

Preliminary comparison of line-field confocal optical coherence tomography and reflectance confocal microscopy for ex vivo skin imaging



C. Lenoir¹ | J. Malvehy¹ | S. Puig¹ | P. Rosès¹ | A.C. Rivas¹ | J. Perez-anker¹

¹. Dermatology Department, Hospital Clinic de Barcelona, Universitat de Barcelona, IDIBAPS, Barcelona, Spain.



Introduction

Accurate imaging of surgical margins is essential in the management of skin cancer to ensure complete removal of malignant tissues and reduce the risk of recurrence. Reflectance Confocal Microscopy (RCM) has been widely used for high-resolution imaging of skin lesions, in vivo as well as ex vivo for margin control after excision of skin cancer lesions.¹ Line-field Confocal Optical Coherence Tomography (LC-OCT) is a novel imaging technique that offers high-resolution, vertical histology-like three-dimensional (3D) visualization of skin structures. It has already been used and studied for in vivo imaging of the skin² and we believe that its technical abilities could be particularly valuable in evaluating the presence and extent of skin cancer infiltration in an ex vivo setting.

Objective

The objective of this introductory poster is to visually compare the features observed with LC OCT and RCM by imaging the exact same areas in healthy and pathological skin tissue

Methodology

One ex vivo skin sample comprising healthy tissue as well as areas infiltrated by a micronodular basal cell carcinoma (BCC) was selected for initial imaging comparisons. The sample was stained with a combination of acetic acid, alcohol, and acridine orange to enhance the visualization of specific cellular structures and it was then imaged using both LC-OCT and RCM. The imaging protocol for LC-OCT included acquiring scout views and 3D mosaic images to provide a detailed visualization of skin tissue architecture in three dimensions, then selecting the most appropriate depth level to display a full two-dimensional mosaic. For RCM, both reflectance and fluorescence channels were used to obtain a high-resolution, two-dimensional mosaic, digitally stained to resemble an HE histology slide. The images were then analyzed as we focused on correlating the exact same areas within each sample with both modalities, allowing for a direct comparison of the features observed.

Results

Initial imaging comparisons demonstrated that both LC-OCT and RCM successfully visualized key histological features of healthy as well as pathological skin structures in our sample. As shown in the first figure, both RCM and LC-OCT displayed cellular resolution with the ability to observe keratinocytes, assess their regular organization in the epidermis and in the bulb, as well as the stroma around filled with fibroblasts and lymphocytic cells. LC OCT's 3D imaging capability provided a comprehensive visualization of annexal structures like a hair follicle and its surrounding. Both mosaics were successfully superimposed to cover the exact same area of the sample, demonstrating a strong correlation between the two imaging techniques as shown in the second figure. The tumor remnants were observed in both techniques, as basaloid structures dispersed in the dermis in RCM whereas they appeared as dense hyperreflective nests in LC-OCT.

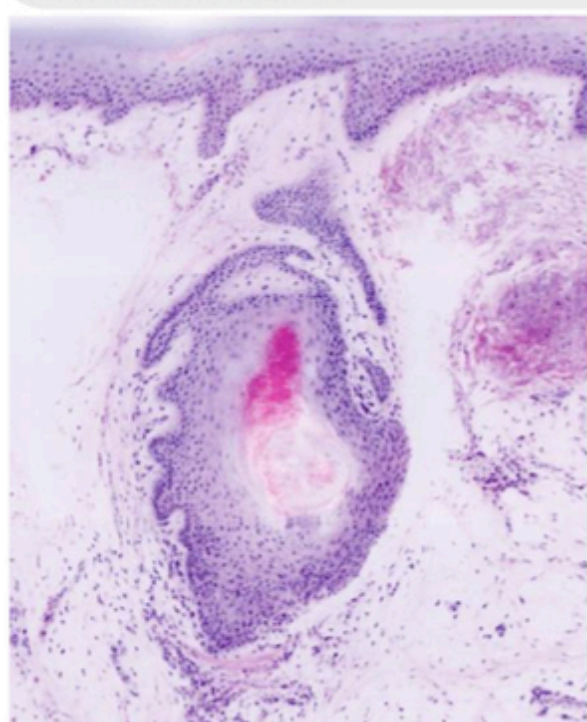


Figure 1. Ex-vivo RCM (left) and LC-OCT tridimensional visualization (right) of a hair follicle

