Methods of Melanoma Detection

Sancy A. Leachman, Pamela B. Cassidy, Suephy C. Chen, Clara Curiel, Alan Geller, Daniel Gareau, Giovanni Pellacani, James M. Grichnik, Josep Malvehy, Jeffrey North, Steven L. Jacques, Tracy Petrie, Susana Puig, Susan M. Swetter, Susan Tofte and Martin A. Weinstock

Abstract

Detection and removal of melanoma, before it has metastasized, dramatically improves prognosis and survival. The purpose of this chapter is to (1) summarize current methods of melanoma detection and (2) review state-of-the-art detection methods and technologies that have the potential to reduce melanoma mortality. Current strategies for the detection of melanoma range from population-based educational campaigns and screening to the use of algorithm-driven imaging technologies and performance of assays that identify markers of transformation.

S.A. Leachman (✉)
Department of Dermatology and Knight Cancer Institute, Oregon Health and Science University, 3303 SW Bond Avenue, CH16D, Portland, OR 97239, USA
e-mail: leachmas@ohsu.edu

P.B. Cassidy
Department of Dermatology and Knight Cancer Institute, Oregon Health and Science University, 3125 SW Sam Jackson Park Road, L468R, Portland, OR 97239, USA
e-mail: cassidyp@ohsu.edu

S.C. Chen
Department of Dermatology, Emory University School of Medicine, 1525 Clifton Road NE, 1st Floor, Atlanta, GA 30322, USA
e-mail: schen2@emory.edu

C. Curiel
Department of Dermatology and Arizona Cancer Center, University of Arizona, 1515 N Campbell Avenue, Tucson, AZ 85721, USA
e-mail: ccuriel@email.arizona.edu

A. Geller
Department of Dermatology, Harvard School of Public Health and Massachusetts General Hospital, Landmark Center, 401 Park Drive, 3rd Floor East, Boston, MA 02215, USA
e-mail: ageller@hsph.harvard.edu

H.L. Kaufman and J.M. Mehnert (eds.), *Melanoma*,
Cancer Treatment and Research 167, DOI 10.1007/978-3-319-22539-5_3

This chapter will begin by describing state-of-the-art methods for educating and increasing awareness of at-risk individuals and for performing comprehensive screening examinations. Standard and advanced photographic methods designed to improve reliability and reproducibility of the clinical examination will also be reviewed. Devices that magnify and/or enhance malignant features of individual melanocytic lesions (and algorithms that are available to interpret the results obtained from these devices) will be compared and contrasted. In vivo confocal microscopy and other cellular-level in vivo technologies will be compared to traditional tissue biopsy, and the role of a noninvasive "optical biopsy" in the clinical setting will be discussed. Finally, cellular and molecular methods that have been applied to the diagnosis of melanoma, such as comparative genomic hybridization (CGH), fluorescent in situ hybridization (FISH), and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), will be discussed.

D. Gareau
Laboratory of Investigative Dermatology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
e-mail: daniel.gareau@rockefeller.edu

G. Pellacani
Department of Dermatology, University of Modena and Reggio Emilia, Via del Pozzo 71, Modena, Italy
e-mail: giovanni.pellacani@unimore.it

J.M. Grichnik
Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Room 912, BRB (R-125), 1501 NW 10th Avenue, Miami, FL 33136, USA
e-mail: grichnik@miami.edu

J. Malvehy · S. Puig
Melanoma Unit, Dermatology Department, Hospital Clinic Barcelona, Villarroel 170, 08036 Barcelona, Spain
e-mail: jmalvehy@clinic.ub.es

S. Puig
e-mail: spuig@clinic.ub.es

J. North
University of California, San Francisco, 1701 Divisadero Street, Suite 280, San Francisco, CA 94115, USA
e-mail: jeffrey.north@ucsf.edu

S.L. Jacques
Department of Biomedical Engineering and Dermatology, Oregon Health and Science University, 3303 SW Bond Avenue, CH13B, Portland, OR 97239, USA
e-mail: jacquess@ohsu.edu

Keywords

Melanoma detection · In vivo confocal microscopy · Hyperspectral imaging · Melanoma screening · Dermoscopy · Gene expression profiling · Optical coherence tomography · Melanoma prevention · Atypical nevi · Melanoma digital photography · Optical biopsy · Quantitative real-time PCR (qRT-PCR) · Fluorescence in situ hybridization (FISH)

Contents

1 Background 54
2 Population-Level Approaches to Early Detection 55
2.1 The Role of Public Health Campaigns in the Early Detection of Melanoma 55
2.2 Effects of Skin Self-examination and Clinician Skin Examination on Early Melanoma Detection 58
3 The Role of Clinical Examination-Based Detection Methods for Melanoma 61
3.1 The Comprehensive Skin Examination 61
3.2 Use of Longitudinal Photography for Early Detection of Melanoma 62
3.3 Use of Dermoscopy as an Adjunct to Skin Examination 66
4 Advanced Technological Methods for Melanoma Detection 72
4.1 Beyond Visual Dermoscopy: Machine Vision in Melanoma Detection 72
4.2 In Vivo Confocal Microscopy 76
4.3 Optical Coherence Tomography 82
4.4 Electrical Impedance and Ultrasound Detection Technologies 85
4.5 Molecular Assays for the Detection of Melanoma 90
4.6 Fluorescent In Situ Hybridization (FISH) 91
4.7 Comparative Genomic Hybridization (CGH) 92

T. Petrie
Department of Biomedical Engineering, Oregon Health and Science University,
3303 SW Bond Avenue, CH13B, Portland, OR 97239, USA
e-mail: petrie@ohsu.edu

S.M. Swetter
Department of Dermatology/Cutaneous Oncology, Stanford University, 900 Blake Wilbur Drive, W3045, Stanford, CA 94305, USA
e-mail: sswetter@stanford.edu

S. Tofte
Department of Dermatology, Oregon Health and Science University, 3303 SW Bond Avenue, CH16D, Portland, OR 97239, USA
e-mail: toftes@ohsu.edu

M.A. Weinstock
Departments of Dermatology and Epidemiology, Brown University,
V A Medical Center 111D, 830 Chalkstone Avenue, Providence, RI 02908, USA
e-mail: maw@brown.edu

4.8 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and Next-Generation Sequencing 93
5 Conclusion 95
References 95

1 Background

Incidence and mortality rates for melanoma have increased steadily since the 1900s. Melanoma estimates for 2014 in the US include 76,100 new invasive and 63,770 new in situ cases, along with 9710 anticipated deaths [190]. The probability of developing melanoma from birth to death is now estimated to be 1 in 34 in men and 1 in 53 in women [190]. In the latest version of the AJCC staging guidelines [25], a total of 38,918 cases of melanoma are staged: 18,370 (47.2 %) Stage I; 9269 (23.8 %) Stage II; 3307 (8.5 %) Stage III; and 7972 (20.5 %) Stage IV. The 5- and 10-year survival rates decrease with advancing stage. For example, 10-year survival rates for localized (Stages I and II) melanoma range from 93 % for Stage IA to 39 % for Stage IIC. Reported ten-year survival rates for regionally metastatic (Stage III) disease range from 68 % for Stage IIIA to 24 % for Stage IIIC, and the 10-year survival rate for Stage IV disease is only 10–15 % [25]. It is not possible to determine from these data how many lives might have been saved if patients with metastatic disease had been detected at Stage IA. However, a "back of the envelope" estimate would suggest that of the almost 8000 Stage IV patients diagnosed, only about 10 % (800) would be predicted to be alive at 10 years. If 50 % of these 8000 patients had been diagnosed earlier with Stage IA disease, the estimate of living patients at 10 years would be increased to approximately 4120 (4000 patients X 10 % + 4000 X 93 %), potentially saving 3320 lives. This rough estimate is only intended to be illustrative of the concept that ***early detection of melanoma has the potential to dramatically reduce death due to this disease***.

In addition to mortality increases, the cost of treatment of melanoma increases dramatically with the stage of disease [104]. Six independent studies of the cost of melanoma treatment in the US conclude that the direct cost of melanoma care increases with increasing stage of disease [11, 57, 108, 188, 201, 213]. A summary of annual per-patient medical costs for melanoma in 2010 US dollar equivalents published by Guy et al. demonstrated a range from $2169 to $31,032/year to treat local disease, $31,778 to $69,006/year to treat regionally metastatic disease, and $34,103 to $152,244/year to treat distant metastatic disease [104]. These numbers do not take into consideration the escalation of cost for metastatic disease in the current era of targeted immunotherapies, which cost approximately $60,000–$120,000 per course of therapy for the drug alone, and do not include the costs of administration and management of side effects or associated hospitalization costs. Detection of melanoma at the earliest stages has the potential for substantial reduction in healthcare costs.

The capacity of early melanoma detection to save lives and dollars will depend on the application of a variety of detection methods. In this chapter, a full spectrum of detection methods will be reviewed, including (1) population and public health approaches, (2) skin cancer screening and self-skin examination approaches, (3) photographic methods,

(4) dermoscopic methods, (5) spectral imaging methods, (6) in vivo confocal microscopy methods, (7) optical coherence tomography methods, (8) electrical impedance and ultrasound detection methods, and (9) molecular methods to improve the diagnosis of melanoma. Each detection method has costs and opportunities associated with application to patient care, and the ultimate goal is to optimize the use of each method in a context that will provide the greatest reduction of melanoma-related death at the lowest cost.

2 Population-Level Approaches to Early Detection

2.1 The Role of Public Health Campaigns in the Early Detection of Melanoma

Screening for melanoma generally occurs in two venues—clinic-based (performed by dermatologists or primary care physicians) and mass screening, often led by the American Academy of Dermatology [83, 85] or similar organizations such as Euromelanoma in Europe [196]. A third form of melanoma screening that has recently emerged combines screening and educational awareness, including publicity on behavioral risks and training of physicians in behavior modification skills, and takes place in a given state or workplace. These population-based programs bear some resemblance to large-scale, statewide public health efforts to reduce rates of smoking in Massachusetts and California, which led to dramatic decreases in smoking rates and concomitant reduction of tobacco-related disease [122, 176].

American Academy of Dermatology Mass Screening Programs

The American Academy of Dermatology has conducted free skin cancer screening programs since 1985. Although screenees that are identified as possibly having a melanoma are not frequently followed up for histologic outcome, among the 242,374 skin cancer screenings conducted during the period 1992–1994, 363 screenees had histologically proven melanoma. Middle-aged and older men (age ≥50 years) comprised only 25 % of persons screened but comprised 44 % of those with a confirmed diagnosis of melanoma. The overall yield of melanoma (the number of confirmed diagnoses per the number of screenees) was 1.5 per 1000 screenings compared with a yield of 2.6 per 1000 screenings among men age ≥50 years. The yield was improved further for men age ≥50 years who reported either a changing mole (4.6 per 1000 screenings) or skin types I and II (3.8 per 1000 screenings) [83, 85]. The authors of AAD studies and those from the Euromelanoma screening programs agreed that the yield of mass screening for melanoma would be improved by outreach to middle-aged and older men, with particular focus on men with changing moles or with skin types I and II. Messages to primary care physicians illustrated that they should be attuned to the risk factors among all of their patients but should be alerted in particular to the heightened risk of melanoma for men age ≥50 years.

Training of Primary Care Physicians

Given that most Americans will not see a dermatologist during their lifetime but make frequent visits to primary care physicians (PCPs), it is necessary to train PCPs

in minimal triage of at-risk individuals requiring further expert dermatologist consultation. To this end, Eide et al. developed a 1- to 2-h interactive, Web-based course in skin cancer detection for practicing board-certified PCPs (http://www.skinsight.com/info/for_professionals/dermatology-education-resources) and evaluated its use and success with 54 PCPs at 2 US sites using pretests, immediate tests, and 6 month posttests [63].

The mean score for appropriate diagnosis and management increased from 36.1 to 46.7 % (odds ratio (OR) 1.6; 95 % confidence interval (CI), 1.4–1.9), with strongest improvement found for benign lesions from 32.1 to 46.3 % (OR 1.9; 95 % CI, 1.6–2.4). Dermatology referrals for suspicious lesions or new visits by participants' patients decreased at both sites after the course (from 630 to 607 and from 726 to 266, respectively) [63]. Ongoing efforts are underway to train medical students in the basic elements of the skin cancer screening examination [82].

Status of Major Skin Cancer Screening Efforts

In 2009, the United States Preventive Services Task Force (USPSTF), utilizing studies through 2006, concluded that the current evidence for skin cancer screening is insufficient to assess the balance of benefits and harms of the service. Since 2006, two major non-randomized studies have demonstrated the potential benefit of screening and education. At the same time, the USPSTF has given greater attention to a balance of evidence from observational studies and randomized studies rather than to the latter alone. Mounting evidence of the benefit to harm ratio is of crucial importance as preventive services that have a rating of A (high) or B (moderate) from the USPSTF will be relevant to the application of the Affordable Care Act [186].

In response to apparently high rates of melanoma at the Lawrence Livermore National Laboratory (LLNL), Schneider et al. designed an educational campaign to promote self-examination and targeted screening. This first non-randomized study monitored thickness and crude incidence of melanomas detected during three phases of increasing melanoma surveillance. These periods were as follows: (1) pre-awareness (1969–1975), (2) early awareness of increased melanoma risk (1976–1984), and (3) screening program (1984–1996). Crude incidence of melanomas thicker than 0.75 mm decreased during the 3 periods from 22.1 to 15.13 to 4.62 cases per 100,000 person-years ($p = 0.001$ by chi-square for trend) with the larger decrease from the active screening program. No eligible melanoma deaths occurred among LLNL employees during the screening period, whereas the expected number of deaths was calculated to be 3.39 deaths ($p = 0.034$) [182].

In the second non-randomized study, fueled by a large public awareness initiative, more than 360,000 residents of Schleswig-Holstein (a northern state in Germany) ages 20 and above received full-body skin examinations from dermatologists and trained PCPs. PCPs were trained in mandatory 8-hour programs and reimbursed the equivalent of $25 for screening and recording of the skin cancer examination. Twenty-seven percent of female and 10 % of male residents received screenings between July 1, 2003, and June 30, 2004. Incidence and mortality rates

for Schleswig-Holstein and adjacent regions were compared for the period 2000–2009, encompassing a period prior to screening, during screening, and post-screening. Incidence rates were greater as recorded in the Cancer Registry of Schleswig-Holstein than Saarland (control state), and mortality rates dropped an estimated 45 % for men and women, while adjacent areas such as Denmark and the rest of Germany experienced little change during this 10-year period [64, 118, 205].

On the heels of this statewide effort, a nationwide screening program with no preceding educational campaign is currently taking place in Germany. Reports indicate that more than 75 % of primary care physicians have received the same 8-h training program and more than 30 million screenings have taken place (2008–2013). Comparisons in incidence and mortality rates between Germany and their nine adjacent countries are being planned.

Potential Harms of Skin Cancer Screening

While demonstrating screening-associated reductions in mortality is paramount, the USPSTF is also interested in assessing the potential harms associated with melanoma screening. Such harms may include pre- or post-screening anxiety, embarrassment encountered during screening, unnecessary excisions, costs, and scarring associated with biopsies and excisions. Future research should seek to assess harms from skin cancer screening in large-scale efforts such as the nationwide campaign in Germany or in healthcare system-led efforts currently underway at the University of Pittsburgh Medical Center.

With respect to data on screening anxiety, a study of 324 patients undergoing investigation of a suspicious skin lesion in the UK at a Pigmented Lesion Clinic consented to complete a baseline and 6-month survey. Using recognized cutoff scores, 27 % of women reported clinically high levels of anxiety at the time of clinic arrival, in comparison with 10 % of men ($p < 0.0001$). Patients given an immediate benign post-clinical diagnosis reported a reduction in anxiety ($p < 0.0001$), but patients requiring a biopsy reported elevated levels of anxiety. Approximately 30 % of these biopsy patients reported clinically high levels of anxiety both before and after diagnosis [8].

Expanding Population-Based Approaches to High-Risk States

Statewide efforts in the US and elsewhere are needed to replicate findings from Schleswig-Holstein. In particular, in states with high melanoma mortality rates, prescreening campaigns could be launched to promote public awareness of the importance of skin self-examination and physician examination. Cancer registry and vital statistics data can be utilized to compare states with high screening penetration versus unscreened states, and routinely administered surveys such as the Behavioral Risk Factor Surveillance System can measure awareness, exposure to screening information, intentions to screen, self-efficacy for skin cancer screening, and actual practice of the skin self-examination and receipt of a skin cancer screening. Additionally, cost studies should be incorporated.

2.2 Effects of Skin Self-examination and Clinician Skin Examination on Early Melanoma Detection

Multiple studies support the value of early detection of melanoma through skin self-examination (SSE) and clinician skin examination, though evidence that this translates into reduced population-based melanoma mortality has thus far been insufficient for the USPSTF to recommend skin screening as part of primary care practice. Ample data suggest that melanomas detected by clinicians through directed skin examinations or during the course of routine physical examinations (e.g., "opportunistic screening") are thinner than those found by patients or their significant others [5, 66, 116, 125, 197]. In an analysis of 9 worldwide studies of over 7500 patients, a mean decreased tumor thickness of 0.55 mm was found when comparing melanomas initially detected by physicians versus by patients or significant others [200]. Thus, peer-reviewed data and observational evidence support the efficacy of SSE in detecting thinner melanomas and reducing mortality.

Skin Self-Examination

In 1996, Berwick et al. [32] reported a 63 % reduction in lethal or advanced melanoma associated with SSE in a population-based, case-control study of Connecticut residents. The mean thickness of melanomas was reduced, though not significantly, in the 15 % of patients who performed SSE, compared with those who did not (OR 0.58; 95 % CI, 0.31–1.11). Subsequent analysis of the study population at a median of 5.4 years demonstrated lower risk of death from melanoma in patients with increased skin screening practices (inferred from a combination of skin awareness, SSE, and physician skin examination), although reported SSE itself was not associated with reduced melanoma mortality [31]. A 2003 study found that regular performance of SSE was associated with a significantly reduced likelihood of melanomas >1 mm at diagnosis (covariate-adjusted OR 0.65; 95 % CI, 0.45–0.93), although details regarding the thoroughness and frequency of SSE were not reported [47].

Improved understanding of the effectiveness of SSE has been hindered by variable study definitions of SSE, including the number or percent of body sites examined and the frequency and method of examination [152, 209] and the small number of studies examining the reported benefits of techniques such as the use of photographs to supplement SSE [153]. In a study of 321 recently diagnosed cutaneous melanoma patients, Pollitt et al. [163] showed that the thoroughness of SSE, as measured by the number of body sites examined and use of a picture aid illustrating a melanoma, was the best predictor of reduced melanoma thickness, with thinner tumors observed in patients who frequently examined at least some of their skin in the year prior to melanoma diagnosis (OR 2.66; 95 % CI, 1.48–4.80). The effect of SSE was even greater in men and in older patients (>60 years).

Despite the potential benefit of self-inspection of the skin for early melanoma detection, the prevalence of SSE in the general population is low. It is estimated that only 10–25 % of individuals in the US practice regular, thorough SSE [210]. Effective self-identification of melanoma is dependent on several factors, including

increased awareness and knowledge of SSE practices, health provider teaching of SSE to patients, and consistent performance of SSE by patients [53]. The American Cancer Society recommends thorough SSE of all body areas, including the back, back of the legs, and scalp [192] areas that are typically difficult to self-inspect. While the USPSTF described insufficient evidence to recommend SSE for the general population in its 2009 report, the potential benefit in high-risk groups such as older men was noted [186].

