

Chapter 2: Diagnosis of Cutaneous Malignant Melanoma

Introduction:

This chapter will look at the current practice and techniques that clinicians commonly employ in making or refuting the diagnosis of malignant melanoma. There will be a discussion on several of the new technologies currently being developed and a final section will follow this on the basic science of spectrophotometric intracutaneous analysis.

2.1 Diagnostic Accuracy, Specificity & Sensitivity:

Before discussing the merits and shortfalls of any diagnostic test, it is necessary to define and clarify the terminology used in the literature to objectively assess and measure their usefulness and allow comparison. Diagnostic accuracy (synonym: *Validity*) can be defined as 'a measure of the capacity of a test to give a true result' [Farmer & Miller, 1991]. In addition, the description of the accuracy of a test can be further qualified as the ability to correctly detect the presence or absence of a disorder or place a subject correctly on a continuous scale of measurement. The diagnosis of melanoma is not a continuous measurement - the disease is present or absent. Thus, the validity of tests employed for the diagnosis of melanoma is described in terms of *Specificity* and *Sensitivity*. Specificity can be defined as the ability of a test to reject those without the disease. Sensitivity can be defined as the ability of a test to pick up those patients with the disease [Farmer & Miller, 1991]. Alternatively, sensitivity can be defined as the proportion of people with the disease *and* a positive test result whereas specificity can be defined as the proportion of people without the disease *and* a negative test result [Campbell & Machin, 1999]. Both values are described in terms of a percentage and Table 2.1 summarises how these values are calculated.

Table 2.1 Specificity & Sensitivity

	With Disease	Without Disease	Total
Test Positive	True Positive A	False Positive B	A+B
Test Negative	False Negative C	True Negative D	C+D
Total	A+C	B+D	A+B+C+D

Specificity = $D / (B+D)$ = True Negatives / All without the disease

Sensitivity = $A / (A+C)$ = True Positive / All with the disease

Sackett *et al.* (1998) describe a useful mnemonic for remembering these terms and their application clinically, namely SPIn and SNOuT, and is based on a test that has either 100% specificity and sensitivity. In a test that is 100% specific, there are no false positives and a positive finding means that the diagnosis is certain - **Specificity, Positive, count In**. In a test that is 100% sensitive, there are no false negatives and the lack of a finding means that the diagnosis is absolutely refuted - **Sensitivity, Negative, count Out**.

Campbell & Machin (1999) explain the benefit of describing diagnostic tests in terms of their specificity and sensitivity, namely that 'they will yield consistent results for the diagnostic test in a variety of patient groups with different disease prevalence...sensitivity and specificity are characteristics of the test, not the population to which the test is applied.' However, they go on to explain that if, in practice, the disease is very rare then the accuracy with which the sensitivity can be estimated is limited.

2.2 Clinical Diagnosis:

2.2.1 History:

The first thing the clinician would usually do is to ask the patient how the lesion came to their attention. Most of the time, patients notice the change in the lesion themselves but one study found that nearly 20% of the time, the mole was singled out coincidentally by the patient's relatives or partner or by the family doctor [du Vivier *et al.*, 1991b]. MacKie (1985) analysed the presenting symptoms and changing features of patients who attended her department. These were correlated with the histopathological diagnosis of invasive melanoma and a seven-point checklist was devised - Table 2.2a - that formed the basis of a booklet distributed to family doctors. This checklist was meant to be a guideline for General Practitioners in order to separate melanoma from benign pigmented lesions and those that had three or more features were to be regarded as suspicious. However, there were criticisms of the list [Keefe *et al.*, 1990; du Vivier & Higgins, 1991a] and as a result this checklist was revised - table 2.2b [MacKie, 1990]. This revision took account of the fact that the checklist was tending to describe superficial spreading melanomas and was passing nodular melanomas as benign. Conversely, seborrheic keratoses were increasingly likely to be scored as malignant.

Table 2.2a Original seven-point checklist

1. Sensory change or itch
 2. Diameter \geq 1cm
 3. Growth of the lesion
 4. Irregular edge
 5. Irregular pigment
 6. Inflammation
 7. Bleeding, crusting or oozing
-

Table 2.2b Revised seven-point checklist

Major signs: (two points each)

1. Change in size
2. Change in shape
3. Change in colour

Minor signs: (one point each)

1. Inflammation
2. Crusting or bleeding
3. Sensory change
4. Diameter \geq 7mm

From the previous chapter (section 1.4.1.3) it can be seen that it is important, additionally, for the clinician to ascertain the risk factors for melanoma that the patient may have. These would include family history of atypical moles and melanoma, sunburn as a child, ease of tanning and previous melanoma.

2.2.2 Examination:

Another checklist, from the American Cancer Society known as the 'ABCDE system', outlines the features that the physician is looking for in the lesion to confirm the diagnosis [Fitzpatrick *et al.*, 1988]. These are listed in table 2.3.

Table 2.3 The 'ABCDE' System:

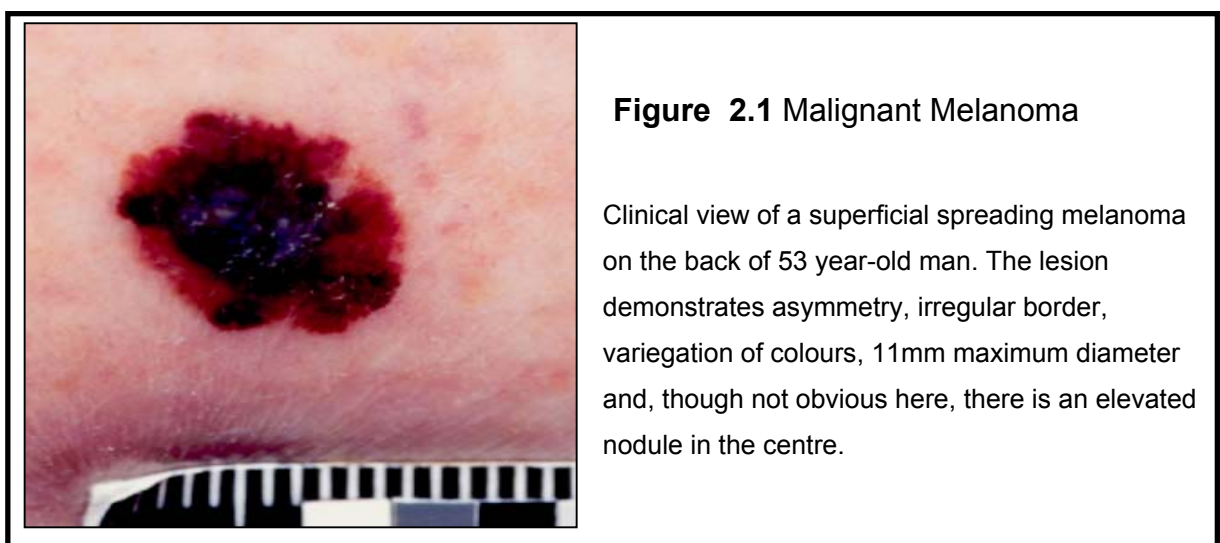
- A - Asymmetry in shape
- B - Border is irregular -scalloped
- C - Colour - variegated and several shades
- D - Diameter - greater than 6mm
- E - Elevation - from surrounding skin

This mnemonic was devised to aid clinicians in the diagnosis of early melanoma but it also suffers from the same criticisms as the seven-point checklist, namely identifying superficial spreading melanoma, missing nodular melanomas and including many benign lesions [Hall, 1992; Keefe *et al.*, 1990]. However, it is relatively easy to visualise the underlying pathological process from this *aide memoire*. As the horizontal growth phase spreads it does so in an irregular fashion, hence the asymmetry, irregular border and diameter. With the onset of invasion and the vertical growth phase, the tumour develops a nodule - often with destruction of the overlying skin lines - and, as will be discussed later, melanin at various depths in the skin produces different hues of colour.

For completeness, the examining physician would clinically assess the regional lymph nodes, liver and chest for evidence of metastatic spread.

2.2.3 Accuracy

The clinical diagnosis of malignant melanoma is acknowledged as challenging by most authors and has provoked much debate [Curley *et al.*, 1989; Grin *et al.*, 1990; MacKie, 1992; Morton & MacKie, 1998; Grant-Kels *et al.*, 1999]. Diagnostic accuracy was stated at being



between 50 and 80%. Grin and colleagues (1990) demonstrated a small improvement in accuracy with time (60% v. 64%) and Morton & MacKie (1998) demonstrated a significant improvement in accuracy with an examiner of 10 years experience or more (60% v 80%). Other differences in accuracy may probably be accounted for in study design.

Perednia *et al.* (1992) highlighted three key phases in the diagnostic process and sought to explain where the errors may occur. First, the examining physician must pick out a suspicious lesion from the surrounding skin. Second, having identified an interesting lesion, the examiner must assess its features for irregularity, variegation and others as outlined above. Finally, the examiner has to formulate a differential diagnosis given the information they have accumulated. From this diagnostic pathway it is apparent that one process follows on from another - if the examining clinician fails to register a suspicious lesion, then the discriminating features will not be sought and a differential diagnosis cannot be arrived at. This has important consequences when screening a population for melanoma but in clinical practice, it is usually the patient that initiates that first step [du Vivier *et al.*, 1991b]. In their study, Perednia & colleagues found a reproducibility (defined as making the same decision given the same stimulus on different occasions) of approximately 80% amongst 15 observers in making that first step. Work elsewhere has assessed clinicians' ability to recognise features such as irregularity and variegation [Breitbart *et al.*, 1989; Claridge *et al.*, 1992] - step two in Perednia's algorithm - and this was measured at approximately 80%. Given the multi-step nature of arriving at a diagnosis, Perednia and colleagues (1992) hypothesise that the diagnostic accuracy of clinical examination is a function of the reproducibility scores for observation. They estimate a figure of 61% and this correlates well with the observed figures detailed above.

In chapter one, the natural history and prognosis of melanoma were discussed in detail and the clear message is that, if detected early, melanoma is eminently curable and if detected late then the prognosis is uniformly dismal. Given the low diagnostic accuracy of even the experts in detecting early melanoma [Curley *et al.*, 1989], the relatively large error in diagnostic reproducibility [Perednia *et al.*, 1992] and the implications of missing the diagnosis, the opportunity arises for diagnostic techniques to be pioneered and developed to aid the clinician.

2.2.4 Screening and Public Education:

In order for screening to be effective it has to fulfil certain criteria [Farmer & Miller, 1991]. In essence, the population (at risk) should be defined, the natural history of the disease should be known and the diagnostic test should be accurate, acceptable to the patient and pick up the disease at a point where intervention will make a difference to survival. Finally, the

screening should be cost effective. At face value, melanoma would seem to be an ideal disease to screen for - it is a significant health problem, the natural history is well known, prognosis is related to Breslow thickness at diagnosis, simple surgery cures early melanomas and screening is painless and cheap [Rampen *et al.*, 1992]. However, there is a lack of data to confirm the benefits of screening that is mainly due to inherent problems in experimental design. Koh *et al.* (1991) point out that, in order to demonstrate reduced mortality, two randomised groups, each containing several hundred thousand patients, would need to be followed up for many years. The challenge of undertaking such a study would be immense. Second, as previously discussed the epidemiology of melanoma appears to be changing worldwide so that it may be difficult to disentangle the effect of the increasing incidence of thinner (less malignant) lesions from any real advantages of screening.

In the United Kingdom, public education takes the form of leaflets, posters and more recently websites [CRC Website] that inform the reader of the signs and symptoms described in the seven-point checklist and advise them to seek the advice of the family doctor if there are any concerns. As a result, some claimed [Doherty & MacKie, 1988] an increase in the number of thin melanomas detected. Other groups did not find this on analysing their experiences [Williams *et al.*, 1990]. However, all agreed that the effect of the publicity campaigns is to dramatically increase the number of referrals to the local dermatology departments. Thus, public education campaigns do succeed in raising public awareness of melanoma but whether they assist in the early detection of melanoma remains debatable.

