CAPILLARY BLOOD FLOW MONITORING USING LASER SPECKLE CONTRAST ANALYSIS (LASCA)

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ABSTRACT
Coherent light scattered from an ensemble of moving scatterers produces a time-varying speckle pattern. The intensity fluctuations observed in a single speckle can be regarded either as a time-varying interference effect or as a Doppler beating effect. Techniques based on each of these approaches have been developed to analyze the fluctuations in an attempt to measure the velocities of the scatterers. Most of these methods measure the temporal statistics of the intensity fluctuations in a single speckle, i.e., at a single point. If a map of the velocity distribution is required, some form of scanning must be introduced. One way of avoiding the need to scan is to make use of the spatial statistics of time-integrated speckle. This is the basis of a technique, already described in the literature, called laser speckle contrast analysis (LASCA). In this article, we present a brief review of the theory linking the intensity fluctuations to the velocity and of the various techniques that have been proposed to measure them. We then describe the present configuration of our LASCA technique and describe some recent developments in our search for a real-time, noninvasive, full-field technique for visualizing capillary blood flow.© 1999 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(99)01801-8]

Keywords laser speckle; time-varying speckle; time-averaged speckle photography; laser Doppler velocimetry; blood flow monitoring.

1 INTRODUCTION
The monitoring of blood flow is an important medical diagnostic tool. In nonsurgical applications, noninvasive optical techniques are feasible in the specific areas of skin capillary flow and retinal flow. The techniques rely on analyzing the intensity fluctuations that occur when the area of interest is illuminated with laser light.

In the case of skin perfusion, potential application areas include dermatology, hematology, the early diagnosis of diabetes and some cardiac diseases, research into the effects of smoking and drugs, and the assessment of burns and other wounds. Information on retinal blood flow might assist in the diagnosis of cancer, glaucoma, and other ophthalmic problems, as well as shedding light on the pathology of brain microvessels. The availability of a real-time, noncontact method for mapping flow or perfusion would be a useful diagnostic aid.

This article presents a brief review of the causes of the light fluctuations and of the various techniques that have been developed to analyze them, and then describes the present state of the art in one of these methods, laser speckle contrast analysis (LASCA).

2 CONCEPTS AND TECHNIQUES
The physics behind the light fluctuations that are observed when moving particles (such as blood cells) are illuminated with coherent (laser) light can be explained in two ways. These are discussed in detail below, but a brief summary now may help to clarify the relationships between the various techniques that are referred to in this article.

The intensity fluctuations can be explained either as a fluctuating random interference pattern or as a beating effect between light of different frequencies. In the first case, the interference pattern is known as laser speckle and the effect as time-varying speckle. In the second case, the different frequencies are explained as Doppler shifts caused by the light being scattered from moving particles. If a separate reference beam (from a stationary reference) is used, the technique is known as heterodyne; if not, it is a homodyne technique. Both effects can be conveniently referred to as laser Doppler.

The various techniques that are described or mentioned in this article relate to these two concepts as follows:
First we shall describe the phenomenon of laser speckle and its properties and the concept of time-varying speckle.

3 LASER SPECKLE

Laser speckle\textsuperscript{1,2} is a random granular pattern produced when laser light illuminates a rough (i.e., nonspecular) object. It is a result of the high degree of coherence of laser light. Two basic types of laser speckle are generally recognized. Image speckle (Figure 1) appears on the image produced by an optical system (including the eye); far-field speckle (Figure 2) is seen in the scattered light falling on a screen placed some distance away from an object illuminated with laser light. Originally regarded as unavoidable and undesirable noise, speckle has become an important tool of optical metrology.\textsuperscript{3} Speckle techniques can measure surface roughness, small displacements, deformations, and vibrations. Many of these techniques are useful in biological and medical measurements.\textsuperscript{4} Laser speckle can even be used to measure visual defects.\textsuperscript{5}

Laser speckle is an interference effect. Consider image speckle first (Figure 1). If the surface is rough compared with the wavelength of the light, rays from different parts of the surface within a resolution cell (the area just resolved by the optical system imaging the surface) traverse 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 intensity at a given point on the image results from the coherent addition of the complex amplitudes associated with each of these rays. If the resultant amplitude is zero, a dark "speckle" occurs at the point, while if all the rays arrive at the point in phase, an intensity maximum is observed. In the case of far-field speckle (Figure 2), rays from all points within the illuminated area contribute to the speckle intensity at any point on the observing screen.

