potential of EVD and DD in identifying ulceration in melanomas has to be further evaluated in larger series. Ex vivo dermoscopy and DD can also help to identify the most suspicious areas for deep invasion in melanomas. These areas often show a white-gray color as sign of regression or a blue-gray aspect owing to the presence of deeper dermal melanin pigment.

By using EVD and DD, we observed more collision lesions (0.07% to 1.07%). This increase may indicate a higher sensitivity of the new method compared with the standard method. Although most of these collision lesions are incidental findings, without clinical or therapeutic consequences, in some of these cases, the identification of a second lesion can be of clinical importance (Figure 1).

Turnaround times decreased in the second period compared with the first period: for nevi (from 2 days to 1 day), melanomas (from 5 days to 2 days), and BCC (from 2 days to 1 day) significantly. In the private dermatopathology laboratory we process tissues twice a day compared with only 1 overnight run in the general pathology hospital laboratory, but this explains only in part the low TATs.

Deeper cuts were required in 6.4% of cases compared with 5.3% before, which is a nonsignificant difference. The need for deeper cuts is low compared with that reported in a previous study (7.6% to 23.3%).[10] With EVD-guided sectioning, most deeper cuts are made to confirm or further examine focal lesions observed on the first cuts, whereas with the standard method, cuts are often blind because of a discordance between the histological findings and the expected findings, based on the clinical information or macroscopy. The exact impact on the accuracy and efficiency of working in an exclusively dermatopathology environment compared with that in a general pathology laboratory is difficult to measure. Nevertheless, we are convinced that EVD and DD may contribute in improving the accuracy and efficiency in dermatopathology. The main limitation of the study is that for the second period the biopsies were processed in a new dermpat lab.

Conclusions

This retrospective evaluation shows that EVD with DD and adapted sectioning of skin tumors, permits a more accurate histologic diagnosis. It allows better margin evaluation, better understanding of lesional architecture, and specific evaluation of remarkable/suspicious areas, either described by the clinician or observed by the laboratory technician. The method is easy to implement in a dermatopathology setting. In our opinion, EVD with DD is a valuable new scientific tool to make clinicopathologic correlations. We believe it is time for the pathologist involved in tumoral skin evaluation and their technical staff to learn dermoscopy and replace random transverse cutting by lesion-specific and DD guided cutting.

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Shaving and curretage

Embedded intact or transect if necessary.
Longitudinal or transverse cut at 3 levels

Punch biopsy

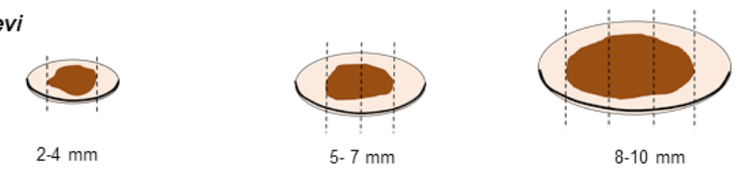
Dotting of center
3 levels until center

Dotting of focal lesion on EVD
3 levels up to dotted area

Excisional biopsy

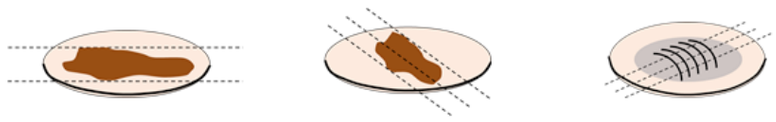
General orientation : horizontal transverse varnish border line and inking with green/black of bottom side

Nevi



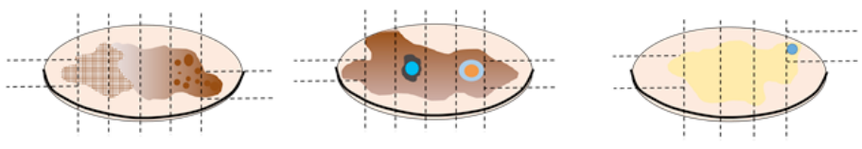
2-4 mm | 5- 7 mm | 8-10 mm

Oriented bread-loaf sectioning without processing of tips



Longitudinal, tangentional or perpendicular (acral nevi) sectioning in elongated lesions

Atypical melanocytic lesions, melanomas and non-melanoma skin cancer



Oriented bread loaf sectioning targeted to different patterns seen on EVD

Focal lesions seen on EVD are marked with nail varnisch dot. Different colors can be used

Chapter V Application of derm dotting in oral and maxillofacial surgery

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Original Article

Application of derm dotting in oral and maxillofacial surgery

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ABSTRACT

Purpose: Derm dotting is a new method of marking skin biopsies, and it is used by dermatopathologists to identify most diagnostic tissues on a microscopic slide. This method uses nail varnish to mark specific lesions and suspected section planes, or to orient skin resections. The nail varnish is resistant to different tissue processes, discernible while cutting the tissue block, and easily recognizable under the microscope. We examined the effectiveness of derm dotting on intraoral tissues.
Methods: We used the derm-dotting technique on the intraoral tissues of nine patients who underwent resection of a squamous carcinoma. We also tested this method on frozen sections.
Results: In all cases of resected tissue, the nail varnish was visible under gross examination, traceable while cutting the tissue block, and clearly visible in the definitive sections. The dots were preserved in the frozen sections, but they were lost in half of the decalcified tissues.
Conclusions: Derm dotting is an inexpensive, simple method that can replace the stitching technique used by surgeons to orient specimens. The stitches have to be removed by the pathologist, therefore possibly creating an artifact in the biopsy. The varnish dots or lines can be used to orient the specimen. In addition, the dots can easily mark suspect borders or areas of interest to be examined by the pathologist, using different colors, if desired. With derm dotting, the pathologist receives a more representative slide enabling a more accurate clinicopathological correlation.

1. Introduction

In modern medicine, biopsies are essential in making accurate treatment plans. Physicians rely on histological diagnosis to make decisions regarding surgery, chemotherapy, radiotherapy, or combinations of treatment. New technologies have been developed to help diagnose intraoral pathologies. One of the techniques for the earliest possible detection of oral precancerous lesions is direct oral microscopy, which involves obtaining biopsies according to colposcopic criteria [1,2]. When resecting a carcinoma, the surgeon has to communicate to the pathologist key information on the location, position, orientation, or width of the resection margins of the specimen. Surgeons currently use stitches to mark or orient the biopsy. When the biopsy arrives at the pathology department, technicians have to remove the stitches so that the microtome knife is not damaged, and the removed stitches leave an artifact with loss of tissue. Other surgeons do not mark the specimen, and they make only limited comments and drawings on the application form. Thus, new methods are needed to indicate to the pathologist the relevant parts of the tissue to examine and improve the accuracy of the histological report because a pathologist only examines a random 2% of the delivered tissue [3]. Therefore, it is very important that the proper areas to be examined are identified. The ideal method would be inexpensive, safe for the specimen, practical for the pathologist, and fast and easy for surgeons to apply.

Several different marking techniques have been proposed in the literature. Artist pigments are used to mark the resection borders of breast biopsy specimens [4]. In a cone biopsy of the ectocervix–endocervix transition zone, ink can be applied to areas of interest [5]. In pneumology, the bronchoscopist injects either carmine dye or methylene blue into the place of interest before using video-assisted thoracoscopic surgery (VATS) for resection [6–8].

In 2013, Haspeslagh et al. [9] described a new method called ex vivo dermoscopy with derm dotting. They discovered that nail

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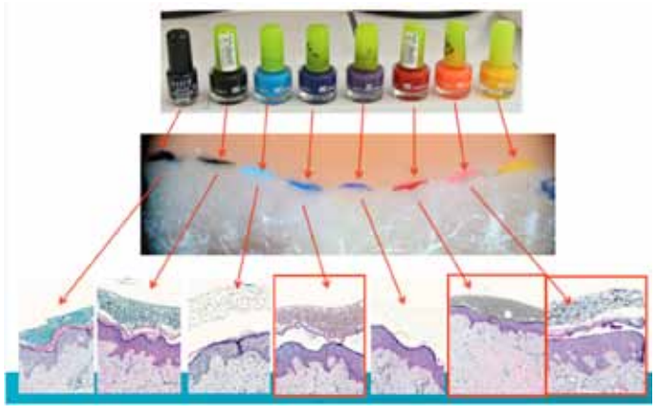

Fig. 1. Different types of colors can be distinguished by microscopy as different types of granular gray. In the middle, we see the different colors in the tissue block before processing (trimming).
Reproduced with permission from M. Haspeslagh.

varnish adheres to skin, is resistant to tissue processing, and can be detected under the microscope. Varnish dots can be used to mark specific areas to be examined by the pathologist or for orientation of the specimen. The nail varnish does not damage the tissue, and different types of nail varnish or different colors applied on the same biopsy can be distinguished on the tissue block and under the microscope. The different varnish colors are discernible under the microscope as different granular gray areas. In this study, we examined whether this derm-dotting technique is also applicable for lesion marking or orientation after resection of intraoral lesions. Our aim was to determine whether this nail varnish method can eventually replace the current, nonoptimal method of using stitches.

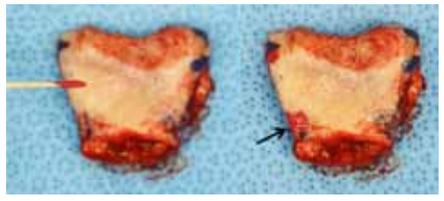

Fig. 2. Use of derm dotting on a lower lip specimen with squamous cell carcinoma. A combination of red and blue dots, applied with a toothpick, orients the specimen. A bigger red dot marks the place where the tumor free margin was clinically smallest.

2. Materials and methods

This experimental study was performed over a period of 3 months. The study complied with the principles laid down in the Declaration of Helsinki. The study was approved by the ethical committees of UZ-Leuven, Belgium (Ethical no: B322201319028). All nine subjects agreed and signed informed consent before being included in the study.

The method of derm dotting uses a simple, inexpensive nail varnish to mark the biopsy. In this study, we used the nail polish brand 2B Mega Colors. We used color 94 (Mat dark blue), 03 (American rose), 25 (Fluo yellow), 55 (Fluo red), and 04 (Cerise red). These colors were selected after testing many types of nail varnish on skin under a microscope (Fig. 1). Derm dotting was tested on 15 specimens of nine squamous carcinomas. Six out of the nine patients were male and the mean age was 67 years (Table 1). A primary resection with macroscopic free margins was performed in these patients. These resection specimens were placed on a surface next to the operation table. After completion of the surgery, the surgeon oriented the specimen using different colors of nail varnish dots for each side of the specimen. The small dots were marked on the tissue with a toothpick (Fig. 2), while the nailbrush was used for larger marks. Different colors were used to mark suspected resection borders or areas to be examined by the pathologist. After application, the nail varnish was left to dry for 3–5 min. Then, the specimen was placed in a closed biopsy tube (65 or 125 ml) with or without formol. After a maximum of 5 days, the pathologist checked whether the dots were still present on the specimen. In two patients, small fragments were taken for frozen sections, which were also marked with nail varnish for evaluation (Fig. 3). During this small study, different anatomic sites were examined, including the mucosa of the tongue, lip, cheek, uvula, mouth floor, and pharynx (Fig. 4). In addition, different types of tissue were examined, namely mucosa, fat tissue, submandibular, and sublingual gland tissue.

Table 1
Study population according to age and gender.

	Enrolled patients								
Number	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Gender	Male	Male	Female	Male	Female	Female	Male	Male	Male
Age (mean 67 years)	59	59	57	66	78	52	73	80	79

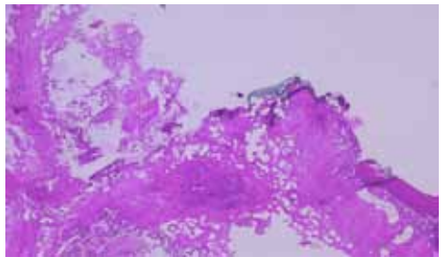

Fig. 3. Frozen section of a pharynx with a clearly visible gray granular layer of nail varnish, which was originally blue nail varnish.

When the specimen arrived at the pathology department, the technician trimmed through the tissue block until reaching the colored dot; then, the dotted sections were mounted on the microscopic slides. Specimens for definitive sections were divided into two groups. One group of specimens did not contain bone and underwent the normal histological processing as described (11 of 13 specimens). The other group included resection specimens with bone (two specimens), which were decalcified before the histological processing. On the definitive section, the pathologist traced the dots under the microscope, photographed the dotted areas, and made comments in the report.

3. Results

The results of our study are summarized in Table 2 for each type of section and specimen. Dots were placed in 15 specimens of squamous cell carcinoma. All of these dots were clearly visible in the laboratory before processing and during the cutting of the tissue block. The fluorescent orange and red varnishes were best seen in the tissue block. In the definitive sections without bone, all the dots were visible under the microscope and mentioned in the protocol by the pathologist, except for one case where the reporting pathologist did not mention the absence or presence of the marking dot (Fig. 4). The dots were also visible in the tissue block of the two frozen sections while cutting the slide and were also visible under the microscope (Fig. 3). In two of the four specimens that were decalcified before routine processing because of the presence of bone, the varnish mark was lost.

4. Discussion

This small study examined whether the method of derm dotting, described by Haspeslagh [9] in skin biopsies, was applicable in the field of maxillofacial surgery. In all 15 examined specimens, the varnish dots were clearly visible to the pathologist and technicians, thus enabling proper cutting and embedding of the biopsies. The different dots allowed a suitable orientation in the tissue blocks and during microscopic examination. The dotting method with varnish can replace the stitching technique or other techniques such as primitive drawings, or the use of alcian blue solution [10], colored gelatins [11,12], acrylic pigments [13], and Typp-ex fluid for marking the resection margins [14,15]. Indian ink is not very satisfactory due to the long drying time and the tendency of the ink to spread [16]. Acetone or Bouin's solutions have been used recently to aid in the drying of Indian ink [17–19]. In addition, other special color dyes in current use for marking section planes cannot be used to mark small areas because they also run out [20].

