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Coherent anti-Stokes Raman scattering and two photon excited fluorescence for neurosurgery

Bernd F.M. Romeike[a,*], Tobias Meyer[b], Rupert Reichart[c], Rolf Kalff[c], Iver Petersen[a], Benjamin Dietzek[b,d], Jürgen Popp[b,d]

[a] Institute of Pathology, Jena University Hospital, Friedrich-Schiller-University, Erlanger Allee 101, D-07740 Jena, Germany
[b] Institute of Photonic Technology (IPHT) Jena e.V., Albert-Einstein-Straße 9, D-07745 Jena, Germany
[c] Clinic for Neurosurgery, Jena University Hospital, Friedrich-Schiller-University, Erlanger Allee 101, D-07740 Jena, Germany
[d] Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena, Helmholtzweg 4, D-07743 Jena, Germany

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ABSTRACT

Objective: There is no established method for *in vivo* imaging during biopsy and surgery of the brain, which is capable to generate competitive images in terms of resolution and contrast comparable with histopathological staining.
Methods: Coherent anti-Stokes Raman scattering (CARS) and two photon excited fluorescence (TPEF) microscopy are non-invasive all optical imaging techniques that are capable of high resolution, label-free, real-time, nondestructive examination of living cells and tissues. They provide image contrast based on the molecular composition of the specimen which allows the study of large tissue areas of frozen tissue sections *ex vivo*.
Results: Here, preliminary data on 55 lesions of the central nervous system are presented. The generated images very nicely demonstrate cytological and architectural features required for pathological tumor typing and grading. Furthermore, information on the molecular content of a probe is provided. The tool will be implemented into a biopsy needle or endoscope in the near future for *in vivo* studies.
Conclusion: With this promising multimodal imaging approach the neurosurgeon might directly see blood vessels to minimize the risk for biopsy associated hemorrhages. The attending neuropathologist might directly identify the tumor and guide the selection of representative specimens for further studies. Thus, collection of non-representative material could be avoided and the risk to injure eloquent brain tissue minimized.

1. Introduction

Label free intravital microscopy holds great promise to improve tissue sampling, *i.e.* reducing the risk of procuring non-representative specimens, and minimizing the risk of bleeding by visualization and avoiding destroying blood vessels, especially during stereotactic surgery or other minimal invasive central nervous system surgery. Furthermore, where applicable, neurosurgeons could be guided to borders of lesions for exact orientation and to avoid excessive resection of functional or eloquent brain tissue during surgery by precise boundary detection.

Especially, the application of coherent anti-Stokes Raman scattering (CARS) [1] and tissue intrinsic two photon excited fluorescence (TPEF) [2] microscopy are promising tools which enable intraoperative imaging of histological structures without staining the tissue. The generated images provide a detailed insight into the molecular composition of the sample. CARS is widely used for visualizing the distribution and concentration of lipids [3], while TPEF enables displaying the spatial distribution of autofluorescing molecular species like elastin, keratin or NAD(P)H [4], thus offering molecular selective imaging. The techniques allow for spatially highly resolved imaging even of single cells. The penetration depth in tissue is a few 100 μm which would provide a sufficient security margin for intraoperative guidance in order to avoid damaging functional structures, *e.g.* blood vessels. The pulsed illumination in nonlinear imaging allows investigation of the sample at low average power thus avoiding photodamage of the tissue but providing good image contrast.

* Corresponding author at: Institute of Pathology, Neuropathology Section, Jena University Hospital, Friedrich Schiller University, Erlanger Allee 101, D-07747 Jena, Germany. Tel.: +49 3641 9 3247 55; fax: +49 3641 9 3247 52.
E-mail address: bernd.romeike@med.uni-jena.de (B.F.M. Romeike).

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In vivo CARS imaging has already been demonstrated even at video rate and proved especially valuable in displaying the distribution of lipids [5]. This makes CARS especially interesting for the study of lipid rich brain tissue. However, for *in vivo* applications the technology has to be safe and non-destructive. Cell line and animal studies could so far show that CARS application *in vivo* is safe with no or negligible photodamage [6,7]. CARS microscopy has been successfully used *ex vivo* to image mice brain [8,9] and various tumors, among them also brain tumors [8,10–12]. CARS was also combined with further imaging techniques on small sets of samples [13].

Here, we present preliminary data from a systematic study on a set of various specimens from the central nervous system *ex vivo* in order to exploit the potential of a combination of CARS and TPEF for multimodal *in vivo* imaging and to determine possible limitations.

2. Materials and methods

2.1. Materials

We prospectively collected consecutive brain biopsy specimens of patients operated at the Clinic for Neurosurgery, Jena University Hospital with the permission of the ethics committee. Informed consent for experimentation was obtained by all patients. For diagnostic work-up all specimens were submitted to a special trained neuropathologist (BFMR), where applicable pathological typing and grading of the specimens was performed according to the current World Health Organization (WHO) Classification of Tumors of the Central Nervous System [14]. Intraoperative diagnosis was based on giemsa stained cytological smear preparations and hematoxylin and eosin (H&E) stained cryostat sections. Final diagnoses were confirmed by standard formalin fixed paraffin embedded tissue with standard stains including immunohistochemical methods.