4 THE STATISTICS OF LASER SPECKLE

Laser speckle is a random phenomenon that can only be described statistically. Goodman\textsuperscript{6–9} and Goldfischer\textsuperscript{10} were the first to derive the statistics of laser speckle patterns in detail. Only the more relevant results are quoted below.

Consider first speckle produced under "ideal" conditions. Coherent light illuminates a perfectly diffusing surface whose surface height variations are Gaussian. Both the coherence length of the light and the size of the scattering area are much larger than the path differences caused by the roughness of the surface and many scattering centers contribute to the production of the speckle pattern. The resulting speckle pattern, in the terminology of Pedersen,\textsuperscript{11} is "fully developed."

It is convenient to divide the statistics of speckle patterns into first- and second-order statistics. The first-order statistics describe the properties of a speckle pattern point by point. The assumption of Gaussian statistics leads to a negative exponential probability density function of intensity.\textsuperscript{5} One of the properties of such a distribution is that the standard deviation of the intensity, $\sigma$, is equal to the mean intensity

$$\sigma = \langle I \rangle. \quad (1)$$

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. (Throughout this article, when we refer to speckle contrast, we mean this ratio.) We therefore have

$$\text{Speckle contrast} = \sigma / \langle I \rangle.$$  \hspace{1cm} (2)

Hence for a fully developed speckle pattern the contrast is defined to be unity

$$\sigma / \langle I \rangle = 1.$$  \hspace{1cm} (3)

"Partially developed" speckle patterns exhibit lower speckle contrast, with $\sigma / \langle I \rangle$ less than 1.

The reduction in speckle contrast can be due to a number of causes. The most obvious are a reduction in the coherence of the light source or a reduction in the roughness of the surface. Other causes might be the addition to the speckle pattern of a uniform background of light, which may be either coherent with the speckle pattern or completely incoherent.

The second-order statistics of a speckle pattern describe how rapidly the intensity varies from point to point in the pattern. They thus give an indication of the size of the speckles and the distribution of speckle size in the pattern. The most common functions used to portray the second-order statistics of speckle patterns are the (spatial) autocorrelation function of intensity and its Fourier transform, the power (or Wiener) spectrum.8 An analysis of these second-order statistics shows that the speckle size is determined entirely by the size of the aperture of the optical system used (for image speckle) or by the size of the illuminated area on the scattering surface (for far-field speckle). In fact, the minimum speckle diameter is equal to the diameter of the central maximum in the far-field diffraction pattern of the relevant aperture. In the case of a circular aperture, this is the well-known Airy disk.

5 Time-Varying Speckle

When an object moves, the speckle pattern 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 scatterers, such as particles in a fluid. An individual speckle appears to "twinkle" like a star. This effect also occurs when a far-field speckle pattern is scanned across a small detector (smaller than a single speckle) by moving the scattering object producing the speckle pattern.

Time-varying speckle is frequently observed when biological samples are observed under laser-light illumination. Examples reported in the literature include various botanical subjects12–15 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.12,13 One of the most important potential applications arises when the fluctuations are caused by the flow of blood. This was recognized by Stern16 over two decades ago.

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.

6 Laser Doppler and Photon Correlation

Two other technologies have also been developed to analyze these intensity fluctuations. Light scattered from a moving scatterer has its frequency shifted by the well-known Doppler effect. In the case of quasirandom movement, such as occurs in diffusion processes, the returning light has a frequency spread that reflects the velocity spread of the scatterers. Light of slightly different frequencies mixes to give a beat frequency ("self-beating") equal to the difference between the two frequencies. Hence the result at the detector is a fluctuating light signal with a frequency spread that is related to the velocity distribution of the scatterers. The usual way of analyzing this fluctuating signal is to use a digital autocorrelator. This gives the autocorrelation function of the fluctuations, which is the Fourier transform of the frequency distribution. The technique was originally known as intensity fluctuation spectroscopy (or light beating spectroscopy).17,18 However, the use of photon-counting techniques at the low light levels often observed has resulted in the name photon correlation spectroscopy becoming more usual.