2.3 Skin Surface Microscopy:

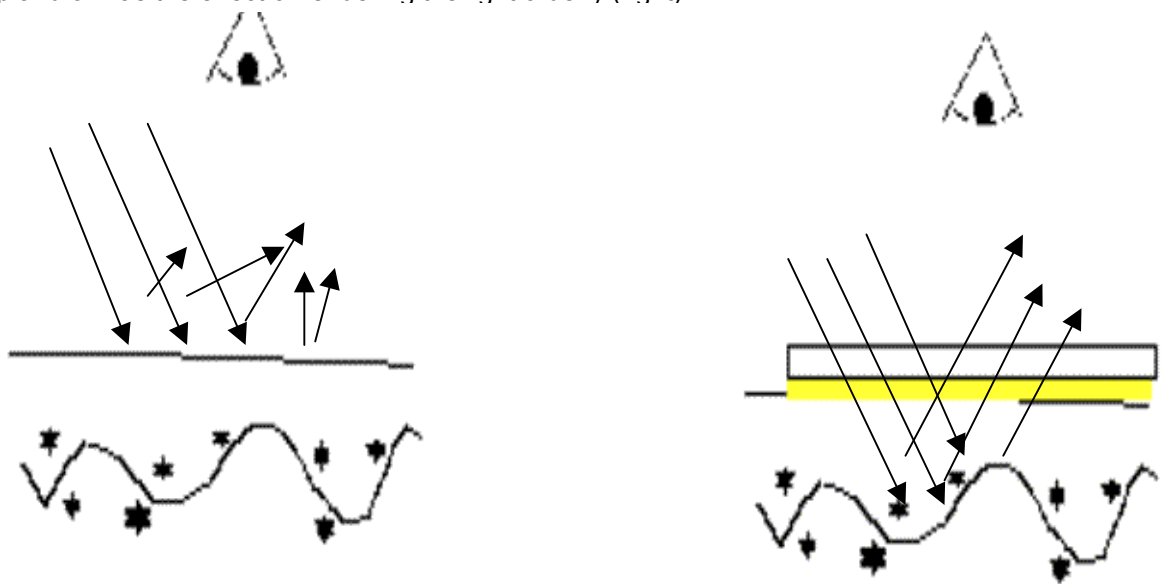
Skin surface microscopy has many synonyms, namely - epiluminescence microscopy (ELM), dermoscopy, dermatoscopy, oil emersion microscopy, *in vivo* cutaneous surface microscopy. Historically, the technique is derived from the colposcope - a surface microscope used in gynaecology to diagnose the presence of cervical carcinoma. The pioneer of this technique, Hinselmann (1933), suggested in a paper that the colposcope could be used in the diagnosis of pathological skin disorders. However, this idea was generally ignored until, in the USA, Goldman (1951) described, for the first time, the use of an operating microscope to examine pigmented skin lesions and this was followed in the UK by MacKie (1971a,b) who described the use of surface microscopy for the pre-operative diagnosis of pigmented skin lesions. Again, little attention was paid to these descriptions until the 1980's when the importance of the use of surface microscopy to help differentiate benign from malignant pigmented skin lesions was emphasised [Fritsch & Pechlaner, 1980; Perhamberger *et al.*, 1987; Steiner *et al.*, 1987;]. In 1990, Bahmer *et al.* published the first paper to standardise the terminology used to describe features seen in surface microscopy of pigmented skin lesions and correlated them with their underlying histology. Since then, a whole wealth of literature, papers and textbooks

have been published regarding this technique and this section will attempt to summarise them and comment on the strengths and weaknesses of surface microscopy.

Initially, workers used a microscope similar to an operating microscope at various powers of magnification to perform surface microscopy and assess pigmented skin lesions [MacKie, 1971a,b; Perhamberger et al., 1987]. However, this is a cumbersome technique and hand-

Figure 2.2: Skin Surface Microscopy:

The rough stratum corneum renders the incident light diffuse and most of the light is reflected at the surface as a result of the difference in refractive index between air and the skin (left). Adding a cover slip and oil has the effect of rendering the light orderly (right).



held devices, similar to an ophthalmoscope or auroscope, have been available and widely used for over a decade that allow the clinician to perform skin surface microscopy at ten-times magnification [Menzies et al., 1996a]. Fundamentally, the technique renders the stratum corneum invisible by either employing oil or polarised light with magnification at the skin surface. As a result, the light that is normally rendered diffuse at the interface between the rough stratum corneum and air becomes ordered (fig 2.2). This allows the observer to examine structures at or near the dermoepidermal junction. The task of the observer is to interpret the features returned from the lesion by relating them to the correlating histology and thereby arriving at a diagnosis.

2.3.1 Morphological Features

The features seen in surface microscopy can be divided into two broad categories namely those relating to colour & pigmentation and those relating to structural morphology.

2.3.1.1 Colour & Pigmentation

When light enters the skin, it interacts in a complex manner with the chromophores that mainly absorb it and the collagen that mainly reflects it. The main chromophores (syn. pigments) found in the skin are melanin and haemoglobin and, to a lesser extent, carotene and bilirubin. The colour remitted back to the skin surface is a function of not only the concentration of these chromophores but also their exact location in the skin in relation to the scattering collagen and the wavelength of the light that impinges on them. This fact forms the fundamental basis of spectrophotometric intracutaneous analysis and, as such, will be discussed in much greater depth in the relevant section of this chapter. Suffice it to say that, melanin and haemoglobin are the two chromophores that are assessed with skin surface microscopy.

The colour remitted from melanin appears black in the superficial epidermis, brown at the dermoepidermal junction and blue-grey in the papillary dermis [Menzies *et al.*, 1996a]. In the superficial epidermis, the majority of the light across the visible spectrum is absorbed that is entering or returning from the skin and the area appears black. In the papillary dermis, light from the blue-end of the visible spectrum (short wavelength) is reflected back to the surface by the collagen fibres before it can be absorbed by the melanin whereas visible light from the red-end (long wavelength) is absorbed [Cotton, 1998: Chap. 4]. Thus the area appears blue. This is known as the Tyndall effect and is purely an *in vivo* phenomenon of human skin, as intradermal melanin appears brown when viewing on a prepared histology slide with the light microscope [Mooi & Krausz, 1992]. The Tyndall effect, the remission of light through a scattering medium according to its wavelength, also accounts for other natural phenomena such as the sky appearing blue.

The number of colours seen within the lesion using surface microscopy has diagnostic relevance. According to Menzies *et al.* (1996b), when pigmented lesions are specifically graded for six colours namely tan, dark brown, blue, grey, black and red/blue, then the presence of five of six colours has a specificity of 92% and a sensitivity of 53%. In other words, if present, the feature of 5 or 6 colours confirms the diagnosis of melanoma 92% of the time but conversely only 53% of melanomas have 5 or 6 colours. Similarly, if the clinician sees only one colour using skin surface microscopy then this feature has a sensitivity of 0% - no melanomas have one colour [Menzies *et al.*, 1996b]. The blue/grey colour seen by the

clinician can also be qualified. Specifically if the blue/grey has a hazy quality to it termed 'blue/grey veil' by Bahmer *et al.* (1990) or 'blue-white veil' by Menzies *et al.* (1996a), then according to the latter this represents "superficial fibrosis with melanophages and/or pigmented malignant cells in the papillary dermis". This feature (fig 2.3) is very specific for melanoma (97%) and is described by Menzies *et al.* as "the single most significant surface microscopic finding of invasive melanoma".

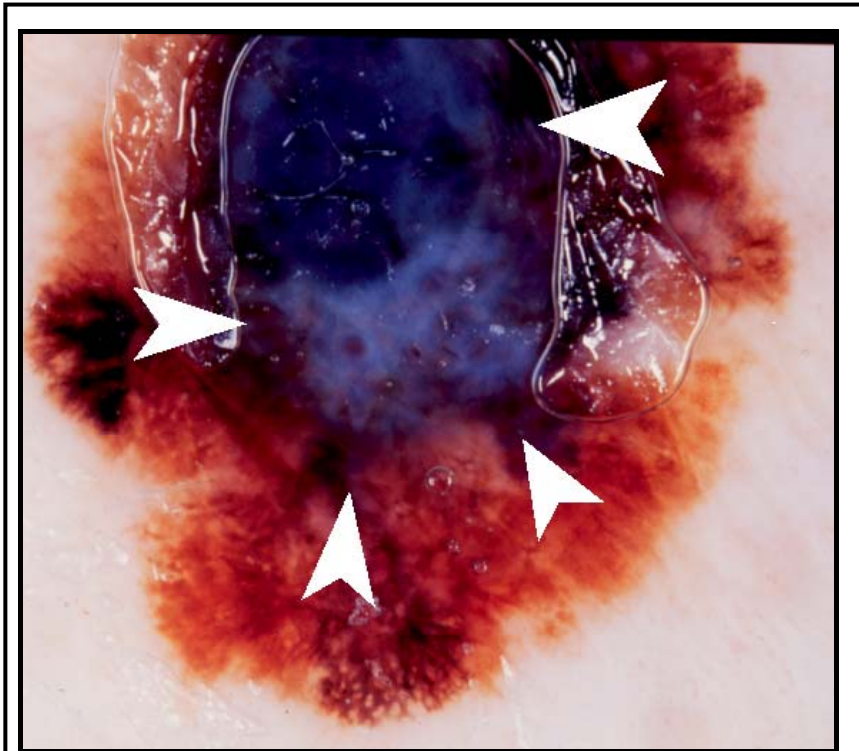


Figure 2.3

Surface microscopy of superficial spreading melanoma - blue/grey veil is arrowed

2.3.1.2 Structural & Morphological Features

There are dozens of morphological features that have been described in the literature - most are subtle variations on a theme and are of limited practical application and to discuss each one in turn would be inappropriate in the context of this thesis. Therefore, there will follow a short discussion of the most important features and the histological correlation as described by Bahmer et al.(1990)

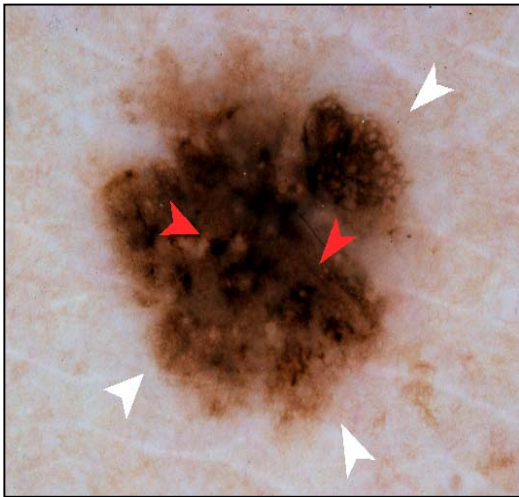


Figure 2.4

Junctional naevus. The pigment network can be clearly seen like a spider's web at the peripheries and is broadened at 1 o'clock (white arrows). The junctional naevus cell nests form the black globules (red arrows).

Structural features are formed by the aggregation of pigmented cells or pigment-containing microstructures such as blood vessels. In effect, we are viewing the microstructure of the lesion in detail an order of magnitude greater than with the naked eye and an order of magnitude less than with the light microscope. An analogous situation would be the description of the structure of a protein molecule. The primary structure is the amino acid sequence and the secondary structure of the protein molecule is the helix this sequence makes. The tertiary structure of the protein molecule is the folding and linking of the amino

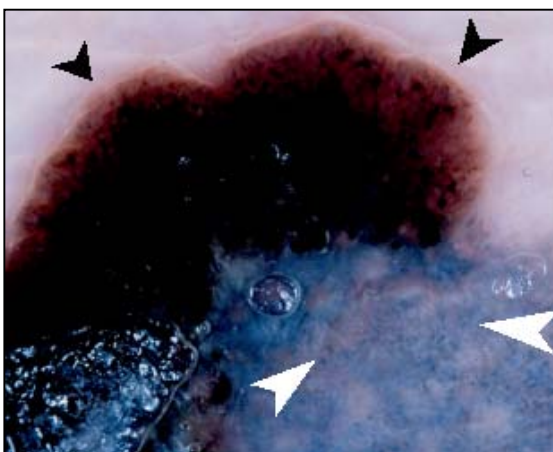


Figure 2.5

Large superficial spreading melanoma. Peripheral black dots represent Pagetoid spread (black arrows) and blue-black 'pepper-like' dots represent melanophages (white arrows).

acid sequence to produce a three-dimensional structure. Finally, some molecules, for instance haemoglobin are a combination of three-dimensional units and this is their quaternary

structure. In a similar manner, the visible structures of a pigmented skin lesion could be thought of as having a primary unit - melanin or haemoglobin; a secondary structure - the object that contains the pigment such as a melanocyte or red blood cell; a tertiary structure - a collection of pigmented secondary structures visible with dermatoscopy that forms a recognisable morphological feature; and a quaternary structure - the pigmented lesion visible with the naked eye. This is a concept of describing the architecture of pigmented skin lesions that the author would like to introduce in this thesis as it would help to categorize the level of magnification or resolving power of any imaging technique being described [Moncrieff, 2001a]. The categories that have been described approximate to a logical and logarithmic progression in magnification, namely one-times, ten-times, one hundred-times and one thousand-times magnification seen using the naked eye, a hand-held microscope, a light microscope and an electron microscope respectively [Mooi & Krausz, 1992; Menzies *et al.*, 1996a].

In diagnosing melanocytic skin lesions, the most important structure is the pigment network.



Figure 2.6

Superficial spreading melanoma. Pseudopodia spreading out into the normal skin (black arrow) and the broadened pigment network is breaking down into branched streaks (white arrow). The network breaks down into radial streaming (red arrow).

