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TOPICAL REVIEW

Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging

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Abstract

Laser Doppler velocimetry uses the frequency shift produced by the Doppler effect to measure velocity. It can be used to monitor blood flow or other tissue movement in the body. Laser speckle is a random interference effect that gives a grainy appearance to objects illuminated by laser light. If the object consists of individual moving scatterers (such as blood cells), the speckle pattern fluctuates. These fluctuations provide information about the velocity distribution of the scatterers. It can be shown that the speckle and Doppler approaches are different ways of looking at the same phenomenon. Both these techniques measure at a single point. If a map of the velocity distribution is required, some form of scanning must be introduced. This has been done for both time-varying speckle and laser Doppler. However, with the speckle technique it is also possible to devise a full-field technique that gives an instantaneous map of velocities in real time. This review article presents the theory and practice of these techniques using a tutorial approach and compares the relative merits of the scanning and full-field approaches to velocity map imaging. The article concludes with a review of reported applications of these techniques to blood perfusion mapping and imaging.

Keywords: laser Doppler, laser speckle, time-varying speckle, medical imaging, blood flow, perfusion

1. Introduction

This article presents a review of laser Doppler velocimetry and related techniques, with emphasis on their use for mapping blood perfusion in tissues. The approach is tutorial, though a knowledge of the basic physics of light interference and the Doppler effect is assumed. The

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use of mathematics is kept to the absolute minimum consistent with an understanding of the basic techniques and their application.

The Doppler effect has been known since the middle of the nineteenth century (Doppler 1842). It explains the change in frequency of a wave when there is relative movement between the source of the wave and an observer. As this frequency change depends on the relative velocities of the source and the observer, the effect can be used to measure velocities.

The laser was invented in 1960 and was being applied to Doppler techniques within a few years of this. The use of laser Doppler to measure blood flow has been investigated since the early 1970s and is now a well-established medical tool. Its advantage over other techniques is that it is non-contact and (often) non-invasive.

'Speckle' is a random interference effect that only came into prominence with the invention of the laser. It will be discussed in more detail below. Laser speckle was first regarded as a nuisance—it severely limits resolution when laser light is used, for example in holography. However, potential applications of laser speckle were soon being developed, including the use of time-varying speckle patterns to detect and measure movement. Although the approach of the speckle techniques seems to be completely different from that of Doppler methods, a mathematical analysis shows that the two approaches are, in fact, identical. Nevertheless, the two techniques have tended to develop separately. Time-varying speckle has been applied to the monitoring of blood flow, mainly in the retina and the capillaries, since the mid-1970s.

In principle, both Doppler and speckle techniques measure the blood velocity at a single point. Many reviews, even books, have been written on these single-point methods (Abbiss *et al* 1974, Durrani and Greated 1977, Shepherd and Öberg 1990) and this paper will not attempt to emulate these. There is sometimes, however, a clinical requirement to have a map of blood flow, especially capillary blood flow, over an extended area of the body. Most researchers have addressed this, in both the Doppler and the speckle techniques, by scanning the focused laser beam over the area of interest, measuring at each point in turn. There is, however, a possibility of using a full-field speckle technique that gives the velocity map in real time. It is on these mapping techniques, both scanning and full-field, that this paper will concentrate, once the basic techniques and implementations have been described.

2. Background physics

2.1. Laser speckle

In the early 1960s the inventors and first users of the laser had a surprise. When laser light fell on a matt surface such as paper or unpolished metal or glass, they saw a high-contrast grainy pattern on which it was difficult to focus. At first they called the effect 'granularity' (Rigden and Gordon 1962), but soon the name *speckle* became more popular. Figure 1 shows a typical *speckle pattern*.

In the early days of lasers, speckle was regarded purely as a nuisance: it severely affected resolution when laser light was used, for example in holography, and much effort was directed towards reducing speckle in images formed in laser light. However, it was not long before scientists started to study speckle for its own sake (Dainty 1975) and to develop practical applications of the phenomenon.

Shining a narrow laser beam onto a surface and looking at the scattered light falling on a screen some distance away also produces a speckle pattern. This type of speckle pattern is referred to in this paper as *far-field speckle*, while the speckle observed on an illuminated surface is called *image speckle*. Figures 2 and 3 illustrate the formation of these two types of speckle pattern.

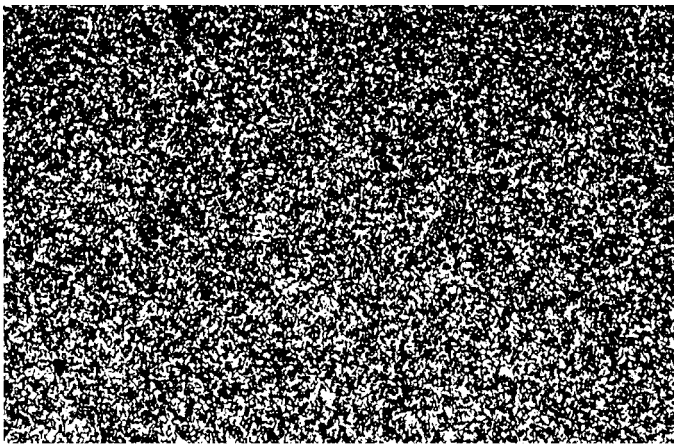

Figure 1. A typical laser speckle pattern.

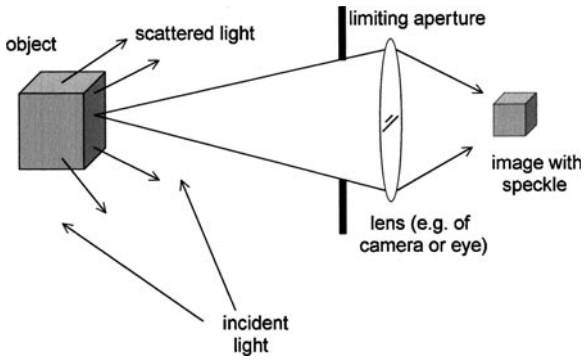

Figure 2. The formation of image speckle.

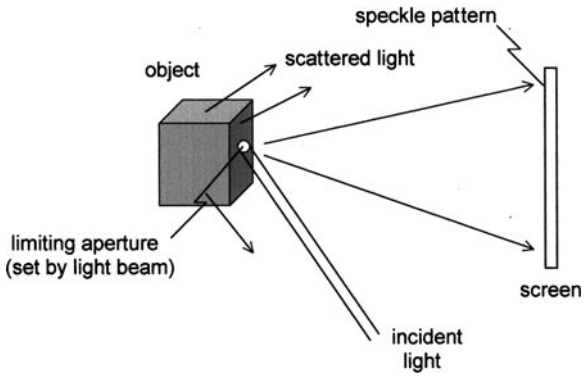

Figure 3. The formation of far-field speckle.

Laser speckle is an interference pattern produced by light reflected or scattered from different parts of the illuminated surface. Consider image speckle first. If the surface is rough (surface height variations larger than the wavelength of the light used), light from different parts of the surface within a resolution cell (the area just resolved by the optical system imaging

the surface) traverses different optical pathlengths to reach the image plane. In the case of an observer looking at a laser-illuminated surface, the resolution cell is the resolution limit of the eye and the image plane is the retina. The resulting intensity at a given point on the image is determined by the algebraic addition of all the wave amplitudes arriving at the point. If the resultant amplitude is zero, because all the individual waves cancel out, a dark speckle is seen at the point, while if all the waves arrive at the point in phase, an intensity maximum is observed. In the case of far-field speckle (figure 3), light from all points within the illuminated area contributes to the speckle intensity at any point on the observing screen.

Laser speckle is a random phenomenon and can only be described statistically. Goodman (1975) has developed a detailed theory, but for this paper only one result is of major importance. This is an expression for the contrast of a speckle pattern. Assuming ideal conditions for producing a speckle pattern—single-frequency laser light and a perfectly diffusing surface with a Gaussian distribution of surface height fluctuations—it can be shown that the standard deviation of the intensity variations in the speckle pattern is equal to the mean intensity. In practice, speckle patterns often have a standard deviation that is less than the mean intensity, and this is observed as a reduction in the contrast of the speckle pattern. In fact, it is usual to define the *speckle contrast* as the ratio of the standard deviation to the mean intensity:

$$\text{speckle contrast } K = \frac{\sigma}{\langle I \rangle} \leqslant 1. \tag{1}$$

In passing, we should mention that the scale of the speckle pattern—the size of an individual speckle—has nothing to do with the structure of the rough surface producing it. It is determined entirely by the aperture of the optical system used to observe the speckle pattern (for image speckle) or by the area illuminated (for far-field speckle). A detailed account of this phenomenon is outside the scope of this paper, but it is important to note that if a camera is used to photograph a speckle pattern, it is the setting of the aperture stop that will determine the speckle size! This can have a serious implication if it is desired to use the aperture stop to control the exposure of the photograph.

Laser speckle techniques have been used in a variety of optical metrology techniques, including displacement, distortion and strain measurement, surface roughness assessment and velocity measurement. Some of these applications have been in the biomedical field.

2.2. Time-varying speckle

When an object moves, the speckle pattern it produces changes. For small movements of a solid object, the speckles move with the object, i.e. they remain correlated; for larger motions, they *decorrelate* and the speckle pattern changes completely. Decorrelation also occurs when the light is scattered from a large number of individual moving scatterers, such as particles in a fluid. An individual speckle appears to 'twinkle' like a star.

Time-varying speckle is frequently observed when biological samples are observed under laser-light illumination. Examples reported in the literature include various botanical subjects (Briers 1975, 1978, Oulamara *et al* 1989, Xu *et al* 1995) and the phenomenon is attributed to the flow of fluids inside the plant, or even to the motion of particles within the cells of the plant. One of the most important potential applications, first recognized by Stern (1975), arises when the fluctuations are caused by the flow of blood.

It is reasonable to assume that the frequency spectrum of the fluctuations should be dependent on the velocity of the motion. It should therefore be possible to obtain information about the motion of the scatterers from a study of the temporal statistics of the speckle fluctuations. This is the basis of the study of time-varying speckle, many of whose applications have been in the biomedical field.