When the motion is directional, whether of a solid object or of a fluid, there is again a Doppler frequency shift of the scattered light. In this case, however, all or most of the light is subject to the same frequency shift. It is therefore usual to introduce a local reference beam and to use heterodyning to analyze the signal: the returning frequency-shifted light beats with the unshifted reference beam to give the difference frequency which is directly related to the velocity of the object. This is the basis of the well-established technique of laser Doppler velocimetry.19 If there is a range of velocities associated with the flow, because different scatterers are traveling with different velocities, then there will be a corresponding range of Doppler shifts and hence of beat frequencies: in other words, there will be a Doppler spectrum. Self-beating will also occur in this case, leading to the beat frequency spectrum illustrated in Figure 3. There is a spread of frequen-
cies around zero, representing the self-beating effect, and a separate Doppler signal, also with a spread of frequencies, representing the heterodyne effect. Provided the flow velocity is large compared with the random movements of the individual scatterers, the two peaks will be separated, as illustrated. (The negative side of the zero-centered peak in Figure 3 is shown by a dashed curve because these “negative” frequencies are not, of course, detected as such: positive and negative velocities produce the same self-beat frequency at the detector. In practice, therefore, these “negative” frequencies are detected as the corresponding positive frequencies.)

It is by no means intuitively obvious that the Doppler and speckle 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 speckle explanation, on the other hand, involves the interference of two waves of the same frequency and the detection of the temporal change in the resultant intensity at a point in space. Nevertheless, analysis shows that the detected frequency is the same in both cases and the two approaches are, in fact, just different ways of looking at the same phenomenon.20

7 RELATING THE INTENSITY FLUCTUATIONS TO VELOCITIES

Before considering the case of fluid flow, it is useful to recall the theory of laser Doppler applied to the motion of a solid object. In this case, instead of a velocity distribution there is only a single velocity. The light reflected from the moving object is Doppler shifted compared with the reference beam (the local oscillator in Doppler parlance), and hence there is a single beat frequency detected (rather than a frequency spectrum). It is easily shown that the relationship between the beat frequency $\delta \nu$ and the line-of-sight velocity component $u$ is

$$\delta \nu = \frac{2u}{c} \cdot \nu$$

(4)

where $\nu$ is the (unshifted) frequency of the laser light used and $c$ is the velocity of light. The measurement of $\delta \nu$ therefore gives directly the line-of-sight velocity of the moving object.

It might appear at first sight that the case of fluid flow should be equally simple: measure the mean frequency in the scattered light and use Eq. (4) to calculate the mean line-of-sight velocity. However, the matter is complicated by the possibility of self-beating and the existence of a second peak around zero frequency, as indicated in Figure 3. Measurement of the mean frequency in this case would not yield the correct answer for the mean velocity. If the peaks are completely separated, as shown in Figure 3, high-pass filtering before calculating the mean frequency might yield the correct answer, but in many cases the two peaks of Figure 3 will overlap. Hence, in general, a more complicated procedure is necessary to extract velocity information from the frequency spectrum.

Now consider the case where there is no directional flow but a random distribution of velocities in the fluid. This occurs, for example, when diffusion processes are being investigated. It is now useful to extend the concept of first- and second-order statistics to the time domain and to speckle fluctuations. So far as the first-order statistics are concerned, the ratio of the standard deviation to the mean intensity then represents the depth of modulation of the fluctuations and in an ideal case is again equal to unity. (Note the parallel here with the spatial concept of speckle contrast: both are defined in the same way and the depth of modulation can be regarded as the temporal analog of contrast.) If the depth of modulation is less than one, this may indicate the presence of some background illumination (coherent or ambient) or of some stationary scatterers in the field of view. Of more interest, however, are deliberate changes to these first-order statistics caused by integrating or differentiating the original signal. The second-order statistics are the frequency spectrum or its Fourier transform, the autocorrelation function (but in the time domain, of course, rather than the spatial domain). The latter is measured directly by the technique of photon correlation spectroscopy.

Calculation of the autocorrelation function involves taking the time-varying signal $I(t)$, multiplying it by itself at different delays or lags $\tau$, and integrating that product over the entire signal:

$$G(\tau) = \int I(t)I(t+\tau)dt.$$  

(5)

For a nonperiodic signal, the resulting curve of $G$ against $\tau$ decreases monotonically to zero (which it reaches when the signal is completely decorrelated). In practice, normalizing factors ensure that the autocorrelation function (then strictly called the autocovariance) at zero lag is equal to unity: a cor-

**Fig. 3** Doppler broadening (homodyne) and a separate heterodyne Doppler signal.
relation time $\tau_c$ can then be defined as the value of $\tau$ at which $G$ has fallen to 0.5 (or sometimes $1/e$ or $1/e^2$). The faster the average velocity of the scatterers, the more quickly the curve falls and the lower the value of $\tau_c$. It is then possible to define a characteristic velocity of the scatterers $u_c$ that is inversely proportional to $\tau_c$:

$$u_c = \frac{k}{\tau_c}.$$  

(6)

The factor $k$ depends on the model used for the velocity distribution.

