A Laser Doppler Anemometer for measuring blood cell velocities in perpendicular capillary loops.

1 Introduction

There are several methods available for measuring blood cell velocity within single capillaries: flying spot, frame to frame, and photometric correlation. They are all image based techniques and can only measure flows in vessels lying parallel to the surface. Since the measurement is derived from the image, good, high contrast images are necessary. This is not always possible to obtain in many subjects and using standard video equipment restricts the maximum range of measurement to the order of 2 mm/s.

Measuring velocities using the laser Doppler technique is well established and there are many applications in the study of fluid flow. The technique was first demonstrated in 1964 (YEH *et al.*, 1964). Because of the relatively high cost of the necessarily precise optics, its use is generally restricted to measurements which would otherwise be impossible or impracticable. Typical applications are (DRAIN, 1980):

Studies of velocity and turbulence profiles of liquids and gases in complex systems, such as gas turbines, engine coolants, piston engine combustion.
Studies of boundary layer flows near walls.
Measurement of fluid dynamic properties.
Investigating aerodynamics in wind tunnels.
Supersonic boundary layers.
Measurement of remote wind speed.
Studies of trailing vortices from aircraft.
Measurement of velocity of solid surfaces.
Measurement of flows within living cells.

The application of laser Doppler to measuring flows in microscopic biological vessels has been published by several groups. The first *in vivo* application was the measurement of the velocity of blood cells in an 80 micron diameter retinal artery of an albino rabbit (RIVA *et al.*, 1972), using laser light backscattered at a known angle. In 1974 Mishina *et al.* described a dual beam Laser Doppler Microscope (MISHINA *et al.,* 1974). This was used to demonstrate the measurement of velocity in a 70 micron diameter venule in the web of a frog's foot by transmission through the tissue (MISHINA *et al.,* 1976). Another laser Doppler microscope anemometer (EINAV *et al.*, 1975) was used to measure velocity profiles in arterioles 65-98 microns in diameter. This system used the beam scattered at a known angle through the tissue to detect blood cell velocity. The measurements compared well with those from a calibrated particle velocity meter technique (a method similar to the flying spot technique). Later Einav *et al.* Described a fringe mode transmittance laser Doppler microscope. Measurements of the velocity profile across a rectangular channel gave good accuracy and reproducibility better than 1% (EINAV *et al.* 1988). All the above techniques depend on the angle of incidence or the angle of scattering of the laser beam. Some of the practical problems this entails *in vivo* are discussed by Born *et al.* (BORN *et al.*, 1978).

All the above techniques measure velocities parallel to the tissue surface, which like the imaging techniques restricts measurements to such sites as the nailfold or forearm.

Following on from the first instrument of Riva *et al.*, was the development of laser Doppler perfusion monitors (STERN, 1975; HOLLOWAY *et al.*, 1977; NILSSON *et al.*, 1980). In these systems the laser light is delivered and collected by two optic fibres. The laser light radiates out into the tissue is diffusely scattered and shifted by the flows in the multitude of vessels within the measuring volume of about 1 cubic mm. These systems produce an exponentially shaped Doppler shift spectrum which is processed to give arbitrary values proportional to average velocity and average blood cell concentration (BONNER *et al.*, 1981). The output requires linearisation (NILSSON, 1984) to correct for changes in blood cell concentration. The linearisation is only effective for part of the physiological range typically encountered. The output is dependent on the morphology of the tissue, i.e. the distribution of vessel dimensions and directions.

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The aim of the CAM1 Capillary Anemometer was therefore to develop an instrument which overcame the limited velocity range, image quality, and limited tissue sites of the imaging techniques, the high cost and complexity of the dual beam laser Doppler microscopes, and the unknown probe volume, arbitrary values, and non-linearity of the laser Doppler perfusion monitors.