However, a population-based telephone survey in Queensland, Australia, demonstrated that only 20 % of men 50 years or older examined the skin of their whole body at least once in the past year [6]. For higher risk populations, various educational programs have successfully increased SSE performance [153, 173, 174]. Other studies have demonstrated that patient and partner intervention with specialized information, such as using videos and telephone reminders, may increase the prevalence of SSE [113]. Other interventions, such as use of mole-mapping images during self-examination, can increase the accuracy of SSE [50].

Efforts have been made to better understand the psychosocial factors that affect skin examination behaviors in an attempt to identify mechanisms to improve compliance with skin examination recommendations. In an international Web-based survey of the general population, it was demonstrated that self- and professional skin examinations were associated with (1) a perceived risk of developing melanoma; (2) perceived benefits of, and barriers to, skin examination; and (3) perceived confidence in one's ability to engage in screening. Additionally, among those with no history of melanoma, higher cancer-related worry was associated with greater frequency of SSE [117]. Self-skin examination practices and seeking of physician skin examinations have also been evaluated in high-risk *CDKN2A/p16* mutation carrying families [21, 22, 199]. These studies confirm a relatively low baseline compliance with skin examination recommendations, despite counseling of risk. However, following provision of genetic test results and counseling, unaffected carriers demonstrated a significantly improved compliance in skin examination practices for as long as two years following the test reporting session. These data suggest that individually tailored risk messaging may improve compliance with early detection recommendations.

Clinical Skin Examination

While numerous worldwide studies have demonstrated that physician detection of melanoma is associated with thinner tumors at diagnosis [200], no randomized trials have established the efficacy of clinician screening for melanoma on mortality reduction. The 2009 USPSTF statement found insufficient evidence to recommend for or against routine skin cancer screening of the general population by primary care providers [186]. Since that time, however, evidence for improved melanoma outcomes with clinician skin screening was reported in a population-based case-control study by Aitken et al. of Queensland residents aged 20–75 years with histologically confirmed first primary invasive melanoma diagnosed between January 2000 and December 2003 [5]. This study demonstrated a 14 % lower risk of being diagnosed with a thick (>0.75 mm) melanoma following a clinician skin

examination within 3 years of diagnosis (OR 0.86; 95 % CI, 0.75–0.98). The decrease in risk was greatest for the thick melanomas (risk reduction 40 % for lesions ≥3 mm), resulting in a projected 26 % fewer melanoma deaths in screened cases versus unscreened cases within 5 years.

A subsequent US study of 566 adults with invasive melanoma assessed the role of physician skin examination in the year prior to diagnosis and found that men over age 60 appeared to benefit the most from this practice [198]. Thinner tumors (≤1 mm) were significantly associated with physician discovery ($p \leq 0.0001$), though this was reported by only 19 % of patients. However, patients who had a full-body skin examination by a physician in the year prior to diagnosis were more than twice as likely to have a thinner melanoma (OR 2.51; 95 % CI, 1.62–3.87), largely due to the effect of the physician skin examination in men >60 years, who had four times the odds of a thinner melanoma (OR 4.09; 95 % CI, 1.88–8.89).

These studies, along with the aforementioned Lawrence Livermore National Laboratory cohort time series, the German SCREEN population-based time series, and ongoing nationwide skin screening program in Germany (discussed in Sect. 2.1), suggest that integration of the skin examination into a routine physical assessment by primary care providers may be a practical strategy for reducing skin cancer mortality. Clinician skin examination should be synergistic with skin self-examination for early melanoma detection. However, the documented prevalence of annual clinician skin examination ranges from only 8–21 % and varies according to the type of health provider [7, 114, 129, 175, 180]). Studies have demonstrated that dermatologists are significantly better than non-dermatologist physicians at diagnosing melanoma [92], although most suggest that tumor thickness does not appear to substantially differ by provider type. Since Americans make an average of 1.7 visits to primary care providers each year, they can serve as an important source of skin cancer diagnosis and triage.

Indeed, most physician-detected melanomas are found by primary care providers, not dermatologists, a statistic related to national shortages in the dermatology workforce, and primary care providers perform the initial biopsy of 1.4–13 % of all melanomas [98]. Therefore, dermatologists and primary care providers must work in tandem to promote early melanoma detection. However, published data suggest that primary care providers in the US may not be adequately trained to identify early skin cancer [84, 148]. Many physicians have minimal exposure to skin cancer examination practices during medical school and residency, resulting in lack of knowledge and confidence in skin cancer diagnosis and effective patient assessment, thereby creating barriers to routine skin exams by primary care providers.

Factors that promote and/or prevent skin cancer screening among US primary care providers and dermatologists were evaluated in a survey study of >1600 randomly selected physicians [189]. More dermatologists (81 %) reported performing whole body skin examination on patients than did family practitioners (59.6 %) ($p < 0.05$) or internists (56.4 %) ($p < 0.05$). Among all physicians, time constraints, competing comorbidities, and patient embarrassment were reported as the top 3 barriers to performing full skin examinations. Factors that facilitated skin

screening among all physicians included having patients at high risk for skin cancer, patient demand for complete examination/mole check, and the influence of specialty medical training.

Effective educational and training programs are essential to increase the efficacy and implementation of comprehensive skin examinations by healthcare providers in at-risk populations. A number of Web-based educational programs have been designed for this purpose [63], including a 1.5-h Web-based, interactive training program called INFORMED (INternet-based program FOR Melanoma Early Detection), available at http://www.skinsight.com/info/for_professionals/skin-cancer-detection-informed/skin-cancer-education. As mentioned above, a recent US study evaluated the effect of INFORMED on 54 primary care providers at two integrated healthcare delivery systems on practice patterns, including referral or visits to dermatology and skin biopsies during the six months following training [63]. Scores for appropriate diagnosis and management increased from 36 % pre-training to 47 % post-training (OR 1.6; 95 % CI 1.4–1.9), with greatest improvement for benign skin lesions. Rates of dermatology utilization decreased without any change in biopsies performed or skin cancers diagnosed, suggesting that primary care provider training in skin cancer did not increase specialty referrals or over-biopsy/treatment, likely due to the improvement in diagnosis and management of benign lesions.

3 The Role of Clinical Examination-Based Detection Methods for Melanoma

3.1 The Comprehensive Skin Examination

A screening examination for skin lesions by a trained practitioner takes only a few minutes and can reveal melanomas in areas not easily viewed by the patient such as the back and posterior legs. The clinician skin examination allows for the assessment of melanoma risk factors such as fair skin phenotype/sun sensitivity, increased nevus count, and/or clinically atypical nevi. It requires few materials, namely adequate examination lighting and possible use of a magnifying lens or dermatoscope, though this latter tool requires appropriate training. The INFORMED Web-based curriculum provides clinical guidance for early detection of melanoma (as well as other common skin cancers) by primary care providers, although it is available for the lay public to use, as well.

For either self-examination or clinician examination, it is important to be systematic and thorough. One suggested physical examination procedure uses a standard sequence of “down and back” (down the anterior body, then back up the posterior). A specific algorithm for clinicians is as follows: (1) examination of the

face and rest of the head and neck while the patient is sitting on the examination table; (2) examination of the scalp, which is particularly important in men with thinning hair; (3) examination of all surfaces of the arms and hands; (4) having the patient to lie down on his/her back for viewing of the chest, abdomen, anterior thighs, anterior legs, dorsal feet, soles, and toe webs; and (5) having the patient turn over for examination of the calves, posterior thighs, buttocks, and back if this permits optimal examination over a standing position. The upper body could also be examined when the patient is sitting or standing. Additionally, an examination of the genitals should be offered to patients as part of the total body skin examination. Physician inspection of skin, especially high-risk melanoma sites (trunk for men, legs and trunk for women), should be encouraged during routine examinations, and simply looking at the back (the site of over 30 % of melanomas in men) would be a useful first step in promoting early melanoma detection. Early detection of a melanoma during a clinical examination can be lifesaving as well as a highly rewarding experience for the provider. Increasing the efficacy and implementation of clinician skin examination, skin self-examination, and targeted population screening may provide the greatest immediate impact the medical community can have on reduction of melanoma mortality.

3.2 Use of Longitudinal Photography for Early Detection of Melanoma

It is often a challenge for a clinician to distinguish an early cutaneous melanoma from an atypical but benign nevus during the clinical examination. The overlap of benign and malignant clinical features may lead to overlooking melanoma and/or excising an excessive number of benign lesions [130]. This clinical scenario applies to both unaided visual and dermoscopic examinations [120, 164].

Digital photography optimizes the monitoring of skin lesion features over time through clinical comparison with baseline and serial photographic documentation. Total body digital photography (TBDP) has been shown to be helpful in the detection of changes in shape, color, or surface eventually occurring in any lesion, and for the identification of new or regressing lesions aided by baseline and subsequent imaging sessions (Fig. 1) [26, 97, 105, 132, 172, 208]. This technique is particularly helpful in the surveillance of individuals with numerous melanocytic nevi, including but not limited to atypical mole syndrome, or other high-risk cohorts such as patients with a personal or family history of multiple cutaneous melanomas, xeroderma pigmentosum, or patients undergoing metastatic melanoma treatment with B-Raf inhibitors.

One metric of the utility of TBDP is in the benign to malignant biopsy ratio. Feit et al. [69] reported 93 lesions biopsied in 576 patients undergoing TBDP. Twenty-seven (35 %) of 77 melanocytic lesions were diagnosed as melanoma, translating to a benign to malignant ratio of 3:1. Banky et al. reported similar benign to malignant ratios using TBDP [26]. These ratios compare very favorably

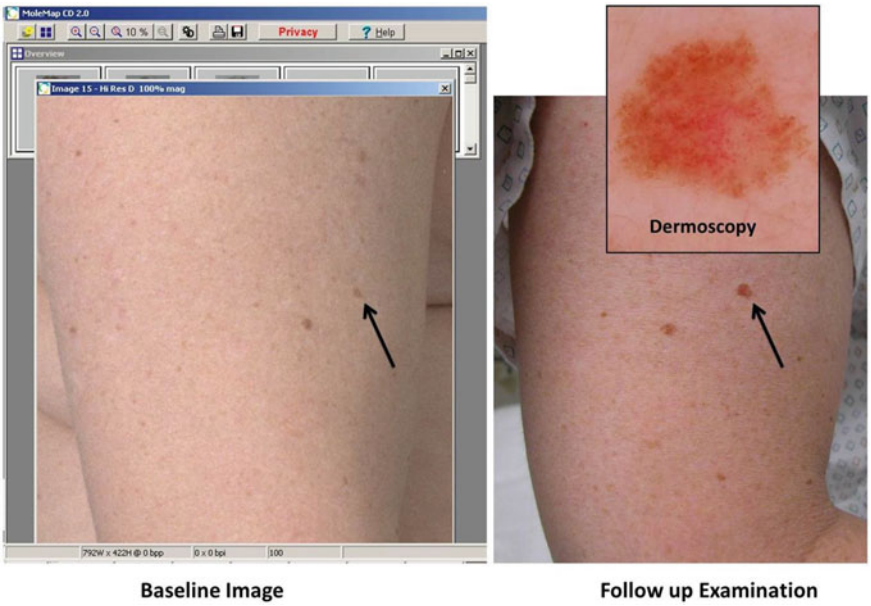


Fig. 1 Total body digital photography is helpful in detecting changes in shape, color, or surface in any lesion. Total body images are obtained for patients at high risk from melanoma, and these patients are then on follow-up. The lesion on the patient's right arm appeared distinctly different than his/her other moles. In addition, some erythema was noted on the subsequent visit (*right panel*). The follow-up dermoscopic image (*inset*) was not entirely diagnostic for melanoma. However, when compared to the baseline image (*left panel*), the lesion had clearly changed. The observed interval change increased the clinical concern for melanoma prompting a skin biopsy. Histological evaluation confirmed the diagnosis of in situ melanoma

with the ratios of 12:1 or 30:1 reported for unaided examination by dermatologists and general physicians, respectively [26]. Of note, while the benign to malignant ratio of biopsies is a useful indicator of diagnostic accuracy, it is also somewhat dependent on the patient mix seen by each individual physician (e.g., a dedicated pigmented lesion clinic in an academic dermatology department will have a different patient mix than that of a primary care provider). Another benefit to TBDP is that the melanomas detected when using this technique tend to be thinner. Banky found that 44 % of melanomas were in situ (vs. 35 % in regional controls) and that the median thickness of the invasive tumors was 0.39 mm (vs. 0.60 mm). Lastly, usage of TBDP can prevent unnecessary biopsies as well as decrease patient's worry. Hanrahan et al. conducted a randomized prospective trial evaluating the effect of photography in the hands of PCPs and found that while there was no difference in melanoma detection, fewer benign pigmented lesions, such as seborrheic keratoses, were removed when using photography [106]. Risser et al. [172] found no difference in the rate of biopsy in a pigmented lesion specialty clinic when

photographs were used, but pointed out that the benefit from TBDP most likely lies in patients who are not already "de-moled," with most of the atypical nevi removed prior to the photographs.

Sequential digital dermoscopy imaging (SDDI) involves the capture and assessment of successive dermoscopic images separated by an interval of time and can include single or multiple melanocytic lesions that warrant surveillance for suspicious changes. This imaging is performed in two settings: short-term digital monitoring (usually over a period of 3 months) for suspicious melanocytic lesions and long-term surveillance (in most instances at intervals of 6–12 months) [143]. A recent meta-analysis grouped both short- and long-term SDDI together and showed that the number of lesions needed to monitor one detected melanoma ranged from 31 to 1008 depending on the clinical setting (lower numbers of lesions were needed to find a melanoma with short-term monitoring) [179]. For every additional month of monitoring, one additional melanoma was detected, with the chances to detect a melanoma during surveillance shown to increase as the length of follow-up extended. Furthermore, the proportion in situ melanoma and thin melanomas detected by SDDI were higher than expected in the general population. Taken together, the literature suggests that SDDI allows for the detection of at least a portion of dermoscopically featureless or otherwise occult melanomas. When used in high-risk patients or on individual suspicious melanocytic lesions, SDDI demonstrates a significant clinical impact with melanomas detected exclusively using SDDI in 34–61 % of these patients (Fig. 2).

The combined use of TBDP and digital dermoscopy, also known as the "two-step method of digital follow-up," has been primarily implemented for the surveillance of patients at high risk for cutaneous melanoma [139]. This method has been proposed as a more sensitive strategy in cutaneous melanoma screening, by allowing not only the detection of dermoscopic changes over time but also detection of macroscopic changes and the occurrence of new lesions not previously identified for follow-up [178].

In the largest retrospective "two-step method" study published to date, 1152 lesions were excised during the surveillance of 779 monitored lesions in 618 patients at high risk for melanoma. A total of 98 melanomas were detected: 60 in the monitored lesions and 38 among the 373 lesions that were new or undetected on previous TBDP. The most frequent dermoscopic changes detected were asymmetric enlargement in almost 60 % ($n = 418$), focal changes in structure in 197 (27 %), and pigmentation in 122 (17 %), the latter two identified more frequently in melanomas than in nevi ($p < 0.001$). No significant differences were detected between dermoscopic and histopathological characteristics of the melanomas in each group, with a considerable proportion of melanomas misclassified as benign in both groups (26.3 and 38.3 %, respectively). Almost 40 % of cutaneous melanomas diagnosed in the study corresponded to lesions that were not under dermoscopic surveillance [177].

Challenges in the selection of lesions for SDDI include the variability in the expertise of the clinicians, the heterogeneous appearance of the lesions, and a broad

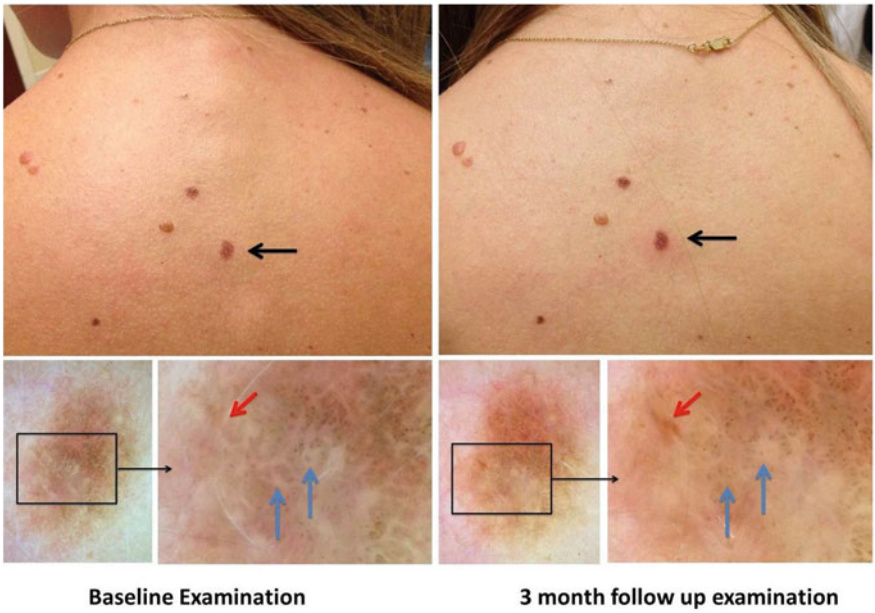


Fig. 2 Sequential digital dermoscopy imaging has significant clinical impact when used in high-risk patients or on individual suspicious melanocytic lesions. Dermoscopic monitoring may be used in limited circumstances when it is unclear whether a lesion is problematic. In this case, the lesion had negative network features but otherwise clinically appeared benign in a patient who had not noted changes and wished to avoid biopsy. At 3-month follow-up, architectural changes were noted dermoscopically. The red arrow points to a new structure, and blue arrows indicate one area where changes have occurred in the distribution of globules and negative network structures. The lesion proved to be a Breslow's 0.45-mm melanoma

range of risk of cutaneous melanoma development across high-risk cohorts. Some authors have suggested an individualized surveillance plan, with digital dermoscopy performed at follow-up intervals of 3 months for patients with familial multiple mole and melanoma (FAMMM) syndrome and 6–12 months (depending on additional risk factors) for those with atypical mole syndrome [12].

Another challenge is represented by patients' compliance. During SDDI, the risk of missing a melanoma (estimated in approximately 4 % of patients) [179] during the baseline visit should be considered relative to the benefit of a more accurate diagnosis at the follow-up examination, with consequent detriment of sensitivity at baseline compensated by higher overall specificity [119]. However, most melanomas detected during follow-up in patients with multiple nevi were false negatives in the clinical and dermoscopical examination at the first visit [178]. In this context, the lack of patient compliance should be carefully considered, since low adherence to digital dermoscopy follow-up could compromise the efficacy of this approach [17].

Given the above listed benefits of longitudinal use of TBDP and dermoscopy, one may wonder why the technology is not utilized more widely. In 2010, Rice

et al. [170] surveyed academic pigmented lesion centers and found that approximately 67 % of the 49 respondents utilized photography to monitor pigmented lesions. The rate is lower for dermatologists who do not practice in pigmented lesion centers. The primary reason for not using photography in the study was logistical constraints. There is no doubt that adjuvant diagnostic tools add more time to the already busy clinic, and they may take additional training by both the dermatologist and staff to efficiently and effectively incorporate these useful imaging modalities into the daily workflow. Incentives for providers to utilize such tools should be considered, particularly for patients who are at risk for melanoma.

