

## In Vivo Measurement of Hypoxia in Brain Tumors by qBOLD MRI Methods

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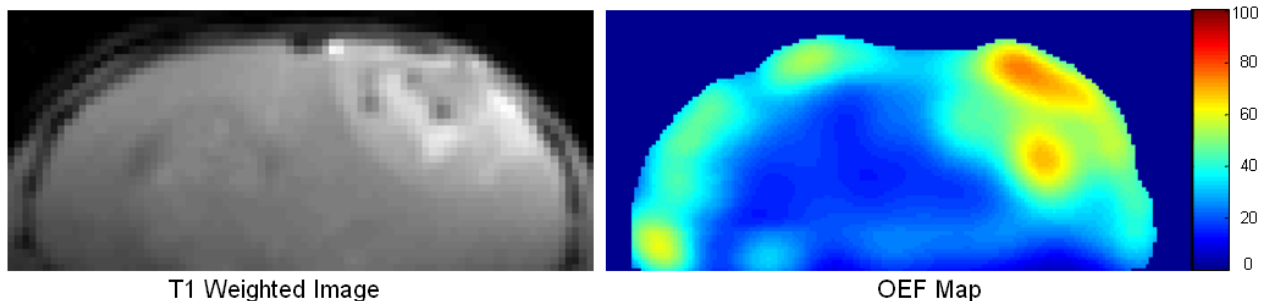
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**Introduction:** Tumor hypoxia and its downstream effects are of considerable interest in both basic and clinical oncology research due to their negative impact on response to various cancer therapies and promotion of metastasis. Diagnosing tumor hypoxia non-invasively could provide a significant advancement in cancer treatment and will be key to implementing emerging targeted therapies that will treat cancer while minimizing the negative impacts of this hypoxia [1,2]. While a variety of techniques have been proposed to measure hypoxia non-invasively, none have been established clinically. Our approach to quantifying tumor hypoxia is based on a recently proposed Quantitative Blood Oxygenation Level Dependent (qBOLD) model of BOLD contrast in MRI [3]. Here we report the results of an initial study of tumor hypoxia in a mouse model of high-grade glioma.

**Methods:** All experiments were performed on a dedicated 4.7 T Varian small-animal MRI scanner using a 2.5 cm ID quadrature birdcage RF coil. All surgical procedures were conducted under the guidelines of the Washington University Institutional Animal Care and Use Committee. Mouse DBT glioblastoma cells were implanted into the brains of female Balb/c mice as previously described [4]. Briefly, cells were plated in T-75 culture flasks for incubation in a 5% CO<sub>2</sub> humidified atmosphere at 37 °C and used at low passages. Mice were anesthetized with intraperitoneal 25 mg/kg ketamine, 5 mg/kg xylazine, and 2.5 mg/kg acepromazine prior to intracranial DBT cell implantation. After a midline scalp incision, a 1-mm cutting burr was used to make a craniostomy over the cortex. Mice were secured in a stereotactic frame and 5 µl of DBT tumor cell suspension ( $5 \times 10^4$  cells) were aspirated into a Hamilton syringe attached to the frame. The syringe was inserted into the brain and the tumor cells were injected over three minutes. The craniostomy hole was secured with bone wax and the incision was closed with a dermal adhesive.

Prior to each imaging experiment, mice were anesthetized with isoflurane/O<sub>2</sub> [3% (v/v)], and maintained on isoflurane [1.5% (v/v)] in a gas mixture of 20%O<sub>2</sub> /80% N<sub>2</sub> throughout the experiment. A 3D version of gradient echo sampling of spin echo sequence (GESSE) [3] was employed. Data acquisition was performed using the following acquisition parameters: FOV 18x18x18 mm<sup>3</sup>, sampling matrix of 64x64x32, TR of 200 msec, NEX of 8. The spin echo occurs at the 11<sup>th</sup> of 31 echoes (56 msec after excitation). All 3D MR data were filtered by a Hanning filter to improve SNR and to reduce the Fourier leakage. The MR signal was analyzed using a qBOLD model [3] that includes signals from tissue, deoxygenated blood, and CSF. In particular, the free induction decay of MR signal from the brain tissue was described in terms of the BOLD model [5] and the signal from the deoxygenated blood was modeled as originating from a network of randomly oriented cylindrical blood vessels [6].

**Results and Discussion:** Mice were imaged using the GESSE sequence at days 4, 11, 14, and 17 following injection of the DBT cells. The figure below shows a representative single slice from a contrast-enhanced T1-weighted image (left) and map of the estimated oxygen extraction fraction (OEF) of a mouse on day 14 following injection of DBT cells. The color bar on the right shows OEF in %. Tumor is clearly visible in the upper right portion of the brain; while a clear elevation in OEF is seen in the tumor area, corresponding to tumor hypoxia. Similar results were obtained for all other mice imaged in this study, with regions of hypoxia correlating closely in both size and location with observed tumors. These results serve to establish the feasibility and importance of qBOLD-based methods for quantitatively measuring hypoxia in developing tumors in mouse models of brain tumors. We anticipate that these methods can be readily translated to clinical studies of patients with high-grade brain tumors.



In patients with tumors, the potential to non-invasively identify and image regions of hypoxia is of particular interest because, as tumors outgrow their blood supply, hypoxic regions develop within the tumor. These hypoxic regions may be relatively less sensitive to traditional therapeutic interventions. Identification of these heterogeneous regions of relative hypoxia within tumors *in vivo* could have significant implications for the clinical management of patients by allowing specific targeting of different types or intensities of therapy to these hypoxic areas. Our qBOLD-based method is well suited for measuring hypoxic regions because lack of oxygen supply leads to elevated OEF. Hence, enhanced OEF contrast in growing tumors can serve as an imaging based biomarker for tumor hypoxia.

**Conclusion:** We report the first measurement of hypoxia/OEF in tumors using the qBOLD method. The technique allows *in vivo* monitoring of oxygenation in growing tumors, and might have significant impact on the study of tumor growth and the development of pre-clinical therapeutics in small-animal models of brain tumors and on the diagnosis and treatment of human tumors.

**References:** [1]. Davda and Bezabeh, *Cancer Metastasis Rev*, 2006. 25(3): p. 469-80 [2] Tatum, et al., *Int J Radiat Biol* 82(10): p. 699-757; [3] He and Yablonskiy, *MRM*, 2007. 57(11): p. 115-126. [4] Jost, et al., *Neurosurgery*, 2007, 60(2): p. 360-371; [5]. Yablonskiy and Haacke, *MRM*, 1994. 32(6): p. 749-763. [6] Sukstanskii and Yablonskiy, *JMR*, 2001. 151(1): p. 107-117.