

MEASURING BLOOD FLOW: TECHNIQUES AND APPLICATIONS - A REVIEW

A.K. Jayanthi, N. Sujatha* & M. Ramasubba Reddy

Biomedical Engineering Group, Department of Applied Mechanics,

Indian Institute of Technology, Madras, Chennai-600036, India

Email: nsujatha@iitm.ac.in

ABSTRACT

An adequate amount of blood supply is necessary for the proper functioning of all body organs as blood carries all the nutrients and oxygen that our body needs to stay healthy. Various diseases cause an impaired supply of blood to the organs. The measurement of the blood flow can therefore provide essential information for the diagnosis of diseases. Since changes in blood flow occurs with the very initial stage of disease, with a fast, reliable and non-invasive blood flow measurement technique, the physicians would be provided with new options for early disease diagnosis. Beginning with brief overview of early methods of blood flow measurement, this article reviews the recent blood flow measuring techniques in detail and ends with suggestions for future research in related areas.

Keywords: *Blood flow, Laser Doppler, Speckle, Plethysmography, Ultrasound, PC-MRI, PET*

1. INTRODUCTION

Blood volume accounts for 7% of human body weight [1] with an average density of approximately 1.054g/cm^3 [2] and is a specialized fluid consisting of red blood cells, white blood cells and platelets suspended in a fluid medium known as blood plasma. An average adult has a blood volume of roughly 5 litres [1]. The blood conveys oxygen from the lungs to the tissues, carbondioxide from tissues to lungs, supplies nutrients to the tissues, removes metabolic end products and performs immunological functions. Additionally, blood regulates pH and body temperature and maintains the water content of the tissues [1]. Diseases / disorders of the blood that commonly affect human beings can involve any or all of the constituent blood cells. Other types of blood diseases affect the proteins and chemicals in the plasma that are responsible for clotting.

One of the best indicators of a patient's health is the concentration of oxygen and other nutrients in cells which is the primary measurement any physician would like to acquire from a patient and closely relates to the amount of blood flow [3]. Research on the various measuring techniques of blood flow has a direct impact on our improved understanding and management of human health. Measurement of blood flow and changes in blood volume are of vital importance and provide essential information for the diagnosis of diseases with impaired blood flow.

The measurement of blood flow and its related parameters have been evolved through a number of techniques involving different principles. From the start of 19th century, till now, a number of attempts have been made to modify the existing methods as well as establishing novel concepts in this field. This article is an attempt to summarize the status of blood flow measuring techniques available to date, giving emphasis to the recent developments. Although reviews regarding selected techniques on blood flow measurement is available in literature [4, 5, 6], an attempt to enlist the available techniques for blood flow measurement with an emphasis on the recent developments in the field is the first of its kind. The early methods of blood flow measurements are described briefly followed by recent advancements in this field such as Doppler ultrasound, Phase contrast magnetic resonance imaging, Positron Emission Tomography, Photoplethysmography, laser Doppler and laser speckle techniques. The principle behind each method is discussed followed by the state of the art involving that method for the measurement of blood flow parameters. The pros and cons of all methods are discussed and compared with each other. Special reference is given to perfusion measurement and the related techniques involving tissue perfusion are discussed in detail. The article ends up with directions for future research in the field of blood flow measurement that opens up wide range of applications in biomedical field.

2. EARLY METHODS FOR BLOOD FLOW MEASUREMENT

The history of blood flow measurement started from the mid of 19th century when the ends of a known volume air tight U-tube was connected to a blood vessel to measure the rate of arterial blood flow according to the Poiseuille formula [7]. Several modifications done on this direct recording flowmeters are described in the literature [7]. Although these methods were applied for estimating the volume rates of blood flow in the limbs, kidneys, head and

abdominal viscera of average size laboratory animals, cannulation of the blood vessel and the resistance offered by these methods to the actual blood flow were considered to be the disadvantages of such measurements. Collection of venous outflow in a graduated cylinder over a known time interval was also another method used in early times to estimate the flow, which was subjected to automation in its successive modifications [8]. Introduction of drop recorders helped measurement of flow rates lower than 2ml/min [9]. The disadvantages of drop recorders such as blood loss and reaction at the electrodes were overcome by the design of closed circulatory system and use of photoelectric method for the measurement of flow. Effects caused by air in the measuring chamber in the rate of drip were also eliminated by replacing air with silicone fluid, thus providing a regular drip rate [10].

In yet another attempt, the time taken by a deliberately introduced bubble to move a known distance along a glass tube having similar diameter as the bubble through which blood is allowed to flow was estimated to provide a measure of the flow velocity [11, 12]. Bubble flowmeters were known for their simplicity, reliability, low resistance to flow, and mostly linear calibration yet with the disadvantages such as cannulation, anticoagulants and the possibility of slip between the bubble and blood [11, 12].

