Fluorescence confocal microscopy for rapid evaluation of EUS fine-needle biopsy in pancreatic solid lesions

Serena Stigliano, MD, PhD,[1] Anna Crescenzi, MD,[2] Chiara Taffon, MD,[2] Gianmarco Marocchi, MD,[3] Francesco Maria Di Matteo, MD[3]

Fluorescence confocal laser microscopy (FCM) allows imaging of tissues in the fresh state, with minimal preparation and without any damage, distortion, or loss of tissue.[1-4] It requires a fluorescent dye applied to the unfixed tissue sample. Two lasers of different wavelengths create 2 distinct images: a fluorescence image and a reflectance image. The device's software uses an algorithm to translate the acquired image information into colors that resemble hematoxylin and eosin. The pseudo-colored images contain similar information to conventional histology and can be examined at any magnification up to 550-fold (Fig. 1).[4-10]

We recently investigated its use in the evaluation of samples obtained with EUS fine-needle biopsy (EUS-FNB) in pancreatic solid lesions. We demonstrated a relevant performance in predicting the sample adequacy of EUS-FNB of pancreatic lesions (92.6%). The sensitivity of FCM was 100%, specificity 66.7%, accuracy 97%, positive predictive value 97%, and negative predictive value 100%. There was good agreement between the FCM diagnosis and the final histologic diagnosis with high diagnostic performances (Cohen's κ coefficient, 0.95).[11]

With the present video we aim to illustrate in detail the steps of this technique (Video 1, available online at www.giejournal.org). After the FNB, the specimen obtained is expelled and placed directly in a dedicated scaffold (Cytomatrix; UCS Diagnostics. Rome, Italy) (Fig. 2). A drop of ethanol is applied on the sample and the liquid is drained away owing to the porous structure of the matrix, followed by the application of a drop of acridine-orange for 20 seconds and washing with saline. The Cytomatrix is then put on a specific microscopic slide and covered with a second slide before the introduction into the slot of the machine (Fig. 3).

Abbreviations: EUS-FNB, EUS fine-needle biopsy; FCM, fluorescence confocal laser microscopy.

2468-4481
https://doi.org/10.1016/j.vgie.2022.11.010

Operative Endoscopy Department, Campus Bio-Medico University Hospital, Rome, Italy (1), Pathology Unit, Campus Bio-Medico University Hospital, Rome, Italy (2), Operative Endoscopy Department, Campus Bio-Medico University Hospital, Rome, Italy.

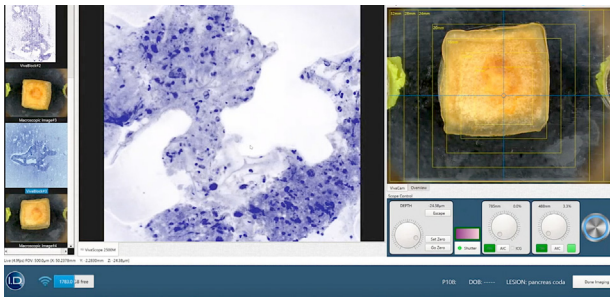

Figure 1. Fluorescence confocal laser microscopy software uses an algorithm to translate the acquired image information into colors that resemble hematoxylin and eosin.

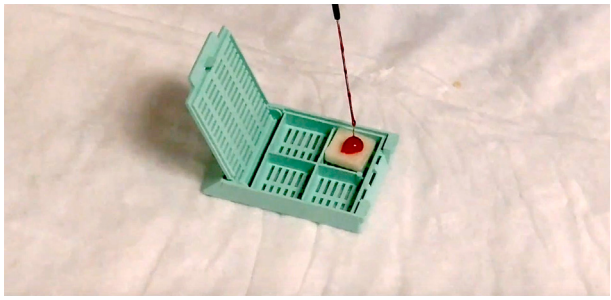

Figure 2. Fine-needle biopsy specimen is expelled and placed in a dedicated scaffold.