The new method of derm dotting is easy to use, and different parts of the resection specimen can be pointed out with different colors without spreading (Fig. 2). Proper orientation, section margins, or areas of interest of the biopsy can be easily communicated with the pathologist. In addition, from a material and cost point of view, stitches are considerably more expensive than a standard bottle of nail varnish. Furthermore, with this new method, there are no artifacts (no holes from stitches) as the nail varnish actually lies on the specimen without damaging it (Figs. 1, 3 and 4). The resins of the varnish responsible for adhesion to the intraoral tissue do not interfere with the cellular detail of the tissue [9]. The small varnish brush can be used to mark lines along a specific margin of the sections. After orientation and proper cutting of the specimen, the technician can cut the tissue block directly at the colored area, and therefore fewer slides have to be prepared and examined. The fluorescent orange and red varnishes are best seen in the tissue block. As with margin inks, blue and black nail varnish gave the best results under the microscope [11]. During the processing stage, the varnish color changes into different granular types of gray (Fig. 1), but an experienced eye can still easily distinguish between each shade of gray. This is similar to other tissue-marking dies where the colors are changed or even lost after routine processing and decalcification [21].

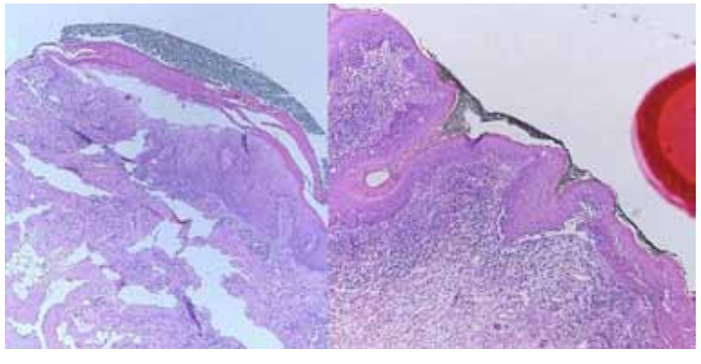

Fig. 4. Definitive sections of lip (a) and cheek (b) tissue with the granular gray nail varnish layer on top of the specimen without damage to the tissue.

Table 2
Types of sections and tissues with results if dot is microcopically visible or not after processing phase.

Sections	Dots microsopically visible after processing phase									
	Examined tissue	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Frozen sections	Uvula		Yes							
	Lip							Yes		
Standard sections	Tongue	Yes								Yes
	Lip							Yes		
	Cheek						Yes (bone)			
	Uvula		Yes							
	Mouth floor			Not reported	No (bone)	Yes (bone)				
	Pharynx		No (bone)							
	Fat tissue neck dissection	Yes							Yes	
	Glandula sublingualis	Yes								
	Glandula submandibularis								Yes	
	EXTRA: Bone in specimen	–	+	–	+	+	+	–	–	–

Finally, while examining the slide, the pathologist is able to control the right area internally, which allows a more confident and easier diagnosis and results in less demand for deeper cuts. With the varnish marks, the pathologist can easily reconstruct and orient the different slides of the specimen.

The method also seemed to work with frozen sections where one important area can be pointed out (Fig. 3 and Table 2). By contrast, the dot was lost in half the cases (two of four) when the definitive section was decalcified before processing because of the included bone in the resection specimen. The exact reason is unclear, but this study was conducted at a university hospital where different pathologist-trainees examined different cases. Not all of them might have looked for the dots with the same level of interest.

Finally, we believe this method can also be applied in other types of surgery. Therefore, we plan to test this method in non-oral surgically resected specimens.

5. Conclusion

The derm-dotting method with nail varnish is an inexpensive, easy marking system that can replace the use of stitches, dyes, or other creative techniques for orientation and marking of oral surgical specimens. We believe that this method can improve the quality of the clinicopathological communication between the surgeon and the pathologist. With this method, the pathologist is also more confident that the proper areas to be examined are identified. In addition, it could be useful to complement this new method with a standardized common color code system for communicating with the derm-dotting technique.

Conflict of interest

None of the authors has financial funding of conflict of interest.

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Chapter VI Application as scientific tool: Rosettes and other white shiny structures in polarised dermoscopy: histological correlate and optical explanation

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SHORT REPORT

Rosettes and other white shiny structures in polarized dermoscopy: histological correlate and optical explanation

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Abstract

Background Rosettes are a specific form of a white shiny structure seen with polarized dermoscopy. The precise morphological correlate and optical explication are not known.

Objective To estimate the frequency of rosettes in *ex vivo* dermoscopy and to find explication and morphologic correlate of this dermoscopic feature.

Methods A series of 6108 consecutive skin biopsies were examined with *ex vivo* dermoscopy and when rosettes were present serial transverse sections with polarization were examined.

Results In this series of 6108 consecutive skin biopsies, rosettes were found on *ex vivo* dermoscopy in 63 cases. When multiple we observed that they are always oriented at the same angle. Transverse sections with polarization of these lesions proved that smaller rosettes are mainly caused by polarizing horny material in adnexal openings, and larger rosettes by concentric perifollicular fibrosis.

Conclusions Rosettes are an optical effect of crossed polarization by concentric fibrosis or horny material and hence are not lesion-specific.

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Conflicts of interest

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Rosettes are peculiar structures only observed with polarized dermoscopy. They are defined as four white points, arranged as a four leaf clover.[1] Since recently the term 'four-clod dots' is used for these structures. First believed to be specific for actinic keratosis and squamous cell carcinoma, they are not lesion-specific and are described in many lesions.[2] They are a form of so-called white shiny structures, as are white shiny lines and white shiny areas.[3] In contrast to shiny lines and areas, that are probably caused by fibrotic dermal changes,[3–7] the exact tissue correlate of rosettes is unknown. Interaction of the polarized light with narrowed or keratin filled adnexal openings has been suggested as the morphological correlate.[2] Others suggested that rosettes correspond to an alternating focal hyperkeratosis and normal corneal layer and keratin filled openings.[8]

Table 1 Lesions with rosettes on *ex vivo* dermoscopy in a series of 6108 consecutive skin biopsies

BCC	17/988 (1.7%)
SCC	12/291 (4.0%)
DF	7/116 (6.0%
Naevus	14/1880 (0.7%)
Melanoma	2/135 (1.4%)
Scar	2/31 (6.4%)
Cyste	2/359 (0.5%)
Inflammatory	5
Molluscum	1/17 (5.9%)
Dilated pore	1/2 (50%)
Total	63/6108 (1.0%)

BCC, basal cell carcinoma; SCC, squamous cell carcinoma; DF, dermatofibroma.

Liebman found white shiny structures (crystalline structures) in 38.7% of a series of 538 retrospectively examined *in vivo* cases[2] of melanoma, basal cell carcinoma, squamous cell carcinoma, actinic keratosis and lichen planus-like keratosis. In a comparative study of 102 consecutive tumoral skin lesions seen by one dermatologist, we found crystalline structures in 42.3% of the *ex vivo* dermoscopy images compared to 34.7% of the *in vivo* images. In *ex vivo* setting white structures are more apparent than on the *in vivo* image.[9]

Since we routinely use *ex vivo* dermoscopy with polarized light as part of the macroscopic examination of skin biopsies, we regularly observe rosettes. They vary in diameter from 0.20 to 0.5 mm. Liebman *et al.*[3] found that rosettes were significantly more likely to be observed in actinic tumours than other lesions. In her study Liebman found rosettes in 46.3% of actinic keratosis. This was confirmed by Lee who found rosettes in 38.2% of actinic keratosis.[10] We found in 63 (1%) cases rosettes on our *ex vivo* image in a series of 6108 consecutive skin biopsies received at the same dermatopathology lab (Table 1). We noticed them mainly in basal cell carcinomas and, squamous cell carcinoma. We also saw them in naevi, and melanomas. Furthermore, we also found them in dermatofibromas and recently we saw multiple rosettes in a punch biopsy taken for an urticarial dermatitis, in a scar and for cicatricial alopecia of lichen planopilaris type. This confirms that rosettes are not lesion specific and can be seen in various cutaneous lesions, even in inflammatory diseases. In lesions resected on actinic damaged skin many of the observed rosettes were seen in the non-lesional part of the skin.

While observing these structures, when multiple, we noticed that the rosettes were always oriented at the same angle (Fig. 1).

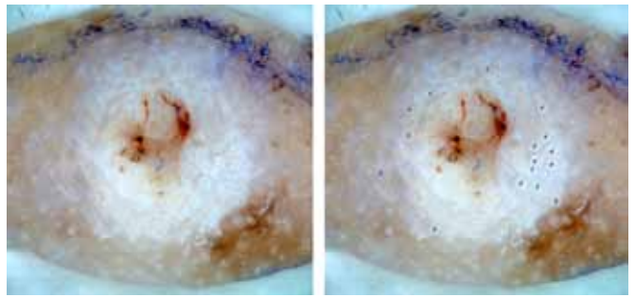

Figure 1 Basal cell carcinoma with numerous rosettes all oriented at same angle (*ex vivo* polarized dermoscopy image).

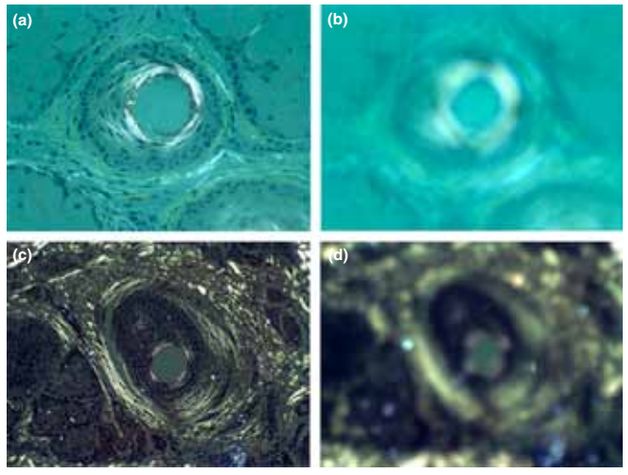

Figure 2 Transverse cuts with crossed polarization of infundibular keratin layer resulting in four-segmented concentric structure (a), acquiring features similar to a rosette when seen out of focus (b). Intrafollicular concentric keratin and outer perifollicular fibrosis (c) creating double rosette when seen out of focus (d).

The angle is preserved for all rosettes visualized by the same dermoscope, even in different lesions and in different patients. The orientation is retained when rotating the dermoscope around an axis perpendicular to the skin surface. The observation of these very regular and symmetric structures, reminded us of the so-called 'Maltese crosses' (birefringent crystals with a symmetrical black cross), caused by polarization of starch crystals. It is well known that they are caused by conoscopic interference, an optical phenomenon occurring when using crossed polarization.[11] Crossed polarized light is produced when a polarizer and an analyser are inserted in the optical path at right angles to each other. Most standard microscopies are equipped with these polarization filters.

Many authors searched for the morphologic correlate of this phenomenon by comparing the dermoscopy with corresponding horizontal sections obtained with *in vivo* reflectance confocal microscopy.[8] We thought that transverse histological sections might help us better to identify these rosettes. In transverse sections through the residual material of these cases we could observe a similar optical effect. We saw a polarizing four-segmented concentric structure, always with the same orientation of the two axes. The smaller structures (0.1–0.2 mm) were caused by polarization of concentric horn material in follicular and even in some eccrine ducts at the infundibular level of the biopsy (Fig. 2a,b). The larger rosettes (0.3–0.5 mm) were caused by concentric fibrosis around the follicles. Bringing the slide out of focus under the microscope created a picture very similar to the rosettes observed in dermoscopy. This convinced us that we were actually looking at the same optic phenomenon. In some cases, we could even find a double rosette: an inner rosette by circular follicular horny material, an outer rosette by perifollicular concentric fibrosis (Fig. 2c,d). Using the same methodology we could also demonstrate that white shiny streaks are indeed mainly caused by polarization of thickened hyaline fibrous bundles. In many dermatofibromas with a stellar aspect on dermoscopy these radial shiny lines are easily identified as polarizing hyaline fibrous bands on transverse cuts.

In conclusion, we describe rosettes and white shiny streaks in *ex vivo* dermoscopy. Rosettes are an optical effect of crossed polarization (so-called Maltese cross) that can be observed in many tumoral and also inflammatory skin lesions and hence are not lesion specific. They are commonly seen in actinic skin. Transverse sections prove that smaller rosettes are mainly caused by polarizing horny material at infundibular level in adnexal openings and larger rosettes mainly by concentric perifollicular fibrosis. As already suggested by others white shiny streaks correspond to polarization of fibrotic changes in the dermis.

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Chapter VII General discussion and future perspectives

Ex vivo dermoscopy (EVD) is a valuable diagnostic tool that was first introduced in dermatopathology in 2007 by Scope et al [21]. We compared in detail ex vivo dermoscopy (EVD) images with in vivo dermoscopy (IVD) images for the evaluation of skin tumours. In general, EVD images are broadly similar to IVD images and most of the structures and colours observed on IVD, can also be recognized on EVD images [24]. In some cases, EVD may reveal increased blue and white colours and a loss of red. Because of the high quality of EVD images, they are very useful to visualize small lesions, to detect focal alterations in skin tumours, to evaluate margins and to guide tissue sectioning. EVD images of pigmented lesions provide a sharp and detailed picture, allowing an excellent orientation and a guided cutting of the lesions. They also allow us to easily identify suspicious superficial section margins.

To find a way to mark focal areas or suspicious section margins without introducing tissue damage or artefacts, we introduced the derm dotting (DD), a procedure based on the use of nail varnish [25]. We evaluated the usefulness of EVD with DD on pathological section margin assessment and diagnostic accuracy. In addition, we studied the impact on time by comparing turnaround time (TAT) and additional cuts [26].

EVD with DD proved to be a reliable method for the evaluation of section margins in non-melanoma skin cancers, resulting in a significant increase in the detection of positive section margins from 8.4% to 12.8%, compared to the standard method of tissue sectioning.

In 27.7% of the 2808 excised naevi we included in our study, many of which were clinically flat and atypical, EVD with DD led to a more specific clinicopathological subtyping, compared to 10.3% using the standard method. EVD and adapted cutting contributes to a better understanding of the specific architecture of melanocytic lesions. In fact, some of the specific clinicopathological subtypes are almost impossible to diagnose without dermoscopic information. Focal areas of fibrosis/sclerosis are a histopathological feature that may cause concern for a clinician and may be a reason to excise a naevus. These areas, however, are difficult to trace without EVD with DD. A correct interpretation of fibrosing/sclerosing naevi is important in order to exclude regressive melanomas. This distinction can be very difficult. Naevi that have been removed because of the presence of a new suspicious black, blue, brown, yellow or white area, can easily be marked at this focus and diagnosed readily without the need for deeper cuts. The dotting prevents us from missing the area of concern in the microscopic slides.