2.3. The Doppler effect

The Doppler effect is the change in frequency that occurs when there is relative movement between the source of a wave and a detector. It occurs because the waves emitted by the source are either compressed (if the source and detector are moving towards each other) or spread out (if they are moving away from each other). The most familiar everyday example is the drop in pitch (which is determined by frequency) as a sound source moves past us. A particularly good example is an emergency vehicle with its siren sounding.

The Doppler effect also occurs with light. There is a problem, however. The frequencies of light waves are very high and difficult to measure directly. This problem is solved by using the phenomenon of 'beats'—the effect that is produced when two waves of slightly different frequency are superimposed. As the two waves come into and out of phase, they alternately add and cancel. The result is the detection of a frequency that is equal to the difference in frequency between the two waves.

By mixing the Doppler-shifted wave with a reference wave of the original frequency, a beat frequency is produced that is much lower than either of the two constituent waves and is therefore much easier to measure. As this beat frequency is equal to the difference between the two frequencies, it is hence equal exactly to the frequency shift produced by the Doppler effect.

It can be shown that the relationship between the frequency change and the relative velocity of source and detector is given by

$$f' - f = \frac{v}{c - v} f \tag{2}$$

where f is the original frequency of the light, f' is the shifted frequency, v is the relative velocity of the source and the detector and c is the velocity of the wave. In the case of light waves, the velocity of light c is usually much larger than the velocities being measured and equation (2) can be simplified to

$$f' - f = \frac{v}{c} f. \tag{3}$$

Hence the frequency shift, and therefore the beat frequency when the Doppler-shifted light is mixed with a reference beam of the original frequency, is proportional to the velocity being measured. All we need in addition is the original frequency and the velocity of light.

2.4. Summary of the physics

We now have all the physics necessary to understand the techniques described in this paper for measuring the velocities of particles in fluids (such as blood). The motion of a light source, or of an object reflecting or scattering the light, leads to a change in the frequency of the light by the Doppler effect. Measuring this frequency change provides a means of measuring the velocity of the object. However, because the frequency of light waves is so high, it is not feasible to measure the frequency change directly. Instead, the reflected (or scattered) Doppler-shifted light is mixed with the original light so that a beat frequency is detected. This beat frequency is equal to the frequency shift and hence proportional to the velocity of the object.

Another approach to measuring the velocities of particles in fluids is to use laser speckle. When the illuminated object is a moving fluid, the speckle pattern fluctuates, leading to so-called time-varying laser speckle. These intensity fluctuations can also be used to measure the velocity of the scattering particles.

3. Doppler techniques for measuring velocities

3.1. Reference beam method

As mentioned in section 2.3, the frequencies of light waves are much too high to be measured directly. Because of this, the basic implementation of the laser Doppler technique to measure fluid velocities is to illuminate part of the flow field with laser light, collect some of the light reflected from it, mix it with the original light and measure the resulting beat frequency (Yeh and Cummins 1964). (Readers familiar with radio engineering will recognize this as a *heterodyne* technique. Physicists with an optics background might prefer to regard it as an *interferometry* method, with the original light serving as a *reference beam*.) The beat frequency is directly proportional to the velocity of the particles in the fluid. However, we do need one small change from the theory given in section 2.3. There we assumed that the object was a *source* of waves. If it is merely a reflector of waves sent out by a source near the observer, then we need to take into consideration the fact that the object is moving with respect to the incoming wave as well as with respect to the detector. This has the effect of doubling the frequency shift compared with that predicted by equation (3) for a self-luminous moving source and we have

$$f' - f = \frac{2v}{c} f. \tag{4}$$

Let us rewrite this equation to give the velocity in terms of the beat frequency. For convenience, let us write the beat frequency (same as the Doppler frequency shift) as Δf

$$f' - f = \Delta f.$$

We can then rearrange equation (4) to give

$$v = \frac{c}{2} \frac{\Delta f}{f}.$$

Using the relationship $c = f\lambda$, where λ is the wavelength, gives

$$v = \frac{\lambda}{2} \Delta f. \tag{5}$$

This is the basic equation of laser Doppler techniques. Remember that v is the velocity of the object (or more correctly the relative velocity of the object with respect to the observer) *measured along the line of sight*. If the object is moving in some other direction, what is measured is the *component* of the velocity *along the line of sight*.

In practice, it is often not necessary to use a separate reference beam. Enough light will be reflected or scattered from stationary objects in or around the probe volume to provide an unshifted reference beam to beat with the frequency-shifted light. Using such 'parasitic' scattering (Abbiss *et al* 1974) has the advantage that it will have traversed practically the same path as the frequency-shifted light, which avoids problems due to air turbulence, etc. (In interferometry, this would be called a *common-path* technique and is used for the same reason.)

3.2. Two-beam (or Doppler difference) method

The bringing together of the light scattered from the moving object and the reference beam is not trivial and for many applications a slightly different technique is better. This uses two laser beams that cross at an angle in the volume where the velocity is to be measured (Rudd 1969). A typical set-up is illustrated in figure 4.

Consider a particle moving with velocity v in the direction shown. The components of this velocity parallel to the two laser beams are $v \sin\theta$ and $-v \sin\theta$. (It is clear that for the

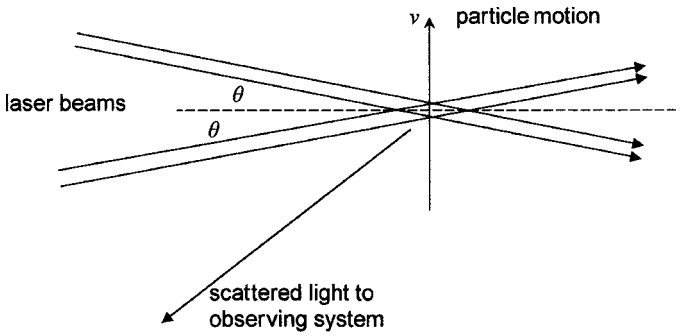

Figure 4. Two-beam (Doppler difference) system.

beam coming in from the upper left, the component of particle velocity is in the direction opposite to the direction the light is travelling, whereas for the other beam it is in the same direction: this accounts for the difference in sign.) Hence there will be a frequency *increase* when the upper beam is reflected from the particle and a corresponding *decrease* (and of the same magnitude) when the lower beam is reflected.

It is clear from the arguments expressed above that the frequency change in each case will be given by equation (3), provided we replace the velocity by the velocity *component* $v \sin\theta$

$$\Delta f = \frac{v \sin\theta}{c} f.$$

The *difference* in frequency between the two beams reflected by the particle is twice this (as the two frequency shifts are equal but of opposite sign)

$$\Delta f = \frac{2v \sin\theta}{c} f. \tag{6}$$

As in the reference-beam case (see section 3.1), there will also be a frequency shift caused by the velocity of the particle with respect to the reflected light travelling towards the observer. However, this will be the same shift for each beam and hence will not contribute to the frequency difference observed. Equation (6) therefore represents the total frequency difference (and hence beat frequency) produced by the motion of the particle.

This also means that the two-beam technique is independent of the direction of view. This has an important advantage: it means that a lens with a high numerical aperture can be used to collect the light scattered by the moving particles and hence improve efficiency. (Note that this is *not* the case with the reference-beam technique of section 3.1. In that arrangement, it is necessary to place a small aperture in front of the detector so that the measurement is being made in a single direction.)

As before (see equation (5)), we can rearrange equation (6) to give an expression for the velocity of the particle in terms of the beat frequency observed:

$$v = \frac{\lambda}{2 \sin\theta} \Delta f. \tag{7}$$

Note that we have assumed that the particle is travelling at right angles to the bisector of the two incident laser beams. If the particle is travelling in any other direction, what is measured is the *component* of the velocity in this direction.

In other words, to determine the velocity of the particle (or at least its component in a certain direction), all we need to know is the angle between the two laser beams and the (original) wavelength of the laser light, and to measure the beat frequency observed.

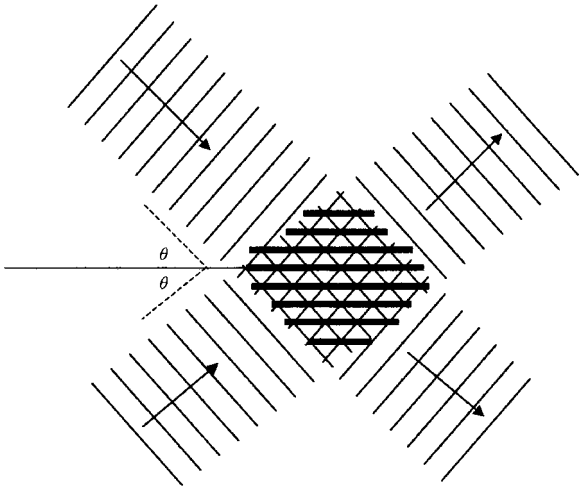

Figure 5. Interference explanation of the two-beam Doppler technique.

The beat frequency mentioned above (and in section 3.1) is observed as a fluctuation in the light scattered by the moving particles. There is another way to look at this intensity fluctuation. Two laser beams crossing at an angle will interfere as shown in figure 5.