3.3 Use of Dermoscopy as an Adjunct to Skin Examination

Dermoscopy, also known as dermatoscopy or epiluminescence microscopy, is a noninvasive technique that uses a dermatoscope for the diagnosis of skin tumors and other skin diseases [15, 19]. Evidence-based data of the highest level support the use of dermoscopy in the diagnosis of skin cancer, including melanoma, basal cell carcinoma, and squamous cell carcinoma [24, 121, 204]. Because of the strength of this data, most clinical guidelines in melanoma include dermoscopy as an essential tool for the examination of pigmented and non-pigmented skin tumors to detect melanoma [23, 78]. This tool is most relevant and useful in clinically equivocal cases and in the early recognition of difficult-to-recognize melanomas [20, 46, 164] (Figs. 3, 4, and 5). Dermatologists worldwide are familiar with dermoscopy, though effective application of the technique requires training [34, 154] and experience. In a prospective randomized study by Argenziano et al. [18], a 4-hour course on dermoscopy increased the ability of trainees to detect skin cancer, including melanoma, in actual clinical settings. The use of dermoscopy has more recently expanded outside the practice of dermatology and is now being incorporated into the diagnostic armamentarium of general physicians. In Australia and Europe, teaching of the method has become standard practice for primary healthcare providers. Moreover, a study in students showed a significant, positive impact in the recognition of melanoma after a short course on dermoscopy [112]. The authors of the study concluded that dermoscopy should be included in medical student education.

Basics of Dermoscopy

Dermoscopy is based on careful observation of the architecture of a selected skin lesion via the use of an optical instrument and illumination. The instrument allows the observer to examine the pigment (melanin, blood, or other) in the epidermis and dermis. With the dermatoscope, it is possible to minimize the reflection of light from the surface of the skin that would otherwise obscure the underlying structures and limit the optimal perception of colors. Two main types of dermatoscopes are available: polarized and non-polarized (depending on their capacity to integrate

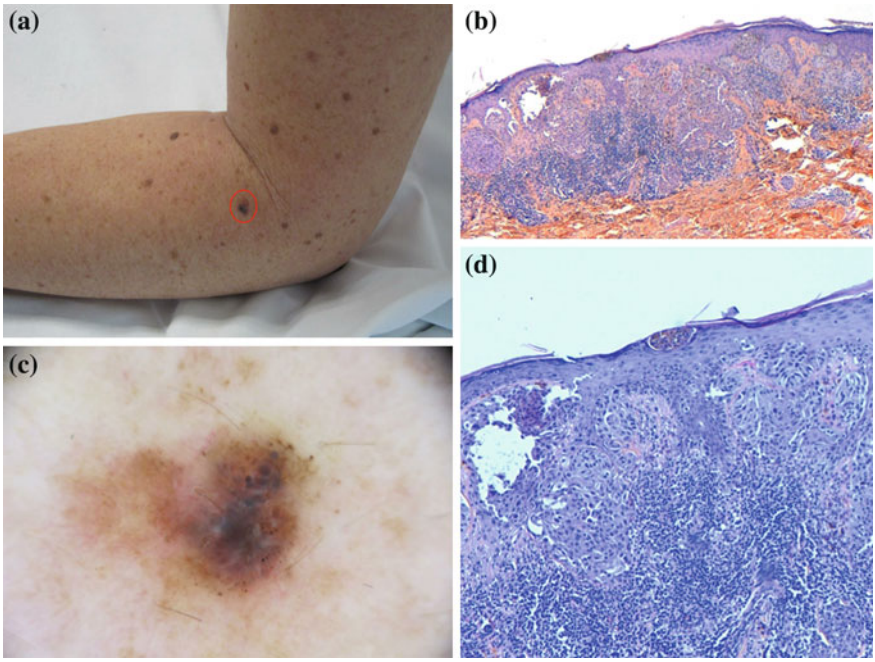


Fig. 3 Clinical, dermoscopic, and histologic images of a superficial spreading melanoma, Breslow thickness 0.9 mm. Clinical image (**a**). Dermoscopy (**c**) shows an asymmetric lesion with multiple colors (*light brown*, *dark brown*, *blue*, *black*, and *white*), global globular pattern, with presence of atypical globules, *black dots*, and *white shiny streaks*. H&E histology ×100 (**b**). The presence of pigmented melanoma nests in the upper epidermis in (**d**, H&E histology ×200) corresponds to black dots in dermoscopy

cross-polarization filters). Non-polarized dermoscopy requires direct contact with the skin and immersion liquid, including water, mineral oil, or gel. Polarized dermoscopy does not require immersion liquid, and contact is not mandatory due to the optical properties of the two polarization filters adapted in the dermatoscope. Although most features observed with polarized and non-polarized dermoscopy are similar, specific differences have been described [207].

In the case of digital dermoscopy, the optical instrument is connected to a video or photographic camera to obtain digital images that can be visualized on a computer screen. Some commercial devices have been introduced for the acquisition and storage of images through the use of dedicated software for clinical practice. With digital dermoscopy devices, follow-up comparisons of atypical lesions are possible in high-risk patients, which have the potential to improve early detection of melanoma and reduction of unnecessary biopsies, particularly in patients with multiple atypical moles [178, 179].

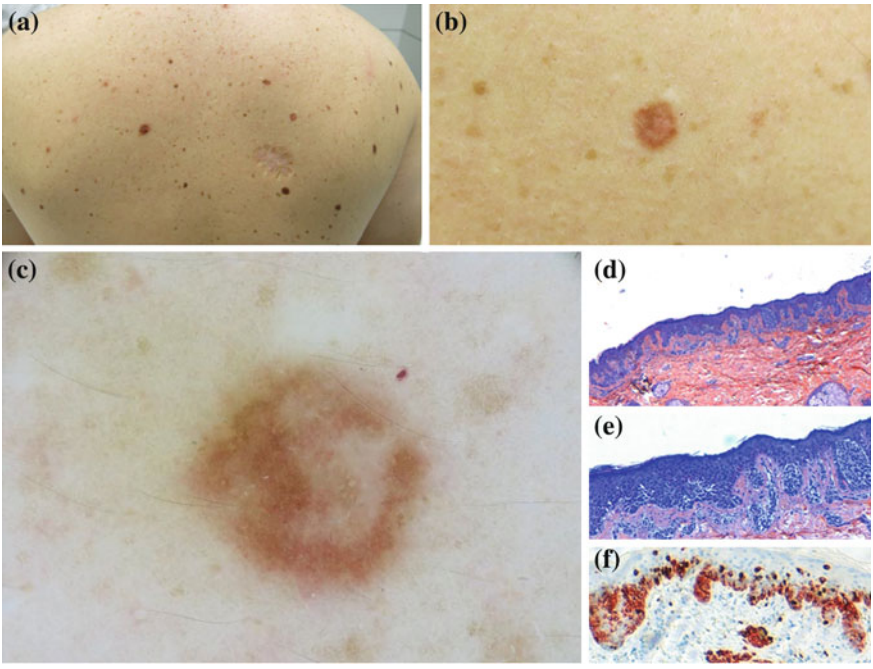


Fig. 4 Clinical, dermoscopic, and histologic images of an early invasive superficial spreading melanoma, Breslow thickness 0.4 mm located on the back. Clinical views in (**a**) and (**b**). Under dermoscopy (**c**), the lesion shows asymmetry, atypical pigment network, few globules irregularly distributed, erythema with some dotted vessels, and short white streaks. H&E histology ×40 (**d**) and ×100 (**e**). The double staining with MelanA and Ki67 shows pagetoid growth and proliferating melanocytes in the dermis (**f**)

Diagnosis with Dermoscopy

A variety of methods for the dermoscopic diagnosis of melanoma and other skin tumors have been proposed by different authors in the last 30 years [16, 33, 39, 193]. These methods use a combination of features of pigmentation, patterns, and structures of the lesion to distinguish between benign and malignant tumors. In general, melanoma exhibits more colors and structures relative to benign lesions, and these features are more likely to be asymmetrically distributed in the lesion. In order to achieve the greatest diagnostic accuracy with dermoscopy, it is essential to be aware of the histopathological correlations associated with the dermoscopic features. The presence or absence of the structures, their distribution in the lesion, and the presence of colors are strictly correlated to pathology with one main difference: Dermoscopy is the examination in the horizontal plane, whereas pathology works in the vertical section of the tissue [137]. It has been postulated that pathology and dermoscopy are particularly complementary for this reason [70]. Dermoscopy has also been effectively utilized to guide gross pathology of melanocytic lesions and for sampling of archived samples in melanoma [137, 185].

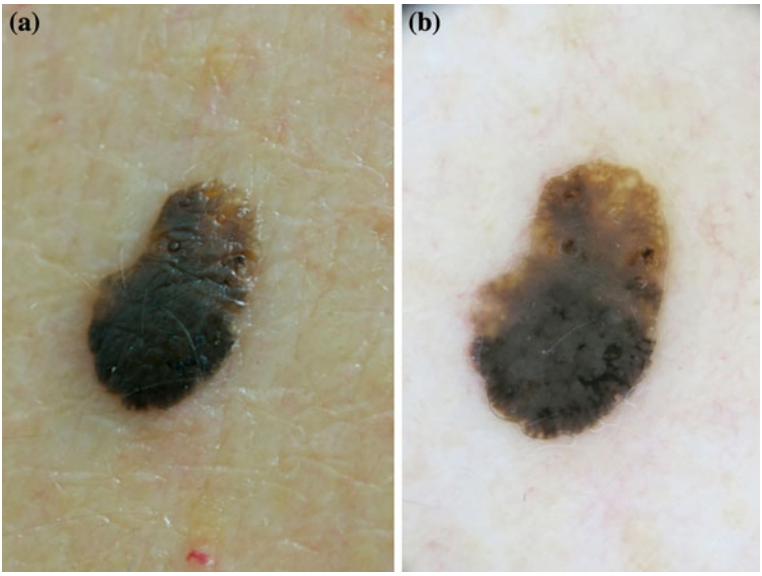


Fig. 5 Clinical and dermoscopic images of a suspicious lesion, asymmetric with black and blue colors. Clinical image (**a**). Under dermoscopy (**b**), the lesion shows abrupt cutoff of the border, presence of fat fingers at the periphery, comedolike openings, and milialike cysts, which favor a diagnosis of benign seborrheic keratosis

Dermoscopic Features in Melanoma

Specific dermoscopic features are associated with melanoma [140]. Depending on the subtype of melanoma and location on the body, different patterns can be observed. In melanomas arising in special locations such as face, acral sites, mucosa, or nails, dermoscopic patterns tend to be influenced by the special anatomy of the skin and the particular growth of the tumors. During the progression of melanoma, new features progressively appear due to the architectural disorganization and bizarre distribution of pigment and vessels associated with neovascularization. In thick tumors, asymmetry in color and structures, presence of multiple colors (blue-gray, brown, black, red, white), complex patterns (combination of many structures in the same lesion), and particular structures characteristic of melanoma are frequent. In contrast, in very early melanomas, dermoscopic features of melanoma tend to be less evident and can be similar to atypical nevi. In Table 1, we summarize the dermoscopic criteria for melanoma.

Recently, dermoscopy utilized in conjunction with other imaging techniques, such as longitudinal digital photography or reflectance confocal microscopy, permitted detection of new melanomas associated with the use of BRAF inhibitors in the treatment of BRAF-mutated metastatic melanomas [60] (Fig. 6).

Table 1 Dermoscopic criteria for melanoma

Pattern	Definition
Dermoscopic global pattern	
Multicomponent pattern	Combination of 3 or more distinctive dermoscopic structures (pigment network, globules, streaks, blotches)
Nonspecific pattern	Pigmented lesion lacking sufficient criteria to meet a reticular, globular, homogenous, or starburst pattern definition
Starburst pattern	–
Multiple colors	The presence of 5 colors in a melanocytic lesion is a sufficient criterion for melanoma diagnosis The combination of black and blue is a criterion for nodular melanoma Pink and red in a melanocytic lesion are suspicious for malignancy
Dermoscopic specific criteria	
Atypical pigment network	Black, brown, or gray network with irregular holes and thick lines
Irregular dots/globules	Irregularly distributed black, brown round to oval, variously sized structures
Irregular streaks (pseudopods and radial streaming)	Irregularly distributed bulbous and often kinked or fingerlike projections seen at the edge of the lesion. They arise from network structures or the body of the tumor. Colors range from tan to black
Blue-whitish veil	Irregular, structureless area of confluent blue pigmentation with an overlying white "ground glass" film. Pigmentation cannot occupy entire lesion and usually corresponds to a clinically elevated part of the lesion
Regression structures	White scarlike depigmentation and/or blue pepperlike granules usually corresponding to a clinically flat part of the lesion
Vascular structures	Irregularly distributed hairpin vessels, dotted vessels, linear irregular vessels, vessels and/or erythema within regression structures
Blotches	Darkly pigmented homogeneous areas irregularly distributed in melanoma
Shiny white streaks (chrysalids)	Short white lines in polygonal distribution only visible with cross-polarized dermoscopy associated with malignancy, with melanoma, and with invasive melanoma
Rosettes	Four white dots in a rhomboidal distribution, arranged as a four-leaf clover, only visible with cross-polarized dermoscopy

(continued)

Table 1 (continued)

Pattern	Definition
Dermoscopic specific criteria/patterns in special locations	
Acral lentiginous melanoma	
Parallel ridge pattern	Pigmentation in the ridges of the fingerprints associated with acral melanoma
Diffuse irregular pigmentation	Geographic pigmentation in different shades of brown with ill-defined border
Lentigo maligna and lentigo maligna melanoma	
Irregular peri-follicular pigmentation	Irregular pigmentation around follicular openings with a c-shape
Granular annular pattern	Blue-gray spots around hair follicles creating an annular pattern
Rhomboidal structures	Confluent pigment around hair follicles that with progression may also invade follicular areas
Isobars	A circle in a circle surrounding a follicular opening
Pink-red rhomboidal structures	Erythema around follicular openings focally seen in early invasive lentigo maligna or amelanotic melanoma

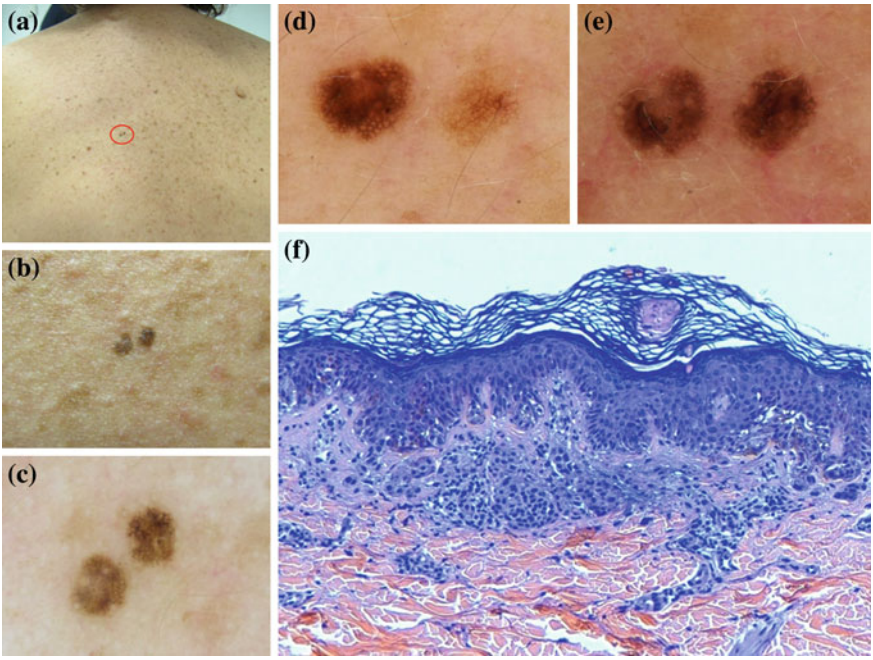


Fig. 6 Clinical, dermoscopic, and histologic images of a cutaneous lesion on a metastatic melanoma patient undergoing treatment with a BRAF inhibitor. Clinical (**a** and **b**) and dermoscopic (**c**, **d**, and **e**) images of two lesions on the back that presented with changes over a 1-month period of digital follow-up. Dermoscopy in November 2013 (**d**) and dermoscopy in December 2013 (**e**). Both lesions were excised. The lesion on the right showed growth and increase in pigmentation and was diagnosed as a severely dysplastic nevus, but melanoma in situ arising in a nevus could not be ruled out. H&E histology ×200 (**f**)

4 Advanced Technological Methods for Melanoma Detection

4.1 Beyond Visual Dermoscopy: Machine Vision in Melanoma Detection

Machine vision here refers to optical imaging and image processing, which extends the vision of the dermatologist. Machine vision is an area of technological growth in dermatology. Optical imaging can use light outside the visible spectrum to see what the eye cannot see. For example, near-infrared light can probe deeply into darkly pigmented lesions that appear black to the eye. Optical imaging can also use visible light with spectral analysis to quantify skin constitution that the eye cannot easily recognize. For example, spectral analysis can distinguish the amount of blood perfusion despite variation in melanin pigmentation or dermal scattering. Analysis of optical images can detect patterns and statistical metrics that are not easily recognized by the brain, for example, the statistics that characterize the branching of melanin pigment networks to recognize melanoma.

Machine vision can be based either (1) on a priori understanding of the mechanism underlying the spectrum or image or (2) on statistical development of a discriminator between two tissue states. The first approach is based on an understanding of how the measurement or image features depend on the tissue composition or structure. The advantage is that variation in observations can be interpreted in terms of variation in tissue composition or structure. The second approach correlates measurements or image features with tissue status, based on a training set of tissue types known by gold standard histopathological, biochemical, or clinical diagnosis to be either in one state or another, for example, normal versus pathological. The advantage is that a discriminator can be identified that optimally discriminates between a normal and pathological state, even when the tissue composition or structure responsible for the discrimination is not understood.

Two aspects of machine vision are illustrated here by examples. They include (1) image acquisition through hyperspectral imaging, reflectance confocal microscopy, and photoacoustic imaging; and (2) image analysis.

Hyperspectral Imaging

The term "hyperspectral imaging" refers to the use of wavelengths beyond the visible spectrum seen by the eye. Images acquired using two or more wavelengths can be algebraically combined to yield a new image sensitive to a particular tissue component. For example, Kollias and Baqer [124] showed that the metric log(R(650 nm)/R(720 nm)), where R denotes reflectance, 650 nm is deep red light, and 720 nm is deeper red light, was proportional to the epidermal melanin content. Thus, two images at these 2 wavelengths can be combined to map the x,y spatial distribution of melanin in the skin.

Ultraviolet A (UVA) light penetrates skin only superficially and is strongly absorbed by melanin. Hence, images of superficial skin structure are acquired ($\sim$upper 100 μm). Near-infrared light penetrates skin deeply and is absorbed by

melanin, blood, and water. Hence, images of the deeper skin structure are acquired (∼upper 1 mm).

Figure 7 shows a multispectral image set acquired using 4 differently colored light-emitting diodes (LEDs) to provide illumination, from invisible UVA to visible deep red. The UVA-illuminated image (Fig. 7 at 364 nm wavelength) clearly displays the superficial pigment, while in longer wavelength images (Fig. 7b–d using blue 488, red orange 598, and deep red 678 nm light), the pigmented lesion fades. Nevertheless, the image taken with deep red light illumination (Fig. 7d) reveals the thicker, denser, and/or deeper pigment.

While melanin absorption is an obvious marker for melanoma, light scattering may also prove useful. Garcia-Uribe et al. [79] have reported that light scattering increases in melanoma (benign nevi < dysplastic nevi < melanoma); hence, light scattering may be an additional metric for discriminating melanoma versus benign nevi.

