Basic Theory and Operating Principles of Laser Doppler Blood Flow Monitoring and Imaging (LDF & LDI).

Introduction

Laser Doppler is a standard technique for microvascular blood flow assessment of skin and other tissues. The strength of the technique is in recording changes in flow over time or differences in flow, comparing two areas of skin or other exposed tissue. For example, to compare blood flow in diseased tissue with flow in adjacent or contralateral healthy tissue, or pre and post appropriate test stimuli.

Operating Principles

The laser Doppler technique measures blood flow in tissues containing the very small blood vessels of the microvasculature, including nutritional blood flow in capillaries close to the skin surface and flow in the underlying arterioles and venules involved in regulation of skin temperature and blood pressure. The tissue thickness sampled is about 1mm and the velocity range measured is typically 0.01 to 10mm/s, and includes flow in capillaries (with diameters of about 10 microns) and in arterioles and venules (up to about 100 micron diameter).

The technique depends on the Doppler principle whereby low power light from a stable, monochromatic laser (Figure 1), e.g. a Helium Neon gas laser or a single mode laser diode, incident on tissue is scattered by moving red blood cells and as a consequence is frequency broadened (Figure 2). The frequency broadened light, together with laser light scattered from static tissue, is photodetected and the resulting photocurrent processed to provide a blood flow measurement. Please note, the average Doppler frequency shift is proportional to the average speed of red blood cells for tissues with a relatively low microvascular density (i.e. skin with low red blood cell concentration for the tissue volume); at higher concentrations, the proportionality deviates due to multiple scattering (Bonner and Nossal, 1981).

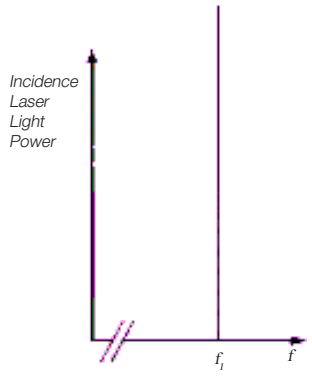


Figure 1: A laser operating in single mode.

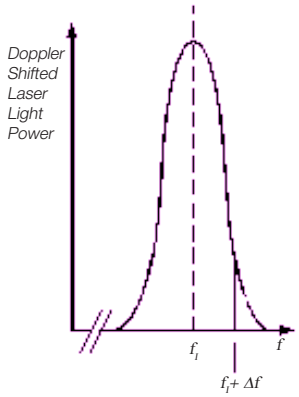


Figure 2: Δf: Doppler shift frequency is typically in the range of 20Hz to 20KHz for a well perfused region of the microcirculation. e.g. finger tips.

Laser light can be directed to the tissue surface either via an optic fibre (Figure 3) or as a light beam (Figure 4). For 'fibre optic' monitors (LDF instruments; moorVMS-LDF) the optic fibre terminates in an optic probe which can be attached to the tissue surface. One or more light collecting fibres also terminate in the probe head and these fibres transmit a portion of the scattered light to a photodetector and the intensity fluctuations are signal processed. Normal fibre separations in the probe head are a few tenths of a mm, consequently blood flow is measured in a tissue volume of about $1mm^3$. When a larger volume of tissue is stimulated to vasodilate or vasoconstrict, or where for example a healing process results in increased

blood flow, the blood flow changes measured by the LDF probe are generally taken to be representative of the larger volume; 'large area' probes and/or averaging measurements taken at multiple sites reduces variance.

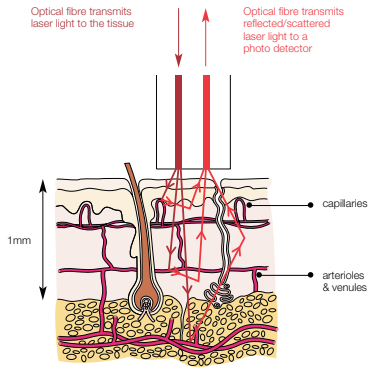


Figure 3: Schematic diagram of LDF probe and light scattering in skin.

In a Laser Doppler blood flow Imager (moorLDI2) the low intensity laser beam is scanned across a tissue surface in a raster fashion or a laser line (moorLDLS, Figure 5) is scanned, both using a moving mirror. There is no direct contact with the tissue being assessed. The basic elements of the moorLDI2 and moorLDLS are shown schematically in the following figures.

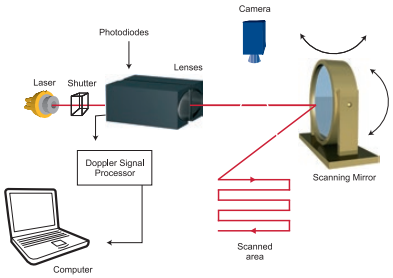


Figure 4: Schematic diagram of single point Laser Doppler Imager.

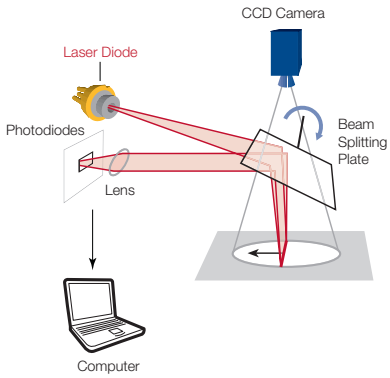


Figure 5: Schematic diagram of line-scan Laser Doppler Imager.

