1 Roles and Applications of Ex Vivo Confocal Microscopy

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1.1 Introduction

Confocal microscopy (CM) was introduced in dermatology in the last decade [1].

It provides a new rapid imaging approach for evaluation of neoplastic and non-neoplastic lesions, enabling bedside pathology [2]. Ex vivoconfocal microscopy (EVCM) acquires images in two modes: reflectance confocal microscopy (RCM) and fluorescence confocal microscopy (FCM). RCM (grayscale) and FCM (grayscale or green scale) images can be visualized separately [3] or in a combined (RCM + FCM) digitally pseudo- color purple and pink images also called digital H&E (DHE) mode as it resembles conventional hematoxylin–eosin (H&E)-stained images [4]. The DHE image is created by combining signal from FCM (nuclear signal) channel which is digitally converted to purple color (similar to hematoxylin stain) and signal from RCM (cytoplasmic and collagen signal) which is digitally converted to pink color (similar to eosin stain). For information about the principles of ECM device, please refer to Chap. 2.

One of the major advantages of imaging with the EVCM device is that it can image fresh tissues without the need for freezing and sectioning. The lack of tissue processing significantly shortens tissue evaluation time from 20 to 45 min (per excision) [5] with conventional frozen section to less than 5 min [6]. Detailed hands-on guide for tissue preparation and EVCM imaging is provided in Chap. 3. Furthermore, this device can be operated with minimal training and doesn't require an expensive laboratory set-up which could aid in reducing the cost of the procedure.

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In this chapter, we will provide a brief overview of applications of EVCM device in dermatology and also in non-dermatological field.

1.2 Applications of Ex Vivo Confocal Microscopy in Dermatology (Table 1.1)

1. *EVCM during peri-operative margin assessment for keratinocytic neoplasm during Mohs surgery*: The use of EVCM has been mostly described during Mohs micrographic surgery (MMS) for rapid evaluation of tumor margins for basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [3, 7]. EVCM has demonstrated an overall high sensitivity (~96%) and specificity (~89%) for detection of BCC [8, 9] and ~95% sensitivity and ~ 96% specificity for SCC detection [10], which is comparable with the standard frozen section analysis. EVCM images of BCC and SCC are shown in detail in Chaps. 7 and 8, respectively.
2. *EVCM for the evaluation of melanocytic lesions:* EVCM has been used to evaluate melanoma and nevi, and their main microscopic features and histopathological correlations have been reported [11]. Also, fluorescent-labeled antibodies such as S100 and Melanin A have been shown to be useful to differentiate melanoma from other tumors such as BCC, much more rapidly than traditional immunohistochemistry and conventional formalin-based tissue fixation [12]. Additionally, EVCM has been used to measure melanoma thickness with a high concordance with conventional histopathology [13]. EVCM images of melanocytic lesions are shown in detail in Chaps. 9, 10 and 11.
3. *Role of EVCM for evaluation for other neoplastic skin lesions:* Features of dermatofibrosarcoma protuberans (DFSP) have been described using EVCM with an

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Table 1.1 Application of ex vivoconfocal microscopy in dermatology

Neoplastic lesions	Non-Neoplastic lesions		Others
	Infectious diseases	Inflammatory diseases	
• Basal cell carcinoma [3, 4, 8, 34–39]	• Mucormycosis [22]	• Pemphigoid [18]	• Skin fillers [40]
• Squamous cell carcinoma [4, 7, 41]	• Aspergillosis [42]	• Vasculitis [19]	
• Melanocytic lesions [6, 11–13, 43]	• Dermatophytosis [44, 45]	• Psoriasis [17]	
• Dermatofibrosarcoma protuberans [14]	• Herpes virus [20]	• Eczema [17]	
• Eccrine syringomatous carcinoma [15]	• Molluscum contagiosum [21]	• Lichen planus [17, 46]	
• Extramammary Paget's disease [16]		• Discoid lupus erythematosus [17, 47]	
• Nail tumors [23, 24]			

excellent correlation with histopathology [14]. This suggests the potential role of EVCM to assess surgical margins of DFSP during Mohs surgery. However, studies need to be conducted to evaluate sensitivity and specificity of EVCM to detect DFSP during Mohs surgery. EVCM features of other less frequent neoplastic skin lesions, such as eccrine syringomatous carcinoma [15] or extramammary Paget's disease [16], have been described. However, the role of EVCM has not been explored for rapid, bedside evaluation of benign lesions, that mimics skin cancers, in clinical setting. In Chaps. 5 and 6, we describe features of common benign lesions including seborrheic keratosis, solar lentigo, epidermal inclusion cyst, lipoma, neurofibroma, and dermatofibroma for the first time.