When imaging pigmented lesions, hyperspectral imaging primarily utilizes photons that have penetrated into the skin, backscattered from the dermis, and transmitted through epidermis to escape at the skin surface. Thus, it is a form of transmission imaging where the light source originates in the dermis. The key

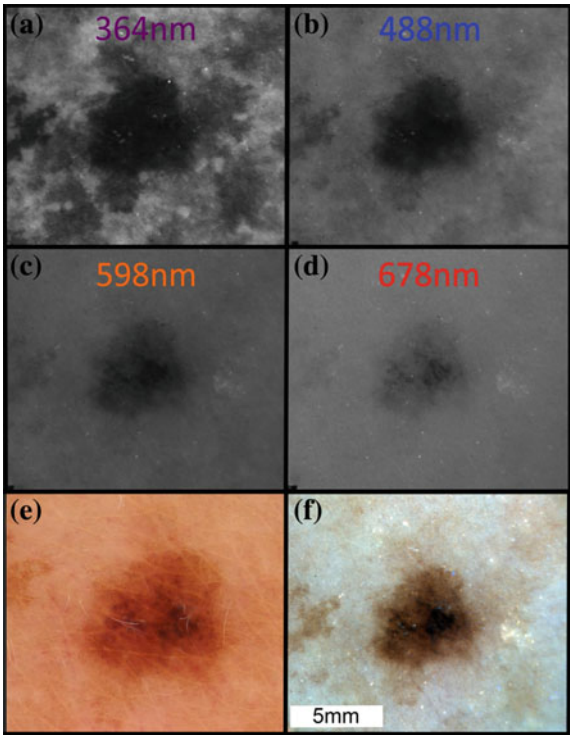


Fig. 7 Multispectral imaging of a common nevus in sun-damaged skin. Imaging at the wavelengths 364 nm (**a**), 488 nm (**b**), 598 nm (**c**), and 678 nm (**d**) with correlating standard dermoscopy (**e**) as well as a reconstruction of the *red/green/blue* image from the multispectral data (**f**) that is calibrated in reflectance units (Images by D. Gareau)

advantages of hyperspectral imaging are its ability to rapidly survey macroscopic fields of view and its relatively low cost.

Reflectance Confocal Microscopy (RCM)

Reflectance confocal microscopy (RCM), or confocal laser scanning microscopy (CLSM), is sensitive to backscatter of light by melanosomes, which are highly reflective [168]. RCM can detect pagetoid melanocytes in the epidermis, which correlate with melanoma, and a disorganized melanosome distribution along the epidermal–dermal junction (DEJ) that sometimes occurs in melanoma [80, 156]. Busam et al. [45] offered a commentary on the strengths and weaknesses of RCM for the detection of melanoma. Scope et al. [184] provided a glossary of terminology for RCM.

Wiltgen et al. [212] studied 50 malignant melanomas and 50 benign pigmented nevi and reported that RCM images of common benign nevi showed more architectural structures and contrast than images of malignant melanoma, which appeared more homogeneous. Guitera et al. [101] described a study of melanoma lesions versus nevi, basal cell carcinoma, and other skin tumors, which cited seven RCM features (cerebriform nests, atypical cobblestone pattern with small nucleated cells in the epidermis, marked cytological atypia, pagetoid cells, and disarranged epidermal layer with no honeycomb pattern) that associated with melanoma. They reported 87.6 % sensitivity and 70.8 % specificity. Braga et al. presented a comparison in six cases of RCM features versus dermoscopic and histopathologic features [40]. Pellacani et al. reported on RCM of 100 melanoma lesions, distinguishing four types of melanoma: (1) “dendritic cell melanomas,” (2) melanomas typified by roundish melanocytes, (3) melanomas characterized by dermal nesting proliferation, and (4) combined-type melanomas [158].

Photoacoustic Imaging (PAI)

Photoacoustic imaging (PAI) uses a focused pulsed laser to deliver light to a focal spot within the skin and detects the sound generated by thermoelastic expansion due to absorption of light. Scanning the laser focus in x, y, and z throughout the volume of a pigmented lesion yields a 3D image of the lesion [68]. Hence, PAI is especially sensitive to melanin, which strongly absorbs light. PAI is a rapidly developing imaging modality that will likely contribute significantly to noninvasive in vivo imaging of the 3D structure of pigmented lesions.

Image Analysis

Automated image analysis can recognize edges for segmentation of tissue types and detect spatial patterns and textures in existing images to yield new images that enhance contrast of optically perturbing structures such as cancer or pigmented lesions. Image analysis algorithms can be applied to dermoscopic images to reveal pathological versus normal morphology or to generate a quantitative end point metric for classification, that is, the percent chance that a pigmented lesion is melanoma.

Early work by Cascinelli et al. [48] quantitatively analyzed image features such as lesion edge, morphology, texture, and color to obtain a positive predictive value of 0.45 and a negative predictive value of 0.95. Subsequent analytical methods included the use of geometries and Burroni's islands of colors [14]. Bauer et al. [30] used such features to obtain a positive predictive value of 0.87 and a negative predictive value of 0.99. Table 2 compares the sensitivities and specificities of recent computational approaches in comparison with algorithms used in dermoscopy with visual inspection.

Wiltgen et al. [212] discussed the classes of image analysis features in RCM images for discriminating malignant and benign melanocytic lesions. Koller et al. [123] studied a large number of RCM images (10,122 test images, after 6147 images in a training set) using CART (Classification and Regression Trees) analysis software (Salford Systems, San Diego, CA). They reported rather poor discrimination of melanoma versus benign nevi, which they attributed to non-standardized image acquisition, and cautioned that better results may rely on standardized acquisition. Gareau et al. [80] reported an image analysis algorithm for using RCM images to detect a disrupted dermal–epidermal junction (DEJ) in melanoma. Kurugol et al. [127] developed an algorithm incorporating texture analysis to use RCM images to localize the DEJ, which may prove useful in identifying a disrupted DEJ.

An example of image analysis is the skeletonization of the pigmented network in dermoscopic images of pigmented lesions. Statistical analysis of the branches in the pigmented network can yield the regularity among branch segments as a quantifiable metric. Figure 8 shows the use of an algorithm to find a diagnostically relevant feature, the coefficient of variation (COV) of branch lengths in the pigmented network. The standard deviation of the branch lengths divided by the average branch length (the COV) is 0.312 for Fig. 8b but 1.077 for Fig. 8d, indicating that the second lesion has more branch length variability. This COV metric is expected to grow with increasing atypical pigmented network features and may prove to be a useful quantitative descriptor indicating a suspicious lesion.

Table 2 Sensitivity (Se) and specificity (Sp) of melanoma detection reported for various algorithms in research studies

	Se (%)	Sp (%)
Pattern analysis [171]	85	79
ABCD [171]	84	75
7-point checklist [171]	78	65
CASH [171]	98	68
Menzies method [171]	85	85
*SIAscopy (European cohort) [65]	50	84
*SIAscopy (Australian cohort) [65]	44	95
*Solar scan [144]	91	68
*Pre-melafind 1 [72]	98	44
*Pre-melafind 2 [72]	91	38

Computer-automated analyses are marked (*). Visual analyses are unmarked

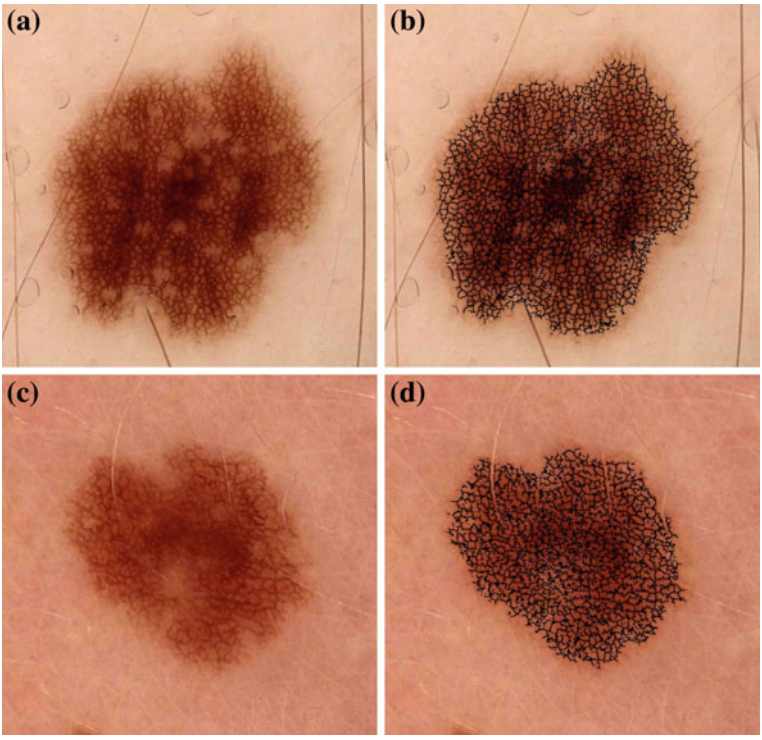


Fig. 8 Computer-automated identification of the pigmented network in two lesions. The original images are (**a**) and (**c**), and the images with computer-identified networks superimposed are (**b**) and (**d**) (Images by D. Gareau)

In summary, optical imaging and image analysis can characterize pigmented skin lesions and contribute to the discrimination of benign nevi, dysplastic nevi, and melanoma. Dermoscopy, hyperspectral imaging, confocal reflectance microscopy, and photoacoustic imaging are examples of image formation. Image analysis can use such images to yield new images based on spectral behavior, spatial patterns, and textures that characterize pigmented lesions.

4.2 In Vivo Confocal Microscopy

Historical Development

The microscope (invented in the 1500s in the Netherlands) was developed by Galileo. Galileo created the first compound microscope in 1625, enabling the discovery of the cell by Robert Hooke in 1665. The LASER, demonstrated in 1960 and reported in 1962 [135], became a powerful tool in combination with the confocal microscope [145], though initial optical sectioning of biological tissue

used white light [161]. Rapid laser scanning microscopy was demonstrated for noninvasive skin imaging in 1995 [168] and improved to video rate in 1999 [166].

The reflectance confocal microscope has potential for clinical translation in dermatology thanks to engineering of rapid polygon scanning, which enabled video rate imaging [166–168] and a stable mechanical interface for skin coupling. The commercialized VivaScope (Caliber ID, Rochester, NY), which has 1 micrometer lateral resolution, 0.75 mm field of view, and temporal resolution of ~10 image frames per second, achieves excellent resolution and contrast in epidermis. Reflectance mode confocal scanning laser microscopy (RCM) enables en-face (horizontal plane) dynamic visualization of cellular and architectural morphology in vivo. The ability to observe cellular details is a key advantage of RCM over other noninvasive skin imaging techniques such as high-frequency ultrasound [115, 126] and magnetic resonance imaging [194]. Recent advances in optical coherence tomography [55] are beginning to improve the resolution to the cellular level. Though promising, these results are not widely confirmed, and it remains to be seen whether optical coherence tomography will undergo the massive clinical translation seen with RCM since 1995.

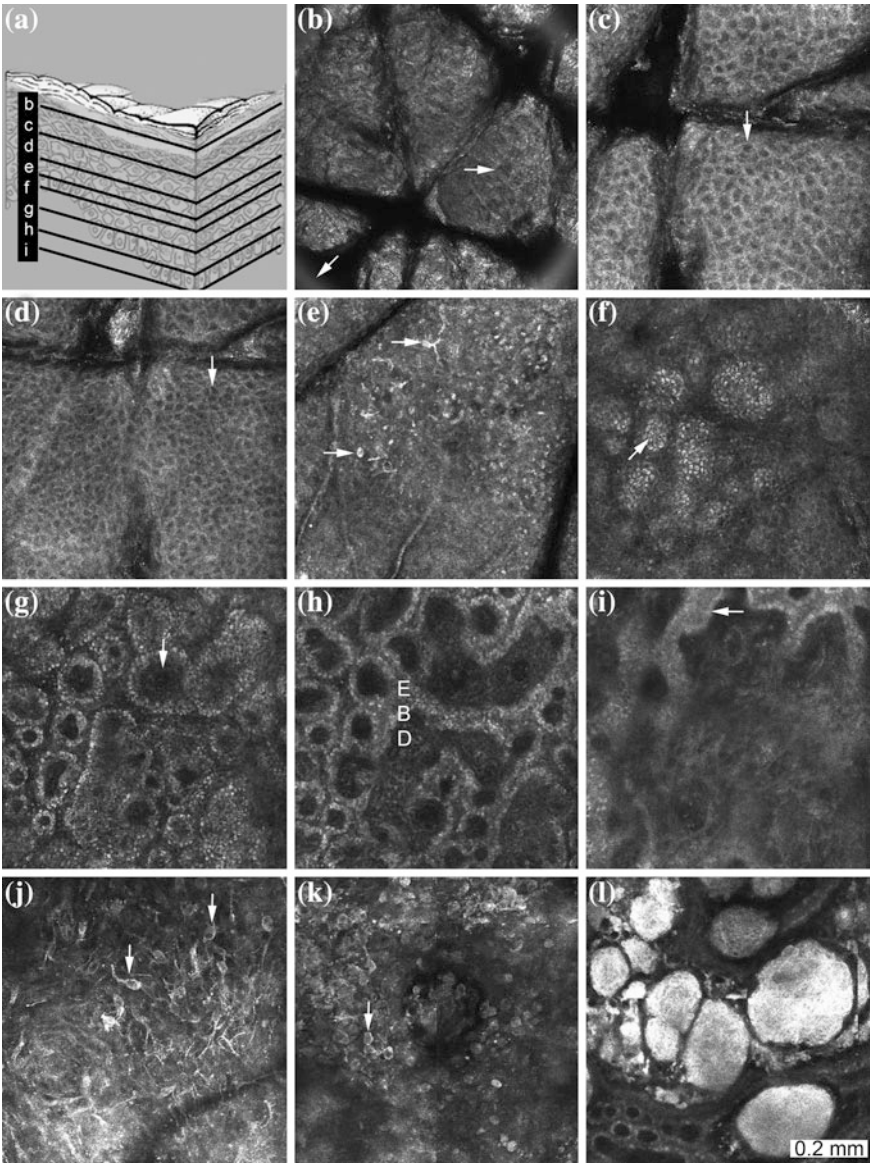
Resolution

RCM implements optical sectioning (instead of conventional physical cryostat sectioning) by measuring the light that reflects off a 1 μm spot where the laser focuses. The 1 μm laser spot size dictates the spatial resolution, which is about 1 μm. The time resolution is about 10 Hertz, which is 10 frames per second on a monitor screen.

Penetration

In RCM, light penetrates to a subsurface focus of the laser and reflects back from that focus out of the skin and into a detector in the microscope. Light that reflects superficially to the focus is eliminated, and so if the focus is too deep, there will be no signal. Because the laser must propagate into the skin and from the subsurface focus back to the detector in the microscope, the depth penetration of RCM is limited to 100–200 μm in human skin at the 830 nm laser wavelength used in the commercial system. Longer wavelengths (e.g., 1064 nm) penetrate more deeply, and shorter wavelengths (e.g., 488 nm) offer superior resolution at the cost of resolution or penetration, respectively. The variability in the imaging penetration depth also depends on natural variation in the concentration of reflective components composing skin such as keratin, collagen, and melanin.

This process is (1) noninvasive, as enabled by the optical sectioning confocal principle; (2) painless because the laser power is <10 milliwatts; (3) safe because the laser wavelength, 830 nm, is near-infrared light that does not damage DNA; and (4) rapid compared to biopsy and histological analysis, requiring a few seconds for the acquisition of single images (up to 0.75 × 0.75 mm) up to 10–20 min for the acquisition of 3–5 full mosaics at different depths covering an area up to 8 × 8 square mm, which is usually needed for melanoma differential diagnosis.



In the commercialized VivaScope® 1500, which is mounted on an articulating arm, a plastic window with an adhesive outer ring is affixed to the skin, enabling large mosaics which effectively increase the field of view from a single 0.75-mm image to up to 8 mm. The handheld version of the device (VivaScope® 3000) only affords a series of 0.75 × 0.75 mm images. Figure 9 shows a representative set of images.

◀ **Fig. 9** Confocal reflectance microscopy images (0.75 × 0.75 mm each) of skin. The layered skin architecture (**a**) is shown in sequentially deeper optical sections (**b–i**). The superficial section (**b**) shows the bright peripheral ring of the plastic window surface; bright, stratified keratin structure; and faint dark nuclei in the stratum granulosum (*arrow*, 25–35 μm diameter). The upper stratum spinosum (**c**) contains keratinocytes (12–25 μm diameter) in a honeycomb pattern. The deeper stratum spinosum (**d**) has a greater number of smaller keratinocytes. Suspected pagetoid melanocytes (**e**) lie above surface of the stratum basale (**f**), where basal cells form a cobblestone appearance. Basal cells (**g**) form bright rings around dim reticulated fibers around *dark circles* that are capillaries (*arrow*). The optical section (**h**) that bisects the rete pegs shows the spinous epidermis (E), basal layer (B), and papillary dermis (D). In the deep optical section (**i**), the collagen dermis that would show individual fibers is blurred. The arrow indicates the deepest basal layer cells. Additional images shown here include pagetoid pleomorphic melanocytes in the epidermis in a melanoma (**j**), junctional aggregates and sheets of pleomorphic malignant melanocytes in a melanoma (**k**), and compact nests of melanocytes in a nevus (**l**). *Note* Confocal images not all from the same patient

Contrast

The appearance of normal skin in RCM is characterized by similarly sized and shaped cells, whether appearing in the honeycomb pattern in the spinous and granular layers or the cobblestone pattern of the basal layer. Additionally, the appearance varies according to Fitzpatrick skin type, sun exposure, age, and physiological condition [110, 131]. The RCM features of a wide range of pathologies [96, 109] have been described in the literature. However, new imaging modalities require training that can be supported by understanding the mechanism of contrast. The mechanism of contrast in reflectance confocal microscopy is the naturally occurring microscopic differences in refractive index that exist in biological tissue. The advantage of this contrast mechanism is that it is endogenous, but the disadvantage is that it is nonspecific, which means pattern recognition is required to interpret the biological meaning of the anatomical features observed in confocal micrographs. Reflectance (optical scattering) occurs when microscopic components of high refractive index (n) lie in surrounding media of low refractive index. One example is keratin ($n1 = 1.40$) in cytoplasm ($n2 = 1.34$). An even stronger scattering component is melanin because its refractive index is $n1 = 1.72$. The difference in refractive index $\Delta n = n1 - n2$ is larger (0.38) for melanin in cytoplasm than (0.06) for keratin in cytoplasm or collagen in the dermis. Therefore, melanin appears brighter than keratin or collagen.

Melanoma Pathology

Clinical melanoma diagnosis is exceptionally challenging, and the use of confocal microscopy has been extensively researched [42, 44, 94, 128, 159]. The key diagnostic RCM melanoma features in the epidermal layers are enlarged atypical cells with pleomorphic morphology including stellate, oval, and fusiform types, nuclei that are enlarged, and coarse dendritic processes. These features have also been reported in clinically amelanotic melanomas [44]. Alteration of the architecture at the dermal–epidermal junction and aggregates of atypical cells clustering into nests at the junction and in the dermis are also clues for melanoma identification [157]. In the case of small melanomas, which are particularly difficult to

diagnose with dermoscopy, one report [165] suggested that RCM microscopic morphologies, such as the presence of at least five pagetoid cells per mm^2, tangled lines within the epidermis, and atypical roundish cells at the dermoepidermal junction, were characteristics of melanoma. The diagnostic sensitivity and specificity of RCM reported in the literature is widely variable because each study reports on a data set selected by particular researchers and has been analyzed by clinicians and pathologists with particular training. Table 3 provides an overview of the published reports.

In clinical practice, RCM should be considered as an adjunct to dermoscopy, since it should be performed on selected lesions, and feature interpretation should consider the dermoscopic background. Systematic use of RCM in a prospective cohort of over 1000 patients reduced the number of excisions needed (as determined by the number of benign lesions removed to find one melanoma) from 14.6 to 6.8, also reducing the number of lesions requiring referral for digital dermoscopy monitoring [160].

