CYTOPLASMIC STREAMING IN CHARA CORALLINA STUDIED BY LASER LIGHT SCATTERING

D. B. SATTELLE* AND P. B. BUCHAN
A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge, England

SUMMARY
An apparatus is described by means of which the power versus frequency spectrum of the photomultiplier current can be obtained for laser light scattered by streaming cytoplasm in the algal cell Chara corallina. A Doppler peak is noted in the spectrum which is abolished when cytoplasmic streaming is arrested by electrical stimulation. For 5 cells of Chara, this simple laser-Doppler velocimeter gave streaming velocities (46.7 μm s⁻¹, s.d. ±4.8 at 20 °C) similar to those obtained for the same cells using the light microscope (44.3 μm s⁻¹, s.d. ±5.3 at 20 °C). A narrow distribution of streaming velocities is indicated. The technique described provides a rapid, quantitative assay of the in vivo rheological properties of cytoplasm.

INTRODUCTION
Quasi-elastic laser light scattering has provided an accurate and rapid method for determining the velocities of flowing fluids (cf. Yeh & Cummins, 1964; Trolinger, 1972; Chu, 1974; Crosignani & Di Porto, 1974). Light scattered by particles undergoing directed motion is Doppler shifted and the size of the frequency shift provides a direct measure of the velocity. The potential of 'laser Doppler velocimetry' for assessing flow properties in biological systems has been demonstrated for cytoplasmic streaming in the alga Nitella (Mustacich & Ware, 1974, 1976; Langley, Piddington, Ross & Sattelle, 1976; Sattelle, Langford & Langley, 1977) and the slime mould Physarum (Newton, Ford, Langley & Sattelle, 1977). The chemotactic swimming behaviour of sperm (Dubois, Jouannet, Bergé & David, 1974) and motile bacteria (Nossal & Chen, 1972, 1973) have also been followed. The technique has in addition enabled measurement of in vivo rheological properties of blood in arteries (Tanaka, Riva & Ben-Sira, 1974) veins (Tanaka & Benedek, 1975) and small vessels in the peripheral circulation (Stern, 1975).

For signal processing of the scattered light detected by a photomultiplier, these studies have utilized such instruments as the spectrum analyser and the photocount autocorrelator in order to extract either the power spectrum of the scattered light or its inverse Fourier transform, the autocorrelation function. We describe a simple signal-processing system for spectrum analysis of scattered laser light, based on the

* Currently Wellcome Travel Fellow of the Medical Research Council of the United Kingdom.
use of 2 phase detectors in quadrature. This technique is applied in a study of cytoplasmic streaming in the alga *Chara corallina*.

**THEORY**

The optical spectrum of laser light is a single delta function at the laser frequency ($\omega_0$). If a scattering particle moves across the incident wavefronts at a constant velocity and in a constant direction, light scattered by the flowing particle will be Doppler-shifted to a frequency other than $\omega_0$ (Fig. 1). When laser light scattered from a flowing fluid is mixed with unshifted light on a photomultiplier the beat frequency gives the Doppler shift from which the flow velocity can be determined. Under these conditions, the photocurrent power spectrum reproduces the optical spectrum with the centre frequency shifted from $\omega_0$ to zero frequency. Photocurrent power spectra for several kinds of motion are illustrated in Fig. 2 (for comprehensive accounts of theoretical considerations see Trolinger, 1972; Chu, 1974; Crosignani & Di Porto, 1974).

For the scattering geometry employed in the experiments described in this report (see Fig. 3) the velocity of a flowing particle ($v$) is given by:

$$v = \frac{2\pi f_D}{q \cos \alpha}$$

(where $f_D$ is the frequency at which the Doppler peak appears in the photocurrent spectrum).
Laser-Doppler study of cytoplasmic streaming

Fig. 2. Photocurrent spectra [power \( S(\omega) \) versus frequency \( \omega \)] derived from light scattered by various kinds of motion of scattering centres. Following square-law detection the optical spectrum is reproduced with \( \omega_0 \) shifted to zero frequency.

power spectrum, \( q = \) the scattering vector and \( \alpha = \) the angle between the direction of flow and \( q \); equation (1) is from Yeh & Cummins (1974). Since

\[
q = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2}
\]

(2)

(where \( n = \) refractive index of the medium; \( \lambda_0 = \) the wavelength of the incident laser light; \( \theta = \) the scattering angle), by substituting in equation (2) we obtain,

\[
v = \frac{f_D \lambda_0}{2n \sin \frac{\theta}{2} \cos \alpha}
\]

(3)

