Intraoperative Optical Biopsy of Brain Tumors: Update on Multigenerational System Experience with Confocal Laser Endomicroscopy

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Introduction

- Confocal laser endomicroscopy (CLE) allows intraoperative “optical biopsy” at a cellular level without tissue processing.
- We report the evolution of this technology and results of clinical and experimental experience assessing a multigenerational blue laser 488nm CLE system.
- The equipment can be used for diagnosis and visualization of various brain pathologies.
Methods

- CLE guided biopsies and resections of tumors were obtained in patients and experimentally in animals
- Animal CLE feasibility studies included rodents injected with GL261, C6 glioma, and human derived GBM, swine and rodent brain injury models
- Fluorophores studied: fluorescein sodium (FNa), acridine orange, acriflavine, sulforhodamine101, 5ALA, cresyl violet, ICG
- Selected experimental gliomas had additional specific antibody labeling
- Total clinical experience with CLE: 237 patients with gliomas, meningiomas, and other CNS pathologies examined ex vivo and in vivo using a Generation 1 CLE and 34 patients using a Generation 2 CLE
Results

- CLE FNa clinical imaging produced an average of $77.7 \pm 46.2$ images per optical biopsy location.
- A first diagnostic image was identified within seconds of CLE use clinically (7-14 images).
- CLE specificity/sensitivity during FNa-guided surgery was equal or better than frozen section (94% / 91% gliomas, 93% / 97% meningiomas).
- Gliomas and brain injuries with visible FNa extravasation were distinguished in over 90% of CLE locations imaged (Sensitivity = 0.86, Specificity = 0.96, PPV = 0.97, NPV = 0.78).
- Generation 2 CLE showed improved image resolution and system operation for detecting tumor signal, rapid image acquisition allowing 3D-volumetric image display.
- Animal and human CLE, including specific antibody labeling, provided clear identification of tumor cells and border, and invading cells.
- Image intensity differences were detectable for PpIX fluorescence between normal brain and tumor areas ($P < 0.01$).
GL261 tumor core after fluorescein injection. Gen 1 (C/D) and Gen 2 (A/B) CLE images show similar tissue architecture pattern. E. Lower left image shows characteristic HE staining of the tumor core.
Tumor border visualized with fluorescein. Rapid ex-vivo LSM image shows gradient of fluorescein diffusion from the tumor to the normal brain. Scale bar is 50 um.
CLE imaging does not show 5-ALA.

A. Representative image of a coronal brain slice with a tumor viewed through operative microscope in BLUE400 mode shows bright red fluorescent signal from the tumor. B. Selected CLE images of GL261 tumor and normal brain in mice 2 h after 5-ALA administration. Normal brain showed no fluorescent signal in all of the cases. Fluorescent signal form tumor was visible only from a few areas and was not consistent across the biopsy locations and across the mice imaged. C. HE stained slice of the brain with a tumor, low magnification. D. Characteristic HE slide of the tumor core shows hypercellular tumor. It represents tumor tissue, from which CLE imaging was performed. E. Quantification of the images from an#27 (n=5 control; n=4 tumor) showed minimal, but yet significant difference in the overall pixel intensities of the selected best images taken from the tumor and normal brain. T-test was used to make comparison between the groups * - p<0.01. Selected images were acquired with similar CLE settings.
Conclusions

• 488nm CLE provides rapid intraoperative information on tissue architecture, atypical cellular features and could significantly improve the surgery pathology workflow

• Gen 2 CLE system provides at least equal quality images compared to Gen 1 CLE, with increased pixel resolution and novel scanning options

• Future avenues regarding precision or theranostics-based surgical management of tumors may involve CLE imaging with specific fluorescent targeted markers