WORKSHOP

Oxygen Imaging in Tissue and Blood Circulation with Two-Photon Phosphorescence Lifetime Microscopy

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Presenter:

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Schedule

- 3 hours
- 4 lectures:

  Lecture 1 (Oxygen imaging)

  Lecture 2 (Two-photon microscopy)

  Lecture 3 (Phosphorescent O_2 nano-probes)

  Lecture 4 (*In vivo* implementation)

Lecture 1

Oxygen Imaging

Outline

- Why oxygen imaging?
- Available methods for oxygen imaging
- Advantages and disadvantages of each method
- Oxygen sensing with phosphorescent lifetime microscopy

Why oxygen imaging?

- O_2 is essential for energy metabolism in every cell in the body.
  - No food: up to 1 month, no water: up to 2 weeks, no oxygen: up to 10 min

- More critical for the brain
  - High rate of oxygen consumption
  - No reserved oxygen in brain
  - Limited neuroregeneration
  - Glucose in the brain enough for several minutes, O_2 enough for just 1 second

- Compromised oxygen delivery to the brain in healthy aging and/or diseases and possible role in neuronal death and neurodegenerative diseases.
  - Dementia
  - Alzheimer's Disease
  - Parkinson's Disease

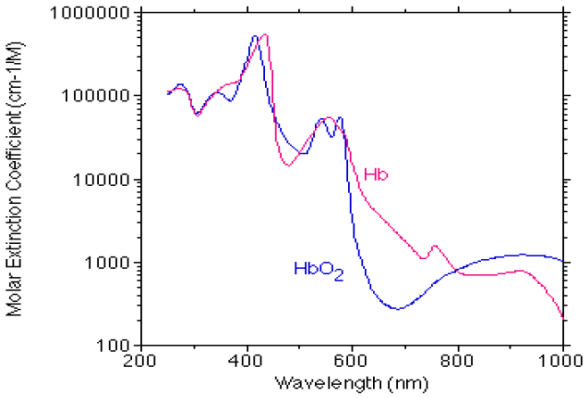
- Cancer therapy
  - Hypoxic tumors
  - More resistant to treatments
  - Diagnosis and optimization of treatment protocols

Available methods for oxygen imaging

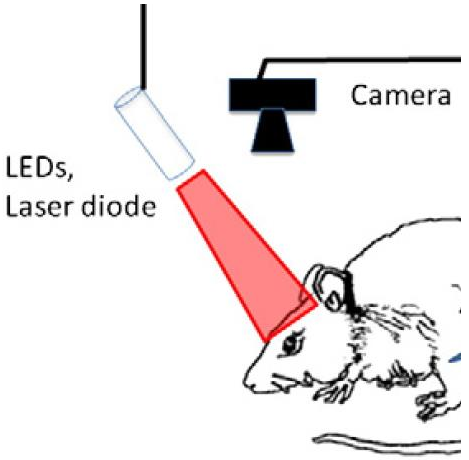
- Optical imaging of intrinsic signals
- Photo-acoustic tomography
- Blood-oxygen-level dependent (BOLD) fMRI
- Positron emission tomography (PET)
- Electron paramagnetic resonance (EPR) oximetry
- Polarographic O2 sensors
- Immunochemical methods
- Methods based on oxygen dependent quenching of phosphorescence

Optical imaging of intrinsic signals

- Based on differential absorption spectra of oxy- and deoxy-hemoglobin
- Beer–Lambert law
- Cannot measure absolute values of Hb or OHb concentrations
- Oxygen saturation (SO_2) = the ratio of oxygenated hemoglobin concentration to total hemoglobin concentration



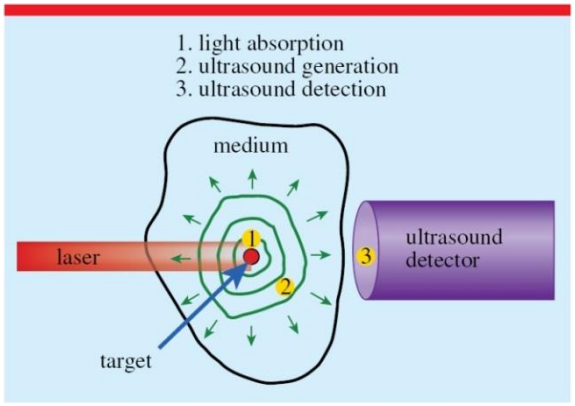
- Non-invasive
- No need for loading of extrinsic O2-sensitive probes
- Excellent temporal resolution (real-time measurements)
- Low cost
- Long penetration depth