In the case of fluid flow, there will be a combination of the above two effects, as illustrated in Figure 3. There will be a frequency spread around zero from the self-beating (homodyne) effect, as well as one around the mean Doppler frequency from the heterodyne effect. If the heterodyne and homodyne effects are both present and of similar magnitude, the autocorrelation function (which is the Fourier transform of Figure 3) will deviate from that of a monotonic fall to zero and will typically fall to a nonzero value. The extraction (and meaning) of $u_c$ will then be much more difficult. In general, there will be a need to resort to a model involving a succession of assumptions and approximations that make absolute measurements very difficult: the techniques are, however, very valuable for relative measurements of flow velocity.

8 Measurement Techniques

Photon correlation spectroscopy is used to study random or quasirandom motion such as Brownian motion, diffusion processes, or motility of organisms. Full analog correlation requires the collection and processing of very large amounts of data. To alleviate this problem, researchers introduced "hard clipping" of the signal, the usual method being to assign a value of 1 to the intensity if it was greater than the average intensity and a value of 0 if it was less. This allowed the design of digital autocorrelators based on a shift register technique and greatly reduced the amount of data processing required. Specialized digital autocorrelators were developed and marketed for a variety of applications. These directly measured the autocorrelation function of the intensity fluctuations and hence a correlation time $\tau_c$ that could be related to a characteristic velocity of the scatterers. Researchers also formulated theories that allowed the conversion of this information directly into diffusion constants. Alternatively, fast Fourier transform algorithms converted the autocorrelation functions into frequency spectra (power spectra).

For directional motion, heterodyning is introduced by adding a reference beam or "local oscillator." The reference beam is usually produced by reflection from a stationary mirror. The beating of this light with the Doppler-shifted light scattered from the moving object produces a difference frequency $\partial \nu$ that depends on the velocity $u$ of the moving object according to Eq. (4). This is the now well-known laser Doppler velocimetry technique.\(^{19}\)

A refinement is the so-called two-beam technique, used mainly for flow measurement. This uses two laser beams to illuminate the area under investigation, the two beams intersecting at a known angle and the intensity fluctuations being observed from a third direction (typically the bisector of the angle between the beams). There is no separate reference beam. The Doppler shift of the light scattered from the moving scatterers is now in general different for each beam, as it depends on the relative velocity of light and scatterer. The two scattered beams therefore beat together to give a difference frequency that can again be interpreted in terms of the velocity of the scatterers. (An alternative interpretation of the technique is to argue that the two laser beams interfere where they cross and produce a pattern of interference fringes whose spacing depends on the angle between the two beams. The scattering particles passing through the fringe pattern will appear brightest when they pass through a maximum fringe intensity and will not be visible when they pass through a minimum: the resulting intensity fluctuations are clearly dependent on the velocity of the particles. Again, it is not immediately obvious that the Doppler and interference interpretations are identical, but a mathematical analysis of the two approaches shows that indeed they are.)

Photon correlation spectroscopy and, more particularly, laser Doppler velocimetry, are well-established techniques and are used routinely in many fields of flow measurement. Nevertheless, their reliance on monitoring the instantaneous intensity of the fluctuations as a function of time and their use of large data sets demand quite sophisticated and therefore expensive equipment. In addition, Doppler measurements are often complicated by the presence of a homodyne signal and again the measures that have to be taken to extract the signal can make the method very complex and expensive.

Researchers approaching the same problems from the viewpoint of time-varying laser speckle have developed several ways of reducing the complexity and therefore the cost of the measurements. For example, Asakura and co-workers introduced the idea of using the ratio of a selected high-frequency component of the fluctuations to a selected low-frequency component\(^{21}\) and the even simpler concept of measuring the mean frequency.\(^{22}\) They applied both these techniques to the measurement of blood flow. The same group has developed several other simplified methods of using these second-order statistics of fluctuating speckle. Other variations aimed at reducing the amount of data processing necessary have involved the introduction of new degrees of freedom. So far it has been assumed that intensity measurements are instantaneous. This, of course, is never achieved in practice and the detector used has a finite inte-
gration time. If this integration time is long compared with the correlation time of the speckle fluctuations, the intensity fluctuations average out and a constant intensity is recorded. At shorter integration times, the depth of modulation is dependent on both the integration time and the velocity of the scatterers. Hence, for time-integrated speckle, the first-order statistics, as well as the second-order, contain information about the velocity of the scatterers. \(^\text{23}\) By purposely using temporal integration, therefore, a new variable (the integration time) is introduced. Another possibility is to use time-differentiated speckle, and again the statistics contain information about the velocities of the scatterers. \(^\text{24–28}\)