Future Directions

Challenges to the clinical utility of RCM include the time required to acquire confocal images, the awkwardness of the physical device, and the fact that confocal images are both difficult and time-consuming to read. The hardware issues are being addressed by investigation of line scanning as a rapid and simpler (i.e., smaller package) alternative to point scanning [4, 62, 81].

Image interpretation remains difficult because of qualitative and quantitative challenges, both of which will likely be eased by computer vision approaches in the future. To qualitatively assess tissue morphology, extensive training is required. Although the number of healthcare professionals trained to read RCM is growing and consensus terminology [184] is taking root, RCM remains slow to expand to its full diagnostic promise in dermatology. Automated computer image analysis has great potential to guide novice readers by illustrating features that it can quantitatively identify through image processing. RCM images should be standardized by the absolute reflectance from the window surface that contacts the skin and then processed digitally to generate metrics that can be numerically compared to a threshold to generate a diagnostic classification such as nevus, dysplastic nevus, or melanoma. Ultimately, it will likely be shown that the threshold will be nebulous and that dysplasia can be quantitatively scored on a scale from benign to malignant. A convenient property of digitally rendered diagnoses is that they can be rapidly evaluated against a set of “melanoma diagnosis threshold” scores to generate the receiver–operator characteristic of the diagnostic. Such a system would allow dermatologists to “dial in” their desired level of conservative tendency when choosing how aggressively to biopsy.

Preliminary works have included automated identification of pagetoid melanoma cells [80], identification of the dermal/epidermal junction [80, 127], and the identification of the honeycomb keratinocyte pattern in the spinous and granular epidermis. Though sensitivity/specificity studies have not yet tested these three

Table 3 Summary of diagnostic sensitivity and specificity reported in the literature

Author	Year	Journal	Nevi/Melanomas	Sensitivity	Specificity	Note
Gerger	2005 [94]	J Invest Dermatol	90/27	88.1–98.1	97.6–98.9	Selected images in clinically equivocal and non-equivocal cases
Pellacani	2005 [157]	J Am Acad Dermtol	65/37	97.3	82.6	Dermoscopically equivocal lesions
Bono	2006 [35]	Br J Dermatol	183/23	83	69	Dermoscopically equivocal small (<3 mm) lesions
Gerger	2006 [95]	Cancer	90/27	90.74	98.89	–
Pellacani	2007 [159]	J Invest Dermatol	215/136	77.9–89.7	69.7–52.1	Dermoscopically equivocal lesions
Gerger	2008 [93]	Br J Dermatol	20/50	97.5	99	–
Guitera	2009 [103]	J Invest Dermatol	203/123	91	68	Dermoscopically equivocal lesions
Segura	2009 [187]	J Am Acad Dermtol	92	86.1–100	57.1–95.3	–
Guitera	2010 [102]	J Invest Dermatol	203/81	93	82	Lentigo maligna and pigmented macules
Curchin	2011 [52]	Australas J Dermatol	29/13	92.3	75	Difficult lesions
Guitera	2012 [101]	J Invest Dermatol	375/216 (+119 BCC)	87.6	70.8	Consecutive dermoscopically equivocal lesions including non-melanoma skin cancers
Stevenson	2013 [195]	Dermatol Pract Concept	5 studies	93	76	Meta-analysis
Alarcon	2014 [9]	Br J Dermatol	172/92	97.8	92.8	Excision rate

characteristics for computer-automated diagnostic purposes, a study that used human analysis [159] achieved sensitivity/specificity of 78/70, 90/59, and 88/52 % based on the three single characteristics, respectively. Future work will undoubtedly develop computer vision metrics that attempt to mimic the human-documented [184] patterns. Perhaps the most exciting potential is that of machine vision as instructive and educational, elucidating morphological patterns that sensitively and specifically detect melanomas that are not easily perceived by humans.

4.3 Optical Coherence Tomography

How It Works and the Gaps It Fills

Optical coherence tomography (OCT) is a safe, fast, noninvasive, cross-sectional in situ imaging system. It is commonly compared to ultrasound because both techniques use reflected waves to reconstruct an image. While ultrasound can measure the time of flight of sound waves, the speed of light is too fast and therefore interferometry methods are used. Interferometry splits a light source into two paths, and differences in the lengths of the two paths cause the two light sources to interfere with each other when recombined. In OCT, one part traverses a *reference* path terminated with a mirror and the other traverses a *sample* path where the tissue structures absorb or scatter the light.

For the near-infrared wavelengths typically used, scattering and not absorption is the dominant effect, resulting in a sufficient number of photons being reflected back. These reflected photons from the tissue sample constructively and destructively interfere with the reflected photons from the reference arm to generate the interference signal. This interference signal can be measured by a variety of sensing devices depending on system design and the principal wavelength of the light source.

Most OCT systems use broadband light sources to create one-dimensional images of reflecting structures in tissue at a given spot. These are sometimes called *axial scans* or *A-scans*. Sets of A-scans are typically acquired in a raster scanning pattern and assembled into 2D cross-sectional images that can then be stacked to form 3D volumes. Capturing multiple A-scans at a single location over time is used to create functional images involving dynamic tissue properties. Measuring blood flow [49] and the mechanical response to vibration [206] are two examples of functional imaging.

Two broad classes of OCT are in use today (Fig. 10): time domain OCT (TD-OCT) and Fourier domain OCT (FD-OCT). TD-OCT was the first to be widely deployed in ophthalmology settings and uses a moving reference arm mirror and a photodetector to identify reflections in tissue samples. FD-OCT measures the interference at each frequency of light and uses the Fourier transform to convert frequency domain measurements into spatial domain values. FD-OCT itself has two principal variants: spectral domain OCT (SD-OCT), which uses a diffraction grating

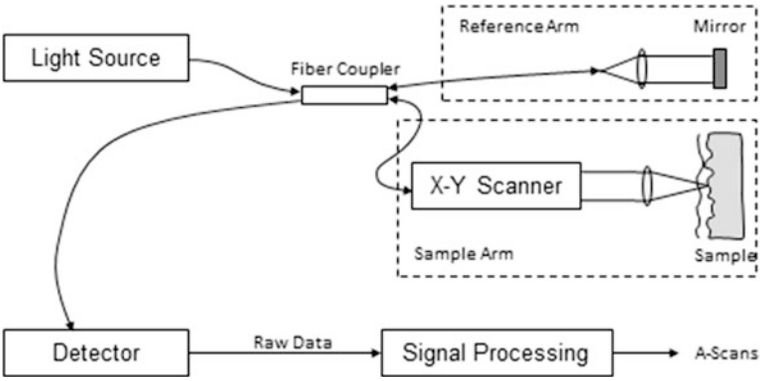


Fig. 10 Generic fiber-based OCT schematic. For TD-OCT and SD-OCT, the light source is usually a super-luminescent diode (SLD), while for SS-OCT, it is usually a tunable laser. In TD-OCT, the reference arm mirror moves; for other types, it is fixed. The detector in SD-OCT is a spectrometer, and in others, it is usually a balanced photodetector. Details of the optics and components, such as optical circulators and polarization controllers, vary significantly between designs and have not been included

and a line scan camera to measure all the frequencies at once, and swept-source OCT (SS-OCT), which uses a tunable light source and a balanced detector to measure each frequency individually. Both FD-OCT methods are faster than TD-OCT and have a better signal-to-noise ratio [58].

Many further variations on these general classes of OCT exist. Polarization-sensitive OCT detects birefringence changes in tissue. Microscopy variants, called optical coherence microscopy (OCM), add transverse resolution enhancements to increased axial resolution abilities. Full-field versions blend the best parts of reflectance confocal microscopy with OCM. These are just some examples of the OCT innovation that continue to grow at a rapid pace.

In all of these designs, the axial resolution is proportional to the square of the center wavelength of the light source and inversely proportional to the bandwidth of the light source: $\Delta z = 2\ \ln(2)\lambda^2/(\pi\Delta\lambda)$ where Δz is the axial resolution and $\Delta\lambda$ is the light source full-width half-maximum (FWHM) bandwidth. Broadband light sources are therefore used to create good axial resolutions. Smaller center or principal wavelengths also contribute to better axial resolution. Most of the initial work in OCT has been based on available light sources and optics developed for the telecommunications industry [181], which means the most common principal wavelengths are around 800, 1300, or 1550 nm. With the increased attention on OCT, new light sources, sensors, and optics are now in development driven by the biological application. However, because of its availability and its ability to penetrate more deeply [13], OCT systems using light sources with a center wavelength of around 1300 nm are most commonly reported in dermatology applications. Axial resolutions for commercially available systems now range from 5.5 to 16 μm.

Lateral resolutions are dictated by the optics of each system. One of the benefits of OCT is the ability to obtain good axial resolution while positioning the optics a distance away from the sample. This is accomplished using low numerical aperture (NA) sample optics and facilitates, for instance, imaging retinas from several centimeters away. For dermatology applications, this creates a trade-off: Low NAs create a more uniform lateral resolution through the tissue sample at depths of up to 2 mm, while higher NAs are used to create better lateral resolution at the expense of imaging depth. For low NA systems, this lateral resolution is typically between 5 and 15 μm [61, 202]. Recent interest in OCM, which uses high NA optics coupled with very broadband light sources, yields both high axial and high lateral resolutions at the expense of depth of field [111]. A variation on this idea is full-field OCT (FF-OCT), which creates en-face images similar to confocal microscopy but with the ability to image more deeply [55]. Recently, a high-definition OCT (HD-OCT) has been introduced with cellular resolution [36]. Different non-melanocytic and melanocytic skin tumors have been described with HD-OCT with histopathological correlation [37, 38, 133, 134].

This ability to image up to 1 mm in HD-OCT and 2 mm with HD of tissue coupled with axial and lateral resolutions measured in microns positions OCT between confocal laser scanning microscopy (CSLM) and high-frequency ultrasound (HFUS) in terms of resolution and depth penetration. OCT has better resolution than HFUS but worse resolution than CSLM. OCT has better depth penetration than CSLM but worse than HFUS.

For more details on how OCT works, see [41, 73, 162].

Strengths/Weaknesses

For dermatology applications, OCT has long been heralded as a potential diagnostic solution that does not require invasive surgery, does not alter the sample morphology, and can be repeated over time for the same suspicious lesion. Unfortunately, the resolution of OCT is still insufficient to replace histopathology where cellular differentiation is required [59]. In the case of HD-OCT, cellular resolution is achieved but with some limitations (compared to confocal microscopy) that are critical in the recognition of melanocytic lesions, such as the possibility of differentiating dendritic cells. At the same time, the field of view compared to confocal microscopy with HD-OCT is reduced to 1.3 mm that in the study of melanocytic lesions makes a diagnostic conclusion difficult. These limitations may be not relevant in the recognition of epidermal tumors including actinic keratosis, squamous cell carcinoma, or basal cell carcinoma. Future integration of OCT, HD-OCT, and confocal microscopy may be very promising due to the complementary information that they allow one to visualize. In the case of OCT, even though the histopathology correlates may be missing, several studies suggest OCT is capable of revealing microstructures in skin that correlate well with known morphological changes introduced by various diseases [51, 141, 146, 147, 215]. Most of the promising research thus far has been in the area of non-melanoma skin cancers. A cautionary study showed basal cell carcinomas could be differentiated from normal skin, but the subtypes could not be discerned [75].

Melanoma detection is still an area of active investigation and will remain so as the resolution of OCT continues to improve. On the one hand, de Giogi et al. [59] attempted to correlate histology, dermoscopy, and OCT on 10 patients without significant success. They concluded that a differential diagnosis between melanoma and benign melanocytic nevus using OCT was not possible. In contrast, another group attempted to characterize melanocytic skin lesions using OCT in vivo as either melanoma or benign nevus. They assessed 92 lesions from 75 patients and carefully validated the results histologically [75]. The most significant differences found were that melanomas showed marked architectural disarray and lacked clear dermoepidermal borders compared to benign nevi. Other differences were identified in the study, and their conclusion was that micromorphologic features visible in OCT have the potential to be used as discriminating features. Caveats included the inability to subclassify the benign nevi and the need for further sensitivity and specificity studies with other types of skin tumors.

Availability/Usability

The general assessment for using OCT to diagnose melanoma is that it is still in the "promising stage" and will continue to improve, but it is not quite ready for clinical practice [142, 147, 155, 191].

At present, OCT is limited to assessment of tissue microstructures and cannot provide cellular features visible in traditional histopathology or CSLM, though it can provide in situ imaging at deeper depths than CSLM. Nevertheless, the clinical community continues to wait for significant advances before adopting OCT as a diagnostic tool. Meanwhile, vendors are beginning to produce tools so providers can at least begin experimenting and learning more about the capabilities and limitations of OCT [74, 142].

OCT and HD-OCT have become a very active area of research, especially in the last decade [216]. There has been a proliferation of novel enhancements and advances, from the significant improvements in light sources and sensing devices to the introduction of new designs and software processing algorithms. Despite the current limitations, most authors continue to be optimistic that continued improvements will finally enable OCT to fulfill its promise as a vital tool in clinical practice.

4.4 Electrical Impedance and Ultrasound Detection Technologies

This section presents an introduction to two modalities for detecting and/or imaging melanoma. These include (1) electrical impedance spectroscopy (EIS) and (2) high-frequency ultrasound (HUS).

Electrical Impedance Spectroscopy (EIS)

The electrical impedance of a tissue largely depends on (1) ions and (2) membranes. More specifically, the key factors are (1) the concentration and mobility of charged ions and (2) the presence of tight junction membranes, cellular membranes, and

macromolecular surfaces against which the ions can move to capacitively store energy. Hence, electrical impedance can characterize both the extracellular matrix and the intracellular matrix within which ions move as well as the membranes (and surfaces) that can support charge separation. The influence of ion mobility will decrease if the tissue is less hydrated, the matrix is denser, or the number of ions is low. The capacitive effect will decrease if the membranes are leaky or there are fewer membranes.

The electrical impedance of skin when measured by topical application of electrodes is dominated by the high resistance of the stratum corneum. Changes in stratum corneum hydration or structure can be followed by electrical measurements at low frequencies (<1 kHz). To measure the living epidermis and dermis, the stratum corneum must be bypassed. Tape stripping can remove the stratum corneum to allow electrical measurements of the underlying skin layers. Alternatively, microneedles serving as electrodes can penetrate the stratum corneum, thereby placing the electrodes in direct contact with the underlying skin layers [100]. The SciBase system uses such microneedles (SciBase Inc., http://www.scibase.se). For the detection of melanoma, the influence of the stratum corneum must be bypassed.

Electrical measurements in tissues have been studied for some time [71, 183, 214]. Plus and minus charges that accumulate on either side of a membrane will store electrostatic energy, which can be described as a capacitance C [Farads]. The movement of ions imparts a loss of energy due to frictional forces that heats the tissue. The mobility of the ions can be described by conductance, and the inverse of conductance is resistance R [ohms]. There is a time constant for charging of a capacitor, τ = RC [s]. If an alternating current (AC) is applied to the skin, the capacitance due to membranes and macromolecular surfaces will charge to 63 % of maximum in a time period of τ seconds. If the frequency (f [Hz] or [cycles/s]) of the AC is low ($f\tau \ll 1$), the capacitance will fully charge. If the frequency of the AC is high ($f\tau \gg 1$), the capacitance will not charge because the ions will just jiggle in place but not move over any appreciable distance. The energy loss due to heating (which is negligible in diagnostic measurements) is maximum when the frequency matches the time constant ($f\tau \approx 1$), since the ions are constantly moving to charge and then discharge the membrane capacitors. So the time constant τ or the center frequency $f_c = \tau/(2\pi)$ is a key parameter that characterizes the frequency dependence of electrical impedance.

There can be a variety of local domains with distinct membrane surfaces and ion mobilities, which have distinct values of $f_{c,\text{domain}}$. Hence, the observed dispersion centered around the f_c of the population of domains will broaden as the heterogeneity of local domains increases. This broadening is described by the factor a, where the capacitive energy storage, expressed as the real permittivity (ε'), behaves proportional to $1/(1 + (f/f_c)^{1-a})$, which equals 1 at low f and zero at high f. Figure 11 shows the behavior of a generic dispersion centered at f_c and the effect of increasing heterogeneity (a increasing from 0 to 0.5). The real permissivity (ε') describes capacitive energy storage, and the imaginary permissivity (ε'') describes the dissipative frictional losses of ion movement.

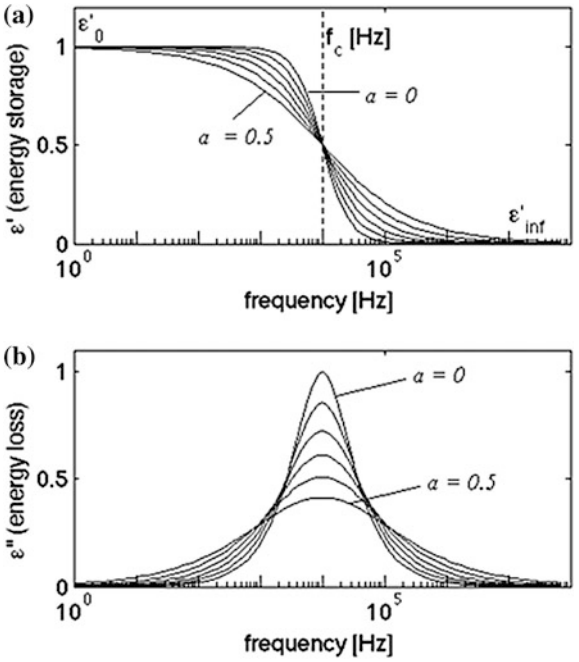


Fig. 11 A generic dispersion of electrical impedance. This example shows a central frequency (f_c) of 10^4 Hz. **a** The real permittivity (ε') is high at low frequencies, where membranes block DC current and capacitive charging of membranes by ions stores energy. At high frequencies, the impedance is low where ions are free to move, but they oscillate so fast and cannot charge the membranes, so energy storage is low. **b** The imaginary permittivity (ε'') shows the energy losses maximizing at the central frequency f_c, where the rate of charging and discharging the membranes by ion movement optimizes the energy dissipation by frictional losses. The factor a characterizes the heterogeneity of the dispersion. Each local domain has its own local unique $f_{c.\text{domain}}$, and a distribution of domains will have a net broadened f_c. As the heterogeneity increases, the a increases from 0 to 0.5

There are several types of capacitive charging with different characteristic f_c, called *dispersions*. The movement of ions up against tight junction membranes or other rather macroscopic membrane surfaces (including electrodes) involves large ion movements through the extracellular matrix, and hence, the time constant of charging (τ) is large and f_c is low, approximately in the 1 Hz to 10 kHz range. This process is called the *α dispersion*. The movement of intracellular ions up against cell membranes and macromolecular surfaces involves shorter range ion movements; hence, the τ is short and f_c is high, approximately in the 1 kHz to 100 MHz range. This process is called the *β dispersion*. The rotation of dipoles, such as bound water, amino acid side groups, and other small molecules, occurs at very high frequencies, approximately in the 100–1000 MHz range, and is called the *δ dispersion*. At ~10 GHz, the rotation of free water occurs and is called the *γ dispersion*.

Most studies on skin are conducted in the frequency range of the α and β dispersions. Figure 12 shows a typical dispersion spectrum of the real permissivity versus frequency for tape-stripped skin. The frequency ranges for the α and β dispersions of skin are much lower than the ranges for soft tissues. The α of skin is $\sim$11 Hz, while the α for soft tissue is in the range 100 Hz to 10 kHz. The β of skin is $\sim$64 kHz, while the β for soft tissue is in the range 100 kHz to 1 MHz. Not shown are data reported using the SciBase system [3], which are similar to the β dispersion data in Fig. 12.