Consider two light beams crossing at an angle 2θ as shown in figure 5. The parallel lines represent the crests of the waves at a particular time. The bold horizontal lines in the area of overlap indicate the locations where the crests of one wave fall on the crests of the other wave. (Inspection of the diagram shows that the troughs of the two waves are also superimposed along these same lines—the troughs are midway between the crests in the two waves.) Hence at these locations, which are lines in the diagram but *planes* if the three-dimensional picture is considered, the two waves are in phase and there will be a maximum light intensity. Midway between these lines the two waves will be in antiphase, with the crest of one falling on the trough of the other, and the two waves will cancel out. Hence there will be an intensity minimum at these points (which also form parallel lines in the diagram, or parallel planes in space). After a time equal to $1/f$ seconds, where f is the frequency of the waves in cycles per second, both waves will have advanced one wavelength (λ) and it is clear that the lines indicating where the two waves are in phase have not moved. Further thought will show that these lines of equal phase *never* move but are fixed in space. Hence they represent a pattern of *interference fringes* in space. It is clear from figure 5 that these fringes are equally spaced and are parallel to the line bisecting the angle between the two waves. But what is their spacing? We won't go through the mathematical derivation here, but simple trigonometry applied to the area of overlap shown in figure 5 leads to the following expression for the fringe spacing s in terms of the wavelength λ of the light and the angle θ between each light beam and the angular bisector of the beams (which is parallel to the fringes):

$$s = \frac{\lambda}{2\sin\theta}. \tag{8}$$

Now consider a particle travelling through this area of overlap. When it is in a bright fringe, it will reflect light to the observer (or detector). However, when it is in a dark fringe, where the two light beams have cancelled out due to their being in antiphase, there will be no light reflected from the particle. Hence the light received from the particle will fluctuate with a regular period as it passes through the interference fringes. To calculate the frequency of

this oscillation is now straightforward. If the velocity of the particle (actually the velocity component perpendicular to the fringes) is v, it clearly travels a distance v in one second. If the fringe spacing is s, this means that it will pass through v/s bright fringes per second and the light will therefore oscillate with this frequency. Replacing s from equation (8) gives the frequency of oscillation (which we shall call Δf for reasons that will quickly become apparent) as

$$\Delta f = \frac{v}{\lambda} 2 \sin\theta.$$

Rearranging this to give the velocity in terms of the oscillation frequency gives

$$v = \frac{\lambda}{2 \sin\theta} \Delta f.$$

This is identical to equation (7). In other words, considering the experiment as one in which two light beams interfere to form a fringe pattern and the intensity fluctuations in the light reflected by the particle are caused by its passing through these fringes gives exactly the same answer as considering the Doppler shifts caused by light reflecting from a moving particle. This is gratifying—indeed it would have been embarrassing if the two approaches had given different answers!—but it is not intuitively obvious that the two approaches are equivalent. In one approach we treated the situation as one in which light has its frequency changed by the Doppler effect and then we *combined two beams of different frequency* to give a beat frequency. In the other, we considered the *interference of two beams of the same frequency* and counted the resulting interference fringes as the particle travelled through them. No frequency shift was involved! Yet it is clear from the fact that both approaches give the same answer that they must be two different ways of looking at the same phenomenon. They are, in fact, equivalent.

Further details of both the two-beam and the reference-beam methods of laser Doppler velocimetry can be found in the literature. Eliasson and Dändliker (1974), for example, have given a thorough, but very theoretical, analysis. She and Wall (1975) have compared different experimental techniques applied to the measurement of flow and turbulence.

3.3. Effect of particle density

The interference-fringe interpretation of section 3.2 is clear when there is only one particle in the area of overlap. What happens when there is more than one? If one particle is in a bright fringe when a second is in a dark fringe, then the variation in the total intensity detected will not be as marked. In other words, the *depth of modulation* is reduced. This leads to a reduction in the strength of the beat signal detected. If there are large numbers of particles in the beam at the same time, it is possible for the signal to be reduced to zero. Hence the two-beam technique is better when there is a low density of particles in the flow (preferably only one in the beam at any time). This restriction is not so severe for the reference-beam technique, which is therefore preferred when the particle density is high.

3.4. Detecting the direction of flow

In the fringe interpretation of the two-beam technique, it is obvious that a particle crossing the fringe pattern in the opposite direction produces the same signal. In the Doppler interpretation (and for the reference-beam technique), the beat frequency measured is the *difference* between two frequencies and there is nothing to indicate which of the two frequencies is higher or lower. Hence in both basic Doppler techniques there is an ambiguity about the direction of flow.

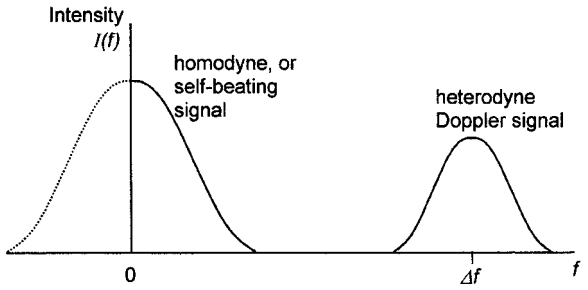

Figure 6. Homodyne and heterodyne Doppler signals.

As reported in an early review by Abbiss *et al* (1974), several methods are available to remove this ambiguity and to determine the direction of motion. One approach is to change the frequency of the reference beam (or of one of the probe beams in the two-beam technique). Whether the beat frequency increases or decreases will determine the direction of the motion. (In practice, the frequency is usually modulated continuously and signal-processing techniques are used to unscramble the signal.) Another method is to use light beams polarized at right angles to each other (to produce *two* sets of fringes in the two-beam technique) and polarization-sensitive detectors.

3.5. Effect of finite velocity distributions

What happens if the particles do not all have the same velocity, but show a distribution of velocities about some mean value? Doppler shifts still occur, but there will now be a spread of frequencies instead of a single Doppler-shifted frequency. However, another phenomenon will now occur. Light from two particles with different velocities will reflect light with different frequencies and these will beat with each other (as well as with the reference beam). In fact, light from all the particles will beat together. This *self-beating* gives rise to a frequency distribution about zero instead of about some finite frequency. (Readers familiar with radio engineering will recognize this as a *homodyne* signal as opposed to the *heterodyne* signal due to the interaction of the Doppler-shifted light with a reference beam.) Hence, if we plot the *spectrum* of intensity in the detected signal against frequency we obtain a picture similar to figure 6. We have a spread of frequencies about a frequency representing the mean velocity of the particles and a spread of similar shape about zero representing the self-beating (homodyne) effect. (Of course, we cannot measure *negative* frequencies, so the part of the spectrum to the negative side of zero is shown in the diagram as dotted. What happens physically, of course, is that particles moving towards and away from the detector give rise to the same beat frequency—the same direction ambiguity discussed in section 3.4.)

3.6. Photon correlation spectroscopy

It is clear that the homodyne self-beating signal is independent of the overall velocity of the moving particles. Hence it also exists when there is *no* mass movement or flow. In other words, it can be used to investigate *random* motion of scattering particles, such as occurs in diffusion.

Consider a fluid containing scattering particles illuminated with laser light. The Doppler shifts of particles moving with different velocities give rise to a range of beat frequencies that are observed as a fluctuating intensity in the light scattered by the particles. An analysis of

these fluctuations provides information about the movement of the particles. Applications include the studies of diffusion rates, Brownian motion and motility of biological organisms.

Traditionally, these phenomena have been investigated by measuring the *autocorrelation function* of the intensity fluctuations (or its close relative, the autocovariance), rather than the frequency spectrum. The technique has been known variously as *light beating spectroscopy*, *intensity fluctuation spectroscopy*, and (more usually in recent times) *photon correlation spectroscopy* (Cummins and Pike 1974, 1977). The autocorrelation function can be visualized as the sliding of a function (for example a plot of intensity against time) over itself along the time axis and the multiplication of the original and shifted signals for each value of shift (or *lag* as it is usually called). It is a measure of the probability that the intensity at a given time will be similar to the value recorded a short time earlier. It is clear that this probability will fall as the time interval (lag) between the two measurements increases. It is also clear that the more rapidly the intensity changes (or the higher the frequency of a fluctuating intensity), the faster will this fall-off in probability be. In fact, there is a *Fourier transform* relationship between the autocorrelation function and the frequency spectrum. Hence the distinction between photon correlation spectroscopy and Doppler is merely one of convenience rather than anything fundamental. The former is used for the homodyne case (and measures the correlation function), while Doppler is reserved for the heterodyne technique (and measures the frequency spectrum).

The time taken for the autocorrelation function to fall to a pre-determined low level is called the *correlation time* and can be used as a defining parameter of the technique. From the above it is clear that a more rapidly changing intensity leads to a shorter correlation time. As the frequency of the intensity fluctuations (the Doppler frequency) is proportional to the mean velocity of the scatterers (see equation (5)), this means that there is a direct relationship between the correlation time and the mean velocity. The exact relationship will depend on the velocity distribution of the scatterers, as this determines the *shape* of the autocorrelation function, but in general the *mean velocity is inversely proportional to the correlation time*.

3.7. Recent developments in laser Doppler techniques

The basic techniques of laser Doppler velocimetry and photon correlation spectroscopy have not changed much since the 1970s. There has been an increase in sophistication, of course, including the application of Doppler techniques to microscopy (Cochrane and Earnshaw 1978, Johnson 1982) and the use of fibre optics. But the main attack has been on the theory, and especially the effect of multiple scattering when there are many scatterers in the beam (Bohren 1987). This has special relevance to blood flow measurements (Stern 1985, Riva *et al* 1989). Monte Carlo techniques have been applied extensively in an attempt to get to grips with the complexities of this (Jentink *et al* 1991, Konák *et al* 1991). There have been specific advances in the application of laser Doppler techniques to the measurement of blood flow, but these will be dealt with later in this paper.

4. Time-varying speckle for measuring velocities

4.1. Relationship between time-varying speckle and laser Doppler

As described in section 2.2, the laser speckle pattern produced by scattering particles in a fluid fluctuates in a random fashion. If the velocity distribution of the scatterers is Gaussian, then the temporal statistics of the fluctuations (and in particular the ratio of the standard deviation to the mean) should be identical to the spatial statistics of an ideal speckle pattern (which also

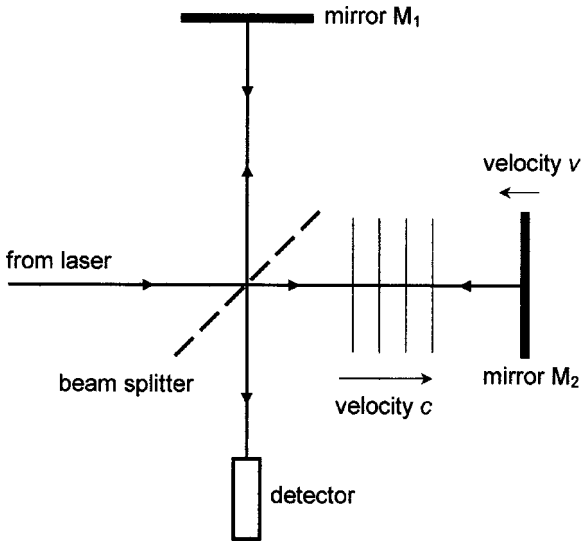

Figure 7. Michelson interferometer with a moving mirror.

assumes Gaussian statistics). In ideal circumstances, this is, in fact, what happens. But when the speckle pattern is being produced by a mixture of moving and stationary scatterers, or of scatterers with varying velocities, this is no longer the case. In fact, the depth of modulation of the speckle intensity fluctuations can give some indication of how much of the light is being scattered from moving scatterers and how much from stationary scatterers. In addition, the frequency spectrum of the fluctuations clearly depends on the velocity distribution of the motion. It should therefore be possible to obtain information about the motion of the object from a study of the temporal statistics of the speckle fluctuations.