Contemporary to the cannulated measurement of blood flow using glass tubes as mentioned in the beginning part, trails were also made for measuring blood flow using dye dilution techniques. The principle involved in this method is available elsewhere [13, 14]. Experiments were carried out using continuous / bolus injection of the dye to the blood stream. Factors affecting the end result using this method include errors in blood sampling procedure, nature of dye infusion and type of dye being used. Typical applications include limb blood flow, portal blood flow renal blood flow, retinal blood flow etc. Injection of radioactive microspheres into the systemic circulation and measuring the concentration variation was also practiced as an indicative of the blood flow [15]. The use of radioactive elements in the blood stream was surely invasive upon repeated testing and subsequently this method has only been utilized to estimate flow in animals [15].

Measuring flow based on the electric conductivity of blood was another alternative to be tried out in the past years. Related theories were developed in early attempts [16], making use of permanent / DC magnets and suffered from disadvantages such as interfering potentials and electrode polarization [16]. AC magnets were used as an alternative, with different excitation waveform shapes such as sinusoidal, square, trapezoidal etc. with respective pros and cons yet with the disadvantage of 'transformer effect' at the electrodes [17]. Alternatively, thermal convection principle was applied to the blood flow studies, which made use of thermal sensors like thermocouples and thermistors [18, 19]. The working principle is that when a hot object is placed in a colder flowing medium it gets cooled due to thermal convection and the rate of cooling is proportional to the rate of flow of the medium. The electrical energy required to maintain the constant temperature of a temperature sensor introduced into the stream is taken as the measure of the blood flow rate which is found out by the help of equations. Most of the times, these sensors will be a part of a bridge circuit such as in hot film anemometer [20]. The concept of quantifying the oxygen content in blood and using it for the measurement of blood flow began with the development of an oxyhemograph, which worked on the principle which was used to measure the circulation time (CT) as fast as few tenth of a second in animals such as albino rats and dogs irrespective of their skin colour from which the blood flow velocity was estimated [21, 22].

Volumetric changes in the blood flow has been achieved earlier times by direct Plethysmography techniques that involved trapping the body part of interest in a fixed pressure chamber and monitoring the volume changes of the chamber occurring due to the changes in blood flow. Different types of transducers were made use, to serve this purpose and the designed plethysmographs were subsequently known by the type of transducer involved. Capacitance plethysmograph which made use of the change in capacitance of the transducer due to the volumetric changes in flow and the impedance plethysmograph which measures small changes in electrical resistance of the region of the body part of interest are only few examples of these types. The down side of this is the slow response time and the accuracy [3].

3. RECENT ADVANCEMENTS

Disadvantages of conventional methods led to the research and development of new techniques in the measurement of blood flow. These techniques are described here in detail which include Doppler Ultrasound Techniques (continuous and pulsed), Magnetic Resonance Imaging, Positron Emission Tomography, and laser based techniques for flow measurement such as laser speckle and laser Doppler.

3.1. Ultrasound Technique

Ultrasound waves (frequency range above 20kHz) have found tremendous application in different fields of medicine such as imaging, localizing tissues as well as in flow measurements. This technique is used for blood flow measurement based on the principle of propagation of ultrasound waves in the tissue. Generally, these ultrasound

systems are categorized as conventional diagnostic ultrasonic imaging systems as well as Doppler ultrasound systems. In conventional imaging, the typically used frequency is in the range of 2MHz to 15MHz [23].

3.1.1 Doppler Ultrasound

Doppler ultrasound is a technique used for making non-invasive measurements of blood flow. The underlying principle is that a frequency shift occurs when sound is reflected from a moving source. To measure the velocity of blood an ultrasound of a specific frequency is transmitted into a blood vessel as shown in figure 1 and the sound that is reflected back from the blood vessel is detected. Due to the movement of blood the sound undergoes a frequency (Doppler) shift that is described by the equation (1) where, $c=1540\text{m/s}$ is the velocity of sound in the test tissue,

$$v_D = \frac{cf_D}{2f_0 \cos\theta} \quad (1)$$

V_D is the blood flow velocity, f_0 is the irradiated ultrasound frequency, f_D is the Doppler shift frequency of the received sound signal and θ is the angle of placement of the transducer with respect to the blood vessel [3]. The transmission of sound signal can be either in the continuous wave (CW) / pulsed wave (PW) mode which will be detailed in the next section.