EVD with DD also permits marking and guided cutting of focal areas, like crusts, erosions, and pigment changes. This can be important for the diagnosis of irritated, traumatized, eczematous and recurrent naevi.

With the use of EVD with DD, the incidence of moderately and severely dysplastic naevi increased from 1.0% to 7.2% and from 0.6% to 1.4%, respectively, which suggests a higher sensitivity of EVD with DD for the identification of areas with significant dysplasia. EVD with DD also helps in identifying and marking a focus of melanoma in a benign naevus or a preexisting naevus in a melanoma. In fact, focal areas with a disturbed network or a blue-grey aspect on EVD, can draw the pathologist's attention to a small melanoma area arising within a melanocytic naevus. Small areas with a residual regular network or conserved globules in a lesion with dermoscopic features of malignancy, can lead to the identification of a pre-existing naevus. With EVD with DD, the presence of residual naevi in melanomas increased from 15.2 to 33.3%. Ulceration is a focal alteration that can be visualized and marked by EVD with DD. This may be useful in identifying tiny areas of ulceration in melanomas. Whether the finding of more ulceration from 24.1% to 31.3% in melanomas thicker than 1 mm with EVD with DD might represent a statically significant factor requires evaluation of a larger series of cases. For melanomas, EVD with DD can also help to identify the area's most suspicious for deep invasion, which are (often) identified by their blue-grey aspect, due to the presence of deeper dermal melanin pigment. Similarly, the white-grey colour in melanomas, represent a sign of regression.
Collision tumours are rare lesions, most often incidentally detected, whose identification rate can be increased by using EVD with DD.
EVD with DD is easy to implement in dermatopathology laboratories with a positive impact on the turnaround time (TAT) . By using EVD-guided sectioning, most cuts are made to confirm or further examine focal lesions, while using the standard method, deeper cuts are often 'blind', because of a discordance between the histological and the clinical or macroscopic findings. The entire impact of EVD with DD on the accuracy and efficiency in dermatopathology is difficult to measure, but we are convinced that EVD with DD is an important improvement compared to random transverse cutting.

We also evaluated the potential usefulness of derm dotting in oral surgery for the resection of squamous cell carcinomas [27]. Moreover, the usefulness of this method was also examined on frozen sections. For these sections too, derm dotting is an inexpensive, simple method that can replace marking by stitches. The varnish dots or lines, using different colours, can be used to orient the specimen, and to mark suspicious section margins or focal areas of interest to be examined by the pathologist with greater care.
In addition to its value in clinical routine dermatopathology, EVD in combination with histopathology can also be used as a scientific tool to explain dermoscopic findings at a microscopic level [28]. We were able to find a histological correlate and optical explanation

for rosettes and other white shiny structures seen in polarised dermoscopy. We could demonstrate that smaller rosettes are mainly caused by polarizing keratin material in adnexal openings, and larger rosettes by concentric perifollicular fibrosis. Rosettes are an optical effect of crossed polarization that can be observed in many tumoural and also inflammatory skin lesions, and therefore, are not specific. White shiny streaks correlate with polarization of fibrotic changes in the dermis.
With EVD with DD we also could demonstrate that yellow dots seen in some naevi correspond to balloon cell nests, identifying balloon cell differentiation as another possible explanation for the appearance of yellow dots in a skin lesion (see atlas ex vivo p 107).

Professional considerations

The method of ex vivo dermoscopy with derm dotting does not only permit a faster and more accurate diagnosis, but it also leads to a different diagnostic approach whereby the diagnosis of a skin tumour becomes a unique intellectual process that integrates ex vivo dermoscopy and microscopic images, in addition to clinical information. This process starts for the dermatopathologist with a 'blind' examination of the microscopic slide (i.e. blinded to clinical and ex vivo dermoscopic information). Next, the first diagnostic impression is correlated with the ex vivo dermoscopic information, which also serves as an internal control. The emerging diagnosis has to fit the EVD-aspect and fully explain eventual dotted and traced focal lesions. When the microscopic information does not match or does not explain all the EVD-information, no definite diagnosis can be made and deeper cuts and further clinicopathological correlation are required.
As such, diagnosing a skin tumour using EVD with DD gives more confidence to the pathologist. Lesions examined at a microscopic level are not only classified, but also individually checked whether they are representative for the whole lesion. The importance of this inherent internal control of the method is difficult to statistically measure, but to us, in a practical dermatopathology context, this is at least as important as the measurable diagnostic advantages.
It cannot be ignored that an additional advantage of EVD with DD is the engagement of laboratory technicians in the diagnostic process. The method demands gifted, motivated technicians with knowledge of dermoscopy, pathology, and dermatology. It might be suggested that this method makes their job less monotonous, more interesting and gives them more responsibility and satisfaction.
Since this method demands for sub-specialization of technicians, it is more difficult to implement in a general pathology lab where dermatopathology is only a minor part of the workload.

As a result of our observations, we propose the following possibilities for (full or partial) integration of EVD with DD:

For larger general pathology labs with a significant dermatopathology supply, we advise to create a special dermatopathology technician team that can be educated in the EVD with DD method.

In labs where full implementation of EVD with DD is not possible, a less extensive form of dermoscopy-guided and targeted processing can be put in place: the referring dermatologist can dot possible diagnostic areas or suspicious margins with nail varnish in vivo, prior to the excision.
We are currently investigating how this could be done. The first results are promising and show that the varnish dot can be applied before the region is anesthetized for resection of the lesion. The dot is resistant to local disinfection and manipulation of the tissue afterwards. When the biopsy arrives at the lab, the dot can be inspected, even removed with nail polish remover to detect the underlying hot spot and to eventually take an EVD. The dot can be redone to target the focal lesion indicated by the clinician.

In the setting where no EVD can be done in the lab, it would be advisable that the dermatologist provides an accompanying in vivo dermoscopic photograph to the pathologist. The lab technician can trace the dot on the microtome and the pathologist can correlate his findings with the IVD to correctly diagnose the lesion.

Apart from the value for tumoural skin disease, implementation of the EVD with DD method may also be useful in cases of inflammatory skin lesions. The routine marking of the center of diagnostic punches of inflammatory skin diseases, allows a direct and controlled examination of the center of the punch. Also, the marking of focal crusts, blood vessels, pigmentation, vesicles, ulcerations, or erosions can guide towards the most affected, most diagnostic area of the inflammatory skin disease. Examples of this are the foci of acantholysis in focal acantholytic disorders, cornoid lamella and the central crust or spongiotic vesicle in an insect bite.
We have the impression that the implementation of the method of EVD with DD intensifies the cross-talk between pathologists and dermatologists: derm dotting can be guided by

the clinical information and an area of concern for the clinician. The report starts with a description of the ex vivo dermoscopic findings, which invites dermatologists to correlate ex vivo data with their in vivo dermoscopy findings. For many dermatologists, this feedback is probably an incentive to valorize dermoscopy as diagnostic tool in their daily diagnostic work. This potential stimulus to use dermoscopy could be a subject for a future study.
Finally, this research work demonstrates that clinicopathological correlation is no longer completely dependent on the information written down by the dermatologist in the pathology request form, but can be, for an important part, within reach of the pathologist.

Scientific perspectives

It would be interesting to compare the concordance between in vivo marking by dermatologists and independent ex vivo marking in a dermatopathology lab. For this purpose, we are planning a retrospective study on a large series of consecutive skin tumours with IVD- and EVD-documentation, coming from the same dermatology practice. Experienced dermatologists will review the in vivo dermoscopy images ‘blindly’ and mark areas of interest, which in their opinion need targeted histology for a correct and complete diagnosis. These selected areas will be compared with the actual areas of interest that were selected by EVD with DD in the dermatopathology lab.
As described in the paper on the diagnostic performance of EVD with DD [26], the implementation of EVD with DD allows us to subtype (or categorize) more than one fourth of the naevi as a type of naevus with special clinicopathological characteristics. This might explain the atypical clinical presentation and the reason for excision. Some of these subtypes are known and well-described, for example hyperpigmented flat naevi.
For other subtypes, the clinicopathological meaning may not be so well understood, for example for ‘actively growing naevi’.
A retrospective analysis of the clinical features of these subtypes detected by EVD with DD (such as age, gender, and localization) could tell us more about their exact nature. It particularly offers a new option to investigate whether they form separate clinicopathological entities with possible prognostic and therapeutic consequences.
For example, fibrosing/sclerosing naevi are detected more frequently with this approach. Moreover, we can subtype them as superficial, focal, perifollicular, and deep sclerosing forms. In these lesions pseudomelanomatous features may be seen.
At the moment, the relevance of this subtyping is unknown, but retrospective or prospective analysis of these lesions might teach us more about the pathways of senescence, natural evolution, or growth arrest of naevi.

Investigation of the group of naevi with inflammation (e.g. halo naevus and lichenoid or eczematous naevus) might inform us about a possible inflammatory pathway involved in the natural evolution, maturation and growth arrest of these lesions.

The concept of dysplastic naevi

Since 1997, when Wallace Clark described the so called ‘dysplastic naevus’ [29], there has been a lot of controversy about the exact nature of this type of mole, and its clinical and histological definition has been the subject to different views [30]. Clinically, these are flat naevi with some features that may resemble melanoma, and on dermoscopy, lesions are well delineated with irregular borders and variation of colours. Since the diagnosis of a ‘dysplastic naevus’ requires histologic evaluation, the term ‘atypical naevus’ is considered more appropriate for the clinical classification. Although a universally accepted definition for dysplastic naevi does not exist, they are retained to be clinically relevant and considered to represent a marker for melanoma development. It is, however, not possible to predict which lesions might progress to melanoma and which ones may serve as predictors of melanoma risk.

Most pathologist still use the term ‘dysplastic naevus’, others prefer ‘atypical naevus’ or ‘Clark naevus’. Regardless of what they are named, they are usually graded into mildly, moderately, or severely dysplastic. Only in the case of severely dysplastic lesions, which are difficult to distinguish from melanoma in situ, a 0.5 cm broader resection is advised. The low-grade lesions are considered benign.

Moles removed as ‘dysplastic naevus’ represent an important fraction of the melanocytic lesions examined by pathologists. Since a universally accepted pathological definition of dysplastic naevi is lacking, the diagnostic term ‘dysplastic naevus’ is used for a broad spectrum of pigmented lesions. First of all, there is a lack of concordance between clinically atypical naevi and histological dysplasia. Moreover, some clinically dysplastic naevi have no histologically dysplastic features, while clinically non-atypical lesions might have. Naevi with some atypia that do not correspond to classic subtypes, such as flat Clark, Miescher, or Unna naevus, are often diagnosed histologically ‘dysplastic’. This because of a prudent reaction not to underdiagnose a potentially malignant lesion. Even, since over diagnosis will usually be without consequences, some of these dysplastic naevi may be incorrectly diagnosed as thin melanomas. However, with good clinicopathological correlation, many of these lesions can be diagnosed as benign reactive, traumatized, inflamed, fibrotic/sclerotic, actively growing, or phenotypically heterogeneous naevi.

In one third of melanomas, a pre-existing naevus is found, mostly of a non-specific congenital type [31]. In a smaller part of melanomas however, the melanoma is arising in a pre-existing flat dysplastic naevus [32], strongly supporting the existence of an authentic dysplastic naevus that may evolve into melanoma. The existence of a genetically intermediate type of naevus has recently been proposed by Shain et al [33]. The criteria to recognize this “authentic” dysplastic naevi and their exact morphological counterpart is unknown.

Apart from molecular analysis, a possible way to identify the exact nature of different types of flat naevi is a clinicopathologic approach. As already mentioned, with EVD with DD-guided clinicopathologic approach, we could make a specific benign clinicopathologic diagnosis in 27.7% of the naevi, most of which were excised as an ‘atypical naevus’. In these lesions, none or only mildly atypical features were seen. A small remaining group of flat naevi was diagnosed as moderately (7.2%) or severely atypical (1.4%). The revision of these lesions allowed us to identify a subtype of naevus with distinctive ex vivo dermoscopic and histopathological features. On EVD, they appeared as flat lesions with an orange-brown peripheral colour and with areas of an irregular broken, or even linear, pigment network and a variable blue-white centre. In most cases, the diameter of these lesions varies from 5 to 10 mm. On histology, these lesions contain a variable number of small to medium sized melanocytes with a finely granular pigmented cytoplasm, sometimes conferring a grey colour. This type of melanocyte corresponds to what has been previously described as ‘pulverocytes’ [34].

These pulverocytes are mainly localized along the asymmetric, junctional shoulders of these lesions. The cases with a blue centre, contain numerous melanophages. The shoulders are composed of irregularly growing melanocytes, linearly arranged along the basal layer of the epidermis or forming small irregularly placed nests. In most of these lesions, the nests show variable horizontal fusion.

The irregular broken up network seen on EVD, can partially be explained by the peculiar linear grey pigmentation of basal epithelial cells. In contrast to the normal basal keratinocytes, where the pigment is in an apical perinuclear position, the dusty pigment is present at the bottom of these basal cells. However, due to hyperpigmentation, the distinction between lentiginous pulverocytes and pigmented epidermal basal cells can be difficult to make. A melan A stain is necessary to identify the exact number of melanocytes, their growth pattern, and the grade of atypia in the shoulders of these lesions.

Interestingly, immunostain for p16 showed a variable loss of the protein in some of these lesions, from scattered cells to the entire pulverocytic component. There might be a correlation between p16 loss and the degree of atypia. Most lesions with severe atypia (lesions with some epidermotropism, irregular fusion of junctional nests and clear random atypia) show areas with complete loss of p16. These data correspond well to the existence

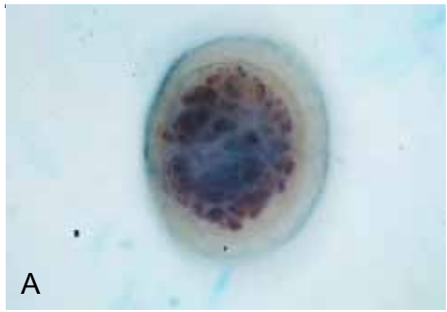
of a so called ‘intermediate lesion’, gradually evolving from naevus into a melanoma [33]. In their study, Shain et al defined the succession of genetic alterations during melanoma progression and identified an intermediate category of melanocytic neoplasia, characterized by the presence of more than one pathogenic genetic alteration and distinctive histopathological features. Homozygous loss of p16 seems to be a late event in this genetic cascade, since it was observed only in fully developed melanomatous lesions. As such, the “pulverocytic orange naevi” could represent a genuine ‘dysplastic naevus’. So far, we collected over 280 of these lesions. Further molecular analysis of these lesions is required to better define their nature and their potential capacity to evolve into melanoma. Moreover, some patients have had multiple atypical pulverocytic naevi and were diagnosed as patients with a dysplastic naevus syndrome or with the so called ‘signature naevi’. We are actually performing a retrospective analysis of clinical features, like gender, age, localization, and personal and/or familial history of these naevi. In this way, we want to examine whether morphologic features can be identified to separate them from other flat naevi and to determine their risk of progression to melanoma.