But laser Doppler techniques also analyse the frequency spectrum of light intensity fluctuations observed when laser light is scattered from moving particles. Are these the same fluctuations? To answer this question we need to simplify the situation and consider first a moving solid object rather than a collection of particles. The experimental arrangement we shall use is a very familiar one in optics—the Michelson interferometer.

Note that in the following analysis the velocity of the moving object is assumed to be very small compared with the velocity of light and relativistic effects are ignored.

In figure 7, one mirror (M_1) is stationary and the other (M_2) can move along the direction of the beam. The component marked 'beam splitter' is a semi-reflecting mirror (for example, coated with a thin layer of aluminium) that is designed to reflect half of the incident light and transmit the other half. When M_2 is stationary, the arrangement is that of a classical Michelson interferometer. (Strictly speaking, as we are using parallel light, the set-up is actually a Twyman–Green interferometer, as developed in the 1920s for testing optical components.) When the two path-lengths (to M_1 and M_2) are exactly equal, or differ by an exact number of wavelengths, the two beams are in phase and add together when they recombine at the beam splitter. The detector will record a maximum of intensity. When the path-lengths differ by half a wavelength (or any odd multiple of half a wavelength), the two beams are in antiphase and cancel out. Hence the detector will record zero intensity.

Now consider the situation when the mirror M_2 moves in the direction shown with a velocity v. The detector now records a fluctuating intensity signal.

The Doppler interpretation of this experiment runs as follows. If the velocity of the wave is c and its frequency is f, this means that in the incident beam f wave-crests occupy a distance c. This would also, of course, be the case after reflection if M_2 was stationary. If, however, M_2 is moving towards the light source with a velocity v, then after reflection these f wave-crests occupy a distance $c-2v$, since M_2 has travelled a distance v towards the incoming wave. The frequency of the reflected wave, as recorded at the detector, is therefore increased to f', where

$$f' = \frac{c}{c-2v} f.$$

At the detector, in Doppler parlance, the reflected wave of frequency f' is mixed with that from a 'local oscillator' (the reference wave) of frequency f, and the beat frequency detected is the difference between the two frequencies:

$$\Delta f = f' - f = f\left(\frac{c}{c-2v} - 1\right) = f\left(\frac{2v}{c-2v}\right)$$

i.e. (since $c \gg v$)

$$\Delta f = f\frac{2v}{c}. \tag{9}$$

Equation (9) gives the 'Doppler frequency' recorded by the detector.

The classical interferometric explanation is as follows. The reflected beam from M_2 interferes with the beam from M_1 (the reference beam). If the two mirrors are exactly perpendicular to the light beams and are perfectly flat, the intensity observed in the detection plane is uniform; if not, the detection plane is crossed by a pattern of interference fringes. In either case, a small detector in that plane records an intensity that depends on the phase difference between the two beams at that point. If M_2 now moves a distance equal to $\lambda/2$, where λ is the wavelength of the light, the optical path difference between the two beams changes by λ and the detector records one complete cycle of interference (say bright–dark–bright). If Δf such cycles are recorded in one second, this implies that M_2 has moved a distance v in that second, where

$$v = \Delta f\frac{\lambda}{2}. \tag{10}$$

v, of course, is the velocity of M_2, and Δf is the frequency of the intensity oscillations recorded by the detector. Using the relationship $c = f\lambda$ and rearranging equation (10) gives

$$\Delta f = f\frac{2v}{c}. \tag{11}$$

which is identical to equation (9).

Hence, as in section 3.2, the same result is obtained whether the phenomenon is regarded as an interference effect or as a Doppler effect. Again, it is by no means intuitively obvious that the two models are identical. The Doppler explanation involves the superposition of two waves of slightly *different* frequencies and the detection of the resulting beat frequency. The interference explanation involves the superposition of two waves of the *same* frequency and the detection of correlations as the path difference between them is changed. Nevertheless, the analysis again shows that the detected frequency is the same in both cases and the two approaches are, in fact, two ways of looking at the same phenomenon.

If the mirror M_2 is replaced by a rough (diffusing) surface, the light scattered from it produces a far-field speckle pattern in the detector plane. This speckle pattern still interferes with the reference beam, though this time it produces a new speckle pattern. The argument used above still applies. A Doppler signal is still recorded by the detector and the velocity of

the moving diffuser is still given by equation (9). Similarly, the interference interpretation is unaffected: the intensity at any point in the speckle pattern fluctuates if M_2 moves and again passes through one cycle for each half-wavelength movement of M_2. Equation (11) is still valid.

If instead of a solid object, M_2 consists of a collection of individual scattering particles, the same arguments apply, except that now there is a range of velocities associated with the scatterers. This results in a spectrum of frequencies being detected rather than a single frequency. Once again, however, both the Doppler and the time-varying speckle approaches can explain the phenomenon and give the same quantitative answers (Briers 1996).

4.2. Analysis of time-varying speckle patterns

Although it is clear from section 4.1 that the intensity fluctuations observed in time-varying speckle patterns are identical to those observed in Doppler experiments, the two fields have tended to develop separately. Only a few papers have recognized their essential equivalence (Briers 1975, Pusey 1976, Fercher 1976, Briers 1996, Dunn *et al* 2001) and some authors still prefer to regard the techniques as essentially different (Konishi and Fujii 1995, Starukhin *et al* 2000). We therefore need to look at the techniques that have been used specifically by workers in the speckle field.

First consider the experimental arrangement usually used. If the detector sees more than one speckle, it will record an average intensity and the amplitude of the fluctuations will be reduced. If several speckles are in the field of view, the averaging will be so severe that a uniform (and constant) intensity will be recorded. Hence it is necessary for the detector to look at a single speckle. This is usually achieved by illuminating a small area of the flow and placing a detector with a small aperture in front of it in the scattered light. Reference to section 2.1 will show that this is the arrangement for observing *far-field speckle* with the detector replacing the screen shown in figure 3. For the detector to record a single speckle, its aperture must be smaller than the average speckle size. As mentioned in section 2.1, the speckle size in far-field speckle is controlled by the size of the illuminated area—the smaller the area, the larger the speckle. In practice, best results are obtained when the diameter of the detector aperture is about one-tenth of the average diameter of a speckle. (Note that the need for a small aperture in front of the detector also arises in the Doppler approach, though for apparently different reasons (limiting the direction of view). A closer examination of the physics shows, however, that the two arguments are, in fact, identical. Again it is a case of two ways of looking at the same problem. The speckle argument may, however, be easier to appreciate and calculate.)

As mentioned in section 3.7, the emphasis of the Doppler researchers in recent years has been on the theory of the technique, especially the effect of multiple scattering and the use of statistical methods. This, and an increasing sophistication in the experimental techniques, has led to laser Doppler being a relatively expensive method to implement. The same is true in general of photon correlation techniques. On the other hand, workers in the field of time-varying speckle have concentrated on the experimental methods and have used a variety of techniques to try to reduce both the complexity and the cost of the equipment.

For example, the above discussion of time-varying speckle has implicitly assumed instantaneous measurements of intensity. This, of course, is never achieved in practice, and the detector used has a finite integration time. If this integration time is long compared with the correlation time of the speckle fluctuations, the intensity fluctuations are averaged out and a constant intensity is recorded. At shorter integration times, the depth of modulation is dependent on the integration time and on the velocity of the scatterers. Hence the depth of

modulation of *time-integrated speckle* as a function of integration time contains information about the velocity of the scatterers (Ohtsubo and Asakura 1976) and the integration time can be used as an additional degree of freedom.

Another possibility is to use *time-differentiated speckle*, and again the statistics contain information about the velocities of the scatterers. Takai *et al* (1979) used frequency analysis, while Fercher (1980) appears to have been the first to recognize the advantages of using the simpler parameter of the standard deviation of the intensity fluctuations (speckle contrast—see section 2.1). A similar approach was later used by Fujii *et al* (1987), who measured the differences in intensity of individual speckles in successive scans as a simpler alternative to the measurement of the complete autocorrelation function of fluctuating speckle. Ruth (1987, 1988) also used differentiation of the speckle intensity. He showed that the velocity is proportional to the mean frequency of the fluctuations and that this in turn is proportional to the root mean square of the time-differentiated intensity.

Frequency analysis and photon correlation techniques (see section 3.6) have also been used by researchers using the time-varying speckle approach. However, many workers have tried to avoid the complexities and expense of these techniques and have developed alternative methods. In their work on measuring blood flow, Asakura's group in Sapporo have used several approaches, which will be described in section 5.2 below.

Finally, it is also possible to use the *spatial statistics*, usually the contrast, of *time-integrated speckle patterns*. This is the basis of a full-field technique known as *laser speckle contrast analysis* or *laser speckle contrast imaging*, which will be discussed in section 6.5 below.

5. Monitoring blood flow at a single point

5.1. Laser Doppler techniques

Laser Doppler velocimetry has been used to measure blood flow velocity for nearly 30 years. Early work was on retinal blood flow (Riva *et al* 1972, Tanaka *et al* 1974) but was soon extended to other tissues (Stern *et al* 1977). The particular difficulties of light scattering from blood, especially the problem of multiple scattering, have been recognized. Various models have been proposed, starting with the pioneer work of Bonner and Nossal (1981).