It is clear from equation (3) that the flow velocity can be determined from a knowledge of the scattering geometry of the experiment and the measurement of \( f_D \). If the flowing scatterers exhibit a range of velocities, the spectral peak centred at \( f_D \) is broadened, the width relating to the distribution of velocities – a narrow peak corresponding to a narrow range of velocities.
MATERIALS AND METHODS

Living material

Internodal cells of Chara corallina (about 5 cm long) were maintained in a physiological medium containing (mM/1.): NaCl, 10; CaCl₂, 0.1; KCl, 0.1. This solution was filtered using a Millipore filter (0.45 μm pore diameter). Cells in which cytoplasmic streaming could be clearly seen with a ×50 stereomicroscope were cleaned with a camel-hair brush and mounted horizontally on short, rigid platinum stimulating electrodes which were in turn held firmly in a micromanipulator (Leitz). Experiments were performed at 20 °C.

Light-scattering apparatus

The apparatus illustrated in Fig. 4 was mounted on a stout wooden table. A 1.5-cm-thick base-plate (1 m × 1 m) was shock-mounted on a tire inner tube. A smaller (0.5-cm-thick) steel plate was supported on the base-plate by rubber stoppers and carried the turntable-mounted, light-scattering arm and a T-shaped table which supported the laser and the glass experimental chamber. The turntable used was the stage of a milling machine. A 5-mW He-Ne laser (Spectra Physics 120) served as an incident light source and was provided with an adjustable mount to facilitate alignment. The plant cell was positioned in the chamber over the centre point of the turntable. The light-scattering arm was constructed to enable ready adjustment to a variety of scattering angles. Although the experiments reported here were carried out at a single scattering angle the angular dependence of the spectral width can be useful in the characterization of diffusive and directed motions (Langley et al. 1976).

The main features of the light-scattering arm are shown in Fig. 5. The laser light scattered from the preparation entered the arm through an iris aperture and was focused in the plane of a pinhole which was situated immediately in front of the face of a photomultiplier tube (Twentieth Century Electronics Type P4283 TIRA). The pinhole was mounted on a vertical,
adjustable X-Y stage. To achieve a single coherence area (iris aperture area) the radii of the aperture and the pinhole are related by

\[ p = \frac{1.22 \lambda_0 z}{2a}, \]

where \( a \) = aperture radius; \( p \) = pinhole radius; \( \lambda_0 \) = wavelength of incident laser light; \( z \) = distance between lens and pinhole (see Jakeman, 1974). From equation (4), a range of pinhole diameters appropriate to a particular aperture diameter were computed. In the experiments reported here an aperture diameter of 0.8 mm and a pinhole diameter of 2.0 mm were employed.

**Fig. 4.** Diagram of the light-scattering apparatus. A tyre inner tube (T) provides shock-mounting for the apparatus which is mounted on a steel base-plate (BP). The turntable-mounted, scattering arm carries the photomultiplier (PMT) and the collection optics. The aperture (A) and the X-Y pinhole adjustment screws (P) are shown. The laser light intensity is attenuated by a neutral density filter (NDF). The glass experimental chamber (C) containing the cell is shown.

**Signal processing**

A block diagram of the main units in the circuitry employed in signal processing is illustrated in Fig. 6. The photomultiplier output was fed into a precision a.c. amplifier (Brookdeal 5022). The output impedance from the photomultiplier was 20 M\( \Omega \) and the input impedance of the a.c. amplifier was 100 M\( \Omega \) shunted by 20 pF. This combination of impedances including stray capacitance did not significantly attenuate the signal over the range 1 to 500 Hz. Following amplification, the signal was fed directly into 2 phase-sensitive detectors (Brookdeal 9421A) whose inputs were connected in parallel. A square wave reference signal was fed into one detector and that signal in quadrature (+90°) was fed into the second detector. The reference signal was taken from a signal source (Brookdeal 9742) which was operated in the external
voltage controlled frequency mode. The frequency of the reference signal was in turn controlled by a ramp waveform generated by a ramp generator (Prosser A341). In most experiments the reference signal was swept through a range of either 1 to 110 Hz or 2 to 220 Hz. The time taken to sweep through the spectrum was about 20 s.

**Fig. 5.** Schematic sectional view of the light-scattering arm of the apparatus. A cell, under saline, is shown in the experimental chamber (C). Light scattered at a given angle is collected via an aperture (diameter = 2a), and focused by the lens on to the pinhole (diameter = 2p) situated immediately in front of the photomultiplier (PMT). When the modified microscope is lowered into the position shown, the mirror deflects the light upwards enabling the investigator to examine the illuminated cell.

**Fig. 6.** Block diagram of the major components in the signal processing system. The ramp generator drives the X-axis and the vector unit \((\sqrt{a^2+b^2})\) drives the Y-axis of a recorder (oscilloscope in this case). PMT = Photomultiplier.