In conclusion, EVD with DD allows adapted sectioning of skin lesions and permits a more accurate histological diagnosis in less time, compared to the standard method of random transverse cutting. It allows better section margin evaluation, better visualization of skin lesions, and specific marking and evaluation of focal or suspicious alterations. The method is easy to implement in a dermatopathology setting.

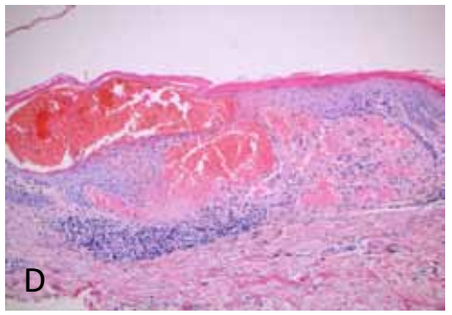
Especially for classification and interpretation of flat naevi, the method adds a new dimension to the clinicopathologic approach of these lesions. Besides a more confident and specific diagnosis, it might lead to more insight in the pathologic correlate of specific features observed in dermoscopy, as well as to a more accurate delineation of specific subtypes of melanocytic naevi.

Chapter VIII Atlas of Ex Vivo Dermoscopy

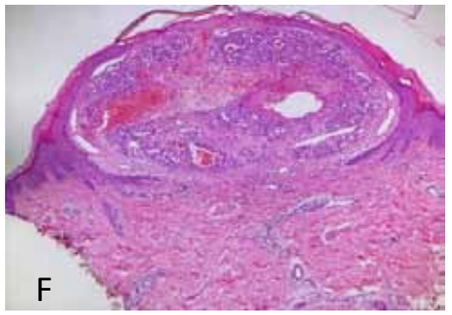
Angioma



The dermoscopic hallmark of hemangiomas is the presence of lacunae corresponding to well demarcated purple blue to brown round to oval areas (A). A thrombosed angioma shows formation of larger brown black area in a blue white veil background (B).

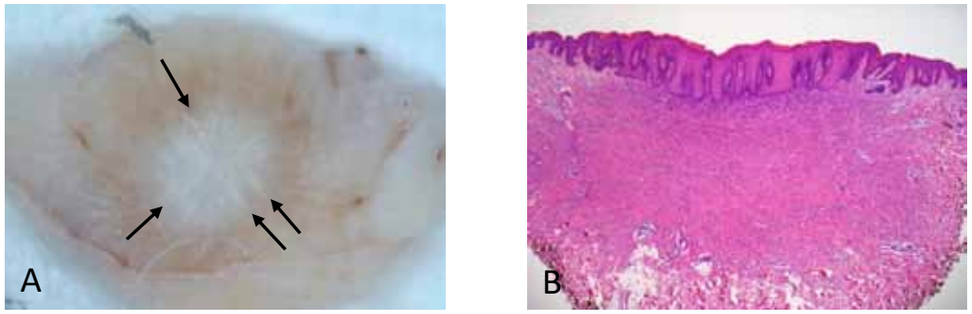


In this angiokeratoma a whitish veil covers partly the vascular lesion, a focally crusted area contains areas of corneal hemorrhage(C). The variable blue white veil corresponds to a hyperplastic and hyperkeratotic epidermis, the lacunae to dilated vessels(D).

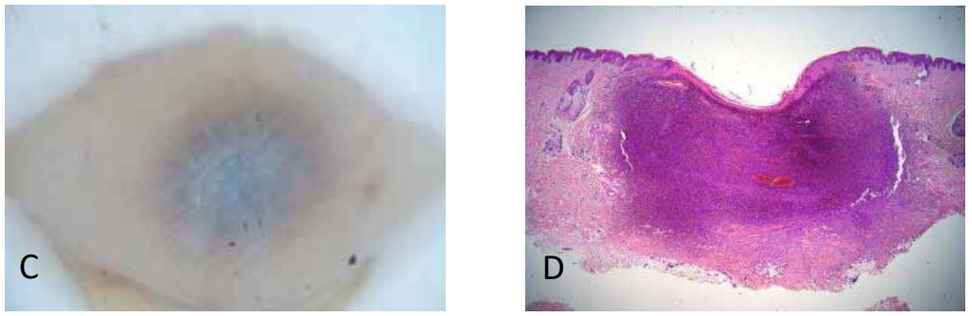


A pyogenic granuloma (lobular angioma) shows a white collarette surrounding a homogeneous orange brown center (E), the collarette corresponds to the formation of a claw like epidermal border around the angioma (F).

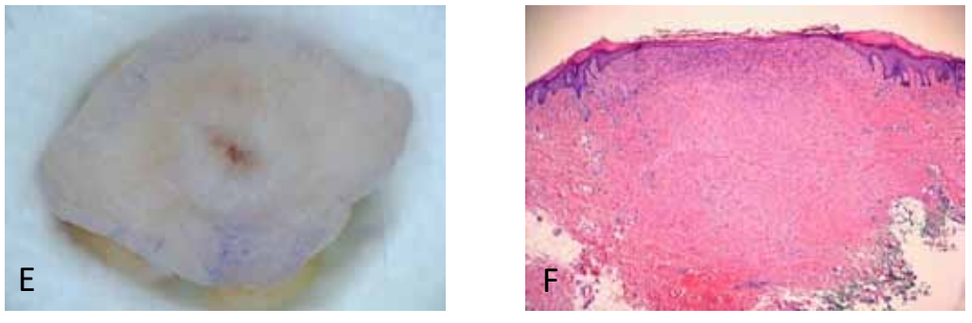
Dermatofibroma



Most dermatofibromas show a white scar like center surrounded by a peripheral brown ring with delicate pigment network (A). Lentiginous pigmentation and elongation of rete ridges creates a delicate peripheral pigment network. Peripheral sclerosis forms chrysalis like, stellar structures (B).

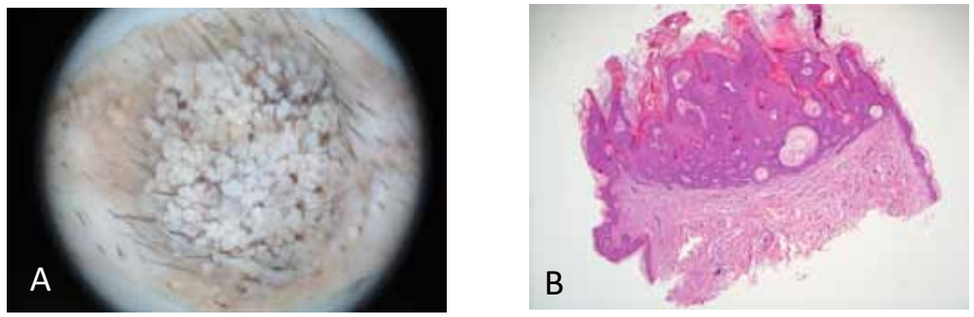


Cellular dermatofibroma with siderophagia forms a blue homogeneous aspect on EVD (C,D).

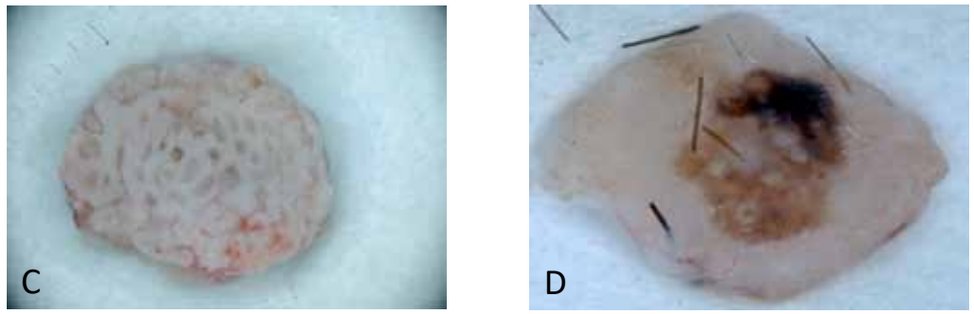


Superficial perforating dermatofibroma with central brown crust (E) formed by a thinned hyperkeratotic epidermis (F).

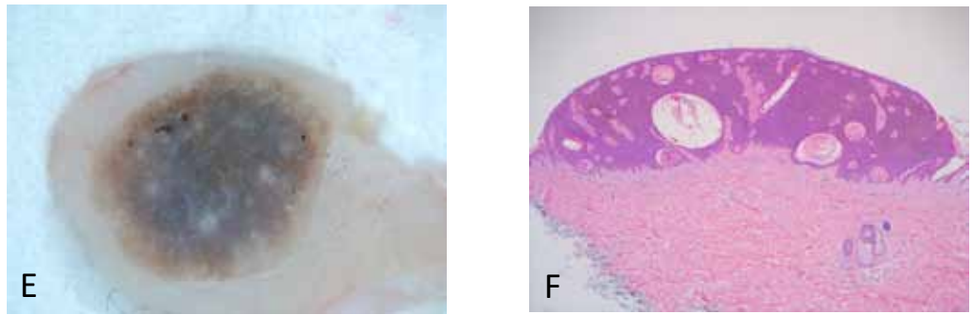
Seborrheic keratosis



Non pigmented hyperkeratotic seborrheic keratosis (A) with papillomatosis and hyperkeratosis of epidermis (B).

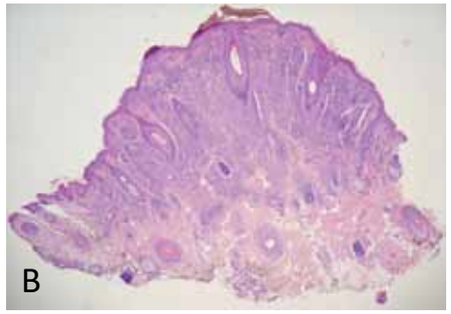


Non pigmented seborrheic keratosis with cerebriform surface (C) and seborrheic keratosis with hyperpigmented part and hypopigmented part (D).

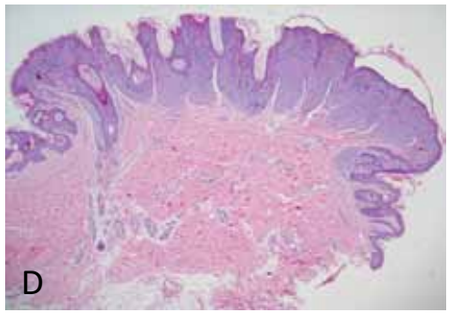


Pigmented acanthotic seborrheic keratosis forming a blown blue plaque without network (E). The comedo like cysts corresponding to pseudo horn cysts on histology (F).

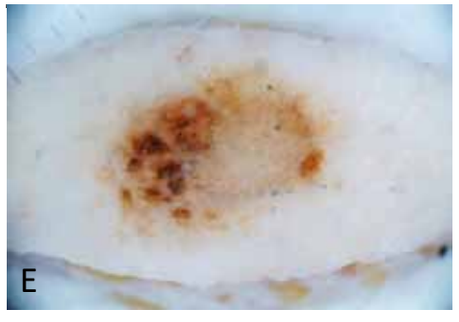
Miescher - Unna naevi

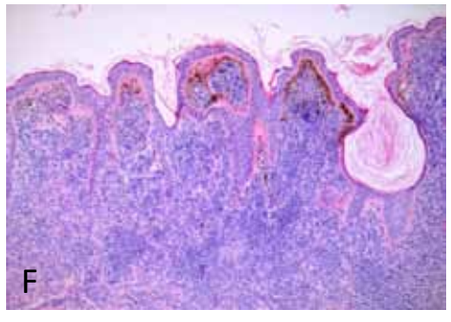


Miescher naevus forming hairy white structureless nodule (A,B).



Unna type with papillomatous nodular appearance (C, D).





Combined lesion with flat part and papillomatous part (E). Cobblestone pigmentation corresponds to pigmented nests of melanocytes in the upper dermis (F).