9 MAPPING FLOW FIELDS

The techniques described in the previous section provide a very useful arsenal for measuring fluid flow, including skin capillary blood flow and retinal blood flow. The sophistication of the measurements will depend on the resources available, and can range from full autocorrelation measurements or Doppler spectrum analysis to simple measurements of the intensity differences between successive readings. However, they all suffer from the limitation that they measure the flow 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. One technique was described by Fujii et al. in their application of time-differentiated speckle to skin capillary blood flow. \(^\text{25}\) They used a linear charge coupled device (CCD) array to monitor simultaneously a line of speckles, and a scanning arrangement to extend this to a two-dimensional area. This technique is also described in a review article by Aizu and Asakura. \(^\text{29}\) More recently, Fujii’s group have applied a scanning version of their time-differentiated speckle technique to the mapping of retinal blood flow. \(^\text{30–32}\) 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 then calculating the differences between successive images. The parameter they use is the ratio of the mean intensity to the intensity difference, a quantity they call “normalized blur” and which they use as a measure of velocity. Experiments on a rabbit eye showed good correlation with invasive methods. \(^\text{31}\)

Scanning has also recently been applied to the laser Doppler technique \(^\text{35,34}\) and commercial scanning Doppler systems are now on the market that can provide full-field monitoring of capillary blood flow over quite large areas of the body. The problem of either processing or storing vast amounts of data demands a compromise between cost and performance and the systems offered suffer from both long scanning times and a loss of spatial resolution. Nevertheless, they are useful as a diagnostic tool and offer easily interpreted false-color maps of blood velocity and/or flow.

Ideally, a full-field technique would avoid the need for scanning. One such technique is the recently developed "global Doppler." \(^\text{35}\) This converts velocity directly to intensity (or false color) 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.

10 A TRUE FULL-FIELD TECHNIQUE: LASER SPECKLE CONTRAST ANALYSIS (LASCA)

A technique developed in the early eighties simultaneously achieved full-field operation (without scanning) and very simple (and cheap) data gathering and processing. “Single-exposure speckle photography” \(^\text{36}\) used the first-order spatial statistics of time-integrated speckle and was originally developed for the measurement of retinal blood flow. \(^\text{37,38}\) The basic technique was simply to photograph the retina under laser illumination, using an exposure time 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 photograph \(^\text{39}\) converted these contrast variations to more easily seen intensity variations. Later work \(^\text{40}\) introduced digital image processing of the speckle photographs, including a color coding of the velocities.

The disadvantage of single-exposure speckle photography is that it is a two-stage process. First the photograph is taken and the film developed, then it is processed (either optically or digitally). This precludes real-time operation and severely limits clinical application.

More recently, we have been developing single-exposure speckle photography into a fully digital, real-time technique for the mapping of skin capillary blood flow. \(^\text{41}\) As the method is no longer photographic, we now call it “laser speckle contrast analysis” (LASCA). \(^\text{42,43}\) The physics on which it is based, however, is exactly the same as the earlier technique. (A closely related technique has recently been used by Dacosta \(^\text{44}\) as a remote method of sensing heartbeats. He uses a TV camera to record the speckle pattern produced by a vein and digitizes it frame by frame, then computes the speckle contrast...
and plots this as a function of time. A minimum in this contrast indicates the occurrence of a heartbeat.

LASCA uses a CCD camera and a frame grabber, and specially developed software to compute the local contrast and convert it to a false-color map of contrast (and hence of velocity). The image is a time-integrated exposure, but at the velocities involved in blood flow, the exposure is short enough (typically 20 ms) to render the technique effectively real time. In the current version (see Figure 4), light from a laser (usually a 40 mW He–Ne laser operating at a wavelength of 633 nm) diverges to illuminate the area of skin under investigation. [Care is taken to ensure that the maximum permissible exposure (MPE) of 2000 Wm$^{-2}$ for long-term exposure is not exceeded.] A CCD camera (currently a Hitachi-Denshi KP-M1 with a 16-mm diam sensing area containing about 440 000 pixels) 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 immediately processes it by built-in software to produce a false-color contrast map indicating velocity variations. The operator has several options at his disposal, including the number of pixels over which the local contrast is computed, the scaling of the contrast map, and the choice of contour colors. The most important of these is the choice of the number of pixels over which to compute the speckle contrast: too few, and the statistics will be questionable, too many and spatial resolution will be lost. In practice, we have found that a square of 7×7 or 5×5 pixels is usually a satisfactory compromise.