This has been described as the 'unifying surface microscopic feature of melanocytic-derived lesions.' [Menzies *et al.*, 1996a]. It is formed by the varying concentration of melanin in the melanocytes in a vertical direction as they lie along the undulations of the dermoepidermal junction and has the appearance of a spider's web. However, an absence of a pigmented network does not exclude a melanocytic lesion. The network can be disturbed by processes affecting the normal contour of the dermoepidermal junction often as a result of neoplastic invasion in melanoma and inflammation in dysplastic naevi and melanoma. When these processes occur, the branching of the network breaks down at certain points and is termed branched streaking in the centre of the lesion [Braun-Falco *et al.*, 1992] and radial streaming at the periphery [Bahmer *et al.*, 1990]. From the edge of the lesion, pseudopodia can

sometimes be seen in malignant melanoma projecting out into the surrounding normal skin. They appear similar to a drumstick and represent the progression of the radial growth phase in a superficial spreading melanoma [Menzies *et al.*, 1996a]. Also in the epidermis, black dots, especially located at the periphery of the lesion, are suspicious of melanoma as they usually represent Pagetoid spread.

In the superficial dermis, brown globules are seen that are consistent with junctional or compound nests. They are usually regularly and concentrically arranged and give the compound naevus a pathognomic 'cobblestone' appearance [Braun-Falco *et al.*, 1992]. With dermal invasion of melanoma there is an immunocytic response with macrophages engulfing melanoma cells, telangiectasia and fibrosis. With the dermatoscope these events are seen as 'pepper-like' blue-grey dots, telangiectasia and scar-like depigmentation with blue-grey veil respectively [Bahmer *et al.*, 1990].

Another useful feature of skin surface microscopy is the easy identification of lesions that can mimic melanoma but are of non-melanocytic origin. This is especially true of eruptive haemangioma that often presents with a history of rapid progression and has a dark, irregular clinical appearance. With skin surface microscopy, the vascular origin of this lesion is revealed by its red/blue lagunes [Menzies *et al.*, 1996a]. Seborrheic keratoses often provide a diagnostic quandary, especially the macular subtype located on the face of an elderly patient that can easily be resolved by skin surface microscopy. Seborrheic keratoses often contain keratin plugs that appear as white, pearl-like structures termed 'milia cysts'.

2.3.2 Diagnostic Accuracy of Skin Surface Microscopy

The most comprehensive and objective assessment of the diagnostic accuracy of skin surface microscopy to date has been the systematic review of the technique undertaken by Justine Mayer (1997). In this review, 579 articles were identified of which only six met the inclusion criteria of being "original studies with formal methods and results sections comparing diagnostic accuracy for skin surface microscopy with another clinical method; the criterion standard was excision biopsy with histopathological examination."

In the review, Mayer was hampered in assessing the validity of the studies due to several shortcomings in design of the experiments, namely "only [one] provided sufficient details to enable the study to be repeated". Other points of issue included "only one study explicitly stated how lesions were chosen for entry into the study", "none provided data on all presenting lesions or followed them up to calculate the true false negative rate", "it was not apparent that the clinical and dermatoscopic diagnoses were independent" and "no study remarked that a lesion was unable to be diagnosed by dermatoscopy".

Eventually, it was concluded that due to the "variability [of the studies] prevented formal meta-analysis and estimation of a single summary statistic for the diagnostic benefit of dermatoscopy." In examining the results of the studies, it was found that where the specificity and sensitivity of the clinician was high for clinical diagnosis then the dermatoscope had little to offer in addition [Cristofolini *et al.*, 1994; Nachbar *et al.*, 1994]. However, using the dermatoscope with equivocal lesions increased the sensitivity for diagnosis between 10 and 27% [Steiner *et al.*, 1987; Binder *et al.*, 1995]. Another interesting aside in the latter paper was that clinicians who received no formal training in the technique performed worse using skin surface microscopy without and before any formal training than if they had attempted to make the diagnosis on clinical grounds alone! However, the specifics of the formal training were not discussed in any detail.

2.4 Newer Techniques for Assessing Pigmented Skin Lesions

In this section there will be a short discussion on other technologies that are being used for the investigation of pigmented skin lesions.

2.4.1 High Frequency Ultrasound

Ultrasound is a well-established technology that has been used in the management of skin disorders since 1979 [Alexander & Miller, 1979]. Scans of normal skin show a thin echo-rich line termed the entry-echo followed by another echo-rich layer consistent with the dermis. Below this layer is the echo-lucent subcuticular fat that has the occasional echo-rich signal consistent with the interlobular septae. At lower frequencies resolution is poor - lateral resolution of 200 microns and axial resolution (section thickness) of 80 microns at 20 MHz [El Gammal *et al.*, 1999]. At 20MHz, it is not possible to resolve all but the largest structures within the skin such as hair follicles but, with the adjunct of Doppler ultrasound, blood flow within the larger vessels of the skin can be determined. At 20 MHz it is possible to measure skin lesions up to 10 mm in depth [Krahn *et al.*, 1998]. This has led to the development of systems with greater frequencies up to 100 MHz where lateral resolution of 27 microns and axial resolution of 7 microns has been claimed [Kaspar *et al.*, 1999]. However, with increasing the frequency of the ultrasound reduces the depth of visualisation into the dermis and this is a significant consideration. Melanomas appear as an echo-lucent region with sharp demarcation of the deep and lateral borders of the lesion that act as good boundaries to enable measurement though the detail that can elucidated within the lesion is minimal [Kaspar *et al.*, 1999]. Regardless of this, high-frequency ultrasound has limited use in the differential diagnosis of pigmented skin lesions [Fuechsel *et al.*, 1999] as it barely resolves the tertiary structure of a pigmented lesion. It may, however, have a role in the pre-operative planning of

clinically obvious lesions where strong correlation between tumour depth measured using ultrasound and Breslow thickness has been claimed [Semple *et al.*, 1995; Lassau *et al.*, 1999]

2.4.2 Optical Coherence Tomography

Optical coherence tomography is a technique first reported in 1991 (Huang D *et al.*, 1991) for the analysis of biological tissues. It is based upon the principle of interferometry. Broadband near-infrared light is passed into the system whereby a coupler directs one portion to the skin and another to a reference mirror. The reflected light from both sources are returned to the coupler and passed to detecting hardware. The light received is processed to images by appropriate hardware and signal-image processing software. This process is analogous to ultrasound but uses infrared light waves instead of acoustic waves [Reiss, 1999].

Axial (section thickness) and lateral resolution of 4 and 7 microns resolving structures less than 10 microns has been claimed. The depth of penetration has been measured at 1mm into the skin though at depths greater than 500microns resolution tails off rapidly due to photon scattering [Schroeder *et al.*, 1999]. The stratum corneum, viable epidermis, and papillary dermis can be differentiated. However, naevus cell nests and other structures can only be visualised with difficulty due to the lack of resolution of this technique and therefore has limited practicality in the diagnosis of melanoma [Pagnoni *et al.*, 1999; Welzel & Frederking 1999]. Therefore this technique resolves down to the tertiary structure of the pigmented lesion. In addition, systems are expensive to purchase initially, require specialist expertise to interpret the data and thus its role in detection and screening of melanoma is limited.

2.4.2 Magnetic Resonance Imaging

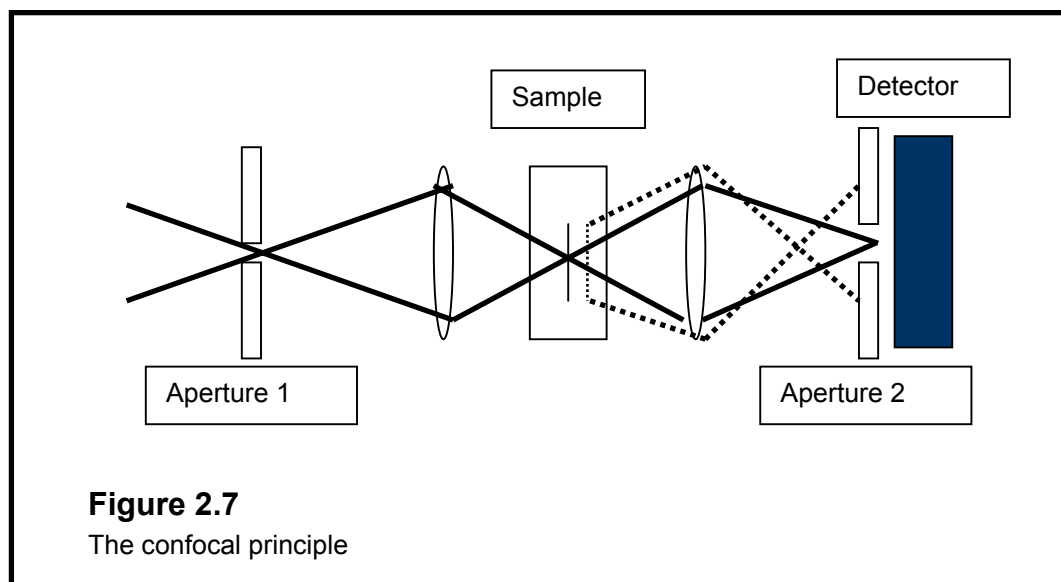
Magnetic Resonance Imaging (MRI) has been used to visualise structures within the skin. The epidermis is demonstrated as a signal-rich area, the dermis as a signal-poor region and within the hypodermis, septations demarcating lobules of fat are easily recognised [Song *et al.*, 1997]. Melanin appears as a bright signal and lesions containing it can be demarcated from the surrounding signal-poor dermis in specimens freshly excised. However, the resolution available has been a limiting factor and again, only the tertiary structure of the lesion can be determined. Authors claim that currently a spatial resolution of 75um is available (Song *et al.*, 1997 & Querleux 1999) allowing visualisation of hair follicles, sweat glands and blood vessels. This, however, adds little information to the diagnosis of melanoma and does not allow the clinician to be able to differentiate between benign and malignant lesions [Takahashi & Kohda, 1992]. One useful feature is the preoperative prediction of the depth of penetration of the melanoma in cases of locally advanced disease that allows for planning of surgical procedures [Ono & Kaneko, 1995]. Cheaper and smaller magnets continue to be designed

but, in balance, high frequency ultrasound and optical coherence tomography are cheaper, more portable and produce better resolutions [Pagnoni *et al.*, 1999].

2.4.4 Confocal Microscopy

The confocal principle allows the visualisation of structures in high resolution through a turbid medium and is demonstrated by the schematic diagram - figure 2.7 [Cullander, 1998]. Light is passed through a narrow aperture and is focused by the condenser lens to a focal point in the specimen. The light is then remitted and passes through a second lens and is focused on a very narrow aperture. This aperture is the critical factor to the system and any scattered or out of focus light cannot pass through (figure 2.7 - dotted line). This is a very powerful resolving technique that can allow the observer to view detail down to the sub-cellular level.

Modifications in the design of the confocal microscope have, for the last decade, made it possible to analyse skin *in vivo* in 'real-time' [Rajadhyaska *et al.*, 1999]. This group has claimed lateral resolutions of 0.5-1.0 micrometres and sectional or axial resolutions of 3-5 micrometres but due to the narrow apertures used the field of view is limited. This means that while the secondary structure of the lesion is visible, perversely the tertiary structure often is not. In addition, scattering of the light by the dermis allows for limited resolution in this layer, though it has been claimed that circulating erythrocytes are visible in the papillary vessels [Rajadhyaska *et al.*, 1999]. Other drawbacks to confocal microscopy include bulkiness of the apparatus, limitation to viewing flat surfaces and the extremities; costliness of the device and the specialist training required to interpret the images produced. However, this technique does allow for excellent resolution of epidermal structures and will certainly be of value in the



assessment of basal cell and squamous cell carcinomata.

2.5 Computer Imaging, Feature Extraction & Artificial Intelligence

It has long been the goal of many research groups to replicate and automate the way a human visualises and examines a lesion. This is a significant task that can be subdivided into three broad categories – image processing, image analysis and image interpretation.

2.5.1 Image Processing

Computer imaging can be defined as ‘the acquisition and processing of visual information by a computer’ [Umbaugh, 1998]. Computer imaging requires two broad and fundamental computing subsystems – computer hardware and computer software. The former is mainly concerned with getting the image into the computer in a format that can be manipulated by the software (digitisation) and the displaying of the information on an output device such as a monitor or printer; the latter is mainly concerned with preparing and processing the image for interpretation by the user on the output device. In systems used for dermatological analysis the format usually begins with a camera or ‘charged couple device’ (CCD) that transmits information to the computer where the image is processed and analysed using dedicated software and finally outputs this information to a monitor for interpretation by a physician.