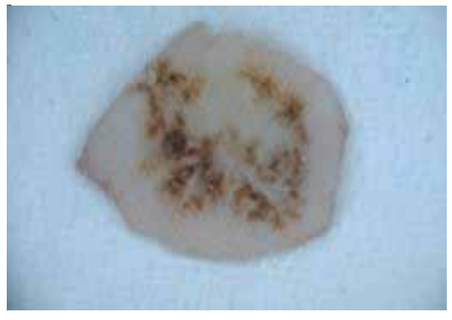
Clinicopathologic subtypes with EVD with DD

Actively growing naevus

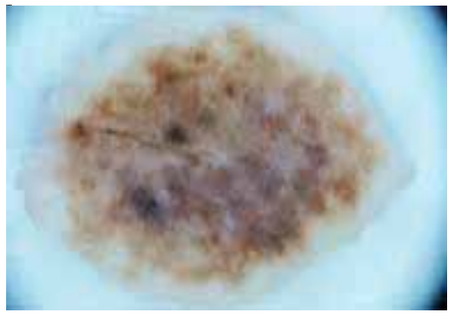
Naevus with perifollicular fibrosis

Meyerson naevus

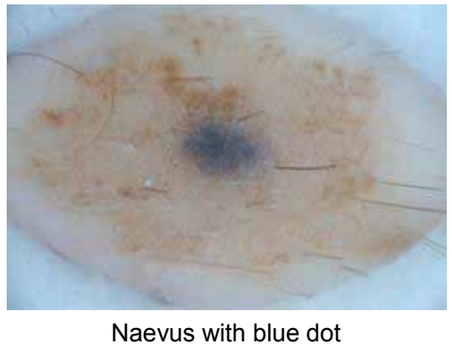
Recurrent naevus



Sclerosing naevus



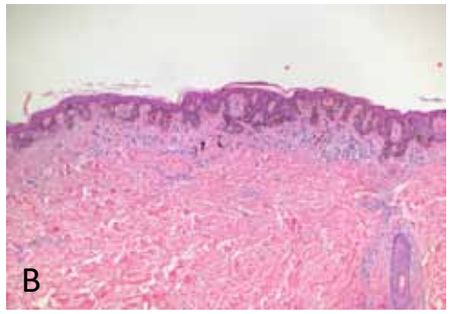
Superficial fibrosing naevus



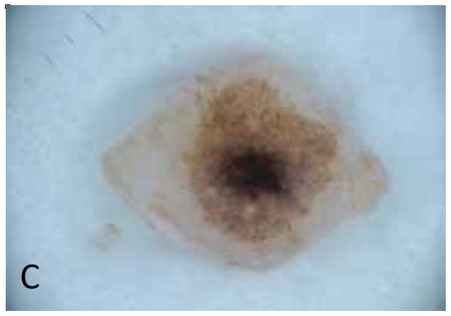
Naevus with blue dot

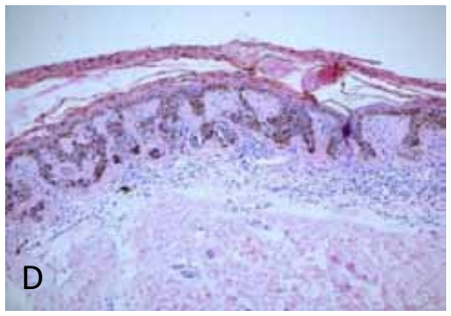
Naevus with asymmetric shoulder

Flat reticular and hyperpigmented naevi



Flat reticular naevi show a network pattern (A) corresponding to pigmented melanocytes arranged in single units or small nests along the elongated rete ridges (B).

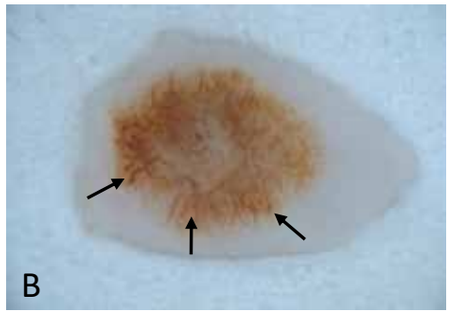


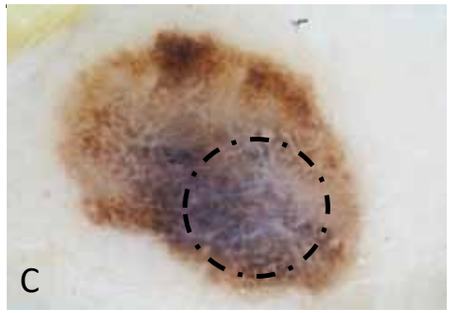


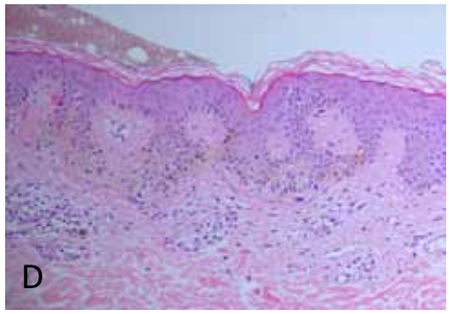
The central hyperpigmentation (C) is often due to a combination of black lamella formation, corresponding to pigmented parakeratosis and a black blotch formed by hyperpigmented seborrheic epidermal reaction (D).

The central hyperpigmentation varies in diameter (E) and can also be patchy reticular (F) with areas of perifollicular depigmentation.

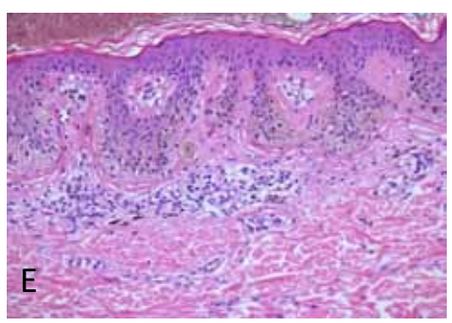
Dysplastic naevi

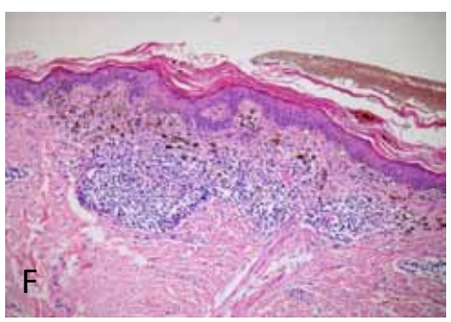






Dysplastic naevi often show some benign dermoscopic patterns associated with one or more melanoma specific patterns like 3 colours (A) or streaks (↗) (B) or blotches with negative network (C).

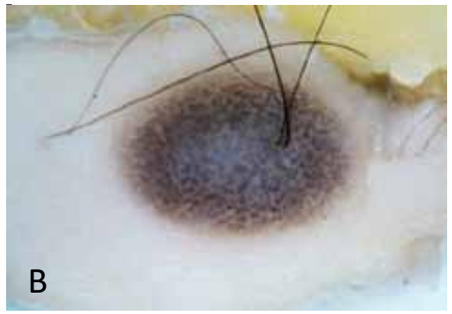




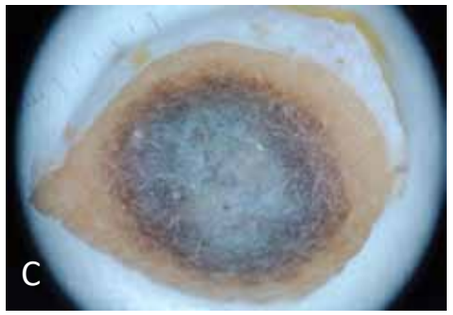
Streaks correspond to horizontally fused junctional nests (D), negative network to areas of confluent lamellar fibroplasia (E) multiple colours to presence of melanin pigment at different levels and epidermal keratotic reaction (F).

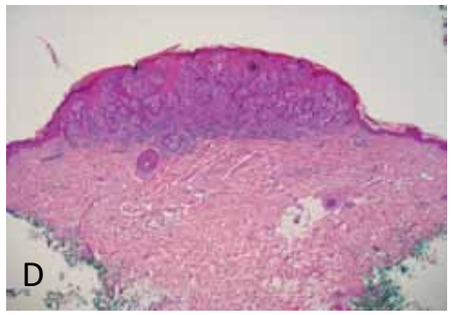
Spitz/reed naevi

Amelanotic Spitz naevus (A).

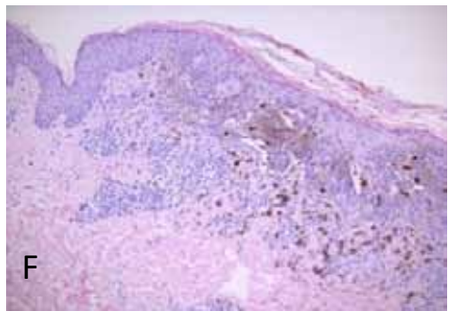


Partially pigmented Spitz naevus (B).



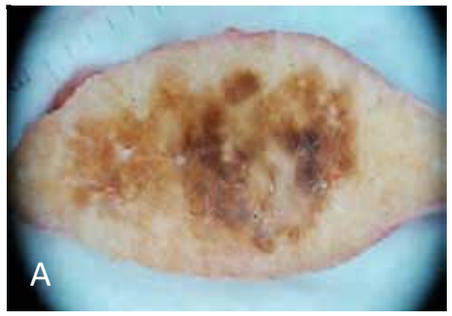


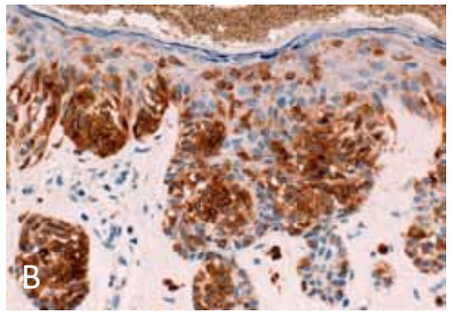
Pigmented Spitz naevus with blue veil (C) formed by hyperkeratotic and hyperplastic epidermis (D).



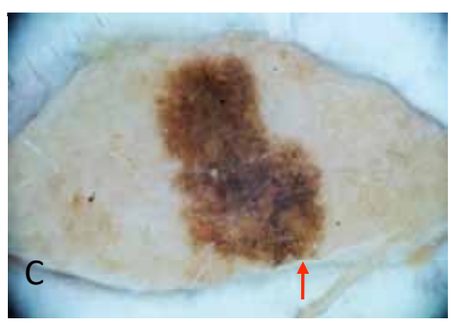
Pigmented Spitz (Reed) naevus with starburst pattern (E) formed by horizontally fusing peripheral junctional nests (F).

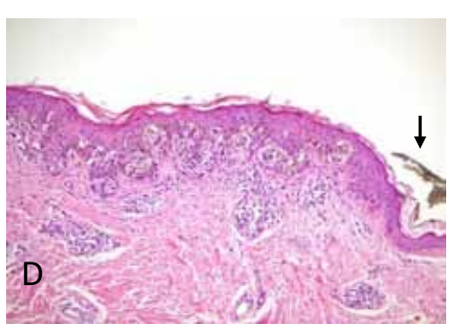
Melanoma in situ



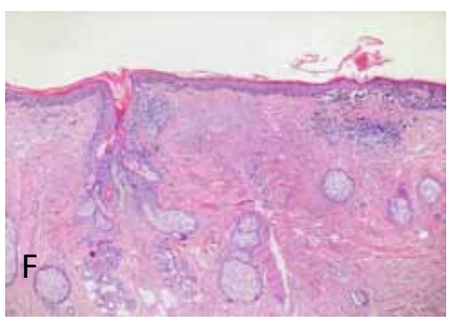


Lentiginous melanoma in situ evolving in atypical naevus of the elderly forming a large flat irregular brown lesion with white structureless areas (A). Melan A of these areas shows junctional growth with surprising epidermotropism of small melanocytes (B).



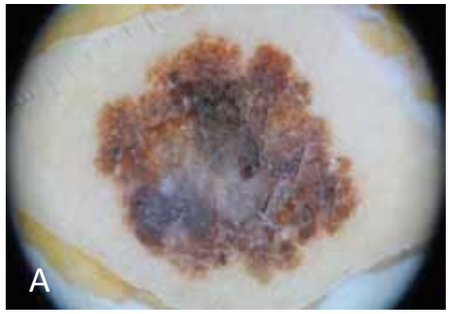


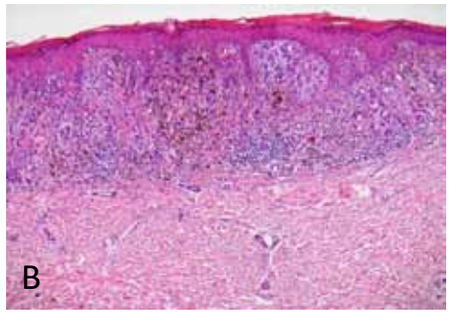
Melanoma in situ flat irregular brown lesion with loss of structure, and suspicious margin (↗) (C) corresponds to pigmented intraepidermal melanocytes (D). Margin is negative (↗).



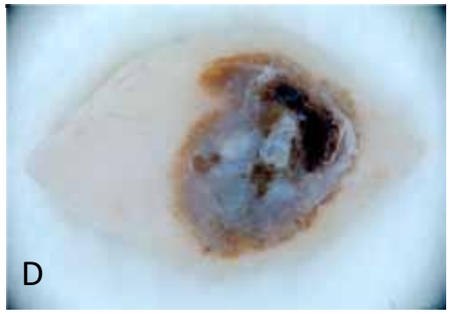
Lentigo maligna with irregular, confluent perifollicular pigmentation and areas of blue grey peppering (E), corresponding to irregular lentiginous and partly nested melanocytic proliferation with perifollicular extension (F).

Melanoma

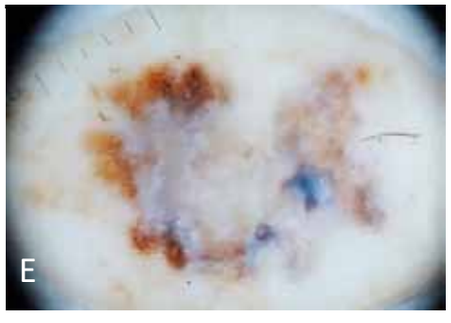


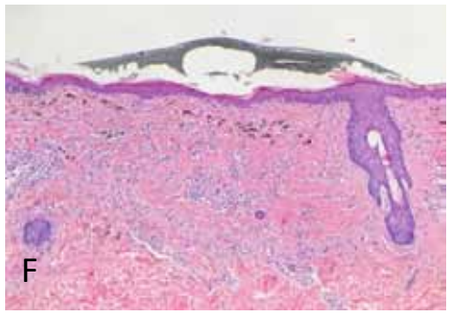


Superficial spreading melanoma forming large asymmetric lesion with extensive loss of network and brown, black, white and blue colour (A). Multiple colours correspond to the presence of melanin at various levels within epidermis and dermis (B).



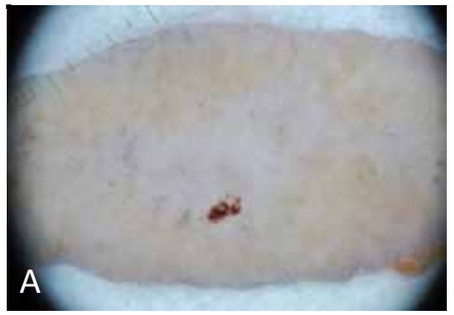
Nodular melanoma with blue veil, irregular globules and areas of negative network (C). Nodular melanoma with 3 colours and ulceration (D).

