

## Near-infrared spectroscopy for the study of biological tissue

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### Photon migration

The propagation of light inside biological tissues can be described in terms of a transport process for photons (the quanta of light). In this perspective, the light source injects a certain number of photons per unit time, per unit volume, per unit solid angle into a specific tissue location and these photons travel inside the tissue along certain trajectories that are determined by the absorption and scattering properties of tissue. The collective motion of photons along these trajectories in tissue is called *photon migration* and can be described with [transport theory and its high-scattering limit of diffusion theory](#).

### Absorption

The main absorbers of near-infrared (NIR) light in blood-perfused tissues are oxy-hemoglobin, deoxy-hemoglobin, and water. Their absorption spectra between 300 and 1300 nm are shown in Fig. 1, which is obtained from compiled absorption data for water (Hale and Querry, 1973) and hemoglobin (Prahl). In Fig. 1, the concentrations of oxy-hemoglobin and deoxy-hemoglobin are assumed to be 50  $\mu\text{M}$ , which is a typical value in blood-perfused tissues. The so-called “medical spectral window” extends approximately from about 700 to 900 nm, where the absorption of light shows a minimum (see Fig. 1). As a result, light in this spectral window penetrates deeply into tissues, thus allowing for non-invasive investigations. The optical penetration depth into tissues is limited, at shorter wavelengths, by the hemoglobin absorption, and at longer wavelengths by the water absorption. Other NIR absorbers in tissues that may be important in particular cases include myoglobin, cytochrome oxidase, melanin, and bilirubin.

The absorption properties of tissue are described by the absorption coefficient ( $\mu_a$ ), which is defined as the inverse of the average distance traveled by a photon before being absorbed. In the NIR, typical values of  $\mu_a$  in tissues range from 0.02 to 0.30  $\text{cm}^{-1}$ . The photon mean free path for absorption thus ranges between about 3 and 50 cm.

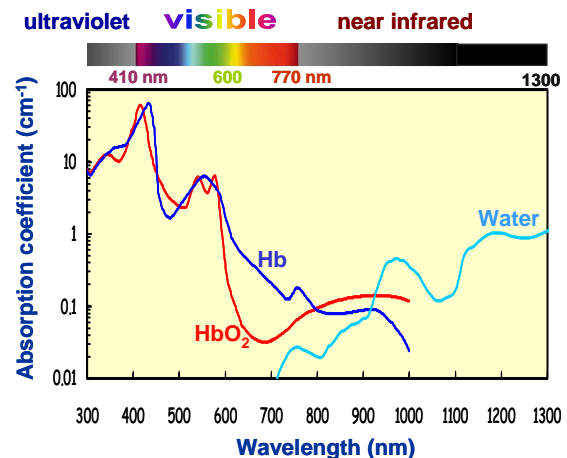


Fig. 1. Absorption spectra of deoxy-hemoglobin (Hb), oxy-hemoglobin (HbO<sub>2</sub>), and water. The concentrations of Hb and HbO<sub>2</sub> are set to 50  $\mu\text{M}$ .

### Scattering

While near-infrared light in the medical spectral window (~700-900 nm) is weakly absorbed in tissue, it is highly scattered similarly to the way that light is highly scattered in fog or in a glass of milk. This scattering problem, which is illustrated in Fig. 2, is at the heart of near-infrared spectroscopy of tissue. The scattering properties of a sample are mainly determined by the size of the scattering particles relative to the wavelength of light and by the refractive index mismatch between the scattering particles and the surrounding medium. In biological tissues, the scattering centers are cells and cellular organelles. In the medical spectral window (700-900 nm), cellular organelles have dimensions comparable to the wavelength, and their index of refraction is relatively close to that of the cytosol and extracellular fluid. As a result, light scattering in tissue is mainly forward directed and shows a weak wavelength dependence.

In strongly scattering media, the scattering properties are usually described by the reduced scattering coefficient ( $\mu_s'$ ), which represents the inverse of the average distance over which the direction of propagation of a photon is randomized. Typical values of  $\mu_s'$  in biological tissues range from 2 to 20  $\text{cm}^{-1}$ . The average distance traveled by a photon in tissues before losing memory of its initial direction of propagation is typically a few millimeters or less.

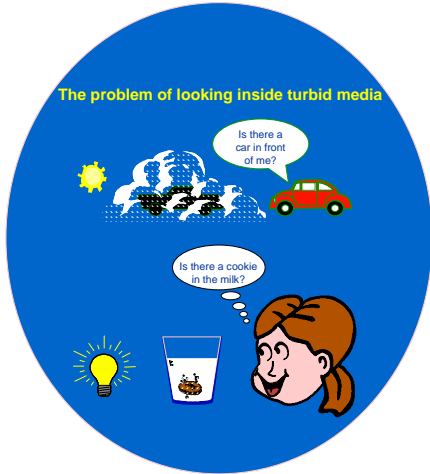


Fig. 2. Pictorial representation of strong light scattering in fog and in a glass of milk. The objective of near-infrared spectroscopy and imaging of tissue is to quantify the optical properties of tissue and identify embedded structures using optical measurements from the outside.

### Diffusion theory

Since in the near-infrared  $\mu_s'$  is typically much larger than  $\mu_a$  for most biological tissues, NIR light propagation in tissue is dominated by scattering and is highly diffusive. Under these conditions, one can use the diffusion equation to model the relationship between the optical energy density ( $U$ ), the absorption coefficient ( $\mu_a$ ), and the reduced scattering coefficient ( $\mu_s'$ ) of tissue:

$$\frac{\partial U(\mathbf{r}, t)}{\partial t} = \frac{v}{3(\mu_s' + \mu_a)} \nabla^2 U(\mathbf{r}, t) - v\mu_a U(\mathbf{r}, t) + S_0(\mathbf{r}, t). \quad (1)$$

In the diffusion equation,  $v$  is the speed of light in tissue, and  $S_0$  is a spherically symmetric source term. Diffusion theory provides a quantitative description of photon migration in tissue.