The hardware used in image processing has important and noteworthy limitations. First the CCD may only deal with ‘grey-scale’ or black-and-white images. In addition, the CCD only has a finite number of photoreceptors or pixels and this places a limitation on the resolution of the image produced and any subsequent analysis. Fluctuations in electrical current within the system and electrical interference will result in erroneous signals being sent to the computer that can be seen in the final image as ‘noise’. Noise-reducing systems, such as those that cool the CCD processing chip, are available with certain cameras that logarithmically increase their expense. Images requiring fine detail analysis tend to involve a large quantity of digital information that place a large burden on the computer hardware such as the processor and system memory. This may result in significant delay from image acquisition to image output to the monitor, ranging from several minutes to many hours depending upon the task required by the user. However, with the exponential improvement in hardware annually, this is becoming less of a problem.

In addition to the problems associated with the acquisition of the image, its output to a monitor may also have several limitations that affect the interpretation of the image by the clinician. Similarly to the CCD, the resolution of the image on the monitor screen depends upon the actual number of pixels the monitor can project [Kundel, 1986]. If a large surface area is being projected, such as a chest x-ray, then high resolutions are required to reproduce the details useful for diagnostic information faithfully. These resolutions may be beyond the physical capability of the monitor and this results in a 'blocky' appearance to the image. In addition, the monitor interprets and displays light in a linear manner derived from the digital data yet the human visual system responds to light and colour intensity in a non-linear manner. This means that a doubling in light intensity of an image by the monitor does not result in the perception of the image being twice as bright by the human. This results in reduced contrast sensitivity and a loss of the perception of fine detail by the user [Kundel, 1986]. This problem extends further into the perception of colour – the non-linear response of cones in the human retina to light makes it difficult for the monitors to reproduce faithfully the 'natural' colours of an image from data acquired using a CCD. However, like most computer hardware, this situation is improving with successive developments in imaging technology.

Fortunately, dermatological lesions are relatively small and so resolution does not often become an issue. However, if images are to be compared for sizes, features and colours then the images must be standardised and calibrated [Hall *et al.*, 1995]. The difference in light exposure can affect the image in terms of contrast and any subsequent image analysis and feature extraction. Hall *et al.* state that 'the majority of current work makes no, or scant, reference to this crucial first step.' They go on to point out that every step used in image acquisition introduces errors and that any image used for processing and analysis must be the best possible representation of the original 'before meaningful and comparable results can be obtained.' In addition to this, there may be unavoidable errors produced by the imaging system that will have to be interpreted and recognised as artefact by the user after the final computer analysis has been performed. A good example of this latter point is flash photography producing 'casts' – areas of bright white resulting from direct reflection from the skin's surface.

2.5.2 Image Analysis & Feature Extraction - Principles

This step involves the manipulation of the 'raw' digital data on the computer by the software to produce an image for output and interpretation by the user or further software on the computer. The principle steps are pre-processing, data reduction and feature extraction [Umbaugh, 1998].

Pre-processing algorithms are used to make the task of data reduction and feature extraction easier and are usually simple in their nature. Examples of pre-processing include the removal of camera 'noise' by using filtering software, honing in on a region of interest to exclude extraneous data by using such techniques as digital subtraction and image cropping, and enhancing images that have poor contrast by using contrast-stretching techniques. When chosen carefully and appropriately, the software required to undertake these techniques is small and efficient and these steps usually make little, or no, impact on the overall processing time.

Data reduction or image quantisation is 'the process of reducing the image data by removing some of the detail information and mapping groups of data points to a single point' [Umbaugh, 1998]. This can be performed on the pixel values themselves whilst retaining the absolute

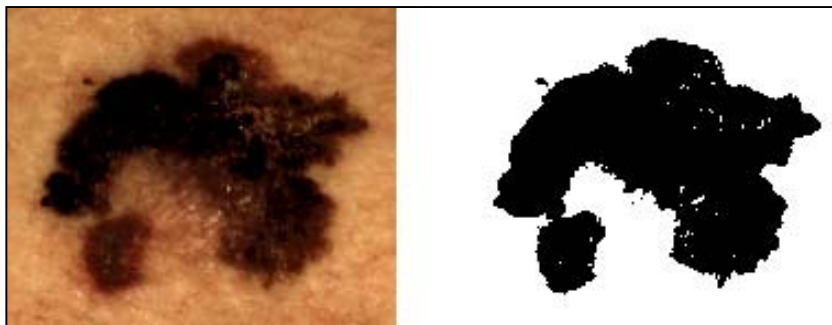


Figure 2.8

Obtaining a silhouette of a melanoma by a simple thresholding technique.

number of pixels, termed *grey-level reduction*, or by reducing the number of pixels whilst retaining image detail – *spatial reduction*. The most common technique to perform grey-level reduction is *thresholding* whereby the user maps all pixels below a certain brightness value to black and all others above to white (fig 2.8). This process produces a two-tone silhouette. When this technique is applied to images of pigmented skin lesions, it is easy to envisage measuring their diameter, area and other features. If more information than a simple two-tone image is required then more subtle and sophisticated techniques (though not much more involving) are available to produce a multi-level or 'quantised' image for further feature extraction. In contrast to grey-level reduction, spatial reduction results in a reduced image size and, again, several simple techniques are available to produce this. When performing this task, if the height and width constraints of the original image are not observed then image distortion occurs, specifically stretching and shrinking along the vertical or horizontal axis.

With the image pre-processed and reduced it may be ready for feature analysis. However, a final step may be required before this can occur – feature extraction. Edge and line detection may be useful in delineating the edge of a lesion from normal surrounding skin. Segmentation

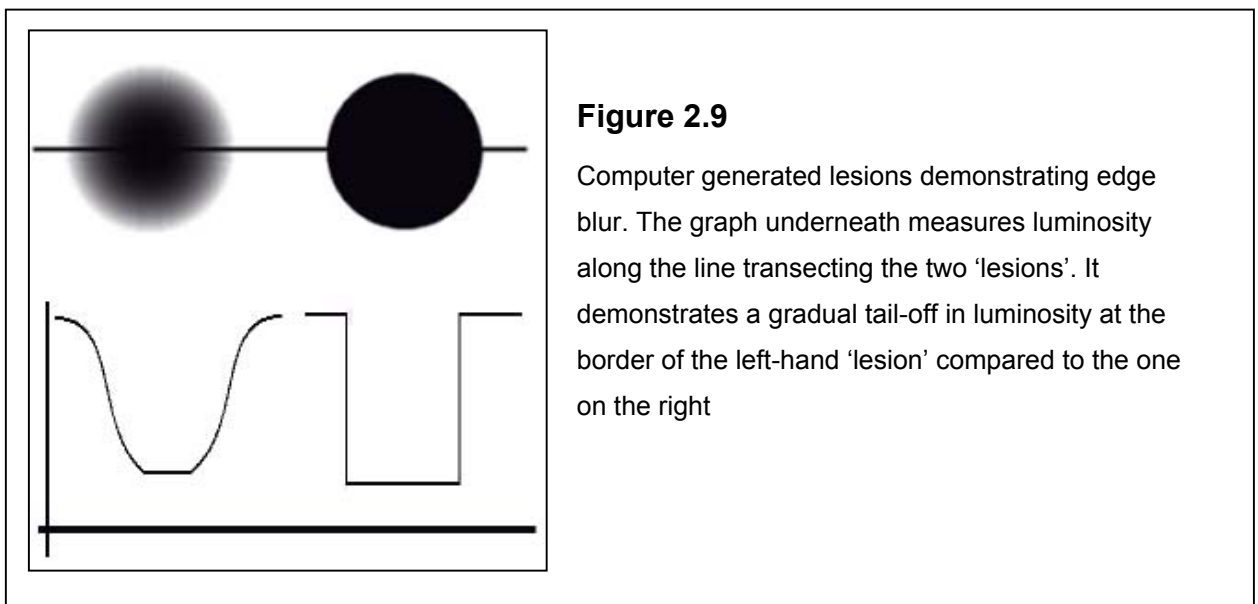
involves the detection of regions that represent meaningful objects within an image and this is useful in automating the analysis of skin surface microscopy images and counting colours within a lesion.

2.5.3 Feature Analysis Applied to Melanoma

Specifically for melanoma, computerised feature analysis would attempt to reproduce and objectively quantify the clinical 'ABCD' parameters of a lesion as described earlier in this chapter (section 2.2.2 & table 2.3). Hall *et al.* (1995) summarised the current research in this area. Before attempting to analyse the 'ABCD' features of a lesion it is necessary to segment it in some way - that is to delineate the lesion from the surrounding normal skin. The most common way to do this is to perform a thresholding algorithm as part of the pre-processing, as shown in figure 2.8.

2.5.3.1 Asymmetry

There are several methods to measure the asymmetry of the lesion [Claridge *et al.*, 1992; Stoecker *et al.*, 1992]. One such method would be to determine its centre of gravity of the



lesion by a computer algorithm and the long and short axis of the lesion and a corresponding ellipse, from this, can be ascertained. The ratio of the long to short axis gives an indication of how stretched out the lesion is and the ratio of the area of the ellipse to the actual area of the lesion gives a measure its 'bulkiness' [Claridge *et al.*, 1992]. Finally, if the lesion is then mirrored in the long axis determined previously, asymmetry could be measured [Stoecker *et al.*, 1992].

2.5.3.2 Border

Border irregularity can be determined using computer algorithms that employ fractal analysis as described by Mandelbrot (1977). In analysing a map of Great Britain and, in particular the length of the coastline, Mandelbrot found that using a smaller and smaller ruler the distance measured actually increased as he was able to measure smaller and smaller indentations of the coastline. The distance measured versus the ruler size plotted on a graph produces a straight-line function and the gradient of this line can be determined. This gradient is termed the *fractal number*. A straight line has a fractal number of one because the distance measured is the same regardless of the ruler size. Conversely, a shape that is infinitely irregular now fills the page and has a fractal number of two – it is now a two-dimensional plane. Therefore the range of irregularity using the fractal number of a lesion border is between 1 and 2. Two studies have employed fractal dimensions to distinguish melanoma from non-melanoma with good specificity and sensitivity (Claridge *et al.*, 1992; Hall, 1992).

Another characteristic of the border of a lesion is either a blurring or a sharp cut-off at its periphery. Edge-blur is recognised as a clinical characteristic of melanoma. When attempting to determine this objectively using a computerised analysis system, it is easy to envisage a graph measuring the luminosity (brightness) along a cross-section of a lesion (fig 2.9) Intuitively, a lesion that has a blurred border will show a gentler rate of change in luminosity at its junction with normal skin than one with a sharp border. In practice, however, hairs and skin-creases at the skin surface confound this approach. Any attempt to filter these features out produces edge-blur itself thus rendering the test less helpful [Hall *et al.*, 1995].

2.5.3.3 Colour

Colour measurement and analysing the colour features within a pigmented lesion is a challenging and difficult undertaking. Intrinsic to this problem is the differences in the processing and response to colour between humans and machines - the colour theory behind this will be discussed in some detail in a later section of this chapter. For instance, it is very difficult for clinicians to describe 'dark brown' or 'blue/black' colours deemed to be important by Menzies *et al.* (1996b) – and to reliably agree on their presence. It would appear that clinicians describe colours in relation to the surrounding skin and to each other within a lesion [Stoecker *et al.*, 1995].

Regardless of the complex underlying theory it is sufficient to say that research groups have claimed some success with computerised colour analysis to discriminate melanoma from benign pigmented skin lesions [Dhawan & Siscu, 1992; Umbaugh *et al.*, 1992 & 1993; Schwindewolf *et al.*, 1993; Stoecker *et al.*, 1995; Schmid & Fischer, 1997; Tomatis *et al.*, 1998]. It is especially important with this analysis that image acquisition is rigorously

standardised and any variation can introduce significant errors in image interpretation. Colour images of melanomas have been analysed for variegation [Stoecker *et al.*, 1995] and absolute colour indices in the RGB and HSV planes [Schwindewolf *et al.*, 1993; Tomatis *et al.*, 1998] with good effect claimed. Segmentation algorithms applied to colour images have been able to separate melanoma from non-melanoma with varying effect [Dhawan & Siscu, 1992, Umbaugh *et al.*, 1993] and segmentation has also been applied to dermoscopic images [Schmid & Fischer, 1997]. A further challenge to this arm of research in feature extraction is the non-standardisation of colour between imaging hardware systems that often requires complex mathematical transformations to the image files to overcome this. Recently steps have been taken to address this problem [Poynton, 1999a].

2.5.3.4 Diameter

In contrast to colour, determining the diameter of a lesion from the image is a simple matter of counting pixels. Images usually have a standard reference of some kind such as ruler to enable this to be determined [Hall *et al.*, 1995]. However, in order to measure the diameter the boundaries of the lesion must be ascertained and this can be performed using methods described in section 2.5.3.2.

