Advances in confocal laser endomicroscopy for neuro-oncological tumor resection: a mini-review of current literature

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Please note: this brief overview does not substitute reading of the original studies cited, but provides an abridged insight that does not necessarily reflect the opinion of notified bodies and/or regulatory authorities. Please consult the original literature in the references list for full information.

Precise identification of tumor areas is crucial in neuro-oncological tumor resection, particularly in the case of infiltrative gliomas.[1]

Conventional neuropathological assessments during neurosurgery are typically based on rapid frozen-section histopathology of small extracted tissue samples using conventional benchtop microscopes. Different staining methods (e.g. hematoxylin and eosin [H&E] staining) or fluorescent dyes are used to provide sufficient image contrast.

A number of neurosurgical studies have indicated a range of benefits of fluorescence-guided microscopy for glial tumor resection.[2] However, conventional fluorescence microscopy does not provide sufficient spatial resolution or sensitivity to effectively visualize the surgical margins of diffuse tumors.

Intraoperative optical-sectioning with handheld probe-based confocal laser endomicroscopy systems provides superior resolution.[3]

From the viewpoint of neuropathologists, *in vivo* CLE may offer several benefits as an adjunct to physical histopathological assessment. First, biopsy quality can be optimized through more accurately targeting diseased tissue. Second, imaging can complement physical biopsy investigations by providing information on the architectural context of the tissue, without visual artifacts that are commonly seen in frozen biopsy tissue.[4] Third, this technology can enable real-time *in vivo* intraoperative neuropathological consultation, providing practical benefits for tumor resection workflow.[1,2,5]

Technology

In CLE a low-power laser is used to selectively visualize the area of interest in a tissue on a specific focal plane, providing an 'optical section'.[6] As with a conventional, benchtop confocal microscope, newer systems adapted for surgical endomicroscopy incorporate a pinhole that eliminates light from outside the desired focal plane (background fluorescence), which enables high spatial resolution during 3-dimensional assessments (in X,Y, and Z), as well as the evaluation of tissue architecture at a cellular level.[5]

CLE requires the administration of fluorescent contrast agents immediately before the surgical procedure. FNa only penetrates into the CNS in areas where the blood-brain-barrier is compromised, as in most gliomas.[1,6] Peer-reviewed studies state that pre-operative intravenous FNa administration allows the physician to assess tissue microstructure, to determine tumor border regions, and to visualize injury to normal brain tissue and brain vasculature.[1,6]

A number of CLE systems are available, with the confocal tip either integrated into an endoscope (e.g. EC3870K by Pentax, Japan) or into a separate probe (e.g. Cellvizio® by Mauna Kea Technologies, France; ZEISS CONVIVO by Carl Zeiss Meditec AG, Germany). Image data from these systems can be evaluated immediately in the OR, or can be streamed for real-time

external evaluation to allow remote neuropathologist support.[6,7] Thus, unlike current standard surgical practice, CLE allows real-time *in vivo* imaging directly in the relevant tissue, supplementing physical biopsies.

Animal model studies

Proof-of-concept studies in a range of animal species (mice, rats and pigs) suggest that CLE with real-time neuropathology assessment could increase efficiency in the brain tumor resection workflow, potentially reducing the time required in the OR and associated costs.[1,7,8] In a laboratory assessment of CLE diagnostic accuracy in a mouse glioma model, neuropathologists were able to differentiate tumor from nontumor tissue with a mean accuracy, specificity, and sensitivity of 90%, 86%, and 96%, respectively, and with high overall interobserver agreement.[9]

Human *ex-vivo* and *in-vivo* studies

A number of human clinical studies including ex-vivo and *in-vivo* evaluations have assessed the utility of CLE for real-time intraoperative evaluation.

The effectiveness of CLE was evaluated in a study based on *ex vivo* human brain biopsies and sections from a number of different tumor types that had distinctive as well as complementary reflectance and fluorescence characteristics.[10]

In a single-center feasibility study with a precursor of the current ZEISS CONVIVO CLE system in 33 patients with a variety of brain tumors (mainly meningiomas and metastatic gliomas), the neurosurgeon-neuropathology team were able to identify high-grade gliomas and vascular neo-proliferation and to define tumor margins intraoperatively.[11]

Supported by findings from pre-clinical, proof-of-concept data from animal studies,[8] these clinical data suggest that CLE with FNa contrast could allow real-time and interactive assessments which may provide improvements in the efficiency of decision making during neuro-oncological tumor resection.[7,12]

Beyond static 2D imaging

In addition to static two-dimensional image analysis, the Z-stack technology of current CLE systems allows intraoperative three-dimensional construction of time series and video loops to enable assessment of blood cell movements inside the brain vasculature and within brain tissue if oozing blood is present.[1,5,13]

Outlook

Overall, evidence from *in vivo* and *ex vivo* clinical studies conducted to date indicates that CLE may allow physicians to assess tumor margins during resection procedures.[1,6] The possibility of real-time *in vivo* intraoperative neuropathological consultation could bring tangible benefits for the brain tumor surgery workflow.[1,7,14]

Please find further details regarding study design and findings in the **original articles listed** in the **reference list** at the end of this document.

References

The publications below are based on the authors' own professional opinions or their study results. They do not necessarily reflect the opinions of ZEISS and may not be in line with the clinical evaluation or intended purpose of our medical devices. Therefore, suitability of clinical application for each recommendation should be carefully assessed by the concerned physician.

