Preclinical Oxygen Imager for Cancer Oxygenation Studies

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Background and Motivation

Oxygen is an important biomarker in cancer biology studies. Tumor hypoxia is one of the most important factors that regulate tumor growth, development, aggressiveness, metastasis, and affects treatment outcome. Tumor hypoxia is spatially heterogeneous. Despite the clear importance of tumor oxygenation, most scientists studying tumor hypoxia or oxygen kinetics do not have easy access to a reliable oxygen imager.

We report the construction of the first commercial preclinical oxygen imager, JIVA-25, based on electron paramagnetic resonance oxygen imaging (EPROI).

EPROI started its history in the 1990-s when few research groups demonstrated oxygen imaging on rodents using continuous-wave EPR (1). This success was further developed with the advent of pulse imaging (2). Recently, the first successful EPROI-based oxygen guided radiation therapy in mice was demonstrated (3).

EPROI is a noninvasive oxygen mapping method with high precision and absolute accuracy (4). EPR detects unpaired electron spins subjected to the constant uniform magnetic field by manipulating them using radio-frequency electromagnetic radiation. For oxygen measurements, JIVA-25 uses an injectable non-toxic soluble contrast agent, trityl (OX063/OX063-D24).

JIVA-25 Oxygen Imager

JIVA-25 (Fig. 1A) is a compact instrument suitable for *in vitro* and small animal *in vivo* oxygen mapping. This is an all-in-one imager providing image acquisition, processing, and analysis under control of O2MView software. O2MView software features easy-to-use interface and performs automatic measurements using built-in algorithms.

JIVA-25 uses a 25 mT permanent magnet with 11 cm access installed on the top of the electronics shelf. The instrument does not require permanent installation and can be relocated. The ~30 cm magnetic field safety radius allows the imager installation in any laboratory space. The active inner volume of the instrument provides vertical and horizontal access suitable for liquid samples and animal measurement.

JIVA-25 uses pulse acquisition at ~700 MHz to produce images with up to 0.5 mm spatial resolution, 1-3 torr absolute pO_2 resolution at hypoxic conditions, and high temporal resolution (within minutes).

Instrument and Accessories

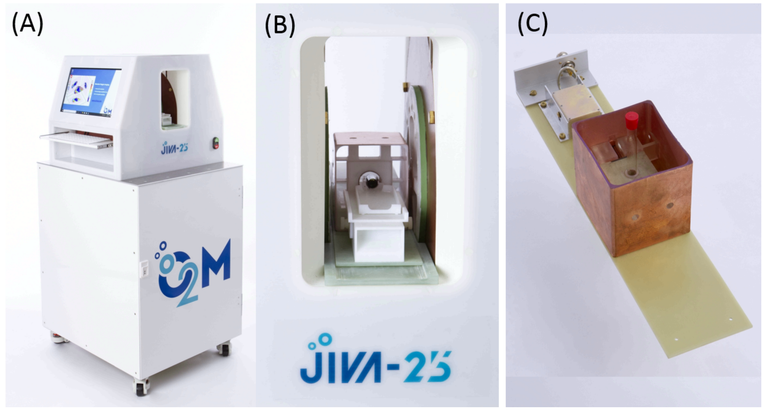

Figure 1. (A) JIVA-25, 25 mT EPROI instrument designed and optimized for 3D oxygen imaging *in vitro* and *in vivo,* (B) Mouse full-body resonator, (C) vertical access resonator for in vitro oxygen imaging

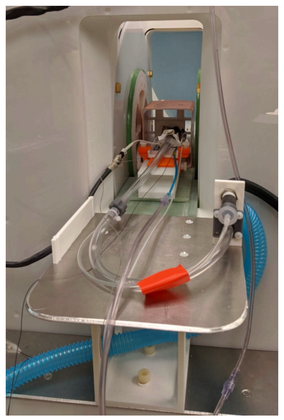

Figure 2. JIVA-25 during a mouse oxygen imaging. Animal anesthetic lines for the bane breathing circuitry is visible at the foreground. The animal is loaded in the animal bed which is mounted on the animal platform.