4. *Role of EVCM for the evaluation of inflammatory skin lesions:* EVCM has a great potential for rapid bedside evaluation of cutaneous inflammatory lesions. Key morphological features of several inflammatory diseases such as psoriasis, lichen planus, eczema, and discoid lupus have been described [17]. Also, the use of immunofluorescence antibodies has been reported to be helpful for the diagnosis of pemphigoid [18] and vasculitis [19] with EVCM device. EVCM images of cutaneous inflammatory lesions are shown in detail in Chaps. 12, 13, 14 and 15.
5. *Role of EVCM for the evaluation of infectious diseases:* EVCM can detect specific morphological features of some infectious skin lesions such as cytopathic effect associated with herpes virus [20] and pox virus that causes molluscum contagiosum [21], and hyperreflective elongated and ramificated fungi structures of mucormycosis [22]. We have covered examples of some common infectious diseases including verruca vulgaris and molluscum contagiosum in Chap. 5.
6. *Role of EVCM for the evaluation of nail tumors:* Lastly, EVCM has shown to be a useful tool for intraoperative diagnosis of malignant nail lesions such as invasive SCC and Bowen´s disease. The diagnosis of these lesions relies on identification of significant nuclear and cytological atypia, which is similar to histopathology [23]. EVCM has also demonstrated its utility for rapid intraoperative diagnosis of melanonychia striata [24] and for the diagnosis of benign epithelial tumors such as onychomatricoma and onychopapilloma [23]. However, larger scale studies showing diagnostic accuracy for nail tumors with EVCM device are lacking.

1.3 Applications of Ex Vivo Confocal Microscopy in Non-Dermatologic Field (Table 1.2)

EVCM provides a rapid approach for imaging tissues compared with conventional histopathology. Thus, EVCM has a great potential to be incorporated into surgical pathology for intraoperative assessment of tumor margins as an alternative to frozen section and for the evaluation of small biopsies such as endoscopic biopsies or core needle biopsies. Although the use of EVCM is not yet standardized for these applications in clinics, many recent studies have reported this technology to be useful for diagnosis of non-dermatological cancers such as prostatic cancer [25–28] and breast cancer [29–33], among others. As this section is out of the scope for this atlas, we have summarized publications in this field in Table 1.2.

Table 1.2 Application of ex vivoconfocal microscopy for non-dermatological cancers

	Authors	Year
Urological Cancers		
Prostatic cancer	• Puliatti et al. [26]	2019
	• Rocco et al. [28]	2020
	• Bertoni et al. [27]	2020
	• Panarello et al. [25]	2020
Bladder cancer	• Sonn et al. [48]	2009
	• Wiesner et al. [49]	2011
	• Bonnal et al. [50]	2012
Renal cancer	• Su et al. [51]	2016
	• Krishnamurthy et al. [52]	2019
	• Phung et al. [53]	2020
Gynecological Cancers		
Breast cancer	• Patel et al. [33]	2012
	• Dobbs et al. [31]	2013
	• Ragazzi et al. [54]	2013
	• Dobbs et al. [29]	2015
	• Abeytunge et al. [30]	2017
	• Krishnamurthy et al. [52]	2019
	• Elfgen et al. [32]	2019
Uterine cervix dysplasia	• Collier et al. [55]	2002
	• Carlson et al. [56]	2005
Gastrointestinal cancers		
Esophagus cancer	• Inoue et al. [57]	2000
	• Yoshida et al. [58]	2007
	• Gorospe et al. [59]	2012
Stomach cancer	• Inoue et al. [57]	2000
	• Yoshida et al. [58]	2007
Colon cancer	• Inoue et al. [57]	2000
	• Yoshida et al. [58]	2007
	• Ragazzi et al. [54]	2013
Liver cancer	• Krishnamurthy et al. [52]	2019
Oral Mucosa Dysplasia	• El Hallani et al. [60]	2013
Respiratory Tract Cancer		
Lung cancer	• Sorokina et al. [61]	2014
	• Krishnamurthy et al. [52]	2019
	• Takemura et al. [62]	2019
Larynx cancer	• Just et al. [63]	2006
Central Nervous System Cancer		
Brain Cancer	• Wirth et al. [64]	2012
	• Snuderl et al. [65]	2013
	• Martirosyan et al. [66]	2016
	• Belykh et al. [67]	2020
	• Belykh et al. [68]	2020
	• Acerbi et al. [69]	2020

(continued)

Table 1.2 (continued)

	Authors	Year
Endocrine Cancer		
Thyroid	• Ragazzi et al. [54]	2013
Others		
Lymph Node Methastasis	• Ragazzi et al. [54]	2013

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