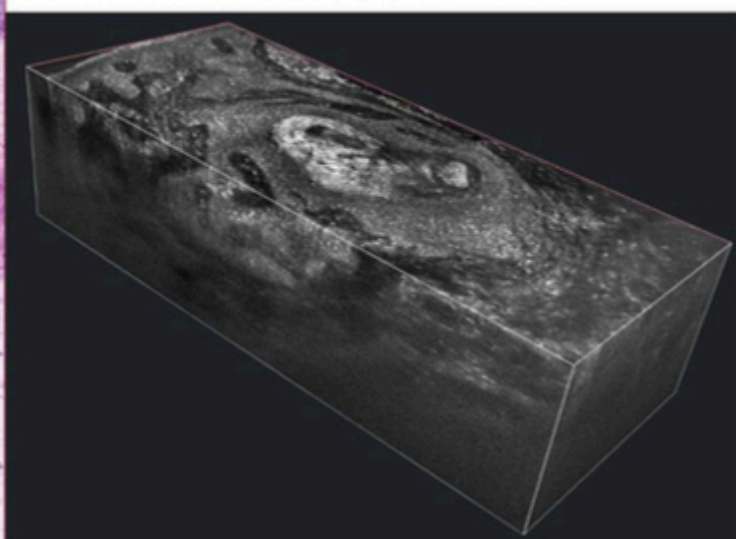
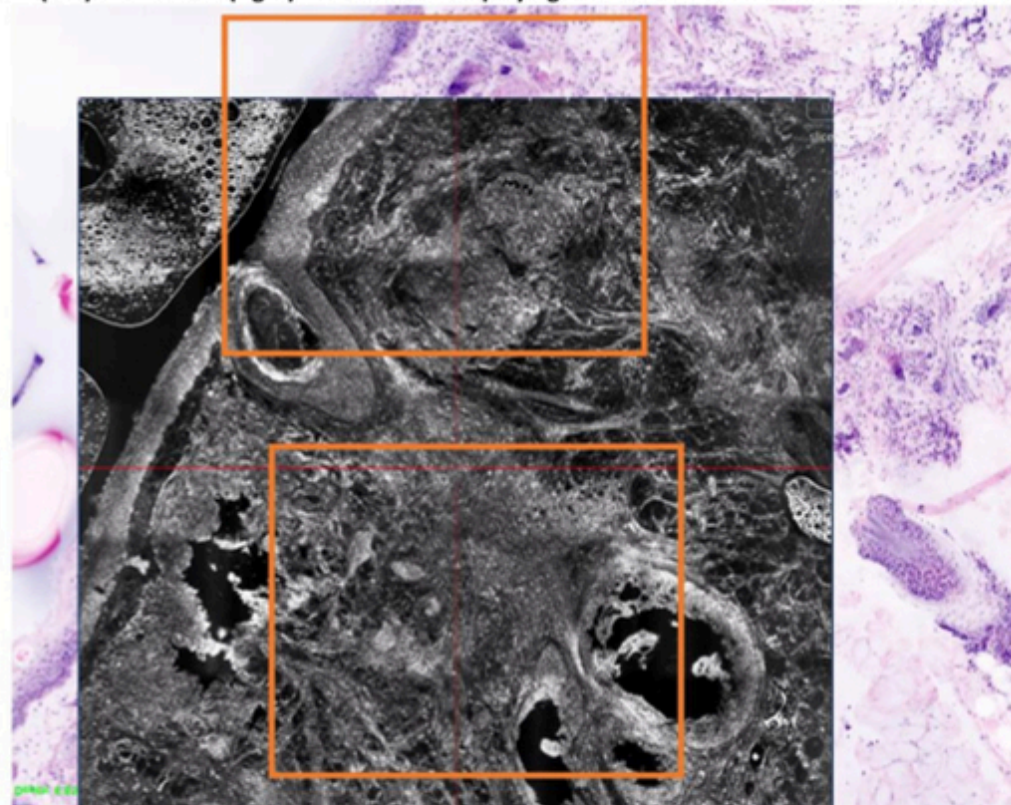
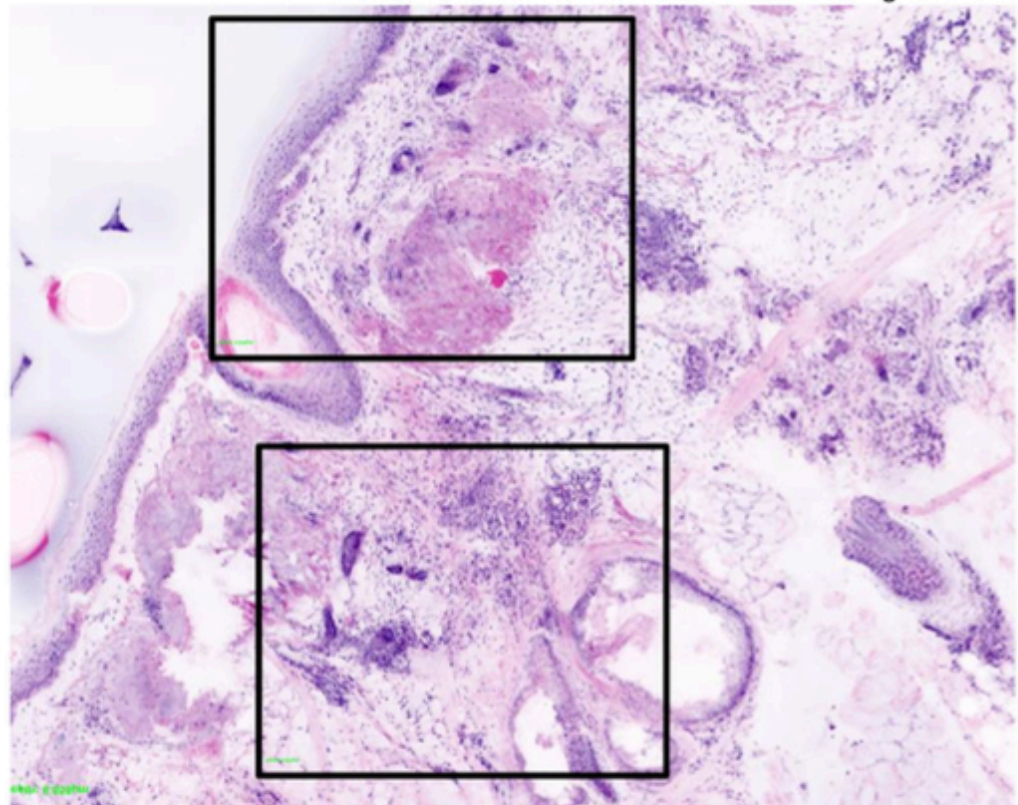