Unstained parallel sections of the diagnostic cryostat sections were subject to CARS, and TPEF. Sections were transferred onto calcium fluoride slides and air dried under ambient conditions. For definite comparison besides the intraoperative parallel cryostat section also the original air dried sections were later stained with hematoxylin and eosin after completion of CARS and TPEF imaging. The studied areas were again searched on the slides and compared to CARS and TPEF images. These areas were again compared with the parallel areas of the initial diagnostic intraoperatively made H&E cryostat section which was then documented and compared with CARS and TPEF images as shown in Figs. 1 and 2.

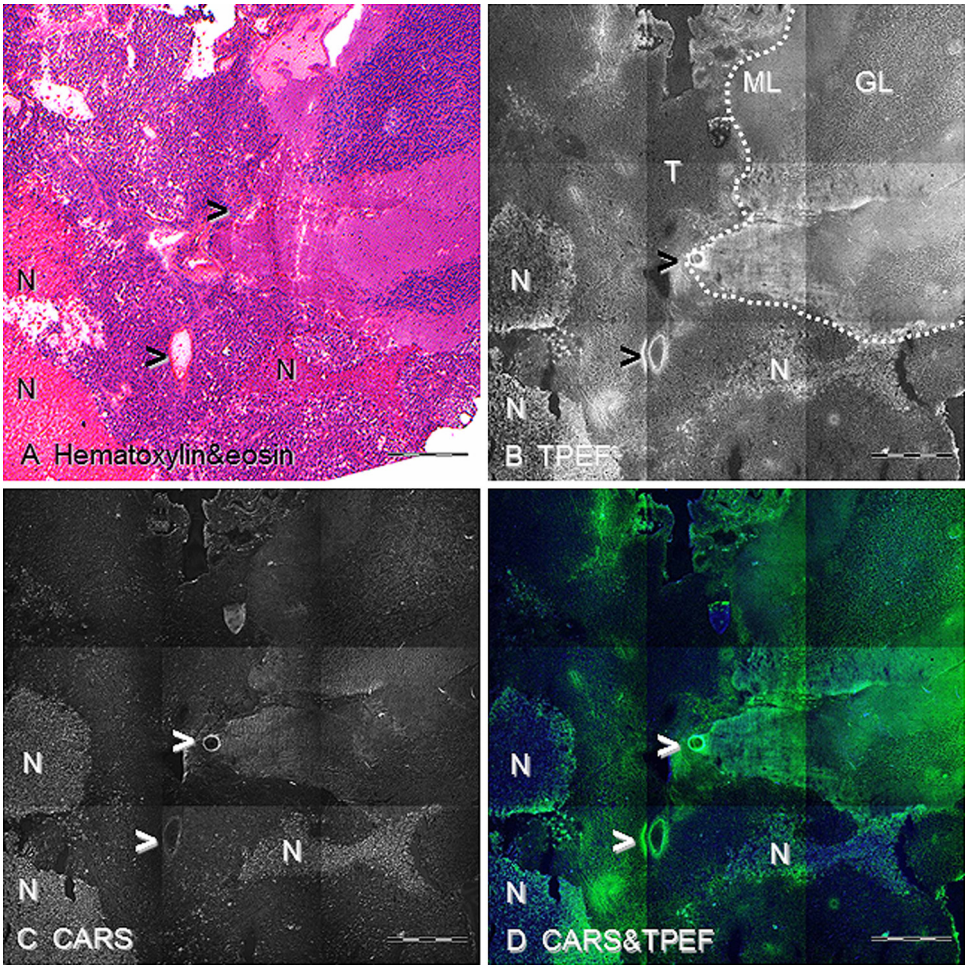

Fig. 1. Low magnification of a cerebellar metastasis of a small cell lung carcinoma, image size 3 mm × 3 mm. (A) Hematoxylin and eosin (H&E) stain shows preexisting cerebellar cortex (upper right), blood vessels (>), tumor and necroses (N). Especially blood vessels are easily detected, even without much experience. (B) The TPEF image highlights the autofluorescing spectrum of preexisting cerebellar molecular layer (ML) and granular layer (GL) vital tumor (T), and necrotic areas (N). There is a clear border between tumor and cerebellum (dotted line). Necrotic tissue contains fluorescing spectrum originating from tissue degradation. (C) Also on the CARS image the cerebellar cortex is sharply demarcated from tumor due to the relative lipid deficiency in tumor tissue with respect to normal brain tissue. Necrotic areas (N) are highlighted indicating higher lipid content and enabling clear differentiation from vital tumor. (D) Combination of CARS and TPEF images.
H&E stain (A), TPEF (B), CARS at 2850 cm^{-1}(C) and combined multimodal CARS-TPEF-imaging (D) displaying CARS in blue and TPEF in green. Scale bar 500 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)