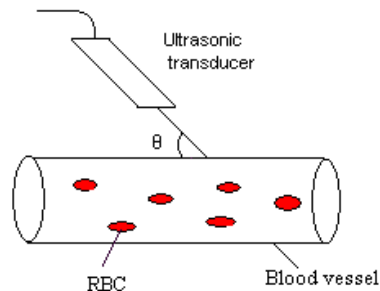


Figure 1. Doppler ultrasound method

3.1.2 Different types of Doppler ultrasound flowmeters

3.1.2.1 Continuous wave Doppler ultrasonic flowmeter

As the name suggests, continuous wave Doppler ultrasound systems use continuous transmission and reception of ultrasound waves. Doppler signals are obtained from all vessels in the path of the ultrasound beam until the ultrasound beam becomes sufficiently attenuated during its propagation along the tissue.

Several studies were carried out based on the principle of Doppler ultrasound using continuous waves. Changes in blood flow velocity in the carotid artery during different stages of physical exercise was studied based on the principle of ultrasound using a 2MHz CW Doppler ultrasonic probe and a Doppler discriminator circuit [24]. The schematic of the measurement system is shown in figure 2. The protocol exercise using a cycle ergometer with transient response and ramp response were conducted. In the Doppler signal discriminator, the Doppler signal and the 2MHz fundamental frequency are detected synchronously. The low-frequency and harmonics noise were then removed so that the Doppler signal is taken out from its range and digitized using an analog/digital converter interfaced to the computer. The blood flow velocity was then calculated using equation (1). In the transient response experiment the maximum blood flow velocity increased initially as the exercise started and then decreased when a constant workload was applied and increased again immediately before the end of the physical exercise. In addition, an arrhythmia was also detected to occur in the subject immediately after exercise. In the ramp response experiment, the blood flow velocity precisely increased during exercise [24].

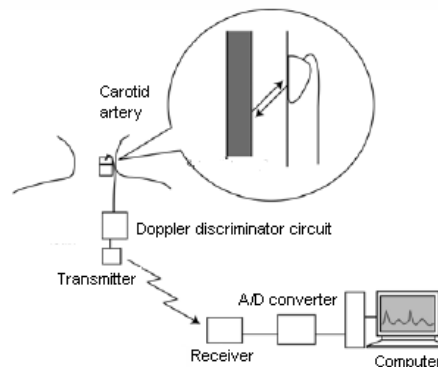


Figure 2. Ultrasound based blood flow measurement system (Reproduced by permission of IEEE, Piscataway, NJ (© 2005 IEEE), from Yamaguchi et al.²⁴)

Following the measurement in a single artery, the variations of the maximum blood flow velocity (MBFV) were evaluated simultaneously in the carotid, brachial and femoral arteries during postural changes [25]. In the experimental setup as shown in figure 3, three different probes collected data from the arteries and variations in MBFV during passive postural changes in supine position, 90° head-down-tilt (HDT) and 90° head-up-tilt (HUT) were measured. MBFV in brachial and femoral arteries were found to increase during supine to HDT posture and were found to decrease during supine to HUT posture, whereas very small changes in MBFV were observed in the carotid artery. The increase and decrease in MBFV are expected to be an effect of venous pressure and a constant MBFV in carotid artery was attributed due to cerebral autoregulation [25].

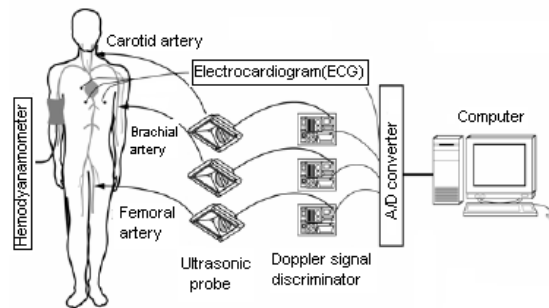


Figure 3. Ultrasound velocimeter system for simultaneous measurement in multiple arteries (Reproduced by permission of IEEE, Piscataway, NJ (© 2004 IEEE), from Azran et al. ²⁵)

Analysis of the dynamics and distribution of the blood circulation in human subjects during exercise was also studied [26]. By changing exercise postures to supine, seating, standing as well as walking on treadmill, and by analyzing the obtained power spectrums, flow velocity in the carotid, brachial and femoral arteries were measured. Lower MBFV values and higher wave propagation time were observed in carotid artery during standing position as compared to supine and sitting position. The carotid artery showed higher flow velocity values in proportion to the work rate during walking on the treadmill [26]. The blood flow measurement during physical exercise was found to give useful data in the early detection of cardiovascular diseases which could not be detected by ECG due to the measurement of changing flow in the carotid artery directly [24].