- Measurement of oxygen saturation of blood rather than actual oxygen content
- Unable to measure tissue pO_2

Photo-acoustic microscopy

- Similar to optical imaging of intrinsic signals
- Photoacoustic effect: formation of sound waves following light absorption
- Modulated light or pulsed light
- Pulsed laser➔ absorption ➔ thermal expansion➔ acoustic waves➔ ultrasound detection
- Based on differential absorption spectra of oxy- and deoxy-hemoglobin



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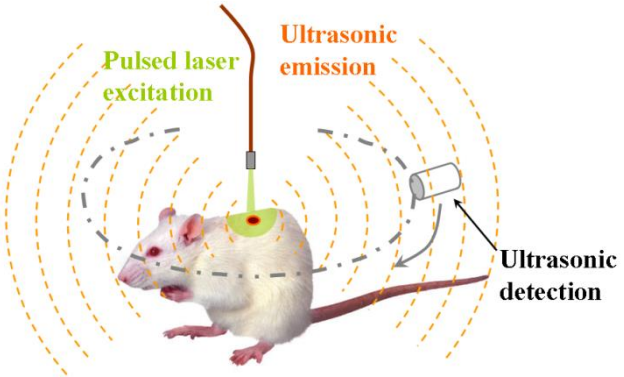

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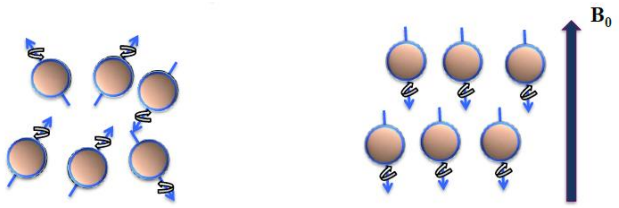
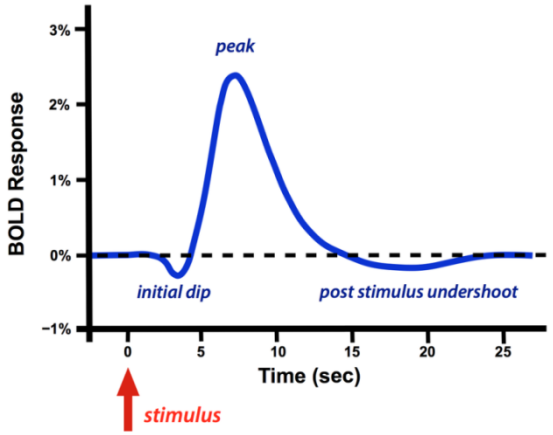
Blood-oxygen-level dependent (BOLD) fMRI

- Based on differential magnetic properties of oxy- and deoxy-hemoglobin
- The oxygenation dependence of the transverse relaxation time of water protons
- Changes in the local magnetic field around the blood vessels



- Non-invasive
- No need for loading of extrinsic O_2-sensitive probes
- Good temporal resolution

- Can only measure **changes** in blood oxygenation
- Low spatial resolution (in the order of 1 mm)
- Unable to measure tissue pO_2

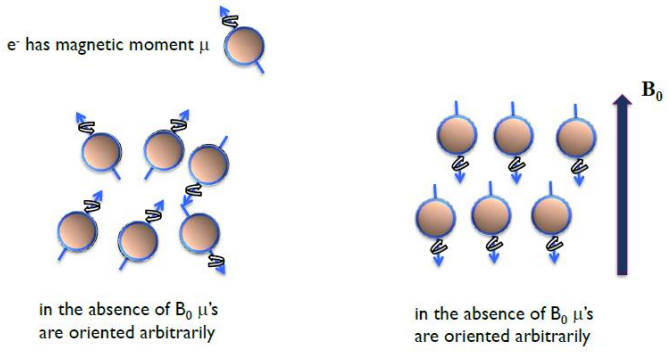


^{19}F MRI

- ^{19}F nuclear magnetic resonance (NMR) relaxation rate of perfluorocarbon (PFC) probes varies linearly with oxygen concentration.
- Unlike conventional MRI (proton imaging), a probe based on PFCs is used
- Absolute values of oxygen concentration
- Requirement of an exogenous probe
- Low spatial resolution (in the order of 1 mm)