Figure 2. Ex-vivo RCM (left) and LC-OCT (right) mosaics both displaying basaloid tumoral remnants in the dermis



Conclusion

This preliminary comparison underscores the capabilities of both LC-OCT and RCM in ex vivo imaging of skin tissue. Both techniques offer high-resolution, two-dimensional images with digital histology-like staining already available in RCM, while LC-OCT has the advantage of being able to provide 3D imaging of skin architecture. These findings underscore the importance of further studies comparing both techniques to expand these observations.

1. Pérez-Anker, J., Toll, A., Puig, S., & Malvehy, J. (2022). Six steps to reach optimal scanning in ex vivo confocal microscopy. *Journal of the American Academy of Dermatology*, 86(1), 188–189. <https://doi.org/10.1016/j.jaad.2021.01.044>
2. Suppa, M., Palmisano, G., Tognetti, L., Lenoir, C., Cappilli, S., Fontaine, M., Orte Cano, C., Diet, G., Perez-Anker, J., Schuh, S., Di Stefani, A., Lacarrubba, F., Puig, S., Malvehy, J., Rubegni, P., Welzel, J., Perrot, J. L., Peris, K., Cinotti, E., & Del Marmol, V. (2023). Line field confocal optical coherence tomography in melanocytic and non-melanocytic skin tumors. *Italian journal of dermatology and venereology*, 158(3), 180–189. <https://doi.org/10.23736/S2784-8671.23.07639-9>