2. Laser Doppler Anemometer

2.1 Optical System

A schematic for the CAM1 is shown in figure 1. A 7 mW 780 nm laser diode is focused by lenses l1 and objective l2. The position of l1 is adjusted so that the waist of the laser beam is in the object plane of the CCD camera and the photodetector. The position of mirror m1 is

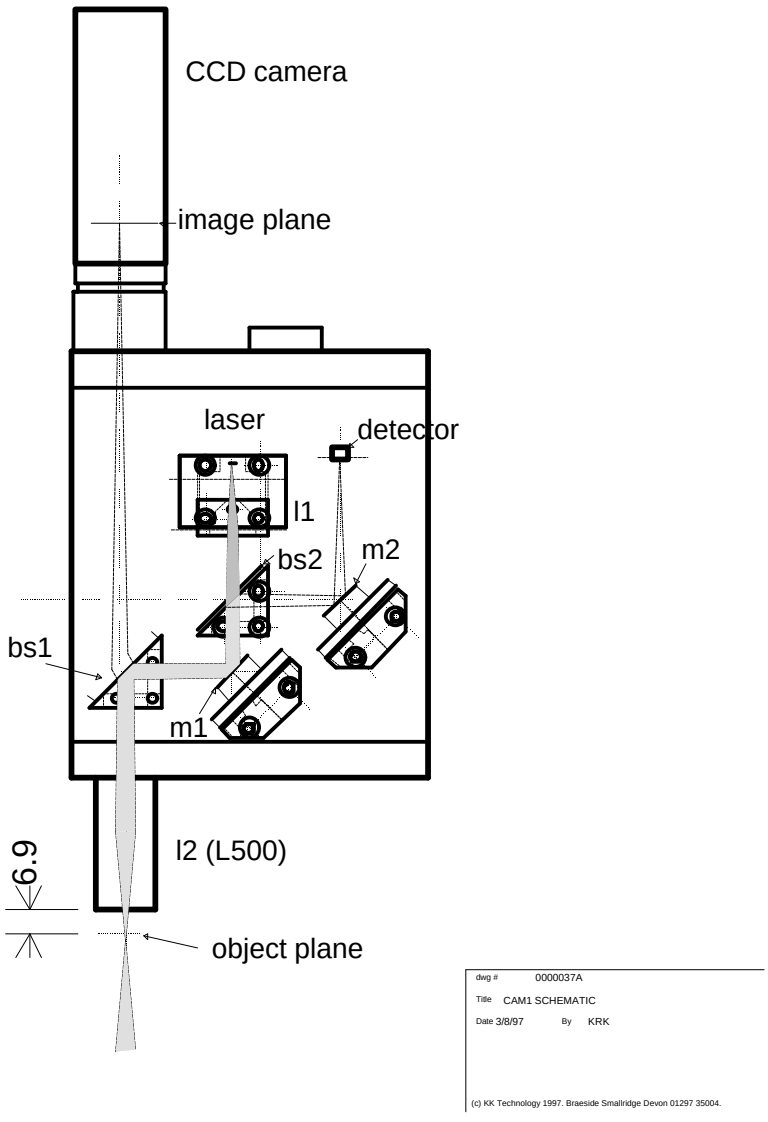

Figure 1

adjusted so that the laser beam is in the centre of the image. The position is set so that the photodetector is aligned with the image of the laser beam focal point. For maximum contrast between red blood cells and the surrounding tissue, green light of 525 nm is used for

illumination for the CCD camera. Beamsplitter bs1 separates the laser light from the green ccd image. The 50:50 beamsplitter bs2 splits the backscattered laser radiation via mirror m2 onto the detector. Laser power at the focal point is typically 0.8 to 1 mW with an elliptical spot of about 5 μm by 10 μm.

When the CAM1 is positioned and focused so that the laser beam is on a vertical arterial or venous limb of a capillary loop, a small fraction of the laser light will be backscattered by blood cells and collected by lens l1. Lens l1 will also collect laser light reflected from the surrounding tissue. Laser light backscattered by a moving blood cell will shift the frequency of the light. The magnitude of the shift will depend on the angle of scatter and the angle of the blood cell velocity. Since the CAM1 collects the reflected light along the same path as the incident light, the frequency shift will only depend on the relative angle, q, between the incident beam and the velocity, V. The frequency shift will be:

$$df = \frac{2nV}{l}\cos q \qquad (1)$$

where n is the refractive index of the medium (the CAM1 uses a value of 1.33) and l is the laser wavelength (780 nm).

