Published in final edited form as:
Conf Proc IEEE Eng Med Biol Soc. 2009 ; 2009: 3231–3233. doi:10.1109/IEMBS.2009.5333600.

Confocal microscopy of skin cancers: Translational advances toward clinical utility

Milind Rajadhyaksha
Dermatology Service, Memorial Sloan-Kettering Cancer Center, 160 East 53rdStreet, New York, NY 10022, 212 610 0832

Milind Rajadhyaksha: rajadhym@mskcc.org

Abstract

Recent advances in translational research in and technology for confocal microscopy of skin cancers, toward clinical applications, are described. Advances in translational research are in diagnosis of melanoma in vivo, pre-operative mapping of lentigo maligna melanoma margins to guide surgery and intra-operative imaging of residual basal cell carcinomas to guide shave-biopsy. Advances in technology include mosaicing microscopy for detection of basal cell carcinomas in large areas of excised tissue, toward rapid pathology-at-the-bedside, and development of small, simple and low-cost line-scanning confocal microscopes for worldwide use in diverse primary healthcare settings. Current limitations and future opportunities and challenges for both clinicians and technologists are discussed.

I. INTRODUCTION

Marvin Minsky invented the confocal microscope in 1957, but the technology for clinical imaging and translation toward noninvasive real-time detection, diagnosis and management of skin cancers has only evolved during the past decade. A confocal microscope consists of a point source of light, condenser and objective lenses, and a point detector. Point illumination is achieved by focusing a point or small source of light into the sample. Point detection is achieved by placing a pinhole in front of the detector. The pinhole collects light emanating only from the focus and blocks out-of-focus light from elsewhere. The point source of light, the spot within the sample and the pinhole lie in optically conjugate focal planes, leading to the “confocal” condition. This arrangement is sensitive mainly to the illuminated spot at the focus and mostly insensitive to out-of-focus light from all other locations. Scanning the spot in the focal plane of the objective lens enables the noninvasive imaging of a thin plane within a thick object – known as optical sectioning. The optical sectioning capability of confocal microscopy is thus an attractive adjunct to physical sectioning. Imaging of thin optical sections is noninvasive, in contrast to the physical sectioning of tissue for conventional histology.

Confocal microscopy enables real time visualization of nuclear and cellular morphology *in vivo*, using endogenous reflectance as a source of contrast [1]. The ability to observe nuclear and cellular detail with high resolution, high contrast and high image quality sets this imaging modality apart from other noninvasive imaging technologies such as optical coherence tomography, magnetic resonance imaging and ultrasound. With the use of high

numerical apertures such as 0.7–1.0, the lateral resolution of reflectance confocal microscopy is typically 0.2–1.0 μm and the optical sectioning is 1–3 μm. The imaging in thin optical sections is quasi-analogous to viewing thin sections of pathology at high resolution and high magnification.

Although the reflectance confocal microscope is able to image with resolution comparable to pathology, there are some important differences between the two. First, confocal images appear in grayscale as opposed to the purple and pink colors seen in hematoxylin and eosin (H&E)-stained pathology. Second, the mode of contrast is different. In H&E-stained pathology, structures that absorb the stains appear dark on a bright background. Viewing H&E pathology represents bright-field imaging with absorbance-based contrast. However, in reflectance confocal microscopy, the background appears dark, while structures with higher reflectance appear bright. This is equivalent to dark-field imaging with scattering-based contrast. Third, although the nominal resolution and optical sectioning of confocal microscopy is similar to that of pathology, the image quality degrades with increasing depth in the tissue. In the deeper dermis levels, strong scattering and aberrations induced by the overlying layers of epidermal tissue cause loss of optical sectioning, loss of resolution and loss of contrast. Since structure-specific stains cannot be used (at least, at present) in human skin, the imaging relies solely on endogenous reflectance for contrast. Unfortunately, image quality degrades with loss of resolution and contrast. By comparison, the observation of physically-prepared thin pathology sections is superior to confocal images obtained *in vivo* because there is no overlying tissue to degrade the image and because of the benefit of stains to enhance contrast in color. Confocal imaging of superficial layers of excised tissue, however, with the use of contrast agents, produces image quality that closely approaches that of pathology.

II. ADVANCES IN TRANSLATIONAL RESEARCH

In parallel with technology development and commercialization, translational research continues to advance for confocal imaging of skin cancers *in vivo* and in excised tissues *ex vivo* [1]. A standardized glossary of terms describing the attributes of melanocytic lesions, as seen in reflectance confocal images, has been developed [2]. The recognition of these attributes shows a high level of inter-observer agreement. A diagnostic algorithm has been developed, resulting in 91% sensitivity and 69% specificity for melanoma diagnosis [3]. A more recent study tested the additive value of reflectance confocal microscopy in melanoma diagnosis [4]. A blinded analysis of dermatoscopic images and, independently, of confocal images from 123 melanomas and 202 clinically suspicious nevi was performed. Confocal imaging showed superior specificity for the diagnosis of melanoma compared to dermatoscopy (68% vs. 32%, respectively), while attaining equally high sensitivity (91% vs. 88%, respectively) for both pigmented and lightly colored lesions. In another study of 152 skin lesions, basal cell carcinomas were detected with sensitivity of 94% and specificity of 78% for the presence or absence of three morphologic features [5].