Clinical EIS Studies of Melanoma

The use of electrical impedance spectroscopy (EIS) to detect skin cancer followed early work by Stig Ollmar on using EIS to detect effects on the skin barrier function. Additionally, the thesis by Åberg is recommended [1]. There is significant inter-subject variability in the parameters that describe the permissivity dispersion of skin sites. Therefore, comparisons are always made relative to a nearby normal skin site on a subject. Investigators using the SciBase system use principal component analysis (PCA) to analyze data, relying on training sets to train the algorithm. For example, the sensitivity to malignant melanoma was reported to be 95 % (59/62) and specificity to be 49 % (72/148) [2]. However, the PCA description does not inform about the variation in tissue structure caused by melanoma. Such clinical studies report only the PCA analysis and do not report the dispersion spectra that might inform about mechanism of contrast. In a recently published multicenter

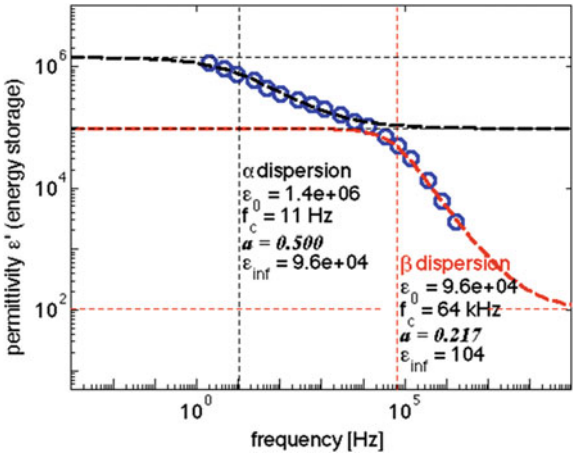


Fig. 12 Measurements of the permittivity of tape-stripped skin show the electrical behavior of the viable skin tissue. The data (*blue circles*) are from [214]. There are two apparent dispersions. In this particular skin site, the α dispersion (*black dashed line*) is centered around 11 Hz (and a potential contribution from polarization of the electrodes is not clear) and is attributed to ions moving through the extracellular space to charge tight junction membranes. The β dispersion (*red dashed line*) is centered around 64 kHz and is attributed to intracellular ion movement to charge cellular membranes. The data measured by the SciBase system (not shown) [3] aligns with the β dispersion data in this figure but with a lower f_c

prospective blinded study with SciBase, 1951 subjects with 2416 lesions were enrolled; 1943 lesions were eligible and evaluable for the primary efficacy end point (including 265 melanomas—112 in situ and 153 invasive melanomas with a median Breslow thickness of 0.57 mm, 48 basal cell carcinomas, and 7 squamous cell carcinomas). The observed sensitivity of Nevisense was 96.6 % (256 of 265 melanomas) with an exact one-sided 95 % lower confidence bound estimated at 94.2 % and an observed specificity of 34.4 % with an exact two-sided 95 % confidence bound estimated at 32.0–36.9 %. The positive and negative predictive values of Nevisense were 21.1 and 98.2 %, respectively. The observed sensitivity for non-melanoma skin cancer was 100 % (55 of 48 BCC and 7 SCC) with an exact two-sided 95 % confidence bound estimated at 93.5–100 % [138].

In summary, EIS is a low-cost and simple measurement for topical assessment of skin and is reported to be responsive to the changes in skin properties caused by melanoma. The ion mobilities of the extracellular and intracellular spaces, the status of membranes that can be charged by ions, and the heterogeneity of local domains contribute to the signals. The use of microneedles for perforating the stratum corneum and serving as electrodes in direct contact with the epidermis is an especially welcome innovation. More reports of the frequency spectra data from the EIS measurements would be welcomed and potentially could elucidate the contrast mechanisms underlying melanoma detection.

Ultrasound detection of melanoma

High-frequency ultrasound (HUS) is another method for detecting melanoma. An ultrasound transducer delivers high-frequency pressure waves to the skin and collects time-delayed reflected waves. The time delay corresponds to the round-trip propagation of the waves to some depth and then back to the surface for detection. Like a radar system, the ultrasound signal can create depth-resolved images. The mechanism of the reflected ultrasound signal is based on the presence of variations in tissue density, which yields variations in the impedance of the tissue that cause reflections. A tissue that is very homogenous will be *anechoic*. A tissuelike dermis has significant fluctuations in mass and hence is highly *echogenic*. The attenuation of ultrasound is very frequency dependent, falling roughly as the square of frequency, f^2. Low-frequency ultrasound can image many centimeters into a tissue. However, ultrasound in the 10–30 MHz range can only image to a depth of $\sim$10 mm. Because of the high frequency, the spatial resolution (10 s of μm) is much better than for low-frequency ultrasound (mm).

The method was first demonstrated as skin ultrasonography [10]. It can detect the thickness of melanoma and is used for detecting melanoma in lymph nodes. But the challenge is to use HUS to discriminate benign from pigmented lesions in situ in the skin.

A study using 20 MHz HUS [107] compared the 25 melanoma lesions (MM) versus 29 basal cell papillomas (BCP) and 15 benign melanocytic nevi (BN). The study compared the echogenicity of the dermis below the tested lesion. MM showed low attenuation of ultrasound such that the high echogenic dermis was clearly seen. In contrast, BCP showed higher attenuation of ultrasound, so there was

an apparent shadow cast into the dermis. The shadowing was reported to correlate most significantly with histological extent of hyperkeratosis, and three cases of non-keratotic acanthotic BCP were noted to be classified as MM by the method. Nevertheless, melanoma was discriminated from BCP with 100 % sensitivity and 79 % specificity. With addition of entry echo line enhancement (EEE), the specificity was improved to 93 %. BN demonstrated patchy shadowing as a result of being keratotic, indicating a more spatially heterogeneous lesion than MM. The specificity for discriminating BN from MM was low (30 %). HUS shows promise for discriminating MM from BCP, while the discrimination of BN and MM needs some improvements.

Toward this end, the use of focused ultrasound that creates an image at a restricted depth was developed so as to increase the sensitivity of the signal contrast between BN and MM [169]. The method is called retroflex transmission imaging (RTI) [99]. In the ultrasound application of RTI to skin, HUS is focused to a small spot near the surface, and the transmitted signal propagates into the tissue volume. The total volumetric backscatter is detected, but the magnitude of this signal is sensitive to the attenuation properties of the small focused spot. Hence, higher contrast for the surface spot is achieved. Scanning the focused spot yields an image that can take advantage of the increased spatial variation in attenuation in BN relative to MM. The method was tested on 25 MM, 24 seborrheic keratosis (SK), and 38 BN. The differentiation of SKs from melanoma showed specificity of 79 % and sensitivity of 100 %. The differentiation of BN and MM showed specificity of 30 % and sensitivity of 100 % without RTI and 55 % specificity with RTI and EEE. This improvement in specificity is encouraging and argues for continued work on novel approaches to HUS imaging.

In summary, HUS is an imaging modality useful for assessing melanoma thickness. It also reveals the anechoic nature of melanoma and its uniformity in attenuation properties.

4.5 Molecular Assays for the Detection of Melanoma

Over the past decade, molecular assessment of the tumor genome has been increasingly utilized as a diagnostic adjunct in the evaluation of histopathologically ambiguous melanocytic tumors. While the majority of melanocytic neoplasms can be accurately diagnosed through routine histopathologic analysis by a properly trained pathologist, a significant minority of cases have conflicting histopathologic features that result in diagnostic discordance, even between expert dermatopathologists [67]. As histopathologic assessment of these difficult tumors often yields equivocal diagnoses, ancillary tests are needed to better direct patient care. Early cytogenetic analysis of melanocytic nevi and melanomas demonstrated that 96 % of unequivocal melanomas have detectable chromosomal gains or losses [28]. In contrast, melanocytic nevi, with the exception of a subset of Spitz nevi with chromosome 11p gain, do not exhibit such aberrations. Chromosomal gains and losses in melanoma are not randomly distributed in the genome. They occur

repeatedly at chromosomal loci that provide a selective growth advantage for the tumor. This fundamental difference in the genomes of melanomas and nevi facilitated the development of molecular assays that can assist in the diagnosis of melanocytic tumors.

4.6 Fluorescent In Situ Hybridization (FISH)

Fluorescent in situ hybridization (FISH) employs fluorescently labeled oligonucleotide probes targeting specific chromosomal loci to assess for copy number changes in tumor cells. Multi-probe FISH assays can simultaneously detect copy number changes at multiple chromosomal loci and are currently clinically available to assist in the diagnosis of melanocytic tumors. Utilizing previous cytogenetic data of recurrent chromosomal aberrations in melanoma, a 4-probe FISH assay was developed in 2009 targeting three loci on chromosome 6 (6q23, 6p25, and CEP6) and one at locus 11q13 that discriminated unequivocal melanocytic nevi from melanomas with a sensitivity and specificity of 87 and 95 %, respectively [89]. Subsequent studies of this probe set involving specific types of melanocytic tumors yielded similar sensitivity and specificity, including:

- Distinguishing nevoid melanoma from mitotically active nevi [91]
- Distinguishing conjunctival melanoma from conjunctival nevi [43]
- Distinguishing metastatic melanoma in lymph nodes from nodal nevi [56]
- Distinguishing blue nevuslike melanoma from cellular blue nevi [76]
- Diagnosing atypical junctional melanocytic proliferations [86, 149]

A lower sensitivity of 47 % was found for discriminating desmoplastic melanomas from sclerosing nevi [87].

Most studies of FISH in melanocytic tumors have involved unambiguous melanocytic nevi and melanomas. However, the clinical utility of FISH as a diagnostic aid is contingent on its ability to discriminate between benign and malignant tumors with ambiguous histopathologic features. Ambiguous spitzoid neoplasms are the most frequent tumors for which FISH is utilized [150]. Other frequent tumor types for which FISH is ordered include atypical blue nevuslike proliferations, dysplastic nevuslike neoplasms, biphasic tumors (i.e., combined nevus or melanoma arising in a nevus), possible nevoid melanoma, and acral or mucosal tumors. Difficulty in collecting large cohorts of ambiguous melanocytic tumors with long-term follow-up has resulted in a paucity of FISH studies in such tumors. The largest study of the chromosome 6 and 11 FISH assay in ambiguous melanocytic tumors yielded a lower sensitivity of 43 % (9/21) for the detection of ambiguous tumors with lymph node or distant metastasis [203], raising concern about the generalizability of prior FISH studies to ambiguous tumors for which the test is clinically used.

A more recent study found increased sensitivity for the detection of spitzoid melanoma with the addition of probes detecting homozygous loss of 9p21 where the *CDKN2A* gene resides [77]. Chromosome 9p21 loss has also been associated with increased risk for metastasis in atypical spitzoid tumors in children [88].

A new FISH assay including probes targeting chromosomal loci 9p21, 6p25, 8q24, and 11q13 reportedly has increased sensitivity and specificity of 94 and 98 %, respectively, in distinguishing unequivocal melanomas and nevi [90].

4.7 Comparative Genomic Hybridization (CGH)

Comparative genomic hybridization (CGH) involves extraction and fluorescent labeling of tumor DNA, with subsequent competitive hybridization against control DNA to metaphase chromosomes or DNA microarrays to detect chromosomal gains or losses. Array-based CGH offers higher resolution of the genome and is considered by many to be the current method of choice in melanoma diagnostics. Compared to FISH, which targets a limited number of chromosomal loci, CGH provides a representative view of the entire genome and thus has greater potential to detect multiple chromosomal aberrations. With the vast majority of melanomas possessing multiple chromosomal gains and losses in their genomes, CGH can provide valuable diagnostic information to assist pathologists in the diagnosis of melanocytic tumors with conflicting histopathologic findings.

Past CGH analysis of melanomas demonstrated different patterns of chromosomal aberrations between melanomas arising in chronically sun-damaged skin, intermittently sun-exposed skin, and acral and mucosal melanomas [54]. Acral and mucosal melanomas arise through a non-UV light-dependent pathway that is characterized by marked genomic instability with chromosomal amplifications. Such amplifications are presented to a much lesser extent in the other two categories. In addition, these amplifications occur early in tumorigenesis in many acral melanomas preceding the invasive stage of disease [151] and can be relatively easily detected with CGH or FISH if the diagnosis is in question.

CGH in Blue, Spitz, and Congenital Melanocytic Nevi and Their Histopathologic Mimics

CGH studies of the spectrum of melanocytic tumors resembling blue nevi, Spitz nevi, and congenital nevi have found unique genomic alterations in these types of neoplasms that assist in differentiating benign and malignant tumors. Cellular blue nevi and the majority of ambiguous blue nevuslike proliferations do not have chromosomal aberrations detectable by CGH [136]. In contrast, blue nevuslike melanomas have ≥3 chromosomal aberrations. Similarly, routine congenital melanocytic nevi do not exhibit chromosomal aberrations. Benign hypercellular proliferations within congenital nevi ("proliferative nodules") can clinically and histopathologically mimic melanoma. CGH analysis of such proliferations shows gain or loss of entire chromosomes, particularly loss of chromosome 7, 9, or 10, which is distinct from the multiple gains and losses of chromosomal fragments that typify melanoma arising in congenital melanocytic nevi [29].

Spitz tumors often exhibit the greatest degree of histopathologic ambiguity. The majority of Spitz nevi do not have detectable chromosomal aberrations by CGH,

excluding a subset of desmoplastic Spitz nevi that harbor *HRAS* mutations and/or isolated gain of chromosome 11p where *HRAS* resides [27]. Another subset of apparently benign spitzoid neoplasms has chromosome 3p21 loss (*BAP1* tumor suppressor gene locus), which can be associated with a *BAP1*-mutant germline syndrome of amelanotic spitzoid nevi, cutaneous and uveal melanomas, and mesothelioma [211]. Chromosomal aberrations other than isolated 3p loss or isolated 11p gain are concerning for spitzoid melanoma, especially if multiple chromosomal gains or losses are detected.

4.8 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and Next-Generation Sequencing

Currently, FISH and CGH are the primary molecular tests clinically available to assist in the diagnosis of histopathologically ambiguous melanocytic tumors. A 23-gene expression signature utilizing qualitative reverse transcriptase polymerase chain reaction (qRT-PCR) has also recently been developed for this purpose. Gene expression measurements from the assay are analyzed to generate a score that reports a lesion as being consistent with a benign nevus or a malignant melanoma. The signature is marketed under the name Myriad myPath™ Melanoma and is undergoing clinical validation to differentiate benign nevi from malignant melanoma across a variety of histological subtypes. Other molecular techniques are currently utilized for prognosis and directing treatment. A qRT-PCR expression profile assay has been developed by Castle Biosciences© as a prognostic tool for predicting metastatic risk in Stages I or II melanoma and is marketed under the test name DecisionDx-Melanoma. Based on analysis of the expression of 31 genes, tumors are classified as low (3 %) or high (69 %) risk for developing metastasis within 5 years. The validation studies for these tests were presented at the 2013 (Castle) and 2014 (Myriad) American Society of Clinical Oncology meetings, but as of June 2014, neither has been published in a peer-reviewed journal.

Next-generation sequencing (NGS) is widely employed in research of melanocytic neoplasia and has many potential exciting uses in the diagnostic setting, but it has not yet transitioned into clinical practice for melanoma diagnostics. Foundation Medicine© currently offers the NGS clinical test FoundationOne™ for solid tumors, which can be used to characterize the spectrum of possible mutations in melanoma and help guide selection of mutation-specific treatments (e.g., BRAF inhibitors for BRAF mutant melanoma). This test uses NGS to sequence all exons of 236 cancer-related genes, including BRAF, HRAS, GNAQ, GNA11, KIT, BAP1, CDKN2A, and PTEN, as well as 47 introns from 19 genes often altered in cancers. However, as mutations in these genes can be seen in both melanomas and nevi, this test does not provide useful diagnostic information for ambiguous melanocytic tumors.

Table 4 compares the tests described above.

Table 4 Clinically available molecular tests for melanocytic tumors

	FISH	CGH	DecisionDx-Melanoma™	FoundationOne™	Myriad myPath™
Clinical use	Diagnostic test	Diagnostic test	qRT-PCR-based prognostic test	NGS mutation detection	qRT-PCR-based diagnostic test
Advantages of technique	– Minimal tissue required – Single cell resolution with intact tumor architecture permitting detection of intratumoral genetic heterogeneity – Ability to detect translocations	– Ability to assess all 23 chromosomes – No proficiency in fluorescent microscopy required	Reportedly provides additional prognostic value beyond the current AJCC staging data	– Single test providing full sequencing of genes associated with melanoma – Can assist in identifying potential treatments for advanced melanomas	– Reportedly provides additional diagnostic value in discriminating benign versus malignant melanocytic lesions
Limitations of technique	– Only visualizes a tiny fraction of the genome – Technical expertise in fluorescent microscopy required	– Limited ability to assess for intratumoral heterogeneity – Potential for dilution of test results from non-neoplastic DNA in sample (e.g., lymphocytes and fibroblasts)	– Limited to early Stages I–II melanoma – New test with little evidence published	– No diagnostic or prognostic value – High cost	– New test with little evidence published – Unclear if diagnostic accuracy is as reproducible in difficult histological cases – No correlation with outcome

All 4 platforms use formalin-fixed, paraffin-embedded tissue samples from routine biopsies. *FISH* fluorescent in situ hybridization, *CGH* comparative genomic hybridization, *qRT-PCR* qualitative reverse transcriptase polymerase chain reaction, and *NGS* next-generation sequencing

5 Conclusion

Effective melanoma early detection methods and technologies are likely to become increasingly available and useful, but one of the key challenges faced in the clinic is the integration of these modalities into current practice. In addition to describing current methods of melanoma detection, which include population-level approaches to early detection, the role of clinical examination-based methods, advanced technological detection methods, and molecular assay detection methods, a secondary purpose of this chapter has been to outline a rational approach to the use of these methods in the context of patient care. A pragmatic approach to maximize the strengths and minimize the weaknesses of the various technologies and methods is needed. We conclude that to be effective, this pragmatic approach must take into consideration logistical clinical issues related to the time, equipment, and expertise requirements for each technology as well as the cost and convenience for the patient. Developing creative strategies to utilize the most appropriate technology or method in the most cost- and time-effective manner is a critical step toward making early detection of virtually all melanomas a reality. Perhaps as never before, through the development of a comprehensive strategy to detect melanoma early, we have the opportunity to reduce suffering secondary to melanoma through early detection. Although there are some rapidly progressive forms of melanoma that are unlikely to be detectable prior to metastasis (e.g., aggressive nodular melanomas), successful application of early detection technologies and methods has enormous potential to save the lives of individuals with melanoma and reduce the costs associated with melanoma treatment.