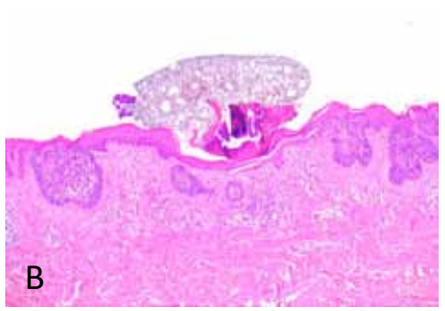




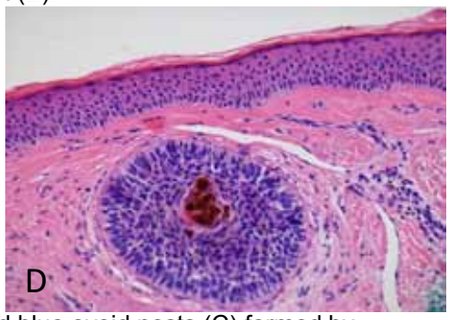
Melanoma with an extensive white gray area of regression and multi-coloured appearance (E). Dotted area of regression corresponds to fibrosis and melanophagia (F).

Basal cell carcinoma

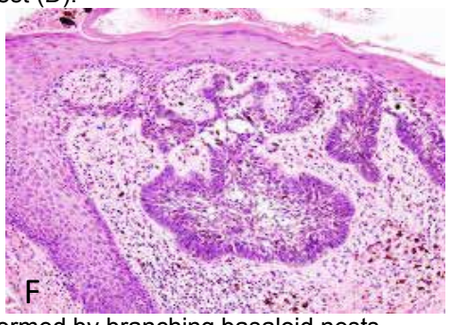




Superficial type with a well delineated white structureless area with dotted scale (A) corresponding to hyper and parakeratotic crust (B).



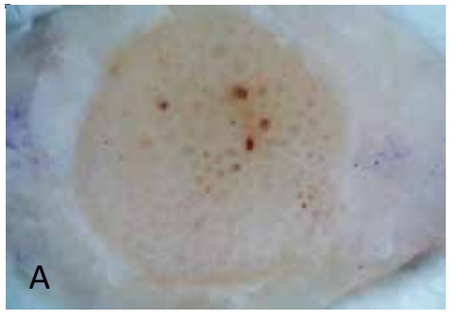
Superficial type with peripheral brown grey and blue ovoid nests (C) formed by melanin pigment in dermal nodular basaloid nest (D).

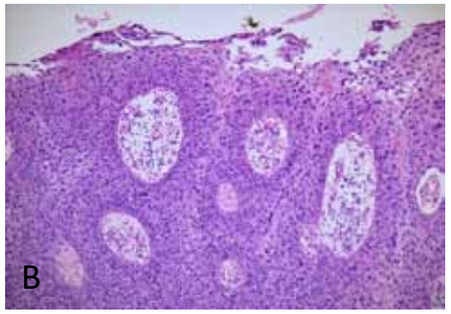


Superficial lesion with leaf like structures (E) formed by branching basaloid nests with intratumoural and peritumoural melanophagy (F).

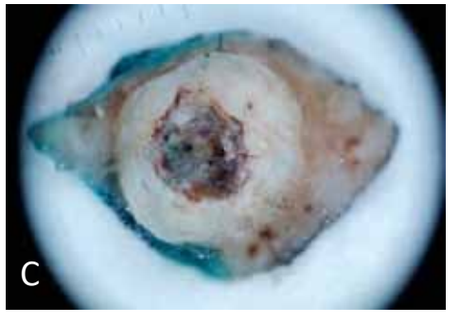
A well delineated nodular lesion with arborifying angiectatic vessels (G), Noduloulcerative lesion with irregular brown black ulcerative areas with presence of vessel structures (H).

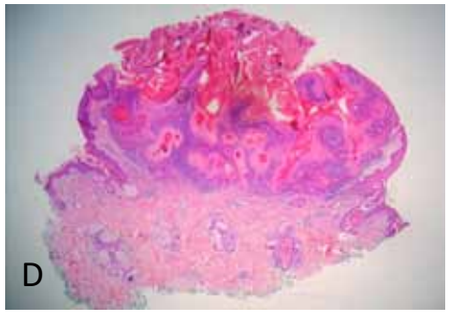
Squamous lesions



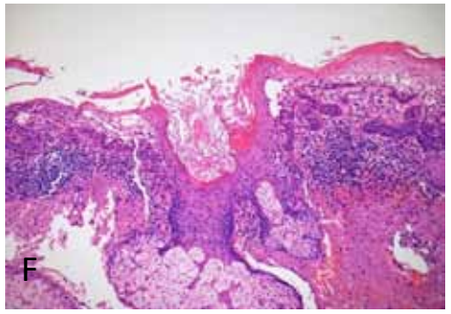


Bowenoid intraepithelial neoplasia with formation of glomeruloid papillary vessels visible on EVD (A,B).





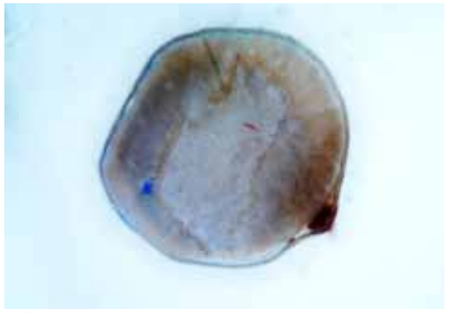
Keratoacanthoma type of squamous carcinoma with umbilicated keratotic aspect (C) corresponding to crateriform growth pattern with formation of surrounding epidermal collarette (D).



Actinic keratosis with crusty aspect and white circles on EVD (E) corresponding to dysplastic budding of epidermis and alternating ortho- and parakeratosis (F).

Special, surprising and unusual cases

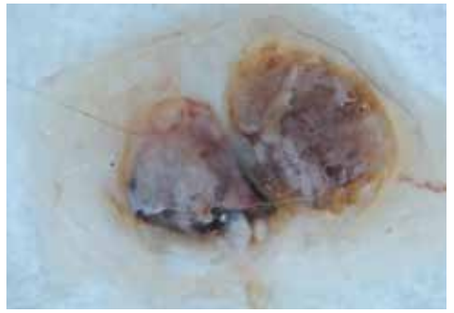
Clear cell acanthoma with vessel component forming string of perls.



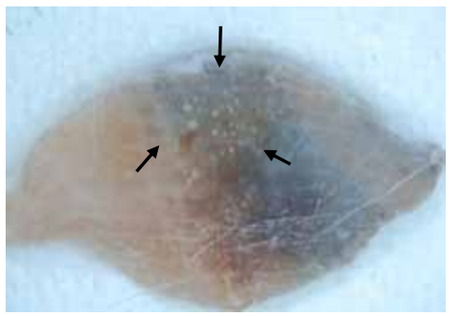
Porokeratosis with railway track forming cornoid lamella.

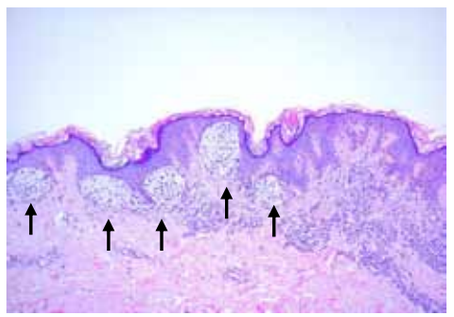
Ingrowing hair.

Tufted folliculitis.



Tunga penetrans: epidermal side shows perforating cloaca (left), dermal side shows numerous eggs (right).





Naevus with yellow dots (↗) corresponding to balloon cell nests.

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Summary

In daily practice, most pathology labs process skin biopsy specimens without access to the clinical and/or dermoscopic images. In the evaluation of skin tumours, this information can be crucial to process and diagnose these lesions correctly. Ex vivo dermoscopy (EVD) was first described as a valuable tool in dermatopathology in 2007. It was reported that EVD can reduce errors by aiding selection of areas in which to perform step sectioning, can help in the diagnosis of ambiguous lesions, and is of help in the evaluation of section margins. More and more smaller lesions are undergoing biopsy, and specific areas of concern are sometimes only visible with dermoscopy. In many dermatopathology labs, this information is not available, and one can assume such areas are often missed with the standard random sectioning technique that examines less than 2% of the tissue. Some dermatologists mark areas of interest for the pathologist with a 1-mm punch or use a suture marking to direct the pathologist to these suspicious areas. However, these methods are not satisfactory because they can create an artifact in the lesion or biopsy specimen. First of all, this thesis describes the development of a new method of processing skin biopsies using EVD, that could overcome the flaws of the actual standard tissue cutting. EVD, in combination with marking of focal or suspected alterations in lesions and of suspected section margins with nail varnish (‘derm dotting’), is a simple and easy method that brings all the necessary information for a complete macro- and microscopical evaluation of skin lesions to the pathologist. The nail varnish is resistant to tissue processing, discernible while cutting the tissue block, and easily recognisable under the microscope. In this thesis, we compared in vivo dermoscopy (IVD) and EVD and validated EVD with derm dotting as a new standard processing method, identifying its advantages in the pathological evaluation of skin tumours. Moreover, this technique was also evaluated in oral and maxillofacial surgery. The method can also be used as a scientific tool for the identification of histopathological correlates of dermoscopic structures. It was used successfully to find a morphological explanation for rosettes and other white shiny structures in polarised dermoscopy and to identify yellow dots as a dermoscopic sign of balloon cell differentiation in naevi.

The **Introduction** gives a general overview of the history of dermoscopy and explains the difference between polarised and non-polarised dermoscopy. In this chapter, we also summarise the history of tissue processing, we describe the standard method of tissue handling and processing, as well as the flaws of this method, especially in dermatopathology.

The aims of this thesis are summarised in **Chapter I**.

Chapter II describes in detail the technique of EVD with derm dotting, a 'new' method for tissue processing and lesion evaluation. The current standard cutting and sectioning protocol is also presented.

In Chapter III, EVD was compared to IVD in the evaluation of skin tumours. One hundred and one consecutive IVD and EVD images of skin tumours were evaluated by four observers (three dermatologists and one dermatopathologist) blinded to the histopathological diagnosis, who independently scored and compared EVD image features (colours, structures, and vessels) with those of the corresponding IVD images. EVD images were generally similar to the corresponding IVD images, although blue and white were observed more often, in respectively 32% and 25% of observations. On the other hand, EVD showed loss of red compared to IVD in 70% of observations. Most structures were well preserved. New areas without structure were found with EVD in 20% of observations, and new crystalline structures were detected in 17% of EVD images. On EVD images, squamae and crusts were lost in 14% and 11% of observations, respectively, while blood vessels in 35% of the images. The main conclusion is that EVD shows similar colours and structures although there is clear loss of red, thereby revealing in some cases blue and white colours that were not visible on IVD. As such, EVD is an important new tool in dermatopathology that may give direction to targeted tissue processing and examination of skin tumours.

In chapter IV, we describe the advantages of ex vivo dermoscopy with derm dotting for the evaluation of skin tumours. 6,526 skin biopsy specimens examined from 2008 to 2010 with a standard method were compared with 8,584 biopsy specimens examined in 2015 using the EVD with DD technique. All specimens from both periods were examined and diagnosed by the same dermatopathologist. Clinicopathological characteristics, added value of EVD with DD, and turnaround times (TATs) were the main outcomes and measures of this study. The use of EVD with DD increased the detection of positive section margins in non-melanoma skin cancer from 8.4% to 12.8%. The most significant increase was seen in Bowen disease, invasive squamous cell carcinoma, and superficial basal cell carcinoma (BCC). With EVD with DD, a specific clinicopathologic diagnosis was made in 27.7% of naevi compared with only 10.3% using the standard method. The incidence of moderately and severely dysplastic naevi increased from 1.0% to 7.2% and from 0.6% to 1.4%, respectively. The detection of ulceration in melanomas thicker than 1 mm increased from 24.0% to 31.3%. The number of naevi-associated melanomas increased from 15.5% to 33.3%. The number of collision lesions from 0.07% to 1.07%. Furthermore, the TAT for naevi decreased from two days to one day, for melanomas from five days to two days, and for BCCs from two days to one day.

In conclusion, EVD with DD with adapted sectioning in a dermatopathology setting allows a more accurate and less time consuming histopathologic diagnosis of skin tumours. These results suggest that pathologists involved in skin tumour evaluation should be encouraged to learn dermoscopy and replace random transverse cutting with lesion-specific and DD-guided cutting.

Chapter V illustrates the use of derm dotting as a marking technique on mucosal surfaces. We used this technique on resection oral specimens of squamous cell carcinomas of nine patients. We also tested this method on frozen sections. In all cases, the nail varnish was visible on gross examination of the resected tissue, it was traceable while cutting the tissue block, and clearly visible in the definitive sections. The dots were preserved in the frozen sections, but they were lost in half of the decalcified tissues.
In conclusion, derm dotting is an inexpensive, simple method that can replace the stitching technique used by oral surgeons to orient specimens. The varnish dots or lines can be used to orient the specimen. In addition, the dots can easily mark suspected borders or areas of interest to be examined by the pathologist, using different colours.

Chapter VI describes the use of EVD as a scientific tool for the identification of histopathological correlates for dermoscopic structures. In particular, we searched for the precise morphological correlate and an optical explanation for rosettes and other white shiny structures in polarised dermoscopy. A series of 6,108 consecutive skin biopsies were examined in EVD and when rosettes were present, serial transverse sections were examined with polarization. Rosettes were found on EVD in 63 cases. When multiple they were always oriented at the same angle. Transverse sections viewed microscopically under polarised light proved that smaller rosettes are mainly caused by polarizing horny material in adnexal openings, and larger rosettes by concentric perifollicular fibrosis. The scientific conclusion of this study is that rosettes are not lesion-specific, but an optical effect of horny material and concentric fibrosis.
Also in naevi with yellow dots on dermoscopy, balloon cell differention was identified as another possible explanation for the dermoscopic appearance of yellow dots in a skin lesion.

Chapter VII, 'General discussion and future perspectives', emphasizes the daily practice influence in dermatopathology and the scientific perspectives of the technique of EVD with DD. Finally, a possible role for EVD in helping to unravel the nature of subtypes of dysplastic naevi is discussed.

Chapter VIII represents the first atlas of ex vivo dermoscopy of the main tumoural skin lesions, with their histopathologic correlates. Diagnostic clinicopathological entities resulting from this dermoscopic, targeted approach in subtyping of naevi are illustrated. Finally, some unusual, surprising or special EVDs are included.