The maximum frequency shift obtainable at any one speed is when q is 0 or 180°. Note that even at an angle of 18° the shift will be down by only 5%.

At the photodetector, Doppler shifted and unshifted laser light will mix to produce an electrical output with an ac component at a frequency of the difference between the shifted and unshifted light, i.e. the at the Doppler shift. The relationship will be 3410 Hz / mms $^{-1}$.

2.2 Electronic system

The CAM1 electronics is shown in figure 2. Output from the photodetector is amplified then bandwidth limited. The bandwidth is limited to 68 Hz to 50 kHz, which corresponds to a velocity range of 0.02 to 14.6 mms^{-1}.

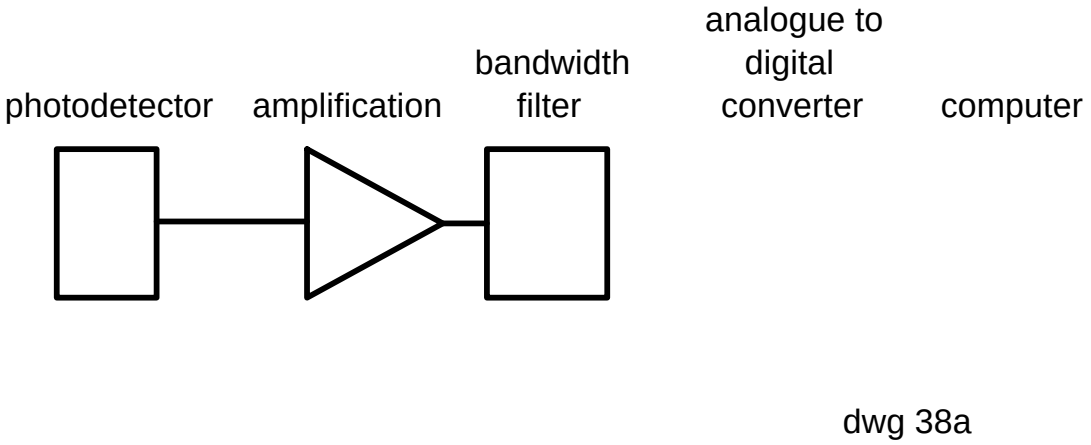

Figure2

An analogue to digital converter (ADC) on an interface card in a personal computer, digitises the signal for processing by the CAM1 software. The ADC samples the signal with 12 bit resolution. The software allows the Doppler 'bandwidth' to be set to 6.25, 12.5, 25, or 50 kHz, corresponding to an ADC sample rate of 12.5, 25, 50, and 100 kHz, and velocity full scale ranges of 1.8, 3.7, 7.3, and 14.6 mm.s^{-1}. The bandwidth into the ADC is fixed, only the ADC sample rate is changed. The 'bandwidth' is therefore determined by the ADC sample rate. Since the 'bandwidth' will always be set to eliminate clipping of the calculated velocity

signal, anti-aliasing filters are not necessary. Data is captured in 512 x 12 bit sample blocks. The interval between blocks can be adjusted but is set by default to 20 blocks per second. The maximum rate is determined by the processing power of the computer, although it is not possible to faster than 24.4 Hz at the lowest bandwidth setting (6.25 kHz) due to the time required to acquire the 512 samples.

2.3 Signal Processing

A radix-4 fast Fourier transform (FFT) is performed on each block of 512 samples. When squared this produces a power spectrum with 256 points. On the lowest 'bandwidth' this gives a resolution of 7.2 $\mu m.s^{-1}$, and 0.057 $mm.s^{-1}$ on the highest bandwidth.
Two methods are provided for determining the velocity from the Doppler shift power spectrum (DSPS). Figure 3 shows two expected types of DSPS. Figure 3a is what would be expected from small capillaries < 25 μm. Figure 3b is what would be expected from a larger vessel parallel to the surface. The large low frequency components in both 3a and 3b are from the amplitude and phase variations as the blood cells pass through the laser beam. For larger vessels, in which the blood flow may have a velocity profile, there will be a wide range of shifts from the low velocities at the edge, to the maximum velocity at the centre.