3.1.2.2 Ultrasound for measuring perfusion

In general, ultrasound techniques are effective in detecting flow velocities in major blood vessels with considerable blood velocity. Capillary flow measurement which is generally known as perfusion or tissue blood flow measurement becomes difficult with ultrasound as the flow velocity reduces, the discrimination / analysis of the reflected spectrum from the noise spectrum becomes difficult. In one of the few efforts, a CW Doppler ultrasound equipment operating at a slightly high frequency of 8.1MHz was used to detect low blood flow velocity in the microcirculation in the thumb of a subject [27]. The measurements were taken using the ultrasound probe with and without the target (to account for the noise spectrum) and the results were compared with the readings taken with a disk phantom rotating at a low velocity. The experimental schematic is shown in figure 4. In case of the

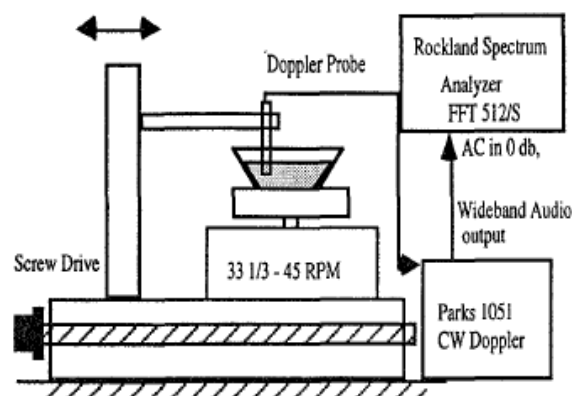


Figure 4. Experimental schematic for CW Doppler ultrasound system for microcirculation measurement (Reproduced by permission of IEEE, Piscataway, NJ (© 1997 IEEE), from David et al. ²⁷)

free probe, the spectrum showed noise spikes at low frequencies (below 20Hz) due to the tremor in the operator's hand. The amplitude of the spectrum from the thumb was higher than that of the free probe in the 20-100Hz region which suggested that additional Doppler shifted energy was being reflected back to the probe, indicating the statistics of the microcirculation [27].

Although CW system is a convenient technique, the system is unable to resolve the target positions along the direction of propagation of the sound beam through the tissue and is also unable to separate Doppler signals arising from different points along the transmitted ultrasound beam.

3.1.2.3 Pulsed wave Doppler ultrasound technique

Pulsed Wave (PW) Doppler systems use a transducer that alternates transmission and reception of ultrasound to and from the human tissue. An ultrasound pulse transmitted into the tissue travels for a given time until it is reflected back by a moving red blood cell. It then returns to the transducer over the same time interval but at a shifted frequency. The Doppler shift in frequency is measured and using equation (1) the blood flow velocity is calculated.

Transcutaneous pulsed Doppler ultrasound technique was used to measure the blood flow velocity in the Superior Mesenteric Artery (SMA) in 25 healthy newborn infants to investigate the response of intestinal blood flow due to feeding [28]. Duplex scan examination of the SMA was performed in a timed sequence in relation to food intake (fasting, 15-45-90 min following feeding). The Doppler flow signal was analyzed using an integral fast Fourier transform processor and the peak blood velocity (V_p), the pulsatility index (PI) and the mean velocity (V_m) were calculated. The analysis of the flow patterns before and after feeding showed an increase in the V_p and V_m with a corresponding decrease in the PI after feeding. PI refers to the calculation of Doppler measurements of systolic and diastolic blood velocities in the uterine, umbilical or foetal circulations [28]. This technique is rapidly becoming a common clinical procedure in non-invasive neonatal diagnosis. Similar works were carried out in adults to measure Superior Mesenteric Blood Flow (SMBF) in 70 healthy adult subjects of both sexes [29]. The subjects had an overnight fasting and after resting for 30 minutes in a supine position the Doppler ultrasound scanning of the upper abdomen was carried out. From the Doppler shift signals the mean value of SMBF rate was calculated [29]. This method was useful in studying the gastrointestinal blood flow in normal and diseased conditions.

Pulsed ultrasound is also used to assess the arteries located in the base of the brain (Transcranial Doppler Sonography, TDS), to find out any narrowing or blockage in the arteries that may decrease or stop the flow of blood to the brain leading to a stroke [30]. A small hand-held, wand-like probe covered with a conductive gel as shown in figure 5 will be placed at various areas on the head, usually at each temple, over each eye, and at the base of the skull. The Doppler shift in the frequency was found and the spectral analysis was done from which the blood velocity and the pulsatility index of the flow within the blood vessel was calculated [31, 32]. The painless procedure takes about 20 to 60 minutes after which normal activity can be resumed immediately [30].