2.5.4 Artificial Intelligence (AI)

Artificial intelligence (AI) is a rapidly progressing and developing field. It has application not only in medicine, but also in the financial and manufacturing sectors of industry worldwide. An in-depth discussion of AI is beyond the scope of this thesis but a short, concise and well-written introduction can be found by reading the paper by Umbaugh *et al.* (1991). Essentially, AI systems attempt to produce consistent and reproducible predictions of outcome by either reproducing rules developed by clinicians as a result of their own experiences, namely *Heuristics*, or by producing a set of rules by machine automation that result in an expert system that the human user may not understand, namely *Formal Induction* and *Neural Networks*. In developing the expert system a training set is used to develop the algorithms and a second, separate set is used for testing its robustness. This is an important feature in the design of a study that Umbaugh *et al.* point out 'is often neglected in practice' and may lead to 'results that are biased and possibly meaningless.'

AI systems would appear to be an attractive solution to analysing pigmented lesions and this line of research has culminated in several systems being developed that combine measurements through the use of neural networks to arrive at a diagnosis from both non-dermoscopic and dermoscopic images [Ercal *et al.*, 1994; Binder *et al.*, 1998; Menzies *et al.*, 1997 & 1999]. These automated systems claim results that approach or equal human experts. However, there are several drawbacks with AI systems, in general. Usually the

training set has to be large if several 'input' features are to be effectively and robustly analysed and this explains why the DANAOS project (Diagnostic and Neuronal Analysis of Skin Cancer: Bochum, Germany) is undertaking a massive, Europe-wide, multi-centre trial [Menzies, 1999]. As a direct result, training of a network takes a long time and there is no guarantee that the system will work or that all the correct inputs have been used in the first place. AI systems are very sensitive to differences in calibration so that changes in illumination, exposure or the scale of the image size will produce large and insurmountable errors – methods of image standardisation must be very exacting. Further problems exist with 'local minima'. This is a difficult concept but can be described by the following analogy. Imagine that the computer is on the top of a mountain and you want it to get to base-camp at the foot of it. To tell a computer to navigate to the camp a reasonable instruction would be to 'keep going down until you can go no further.' However, when the computer encounters a localised depression or hollow in the landscape it has reached a point where it can go no further but still has not reached the ultimate destination – it is trapped. Strategies have been developed to avoid this, namely simulated annealing that, returning to our analogy, cause the computer to jump backwards or upwards and to look around (whilst in the air!) for an alternative route home. Finally, AI systems remain encapsulated – a 'black box'- where the pathway to the output is neither known nor understood. Extending this point further, any breakdown in function cannot be retrieved except by retraining the system with the attending time-penalty. Specific to the diagnosis of melanoma, Menzies (1999) points out a further potential problem with automated systems: 'the ELM features of thicker nodular melanomas are significantly different than those of thin superficial spreading melanoma. For this reason, algorithms based on in situ and thin invasive melanoma may not perform optimally on thicker lesions.'

2.6 Spectrophotometric Intracutaneous Analysis

This section of this chapter will explain in detail the technique of spectrophotometric intracutaneous analysis. In order to do so it is first necessary to discuss the physics of light and colour theory and the optics of human skin.

2.6.1 Light & Colour Theory

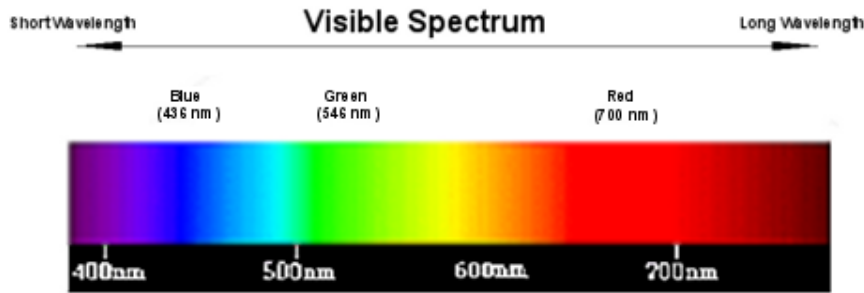
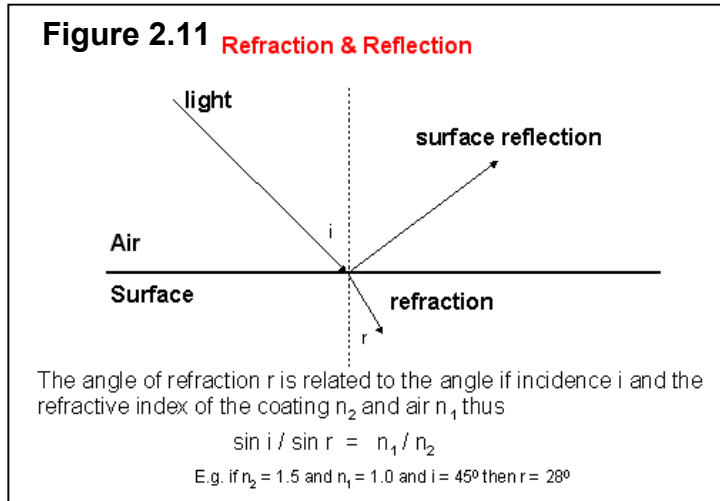


Figure 2.10

Spectral frequency diagram

Visible light is part of the electromagnetic spectrum and thus it has a frequency and a wavelength. The latter extends from approximately between 400nm (violet & blue) to 750nm (red) in the electromagnetic spectrum figure 2.10 [Cotton, 1995] and this range is termed the 'visible spectrum.' When light strikes a surface there are two outcomes that can occur as a result of the change in refractive index of air and that surface (figure 2.11). First, light can be reflected and is called *surface* or *specular reflection*. Second the light may pass through the surface and penetrate into the body of the material where an increase in the refractive index will cause light to slow and therefore deviate – *refraction*. If the material is a non-homogeneous mixture then the light may be reflected off particles – *scattered* - and even returned back to the air and this is termed *remittance*. Any light that passes completely



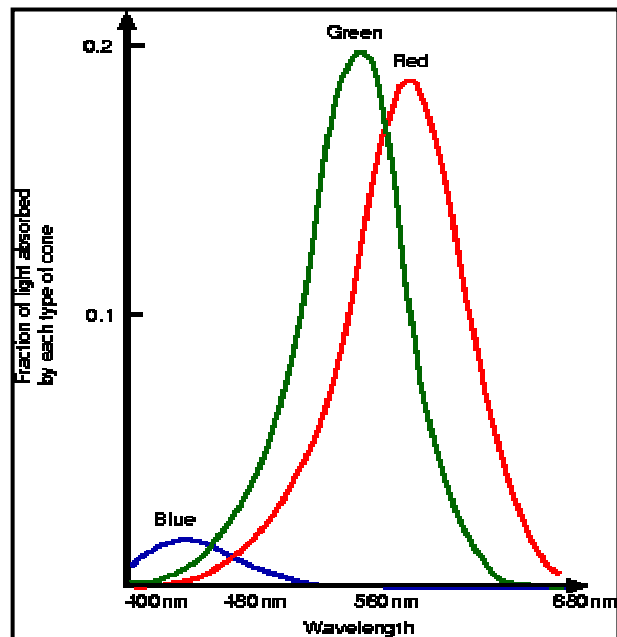
through the material is *transmitted* and light that is neither scattered nor transmitted is *absorbed*. Materials can absorb light in a number of ways but usually the absorption properties of the material are wavelength-specific. The molecules can transform the light into heat and electrical energy or

sometimes the energy can be *re-emitted* at a longer wavelength and fluorescence and phosphorescence are examples of this. The manner in which light is scattered depends upon the size of the particles that is causing this to happen. When the particles are small in relation to the light, usually less than 1000nm, then the scattering is termed *Rayleigh scattering* that is wavelength specific and varies inversely with the fourth power of the incident light. This means that blue light is scattered far more than red. Conversely, when the particles are large, the light is scattered in a feed-forward manner and this is termed *Mie scattering*. At this point it

is convenient to define the term *Chromophore* that is loosely used to describe a molecule that absorbs light, though 'strictly speaking, the term chromophore relates to the conjugated multiple bonded (unsaturated) atoms of the molecule in question as these are responsible for its absorption properties' [Young, 1997]. These terms and principles are important when we come to consider the optics of human skin later in this section.

Light strikes the retina of the human eye and photoreceptors, called cones (approximately 6-7 million in number), are stimulated. There are three types of cone that respond to the visible spectrum as a function of the wavelength of the incident light in the blue, green and red regions (fig 2.12). It is these receptors that allow us to perceive colour by converting light into nerve impulses and this is termed *photopic vision*. Further processing takes place on this raw information at various stages along the 'visual pathway', from retina to visual cortex before the conscious perception of colour and much of this has yet to be elucidated by research.

Figure 2.12
Spectral response curves for human cone cells

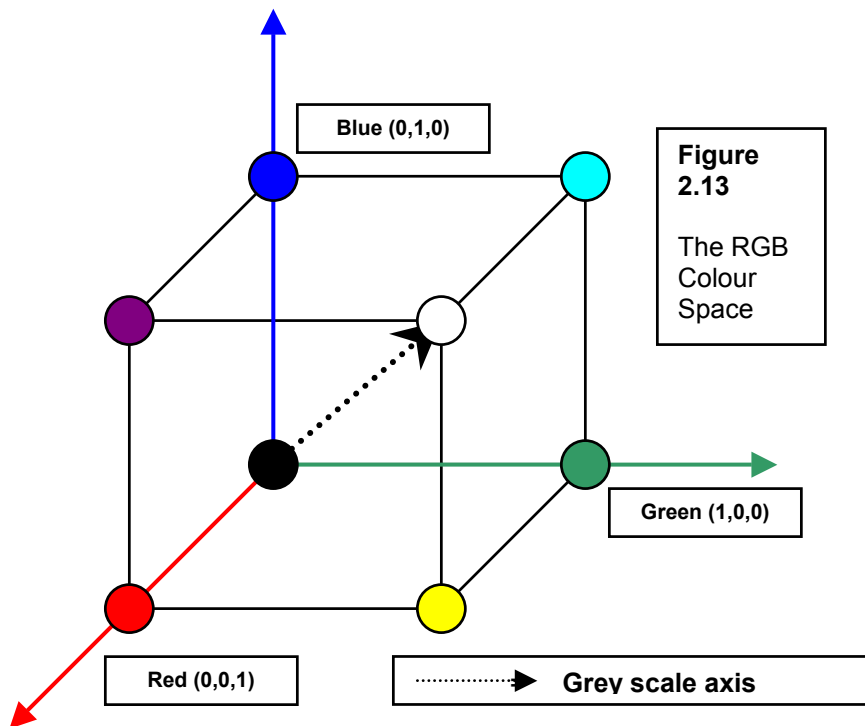


The 'Hue' of a colour is determined by its dominant wavelength and allows us to name it i.e. red, blue, green, yellow and so on. The 'Saturation' of a colour is determined by the excitation purity and characterises it as either pale or vivid. In reality, this quality is inversely proportional to the amount of white light mixed with the hue - a fully saturated colour has no white light. Together, the two variables hue and saturation make up the 'chromaticity' of a colour. Finally, the 'intensity' of a colour is determined

by the energy of the light and is a physical variable. *Luminance* is a function of intensity with area and its unit of measurement is the candela (cd). These two terms are in distinction to the *brightness* of a colour that is a function of human perception and is therefore a psychological variable. *Achromatic* light has no colour so that intensity is its only attribute and can be seen as 'grey-scale' by humans [Poynton, 1999a & 1999b]. Humans perceive brightness in a non-linear and logarithmic fashion. This means that only when a light source has approximately 20% of its luminance do we perceive it as being half as bright. This also means that humans

cannot detect a difference in luminosity if the sources differ by less than 1% and this in turn implies that humans can only detect 100 shades of grey [Poynton 1999a & 1999b]

A fundamental concept of colour science is the Young-Helmholz theory of trichromacy, proposed in 1801, that states:



‘Any colour can be formed by combining three properly chosen primary colours. Any three primary colours can be used as long as none can be formed from a combination of the other two.’

This theory of colour perception would imply that a mix of three

primaries could obtain any colour [Cotton, 1995]. As a result, several colour models have been devised and developed that provide a standard way of specifying a colour. Invariably a 3-dimensional ‘colour space’ is defined containing all constructible colours and a specific colour can be obtained and defined using a vector to arrive at a specific coordinate within this space. When a colour space is represented in this manner it is termed a *tristimulus space* and the values returned from each of the orthogonal primary colour axes are called *tristimulus values*. Each colour model is orientated towards hardware or software systems.