In some medical establishments, laser Doppler is now a routine tool for monitoring blood flow at a single point. Various refinements have been introduced, such as the simultaneous use of two wavelengths (Duteil *et al* 1985, Obeid *et al* 1988, Gush and King 1991). Penetration of tissue is highly wavelength-dependent, with infrared light penetrating the furthest (several mm), red about 1–2 mm and green and blue hardly at all (typically 0.15 mm for green light). The choice of wavelength, therefore, can determine to what depth the flow is being sampled. A recent study investigates the Doppler shift as a function of the laser beam radius, the radius of the blood vessel, and the depth of the vessel in the tissue, with the aim of developing a method for the absolute measurement of blood-flow velocity (Starukhin *et al* 2000).

The book edited by Shepherd and Öberg (1990) includes a comprehensive review of laser Doppler techniques in monitoring blood flow up to the late 1980s. In addition, Obeid *et al* (1990) have written a critical review and Barnett *et al* (1990) a more specific intercomparison of two commercial systems. At least two reviews of techniques for measuring retinal blood flow have recently been published (Feke *et al* 1998, Aizu and Asakura 1999).

Other recent applications include dental diagnosis (Roebuck *et al* 2000), assessing the vitality in sinus bone grafts (Wong 2000), estimating angiogenic and anti-angiogenic activity (Kragh *et al* 2001), measuring jejunal perfusion during liver transplantation (Ronholm *et al*

2001), the early detection of cirrhosis (Domland *et al* 2001), assessing diabetic alterations in microcirculation (Meyer *et al* 2001), studying the haemodynamics of the optic nerve head (Pournaras and Riva 2001) and the diagnosis of deep vein thrombosis (de Graaf *et al* 2001).

A recent development that should be mentioned is the combination of Doppler with the new technique of optical coherence tomography (OCT). An account of the latter is outside the scope of this paper, but in essence it is a method of investigating properties at a predetermined distance by using low-coherence light sources and variable-path reference beams. When combined with Doppler, it is possible to measure blood flow at specific depths inside tissue. Many papers are being written on this method, but a feel for its possibilities can be found in the works of Chen *et al* (1998) on the investigation of blood flow dynamics following pharmacological intervention and photodynamic therapy, Kehlet Barton *et al* (1999) on the three-dimensional reconstruction of blood vessels and Nelson *et al* (2001) on blood flow in port-wine stain.

Many other examples of laser Doppler blood-flow measurement can be found in the literature, but it is not the purpose of this paper to report in depth on point-wise measurements. The present paper concentrates mainly on techniques for mapping blood flow over extended areas of the body.

5.2. Time-varying speckle

The group that has carried out most work on blood flow measurement using time-varying laser speckle is that led by Asakura in Sapporo. They started by using spectrum analysers to measure the power spectrum of the fluctuations. They found that the gradient of the power spectral distribution, conveniently expressed as the ratio of the high-frequency component to the low-frequency component, is an indicator of the mean velocity (Fujii *et al* 1985). They used band-pass filters to facilitate the calculation of this ratio and applied the technique to measure blood flow in the skin, for example at the palm, cheek, chest and leg. Later work showed that this approach was not always applicable, and the mean frequency of the oscillations was used as the velocity indicator instead (Aizu *et al* 1987). The mean frequency was also used in later work on blood flow in the retina and choroid of rabbits. The Sapporo group has also used photon correlation techniques to measure blood flow, for example in the ocular fundus of human subjects and at selected points on the retina (Aizu *et al* 1989, 1990a, 1990b, 1992). All this work, including its relationship to other work, has been reviewed by Aizu and Asakura (1991). They also coined the word 'bio-speckle' to describe the time-varying speckle produced by living organisms.

Fujii and co-workers built on the work of the Sapporo group by using time-differentiated speckle (see section 4.2) to measure blood flow in the skin (Fujii *et al* 1987). They measured the differences in intensity of individual speckles at successive times and computed the ratio of the mean intensity to the intensity difference. This parameter, which they called 'normalized blur', is used as a measure of velocity.

In Europe, Ruth (1987, 1988, 1990) also used time-differentiated speckle to measure skin blood flow. Ruth used optical fibres to introduce the laser light to the skin and to collect the scattered light. He combined an earlier theoretical result of Bonner and Nossal (1981), that the mean blood flow in capillaries is proportional to a value that involves differentiation of the intensity, with the concept that the signal detected is the superposition of two fluctuating speckle patterns, one from the moving blood and the other from the skin, which may itself be subject to involuntary movements. He presented the results of skin blood flow measurements at various points on the body (Ruth 1990). Since the method is truly non-contacting, measurements in the neighbourhood of wounds are possible. Ruth also extended this work to studies of the

dynamics of skin blood flow (Ruth 1994) and to measurements on patients with peripheral arterial occlusive disease (PAOD), diabetics and smokers (Ruth *et al* 1993).

Papers applying time-varying speckle to single-point blood flow measurements are still appearing in the literature. A typical example is the work of Ozdemir *et al* (2000), who used self-mixing laser diodes in a technique that used the mean frequency of the intensity fluctuations for the relative evaluation of blood flow in human tissue.

6. Mapping tissue blood perfusion

6.1. Perfusion or flow?

The techniques described in sections 3–5 provide a very useful arsenal for measuring fluid velocity, including blood velocity. The sophistication of the measurements will depend on the resources available. However, they all suffer from the limitation that they measure the velocity only at a single point—they are not 'full-field' techniques. If a map of velocity distribution is required, some method of scanning the area of interest is necessary. Such a map is of particular importance if blood flow is to be used as a diagnostic tool.

The structure of the microvasculature is extremely complex, with the individual capillaries so convoluted that the blood flow at any given point in any given capillary could be in any direction. The distribution of directions is more or less uniform over all possible directions, and there will be just as much flow towards as away from the detector. In addition, the velocity of flow will be greater in the larger blood vessels and smaller in the narrower ones, and in the larger ones there will also be a velocity variation across the vessel. All this means that in any area of the microvasculature there will be a random distribution of line-of-sight velocities, with an average of zero. This is very similar to a diffusion process, for which, as discussed in section 3.6, the techniques of photon correlation spectroscopy were developed. By measuring the correlation time, a measure of the average line-of-sight velocity (or, more correctly, *speed*, as the direction of the motion is not determined) can be obtained. Similarly, measurement of the distribution of Doppler-shifted frequencies will also yield this average speed. However, since nothing can be deduced about the direction of the motion, it is more usual to talk about these techniques measuring *perfusion* rather than flow.

Strictly speaking, both *flow* and *perfusion* imply the *amount* of fluid being moved per unit time rather than the mere velocity. Both the Doppler and the time-varying speckle techniques essentially measure velocity. Some Doppler systems use the strength of the Doppler signal at a given Doppler frequency to provide information about the actual amount of fluid giving rise to the signal at this frequency, and use the product of this and the velocity computed from the Doppler frequency to give a measure of flow or perfusion. However, other factors also affect the signal strength (the proportion of light scattered from neighbouring stationary tissue, for example), and in the opinion of many authors it is safer to use Doppler and speckle techniques for relative rather than absolute measurements (Briers *et al* 1999, Dunn *et al* 2001).

6.2. Scanning techniques: time-varying speckle

Fujii *et al* (1987) described a scanning technique in their application of time-differentiated speckle to skin capillary blood flow. They used a linear CCD array to monitor simultaneously a line of speckles, and a scanning arrangement to extend this to a two-dimensional area. Fujii's group have also mapped retinal blood flow (Fujii 1994, Tamaki *et al* 1994, Konishi and Fujii 1995). They produce a microcirculation map of the retina by illuminating the retina with light from a diode laser, scanning and storing the speckle images and calculating the differences

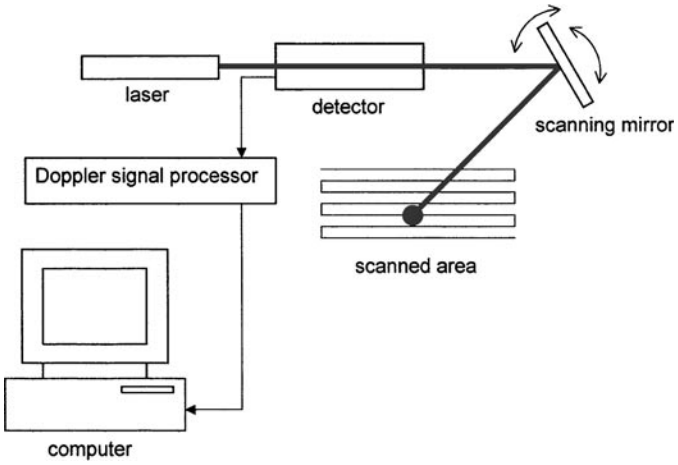

Figure 8. Schematic diagram of laser Doppler imager.

between successive images to produce their 'normalized blur' parameter. Experiments on a rabbit eye showed good correlation with invasive methods (Konishi and Fujii 1995).

6.3. Scanning techniques: Doppler

Scanning has also been applied to the laser Doppler technique (Essex and Byrne 1991, Wårdell *et al* 1993). Commercial scanning Doppler systems (or *laser Doppler imagers*) are now on the market that can provide false-colour maps of capillary blood flow over quite large areas of the body (Huang *et al* 1996, Nilsson 1997). The tissue sampling depth is typically from 1 to 2 mm (determined by the penetration depth of the laser wavelength used) and the velocity spectrum is in the range 0.01–10 mm s^{-1}. Scans of areas up to 500 mm $\times$ 500 mm can be made with a resolution of 256 $\times$ 256 pixels. A typical experimental arrangement is shown in figure 8.

Some critical assessments of these systems have been carried out. Harrison *et al* (1993) used the tuberculin reaction as a model in a preliminary assessment of laser Doppler imagers. More recently, Kernick and Shore (2000) measured the effect of scanner head height, avascular skin thickness and haemocrit. They also took into account the contribution of the signal obtained from skin when flow is arrested.

Laser Doppler imagers have already been used in many medical and surgical situations. The following selection will give some idea of the range of applications that have already been reported.

6.3.1. Inflammation. Ferrell *et al* (1997) measured perfusion in ligaments and Clough *et al* (1998) investigated the effects of intra-dermal injection of histamine. Herrick and Clark (1998) have reviewed the use of the technique in the assessment of vasospastic disorders.