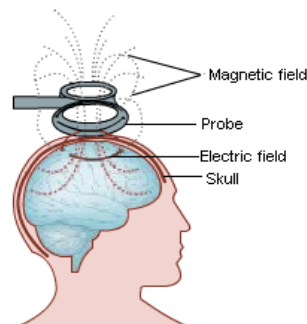


Figure 5. Transcranial Doppler Sonography

Blood flow velocity in the middle cerebral artery was recorded continuously using the TDS technique during a standard electroencephalogram (EEG) for 30min duration in 51 children with different types of epilepsy [33]. A special holding device incorporating a 2MHz pulsed Doppler probe was fixed by elastic bands to the temporal bone of the skull. Ultrasound intensity of the order of 70-80mW/cm² was used for the study. The Doppler readings obtained were analysed by fast Fourier real time frequency analysis technique. The results showed a direct relationship of mean blood velocity to the occurrence of tonic seizures/tonic-clonic seizures in the subject which is attributed to the changes in the arterial blood pressure and cerebral metabolism in the presence of distributed autoregulation [33].

3.1.3 Ultrasound for detecting 3D velocity vector

For the ultrasound systems mentioned previously, only the axial component of the true velocity vector was estimated, whereas the determination of 3D velocity vector was possible by using multiple ultrasound beams. The experiments were carried out using a prototype model (6.5mm diameter dialysis tube) where the blood cell scatterers

were tracked as they moved between three ultrasound beams generated by three piezoelectric transducers [34]. The three ultrasound beams are directed and focused by a tilted lens and a parallel, focused and overlapping beam at a desired focal distance is formed (figure 6). The overlapping of the beam is necessary to ensure that irrespective of the flow direction, the cells will always pass through at least two of the three beam pairs. A 3-D velocity vector algorithm was developed which tracked the movement profile of the cells from beam to beam. Theoretical details and simulation results are also available [35].

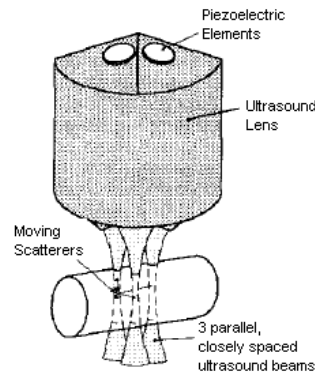


Figure 6. 3-D flow velocity vector measurement concept using Ultrasound (Reproduced by permission of IEEE, Piscataway, NJ (© 1995 IEEE), from Ilmar et al.³⁴)

In general, Ultrasound has been used to measure instantaneous value of flow of blood. Ultrasound can be beamed through the skin, thus making transcutaneous flowmeters practical and advanced types of ultrasonic flowmeters can also measure flow profiles. The instrument cannot be calibrated in absolute units but the sensitivity can be standardized.

3.2 Phase contrast magnetic resonance imaging

Phase contrast magnetic resonance imaging (PC-MRI) is another useful method for the measurement of blood flow using velocity induced phase shifts to distinguish between flowing blood and surrounding tissue. A slice of tissue is excited by the application of a RF pulse followed by two equal but opposite bipolar gradients. Protons moving along the direction of the bipolar gradients experience phase shifts, while the stationary tissue does not. The phase shift depends upon how far the flowing protons have travelled between the application of the bipolar gradient pulses [36, 37]. Faster flowing protons will undergo greater phase shifts and hence a measure of the phase shift gives information about the blood flow velocity.

PC-MRI was used to measure the 3D blood flow and the mean velocity in the main, right and left pulmonary arteries of seven healthy volunteers [38]. The moving red blood cells in the blood produce a phase shift of the MRI signal which is used to create images where the pixel intensity is proportional to velocity in each pixel in a single selected direction. The total flow over one cardiac cycle in the main pulmonary artery, right pulmonary artery and left pulmonary artery were estimated [38]. The axial velocity profiles in the main pulmonary artery showed smaller velocities along the inferior wall of the vessel. The magnitude and directional patterns of flow, assists in understanding phenomena such as thrombus formation (a blood clot formed within a blood vessel and remaining attached to its place of origin), atherosclerosis (fatty deposits inside the arterial walls narrowing the arteries) and changes in endothelial cell structure.

Measurement of blood velocity using PC-MRI technique can also be used for high resolution measurements in the heart or in any part of the circulatory system without the need for catheterization or ionizing radiation [39]. It plays an important role in the evaluation of patients with circulatory disorders. The technique has been tested both with phantoms and on the heart of volunteers using a GE 1.5T scanner. The volume flow per one stroke in the descending aorta and the corresponding cardiac output was measured [39].