As the purpose of this article is to report refinements to a technique that has already been published in this journal, and as these refinements refer to the analysis of the images rather than to any improvements in the images, we do not present any new LASCA images here. The reader should consult Ref. 42 for examples of LASCA images.

### 11 Theory of LASCA

The principle of LASCA is very simple. A time-integrated image of a moving object displays blurring. In the case of a laser speckle pattern, this appears as a reduction in the speckle contrast. This occurs whatever the “movement” of the speckle. For random velocity distributions, each speckle fluctuates in intensity. For lateral motion of a solid object, on the other hand, the speckles also move laterally and become “smeared” on the image—but a reduction in speckle contrast still occurs. For fluid flow, the situation might be a combination of both these types of movement. In each case, the problem for quantitative measurements is the establishment of a relationship between the speckle contrast and the velocity (or velocity distribution).

It is clear that there must be a link between the velocity and the amount of blurring. The higher the velocity, the faster are the fluctuations and the more blurring occurs in a given integration time. By making certain assumptions, the following mathematical link can be established between the speckle contrast and the temporal statistics of the fluctuating speckle:

$$\sigma_s^2(T) = (1/T) \int_0^T C_t(\tau) \, d\tau,$$  \hspace{1cm} (7)

where $\sigma_s^2$ is the spatial variance of the intensity in the speckle pattern, $T$ is the integration time, and $C_t$ is the autocovariance of the temporal fluctuations in the intensity of a single speckle. [The autocovariance is a normalized version of the autocorrelation function of Eq. (5)].

This equation establishes the relationship between LASCA and those techniques that use the intensity fluctuations in laser light scattered from moving objects or particles. LASCA measures the quantity on the left-hand side of Eq. (7); photon correlation spectroscopy, laser Doppler, and time-varying speckle techniques measure the quantity on the right-hand side. [It is also worth noting that LASCA uses image speckle, whereas most of the temporal techniques use far-field speckle. However, this does not detract from the fundamental equivalence of the two approaches expressed in Eq. (7)].

Provided the assumptions made in establishing Eq. (7) are valid, LASCA is now on an equal footing with all the temporal techniques (photon correlation, Doppler, time-varying speckle) so far as linking the measurements to actual velocities is concerned. All the techniques now allow the correlation time $\tau_c$ to be determined. In the case of photon correlation, this parameter is measured directly. In the case of LASCA, some further assumptions must be made in order to link the measurement of speckle contrast (defined as $\sigma_s^2/(I)$) with $\tau_c$. Various models can be used, depending on the type of motion being monitored. For the case of a
Lorentzian velocity distribution, for example, the equation becomes\(^{36}\)

\[
\sigma_s / \langle I \rangle = \left[ \left( \tau_c / 2T \right) \left( 1 - \exp \left( -2T / \tau_c \right) \right) \right]^{1/2}.
\]  

(8)

In the case of laminar flow (or the lateral motion of a solid object), the speckles move with the object (for short distances). Hence the temporal autocovariance \(C_s(\tau)\) is identical with the spatial autocovariance of the speckle pattern \(C_s(\xi)\) under the transformation \(\xi \rightarrow u\tau\), where \(u = \) velocity. By using accepted expressions for \(C_s(\xi)\), this in turn leads to\(^{37}\)

\[
\sigma_s / \langle I \rangle = \left[ \left( \tau_c / 3.83T \right) \int_0^{3.83T/\tau_c} \left\{ 2J_1(x) / x \right\} dx \right]^{1/2}.
\]  

(9)

The constant 3.83 relates, of course, to the first zero of the \(J_1\) Bessel function and arises because \(\tau_c\) has been taken, in this case, as the value of \(\tau\) when \(J_1\) first reaches zero. \(J_1\) appears because \(C_s(\xi)\) is determined by the relative aperture, assumed circular, of the lens used to record the speckle pattern.