References

1. Åberg P (2004) Skin cancer as seen by electrical impedance. Ph.D. thesis dissertation, Karolinska Institutet, Stockholm http://publications.ki.se/xmlui/bitstream/handle/10616/40085/thesis.pdf?sequence=1. Accessed 27 Feb 2014
2. Åberg P, Birgersson U, Elsner P et al (2011) Electrical impedance spectroscopy and the diagnostic accuracy for malignant melanoma. Exp Dermatol 20(8):648–652
3. Åberg P, Geladi P, Nicander I et al (2002) Variation of skin properties within human forearms demonstrated by non-invasive detection and multi-way analysis. Skin Res Technol 8(3):194–201
4. Abeytunge S, Li Y, Larson B et al (2013) Confocal microscopy with strip mosaicing for rapid imaging over large areas of excised tissue. J Biomed Opt 18(6):61227
5. Aitken JF, Elwood M, Baade PD et al (2010) Clinical whole-body skin examination reduces the incidence of thick melanomas. Int J Cancer 126(2):450–458
6. Aitken JF, Janda M, Lowe JB et al (2004) Prevalence of whole-body skin self-examination in a population at high risk for skin cancer (Australia). Cancer Causes Control 15(5):453–463
7. Aitken JF, Youl PH, Janda M et al (2006) Increase in skin cancer screening during a community-based randomized intervention trial. Int J Cancer 118(4):1010–1016
8. Al-Shakhli H, Harcourt D, Kenealy J (2006) Psychological distress surrounding diagnosis of malignant and nonmalignant skin lesions at a pigmented lesion clinic. J Plast Reconstr Aesthet Surg 59(5):479–486

9. Alarcon I, Carrera C, Palou J et al (2014) Impact of in vivo reflectance confocal microscopy on the number needed to treat melanoma in doubtful lesions. Br J Dermatol 170(4):802–808
10. Alexander H, Miller DL (1979) Determining skin thickness with pulsed ultra sound. J Invest Dermatol 72(1):17–19
11. Alexandrescu DT (2009) Melanoma costs: a dynamic model comparing estimated overall costs of various clinical stages. Dermatol Online J 15(11):1
12. Altamura D, Avramidis M, Menzies SW (2008) Assessment of the optimal interval for and sensitivity of short-term sequential digital dermoscopy monitoring for the diagnosis of melanoma. Arch Dermatol 144(4):502–506
13. Anderson RR, Parrish JA (1981) The optics of human skin. J Invest Dermatol 77(1):13–19
14. Andreassi L, Perotti R, Rubegni P et al (1999) Digital dermoscopy analysis for the differentiation of atypical nevi and early melanoma: a new quantitative semiology. Arch Dermatol 135(12):1459–1465
15. Argenziano G, Albertini G, Castagnetti F et al (2012) Early diagnosis of melanoma: what is the impact of dermoscopy? Dermatol Ther 25(5):403–409
16. Argenziano G, Catricala C, Ardigo M et al (2011) Seven-point checklist of dermoscopy revisited. Br J Dermatol 164(4):785–790
17. Argenziano G, Mordente I, Ferrara G et al (2008) Dermoscopic monitoring of melanocytic skin lesions: clinical outcome and patient compliance vary according to follow-up protocols. Br J Dermatol 159(2):331–336
18. Argenziano G, Puig S, Zalaudek I et al (2006) Dermoscopy improves accuracy of primary care physicians to triage lesions suggestive of skin cancer. J Clin Oncol 24(12):1877–1882
19. Argenziano G, Soyer HP, Chimenti S et al (2003) Dermoscopy of pigmented skin lesions: results of a consensus meeting via the Internet. J Am Acad Dermatol 48(5):679–693
20. Argenziano G, Zalaudek I, Ferrara G et al (2007) Dermoscopy features of melanoma incognito: indications for biopsy. J Am Acad Dermatol 56(3):508–513
21. Aspinwall LG, Leaf SL, Dola ER et al (2008) CDKN2A/p16 genetic test reporting improves early detection intentions and practices in high-risk melanoma families. Cancer Epidemiol Biomarkers Prev 17(6):1510–1519
22. Aspinwall LG, Taber JM, Leaf SL et al (2013) Melanoma genetic counseling and test reporting improve screening adherence among unaffected carriers 2 years later. Cancer Epidemiol Biomarkers Prev 22(10):1687–1697
23. Australian Cancer Network Melanoma Guidelines Revision Working Party (2008) Clinical practice guidelines for the management of melanoma in Australia and New Zealand. Cancer Council Australia and Australian Cancer Network, Sydney and New Zealand Guidelines Group. https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/cp111.pdf. Accessed 21 May 2014
24. Bafounta ML, Beauchet A, Aegerter P et al (2001) Is dermoscopy (epiluminescence microscopy) useful for the diagnosis of melanoma? Results of a meta-analysis using techniques adapted to the evaluation of diagnostic tests. Arch Dermatol 137(10):1343–1350
25. Balch CM, Gershenwald JE, Soong SJ et al (2009) Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27(36):6199–6206
26. Banky JP, Kelly JW, English DR et al (2005) Incidence of new and changed nevi and melanomas detected using baseline images and dermoscopy in patients at high risk for melanoma. Arch Dermatol 141(8):998–1006
27. Bastian BC, LeBoit PE, Pinkel D (2000) Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features. Am J Pathol 157(3):967–972
28. Bastian BC, Olshen AB, LeBoit PE et al (2003) Classifying melanocytic tumors based on DNA copy number changes. Am J Pathol 163(5):1765–1770
29. Bastian BC, Xiong J, Frieden IJ et al (2002) Genetic changes in neoplasms arising in congenital melanocytic nevi: differences between nodular proliferations and melanomas. Am J Pathol 161(4):1163–1169

30. Bauer P, Cristofolini P, Boi S et al (2000) Digital epiluminescence microscopy: usefulness in the differential diagnosis of cutaneous pigmentary lesions. A statistical comparison between visual and computer inspection. Melanoma Res 10(4):345–349
31. Berwick M, Armstrong BK, Ben-Porat L et al (2005) Sun exposure and mortality from melanoma. J Natl Cancer Inst 97(3):195–199
32. Berwick M, Begg CB, Fine JA et al (1996) Screening for cutaneous melanoma by skin self-examination. J Natl Cancer Inst 88(1):17–23
33. Binder M, Kittler H, Steiner A et al (1999) Reevaluation of the ABCD rule for epiluminescence microscopy. J Am Acad Dermatol 40(2 Pt 1):171–176
34. Binder M, Puespoeck-Schwarz M, Steiner A et al (1997) Epiluminescence microscopy of small pigmented skin lesions: short-term formal training improves the diagnostic performance of dermatologists. J Am Acad Dermatol 36(2 Pt 1):197–202
35. Bono A, Tolomio E, Trincone S et al (2006) Micro-melanoma detection: a clinical study on 206 consecutive cases of pigmented skin lesions with a diameter < or = 3 mm. Br J Dermatol 155(3):570–573
36. Boone M, Jemec GB, Del Marmol V (2012) High-definition optical coherence tomography enables visualization of individual cells in healthy skin: comparison to reflectance confocal microscopy. Exp Dermatol 21(10):740–744
37. Boone MA, Norrenberg S, Jemec GB et al (2012) Imaging of basal cell carcinoma by high-definition optical coherence tomography: histomorphological correlation. A pilot study. Br J Dermatol 167(4):856–864
38. Boone MA, Norrenberg S, Jemec GB et al (2014) High-definition optical coherence tomography imaging of melanocytic lesions: a pilot study. Arch Dermatol Res 306(1):11–26
39. Bowling J, Argenziano G, Azenha A et al (2007) Dermoscopy key points: recommendations from the international dermoscopy society. Dermatology 214(1):3–5
40. Braga JC, Macedo MP, Pinto C et al (2013) Learning reflectance confocal microscopy of melanocytic skin lesions through histopathologic transversal sections. PLoS ONE 8(12): e81205
41. Brezinski ME (2006) Optical coherence tomography: principles and applications. Academic Press, Amsterdam
42. Busam KJ, Charles C, Lohmann CM et al (2002) Detection of intraepidermal malignant melanoma in vivo by confocal scanning laser microscopy. Melanoma Res 12(4):349–355
43. Busam KJ, Fang Y, Jhanwar SC et al (2010) Distinction of conjunctival melanocytic nevi from melanomas by fluorescence in situ hybridization. J Cutan Pathol 37(2):196–203
44. Busam KJ, Hester K, Charles C et al (2001) Detection of clinically amelanotic malignant melanoma and assessment of its margins by in vivo confocal scanning laser microscopy. Arch Dermatol 137(7):923–929
45. Busam KJ, Marghoob AA, Halpern A (2005) Melanoma diagnosis by confocal microscopy: promise and pitfalls. J Invest Dermatol 125(3): vii
46. Carli P, De Giorgi V, Argenziano G et al (2002) Pre-operative diagnosis of pigmented skin lesions: in vivo dermoscopy performs better than dermoscopy on photographic images. J Eur Acad Dermatol Venereol 16(4):339–346
47. Carli P, De Giorgi V, Palli D et al (2003) Dermatologist detection and skin self-examination are associated with thinner melanomas: results from a survey of the Italian Multidisciplinary Group on Melanoma. Arch Dermatol 139(5):607–612
48. Cascinelli N, Ferrario M, Bufalino R et al (1992) Results obtained by using a computerized image analysis system designed as an aid to diagnosis of cutaneous melanoma. Melanoma Res 2(3):163–170
49. Chen Z, Milner TE, Srinivas S et al (1997) Noninvasive imaging of in vivo blood flow velocity using optical doppler tomography. Opt Lett 22(14):1119–1121
50. Chiu V, Won E, Malik M et al (2006) The use of mole-mapping diagrams to increase skin self-examination accuracy. J Am Acad Dermatol 55(2):245–250

51. Coleman AJ, Richardson TJ, Orchard G et al (2013) Histological correlates of optical coherence tomography in non-melanoma skin cancer. Skin Res Technol 19(1):10–19
52. Curchin CE, Wurm EM, Lambie D et al (2011) First experiences using reflectance confocal microscopy on equivocal skin lesions in Queensland. Australas J Dermatol 52(2):89–97
53. Curiel-Lewandrowski C, Chen SC, Swetter SM (2012) Screening and prevention measures for melanoma: is there a survival advantage? Curr Oncol Rep 14(5):458–467
54. Curtin JA, Fridlyand J, Kageshita T et al (2005) Distinct sets of genetic alterations in melanoma. N Engl J Med 353(20):2135–2147
55. Dalimier E, Salomon D (2012) Full-field optical coherence tomography: a new technology for 3D high-resolution skin imaging. Dermatology 224(1):84–92
56. Dalton SR, Gerami P, Kolaitis NA et al (2010) Use of fluorescence in situ hybridization (FISH) to distinguish intranodal nevus from metastatic melanoma. Am J Surg Pathol 34 (2):231–237
57. Davis KL, Mitra D, Kotapati S et al (2009) Direct economic burden of high-risk and metastatic melanoma in the elderly: evidence from the SEER-Medicare linked database. Appl Health Econ Health Policy 7(1):31–41
58. De Boer JF (2008) Spectral/Fourier domain optical coherence tomography. In: Drexler W, Fujimoto JG (eds) Optical coherence tomography: technology and applications. Springer, Berlin
59. de Giorgi V, Stante M, Massi D et al (2005) Possible histopathologic correlates of dermoscopic features in pigmented melanocytic lesions identified by means of optical coherence tomography. Exp Dermatol 14(1):56–59
60. Debarbieux S, Dalle S, Depaepe L et al (2013) Second primary melanomas treated with BRAF blockers: study by reflectance confocal microscopy. Br J Dermatol 168(6):1230–1235
61. Drexler W (2004) Ultrahigh-resolution optical coherence tomography. J Biomed Opt 9 (1):47–74
62. Dwyer PJ, DiMarzio CA, Rajadhyaksha M (2007) Confocal theta line-scanning microscope for imaging human tissues. Appl Opt 46(10):1843–1851
63. Eide MJ, Asgari MM, Fletcher SW et al (2013) Effects on skills and practice from a web-based skin cancer course for primary care providers. J Am Board Fam Med 26(6): 648–657
64. Eisemann N, Waldmann A, Geller AC et al (2014) Non-melanoma skin cancer incidence and impact of skin cancer screening on incidence. J Invest Dermatol 134(1):43–50
65. Emery JD, Hunter J, Hall PN et al (2010) Accuracy of SIAscopy for pigmented skin lesions encountered in primary care: development and validation of a new diagnostic algorithm. BMC Dermatol 10:9
66. Epstein DS, Lange JR, Gruber SB et al (1999) Is physician detection associated with thinner melanomas? JAMA 281(7):640–643
67. Farmer ER, Gonin R, Hanna MP (1996) Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol 27(6):528–531
68. Favazza CP, Jassim O, Cornelius LA et al (2011) In vivo photoacoustic microscopy of human cutaneous microvasculature and a nevus. J Biomed Opt 16(1):016015
69. Feit NE, Dusza SW, Marghoob AA (2004) Melanomas detected with the aid of total cutaneous photography. Br J Dermatol 150(4):706–714
70. Ferrara G, Argenziano G, Soyer HP et al (2002) Dermoscopic and histopathologic diagnosis of equivocal melanocytic skin lesions: an interdisciplinary study on 107 cases. Cancer 95 (5):1094–1100
71. Foster KR, Schwan HP (1989) Dielectric properties of tissues and biological materials: a critical review. Crit Rev Biomed Eng 17(1):25–104
72. Friedman RJ, Gutkowicz-Krusin D, Farber MJ et al (2008) The diagnostic performance of expert dermoscopists vs a computer-vision system on small-diameter melanomas. Arch Dermatol 144(4):476–482

73. Fujimoto J, Drexler W (2008) Introduction to optical coherence tomography. In: Drexler W, Fujimoto JG (eds) Optical coherence tomography: technology and applications. Springer, Berlin
74. Gambichler T, Moussa G, Sand M et al (2005) Applications of optical coherence tomography in dermatology. J Dermatol Sci 40(2):85–94
75. Gambichler T, Orlikov A, Vasa R et al (2007) In vivo optical coherence tomography of basal cell carcinoma. J Dermatol Sci 45(3):167–173
76. Gammon B, Beilfuss B, Guitart J et al (2011) Fluorescence in situ hybridization for distinguishing cellular blue nevi from blue nevus-like melanoma. J Cutan Pathol 38(4): 335–341
77. Gammon B, Beilfuss B, Guitart J et al (2012) Enhanced detection of spitzoid melanomas using fluorescence in situ hybridization with 9p21 as an adjunctive probe. Am J Surg Pathol 36(1):81–88
78. Garbe C, Peris K, Hauschild A et al (2012) Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline–Update 2012. Eur J Cancer 48(15):2375–2390
79. Garcia-Uribe A, Zou J, Duvic M et al (2012) In vivo diagnosis of melanoma and nonmelanoma skin cancer using oblique incidence diffuse reflectance spectrometry. Cancer Res 72(11):2738–2745
80. Gareau D, Hennessy R, Wan E et al (2010) Automated detection of malignant features in confocal microscopy on superficial spreading melanoma versus nevi. J Biomed Opt 15 (6):061713
81. Gareau DS, Abeytunge S, Rajadhyaksha M (2009) Line-scanning reflectance confocal microscopy of human skin: comparison of full-pupil and divided-pupil configurations. Opt Lett 34(20):3235–3237
82. Garg A, Wang J, Reddy SB et al (2014) The Integrated Skin Exam film: an educational intervention to promote early detection of melanoma by medical students. J Am Acad Dermatol 70(1):115–119
83. Geller AC, Sober AJ, Zhang Z et al (2002) Strategies for improving melanoma education and screening for men age > or = 50 years: findings from the American Academy of Dermatological National Skin Cancer Sreening Program. Cancer 95(7):1554–1561
84. Geller AC, Venna S, Prout M et al (2002) Should the skin cancer examination be taught in medical school? Arch Dermatol 138(9):1201–1203
85. Geller AC, Zhang Z, Sober AJ et al (2003) The first 15 years of the American Academy of Dermatology skin cancer screening programs: 1985–1999. J Am Acad Dermatol 48(1):34–41
86. Gerami P, Barnhill RL, Beilfuss BA et al (2010) Superficial melanocytic neoplasms with pagetoid melanocytosis: a study of interobserver concordance and correlation with FISH. Am J Surg Pathol 34(6):816–821
87. Gerami P, Beilfuss B, Haghighat Z et al (2011) Fluorescence in situ hybridization as an ancillary method for the distinction of desmoplastic melanomas from sclerosing melanocytic nevi. J Cutan Pathol 38(4):329–334
88. Gerami P, Cooper C, Bajaj S et al (2013) Outcomes of atypical spitz tumors with chromosomal copy number aberrations and conventional melanomas in children. Am J Surg Pathol 37(9):1387–1394
89. Gerami P, Jewell SS, Morrison LE et al (2009) Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. Am J Surg Pathol 33(8): 1146–1156
90. Gerami P, Li G, Pouryazdanparast P et al (2012) A highly specific and discriminatory FISH assay for distinguishing between benign and malignant melanocytic neoplasms. Am J Surg Pathol 36(6):808–817
91. Gerami P, Wass A, Mafee M et al (2009) Fluorescence in situ hybridization for distinguishing nevoid melanomas from mitotically active nevi. Am J Surg Pathol 33 (12):1783–1788

92. Gerbert B, Maurer T, Berger T et al (1996) Primary care physicians as gatekeepers in managed care. Primary care physicians' and dermatologists' skills at secondary prevention of skin cancer. Arch Dermatol 132(9):1030–1038
93. Gerger A, Hofmann-Wellenhof R, Langsenlehner U et al (2008) In vivo confocal laser scanning microscopy of melanocytic skin tumours: diagnostic applicability using unselected tumour images. Br J Dermatol 158(2):329–333
94. Gerger A, Koller S, Kern T et al (2005) Diagnostic applicability of in vivo confocal laser scanning microscopy in melanocytic skin tumors. J Invest Dermatol 124(3):493–498
95. Gerger A, Koller S, Weger W et al (2006) Sensitivity and specificity of confocal laser-scanning microscopy for in vivo diagnosis of malignant skin tumors. Cancer 107 (1):193–200
96. González S, Gill M, Halpern AC (2008) Reflectance confocal microscopy of cutaneous tumors: an atlas with clinical, dermoscopic, and histological correlations. Informa Healthcare; distributed in North and South America. Taylor & Francis, London
97. Goodson AG, Florell SR, Hyde M et al (2010) Comparative analysis of total body and dermatoscopic photographic monitoring of nevi in similar patient populations at risk for cutaneous melanoma. Dermatol Surg 36(7):1087–1098
98. Goulart JM, Quigley EA, Dusza S et al (2011) Skin cancer education for primary care physicians: a systematic review of published evaluated interventions. J Gen Intern Med 26 (9):1027–1035
99. Green PS, Arditi M (1985) Ultrasonic reflex transmission imaging. Ultrason Imaging 7 (3):201–214
100. Griss P, Enoksson P, Tolvanen-Laakso HK et al (2001) Micromachined electrodes for biopotential measurements. J Microelectromech Syst 10:10–16
101. Guitera P, Menzies SW, Longo C et al (2012) In vivo confocal microscopy for diagnosis of melanoma and basal cell carcinoma using a two-step method: analysis of 710 consecutive clinically equivocal cases. J Invest Dermatol 132(10):2386–2394
102. Guitera P, Pellacani G, Crotty KA et al (2010) The impact of in vivo reflectance confocal microscopy on the diagnostic accuracy of lentigo maligna and equivocal pigmented and nonpigmented macules of the face. J Invest Dermatol 130(8):2080–2091
103. Guitera P, Pellacani G, Longo C et al (2009) In vivo reflectance confocal microscopy enhances secondary evaluation of melanocytic lesions. J Invest Dermatol 129(1):131–138
104. Guy GP Jr, Ekwueme DU, Tangka FK et al (2012) Melanoma treatment costs: a systematic review of the literature, 1990-2011. Am J Prev Med 43(5):537–545
105. Halpern AC (2003) Total body skin imaging as an aid to melanoma detection. Semin Cutan Med Surg 22(1):2–8
106. Hanrahan PF, D'Este CA, Menzies SW et al (2002) A randomised trial of skin photography as an aid to screening skin lesions in older males. J Med Screen 9(3):128–132
107. Harland CC, Kale SG, Jackson P et al (2000) Differentiation of common benign pigmented skin lesions from melanoma by high-resolution ultrasound. Br J Dermatol 143(2):281–289
108. Hillner BE, Kirkwood JM, Agarwala SS (2001) Burden of illness associated with metastatic melanoma: an audit of 100 consecutive referral center cases. Cancer 91(9):1814–1821
109. Hofmann-Wellenhof R, Pellacani G, Malvehy J et al (eds) (2012) Reflectance confocal microscopy for skin diseases. Springer, Berlin
110. Huzaira M, Rius F, Rajadhyaksha M et al (2001) Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. J Invest Dermatol 116(6):846–852
111. Izatt JA, Choma MA (2008) Theory of optical coherence tomography. In: Drexler W, Fujimoto JG (eds) Optical coherence tomography: technology and applications. Springer, Berlin
112. Jaimes N, Dusza SW, Quigley EA et al (2013) Influence of time on dermoscopic diagnosis and management. Australas J Dermatol 54(2):96–104