"Melanoma writes its message on the skin with his own ink, and it is there for all to see. Unfortunately, some see, but do not comprehend"

- Naeville Davis, 1978 -

Ten reasons to use EVD with DD

1. EVD with DD significantly improves your **diagnostic accuracy**.
2. EVD with DD makes you **understand** why the lesion has been resected.
3. EVD with DD increases your **confidence** when diagnosing difficult lesions.
4. EVD with DD identifies diagnostic foci permitting **targeted** processing.
5. EVD with DD identifies **suspicious borders**.
6. EVD with DD identifies **histologic correlate** of dermoscopic structures.
7. EVD with DD reduces **unnecessary deeper cuts**.
8. EVD with DD is **cheap and easy** to apply.
9. EVD with DD **triggers lab technicians** who become part of the diagnostic process.
10. EVD with DD stimulates **clinicopathologic cooperation** with the dermatologist.

Samenvatting

In de meeste laboratoria voor pathologie worden huidbiopsieën verwerkt en geëvalueerd zonder informatie over klinische en/of dermoscopisch beelden. Bij de evaluatie van huidtumoren kan deze informatie nochtans erg belangrijk zijn om deze letsels correct te verwerken en te diagnosticeren. In 2007 werd er gepubliceerd dat ex vivo dermoscopie (EVD) een belangrijke aanwinst kan zijn voor dermatopathologie. Er kon namelijk worden aangetoond dat EVD het aantal fouten in de diagnostiek kan verminderen, aangezien het zowel een hulpmiddel is bij het selecteren van zones waarvan histologische coupes moeten worden gemaakt, als bij de diagnose van moeilijke of 'dubbelzinnige' letsels. Daarnaast kan het belangrijk zijn bij de evaluatie van snijranden. Op vandaag worden er steeds meer biopsieën genomen van kleinere letsels, die soms enkel zichtbaar zijn bij dermoscopie. In vele pathologie-laboratoria is de informatie over klinische en/of dermoscopisch beelden echter niet beschikbaar en kan er daarom verondersteld worden dat deze kleinere verdachte letsels vaak gemist worden bij het gebruik van de klassieke random, blinde sectietechniek, waarbij minder dan 2% van het weefsel wordt onderzocht. Sommige dermatologen markeren deze belangrijke zones echter met een 1-mm-pons of met een draad, wat aan de patholoog de mogelijkheid geeft om de belangrijke zones te traceren. Het nadeel van deze methodes is wel dat ze een artefact creëren in het letsel of in de biopsie.

Deze thesis beschrijft de ontwikkeling van een nieuwe methode voor het verwerken van huidbiopten, waarbij gebruikt wordt gemaakt van EVD, zonder de tekortkomingen van de oude methode. EVD in combinatie met het markeren met nagellak - derm dotting (DD) - van focale of verdachte veranderingen in letsels en van verdachte snijranden, is een eenvoudige methode, waarbij alle informatie ter beschikking wordt gesteld aan de patholoog die nodig is voor een volledige evaluatie van een huidletsel. De nagellak blijft aanwezig na behandeling van het weefsel in de weefselprocessor, is zichtbaar in de paraffineblok en is gemakkelijk herkenbaar onder de microscoop. Een ander voordeel van DD is dat deze techniek ook eenvoudig kan worden gebruikt om structuren en snijvlakken te markeren op slijmvliezen. In deze thesis wordt in vivo dermoscopie (IVD) vergeleken met EVD en wordt deze nieuwe methode, genaamd 'ex vivo dermoscopie met derm dotting', wetenschappelijk gevalideerd. Daarnaast wordt er gezocht naar potentiële voordelen van EVD met DD ten opzichte van de standaard toegepaste techniek (met de standaard klinische informatie) bij de dermatopathologische evaluatie van huidbiopten. Tenslotte kan EVD met DD ook gebruikt worden als wetenschappelijke methode om het morfologische substraat van dermoscopische structuren te identificeren, zoals rozetten of andere witte glanzende structuren bij dermoscopie met gepolariseerd licht en naevi met gele dots, die worden veroorzaakt door nesten met ballon-cel differentiatie.

In de **introductie** wordt een algemeen overzicht gegeven van de geschiedenis van de verwerking van weefsels door middel van microscopisch onderzoek en wordt eveneens de 'oude' methode van weefselverwerking uitgelegd. In dit hoofdstuk vatten we ook de geschiedenis van de dermoscopie samen en bespreken we het verschil tussen dermoscopie met niet-gepolariseerd en met gepolariseerd licht.

De specifieke doelstellingen van deze thesis worden samengevat in **hoofdstuk I.**

Hoofdstuk II geeft een gedetailleerde beschrijving van de nieuwe methode 'ex vivo dermoscopie met derm dotting' en van de manier waarop huidbiopten met behulp van EVD met DD in een laboratorium verwerkt worden.

In **hoofdstuk III** worden ex vivo en in vivo dermoscopie in detail met elkaar vergeleken op vlak van de dermatopathologische evaluatie van huidtumoren. Hiervoor werden 101 corresponderende IVD- en EVD-beelden van dezelfde huidtumoren gebruikt. Vier waarnemers (drie dermatologen en één dermatopatholoog) hebben onafhankelijk van mekaar, zonder kennis van de histopathologische diagnose, zowel de kleuren, de structuren als de bloedvaten zichtbaar op de EVD-beelden vergeleken met deze op de overeenkomstige IVD-beelden. In totaal werden 404 waarnemingen van 101 beelden geanalyseerd. Algemeen kan worden gesteld dat de EVD-beelden sterk op de overeenkomstige IVD-beelden leken, hoewel ze in kleur toch een aantal verschillen toonden: de beelden waren vooreerst beduidend donkerder. De EVD-beelden toonden nieuwe zones met een blauwe kleur in 32% van de gevallen en nieuwe zones met wit in 25% van de gevallen; verlies van rood werd gezien in 70% van de waarnemingen. De meeste structuren werden goed bewaard, maar ook hier werden kleine verschillen waargenomen. Nieuwe structuurloze zones werden gevonden in 20% van de EVD-beelden en nieuwe kristallijne structuren in 17% van de EVD-beelden. Op de EVD-beelden werd er een verlies vastgesteld van schilfers en korstjes in respectievelijk 14% en 11% van de observaties. Er was verlies van bloedvaten in 35% van de EVD-beelden. De belangrijkste conclusie van dit hoofdstuk is dat EVD een nieuwe methode is in de dermatopathologie, die een belangrijke rol kan spelen bij gerichte weefselverwerking en bij gericht onderzoek van huidtumoren.

In **hoofdstuk IV** worden de voordelen van het gebruik van EVD met DD beschreven bij de evaluatie van huidtumoren. Om dit op een objectieve manier te kunnen onderzoeken, werd de diagnostische performantie van de standaardmethode van weefselverwerking van 2008 tot 2010 onderzocht op 6.526 huidbiopsieën en vergeleken met 8.584 biopsieën onderzocht met EVD met DD in 2015. De biopsieën van 2008 tot 2010 werden verwerkt en onderzocht in een

algemeen laboratorium voor pathologie in een ziekenhuis; de biopsieën van 2015 in een privaat gespecialiseerd dermatopathologie-laboratorium. De biopsieën van beide perioden werden bekeken door dezelfde dermatopatholoog. De belangrijkste uitkomstvariabelen van deze studie waren clinicopathologische karakteristieken, het nut van EVD met DD en doorlooptijden. Het gebruik van EVD met DD deed de detectie van positieve snijranden in niet-melanoma-huidtumoren stijgen van 8,4% naar 12,8%. De meest significante toename werd gezien bij de ziekte van Bowen, bij invasief plaveiselcelcarcinoma en bij superficieel basocellulair carcinoma (sBCC). Met EVD met DD was het mogelijk een specifieke clinicopathologische diagnose te stellen bij 27,7% van de naevi, wat beduidend meer is dan 10,3% met de standaardmethode. De diagnose van matig tot ernstig dysplastische naevi nam toe van respectievelijk 1,0% tot 7,2% en van 0,6% tot 1,4%. De detectie van ulceratie bij melanomen met een dikte van meer dan 1 mm steeg van 24,0% tot 31,3%. Het aantal melanomen met een geassocieerde naevus nam toe van 15,5% tot 33,3%. Het aantal samengestelde huidtumoren van 0,07% tot 1,07%. De doorlooptijd van naevi daalde van twee dagen naar één dag, voor melanomen van vijf naar twee dagen, en voor BCC's van twee dagen naar één dag. De conclusie van dit hoofdstuk is dat EVD met DD het maken van gerichte coupes mogelijk maakte en dat deze techniek, in de context van een dermatopathologie-laboratorium, een meer accurate histopathologische diagnose van huidtumoren toelaat, die bovendien minder tijd in beslag neemt. Deze bevindingen suggereren dat pathologen die huidtumoren onderzoeken, aangemoedigd zouden moeten worden om dermoscopie aan te leren en om random dwarse snedes te vervangen door letsel-specifieke en DD-gerichte snedes.

Hoofdstuk V illustreert het gebruik van derm dotting als een techniek om markeringen aan te brengen op slijmvliezen. Om dit te bestuderen werd deze techniek toegepast op resectiestukken met plaveiselcelcarcinomen, geresecteerd bij negen patiënten. Er werd eveneens nagegaan of deze methode gebruikt kan worden op vriescoupes. In alle gevallen was de nagellak macroscopisch duidelijk zichtbaar. De nagellak bleef bovendien goed zichtbaar in de paraffineblok en was eveneens zichtbaar op de microscopische coupes. De nagellak-markeringen bleven ook zichtbaar op vriescoupes, maar gingen echter verloren in de helft van de ontkalkte weefsels. De conclusie van dit hoofdstuk is dat DD een goedkope en eenvoudige methode is die het gebruik van hechtingen - aangebracht door chirurgen - om resectiestukken te oriënteren kan vervangen. De nagellak-dots of -lijnen kunnen gebruikt worden om het resectiestuk te oriënteren. Bovendien kunnen verdachte snijranden of zones die de speciale aandacht van een patholoog vereisen, door het gebruik van verschillende kleuren nagellak, op een eenvoudige manier gemarkeerd worden.

Hoofdstuk VI beschrijft EVD als een wetenschappelijke methode voor de identificatie van histopathologische structuren die dermoscopisch waarnemingen kunnen verklaren. Meer bepaald werd er gezocht naar een morfologisch correlaat en een optische verklaring voor rozetten en andere witte glanzende structuren bij dermoscopie met gepolariseerd licht. Hiervoor werd een reeks van 6.108 opeenvolgende huidbiopsieën onderzocht met EVD. Wanneer rozetten aanwezig waren, werden dwarse seriële coupes onderzocht door middel van microscopie onder gepolariseerd licht. Met behulp van EVD werden rozetten gevonden in 63 gevallen. Wanneer multipele rozetten gezien werden, waren deze steeds georiënteerd in dezelfde richting. Dwarse coupes die microscopisch werden bekeken onder polariserend licht, toonden aan dat de kleinere rozetten vooral veroorzaakt worden door dubbelbrekend hoornmateriaal in de openingen van adnexen. Grotere rozetten worden daarentegen veroorzaakt door concentrische perifolliculaire fibrose. Het wetenschappelijk besluit van deze studie is dat rozetten geen specifieke letsels zijn, maar een optisch effect van hoornmateriaal en concentrische fibrose. De studie identificeerde eveneens ballon-cel nesten in naevi als oorzaak van gele globules op dermoscopie.

Hoofdstuk VII benadrukt de professionele invloed van EVD met DD op dermatopathologie als sub-specialisme en de mogelijke toekomstige wetenschappelijke toepassingen van deze techniek. Op het einde van dit hoofdstuk wordt de mogelijke rol van EVD besproken bij het ontrafelen van de aard van verschillende subtypes van dysplastische naevi.

Hoofdstuk VIII bevat een eerste aanzet tot een atlas met een overzicht van EVD beelden en de histologische correlaten van de voornaamste tumorale huidletsels. Hierbij worden ook clinicopathologische subtypes van naevi geïllustreerd die voortkomen uit deze gerichte correlatie met de EVD informatie. Tenslotte worden ook een aantal speciale, verrassende of ongewone EVD's in deze atlas opgenomen.