3.3 Positron Emission Tomography Imaging

Positron Emission Tomography (PET) is a non-invasive imaging modality for obtaining in-vivo cross sectional images of the human body. In this technique a very small amount of labelled compound (called radiopharmaceutical or radiotracer) is introduced into the patient usually by intravenous injection and after an appropriate uptake period, the concentration of the tracer in the tissue is measured by the scanner. The radionuclide decays by emitting a positron. The emitted positron almost immediately combines with an electron and annihilates each other to emit two gamma rays at 511keV in opposite directions [3] which penetrates the surrounding tissue and are recorded by a circular array of detectors. The spatial distribution of the radioactivity within the subject for a selected plane is

reconstructed and the resulting image is displayed on the monitor. Thus, PET provides a non-invasive regional assessment of the biochemical processes necessary for the proper functioning of the organ being imaged.

Regional Myocardial Blood Flow (MBF) was measured accurately and non-invasively using ^{13}N -Ammonia dynamic PET imaging and factor analysis of dynamic structures (FADS) in ten dogs [40]. The MBF estimate results correlated well with the MBF estimates obtained by the two compartment model and microscopic technique and the MBF parametric image had better image quality and lower noise level when compared to those generated by Patlak graphical analysis method [40].

PET with H_2^{15}O was used for measuring regional blood flow to the hind limb skeletal muscle of six anesthetized dogs [41]. Local infusion of adenosine was done to increase blood flow and a hydrooccluder was positioned on the femoral artery to reduce blood flow. The readings obtained by PET imaging were compared with those measured by using radioactive microspheres. The basal flow to the hind limb skeletal muscle was measured as $3.83 \pm 0.36 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ which was in excellent agreement with the microsphere data given by $3.73 \pm 0.32 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$. The local infusion of adenosine increased the flow to as high as $30 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ and the PET and microsphere data were highly correlated over the entire flow range [41].

The measurement of blood flow in tumor cells gave information about the uptake of oxygen into the tumor cells in relation to delivery of oxygen through blood and is very important in understanding the pathophysiology and in therapy response [42]. The role of PET scans in measurement of cerebral blood flow is well discussed in [42].

3.4 Role of Optics in blood flow measurement

The advantages of optical imaging are high image resolution, relative low cost (compared to MRI, PET), noninvasive and no physical contact. Photoplethysmography is one of the most popular method for measuring the flow volume changes which is based on optical absorption of arterial blood and is most commonly used in current clinical practice. Other important optical techniques for monitoring blood flow are based on the frequency shift of lasers (Doppler effect) and the temporal statistics of time-varying laser speckle. Accordingly they are termed as laser Doppler and laser speckle methods.

3.4.1 Photoplethysmography

Referring the Plethysmographic principles detailed in section 2 of this article, it can be seen that different methods can be adopted to perform plethysmographic measurements such as impedance, direct and indirect methods. The recent advancement in this field is the use of optical techniques in plethysmography, referred to as PhotoPlethysmoGraphy (PPG). The PPG makes use of a laser/light emitting diode(LED) source of selected wavelength (usually in the red/NIR range) to illuminate the tissue and a photodetector to measure the small variations in light intensity associated with changes in perfusion in the catchment volume [43]. PPG has been used to measure microvascular blood flow and tissue viability. The potential of the PPG signal was taken as an objective measure of the healing potential of tissue [44]. If the PPG signal was pulsatile then healing was indicated with conservative management, if non-pulsatile then healing was likely to fail.

The role of the wavelength of the LED(used in the PPG) in perfusion measurement has also been taken up and found successful [45]. The differences in the tissue occlusion pressure has been demonstrated as a function of PPG LED wavelength. This finding helped in developing better methods to predict who might be at risk of getting sores [45]. PPG can also be used to measure the healing potential of ulcers in the skin by measuring the pulsatile cutaneous blood flow. It was found that healing ulcers demonstrated good pulsatile waveforms in cutaneous blood within the immediate vicinity of the ulcer. Cutaneous blood flow around the ulcer was seen to be nonpulsatile if the ulcer did not display evidence of healing [46]. A pulsed PPG operating with different light frequencies made it possible to detect simultaneous flow variations at different levels of dermis/subcutis corresponding to the subpapillary and deep vascular plexi [47]. Despite its simplicity the origin of the different components of the PPG signal are still not fully understood [43].