Melanoma on sun-damaged skin of the face tends to be widespread below the surface and poorly delineated, requiring multiple biopsies to determine the cancer's margins. Pre-operative mapping of the margins of melanomas is important for two reasons: first, despite

the high cure rates of excision, there is a high rate of local recurrences. Secondly, the sub-clinical margins – i. e., margins below the skin surface that are not seen by the clinician - may extend for several centimeters beyond the visible edges, in the form of thin root-like extensions. Pre-operative confocal mapping of lentigo maligna melanomas and amelanotic melanomas has proven successful for guiding precise surgery, with the post-operative margins appearing clear and correlating well with the post-operative pathology. The imaging has proven useful for determining the subclinical sub-surface margins of large and widespread superficial melanomas [6, 7]. In 27 cases performed thus far, the imaging has saved patients from a large number of otherwise necessary “where-is-the-margin?” biopsies on sensitive sites such as the scalp, cheek, chin and thigh. The improved accuracy of excisions has benefitted patients by preserving normal tissue and reducing the number of repeat surgeries.

In addition, intra-operative imaging of shave biopsy-sites on 50 patients shows the feasibility to image the peripheral and deeper margins of surgical wounds, and to detect residual foci of basal cell carcinoma and squamous cell carcinoma. In the long-term, such imaging may guide the excision of these cancers during Mohs surgery intra-operatively, directly on the patient.

III. ADVANCES IN TECHNOLOGY

In parallel, new developments in technology include confocal mosaicing microscopy to rapidly image large areas of excised tissue *ex vivo* and line-scanning confocal microscopes as smaller, simpler and lower-cost alternatives to current point-scanning instrumentation.

Confocal mosaicing microscopy, with the use of acridine orange to stain nuclei in fluorescence, shows potential for detection of basal cell carcinomas in 15×15 mm-large excisions from Mohs surgery [8, 9]. Creating a mosaic requires only 5–9 minutes, compared to 20–45 minutes for preparation of frozen pathology. (In other surgical settings, preparation of frozen or permanent pathology can take hours to days or, in some tissues, not possible.) In a blinded evaluation of 45 mosaics by two Mohs surgeons, basal cell carcinomas were detected with 97% sensitivity and 89% specificity [10, 11]. Further work is in progress, to combine reflectance mosaics with fluorescence mosaics, followed by digital staining to mimic the purple-and-pink colored absorbance-based contrast and appearance of pathology [12]. Mosaicing with digital staining may enable rapid pathology-at-the-bedside in large areas of excised tissue to potentially guide surgery.

A current barrier to widespread clinical translation is that point-scanning confocal microscopes are relatively large and expensive. For confocal microscopy to succeed in diverse clinical settings worldwide, the instrumentation must be made smaller, simpler, more robust with repeatable performance, and low-cost. Toward creating low-cost technology for worldwide use in primary healthcare settings, line-scanning confocal microscopes are being developed with the use of linear complementary metal oxide semiconductor and charge coupled-device (CMOS and CCD) array detectors and field programmable gate array (FPGA)-based control electronics. We have shown feasibility with two laboratory prototypes, a divided-pupil and a full-pupil configuration [13–15]. While the

divided-pupil offers strong rejection of multiply-scattered out-of-focus light and strong contrast, as needed in scattering human tissues, the full-pupil offers better optical sectioning and resolution. Each prototype consists of only 8–10 optical components in simple configurations that are relatively easy to assemble, with total hardware costs of only $15,000. Preliminary results demonstrate optical sectioning, resolution and imaging of nuclear detail in human epidermis *in vivo* that appears to be competitive with today's state-of-the-art point-scanning microscopes.

IV. FUTURE: CHALLENGES AND OPPORTUNITIES

As the translation of reflectance confocal microscopy from the research and commercial worlds into the clinic (i. e., bench to bedside) progresses, there are both opportunities and challenges for both clinicians and technologists.

The opportunities and challenges for clinicians include the ability to observe large volumes of tissue in real-time, image understanding and interpretation of grayscale images without the benefit of structure-specific contrast agents (mainly for *in vivo* but not necessarily for *ex vivo*), strategies to improve sensitivity and specificity, using the imaging to guide biopsy and pathology, screening of early precancers and determining new paradigms for clinical and pathological utility.