JIVA -25 Accessories

The flexibility of the instrument is dependent on its accessories. JIVA-25 is equipped with replaceable resonator platforms. The variety of the resonators with horizontal and vertical access orientations are available (Figs. 1B and 1C). The most common resonator diameters are 10 mm and 16 mm for *in vitro* measurements, 19 mm for mouse limbs, and 25 mm for mouse full body measurements. A 40 mm diameter resonator for full body rat imaging is in the development.

Other accessories available for animal imaging are: animal bed with registration aid, animal platform, and animal loading support with anesthesia connectors compatible with the most Vetamac anesthetic systems (Fig. 2A). Respiratory monitor and temperature control units are in development.

For *in vitro* measurements, the accessories include temperature and gas mixture stabilized bioreactor chamber.

Results

Fig. 3 shows a typical oxygen image of a mouse tumor grown on the animal hind leg. Image data acquisition is 5 minutes (828 projections, gradient 0.75G/cm).

The concentration of spin probe in tissues of 0.15mM is optimal for imaging, however even 50μM is sufficient. In mice this concentration is achieved by the tail iv injection of ~200 μL of 70 mM OX071 spin probe solution. The probe clearance time is 15-25 min.

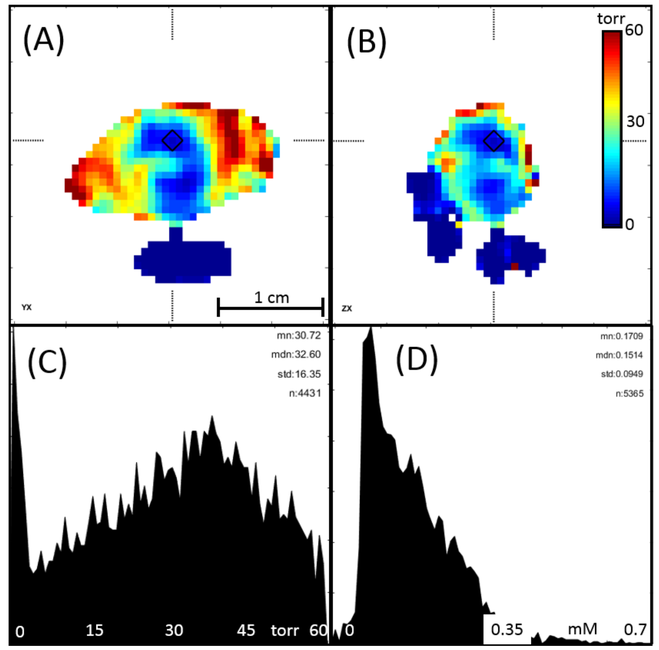

Figure 3. (A,B) Sagittal and axial slices of an oxygen image of a tumor grown on the mouse leg. Histograms of (C) pO_2 (D) spin probe concentration. Dark blue hypoxic areas are inside the tumor core. Red oxygenated areas belong to muscle and other tissues. Four cylinders with 0 torr oxygen are fiducial tubes used for image registration. The tubes are filled with the deoxygenated trityl. Image duration 5 minutes. Spatial resolution ~1 mm.

References:

1 Halpern HJ et al. Proc Natl Acad Sci USA 1994;91:13047-51.; Zweier JL et al. J Bioenerg Biomembr 1991;23:855-71. Kuppusamy P et al. Proc Natl Acad Sci USA 1994;91:3388-92.

2 Krishna, M. C. et al Radiation Research 177(4): 376-386; Mailer, C., H. J. Halpern et al. 2006. Magn. Reson. in Med. 55(4): 904-912.

3. Epel B, Halpern HJ et al. Oxygen-guided radiation therapy. Int J Radiat Oncol Biol Phys. 2019;103(4):977-84.

4. Epel B, Kotecha M, Halpern HJ. J Magn Reson. 2017;280:149-57.

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