### Absorption spectroscopy

Because the absorption coefficient of tissues is due to a number of chromophores (oxy-hemoglobin, deoxy-hemoglobin, water, cytochrome oxidase, melanin, bilirubin, lipids, etc.), multi-wavelength measurements are needed to determine the relative contributions of each chromophore. The basic idea is that the contribution to  $\mu_a$  from the  $i$ -th chromophore can be written as the product of

the extinction coefficient ( $\epsilon_i$ ) times the concentration ( $C_i$ ) of that chromophore. As a result, in the presence of  $N$  chromophores, the absorption coefficient  $\mu_a$  at wavelength  $\lambda_i$  is given by:

$$\mu_a(\lambda_j) = \sum_{i=1}^N \epsilon_i(\lambda_j) C_i. \quad (2)$$

If the extinction spectra  $\epsilon_i(\lambda)$  of all  $N$  species are known, the concentrations  $C_i$  can be determined by measuring  $\mu_a$  at  $N$  or more wavelengths. This approach requires that  $\mu_a$  be measured independently of  $\mu_s'$ .

### Tissue oximetry

The absorption spectra of tissues can often be well described by considering only three chromophores, namely oxy-hemoglobin, deoxy-hemoglobin, and water. For example, Fig. 3 shows the spectra of pulsatile absorption associated with the arterial pulsation (top panel) and with the background absorption (bottom panel), as measured on the forehead of a human subject. The lines in Fig. 3 are the best fit absorption spectra corresponding to a linear combination of the water, oxy-hemoglobin, and deoxy-hemoglobin extinction spectra. In the fits, the water concentration (by volume) is assumed to be 80%, while the concentrations of oxy-hemoglobin ( $[\text{HbO}_2]$ ) and deoxy-hemoglobin ( $[\text{Hb}]$ ) are the fitting parameters. The blue lines refer to the case of the subject breathing a fraction of inspired oxygen ( $\text{FiO}_2$ ) of 21% (room air), while the red lines refer to a reduced  $\text{FiO}_2$  of 10%. The oxygenation values associated with the pulsatile absorption spectrum (arterial saturation) are 98% for  $\text{FiO}_2$  of 21% and 94% for  $\text{FiO}_2$  of 10%. The oxygenation values associated with the background absorption spectrum (tissue saturation) are 75% for  $\text{FiO}_2$  of 21% and 72% for  $\text{FiO}_2$  of 10%. The corresponding background values of the tissue concentration of oxy-hemoglobin and deoxy-hemoglobin are 30 and 11  $\mu\text{M}$ , respectively.

It is possible to measure the oxygen saturation of hemoglobin in tissues using just two wavelengths. The possibility of using dual wavelength optical measurements for blood oximetry have been known for a long time (Millikan, 1942), and it is currently exploited by pulse oximeters to measure the arterial saturation (Mendelson, 1992).

The two wavelengths  $\lambda_1$  and  $\lambda_2$  for near-infrared oximetry are usually chosen such that  $\lambda_1 < \lambda_{\text{iso}} \leq \lambda_2$ , where  $\lambda_{\text{iso}}$  is the NIR isosbestic wavelength at which the extinction coefficients of oxy- and deoxy-hemoglobin have the same value ( $\lambda_{\text{iso}}$  is about 800 nm as can be seen in Fig. 1). This choice maximizes the sensitivity of the optical measurement to changes in the tissue oxygenation. The measurement of  $\mu_a$  at two wavelengths translates Eq. (2) into a linear system of two equations (one per each

wavelength) in two unknowns (the concentrations of oxy-hemoglobin and deoxy-hemoglobin). Its solution yields the oxy- and deoxy-hemoglobin concentrations, and the tissue hemoglobin saturation can be computed as  $StO_2 = [HbO_2]/([Hb]+[HbO_2])$ .

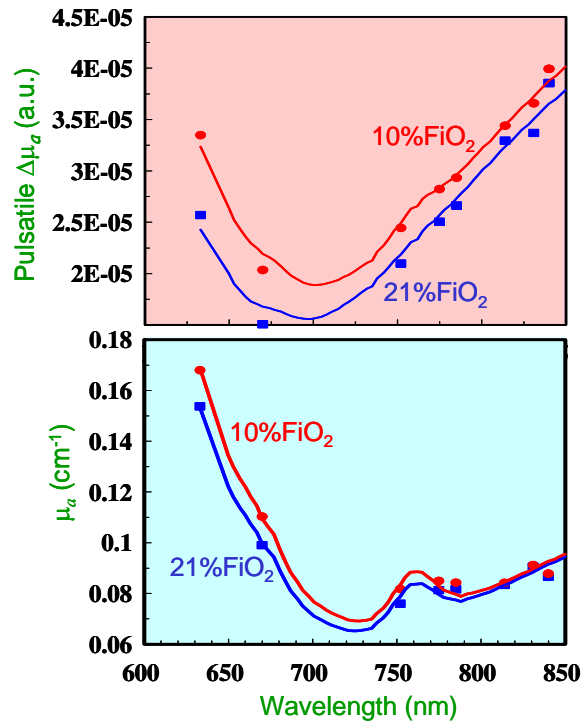


Fig. 3. Pulsatile (top) and background (bottom) near-infrared absorption spectra measured on the forehead of a human subject breathing a fraction of inspired oxygen ( $FiO_2$ ) of 21% (blue lines) and a reduced  $FiO_2$  of 10% (red lines).

#### Acknowledgements:

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#### References:

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