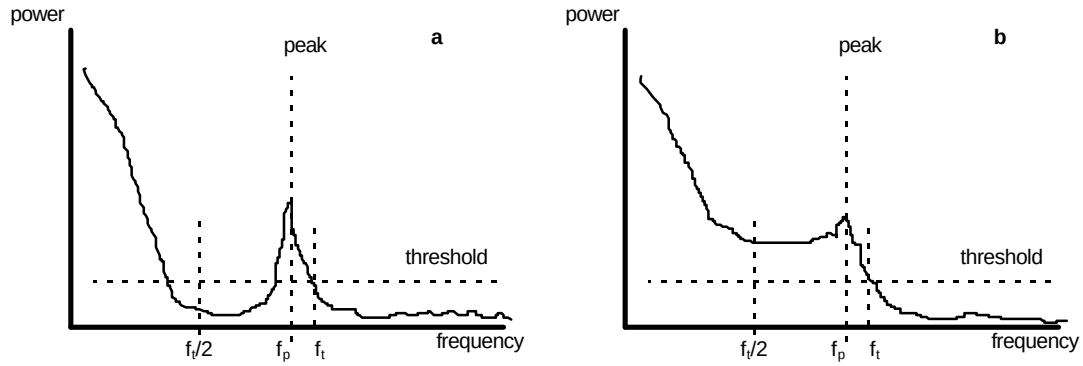

Figure3

The first method works well with small capillaries which usually produce a well defined narrow peak in the DSPS. In this method the software searches for a peak in the DSPS. A user defined threshold is used to ignore the background noise. f_t is set to the maximum frequency component that exceeds the threshold. The maximum power is then searched for in a section of the PSDS between $f_t/2$ and f_t. The capillary blood cell velocity (CBV) can then be calculated from f_p and equation 1.
The second method detects the highest frequency component f_t which exceeds the user defined threshold. This is more suitable to the DSPS of larger vessels as shown in figure 3b. However this later method can be sensitive to the threshold level set by the operator, and the signal strength, if the cut off at f_p is not steep.

3 RESULTS

A sample of Doppler shift signal from a capillary in the dorsal distal phalangeal area of the middle finger of the left hand is shown in figure 4. The high frequency Doppler shift can be clearly seen, along with low frequency amplitude variations due to the passage of blood cells through the probing volume.

The resulting DSPS from this signal is shown in figure 5, with a well defined narrow peak. The power scale is uncalibrated. Figure 6 shows how the DSPS is displayed by the CAM1 software. Frequency is shown on the y axis, with time along the x axis. Power within each frequency component is shown as a grey level, with white for full scale, and black for zero power. The data shown in figures 4 and 5 are taken from one sample point at about the 44 seconds point in figure 6. The highly pulsatile nature of the CBV in capillaries can be seen.

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During the recording of figure 6, the subject was not clamped sufficiently, so that small movements have caused brief periods in the recording where the signal strength is very weak. The calculated CBV trace is shown in figure 7.