Electron paramagnetic resonance (EPR) oximetry

- A Magnetic resonance method
- Similar to MRI, but transition of electrons (instead of protons) between two energy levels is observed.
- Spin probes: broadening of EPR spectrum with O_2 concentration



- Absolute values of O_2 concentration
- Minimally invasive

- Requirement of an exogenous probe
- Low spatial resolution (in the order of 1 mm)
- Poor SNR
- Long acquisition times (Low temporal resolution)

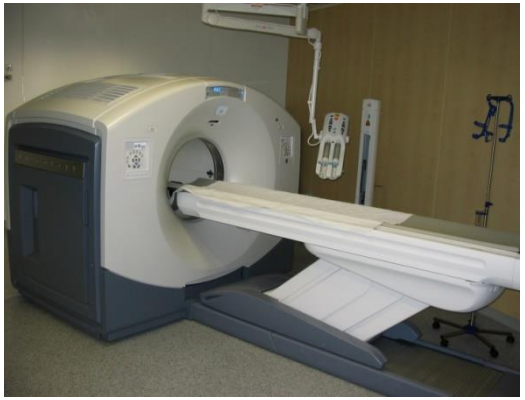
Ahmad and Kuppusamy (2010) *Chem. Rev.*, *110,* 3212.

Immunochemical methods

- Hypoxia markers such as 2-nitroimidazoles
- Maximum binding to severely hypoxic cells, increased inhibition with increasing oxygenation
- Allowing tissue pO_2 imaging
- Cannot measure real-time tissue pO_2
- Dependence of binding on factors other than pO_2 (level of tissue perfusion, the amount of reductases in the tissue) ➔ Difficulty in quantification of the results
- Nonoxygen-dependent metabolism

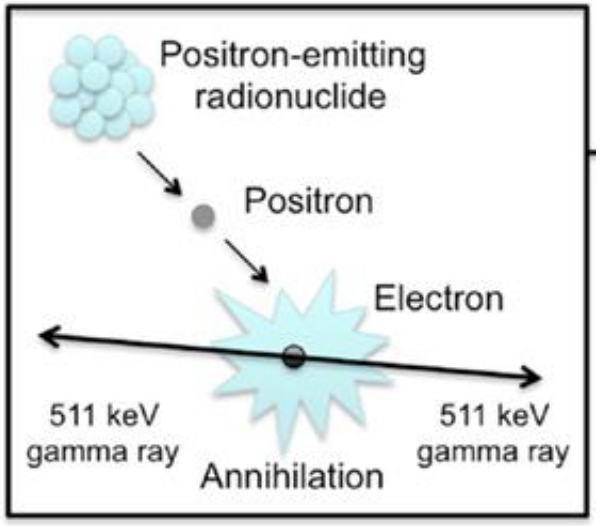
Positron emission tomography (PET)

- A nuclear medicine functional imaging technique
- Short-lived radiotracers
  (Fluorine-18, half-time~110 min)



- Injection of the radiotracer
- Perfusion in tissue
- Imaging

- Positron emission decay of the radiotracer
- Travel of emitted positron in tissue for a short distance (typically <1 mm)
- Kinetic energy loss
- Interaction of decelerated positron with an electron (annihilation)
- Production of a pair of gamma photons moving in opposite directions
- Coincident detection of the pair of photons with a scintillator



Positron emission tomography (PET)

- Fludeoxyglucose (FDG), an analogue of glucose: tissue metabolic activity
- Hypoxia imaging: Similar to immunochemical methods
  2-nitroimidazole compounds (18F-FMISO, 18F-FAZA)
- Accumulation of the tracer in cells is influenced by the O_2 level
- Tissue pO_2
- 3D
- Minimally invasive
- Difficulty in quantification of the results
- Low spatial resolution (in the order of 5 mm)
- Limited by the availability and cost of cyclotrons to produce short-lived radionuclides