The amplitude of the first phase detector output voltage was proportional to the amplitude of the photomultiplier signal at the frequency being swept at any given moment. The output of the second phase detector was the same but in quadrature with the first. The component values of the signal at any moment were therefore present in rectangular form (i.e. \(a+jb\)). The final unit in the system (Brookdeal \(\sqrt{a^2+b^2}\)) changes the information from rectangular into polar form (i.e. \((a^2+b^2)^{1/2} \Delta \phi\)). This signal was then fed into the Y input of a Tektronix 565 storage oscilloscope whose timebase (X input) was driven directly from the ramp generator.
the same generator that controls the reference signal. The resulting display was a frequency spectrum of the photomultiplier current $i(\omega)$ which was squared to give the power spectrum $|S(\omega)|^2$.

**Experimental procedure and calibration**

The mounted internodal cell was positioned in the attenuated beam and moved along the beam until the image of the illuminated region appeared centrally located in the microscope of the scattering arm. Final positioning was achieved using the X-Y adjustments of the pinhole to obtain maximum intensity. In some experiments an audio-amplifier was connected to the output of the precision a.c. amplifier and fed into a loudspeaker, thereby facilitating alignment procedures. Throughout all experiments the mounted cell could be observed using a stereo-microscope ($\times 50$ magnification) mounted above the preparation chamber. It was therefore possible to ascertain whether or not a cell was streaming, and if so to estimate the velocity of the larger cytoplasmic particles.

**RESULTS**

**Spectrum from streaming cell**

The power spectrum $|S(\omega)|$ was derived first of all from light scattered by the platinum electrodes which served as a mount for the cell. The power spectrum was essentially flat over the range 2–200 Hz (Fig. 7A). A small amount of curvature was detected in the frequency range 2–10 Hz which may have been the result of scatterers present in the physiological medium. Light was next scattered from a cell observed to be streaming. The cell was positioned so that none of the scattered light originated from the electrodes. No specific local oscillator was introduced in such experiments.

![Fig. 7](image_url)

*Fig. 7. Photocurrent power spectra $|S(\omega)|$ versus $\omega$ derived from laser light scattered by: A, platinum electrodes serving as a mount for the cell; B, cell of *Chara corallina* with cytoplasm streaming; and C, cell of *Chara corallina* during temporary arrest of cytoplasmic streaming induced by electrical stimulation.*
and scattering from the plant cell wall provided unshifted light. The spectrum of light scattered by a streaming cell contained a substantial 'very low frequency' (VLF) component and in addition a clearly defined peak (Fig. 7B). With the cell aligned so that streaming was in the same plane as the scattering vector and with $\theta = 60^\circ$; $\alpha = 25^\circ$ (see Fig. 3, p. 636), this peak was centred at 80 Hz.

**Spectrum from non-streaming cell**

The spectrum of light scattered by the same cell, in the same orientation but this time with streaming arrested by the application of a single depolarizing pulse (5 V, 2 ms duration, square wave) is shown in Fig. 7C. The VLF component was still present when streaming ceased but the peak at 80 Hz noted in the spectrum during streaming was no longer detectable. From these observations it seems reasonable to conclude that this peak in the spectrum is the result of light that has undergone a frequency (Doppler) shift as a result of being scattered by particles in the streaming cytoplasm.

**Streaming velocities from laser light scattering and light microscopy**

Using data shown in Fig. 7 from a single cell it is possible using equation (3) to calculate the streaming velocity. In this case with $f_D = 80$ Hz, $\lambda_0 = 6.328 \times 10^{-6}$ cm; $\theta = 60^\circ$, $\alpha = 25^\circ$, and $n$ taken to be 1.34, a streaming velocity of 42.0 $\mu$m s$^{-1}$ was calculated. For 5 cells of *Chara corallina* streaming velocities at 20°C were obtained using both laser light scattering and by timing the movements of individual cytoplasmic particles observed in the stereomicroscope over known distances. An average velocity of 46.7 (s.D. ± 4.8) $\mu$m s$^{-1}$ was calculated from laser light-scattering data, and an average velocity of 44.3 (s.D. ± 5.3) $\mu$m s$^{-1}$ was obtained by direct observation of streaming particles. Unless the choice of the value for the refractive index in the analysis of the light-scattering data is seriously in error, it therefore appears that the 2 methods give comparable results when applied to the same material.