6.3.2. Healing processes. Newton *et al* (1999) used laser Doppler imaging to investigate a dermal replacement therapy for ulcer healing in diabetics. Perfusion increases at the bases of the ulcers were observed and this was thought to reflect angiogenesis in newly formed granulation tissue. Gschwandtner *et al* (1999) have measured perfusion in ischaemic ulcers. Nixon *et al* (1999) investigated tissue blood flow patterns in patients at risk from pressure sores.

Kalka *et al* (2000) studied the effects of new blood vessel formation over a 28-day period of treatment with angiogenic cytokines. These experiments showed a marked improvement in blood flow recovery and significant reduction in the rate of limb loss.

6.3.3. Burn assessment. The clinical accuracy for diagnosis of burn depth, within a mixed depth burn, is between 65 and 75%. This is boosted to 100% (in the absence of infection) when laser Doppler imaging is used: high perfusion corresponds to superficial dermal burns, which heal with dressings and conservative management; burns with low perfusion require surgical management (Niazi *et al* 1993, Brown *et al* 1998, Kloppenberg *et al* 2001). Pape *et al* (2001) have recently published an audit of the technique in the assessment of burns of intermediate depth.

6.3.4. Intra-operative measurements. A London team has investigated gastric blood flow during oesophagectomy (Boyle *et al* 1998) and colonic perfusion during colonic resection (Boyle *et al* 2000). In both cases they reported significant falls in perfusion. Hajivassiliou *et al* (1998) also measured colonic perfusion and reported a significant improvement over the conventional (single-point) Doppler technique for the routine clinical prediction of ischaemic injury of the bowel. Experiments on rats have demonstrated the effectiveness of laser Doppler imaging during flap surgery (Fitzal *et al* 2001).

6.3.5. Dermatology. Dermatology is an obvious application area for laser Doppler imaging. Quinn *et al* (1991) used it to observe allergic contact hypersensitivity, irritant reactions and inflammatory responses of the skin. Fullerton and Serup (1995) measured skin irritation. Psoriasis plaque and its responses to PUVA, UVA and calcipotriol have also been investigated (Speight *et al* 1993, Speight and Farr 1994a, 1994b). Laser Doppler imaging has also been used in patch tests (Bjarnason and Fischer 1998, Bjarnason *et al* 1999), in the diagnosis of malignant skin tumours, including melanoma (Stücker *et al* 1999), and in the post-operative assessment of malignant skin tumours following photodynamic therapy (Wang *et al* 1997).

6.3.6. Physiology. Research into microvascular mechanisms is another obvious application for the technique and there have been several experimental and clinical studies. Several workers have studied the vasodilatory response to stimuli (Butler *et al* 1997, Mayrovitz *et al* 1997) and to iontophoresis (Algotsson *et al* 1995, Morris *et al* 1995, Morris and Shore 1996). An important application of this is in the diagnosis of diabetes (Morris *et al* 1995). Diabetes can also be detected by using laser Doppler imaging to assess impaired sympathetic nerve regulation (Bornmyr *et al* 1998, 1999). Newton *et al* (2000) measured the response to the intradermal injection of drugs, and Kubli *et al* (2000) used the technique to assess endothelial function as a possible early diagnosis of cardiovascular disease. Several groups have monitored allergic reactions (Stücker *et al* 1995, Möller *et al* 1999). Stücker *et al* (2001) have investigated the variation of cutaneous microcirculation over different parts of the body. Other physiology applications have included an assessment of liver perfusion during transplantation (Seifalian *et al* 1993). Animal experiments have included measurements of the cerebral circulation in rats (Lauritzen and Fabricius 1995, Ances *et al* 1999, Nielsen *et al* 2000) and of blood flow in cortical bone in rabbits (Shymkiw *et al* 2001).

As with classical laser Doppler techniques, there is sometimes an advantage in using two wavelengths. A common combination is the usual 633 nm of the helium–neon laser together with the near-infrared wavelength of 810 nm. This has been used to study arthritis

and inflammation of joints, for example the microvascular effects of tennis elbow (Lockhart *et al* 1997, Ferrell *et al* 2000).

6.4. Full-field techniques: global Doppler

The scanning necessary to apply either laser Doppler or time-varying speckle techniques to map velocity distributions results in the collection of a vast amount of data and usually takes an appreciable time. Typical scan times for laser Doppler imagers, for example, are five minutes. Ideally, a full-field technique would avoid the need for scanning. One such technique is the recently developed 'global Doppler' (Komine 1990, Komine *et al* 1991, Meyers *et al* 2001). This converts velocity directly to intensity (or false colour) by means of the ingenious device of measuring how much of the return light is absorbed by a substance of known absorption/frequency properties. Unfortunately, at the moment resolution problems limit the technique to fairly high velocities and the method is not suitable for biomedical applications.

6.5. Full-field techniques: laser speckle contrast imaging

A technique developed in the early eighties simultaneously achieves full-field operation (without scanning) and very simple (and cheap) data gathering and processing. Originally called *single-exposure speckle photography* (Fercher and Briers 1981), it uses the *spatial* statistics of *time-integrated speckle* (essentially the *speckle contrast*) and was originally developed for the measurement of retinal blood flow (Briers and Fercher 1982). The basic technique was simply to photograph the retina under laser illumination, using an exposure time that is of the same order as the correlation time of the intensity fluctuations. It is clear that a very short exposure time would 'freeze' the speckle and result in a high-contrast speckle pattern, whereas a long exposure time would allow the speckles to average out, leading to a low contrast. In general, the velocity distribution in the field of view is mapped as variations in speckle contrast. Subsequent high-pass optical spatial filtering of the resulting photographs converted these contrast variations to more easily seen intensity variations. Later work (Fercher *et al* 1986) introduced digital image processing of the speckle photographs, including a false-colour coding of the velocities. More recently, this method has been developed into a fully digital, real-time technique for the mapping of skin capillary blood flow (Briers and Webster 1995, 1996, Briers *et al* 1999). As the method is no longer photographic, these authors now call it *laser speckle contrast analysis*, or *LASCA*. Other workers (Dunn *et al* 2001) use the term *laser speckle imaging*. However, this does not indicate that it is a *processed* image, in which speckle contrast variations have been converted into colour variations. On the other hand, it is useful to stress that the technique does produce an *image*. For these reasons, in this paper we shall adopt the term *laser speckle contrast imaging*. (It is worth noting that Dacosta (1995) employed a closely related technique as a remote method of sensing heartbeats. He used a TV camera to record the speckle pattern produced by a vein and digitized it frame by frame, then computed the speckle contrast and plotted this as a function of time. A minimum in this contrast indicated the occurrence of a heartbeat.)

The set-up for laser speckle contrast imaging (Briers and Webster 1996, Dunn *et al* 2001) is straightforward and is illustrated in figure 9. Light from a laser is diverged by a lens to illuminate the area of skin under investigation. A CCD camera images the illuminated area and the image is observed on a monitor. On receiving an instruction from a personal computer, the frame-grabber captures an image and specially developed software processes it to produce a false-colour contrast map indicating velocity variations. The contrast is quantified by the usual ratio of the standard variation of the intensity fluctuations to the mean intensity, $\sigma/\langle I\rangle$

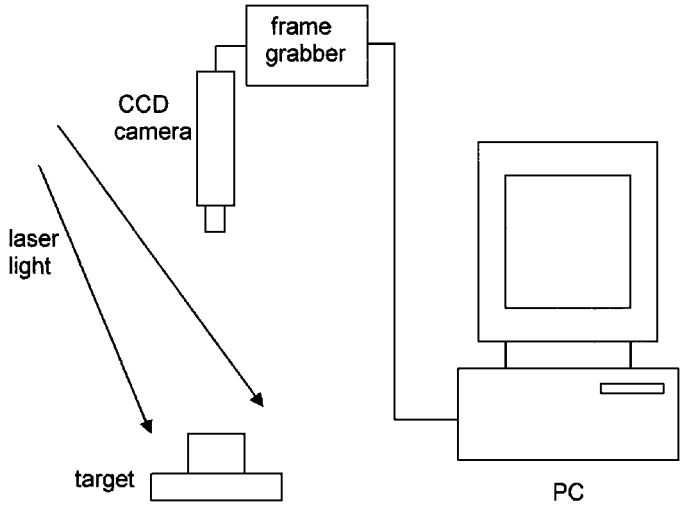

Figure 9. Basic set-up for laser speckle contrast imaging.

(see equation (1)). The image is actually a time-integrated exposure, but at the velocities involved in capillary blood flow, the exposure time is very short (typically 1/50 second). The processing time can be less than one second (He and Briers 1998), making it effectively a real-time technique.

The operator has several options at his disposal. In the LASCA technique (Briers *et al* 1999), this includes the exposure time, the number of pixels over which the local contrast is computed, the scaling of the contrast map and the choice of contour colours. The choice of the number of pixels over which to compute the speckle contrast is important: if they are too few, the statistics will be questionable and if they are too many, spatial resolution will be lost. In practice, it is found that a square of 7×7 or 5×5 pixels is usually a satisfactory compromise.

The principle of laser speckle contrast imaging is very simple. Each speckle is fluctuating in intensity. A time-integrated image therefore shows a reduction in speckle contrast because of the averaging of the intensity of each speckle over the exposure time. (In particular, unless the exposure time is very short, there will be no speckles with zero intensity, and it is the usually large number of 'dark' speckles that gives laser speckle its characteristic high contrast.) Note that because *imaging* is involved, this technique uses *image speckle* rather than the *far-field speckle* used in most of the techniques.