Polarographic O_2 sensors

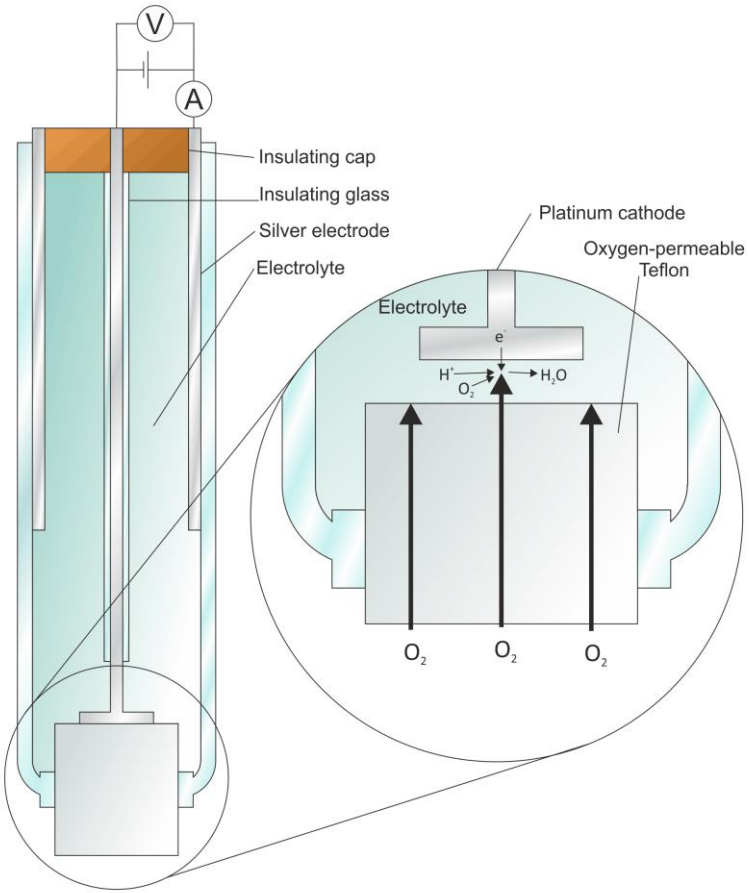
- Clark electrodes: "gold standard" for measuring tissue oxygenation
- Based on electrochemical reduction of oxygen at the cathode:

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$$

- Maximum current depends on the oxygen concentration

- Absolute O_2 concentration
- Allowing tissue pO_2 imaging
- Good temporal resolution

- Invasive
- Low spatial resolution
- O_2 consumption
- Poor SNR at low oxygen concentrations
- Need for repeated calibrations

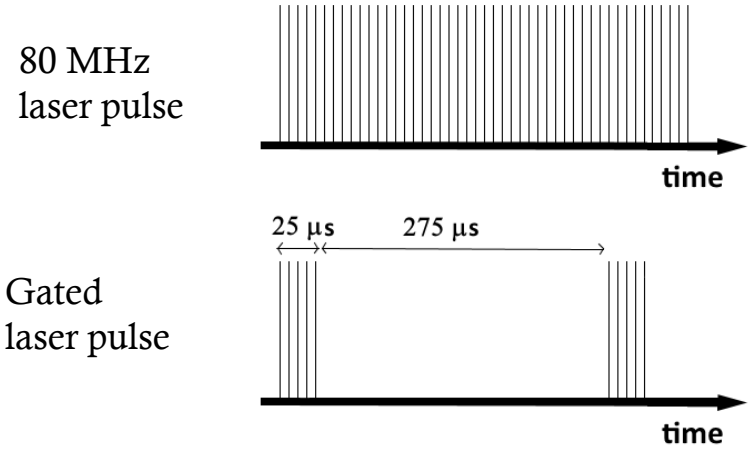


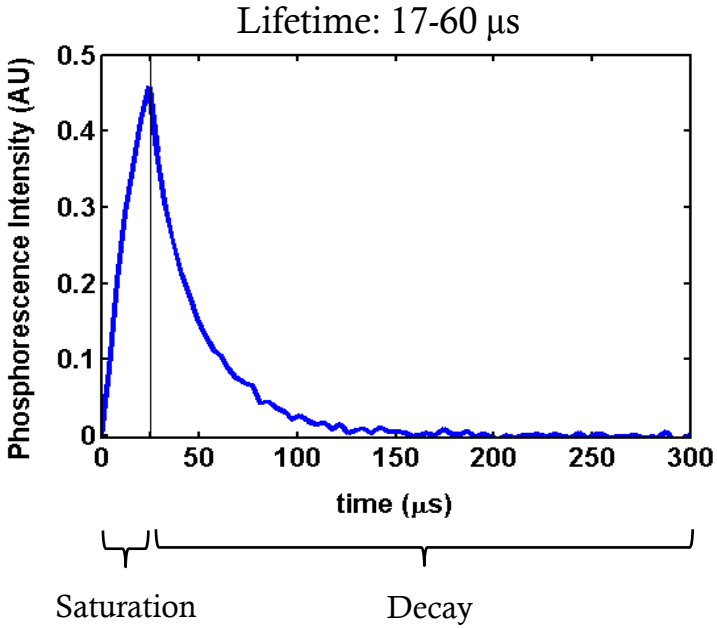
Oxygen dependent quenching of phosphorescence