**DISCUSSION**

A simple apparatus and signal processing system has been devised to enable measurements of spectral changes in cells under different physiological conditions. Novel features of the apparatus include a microscope in the scattering arm which readily enables observation, focusing and alignment of the preparation. In addition a turntable is included which, whilst providing rigid support for the scattering arm, also enables rapid change to a new scattering angle. The signal-processing system which employs 2 phase-sensitive detectors in quadrature to recover the frequency spectrum from the amplified anode current of the photomultiplier provides sufficient resolution to detect spectral differences between streaming and non-streaming states of protoplasm in the algal cell *Chara corallina*. The signal-processing system described here is slower to generate the spectrum than a conventional spectrum analyser but its construction in separate modules provides for other uses of the equipment (e.g. signal averaging).
Laser-Doppler study of cytoplasmic streaming

Using laser-Doppler velocimetry, cytoplasmic streaming in Chara has been detected as a distinct peak in the power spectrum derived from the scattered laser light. This peak is consistently abolished when streaming is arrested by electrical stimulation of the cell. The velocities obtained in this way are comparable with those obtained by direct observations on streaming cytoplasmic particles from the same cells. Streaming speeds reported here for Chara corallina [~ 45 μm s⁻¹ at 20 °C] are similar to those obtained for Chara foetida [42 μm s⁻¹ at 20 °C] (Velten, 1876) and Chara braunii [75 μm s⁻¹] (Hayashi, 1952) using light-microscopical techniques. Velocities of the same order of magnitude have been obtained from cells of Nitella opaca [~ 60 μm s⁻¹] (Langley et al., 1976) and Nitella flexilis [~ 50 μm s⁻¹ at 20 °C] (Mustacich & Ware, 1976). These latter authors have carefully investigated the temperature dependence of streaming and have shown that over the temperature range 4–35 °C, a 1 °C increase in temperature corresponds to a 2–6 μm s⁻¹ increase in streaming velocity.

The spectral peak attributed to cytoplasmic streaming in Chara is comparable with similarly narrow Doppler peaks associated with cytoplasmic flow in Nitella flexilis (Mustacich & Ware, 1974, 1976) and Nitella opaca (Langley et al., 1976). These findings are interpreted in terms of a narrow distribution of velocities for the streaming particles (cf. Fig. 2). Thus a plug-like flow of cytoplasm appears to be a common feature of streaming in Chara and Nitella. It is tempting to postulate that similar rheological properties noted in various algal cells may share common mechanisms of motive force generation. In this context it is of interest to note that microfilaments from both Chara corallina (Williamson, 1974) and Nitella flexilis (Palevitz, Ash & Helper, 1974) react with subfragments of muscle myosin to produce 'arrowhead' filaments indicating that these microfilaments in algal cells are very similar to actin. In addition, it has recently been demonstrated that all actin filaments located in the non-streaming (ectoplasmic) outer layer of cytoplasm have the same polarity (Kersey, 1974). These findings provide an ultrastructural basis for the 'active shearing' mechanism proposed much earlier (for a review see Kamiya, 1959) in which it is envisaged that the interaction of sliding filaments at the interface between the streaming cytoplasmic layer (endoplasm) and the non-streaming layer (ectoplasm) generates the motive force for cytoplasmic streaming. The localization of actin in characean cells does not, however, rule out an alternative view for the generation of motive force which proposes the existence of microfilament bundles anchored in the ectoplasm but extending into the endoplasm where beating movements generate the flow of cytoplasm (Allen & Allen, 1972; Allen, 1974).

Spectral components other than the Doppler peak attributed to cytoplasmic streaming can also be distinguished in the power frequency spectrum derived from light scattered by a Chara cell. The prominent very low frequency (VLF) component is present in both streaming and non-streaming cells. It is suggested that this component can be attributed in part to slow-moving particles located either in the periphery of the flowing endoplasm or in the non-flowing ectoplasm. No significant high-frequency component was detected in these experiments, indicating the absence of particles moving at substantially greater velocities than those reported for cytoplasmic streaming.

The recent advances in knowledge of the biochemistry of the machinery of motility in non-muscle cells (Pollard & Weihing, 1974; Pollard, 1975; Goldman, Pollard...
D. B. Sattelle and P. B. Buchan

(1976) only emphasizes the need for complementary biophysical studies. Laser light scattering provides an objective, rapid, quantitative assay for cytoplasmic streaming. Further laser light-scattering studies of streaming under various physiological conditions and with the streaming in a variety of orientations may lead to a better understanding of the mechanism of this most readily observable of all intracellular motions.

The authors express particular thanks to Professor K. H. Langley (Department of Physics and Astronomy, University of Massachusetts) for helpful discussions during the course of this work. We thank Mr J. W. Rodford for preparing Fig. 3.

REFERENCES


Laser-Doppler study of cytoplasmic streaming


(Received 18 May 1976)