The RGB colour model has three independent axes of intensity representing one of the three primary colours namely red, green and blue. To specify a colour, a triplet coordinate (tristimulus values) is issued specifying the proportions of each of the three primary colours [Cotton, 1995]. This colour space can be represented graphically as a cube (fig 2.13) and it can be seen from this figure that an equal measure of all primaries produces a grey-scale axis. This system is an additive model and represents the mixing or addition of coloured light. Other additive systems include the ‘LMS’ model, where L,M&S stand for long medium and

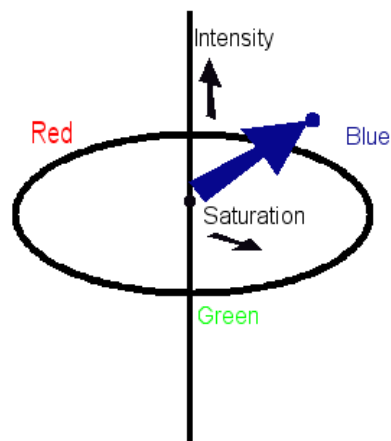


Figure 2.14
HSV colour space

short wavelengths respectively that are defined by the *Commission Internationale de l'Eclairage* (CIE) and this will be referred to again later in this chapter. Additive models are used by televisions and monitors, video cameras and form the basis of many simple file-formats representing graphic images. Conversely, the CMY (cyan-magenta-yellow) model is a subtractive system that corresponds to the absorption of colour such as occurs when light impinges on pigments or paints. The photographic industry and printer devices mainly use this system. The HIS (hue-saturation-intensity) model is a colour space whereby all the hues are represented continuously along the

perimeter of a circle. The radius of this circle is the saturation of the colour and perpendicular to this circular plane is the intensity axis (fig 2.14). Again, a vector that describes a coordinate triplet in this colour-space can represent any colour. Most professional graphical-design software packages use this system, as it is simple, reproducible and intuitive to the human perception of colour described earlier.

2.6.2 The Optics of Human Skin

The path that a ray of light can take through the skin is a complex one: it can be absorbed, reflected, remitted, re-emitted, scattered or transmitted and any combination of these. For a concise and well-written review of the fate of a beam of light on its journey through human skin the reader is directed to the classic paper by Anderson & Parrish (1981). The skin is not a uniform structure and can be subdivided into epidermis and dermis (Chapter 1) and, as one might expect, where the architecture differs so do the optical properties.

2.6.2.1 Optical Properties of the Epidermis

From chapter 1 it can be seen that the epidermis is formed from keratinocytes, the stratum corneum, melanocytes and the epidermal appendages. There is a difference in refractive index between air and the stratum corneum, $n = 1.0$ and $n \sim 1.5$ respectively, and so that typically 5% of the light is reflected back across the entire spectrum uniformly from infrared (1200nm) to ultraviolet (200nm) and this also applies to light returning from deeper regions of the skin. The surface of the skin is not smooth or planar and so light striking it will be not be specular (it does not maintain an image). In addition, the light passing into the stratum corneum will be refracted and made diffuse by this rough surface [Anderson & Parrish, 1981]. Skin surface microscopy alters these events by using a lipophilic and planar surface, oil and a

cover slip for instance, to match the refractive index at the stratum corneum and prevent reflection of the incident light (Fig 2.2 – Section 2.3).

Once in the skin, the light may be scattered or absorbed. Anderson & Parrish (1981) explain that scattering within the epidermis is essentially weak and less than 5% is remitted by to the surface in the Caucasian skin. Thus the major interaction with light seen in the epidermis is absorption. Anderson & Parrish explain that at wavelengths between 200 and 280nm that proteins, nucleic acids and peptide bonds strongly absorb. In addition, urocanic acid, a breakdown product found uniquely in the stratum corneum that is excreted in perspiration, absorbs strongly in the ultraviolet band at 275nm and was thought by some to represent a natural sunscreen [Young, 1997]. However, Anderson & Parrish state, 'In the visible portion of the spectrum, melanin is essentially the only pigment affecting the transmittance of normal human epidermis, giving rise to the wide range of discernable skin colours from "black" to "white".' *In vitro* studies have shown that melanin does not absorb light uniformly across the

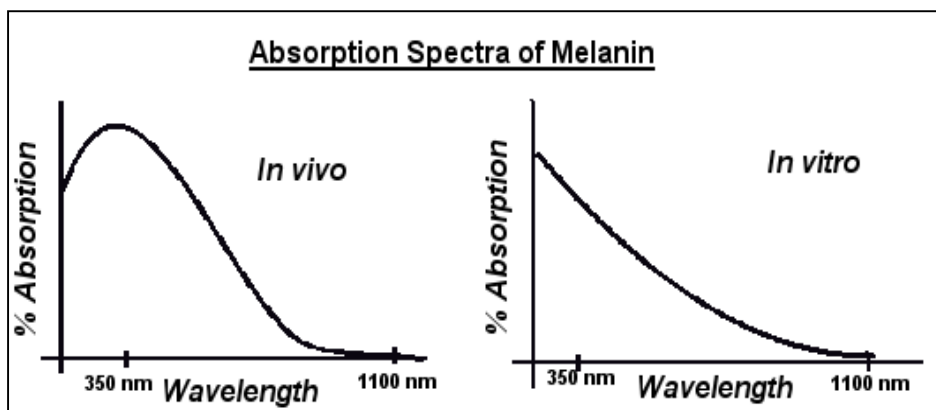


Figure 2.15

Absorption spectra of human melanins *in vivo* and *in vitro*. (Redrawn from text). [Young, 1997; Anderson & Parrish, 1981]

spectrum from 200nm to 1200nm but shows a steady increase at the shorter wavelengths (blue and ultraviolet), is negligible at the near infrared and for wavelengths longer than 1200nm does not affect transmission or remission of optical radiation through the epidermis [Anderson & Parrish, 1981]. Studies of melanin absorption *in vivo* have shown that a maximum absorption peak occurs at 335nm with a sharp tail-off over shorter wavelengths and a gradual decline to the longer wavelengths (fig 2.15) [Young, 1997].

2.6.2.2 Optical Properties of the Dermis

As discussed in chapter 1, the architecture of the dermis can be divided into two distinct layers – the papillary and reticular dermis. The former is typified by loosely packed collagen fibres and capillary loops projecting vertically to the papillary ridges from the subpapillary vascular plexus at the dermo-dermal junction. Experiments on dermis *in vitro* show that absorption of optical radiation is negligible, as the dermis is rendered bloodless. However,

scattering is not uniform and is markedly increased at shorter wavelengths. Anderson & Parrish (1981) state, 'Dermal scattering therefore plays a major role in determining the depth to which radiation of various wavelengths penetrates the dermis and largely accounts for the observations that, in general, longer wavelengths across the UV-visible-near infrared spectrum penetrate the dermis to a greater extent than do shorter wavelengths.' This phenomenon explains the apparent paradox of blue naevi that consist of intradermal melanocytes appearing blue despite melanin absorbing most effectively at this wavelength. Optical radiation is scattered differently in the papillary dermis compared with the reticular dermis. As discussed in section 2.6.1 scattering occurs according to the size of the particle in the scattering medium. In the papillary dermis the collagen fibres are of a small size in comparison with the wavelength of the incident light and therefore produce *Rayleigh scattering* that is wavelength specific but in the reticular dermis the collagen is organised into large-diameter bundles that produce *Mie scattering* in a forward (inward) direction [Cotton, 1998]. This means, in effect, that radiation entering the dermis is remitted by the papillary dermis for most of the visible spectrum whereas near infrared wavelengths are transmitted through to the reticular dermis and beyond with little or no remittance to the epidermis. Therefore, as a broad and simplistic generalisation, the papillary dermis can be considered as the 'reflective' layer of the skin in the visible spectrum.

Pigments found in blood such as oxy- and deoxyhaemoglobin, bilirubin and beta-carotene account for the absorption spectra of the dermis *in vivo* [Anderson & Parrish, 1981]. The absorption spectra of the latter two are similar with a broad absorption band between 400-500nm (mostly blue and some green) giving a yellow appearance to the skin as seen in the clinical situation of jaundice (hyperbilirubinaemia). Oxyhaemoglobin has absorption peaks at 543nm and 576nm whereas deoxyhaemoglobin has one at 554nm – thus green light is maximally absorbed. Absorption of all four pigments rapidly tails off after 600nm [Anderson & Parrish, 1981; Feather *et al.*, 1988].

It can now be seen that light is absorbed and remitted in the dermis according to a complex function of absorption and scattering and that chromophores absorb not only according to the quantity of the molecule and its absorption properties but also the depth at which the chromophore is situated in the dermis determines the range of wavelengths (colours) that the chromophore is exposed to. This is a fundamental point in the understanding of spectrophotometric analysis.

2.6.3 Theoretical Basis of Spectrophotometric Intracutaneous Analysis

As one can envisage, producing a predictive model of skin human colouration from the interactions of chromophores and collagen is a difficult task further complicated by the multilayered architecture of the skin.

2.6.3.1 A model of the epidermis

Light traversing the epidermis can either be reflected or absorbed by melanin with scattering in this layer considered to be negligible. In this situation, Bouguer's law describes the attenuation of light by the melanin;

$$\theta(\lambda, d_m) = e^{-dm(\lambda)} \quad (1)$$

where $\theta(\lambda, d_m)$ is the ratio of transmitted radiation to incident radiation at a given wavelength and path length, d is the path length and $m(\lambda)$ is the spectral absorptivity of the material. Thus for epidermal melanin the intensity of the light emitted from the epidermis, S_e can be shown to be

$$S_e = \theta(\lambda, d_m) s(\lambda) \quad (2)$$

where $s(\lambda)$ represents the intensity of the incident light against wavelength [Cotton, 1998].

2.6.3.2 A model of the dermis

A predictive model of light traversing the dermis needs to account for both scattering produced by the collagen fibres of the papillary dermis and absorption by the chromophores. The Kubelka-Munk theory describes the remittance and transmittance of radiation through an absorbing and scattering medium and is often used as a model to describe the passage of light through the skin (fig 2.16) [Anderson & Parrish, 1981].

It assumes that the inhomogeneous material contains particles that are small in comparison to its thickness and that the incident radiation is diffuse. This is the case for skin where the

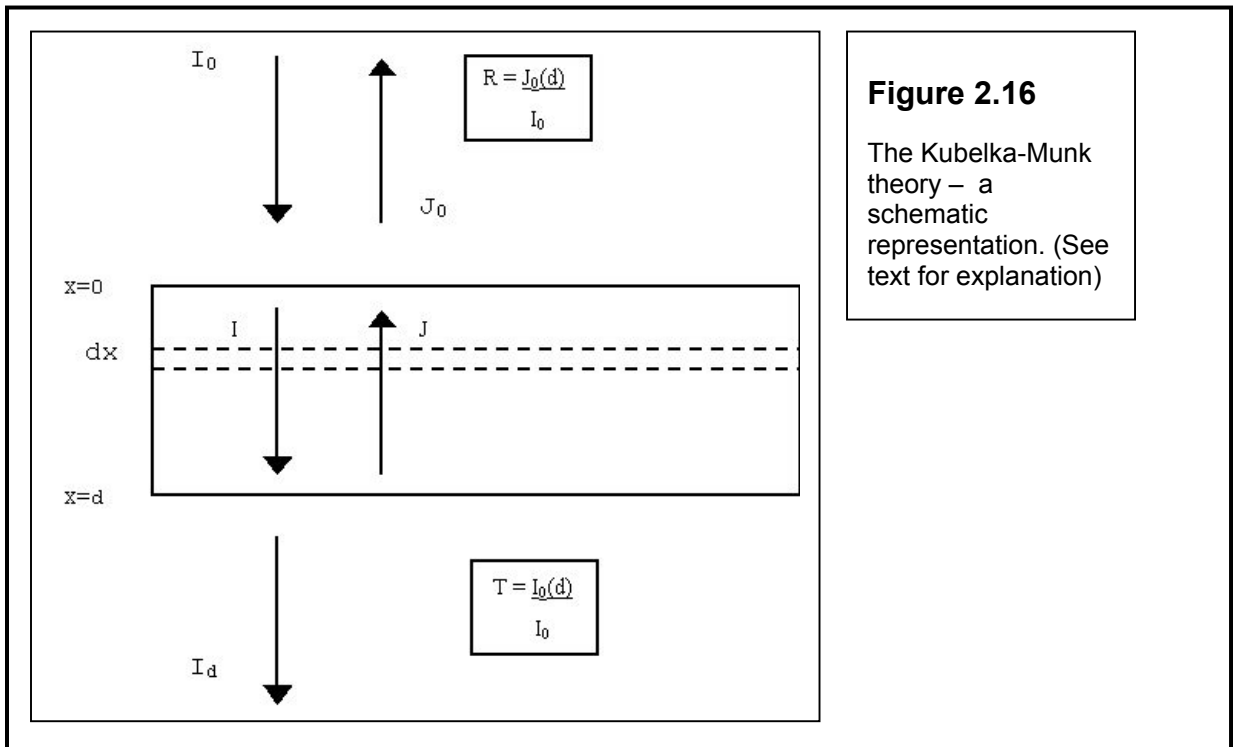


Figure 2.16
The Kubelka-Munk theory – a schematic representation. (See text for explanation)

diameter of the collagen fibrils is a fraction of the dermal thickness and the stratum corneum renders the light diffuse. The theory divides radiation within the sample into 2 opposing fluxes: I describes the radiant flux in the direction of increasing sample depth, d ; and J describes the returning flux as a result of scattering up to the depth d . The remittance, R , and the transmittance, T , can be expressed as

$$R = \frac{J_0(d)}{I_0} \quad \text{and} \quad T = \frac{I(d)}{I_0} \quad (3)$$

In order to compute R and T for a given tissue, the depth d , the fraction of radiation absorbed, α , and the fraction of radiation scattered, ζ , per unit path length of tissue must be ascertained. The constants α and ζ have been ascertained experimentally [Anderson & Parrish, 1981] but are wavelength dependent so that R and T are denoted as $R(\lambda, d, \alpha, \zeta)$ and $T(\lambda, d, \alpha, \zeta)$ respectively. The incident light is spectral in composition and the spectral composition of the remitted, R , and transmitted light, T , are:

$$S_R(\lambda) = R(\lambda, d, \alpha, \zeta) I_0(\lambda) \quad (4)$$

$$S_T(\lambda) = T(\lambda, d, \alpha, \zeta) I_0(\lambda) \quad (5)$$

These equations describe a two-layer system that can be extended for a system of n layers of arbitrary complexity and, provided the thickness and composition of the layers are specified, the total remitted, $S_R(\lambda)$, and transmitted, $S_T(\lambda)$, light can be computed [Cotton *et al.*, 1999]. This model can be used to predict the values of chosen primaries, such as RGB:

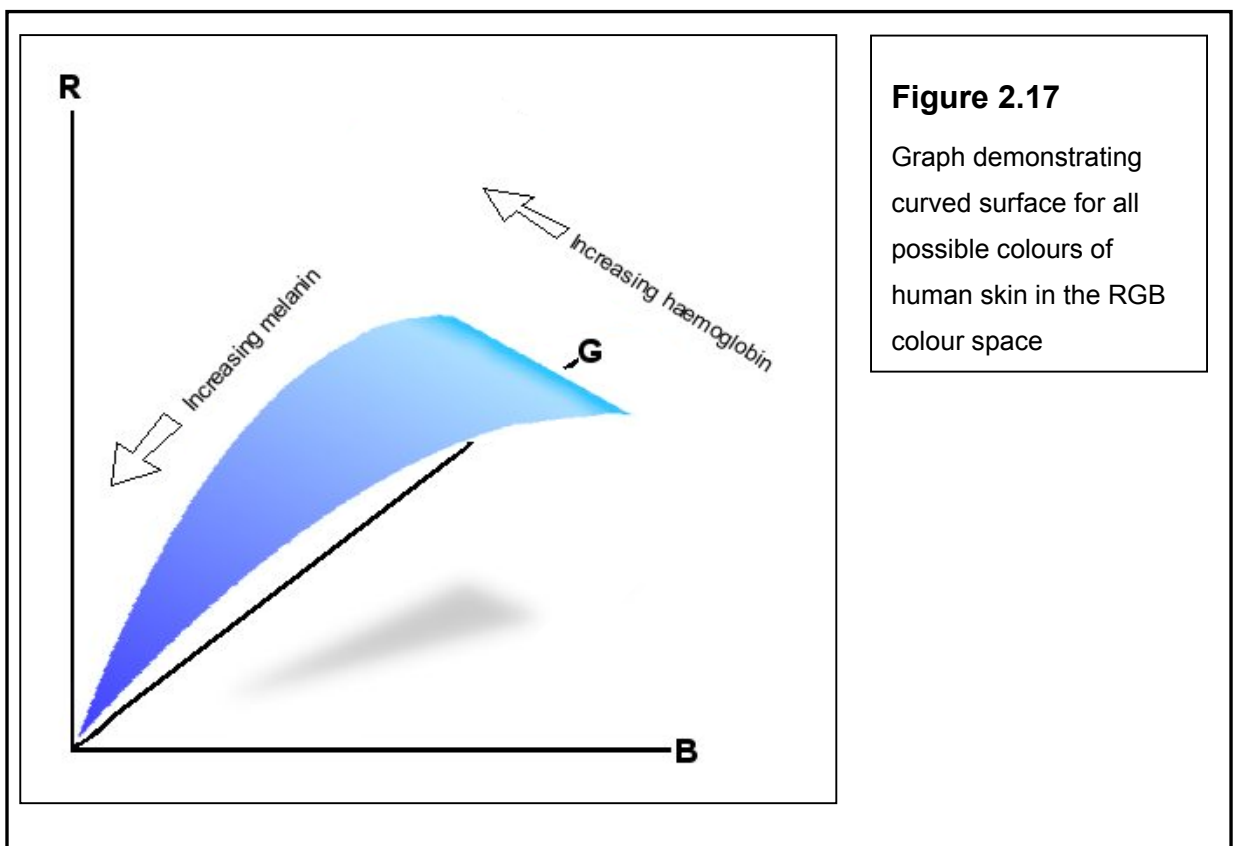
$$r = \int_0^{\infty} R(\lambda, d, \alpha, \zeta) I_0(\lambda) S_r(\lambda) d(\lambda) \quad (6)$$

$$g = \int_0^{\infty} R(\lambda, d, \alpha, \zeta) I_0(\lambda) S_g(\lambda) d(\lambda) \quad (7)$$

$$b = \int_0^{\infty} R(\lambda, d, \alpha, \zeta) I_0(\lambda) S_b(\lambda) d(\lambda) \quad (8)$$

This model will generate skin colouration in terms of RGB primaries but could also be adapted for LMS primaries instead or, indeed, any other set of primaries. In addition, other variables such as oxy/deoxyhaemoglobin and bilirubin concentrations can be factored into the equation so that a comprehensive model of light interaction – transmittance and remittance - is produced.

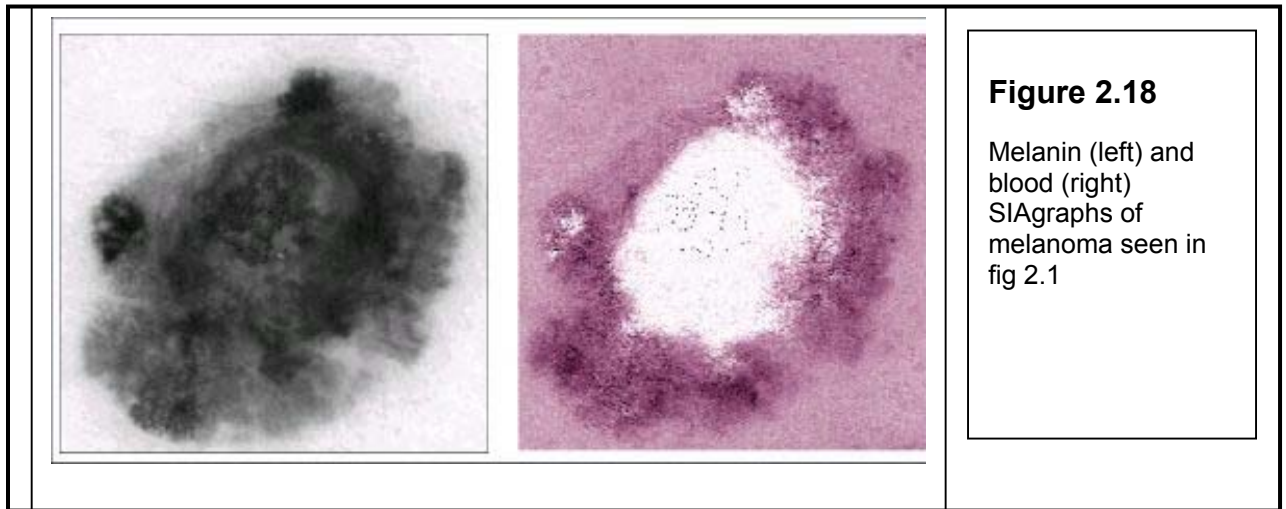
To summarise, a mathematical model can be produced, based on the Kubelka-Munk theory, which predicts light interaction as it is transmitted through the epidermis with some wavelength-specific absorption by melanin. Next the model predicts the wavelength-specific absorption by chromophores, namely haemoglobin and bilirubin, and the wavelength-specific remittance by collagen in the papillary dermis. Finally, the model predicts the absorption of the remitted light from the dermis on its passage through the epidermis. However, the scattering of light in the epidermis is negligible and here the Kubelka-Munk theory is a limited case that reduces to Bouguer's law (equation 1) – as a result all calculations from this model are relative to the dermo-epidermal junction. This point is particularly important when discussing images pertaining to the dermal components of the skin (section 2.6.3.3).



The next intuitive step is to plot the values obtained for the three primaries by varying the physical parameters of the skin, namely epidermal melanin and dermal blood. The resulting graph describes a curved surface that maps all possible colours of normal human skin (fig 2.17).

As shown in the figure, the plane has a (epidermal) melanin axis and a (dermal) blood axis as its bounds. Importantly, this curved plane represents a grid-like surface with each point

uniquely identifying these two parameters and this allows the transformation of a three-dimensional colour space to a two dimensional feature-space so that normal skin colour within

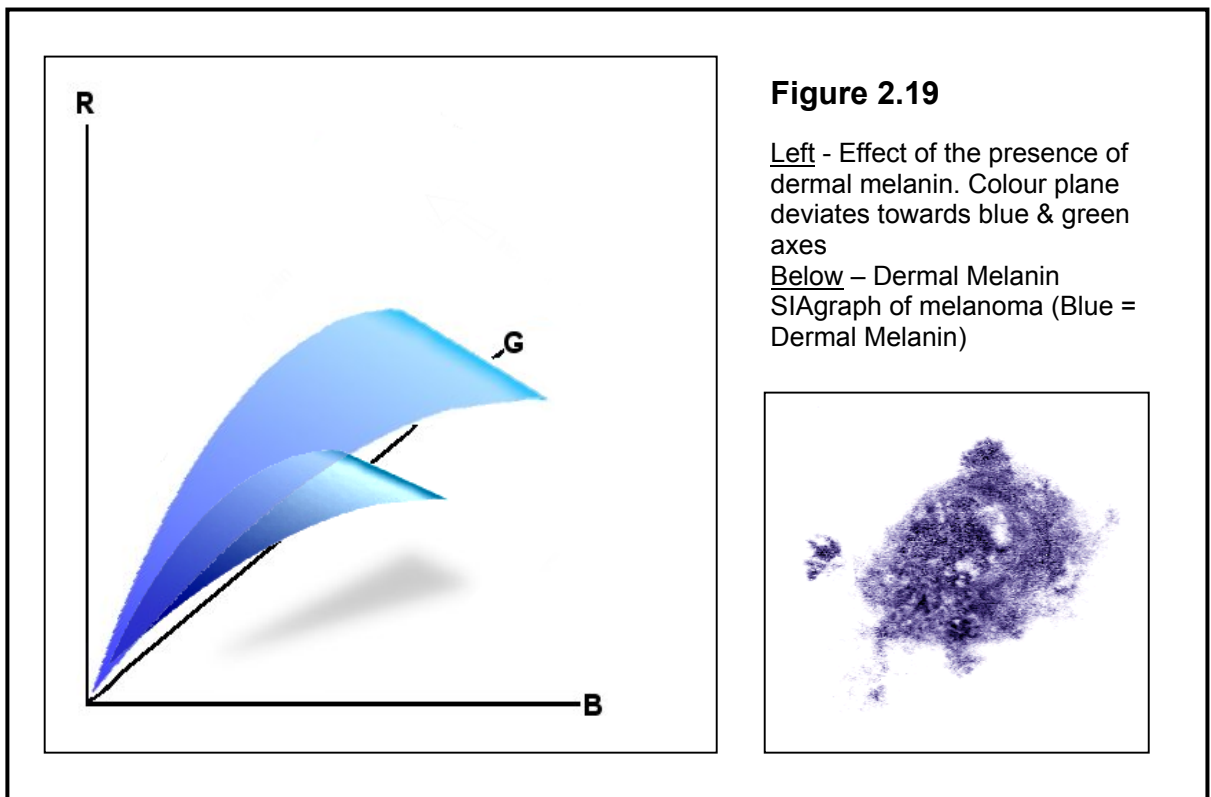


an image can be transformed into separate images showing the amount of blood and melanin content (fig 2.18).