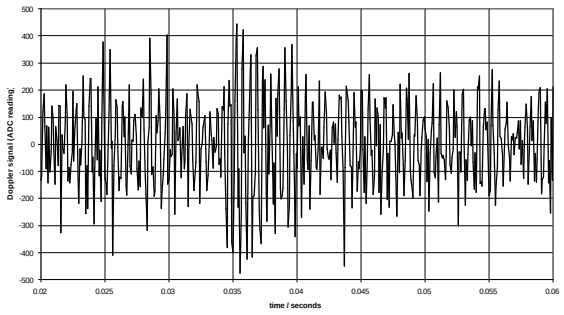

Figure 4

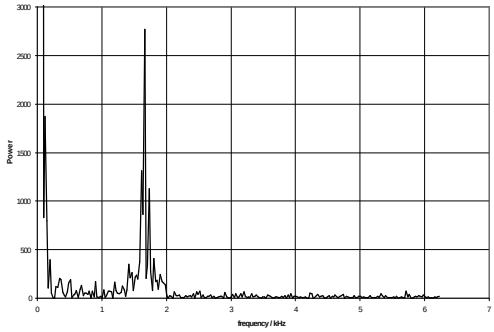

Figure 5

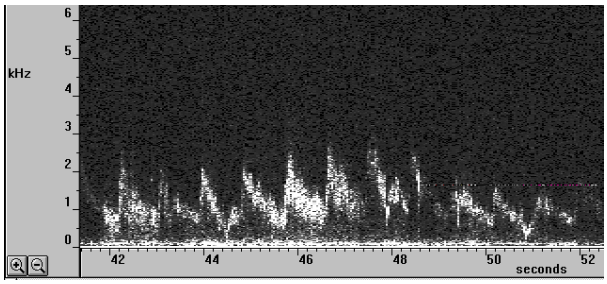

Figure 6

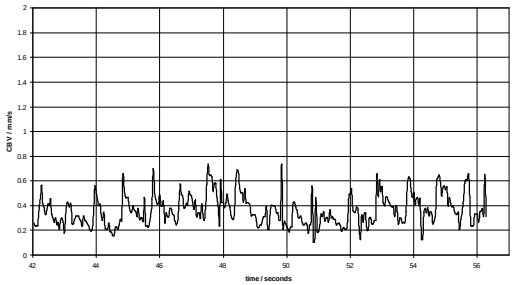

Figure 7

A recording of spontaneous vasomotion effects on CBV in a single capillary in the dorsal distal phalangeal area of the middle finger of the left hand is shown in figure 8. Notice the very regular low frequency period of about 0.1 Hz. Further results using the CAM1 have

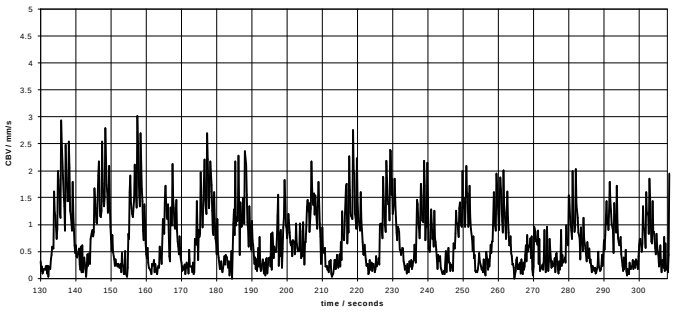

Figure 8

been published elsewhere (MORRIS *et al.*, 1996; STÜCKER *et al.*, 1996; ALTMEYER *et al.,* 1997).

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4 Discussion

As seen in the CAM1 CBV recordings there is a strong pulsatility. This is not possible to follow using the standard frame to frame technique (f-f). In attempts to prove the CAM1 measurements against f-f it was found that the average f-f results were lower than the average CAM1 CBV. By synchronising the two measurements, many of the measurement points for the f-f, were found to occur during diastole, where the CBV is lower. This was probably because any gaps in the erythrocytes during systole (if any) were of too poor a quality to see or use, since they were too blurred. It was also noted that white blood cells travel at a much lower velocity through the capillary than the RBCs. In one subject with peak CBV of about 1 mms^{-1}, white blood cells passed through the narrow capillary at only 0.1 mms^{-1}. For the f-f results these had to be removed even through they were perhaps the most accurate. Since white blood cells provide the best contrast changes, computerised correlation techniques may be strongly influenced these low velocities.
In dual window correlation techniques, the strong vasomotion variations measured in CBV with the CAM1 could cause aliasing problems. The dual window technique requires a few seconds of past data to produce a correlation from. If the averaging period is over half the vasomotion frequency, aliasing will occur.

