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Intra-operative frozen section examination: The need for a rapid non-destructive technique. A near-term potential application of laser scanning confocal microscopy

Intra-operative tissue examination (frozen section) is primarily used for the assessment of the margins of resection in surgical excision of malignancy. Other uses include: determination of tumor type, the presence of tumor in sentinel lymph nodes, confirmation that adequate tissue has been obtained for a complete work-up of an unknown lesion, and less frequently, to look for fungal infection. An answer must be rendered while the patient is still in the operating room under anesthesia, so time is of the essence and pressure is high.

The frozen section process causes considerable damage to the tissue: ice crystal artifacts disrupt and distort cellular morphology and microscopic tissue architecture. This damage renders the tissue suboptimal for diagnostic use after subsequent formalin fixation for permanent histology. Tissue that has been frozen is unsuitable for some special studies that might be useful in diagnosis (such as S-100 IHC in malignant melanoma sentinel nodes). Multiple attempts to obtain a successful full-face section of the tissue mean that the section assessed is not in fact the true en face resection margin, but a tissue plane somewhat interior to that margin. Failure to obtain a complete full-faced section results in sampling errors in margin assessment (tumor is actually present at the margin but out of the plane of the cryostat section). Finally, due to technical inadequacies and time constraints sections of suboptimal quality are examined for the immediate diagnosis: those with folds, tears, chattered, too thick, or incomplete. All of these faults conspire to yield a sub-optimal examination under critical conditions.

One objective is that after the intra-operative examination, the tissue can be submitted intact and undamaged for permanent histology. Consideration must be given to easy decontamination and microbial control. The technique should be capable of displaying tumor and normal nuclei, cytoplasm, collagen, and fungi.

Laser scanning confocal microscopy after staining with acridine orange (or another DNA dye) has the near-term potential to overcome all of these problems. Such an instrument uses fresh un-frozen tissue, just as it is removed from the patient. The surgeon's cut surface of the tissue is pressed against the examination glass and the microscope laser interrogates this surface without cutting, waste or destruction of the tissue. A digital image of the tissue surface is rendered in false color (acridine orange positive nuclei in blue, auto-fluorescent collagen in red – very like an H&E stain). After the intra-operative examination the tissue can be submitted intact and undamaged for permanent histology. Decontamination and microbial control may also be facilitated because biohazardous tissue shavings are not dispersed throughout the instrument and work area. The technique might be adapted to fungal detection by the use of calcofluor white dye.