2.6.3.3 Pathological conditions – dermal melanin and collagen loss

As was discussed in the previous chapter, invasive melanomas and blue naevi often have a blue colouration due to the presence of melanocytes, or, more accurately the melanin they contain, in the papillary dermis. This perturbation in the normal colouration of skin is reflected in the skin model introduced in the previous sections. In figure 2.19 it can be seen that the presence of dermal melanin produces an ‘abnormal’ plane that deviates off towards the blue and green primaries. In addition, this ‘abnormal’ plane has no intersection with the plane of normal skin colouration allowing a transformation into a two-dimensional feature-space that can be represented as an image showing areas of ‘abnormal’ skin. In the presence, of epidermal melanin points in the skin model become metameric and as a result it is impossible to quantify the contribution of each component to the overall colouration of the skin though it is possible to non-specifically state that ‘an amount’ of dermal melanin is present. Therefore, areas where melanin exists in the dermis can be identified as those that do not lie on the planar surface of normal skin colouration and this allows the production of an image segmented in a binary fashion into regions with and regions without dermal melanin. (figure 2.19)

Initial attempts to analyse colour images alone for the presence of dermal melanin failed [Cotton, 1998]. While the skin model identified early invasive melanomas quite effectively other benign lesions, most notably seborrheic keratoses, were being identified as containing



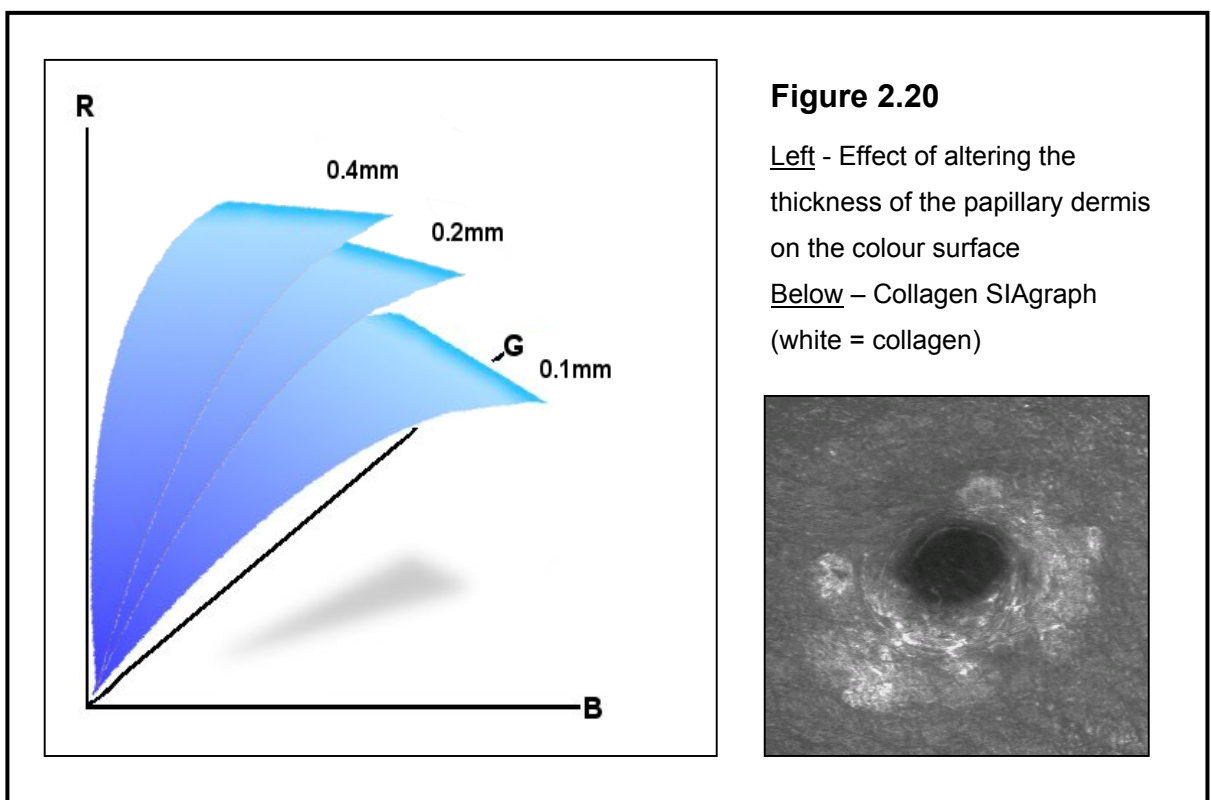
dermal melanin throughout their entirety where this couldn't be the case. The reason for this was that the initial skin model took the parameter of papillary dermal thickness as a constant 200 μ m when, as discussed in the previous chapter, this parameter varies with various lesions (table 1.1). Collagen remits light according to wavelength and thickness such that all blue light is remitted at most thicknesses but more red light is transmitted than remitted the thinner the dermal collagen layer becomes. This means that skin composed of a thin papillary dermis is darker and bluer and the skin composed of a thick papillary dermis is brighter and redder. This fact is also demonstrated by the skin model (figure 2.20) where the colour surface for normal skin descends towards the blue primary for decreasing papillary dermal thickness.

In order to measure the thickness of the papillary dermis it is necessary to extend the model into the infrared. At 600-800nm the absorption of melanin drops to less than one tenth and one hundredth its maximum in the visible spectrum respectively and, at the same time, the remittance of light becomes increasingly sensitive to papillary dermal thickness. At wavelengths between 800-1000nm absorption becomes an order of magnitude less so that papillary dermal thickness is the most significant factor influencing skin colour [Cotton, 1998]. When the remitted values at these primary wavelengths are plotted for varying papillary

dermal thickness a graph is obtained that allows non-invasive measurement of papillary dermal thickness that relates directly to its collagen content. Obtaining this measurement has two desired effects. First it is now possible to transform all values obtained from analysing a lesion to a standardised normal colour skin surface removing the metamerism effect that ruined the initial experiment above. Second this provides another useful parameter in describing the architecture of a lesion and this information can be presented to the clinician as an image. (figure 2.20)

2.6.3.4 The SIAscope

The SIAscope is a device that performs SIA (figure 2.20, <http://www.astronclinica.com>). Light is passed through band-pass filters, through a handset that is in contact with the skin surface



and into the region of skin that is of interest. The light that is remitted is captured using a dedicated charged couple device (CCD) and the signal is then digitised. This information is passed to a computer that runs software that performs the task of SIA. In essence the algorithm takes the two infrared and one RGB measurement for each pixel and compares these values to the skin model to produce four 'SIAgraphs' that are pixel maps depicting the following features: papillary collagen thickness, papillary dermal blood content, total melanin content and papillary dermal melanin content. Dedicated software displays the SIAgraphs on a monitor screen and the whole process takes approximately 15 seconds to perform.

2.7 Summary

This chapter as a continuation of chapter one, described the current situation with regards to clinical diagnosis of cutaneous malignant melanoma (CMM). Clinicians face a difficult task in diagnosing CMM, as it can appear in many different forms especially those that appear similar to benign lesions. Conversely, the clinician also has to contend with the possibility that the lesion they are dealing with is benign though clinically it appears suspicious. Despite history, examination, checklists, mnemonics and several years' clinical experience, it is generally accepted that the diagnostic accuracy of a physician, in terms of specificity and sensitivity, is could be improved (section 2.2.3). This can result in a significant proportion of patients with benign lesions being subjected, unnecessarily, to excision biopsy with the risks that may be incurred by this procedure. Even worse, clinicians risk missing early cutaneous melanoma, when the prognosis for the disease is excellent. Screening and public education may heighten the awareness of the public but it is disputed whether these approaches increase the number of thin melanomas detected whilst it is agreed that they do add to the workload of the clinician [Melia, 1995b].

As a result, diagnostic techniques are being pioneered that assist the clinician in the assessment of pigmented skin lesions. The most commonly applied technique is skin surface microscopy that allows visualisation of the dermo-epidermal junction (section 2.3). To its advantage is the ability to perform this quickly and easily using inexpensive equipment without discomfort to the patient. A systematic review of this technique [Mayer, 1997] found that skin surface microscopy might improve diagnostic accuracy of equivocal lesions when performed by trained clinicians. However, the technique does have shortcomings including the lack of training available and the reliance on the identification of subtle features to make a diagnosis. Other techniques that were discussed (section 2.4) may have a role to play in the diagnosis of melanoma but suffer from some or all of the following problems: lack of resolution, skilled operator dependency, expense, availability and inaccessibility to certain body regions. Humans assess pigmented lesions in a subjective manner and computer image analysis may reduce this error by performing an objective assessment (section 2.5). Artificial intelligence algorithms may be applied to this data though it is not apparent to the operator how the system arrived at that decision.

Spectrophotometric intracutaneous analysis is a new technique that employs a predictive skin model to analyse light remitted from the skin in the visible and infrared spectra and produce images of the constituent chromophores of the epidermis and papillary dermis, namely collagen, haemoglobin and melanin. This technique then presents the clinician with two-dimensional images of these constituents that enable the assessment of the tertiary structure

of the lesion. This thesis will describe the use of SIA in the clinical assessment of pigmented skin lesions and will identify new features that indicate and represent underlying histopathological processes. The hypothesis of this thesis is that these features identified using Spectrophotometric Intracutaneous Analysis can be used in predictive models to diagnose the presence of cutaneous malignant melanoma with a high specificity and sensitivity.

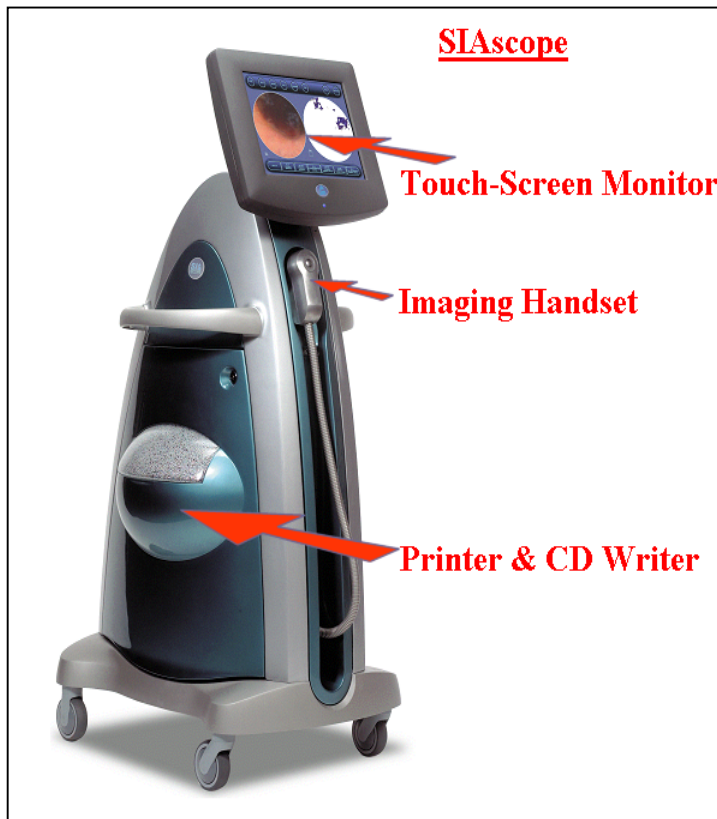


Figure 2.21

Picture of a SIAscope with the salient features indicated. The system measures approximately five feet in height (150 cm)

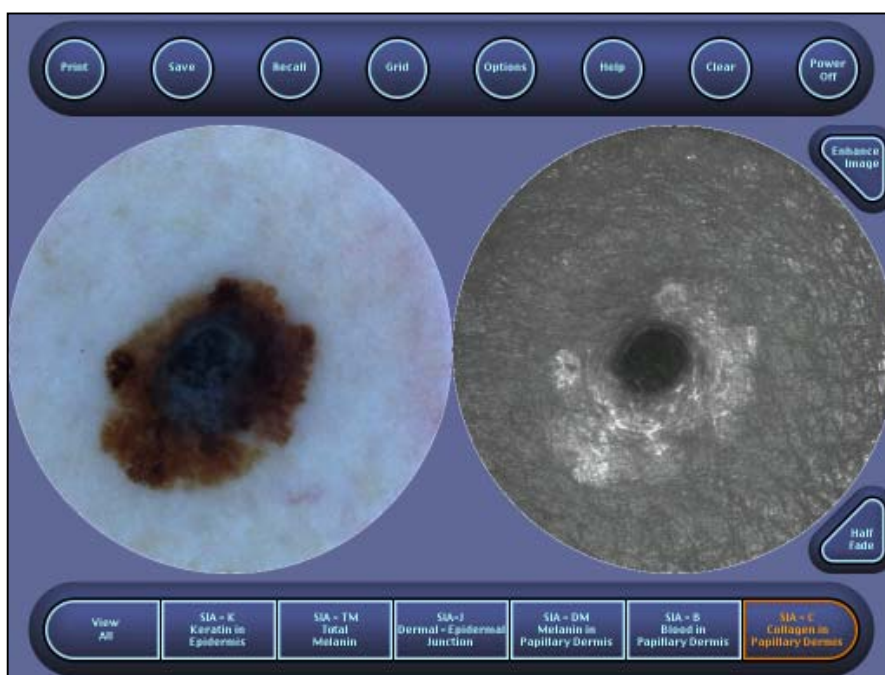


Figure 2.22

Screen shot from the SIAscope of a malignant melanoma (colour view & collagen SIAgraph)