It is clear that there must be a link between the flow velocity and the reduction in contrast. The higher the velocity, the faster are the intensity fluctuations and the more averaging occurs in a given integration time. (In the extreme case of a very long exposure time, of course, the speckle pattern disappears and is replaced by a uniform intensity over the whole field.) In fact, the link is between the speckle contrast and the correlation time (as would be measured by photon correlation spectroscopy—see section 3.6). Based on Goodman's early work on speckle statistics (Goodman 1965), the following equation can be deduced connecting the spatial variance of the intensity of the time-averaged speckle pattern, σ^2, and the time-average of the autocovariance of the intensity fluctuations, C_t:

$$\sigma^2(T) = \frac{1}{T}\int_0^T C_t(\tau)\,\mathrm{d}\tau. \tag{12}$$

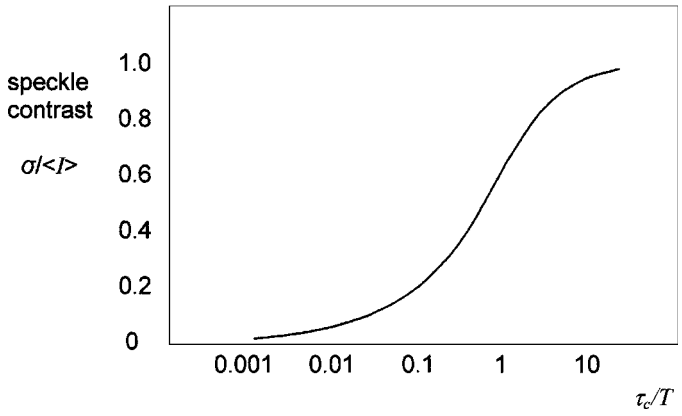

Figure 10. Typical (theoretical) plot of the variation of speckle contrast with the ratio correlation time to integration time for laser speckle contrast imaging.

To make use of this equation, it is necessary to make some assumptions about the velocity distribution of the particles in the flow. In the case of a Lorentzian velocity distribution, for example, the equation reduces to (Fercher and Briers 1981)

$$\frac{\sigma}{\langle I\rangle} = \left[\frac{\tau_c}{2T}\left\{1 - \exp\left(\frac{-2T}{\tau_c}\right)\right\}\right]^{\frac{1}{2}} \tag{13}$$

This equation is plotted, as speckle contrast ($\sigma/\langle I\rangle$) against the ratio of the correlation time (τ_c) to the integration time (T), in figure 10.

Note that the x-axis is plotted on a logarithmic scale. This has been done because the relationship between speckle contrast and the ratio τ_c/T turns out to be an exponential one for most models of velocity distribution. It makes sense to operate in the steepest part of the curve (greatest sensitivity of speckle contrast to changes in correlation time and hence velocity). Taking this as the range of contrast from just under 0.2 to about 0.8, it can be seen that this corresponds to about two orders of magnitude of correlation time (and hence velocity). The influence of the integration time (exposure time) T can also be seen: to stay within the steepest part of the curve, the integration time must be between one-tenth and ten times the average correlation time. Alternatively, the integration time can be used as an additional degree of freedom to select the velocity range being mapped.

The principle of laser speckle contrast imaging has been demonstrated in the laboratory and many of the problems solved (Briers *et al* 1999). The technique has also been applied to the imaging of cerebral blood flow in rats (Dunn *et al* 2001). However, it is at a much earlier stage of development than laser Doppler imaging, which has been commercially available for several years.

6.6. Quantifying the measurements

Equation (12) links the speckle contrast technique with the temporal techniques of Doppler, photon correlation and time-varying speckle. In its more usable forms, such as equation (13) for a Lorentzian velocity distribution, the link is between the speckle contrast in the time-averaged speckle pattern and the correlation time of the intensity fluctuations.

At this point, an apparent limitation of the speckle contrast technique appears. The Doppler technique can measure the frequency *spectrum* and is therefore, at least in theory,

able to deduce the velocity *distribution* of the scattering particles. As mentioned in section 6.1, by weighting the spectrum by the frequency, it should then be possible to measure flow rather than simply velocity. In the speckle contrast technique, on the other hand, only one measure of the correlation time is produced, and hence only an average velocity. Furthermore, the shape of the velocity distribution must be *assumed* in order to produce equations like equation (13).

In practice, however, this is not really such a major disadvantage for the speckle contrast technique. Converting the raw data to velocities is by no means straightforward. The problems include multiple scattering, the difficulty of accurately modelling the complex velocity distributions in the capillaries and the effect of light scattered from stationary tissue, the size of the scattering particles (blood cells in the present case), the shape of the scatterers, non-Newtonian flow, non-Gaussian statistics resulting from a low number of scatterers, spin of the scatterers, etc. Much work is going on into these effects and the question is far from settled (Bohren 1987, Riva *et al* 1989 Konák *et al* 1991, Starukhin *et al* 2000). Several models have been proposed (Rudd 1969, Eliasson and Dändliker 1974, She and Wall 1975, Bonner and Nossal 1981, Stern 1985, Jentink *et al* 1991) and some of these are assumed in the commercial systems on the market. However, because of the uncertainties caused by the complexities of light scattering from blood, it is usual at the moment in all these techniques either to make only relative measurements (e.g. to monitor *changes* in the perfusion), or to rely on calibration rather than on absolute measurements. Tissue phantoms are often used for this (Steenbergen and de Mul 1998), though even these can only be approximations to the actual situation (which is in any case likely to vary between subjects, between different parts of the same subject and between different times even at the same location).

6.7. Comparing scanning and full-field techniques

At the moment there are only two viable techniques for mapping capillary blood flow. Laser Doppler imaging uses a focused laser beam to measure the Doppler frequency at one point at a time and then scans to build up a two-dimensional map of blood flow. Laser speckle contrast imaging is a single-shot, full-field technique that maps the local speckle contrast as an indicator of blood flow.

Both techniques offer a non-invasive and non-contact method of mapping flow fields such as capillary blood flow. Laser speckle contrast imaging uses readily available off-the-shelf equipment and the software operates in a user-friendly way (typically using the Microsoft Windows NT interface). As it is a single-shot technique, with capture and processing taking less than one second (He and Briers 1998), it is a truly real-time method. Typical scanning laser Doppler systems take some minutes to complete a scan. Laser speckle contrast imaging therefore has the advantage of low cost and real-time operation. By sacrificing true real-time operation and using post-processing, the speckle technique offers another advantage over the Doppler method. Dunn *et al* (2001) have taken speckle images at five-second intervals to show the spatio-temporal evolution of blood flow changes in the cerebral cortex of a rat in response to cortical spreading depression. This could easily be speeded up to video rates to produce movies of the dynamic changes in perfusion. (In fact, Dunn *et al* (2001) did use video-rate capture, taking a sequence of 5–10 images, each of 15 ms (1/60 s) exposure, at 30 frames per second, but these sequences were used to improve the statistics by averaging the computed blood flow maps rather than to produce a movie.) It will be difficult for the Doppler technique to achieve video-rate capture.

The main disadvantage of laser speckle contrast imaging is the loss of resolution caused by the need to average over a block of pixels (typically 5×5 or 7×7) in order to produce the spatial statistics used in the analysis. In principle, laser Doppler imaging can operate

on a single pixel. However, in practice sampling is often used in order to speed up the processing, thus reducing the spatial resolution. This often means that the spatial resolution of laser Doppler imaging is in practice actually *lower* than that of laser speckle contrast imaging (Dunn *et al* 2001). Some attempts have been made to develop high-resolution laser Doppler imagers (Linden *et al* 1998), but this is usually at the cost of long scanning times.

There is, however, another complication for the speckle technique. Laser speckle contrast imaging computes the local speckle contrast within a square of pixels, the size of the square being under the control of the operator. The larger the square sampled for each measurement, the better are the statistics. But it is also important to sample a large enough number of *speckles* as well as pixels. In section 4.2, it was explained that the aperture used to restrict the field of view in a far-field time-varying speckle technique must be less than the speckle size and ideally should be about one-tenth the size of a speckle. Although laser speckle contrast imaging uses image speckle rather than far-field speckle, the same restriction applies, with the pixel taking the place of the aperture. However, there is a need to compromise. A speckle size ten times that of a pixel would mean that a 5×5 or 7×7 pixel block would not contain enough speckles to permit a valid contrast calculation. The best compromise is to have the speckle size the same as the pixel size (Dunn *et al* 2001). Thus the speckle size needs to be carefully controlled. This can be done by fixing the aperture of the imaging optics, as this alone determines the speckle size (see section 2.1). But this removes the control on the amount of light entering the camera, as the shutter speed (the other variable available) has already been determined in order to select the range of velocities to be measured. Unless the dynamic range of the camera is very large, this can be a significant restriction and can require the use of either a higher-power laser (thus adding to the cost of the equipment) and/or neutral density filters to ensure usable light levels at the detector.

Table 1. Comparison of four blood perfusion imagers.

	Laser Doppler imagers		Laser speckle contrast imagers	
	Moor Instruments (moorLDI)	Lisca AB (PIM II)	Kingston (LASCA)	Harvard
Wavelength	633, 685, 780 and 830 nm	670 nm	633 nm	780 nm
Depth probed	0.1–2 mm	0.1–1 mm	0.1–1 mm	0.1–1 mm
Laser power	2 mW	1 mW	30 mW[a]	30 mW[a]
Maximum scan area	500 × 500 mm	300 × 300 mm	150 × 150 mm[a]	20 × 20 mm[a]
Pixels displayed	256 × 256	256 × 256	640 × 480	640 × 480
True resolution	256 × 256[b]	64 × 64[c]	128 × 96[d]	128 × 96[d]
Time for image capture or scan	5 min[e]	4 ½ min[f]	1/50 s	1/60 s
Time for capture plus processing	5 min[e]	4 ½ min[f]	1 s	1 s

[a] As the whole area is illuminated in the laser speckle technique, the laser power required will always be higher than that of the scanning laser Doppler imagers. However, the power required can be reduced by having a more sensitive CCD camera or by imaging a smaller area. Likewise, the area that can be imaged depends on the laser power and the camera sensitivity. The areas quoted here are those actually used by the groups concerned.

[b] The moorLDI processes each pixel in the array, at the expense of a relatively slow scan rate.

[c] The Lisca PIM II processes an array of 64 × 64 pixels and interpolates this to obtain the 256 × 256 display.

[d] The quoted resolution for the laser speckle contrast imagers assumes that a 5 × 5 pixel block has been selected for computing the local contrast. If a 7 × 7 block is used, in order to improve the statistics, the true resolution is reduced to 90 × 70 pixels.