REFERENCES

Bollinger A., Fagrell B. (Eds.)(1990): 'Clinical Capillaroscopy'. Hogrefe & Huber Publishers.
Bonner, R. F., Clem, T. R., Bowen, P. D. and Bowman, R. L. (1981): 'Laser-Doppler continuous real-time monitor of pulsatile and mean blood flow in tissue microcirculation', *in* Born G. V. R., Melling A., Whitelaw J. H. (1978): 'Laser Doppler microscope for blood velocity measurements'. Biorheology, 15. pp 163-172.
Chen, S., Chu, B. and Nossal, R. (Eds.): 'NATO advanced study institutes series, Series B physics, (Plenum Press New York, USA) pp 685-701.
Cochrane T., Earnshaw J C., (1978): 'Practical laser Doppler microscopes'. J. Phys. E: Sci. Instrum., Vol 11. pp196-198.
Drain L E. (1980): 'The Laser Doppler Technique'. John Wiley & Sons.
Einav S., Berman H. J., Fuhro R. L., DiGiovanni P. R., Fridman J. D., Fine S. (1975): 'Measurement of blood flow in vivo by laser Doppler anemometry through a microscope'. Biorheology, 12. pp 203-205.
Einav S., Berman H. J., Fuhro R. L., DiGiovanni P. R., Fine S., Fridman J. D. (1975): 'Measurement of velocity profiles of red blood cells in the microcirculation by laser Doppler anemometry'. Biorheology, 12. pp 207-210.
Einav S., Berman H. J. (1988): 'Fringe mode transmittance laser Doppler microscope anemometer: its adaptation for measurement in the microcirculation'. J. Biomed. Eng. 10. pp 393-399.
Einav S., Berman H. J., Dean H. C. (1989): 'Fringe mode reflectance laser Doppler microscope system'. J. Biomed. Eng. 11. pp 57-62.
Eiju T., Matsuda K., Ohtsubo J., Honma K., Shimizu K., (1981): 'A frequency shifting of LDV for blood velocity measurements by a moving wedged glass'. Applied Optics Vol 20. No.22. pp 3833-3837.
Eiju T., Nagai M., Matsuda K., Ohtsubo J., Homma K., Shimizu K., (1993): 'Microscopic laser Doppler velocimeter for blood velocity measurement'. Optical Engineering 32(1), pp15-20.
Holloway, G. A. and Watkins, D. W. (1977): 'Laser Doppler measurement of cutaneous blood flow', *J. Invest. Dermatol.,* **69,** 306-309.
Koyama T., Horimoto M., Mishina H., Asakura T. (1982): 'Measurements of blood flow velocity by means of a laser Doppler microscope'. Optik, 61(4), pp 411-426.
Mishina H., Asakura T., Nagi S., (1974): 'A Laser Doppler Microscope'. Opt. Commun. 11. pp99-102.
Mishina H., Ushizaka T., Asakura T. (1976): 'A laser Doppler microscope'. Optics and Laser

Technology, June. pp 121-127.
Morris S J, Kunzek S, Shore A C. (1996) The effect of acetylcholine on finger capillary pressure and capillary flow in healthy volunteers Journal of Physiology (1996), 494.1, pp 307-313.
Nilsson, G. E., Tenland, T. and ÖBERG, P. Å (1980): 'A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy', *IEEE Trans.,* **BME-27**, 12-19.
Nilsson, G. E. (1984): 'Signal processor for laser Doppler tissue flowmeters', *Med. & Biol. Eng. & Comput.,* **22**, 343-348.
Petrig B L., Riva C E., Grunwald J E., (1984): 'Computer Analysis of Laser Doppler Measurements in retinal Blood Vessels'. Invest. Ophthalmol. Vis. sci. 25(Suppl.), pp7.
Petrig B. L., Riva C. E. (1988): 'Retinal laser Doppler velocimetry: towards its computer-assisted clinical use'. Applied Optics. Vol 27 No 6. pp 1126-1134.
Riva C., Ross B., Benedek G. (1972): 'Laser Doppler measurements of blood flow in capillary tubes and retinal arteries' Investigative Ophthalmology November 1972. pp 936-944.
Stern, M. D. (1975): '*In vivo* evaluation of microcirculation by coherent light scattering', *Nature,* **254,** 56-58.
Stücker M, Baier V, Reuther T, Hoffmann K, Kellam K, Altmeyer P. (1996): Capillary Blood Cell Velocity in Human Skin Capillaries Located Perpendicularly to the Skin Surface: Measured by a new Laser Doppler Anemometer Microvascular Research 52, pp 188-192.
Ushizaka T., Asakura T., (1983): 'Measurements of flow velocity in a microscopic region using a transmission grating'. Applied Optics Vol 22 No 12. pp1870-1874.
Yeh Y., Cummins H. Z., (1964): Applied Physics Letters, 4, pp176-8.

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