Addendum I Curriculum Vitae

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EDUCATION

1965-1972:	Latin Sciences St. Barbara College, Ghent, Belgium
1972-1979:	Medical Doctor KULAK and KUL, Belgium 75-76-77-78 Distinction Promotion '79 Great Distinction
1978-1983:	Degree of Medical Specialist in Pathology Department of Pathology, UZ Leuven, Belgium
1983-1984:	Medical Genetics/Genetic counselling Human Genetics UZ Leuven, Belgium
2005:	Degree in Dermatopathology by International Committee of Dermatopathology and International Society of Dermatopathology

PROFESSIONAL RECORD

1985-2011:	Director Department of pathology, Stedelijk Ziekenhuis Roeselare, Belgium
Since 2008:	Clinical consulent Dermatology Ghent University Hospital, Belgium
Since 2010:	Scientific consulent,Ghent University, Belgium
Since 2011:	Dermatopathologist and founder of Dermpat, Ghent, Belgium

POST GRADUATE courses or training since 2008

2008:	Diagnosis of melanocytic lesions. 5-6 December, Paris, France
2008:	Seminaire d'histopathologie cutanée. 7-9 June, Strasbourg, France
2008:	XXIX Symposium Int. Soc. Dermatopathology. 2-4 October, Graz, Austria
2009:	Perspectives in Dermatopathology. 22-24 May, Friedrichshafen, Germany
2009:	Dermatopathology and beyond it. 17-19 April, Pavia, Italy

2009: Journées dermatologiques. 8-12 December, Paris, France
2009: Dermoscopy advanced course. 21 April, Ghent, Belgium
2010: Belgian week of pathology. 21-24 April, Ghent, Belgium
2010: Seminaire d'histopathologie cutanée. 3-5 June, Strasbourg, France
2010: Summer academy dermatopathology. 12-16 July, Graz, Austria
2011: Dermatopathology 23-29 January, Vars, France
2011: Dermatopathology Symposium. 27-28 April, London, United Kingdom
2011: International Society of Dermatopathology. 1-3 September, Genève, Switserland
2011: Summer academy dermatopathology. 11-15 July, Graz, Austria 2011: Dermoscopy 4 October, Ghent, Belgium
2012: Dermatopathology. 30 January - 4 February, Vars, France
2012: Belgian week of dermatopathology. 18-21 April, Ghent, Belgium
2012: Dermatopathology symposium. 10-12 May, London, United Kingdom
2013: Dermatopathology. 27 January - 2 February, Vars, France
2013: Summer academy dermatopathology. 1-5 July, Graz, Austria
2013: International society of Dermatopathology. 26-28 September, Firenze, Italy
2014: Dermatopathology symposium. 15-17 May, London, United Kingdom
2014: Dermatopathology. 30 September-3 October, Venice, Italy
2014: Belgian week of Pathology. 23 -25 October, Ghent, Belgium
2014: Dermatopathology symposium. 16-18 May, London, United Kingdom
2015: SNNDV. 19-20 November 2015, Rotterdam, The Netherlands
2015: Jounées dermatologiques. December, Paris, France
2015: BADO. 17 January, Brussels, Belgium
2015: Dermatopathology symposium. 13-15 May, London, United Kingdom
2015: Seminaire d'histopathologie cutanée. 25-27 April, Strasbourg, France
2015: Dermatopathology. 21-22 April, Brescia, Italy
2016: Belgian week of Pathology. 12-15 October, Ghent, Belgium
2016: Bullous Disorders. 19-22 June, Groningen, The Netherlands

SCIENTIFIC PRESENTATIONS OR WORKSHOPS

2008 : Co-responsible for the Organisation of the Clinicopathologic Confrontations, University Hospital Ghent, Belgium

2009: Anglo-French-Belgian club of Dermatopathology case report. 29 May, Paris, France
2009: Summer course Dermatopathology European Academy of Dermatology and Venereology: alopecia. 6-10 July
2009: Anglo-French-Belgian club of Dermatopathology: self-healing juvenile mucinosis. 13 November, London, United Kingdom
2010: Clinicopathologic cases in dermatopathology. 9 February, LOK dermatologie, Antwerp, Belgium
2010: Societé Bruxelloise de dermatologie:quand la microscopie éclaire la clinique: epitheloid hemangioendothelioma. 11 February, Brussels, Belgium
2010: AngloBelgoFranco club of Dermatopathology: case report. 7 May, Brussels, Belgium
2010: EADV summer school dermatopathology:melanocytic tumours, 6-10 July 2009
2011: Belgian club of Dermatopathology: case report 7 October 2011 Brussels, Belgium
2011: SNNDV workshop Dermatopathology, 10-11 November, Den Bosh Nederland
2012: SNNDV workshop Dermatopathology. 8-9 November, Antwerp, Belgium
2013: Ex vivo dermoscopy with dermdotting, oral communication 3 February, Vars, France
2013: Organisation AngloFrancoBelgo club of Dermatopathology. 24 May, Ghent, Belgium
2013: SNNDV: Histopathologische correlatie van huidtumouren bij middel van ex vivo dermoscopie en "derm dotting". 7-8 November, Amsterdam, the Netherlands
2014: EADV summer course Dermatopathology. 30 June – 4 July Ghent, Belgium
2014: London Dermatopathology symposium: ex vivo dermoscopie and derm dotting. 15-16 May, United Kingdom
2014: Belgian Week of Pathology Case presentation. 22-25 October, Ghent, Belgium
2015: World congress Dermoscopy: comparison ex vivo and in vivo. 16-18 April. Vienna, Germany

2015: Belgian week Pathology: case presentation. 21-24 October, Ghent, Belgium

2015: London Dermatopathology Symposium: case presentation. 13-15 May. United Kingdom

2016: EADV Spring Convention: diagnostic performance of EVD with DD. 19-22 May. Athens, Greece

2016: Belgian week of Pathology: ex vivo with derm dotting. 12-15 October, Ghent, Belgium

2017: ISDP: case presentation. 28-30 September. Glasgow, Scotland

2018: ISDP: invited speaker: What's new in dermatopathology. 14-15 February. San Diego, USA

MEMBERSHIPS

Belgian Club of Dermatopathology
AngloFrancoBelgo Club of Dermatopathology
Belgian Cutaneous Lymphoma Group
European Association of Dermatology and Venereology
International Society of Dermatopathology

PUBLICATIONS

Haspeslagh M, Hoorens I, Degryse N, De Wispelaere I, Degroote A, Van Belle S, Verboven J, Vossaert K, Facchetti F, Van Dorpe J, De Schepper S, Brochez L.
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Dankwoord

Een Nieuwe Lente een Nieuw Geluid

Twaalf jaar geleden werd ik door Prof. Lieve Brochez van de dienst dermatologie UZ Gent gevraagd om als consulent de dermatopathologie diagnostiek te ondersteunen. Met het plotse en veel te vroege overlijden van het toenmalig diensthoofd Prof. Jean-Marie Naeyaert was de dienst niet alleen een reus in de dermatologie, maar ook hun expertise in dermatopathologie verloren. Het nieuwe diensthoofd, Prof. Jo Lambert, overtuigde mij om vanaf januari 2005 iedere dinsdag bij Prof. Sofie De Schepper op dermatopathologie langs te gaan. Deze wending in mijn professionele loopbaan gaf mij een onverwachte boost, zowel op professioneel als op menselijk vlak. Prof. Sofie De Schepper was net benoemd als staflid en had als opdracht de dermatopathologie van Prof. Jean-Marie Naeyaert over te nemen. Samen ontdekten en ontwikkelden we verder de wondere wereld van de huidbiopten. Met het ganse dermatologie team en in het bijzonder met Sofie klikte het wonderwel. Ik was toen reeds 10 jaar intensief met huidpathologie bezig, maar de academisch-klinische omgeving die mij daar werd aangeboden liet me toe om een grote sprong te nemen in mijn diagnostische vaardigheid. Deze ervaring was een nieuwe lente in mijn professioneel leven. Ik ontdekte er de ware klinische aard van vele inflammatoire huidaandoeningen, volgde vanop de eerste rij de spectaculaire (neven)effecten van nieuwe biologicals en ontdekte er de dermoscopie. Samen met Dr. Katrien Vossaert was Prof. Lieve Brochez actief bezig met de postgraduate opleiding van de Vlaamse dermatologen in deze toen nog vrij nieuwe diagnostische tool. De scherpte en informatieve rijkdom van deze dermoscopie beelden verwonderden mij. Deze verwondering, samen met de frustratie die ik al jaren had over de sensitiviteit van de standaard methode waarmee we huidbiopten verwerkten, werden de kiem van deze doctoraatsthesis. Lieve en Katrien, bedankt om mij in de wondere wereld van de dermoscopie in te leiden. Sofie, bedankt om de vele aangename jaren gezamenlijke clinicopathologische brainstorming en support in mijn wetenschappelijk werk. Jo, bedankt om als toenmalig diensthoofd in mij te geloven en alzo dit academisch avontuur in te leiden.

Al snel ondervond ik dat het als diensthoofd, verantwoordelijk voor de algemene pathologie van het Stedelijk ziekenhuis in Roeselare, moeilijk was om op een kwaliteitsvolle manier algemene pathologie te blijven combineren met mijn steeds groeiende engagement in de huidpathologie. De droom om een zelfstandig laboratorium huidpathologie op te richten bestond reeds vele jaren, maar werd nu een logische nieuwe stap in mijn professionele carrière. Ik verliet in 2011 het ziekenhuis en de algemene pathologie om in mijn geboortedorp Ardooie, samen met laborantes Ine De Wispelaere en Nele Degryse, Dermpat op te starten. Ine, ik wil jou in het bijzonder bedanken om zonder twijfel, onvoorwaardelijk en met vertrouwen

in de goede afloop, je job in het ziekenhuislabo op te geven en voor ons nieuwe Dermpat project in Ardooie te kiezen. Vanaf het begin kon ik op je opgewekt karakter, werklust en trouw rekenen.

Het verhogen van de sensitiviteit van de standaard manier waarop in de meeste laboratoria huidbiopten werden onderzocht, leek ons een uitdaging. We wilden het niet alleen goed, maar ook anders doen. Enkele publicaties over het gebruik van de dermoscoop op reeds uitgesneden letsels (ex vivo) en het enthousiasme van mijn nieuw team in Ardooie, brachten ons vlug een stap verder in de zoektocht naar een nieuwe weefselspecifieke en gerichte onderzoeksmethode. De ex vivo dermoscopie opende voor ons een wondere wereld van focale informatie en letsel heterogeniteit. Samen met Ine en Nele werd naar een markeringsmethode gezocht. Diabetes naaldjes, stiften, kleurstoffen en verfjes werden uitgeprobeerd. Een creatieve ingeving deed ons nagellak uittesten, deze bleek resistent aan het weefselproces, traceerbaar op de microtoom en mooi zichtbaar onder de microscoop. Mijn schoondochter Bieke suggereerde de mooi van de tong rollende ‘derm dotting’ als naam voor dit markeren met nagellak. Ex vivo dermoscopie met derm dotting was geboren. Ine en Nele, bedankt voor het pionieren en het geloof in het vinden van deze nieuw methode.

De publicatie van onze methode in de *American Journal of Dermatopathology* kon op veel reactie rekenen. Internationale collega’s waren erg benieuwd om meer te vernemen. Ik werd geïnviteerd op enkele buitenlandse fora om mijn ex vivo dermoscopie met derm dotting voor te stellen. Mijn enthousiasme over de voordelen van onze methode was echter niet voldoende om hen te overtuigen en terecht werd er om wetenschappelijk bewijs gevraagd van wat ik dagdagelijks aanvoelde. Supporters van het eerste uur: Lieve, Katrien en Sofie droomden mee en motiveerden me om op een systematisch en wetenschappelijke manier deze methodiek te onderzoeken en de diagnostische kwaliteit ervan te evalueren en te vergelijken met de standaard methode. Lieve hielp me om dit onderzoek te structureren en zonder dat ik het besefte ontstond een doctoraatsproject. Tijdens dit traject was het voor mij ook aangenaam en nuttig regelmatig met raad en daad bijgestaan te worden door toenmalige doctoraatstudentes Ines Chevolet en Isabelle Hoorens. Zij hielpen me door de statistische verwerking van onze data. Deze goudmijn van gecodeerde, statistisch verwerkbare data, hebben we te danken aan Jan Verboven. Jan was onze IT-man van het eerste uur en schreef ondertussen reeds meer dan 30 jaar enthousiast mee aan ons pathologie verhaal. Ook assistenten Sven Lanssens, Michael Noë en Julie Lemahieu wil ik bedanken voor de medewerking en inspiratie bij bepaalde studies uit dit doctoraat. In het bijzonder ook dank aan Jeroen Van Hevele, mijn schoonzoon, die even enthousiast als mezelf de nagellak introduceerde in de mond- en kaakchirurgie.

Naast Lieve als promotor, had ik het geluk twee kanjers van copromotoren te hebben die dit onderzoek vanuit morfologische hoek bewaakten. Hun ervaring was voor mij van onschatbare

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Beste Fabio, ons verhaal van vriendschap loopt reeds meer dan dertig jaar. Dat ik je als copromotor zou vragen was reeds beslist vooraleer mijn doctoraatsproject concreet werd. Je geloof in mijn aanvankelijk nog erg intuïtieve en weinig wetenschappelijk suggesties, je rake commentaar op mijn vele drafts, je zin voor humor en vooral je integriteit, eenvoud en trouw brachten me comfort en sterkte tijdens deze onderneming.

Caro Fabio la nostra amicizia dura da più di 30 anni. Che saresti diventuto il mio sostenitore era già stato deciso ancora prima che si aprisse la possibilità di un progetto di dottorato.

La tua fede nelle miei prime ipotesi molto intuitive, poco scientifiche, il tuo commento pertinente sulle miei primi manoscritti, il tuo senso dell'umorismo e soprattutto la tua integrità morale, semplicità e lealtà mi hanno portato conforto e forza durante questa impresa.

Vanaf het begin werden de verschillende stappen die we in dit doctoraat zetten met enthousiasme gedragen door het Dermpat team dat ondertussen snel uitbreidde: Jasmien Taildeman en Eveline Naessens vervoegden de Dermpat ploeg en een verhuis naar Gent werd voorbereid. Jasmien, bedankt om het nieuwe labo aan de Koning Albertlaan mee te bedenken, in te richten en te leiden. Zonder je inzet, stabiliteit, enthousiasme en geloof in ons project zou ik dit doctoraat nooit kunnen afmaken. Eveline, bedankt om je snel vast te bijten in dit project en me geen andere optie te laten dan alles stap voor stap af te werken. Je gevoel voor belangrijk detail en esthetiek waren de sleutel tot de mooie uitgave en verdere organisatie van dit doctoraat. Annemarie Degroote kreeg als assistent bij Dermpat de smaak te pakken en is sinds september 2016 als volwaardig dermatopatholoog verbonden aan Dermpat. Bedankt Annemarie, zonder je charme, werklust en accurate diagnostiek zou er voor mij nooit voldoende ruimte en tijd ontstaan zijn om dit thesis project aan te kunnen. Dermpat blijft ondertussen groeien: Tina Feys en Veronique Clauwaert vervoegden ons team en zijn een garantie dat vanuit Dermpat nog vele nieuwe wetenschappelijke projecten zullen ontstaan en worden uitgewerkt. Tenslotte vervoegde in september 2017 ook dermatologe Karolien Van Den Bossche ons diagnostisch team. Ook Karolien was, als assistente pathologie, reeds betrokken bij een deel van het wetenschappelijk onderzoek van dit doctoraat, waarvoor dank.

Deze toch wel bewogen en drukke doctoraatsperiode had uiteraard ook zijn invloed op mijn familiaal leven. In plaats van een rustig pre-pensionair leventje met veel tijd voor de zoveel andere interesses en activiteiten die ons lief waren, werd gedurende de laatste jaren veel geduld, begrip en inspanning gevraagd aan mijn lieve echtgenote. Beste Kathleen, het is niet altijd makkelijk geweest, maar je hebt ook dit project onvoorwaardelijk gesteund. Ik wil je hiervoor bedanken. Ik geloof en beloof dat er nu terug tijd komt om de vele leuke dingen des levens die we graag samen doen, terug op te pikken. Het waren ook jaren waarin onze vier

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Aan Louise, Alice, Mathilde, Ernest en X.

Marc Haspeslagh - januari 2018

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