[e] By reducing the number of scanned points to 64 × 64, the scan time can be reduced to 40 seconds.

[f] This can be reduced to just over 1 ½ minutes at the expense of image quality.

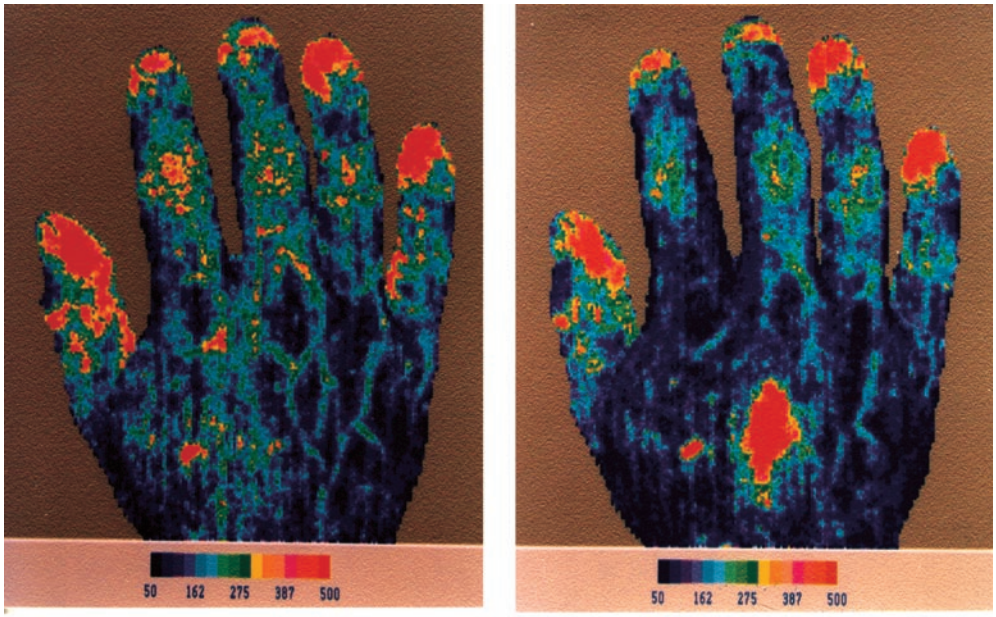

Figure 11. Laser Doppler images, showing perfusion (a) before and (b) after gently scratching a small area on the back of a hand.

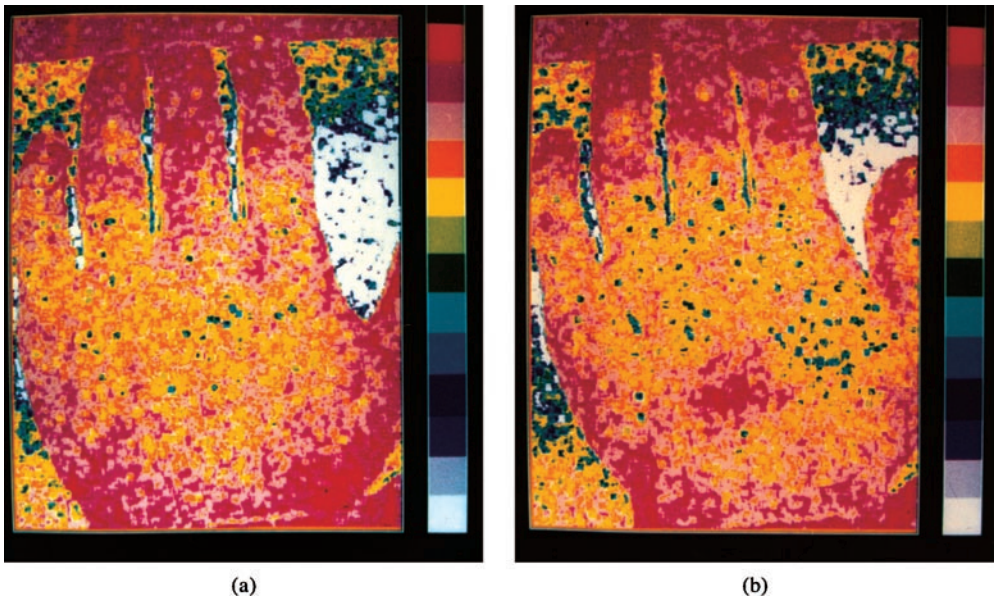

Figure 12. Laser speckle contrast images, showing perfusion (a) before and (b) after gently scratching a small area on the back of a hand.

The other factor to be considered is that laser Doppler imaging is already on the market (and has been for some time), whereas laser speckle contrast imaging is still in the laboratory and will need significant engineering development before it can be commercialized. This may be worth doing, however, for the advantage of truly real-time operation or the ability

Figure 13. Laser speckle contrast image of part of a forearm, showing increased perfusion around a superficial burn (scalded by steam).

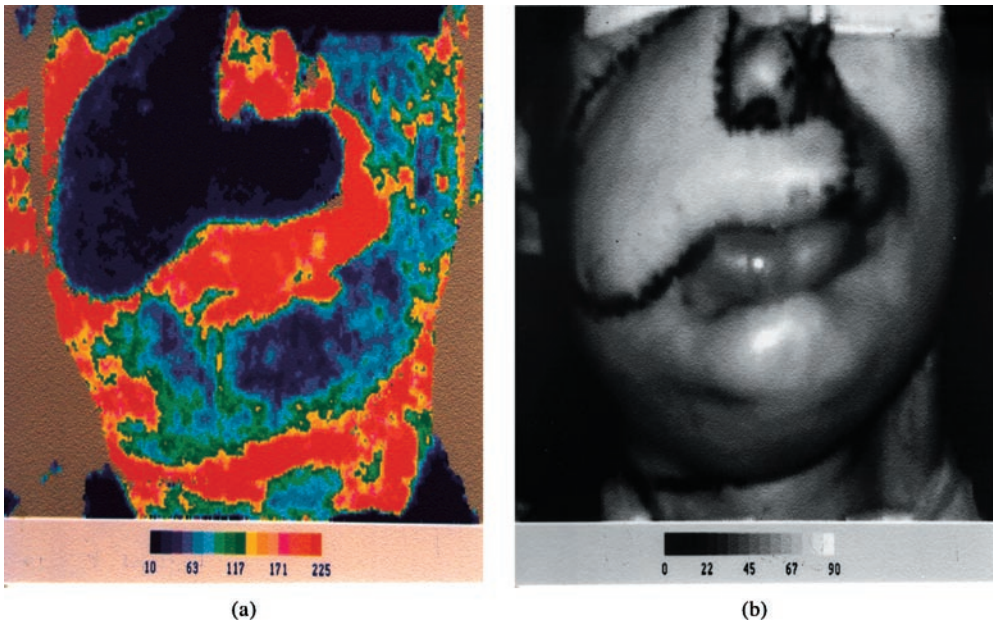

Figure 14. (a) Laser Doppler image of a free flap on the face after surgery, showing much lower perfusion in the flap than in the surrounding tissue. (b) Photograph of the same area, for reference.

to obtain movies of perfusion changes. The manufacturers of laser Doppler imagers have plans to use laser line scanning, together with a linear photodetector array, and faster signal processing to bring scan times down to seconds rather than minutes. Sub-second scan times may be possible using two-dimensional photodetector arrays. If these developments come to fruition, and provided spatial resolution can be maintained, one of the advantages of laser speckle contrast imaging, real-time operation, will have been matched. However, the ability to produce video-rate movies and the inherently lower cost of laser speckle contrast imaging will remain and could provide sufficient incentive to commercialize it. Several workers believe that the speckle technique does have a future and is worth developing further (Aizu and Asakura 1999, Dunn *et al* 2001).

Table 1 compares the properties of two commercial laser Doppler imagers (from Moor Instruments Ltd of England and Lisca AB of Sweden) and two versions of laser speckle contrast imaging (the original LASCA system developed at Kingston University in England (Briers *et al* 1999) and the version used by Dunn *et al* (2001) at the Harvard Medical School in the USA).

6.8. Some examples of full-field blood perfusion imaging

Figures 11–14 show some examples of laser Doppler imaging and laser speckle contrast imaging.

Figures 11 and 12 show the effect of gently scratching the back of the hand to stimulate blood flow. Figure 11 was produced by a commercial laser Doppler imager, while Figure 12 shows the same experiment carried out with a prototype laser speckle contrast imager (Briers and Webster 1996). Both techniques clearly show the increased perfusion, but the greater sophistication of the commercial laser Doppler system is evident (albeit at the expense of a much longer acquisition and processing time).

Figure 13 shows a superficial dermal burn (scald by steam) to the forearm captured by a laser speckle contrast imager, with increased perfusion around the burn.

Figure 14 shows laser Doppler imaging applied to a free flap on the face after surgery (tissue was taken from the patient's back for the graft). As expected, flow is much lower in the flap than in the surrounding tissue.

7. Conclusions

Laser Doppler, photon correlation spectroscopy and time-varying speckle are related techniques that can be used non-invasively to measure capillary blood flow in the skin. They work by analysing the intensity fluctuations in scattered laser light. If a map of blood perfusion is required, there is a choice of the scanning technique of laser Doppler imaging or the full-field technique of laser speckle contrast imaging. Laser Doppler imaging has the advantage of being a fully engineered instrument already in the market-place. Laser speckle contrast imaging has the advantage of being truly real-time and using off-the-shelf components. It is also capable of producing video-rate movies of changing perfusion maps.

Laser Doppler imagers have already proved their worth clinically, and planned improvements may allow real-time operation, which will make them even more attractive to the medical profession. On the other hand, laser speckle contrast imaging can achieve real-time operation at a much lower cost and can produce movies of dynamic changes in blood flow. Ongoing developments may in the future make laser speckle contrast imaging a competitive alternative to laser Doppler imaging.

Because of the complexities of the systems being measured, it will always be difficult to obtain accurate absolute measurements of velocity or perfusion. The attraction of these imaging techniques is therefore mainly in relative measurements, especially the monitoring of changes. This limitation is unlikely to be a major drawback in their use for clinical assessment and diagnosis.

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