[1] Belykh E, Miller EJ, Carotenuto A, Patel AA, Cavallo C, Martirosyan NL, Healey DR, Byvaltsev VA, Scheck AC, Lawton MT, Eschbacher JM, Nakaji P, Preul MC. Progress in confocal laser endomicroscopy for neurosurgery and technical nuances for brain tumor imaging with fluorescein. Front Oncol 2019;9:554.

[2] Belykh E, Martirosyan NL, Yagmurlu K, Miller EJ, Eschbacher JM, Izadyyazdanabadi M, Bardonova LA, Byvaltsev VA, Nakaji P, Preul MC. Intraoperative fluorescence imaging for personalized brain tumor resection: current state and future directions. Front Surg 2016;3:55.

[3] Wei L, Fujita Y, Sanai N and Liu JTC. Toward quantitative neurosurgical guidance with high-resolution microscopy of 5-aminolevulinic acid-induced protoporphyrin IX. Front Oncol 2019;9:592.

[4] Hariri LP. In vivo microscopy: will the microscope move from our desk into the patient? Arch Pathol Lab Med 2015;139:719–720.

[5] Belykh E, Patel AA, Miller EJ, Bozkurt B, Yağmurlu K, Woolf EC, Scheck AC, Eschbacher JM, Nakaji P, Preul MC. Probe-based three-dimensional confocal laser endomicroscopy of brain tumors: technical note. Cancer Manag Res 2018a:10:3109–3123.

[6] Leierseder S. Confocal Endomicroscopy during brain surgery. Laser+Photonics 2018. Available at: https://www.photonik.de/confocal-endomicroscopy-during-brain-surgery/150/21404/363684 [Accessed 2 April 2020].

[7] Fotteler ML, Holl F, Käsbach C, Schlegel J, Swoboda W. Using confocal endomicroscopy for digital biopsy during brain surgery – presentation of a study protocol. In: ICT for Health Science Research 2019, A. Shabo (Shvo) et al. (Eds.), The European Federation for Medical Informatics (EFMI) and IOS Press, Hannover, pp. 237–238.

[8] Sankar T, Delaney PM, Ryan RW, Eschbacher J, Abdelwahab M, Nakaji P, Coons SW, Scheck AC, Smith KA, Spetzler RF, Preul MC. Miniaturized handheld confocal microscopy for neurosurgery: results in an experimental glioblastoma model. Neurosurgery 2010;66:410–417.

[9] Belykh E, Miller EJ, Patel AA, Yazdanabadi MI, Martirosyan NL, Yağmurlu K, Bozkurt B, Byvaltsev VA, Eschbacher JM, Nakaji P, Preul MC. Diagnostic accuracy of a confocal laser endomicroscope for in vivo differentiation between normal injured and tumor tissue during fluorescein-guided glioma resection: laboratory investigation. World Neurosurg 2018b;115:e337–e348.

[10] Snuderl M, Wirth D, Sheth SA, Bourne SK, Kwon CS, Ancukiewicz M, Curry WT, Frosch MP, Yaroslavsky AN. Dye-enhanced multimodal confocal imaging as a novel approach to intraoperative diagnosis of brain tumors. Brain Pathol 2013;23:73–81.

[11] Sanai N, Eschbacher J, Hattendorf G, Coons SW, Preul MC, Smith KA, Nakaji P, Spetzler RF. Intraoperative confocal microscopy for brain tumors: a feasibility analysis in humans. Neurosurgery 2011;68(2 Suppl Operative):282–290.

[12] Martirosyan NL, Eschbacher JM, Kalani MY, Turner JD, Belykh E, Spetzler RF, Nakaji P, Preul MC. Prospective evaluation of the utility of intraoperative confocal laser endomicroscopy in patients with brain neoplasms using fluorescein sodium: experience with 74 cases. Neurosurg Focus 2016;40:E11.

[13] Eschbacher J, Martirosyan NL, Nakaji P, Sanai N, Preul MC, Smith KA, Coons SW, Spetzler RF. In vivo intraoperative confocal microscopy for real-time histopathological imaging of brain tumors. J Neurosurg 2012;116:854–860.

[14] Belykh E, Cavallo C, Gandhi S, Zhao X, Veljanoski D, Izady Yazdanabadi M, Martirosyan NL, Byvaltsev VA, Eschbacher J, Preul MC, Nakaji P. Utilization of intraoperative confocal laser endomicroscopy in brain tumor surgery. J Neurosurg Sci 2018c;62:704–717.

Further references not cited here

Schebesch KM, Rosengarth K, Brawanski A, Proescholdt M, Wendl C, Höhne J, Ott C, Lamecker H, Doenitz C. Clinical benefits of combining different visualization modalities in neurosurgery.
Front Surg 2019;6:56. DOI: 10.3389/fsurg.2019.00056

Izadyyazdanabadi M, Belykh E, Mooney MA, Eschbacher JM, Nakaji P, Yang Y, Preul MC. Prospects for theranostics in neurosurgical imaging: empowering confocal laser endomicroscopy diagnostics via deep learning.
Front Oncol 2018;8:240. DOI: 10.3389/fonc.2018.00240

Peyre M, Clermont-Taranchon E, Stemmer-Rachamimov A, Kalamarides M. Miniaturized handheld confocal microscopy identifies focal brain invasion in a mouse model of aggressive meningioma.
Brain Pathol 2013;23:371–377. DOI: 10.1111/bpa.12039

Wells WA, Thrall M, Sorokina A, Fine J, Krishnamurthy S, Haroon A, Rao B, Shevchuk MM, Wolfsen HC, Tearney GJ, Hariri LP. In vivo and ex vivo microscopy - Moving toward the integration of optical Imaging technologies into pathology practice.
Arch Pathol Lab Med 2019;143:288–298.