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## Fluorescence confocal microscopy for pathologists

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### ABSTRACT

Confocal microscopy is a non-invasive method of optical imaging that may provide microscopic images of untreated tissue that correspond almost perfectly to hematoxylin- and eosin-stained slides. Nowadays, following two confocal imaging systems are available: (1) reflectance confocal microscopy, based on the natural differences in refractive indices of subcellular structures within the tissues; (2) fluorescence confocal microscopy, based on the use of fluorochromes, such as acridine orange, to increase the contrast epithelium-stroma. In clinical practice to date, confocal microscopy has been used with the goal of obviating the need for excision biopsies, thereby reducing the need for pathological examination. The aim of our study was to test fluorescence confocal microscopy on different types of surgical specimens, specifically breast, lymph node, thyroid, and colon. The confocal images were correlated to the corresponding histological sections in order to provide a morphologic parallel and to highlight current limitations and possible applications of this technology for surgical pathology practice. As a result, neoplastic tissues were easily distinguishable from normal structures and reactive processes such as fibrosis; the use of fluorescence enhanced contrast and image quality in confocal microscopy without compromising final histologic evaluation. Finally, the fluorescence confocal microscopy images of the adipose tissue were as accurate as those of conventional histology and were devoid of the frozen-section-related artefacts that can compromise intraoperative evaluation. Despite some limitations mainly related to black/white images, which require training in imaging interpretation, this study confirms that fluorescence confocal microscopy may represent an alternative to frozen sections in the assessment of margin status in selected settings or when the conservation of the specimen is crucial. This is the first study to employ fluorescent confocal microscopy on surgical specimens other than the skin and to evaluate the diagnostic capability of this technology from pathologists' viewpoint.