The opportunities and challenges for technologists include the development of very low-cost confocal microscopes for large-scale translation to the clinic, along with telemedicine networks that will enable images to be evaluated rapidly, endogenous and exogenous sources of contrast (stains), and multimodal instrumentation aimed at providing clinically relevant sensitivity and specificity.

Acknowledgments

This work was supported by grants R43/R44 CA093106 from the NCI/NIH, grants R01EB002715 and R01EB006947 from the NIBIB/NIH, and grants from the Byrne Fund and Geoffrey Beene Cancer Center at Memorial Sloan-Kettering Cancer Center.

The author thanks all his colleagues in both engineering and in dermatology for their involvement and collaborative partnerships in creating and translating this technology.

References

1. Gonzalez, SG.; Gill, M.; Halpern, AC., editors. Reflectance Confocal Microscopy of Cutaneous Tumors – An Atlas with Clinical, Dermoscopic and Histological Correlations. Informa Healthcare; London: 2008. p. 1-6.
2. Scope A, Benvenuto-Andrade C, Agero A-L, Malvehy J, Puig S, Rajadhyaksha M, Busam KJ, Marra DE, Torres A, Propperova I, Langley RGB, Marghoob AA, Pellacani G, Seidenari S, Halpern AC, Gonzalez SG. In vivo reflectance confocal microscopy imaging of melanocytic skin lesions: consensus terminology, glossary and illustrative images. J Amer Acad Dermatol. 2007; 57:644–658. [PubMed: 17630045]
3. Pellacani G, Guitera P, Longo C, Avramidis M, Seidenari S, Menzies S. The impact of in vivo reflectance confocal microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol. 2007; 127:2759–2765. [PubMed: 17657243]

4. Guitera P, Pellacani G, Longo C, Seidenari S, Avramidis M, Menzies SW. In vivo reflectance confocal microscopy enhances secondary evaluation of melanocytic lesions. J Invest Dermatol. 2009; 129:131–138. [PubMed: 18633444]
5. Nori S, Rius-Diaz F, Cuevas J, Goldgeier M, Jaen P, Torres A, Gonzalez S. Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. J Am Acad Dermatol. 2004; 51:923–930. [PubMed: 15583584]
6. Busam KJ, Hester K, Charles C, Sachs DL, Antonescu CR, Gonzalez S, Halpern AC. Detection of clinically amelanotic malignant melanoma and assessment of its margins by in vivo confocal scanning laser microscopy. Arch Dermatol. 2001; 137:923–929. [PubMed: 11453812]
7. Chen CJ, Elias M, Busam K, Rajadhyaksha M, Marghoob AA. Multimodal in vivo optical imaging, including confocal microscopy, facilitates pre-surgical margin mapping for clinically complex lentigo maligna melanoma. British Journal of Dermatology. 2005; 153:1–6.
8. Gareau DS, Li Y, Huang B, Eastman ZM, Nehal KS, Rajadhyaksha M. Confocal mosaicing microscopy in Mohs skin excisions: feasibility of rapid surgical pathology. J Biomedical Optics. 2008; 13:054001.
9. Gareau DS, Patel YG, Li Y, Aranda I, Halpern AC, Nehal KS, Rajadhyaksha M. Confocal mosaicing microscopy in skin excisions: a demonstration of rapid surgical pathology. J Microscopy. 2009; 233:149–159.
10. Karen JK, Gareau DS, Dusza SW, Tudisco M, Rajadhyaksha M, Nehal KS. Detection of basal cell carcinomas in Mohs excisions with fluorescence confocal mosaicing microscopy. British J Dermatology. 2009; 160:1242–1250.
11. Gareau DS, Karen JK, Dusza SW, Tudisco M, Nehal KS, Rajadhyaksha M. Sensitivity and specificity for detecting basal cell carcinomas in Mohs excisions with fluorescence confocal mosaicing microscopy. J Biomedical Optics. 2009 in press.
12. Gareau D. The feasibility of digitally stained multimodal confocal mosaics to simulate histopathology. J Biomed Optics. 2009 accepted.
13. Dwyer PJ, DiMarzio CA, Zavislan JM, Fox WJ, Rajadhyaksha M. Confocal reflectance theta line-scanning microscope for imaging human skin *in vivo*. Optics Letters. 2006; 31:942–944. [PubMed: 16599219]
14. Dwyer PJ, DiMarzio CA, Rajadhyaksha M. Confocal theta line-scanning microscope for imaging human tissues. Applied Optics. 2007; 46:1843–1851. [PubMed: 17356629]
15. Gareau, DS.; Abeytunge, S.; Rajadhyaksha, M. Full-pupil line-scanning confocal microscope for imaging weakly scattering tissues: comparison to divided-pupil. Proceedings of the SPIE Photonics West Biomedical Optics Meeting; 2009. (Technical Abstracts Summary Digest, Paper 7182-06)