These equations relate the speckle contrast to the correlation time \(\tau_c\) for a given integration time \(T\). Models for other types of velocity distribution can be developed,\(^{36}\) but it can be seen that, in principle, it is possible to link the spatial statistics (the contrast) of the time-integrated speckle pattern to the fluctuation frequency of the temporal intensity variations.

From this point on, LASCA shares the same problem as all the temporal frequency measurement techniques—photon correlation spectroscopy, laser Doppler, and time-varying speckle. This is the problem of relating the correlation time \(\tau_c\) to the velocity distribution of the scatterers. It is not straightforward. Problems include the effects of multiple scattering, size of the scattering particles (blood cells in the present case), shape of the scatterers, non-Newtonian flow, non-Gaussian statistics resulting from a low number of scatterers in the resolution cell, spin of the scatterers, etc. The simplest model, ignoring all the above complications, leads to the characteristic velocity \(u_c\) [see Eq. (6)] being defined as follows:\(^{32}\)

\[
u_c = \lambda / 2\pi \tau_c.
\]  

(10)

However, other models, using different approaches to the problems outlined above, lead to significantly different relationships between \(\tau_c\) and the velocity of the scatterers. For example, Bonner and Nossal,\(^{45}\) taking into account the size of the blood cells, predict characteristic velocities 1 1/2 orders of magnitude higher than those given by Eq. (10).

Much work is going on into these effects and the question is far from settled. Because of the uncertainties caused by these factors, it is currently normal practice to rely either on calibration or on relative measurements rather than on absolute measurements. So far as calibration is concerned, several models have been proposed. An excellent brief review of these has been given by Steenbergen and de Mul.\(^{36}\) They vary from simple suspensions of microspheres in water or rotating and stationary disks to complex phantoms that mimic blood and tissue. Steenbergen and de Mul also describe their own version of the latter.

Often, though, relative measurements are all that are necessary in a clinical situation. It is more important to measure or detect changes or differences in blood flow than to make absolute measurements. Examples include monitoring the response of blood flow to changes in external stimuli or detecting differences between different parts of the same body. In such cases, even calibration may not be necessary for the technique to have clinical viability. This avoids not only the complications of relating the contrast measurement to absolute velocities, but also the effects of noise and other uncertainties that may affect the measurements.

**12 PRACTICAL CONSIDERATIONS FOR LASCA**

To measure the temporal statistics of fluctuating speckle patterns, it is necessary to monitor the intensity of a single speckle. In order to do this accurately, the aperture of the detector must be smaller than the average speckle size. Otherwise, some spatial averaging will occur and the first-order statistics will be corrupted. (There will also be some accuracy loss in the second-order statistics, which has implications also for laser Doppler and photon correlation techniques.)

For LASCA, the matter is more complicated. LASCA 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: if the speckles are much larger than the pixels, as suggested above, fewer speckles are sampled. This means that the viable speckle size is more restricted. If it is too small, each pixel samples more than one speckle, leading to speckle averaging and loss of measured contrast. If it is too large, not enough speckles are sampled to ensure good statistics. Thus 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 above). 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...
neutral density filters to ensure usable light levels at the detector.

Another problem that has occurred with LASCA is a failure to realize the full range of contrasts that should theoretically be available. A stationary object should give a speckle contrast of 1. A fully blurred speckle pattern produced by rapidly moving scatterers, should have zero contrast. The Lorentzian model of Eq. (8), for example, predicts the relationship between contrast and the ratio $\tau_c/T$ presented in Figure 5. This suggests that, for a given integration time $T$, the dynamic range of the technique corresponding to contrasts between 0.1 and 0.9 should be about two orders of magnitude in $\tau_c$ (and hence in velocity). In practice, contrasts of only 0.6 were being measured even for stationary random diffusers. We now believe that this problem is caused by the CCD camera. Most CCD cameras are designed to produce a direct current (dc) offset from zero called the pedestal. This is to avoid cutting off the negative peaks produced during any processing. It is formed because of the dark current and other anomalies in the video circuitry of the camera. This does not show as a single line on a histogram of pixel frequency against intensity, but as a "fat zero." This has the effect of shifting the probability density histogram along the $x$ axis with a consequent effect on the standard deviation and hence on the contrast.