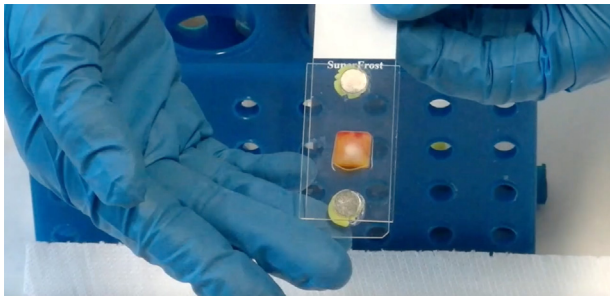

Figure 3. After application of fluorescent dye, the Cytomatrix is put on a dedicated microscopic slide and covered with a second slide before introduction in the slot of the machine.

The machine used is the microscope MAVIG VIVASCOPE 2500 by MAVIG GmbH (München, Germany). The FCM with MAVIG VIVASCOPE 2500 offers the chance

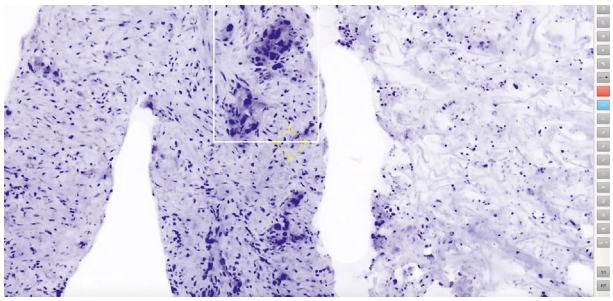

Figure 4. It is possible to mark an area of the digital image and share it for remote consulting with other pathologists.

of rapid analysis of nonfixated, fresh tissue. The possibility of having real-time information about the adequacy of the sample could avoid unnecessary needle passes, shortening the time of the procedure and reducing the risk of adverse events. In addition, the sample used for adequacy assessment undergoes formalin fixation and paraffin embedding that offers the chance of evaluating the same sample with the routine histologic procedures without losing it.

The possibility of producing a digital image allows all the application tools of digital pathology, such as zooming in a high-power field, obtaining measurements of cellular and tissue structures, and saving the image for a re-evaluation (Fig. 4).

This new technique can be successfully applied to cytological and histologic specimens. It provides fast information about the sample adequacy even in small specimens like those obtained with EUS-FNB with good agreement with the final histologic report.

DISCLOSURE

All authors disclosed no financial relationships.

REFERENCES

1. Dhawan AP, D'Alessandro B, Fu X. Optical imaging modalities for biomedical applications. IEEE Rev Biomed Eng 2010;3:69-92.
2. Schulz RB, Semmler W. Fundamentals of optical imaging. Handb Exp Pharmacol 2008;185(Pt 1):3-22.
3. Gibson AP, Hebden JC, Arridge SR. Recent advances in diffuse optical imaging. Phys Med Biol 2005;50:R1-43.
4. Krishnamurthy S, Cortes A, Lopez M, et al. Ex Vivo Confocal Fluorescence Microscopy for Rapid Evaluation of Tissues in Surgical Pathology Practice. Arch Pathol Lab Med 2018;142:396-401.
5. Hartmann D, Ruini C, Mathemeier L, et al. Identification of ex-vivo confocal scanning microscopic features and their histological correlates in human skin. J Biophotonics 2016;9:376-87.
6. Peters N, Schubert M, Metzler G, et al. Diagnostic accuracy of a new ex vivo confocal laser scanning microscope compared to H&E-stained paraffin slides for micrographic surgery of basal cell carcinoma. J Eur Acad Dermatol Venereol 2019;33:298-304.
7. Minsky M. Memoir on inventing the confocal scanning microscope. Scanning 1988;10:128-38.
8. Abeytunge S, Larson B, Peterson G, et al. Evaluation of breast tissue with confocal strip-mosaicking microscopy: a test approach emulating pathology-like examination. J Biomed Opt 2017;22:34002.
9. Karen JK, Gareau DS, Dusza SW, et al. Detection of basal cell carcinomas in Mohs excisions with fluorescence confocal mosaicing microscopy. Br J Dermatol 2009;160:1242-50.
10. Ragazzi M, Piana S, Longo C, et al. Fluorescence confocal microscopy for pathologists. Mod Pathol 2014;27:460-71.
11. Stigliano S, Crescenzi A, Taffon C, et al. Role of fluorescence confocal microscopy for rapid evaluation of EUS fine-needle biopsy sampling in pancreatic solid lesions. Gastrointest Endosc 2021;94:562-8.e1.