3.4.2 Laser Doppler Imaging

Light scattered back from laser irradiated skin provides information on the blood flow in the skin. This is due to the Doppler effect, which causes a frequency shift of light that is scattered by moving red blood cells. This Doppler frequency measured is utilized for the evaluation of the blood flow in the skin. The light from the laser source is delivered to the surface of the skin using a fiber optic cable and the same or a second fiber is used to collect the back scattered light from the skin and returned to a detector. When the incident laser beam is normal to the flow velocity then the Doppler shifted frequency (f) is given by the equation (2) where v is the flow velocity, λ is the wavelength of the laser beam and θ is the angle between the incident and the scattered beam [48]. Once the Doppler shift frequency is measured then the velocity can be calculated.

$$f = \frac{v \cdot \sin \theta}{\lambda} - (2)$$

A laser Doppler velocimeter was first used to measure the blood velocity in retinal arteries of albino rabbits [48]. A He-Ne laser with an output power of 10mW was used and the exposure time for one measurement was 2minutes. The magnitude of the Doppler shifted frequency was measured by optical heterodyning using a photo multiplier tube which is followed by an amplifier, a spectrum analyzer, a squarer and an integrator. The blood flow velocities measured were in the range of 1.1 to 1.8cm/sec [48]. A fundus camera laser Doppler velocimeter has also been used to measure the relative blood velocity in the human optic nerve head [49]. The maximum blood velocity measured was in the range of 0.12 to 0.2 mm/sec.

In-vivo blood flow measurement was conducted on the dissected lateral bony wall of cochlea of normal guinea pigs using a Laser Doppler Flowmeter(LDF) [50]. The output signal of laser Doppler flowmeter was recorded on a strip chart recorder. The effect of the drug norepinephrine administered from the femoral vein was studied without and after placing a dissected piece of bone on the lateral wall of the cochlea. The LDF signal patterns obtained were similar in both the cases but the magnitude of the output signal decreased to about one fourth the original signal. Placement of the bone reduced the magnitude of the light penetrating through the perfused tissue and as a result the blood volume detected had decreased [50].

In another reported work, the bronchial mucosal blood flow was measured in pigs using LDF [51]. The source was a 2mW He-Ne laser with a wavelength of 632.8nm. The viability of the bronchial anastomosis after lung transplantation depends on blood flow from the pulmonary circulation to the bronchial circulation, during the first days after surgery. The standard left thoractomy was made and the bronchial mucosal blood flow was measured, when pulmonary artery, bronchial artery, or both were clamped. The measured bronchial mucosal blood flow diminished by one-third when the pulmonary artery was clamped and by two-thirds when the bronchial artery was clamped which showed that bronchial mucosal blood flow after division of the bronchial circulation is about one-third of the normal value [51]. When both bronchial and pulmonary circulations were closed, a small amount of perfusion could still be measured which was due to the sensitivity of the technique [51].

A laser Doppler scanner technique was used for blood flow imaging in the capillaries under the surface of the skin [52]. Instead of using optical fibres, the laser beam from a 2mW He-Ne laser was moved in a raster pattern over the skin to build up a laser Doppler image the advantage being that the scanner cannot influence the blood flow. The scanning speed was set at a normal speed of 1s per line and low speed of 9s per line. Experiments were conducted on human subjects and the laser Doppler images of the hand and chest of normal subjects were obtained. Using the technique an image of blood flux in the capillaries under the surface of the skin was obtained and it was also possible to detect changes in blood flux resulting from injury, disease and temperature variations [52].

In another study two Doppler blood flow measuring heads each consisting of a diode laser of 5mW, with a wavelength of 685nm or 785nm was used to irradiate the skin tissue and the reemitted light was sampled using two photodiodes placed at a distance of 12mm and at an angle of 45° to the skin surface [53]. The measuring heads were connected to a controller card in the PC and using a fast Fourier transform the frequency spectra was obtained which was then converted into a flow spectrum. Upto four frequency ranges for normalized blood flows could be displayed simultaneously in different colors. The blood flow measured showed different behavior for low and high Doppler frequencies and thus provided the possibility to distinguish between the flow in the superficial dermal plexus and larger micro-capillaries [53].

LDF has also been used to study the optic nerve head (ONH) blood flow in the eye which responds to increased neuronal activity induced by visual stimulation [54]. A fundus camera was modified by replacing the light source (lamps and associated optics) of the camera by 12 light emitting diodes radiating at 770±20nm and a near-infrared laser diode lasing at 810nm was focused on the optic nerve tissue. An optical fibre collected the light from the site illuminated by the laser and an avalanche photodiode was used to convert the light into electric current which was then amplified, digitized and fed to the computer. The blood flow response to diffuse stimulation was recorded. Using the Doppler shift power spectrum the blood velocity, flow and volume were calculated [54]. The instrument was found to be useful in the study of the role of the blood circulation in the pathogenesis of various ocular diseases of vascular origin, such as glaucoma and diabetic retinopathy [54].