Both large areas (a full torso) and small areas (part of a finger) can be scanned enabling the blood flow to be mapped and colour coded images of the blood flow displayed. For moorLDI2, image resolution can vary between 2mm/pixel and 50microns/pixel, depending on distance and model used. Regions of interest can be defined and statistics, histograms and profiles can be calculated and recorded.

Single point measurements give a high temporal resolution (25Hz for moorLDI2; 40Hz for moorVMS-LDF and moorLDI2 in single point mode) enabling rapid blood flow changes to be recorded. Laser Doppler imaging frame rates are slower (depending on size and resolution) but it provides spatial information and can provide average blood flow measurements over large areas, reducing variance. Fibre optic systems can measure at tissue sites not easily accessible to a laser beam and have been used in many applications.

Definitions

The term commonly used to describe blood flow measured by the laser Doppler technique is 'flux': a quantity proportional to the product of the average speed of the blood cells and their number concentration (often referred to as blood volume). This is expressed in arbitrary 'perfusion units' and is calculated using the first moment of the power spectral density. Standardisation of LDF or LDI instrument measurements in perfusion units can be achieved by measuring a flux due to the Brownian motion of particles in a motility standard comprising polystyrene microspheres in water:

$$\text{flux} = k_1 \int_{\omega_1}^{\omega_2} \omega . \frac{P(\omega)}{I^n} d\omega - \text{noise}$$

$\mathbf{k_1}$ is a constant used to scale the raw output to a pre-determined calibration point.

ω is the Doppler shift in angular frequency units.

$\omega = 2\pi\Delta f$ where Δf is the Doppler shift frequency measured in frequency units Hertz

$P(\omega)$ is the optical power density at frequency ω.

$\mathbf{I}$ is the light intensity, used to normalise the signal to help eliminate the effects of gross variations in scattered light intensity (due to laser power differences, skin colour and beam angle of incidence).

$\mathbf{n}$ is an index which normally takes the value 2 if normalisation is being used to compensate for laser power differences. To compensate for tissue reflection changes in imaging a value of n = 1 is a better normaliser.

ω_1 is the lower cut off angular frequency.

$\omega_1 = 2\pi f_1$ Typically f_1 is 20Hz in LDF instruments and 20Hz to 250Hz in LDI instruments depending on scan speed.

ω_2 is the upper cut off angular frequency: it is limited to reduce overall noise.

$\omega_2 = 2\pi f_2$ Typically f_2 is 15KHz for measurement of blood cell speeds up to approximately 6mm/s. For low blood cell speeds (~ 1mm/s), and for an optimum signal to noise ratio, 3KHz can be used. For speeds up to 10mm/s a 22KHz bandwidth is used. (These estimated speeds assume the laser wavelength is 785nm). For a given bandwidth the speed scales with laser wavelengths. The shorter the wavelength the shorter the speed range measured.

Noise is the dark and shot noise components. Dark noise is a constant for an instrument and the shot noise level is proportional to I, the detected light intensity.

Time Constant, Tc, can be applied for single point measurements; a longer Tc is appropriate when monitoring over long periods at low data rates; e.g. use of a 3 second Tc when a data rate of 1Hz is used, so that sampling at various stages of the cardiac cycle are avoided, to avoid 'aliasing'. At high data rates, a short Tc is appropriate during measurement (e.g. 0.1sec) so that spikes, due to tissue/probe movement, are easily recognised; smoothing with a longer Tc can be applied at review but with caution, to avoid spikes influencing results by this 'averaging'.

Movement Artefacts: The effects on LDF and LDI flux of slow movement, e.g. due to respiration, is substantially in the low frequency range. This effect is minimised by use of high-pass filtering; for LDF a cut-off of 20Hz is typical. For LDI, there is the additional movement of laser beam across the skin (single point and line-scan LDI). The effect of this is minimised by high-pass filter and beam speed; 250Hz at 4ms/pixel, 100Hz at 10ms/pixel and 20Hz at 50ms/pixel for moorLDI2.

Depth of Measurement: it is a perennial question and there are several theoretical and simulation studies addressing this (e.g. Fredriksson et al, 2009). However, it is more easily and effectively considered experimentally: laser Doppler images of hand dorsum and volar forearm clearly show the veins under the skin. We can therefore conclude that the technique is sensitive to the full dermal thickness and some depth beyond. Veins are observed for lightly pigmented skin by red LDI (e.g. 632.8nm); in darkly pigmented skin the veins are only visible when the near infrared wavelength is used (e.g. 785nm). The influence of underlying veins on the assessment of skin tissue perfusion responses should be avoided by selecting regions without visible veins.

Penetration depth of gut tissues by LDF was elegantly assessed by Johansson et al, 1987, by additions of unperfused tissue and also by measurements without and with a mirror behind the gut tissue.

Probe Separation influences the depth of tissue probed by LDF (Jakobsson et al, 1993; Gush et al 1984); the wider the separation, the deeper the tissue that contributes to the LD signal. A typical probe separation is 0.5mm for assessing skin blood flow; flow in deeper tissues can be assessed with a 4mm separation probe and a high power laser Doppler (moorVMS-LD1F-HP). The difference between standard and wide separation probes has been observed by Clough et al, 2009, suggesting muscle blood flow was assessed for lean limbs by the wide separation probe.

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