113. Janda M, Neale RE, Youl P et al (2011) Impact of a video-based intervention to improve the prevalence of skin self-examination in men 50 years or older: the randomized skin awareness trial. Arch Dermatol 147(7):799–806
114. Janda M, Youl PH, Lowe JB et al (2004) Attitudes and intentions in relation to skin checks for early signs of skin cancer. Prev Med 39(1):11–18
115. Jemec GB, Gniadecka M, Ulrich J (2000) Ultrasound in dermatology. Part I. High frequency ultrasound. Eur J Dermatol 10(6):492–497
116. Kantor J, Kantor DE (2009) Routine dermatologist-performed full-body skin examination and early melanoma detection. Arch Dermatol 145(8):873–876
117. Kasparian NA, Branstrom R, Chang YM et al (2012) Skin examination behavior: the role of melanoma history, skin type, psychosocial factors, and region of residence in determining clinical and self-conducted skin examination. Arch Dermatol 148(10):1142–1151
118. Katalinic A, Waldmann A, Weinstock MA et al (2012) Does skin cancer screening save lives? An observational study comparing trends in melanoma mortality in regions with and without screening. Cancer 118(21):5395–5402
119. Kittler H, Binder M (2002) Follow-up of melanocytic skin lesions with digital dermoscopy: risks and benefits. Arch Dermatol 138(10):1379
120. Kittler H, Guitera P, Riedl E et al (2006) Identification of clinically featureless incipient melanoma using sequential dermoscopy imaging. Arch Dermatol 142(9):1113–1119
121. Kittler H, Pehamberger H, Wolff K et al (2002) Diagnostic accuracy of dermoscopy. Lancet Oncol 3(3):159–165
122. Koh HK, Judge CM, Robbins H et al (2005) The first decade of the Massachusetts Tobacco Control Program. Public Health Rep 120(5):482–495
123. Koller S, Wiltgen M, Ahlgrimm-Siess V et al (2011) In vivo reflectance confocal microscopy: automated diagnostic image analysis of melanocytic skin tumours. J Eur Acad Dermatol Venereol 25(5):554–558
124. Kollias N, Baqer A (1986) On the assessment of melanin in human skin in vivo. Photochem Photobiol 43(1):49–54
125. Kovalyshyn I, Dusza SW, Siamas K et al (2011) The impact of physician screening on melanoma detection. Arch Dermatol 147(11):1269–1275
126. Kumagai K, Koike H, Nagaoka R et al (2012) High-resolution ultrasound imaging of human skin in vivo by using three-dimensional ultrasound microscopy. Ultrasound Med Biol 38 (10):1833–1838
127. Kurugol S, Dy JG, Brooks DH et al (2011) Pilot study of semiautomated localization of the dermal/epidermal junction in reflectance confocal microscopy images of skin. J Biomed Opt 16(3):036005
128. Langley RG, Rajadhyaksha M, Dwyer PJ et al (2001) Confocal scanning laser microscopy of benign and malignant melanocytic skin lesions in vivo. J Am Acad Dermatol 45(3):365–376
129. LeBlanc WG, Vidal L, Kirsner RS et al (2008) Reported skin cancer screening of US adult workers. J Am Acad Dermatol 59(1):55–63
130. Lindelöf B, Hedblad MA (1994) Accuracy in the clinical diagnosis and pattern of malignant melanoma at a dermatological clinic. J Dermatol 21(7):461–464
131. Longo C, Farnetani F, Ciardo S et al (2013) Is confocal microscopy a valuable tool in diagnosing nodular lesions? A study of 140 cases. Br J Dermatol 169(1):58–67
132. Lucas CR, Sanders LL, Murray JC et al (2003) Early melanoma detection: nonuniform dermoscopic features and growth. J Am Acad Dermatol 48(5):663–671
133. Maier T, Braun-Falco M, Hinz T et al (2013) Morphology of basal cell carcinoma in high definition optical coherence tomography: en-face and slice imaging mode, and comparison with histology. J Eur Acad Dermatol Venereol 27(1):e97–e104
134. Maier T, Braun-Falco M, Laubender RP et al (2013) Actinic keratosis in the en-face and slice imaging mode of high-definition optical coherence tomography and comparison with histology. Br J Dermatol 168(1):120–128
135. Maiman T (1962) Solid state laser and iraser studies. Solid State Electron 4 (Oct): 236–249

136. Maize JC Jr, McCalmont TH, Carlson JA et al (2005) Genomic analysis of blue nevi and related dermal melanocytic proliferations. Am J Surg Pathol 29(9):1214–1220
137. Malvehy J, Aguilera P, Carrera C et al (2013) Ex vivo dermoscopy for biobank-oriented sampling of melanoma. JAMA Dermatol 149(9):1060–1067
138. Malvehy J, Hauschild A, Curiel-Lewandrowski C et al (2014) Clinical performance of the Nevisense system in cutaneous melanoma detection: an international, multi-centre, prospective and blinded clinical trial on efficacy and safety. Br J Dermatol
139. Malvehy J, Puig S (2002) Follow-up of melanocytic skin lesions with digital total-body photography and digital dermoscopy: a two-step method. Clin Dermatol 20(3):297–304
140. Marghoob AA, Malvehy J, Braun RP (2012) Atlas of dermoscopy, 2nd edn. Informa Healthcare, London
141. Marghoob AA, Swindle LD, Moricz CZ et al (2003) Instruments and new technologies for the in vivo diagnosis of melanoma. J Am Acad Dermatol 49(5):777–797; quiz 798–779
142. Marschall S, Sander B, Mogensen M et al (2011) Optical coherence tomography-current technology and applications in clinical and biomedical research. Anal Bioanal Chem 400 (9):2699–2720
143. Menzies SW (2013) Evidence-based dermoscopy. Dermatol Clin 31(4):521–524, vii
144. Menzies SW, Bischof L, Talbot H et al (2005) The performance of SolarScan: an automated dermoscopy image analysis instrument for the diagnosis of primary melanoma. Arch Dermatol 141(11):1388–1396
145. Minsky M (1957) Microscopy apparatus. US patent 3,013,467, 19 Dec 1961
146. Mogensen M, Morsy HA, Thrane L et al (2008) Morphology and epidermal thickness of normal skin imaged by optical coherence tomography. Dermatology 217(1):14–20
147. Mogensen M, Thrane L, Jorgensen TM et al (2009) OCT imaging of skin cancer and other dermatological diseases. J Biophotonics 2(6–7):442–451
148. Moore MM, Geller AC, Zhang Z et al (2006) Skin cancer examination teaching in US medical education. Arch Dermatol 142(4):439–444
149. Newman MD, Mirzabeigi M, Gerami P (2009) Chromosomal copy number changes supporting the classification of lentiginous junctional melanoma of the elderly as a subtype of melanoma. Mod Pathol 22(9):1258–1262
150. North JP, Garrido MC, Kolaitis NA et al (2014) Fluorescence in situ hybridization as an ancillary tool in the diagnosis of ambiguous melanocytic neoplasms: a review of 804 cases. Am J Surg Pathol 38(6):824–831
151. North JP, Kageshita T, Pinkel D et al (2008) Distribution and significance of occult intraepidermal tumor cells surrounding primary melanoma. J Invest Dermatol 128(8): 2024–2030
152. Oliveria SA, Christos PJ, Halpern AC et al (1999) Evaluation of factors associated with skin self-examination. Cancer Epidemiol Biomarkers Prev 8(11):971–978
153. Oliveria SA, Dusza SW, Phelan DL et al (2004) Patient adherence to skin self-examination: effect of nurse intervention with photographs. Am J Prev Med 26(2):152–155
154. Pagnanelli G, Soyer HP, Argenziano G et al (2003) Diagnosis of pigmented skin lesions by dermoscopy: web-based training improves diagnostic performance of non-experts. Br J Dermatol 148(4):698–702
155. Patel JK, Konda S, Perez OA et al (2008) Newer technologies/techniques and tools in the diagnosis of melanoma. Eur J Dermatol 18(6):617–631
156. Pellacani G, Cesinaro AM, Seidenari S (2005) Reflectance-mode confocal microscopy for the in vivo characterization of pagetoid melanocytosis in melanomas and nevi. J Invest Dermatol 125(3):532–537
157. Pellacani G, Cesinaro AM, Seidenari S (2005) Reflectance-mode confocal microscopy of pigmented skin lesions—improvement in melanoma diagnostic specificity. J Am Acad Dermatol 53(6):979–985
158. Pellacani G, De Pace B, Reggiani C et al (2014) Distinct melanoma types based on reflectance confocal microscopy. Exp Dermatol 23(6):414

159. Pellacani G, Guitera P, Longo C et al (2007) The impact of in vivo reflectance confocal microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol 127(12):2759–2765
160. Pellacani G, Pepe P, Casari A et al (2014) Reflectance confocal microscopy as a second-level examination in skin oncology improves diagnostic accuracy and saves unnecessary excisions: a longitudinal prospective study. Br J Dermatol
161. Petran M, Hadravsky M, Egger MD et al (1968) Tandem-scanning reflected-light microscope. J Opt Soc Am 58(5):661–664
162. Podoleanu AG (2005) Optical coherence tomography. Br J Radiol 78(935):976–988
163. Pollitt RA, Geller AC, Brooks DR et al (2009) Efficacy of skin self-examination practices for early melanoma detection. Cancer Epidemiol Biomarkers Prev 18(11):3018–3023
164. Puig S, Argenziano G, Zalaudek I et al (2007) Melanomas that failed dermoscopic detection: a combined clinicodermoscopic approach for not missing melanoma. Dermatol Surg 33 (10):1262–1273
165. Pupelli G, Longo C, Veneziano L et al (2013) Small-diameter melanocytic lesions: morphological analysis by means of in vivo confocal microscopy. Br J Dermatol 168 (5):1027–1033
166. Rajadhyaksha M, Anderson RR, Webb RH (1999) Video-rate confocal scanning laser microscope for imaging human tissues in vivo. Appl Opt 38(10):2105–2115
167. Rajadhyaksha M, Gonzalez S, Zavislan JM et al (1999) In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J Invest Dermatol 113(3):293–303
168. Rajadhyaksha M, Grossman M, Esterowitz D et al (1995) In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol 104(6): 946–952
169. Rallan D, Bush NL, Bamber JC et al (2007) Quantitative discrimination of pigmented lesions using three-dimensional high-resolution ultrasound reflex transmission imaging. J Invest Dermatol 127(1):189–195
170. Rice ZP, Weiss FJ, DeLong LK et al (2010) Utilization and rationale for the implementation of total body (digital) photography as an adjunct screening measure for melanoma. Melanoma Res 20(5):417–421
171. Rigel DS, Russak J, Friedman R (2010) The evolution of melanoma diagnosis: 25 years beyond the ABCDs. CA Cancer J Clin 60(5):301–316
172. Risser J, Pressley Z, Veledar E et al (2007) The impact of total body photography on biopsy rate in patients from a pigmented lesion clinic. J Am Acad Dermatol 57(3):428–434
173. Robinson JK, Turrisi R, Mallett K et al (2010) Comparing the efficacy of an in-person intervention with a skin self-examination workbook. Arch Dermatol 146(1):91–94
174. Robinson JK, Turrisi R, Stapleton J (2007) Efficacy of a partner assistance intervention designed to increase skin self-examination performance. Arch Dermatol 143(1):37–41
175. Rodriguez GL, Ma F, Federman DG et al (2007) Predictors of skin cancer screening practice and attitudes in primary care. J Am Acad Dermatol 57(5):775–781
176. Roeseler A, Burns D (2010) The quarter that changed the world. Tob Control 19(Suppl 1): i3–i15
177. Salerni G, Carrera C, Lovatto L et al (2012) Characterization of 1152 lesions excised over 10 years using total-body photography and digital dermatoscopy in the surveillance of patients at high risk for melanoma. J Am Acad Dermatol 67(5):836–845
178. Salerni G, Carrera C, Lovatto L et al (2012) Benefits of total body photography and digital dermatoscopy ("two-step method of digital follow-up") in the early diagnosis of melanoma in patients at high risk for melanoma. J Am Acad Dermatol 67(1):e17–e27
179. Salerni G, Terán T, Puig S et al (2013) Meta-analysis of digital dermoscopy follow-up of melanocytic skin lesions: a study on behalf of the International Dermoscopy Society. J Eur Acad Dermatol Venereol 27(7):805–814

180. Saraiya M, Hall HI, Thompson T et al (2004) Skin cancer screening among U.S. adults from 1992, 1998, and 2000 National Health Interview Surveys. Prev Med 39(2):308–314
181. Schmitt J (1999) Optical coherence tomography (OCT): a review. IEEE J Sel Top Quantum Electron 5:1205–1215
182. Schneider JS, Moore DH 2nd, Mendelsohn ML (2008) Screening program reduced melanoma mortality at the Lawrence Livermore National Laboratory, 1984–1996. J Am Acad Dermatol 58(5):741–749
183. Schwan HP (1957) Electrical properties of tissue and cell suspensions. Adv Biol Med Phys 5:147–209
184. Scope A, Benvenuto-Andrade C, Agero AL et al (2007) In vivo reflectance confocal microscopy imaging of melanocytic skin lesions: consensus terminology glossary and illustrative images. J Am Acad Dermatol 57(4):644–658
185. Scope A, Busam KJ, Malvehy J et al (2007) Ex vivo dermoscopy of melanocytic tumors: time for dermatopathologists to learn dermoscopy. Arch Dermatol 143(12):1548–1552
186. U.S. Preventive Services Task Force (2009) Screening for skin cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 150(3):188–193
187. Segura S, Puig S, Carrera C et al (2009) Development of a two-step method for the diagnosis of melanoma by reflectance confocal microscopy. J Am Acad Dermatol 61(2):216–229
188. Seidler AM, Pennie ML, Veledar E et al (2010) Economic burden of melanoma in the elderly population: population-based analysis of the Surveillance, Epidemiology, and End Results (SEER)–Medicare data. Arch Dermatol 146(3):249–256
189. Shaikh WR, Geller A, Alexander G et al (2012) Developing an interactive web-based learning program on skin cancer: the learning experiences of clinical educators. J Cancer Educ 27(4):709–716
190. Siegel R, Ma J, Zou Z et al (2014) Cancer statistics. CA Cancer J Clin 64(1):9–29
191. Smith L, Macneil S (2011) State of the art in non-invasive imaging of cutaneous melanoma. Skin Res Technol 17(3):257–269
192. Smith RA, Brooks D, Cokkinides V et al (2013) Cancer screening in the United States, 2013: a review of current American cancer society guidelines, current issues in cancer screening, and new guidance on cervical cancer screening and lung cancer screening. CA Cancer J Clin 63(2):88–105
193. Soyer HP, Argenziano G, Zalaudek I et al (2004) Three-point checklist of dermoscopy. A new screening method for early detection of melanoma. Dermatology 208(1):27–31
194. Stefanowska J, Zakowiecki D, Cal K (2010) Magnetic resonance imaging of the skin. J Eur Acad Dermatol Venereol 24(8):875–880
195. Stevenson AD, Mickan S, Mallett S et al (2013) Systematic review of diagnostic accuracy of reflectance confocal microscopy for melanoma diagnosis in patients with clinically equivocal skin lesions. Dermatol Pract Concept 3(4):19–27
196. Stratigos AJ, Forsea AM, van der Leest RJ et al (2012) Euromelanoma: a dermatology-led European campaign against nonmelanoma skin cancer and cutaneous melanoma. Past, present and future. Br J Dermatol 167 Suppl 2:99–104
197. Swetter SM, Johnson TM, Miller DR et al (2009) Melanoma in middle-aged and older men: a multi-institutional survey study of factors related to tumor thickness. Arch Dermatol 145(4): 397–404
198. Swetter SM, Pollitt RA, Johnson TM et al (2012) Behavioral determinants of successful early melanoma detection: role of self and physician skin examination. Cancer 118(15):3725–3734
199. Taber JM, Aspinwall LG, Leaf SL et al (2013) Partner involvement in conduct of skin self-examinations remains low following CDKN2A/p16 genetic test reporting and counseling. J Am Acad Dermatol 69(5):842–844
200. Terushkin V, Halpern AC (2009) Melanoma early detection. Hematol Oncol Clin North Am 23(3):481–500, viii
201. Tsao H, Rogers GS, Sober AJ (1998) An estimate of the annual direct cost of treating cutaneous melanoma. J Am Acad Dermatol 38(5 Pt 1):669–680

202. Vakoc BJ, Fukumura D, Jain RK et al (2012) Cancer imaging by optical coherence tomography: preclinical progress and clinical potential. Nat Rev Cancer 12(5):363–368
203. Vergier B, Prochazkova-Carlotti M, de la Fouchardiere A et al (2011) Fluorescence in situ hybridization, a diagnostic aid in ambiguous melanocytic tumors: European study of 113 cases. Mod Pathol 24(5):613–623
204. Vestergaard ME, Macaskill P, Holt PE et al (2008) Dermoscopy compared with naked eye examination for the diagnosis of primary melanoma: a meta-analysis of studies performed in a clinical setting. Br J Dermatol 159(3):669–676
205. Waldmann A, Nolte S, Weinstock MA et al (2012) Skin cancer screening participation and impact on melanoma incidence in Germany–an observational study on incidence trends in regions with and without population-based screening. Br J Cancer 106(5):970–974
206. Wang RK, Nuttall AL (2010) Phase-sensitive optical coherence tomography imaging of the tissue motion within the organ of Corti at a subnanometer scale: a preliminary study. J Biomed Opt 15(5):056005
207. Wang SQ, Dusza SW, Scope A et al (2008) Differences in dermoscopic images from nonpolarized dermoscope and polarized dermoscope influence the diagnostic accuracy and confidence level: a pilot study. Dermatol Surg 34(10):1389–1395
208. Wang SQ, Kopf AW, Koenig K et al (2004) Detection of melanomas in patients followed up with total cutaneous examinations, total cutaneous photography, and dermoscopy. J Am Acad Dermatol 50(1):15–20
209. Weinstock MA, Martin RA, Risica PM et al (1999) Thorough skin examination for the early detection of melanoma. Am J Prev Med 17(3):169–175
210. Weinstock MA, Risica PM, Martin RA et al (2007) Melanoma early detection with thorough skin self-examination: the “check it out” randomized trial. Am J Prev Med 32(6):517–524
211. Wiesner T, Obenauf AC, Murali R et al (2011) Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 43(10):1018–1021
212. Wiltgen M, Gerger A, Wagner C et al (2008) Automatic identification of diagnostic significant regions in confocal laser scanning microscopy of melanocytic skin tumors. Methods Inf Med 47(1):14–25
213. Yabroff KR, Lamont EB, Mariotto A et al (2008) Cost of care for elderly cancer patients in the United States. J Natl Cancer Inst 100(9):630–641
214. Yamamoto T, Yamamoto Y (1976) Electrical properties of the epidermal stratum corneum. Med Biol Eng 14(2):151–158
215. Ziolkowska M, Philipp C, Liebscher J et al (2009) OCT of healthy skin, actinic skin and NMSC lesions. Med Laser Appl 24:256–264
216. Zysk AM, Nguyen FT, Oldenburg AL et al (2007) Optical coherence tomography: a review of clinical development from bench to bedside. J Biomed Opt 12(5):051403