- Phosphorescence process:

- Phosphorescence lifetime rather than intensity

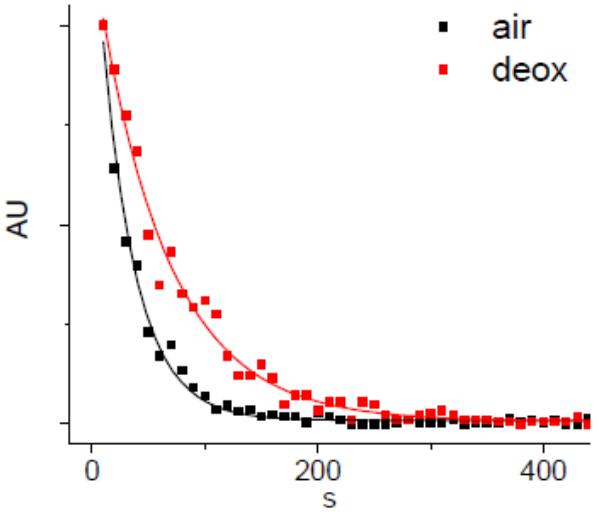
  Gated excitation rather than continuous excitation





Oxygen dependent quenching of phosphorescence

- Collision of oxygen molecules with phosphorescence molecule in the excited triplet state quenches the emitted phosphorescence (energy transfer to O_2)
- Higher oxygen concentration ➔ Higher decay rate of phosphorescence (shorter lifetime)



- Requirement of an exogenous probe

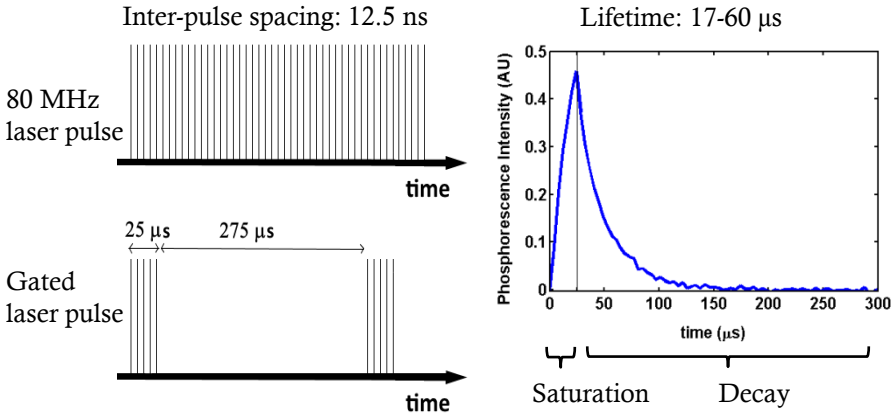
- Absolute measurement of pO_2 in blood and tissue
- Independent of probe concentration
- Real-time measurements
- Minimally invasive
- High spatial resolution

Phosphorescence lifetime two-photon microscopy

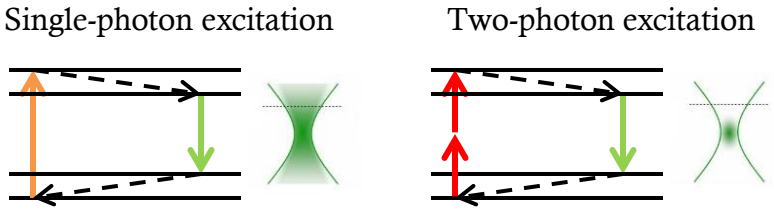
Recent progress: development of **new FRET-based phosphorescent probes that can be combined with two-photon microscopy:**[1,2]

Phosphorescence lifetime imaging

Based on oxygen-dependent quenching of phosphorescence



Two-photon microscopy



3D measurements

Higher spatial resolution

Deeper measurements

Reduced risk of photodamage

[1] Finikova et al. (2008). Chem Phys Chem 9: 1673.

[2] Sakadžić et al. (2010) Nature Methods, 7, 755.