Figure 6(a) shows a histogram of the pixel frequency (i.e., the number of pixels having a specific intensity value) against intensity ("pixel value") when a diffusing metal plate is used as the object. This effectively gives the probability density function (PDF) of the intensity in the image. According to theory, such an object should give a fully developed speckle pattern with a contrast of 1.0 and a negative-exponential PDF. In fact, the image that gave rise to Figure 6(a) had a contrast of 0.41 and the histogram is clearly not a negative exponential. However, inspection of Figure 6(a) shows that the curve to the right of the peak may well approximate a negative exponential. We believe that the part of the histogram to the left of the peak is an artifact introduced into the image by the CCD camera. When this part of the histogram was removed, as in Figure 6(b), the measured contrast of the speckle pattern rose to 0.95, showing that the histogram was indeed now close to a negative exponential. (Note that this device is effectively the same as subtracting a baseline frame, as used by other workers.)

We have applied this correction to an image of a hand illuminated by the laser light. Figure 7(a) shows the histogram before processing and Figure 7(b) after removing the effect of the dark current. The contrast rose from 0.33 to 0.68. This lower contrast (compared with the metal plate) is due to the scatterers in the hand (the red blood cells) being in motion. However, the speckle pattern from a set of moving scatterers should not have the large number of pixels of zero intensity that are present in Figure 7(b). We believe that this effect may be due to the Gaussian intensity profile of the laser beam. Towards the edge of the image, the intensities tend towards zero. Removing the dark current effect forces these low intensities to zero and this is reflected in the histogram. By ignoring the outer edges of the image, this effect can be avoided and histograms similar to Figure 8 obtained.

13 RECENT DEVELOPMENTS WITH LASCA

The above algorithm for removing the dark current effect has been added as an option to the LASCA software. This has resulted in a significant im-

![Fig. 5](image-url) Variation of speckle contrast with the ratio correlation time to integration time for the Lorentzian model.

![Fig. 6](image-url) Histograms showing the probability density function of intensities in speckle on the image of a diffusing metal plate, (a) before and (b) after removing the effect of the dark current.
provement in the dynamic range, with speckle contrasts now covering most of the range 0 to 1 being attainable.

The effect of varying the exposure time (integration time) has also been investigated and times as short as 1 ms have been used. We expected that this short exposure time would “freeze” the speckle pattern observed when human skin is illuminated with laser light, resulting in high contrast. However, the increase in contrast is only small (0.30 for 1 ms, 0.25 for 1/60 s, after removal of the dark current effect). We believe that this is due to the light penetrating far enough into the tissue to pick up larger velocities from larger blood vessels (arterioles rather than capillaries).

We have also investigated the effect of using wavelengths other than 633 nm. There is a need in some clinical situations, for example, eczema and other dermatological problems, to measure the superficial dermal blood flow. This has prompted several groups to use other wavelengths with the laser Doppler technique. Penetration of tissue is highly wavelength dependent, with red light penetrating much further than green light, for example. Preliminary experiments on fingers with LASCA using the 514 nm line of an argon-ion laser failed to produce any evidence of fluctuations caused by blood flow. We interpret this as due to the fact that this wavelength is almost entirely absorbed by red blood cells, so that any light reaching the camera has been scattered from the outer skin layers, where motion is absent. This means that green light can be used to discriminate against gross body movements.

Some other groups involved in laser diagnostics of tissue have used infrared laser light in order to improve the penetration. We have not yet done this, but we believe that it could extend the application of LASCA and will form the basis of some future work.

Parallel with this work on the optics and preprocessing of LASCA, we have also been refining the original software that computes the contrast map. In our earlier publications, we reported a processing time of about four minutes. Hence, although the image was captured virtually instantaneously (with an exposure time typically of 0.04 s), there was a time delay before the processed image was available. By refining the algorithms and the software code, we have now reduced the processing time to less than one second, which means that we now have a genuinely real-time technique. At the same time, we have incorporated the software into a package that operates in the Microsoft Windows environment and have made the operation of LASCA much more user friendly.

14 CONCLUSIONS

Laser Doppler, photon correlation spectroscopy and time-varying speckle are related techniques that can be used noninvasively to measure capillary blood flow in the skin. They work by analyzing the intensity fluctuations in scattered laser light. As they are essentially pointwise methods, some form of scanning must be used if a full-field velocity map of blood flow is required. LASCA achieves this goal in a single shot by utilizing the spatial statistics of time-integrated speckle. The technique produces a false-color map of blood flow without the need to scan.

We have now overcome some of the dynamic range problems encountered in our earlier work and have also reduced the processing time to less than one second. This makes LASCA a truly real-time technique. In addition, as the method is based on standard equipment such as a helium–neon laser, a CCD camera and a personal computer, and the software operates under Microsoft Windows,
the system is very cost effective and simple to operate.

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