The laser Doppler imaging technique has the advantages of being a non-invasive simple to apply technique, providing a continuous or near continuous record but suffers from the disadvantages of limited laser penetration depth, low resolution, longer measurement time, not being a whole field and real time technique and the measurement values are not absolute but relative in nature [55]. The sensitivity of the instrument to a particular blood flow rate in a blood vessel varies with the distance of the vessel from the probe tip and the distribution of blood flow in a tissue through its different layers will not remain constant under different conditions. It was therefore not possible to calibrate laser Doppler flowmeters in absolute units [55].

3.4.3 Laser speckle contrast imaging

The laser speckle technique, over and above the laser Doppler technique offers the advantages of being a real time, non-scanning whole field technique offering higher resolution. The schematic diagram of the experimental setup shown in figure 7 requires only standard hardware components. The light from a laser source illuminates the object of interest. A CCD (Charge Coupled Device) camera images the illuminated area and the speckle pattern is captured by the frame grabber and digitized, then processed on the computer and displayed on the PC monitor.

When fairly coherent light is either reflected from a rough surface or propagates through a medium with random refractive index fluctuations a random intensity distribution pattern is formed which is called as a laser speckle pattern which can be explained only by statistics [56]. The statistical properties of speckle patterns depends both on the coherence of the incident light and the detailed properties of the random surface or medium. In the early days of lasers, the effect was regarded purely as a nuisance, but later scientists started to study speckle and developed practical applications of the phenomenon.

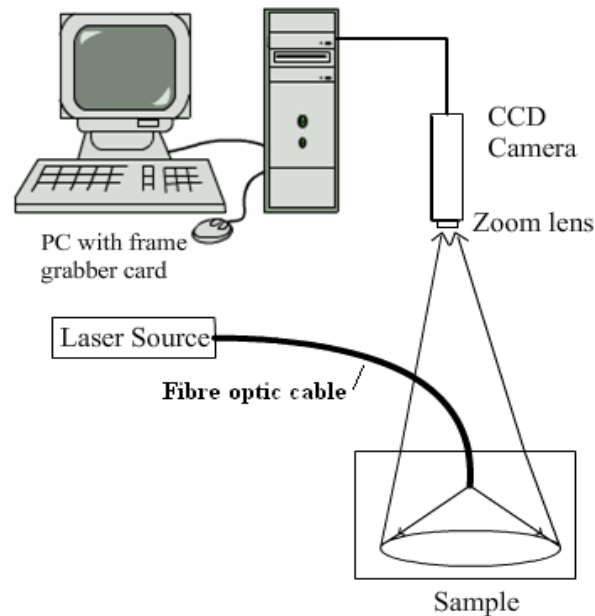


Figure 7. Experimental setup for laser speckle imaging

The intensity of the speckle pattern is not deterministic and can only be described by statistics [57, 58]. Utilising the first order spatial statistics, the property of a fully developed speckle pattern is that the standard deviation of the spatial intensity variations is equal to the mean intensity. In practice, the speckle patterns have a standard deviation that is less than the mean intensity, and hence there is a corresponding reduction in the contrast of the speckle pattern. The expression for the speckle contrast of a speckle pattern [56, 57] is given by equation (3)

$$\text{Speckle contrast } K_s = \frac{\sigma_s}{\langle I \rangle} \quad (3)$$

where σ_s is the standard deviation of the spatial intensity variations in the speckle pattern and $\langle I \rangle$ is the spatial average of the intensity fluctuations. Hence, for a fully developed speckle pattern the contrast is defined to be unity [58] and for a partially developed speckle pattern the speckle contrast is less than 1.

An ideal speckle pattern will have a probability density function of intensity that is negative exponential in nature. It can be shown that the standard deviation of the intensity variations in an ideal speckle pattern is equal to the mean intensity [56, 58]. Thus the contrast of a polarised speckle pattern is always unity [56, 58]. But in practice, a speckle pattern often has a standard deviation less than the mean intensity and this is observed as a reduction in the contrast of the speckle pattern [58].

Goodman [56, 57] related the variance of the spatial fluctuations to the time average of the autocovariance, $C_\tau(\tau)$ of the intensity fluctuations given by equation 4.

$$\sigma_s^2(T) = \frac{1}{T} \int_0^T C_\tau(\tau) d\tau \quad (4)$$

If we assume that it has a Lorentzian velocity distribution (because the capillary network is so convoluted) and no multiple scattering and perfect speckle formation then the speckle contrast is given by equation 5 where, T is the exposure time and τ_c is the correlation time of the intensity fluctuations (i.e. the time taken for intensity to fall to $1/e$ the original value).

$$K_s = \sqrt{\frac{\tau_c}{2T} \left\{ 1 - \exp\left(-\frac{2T}{\tau_c}\right) \right\}} \quad (5)$$

The simplest approach defines the decorrelation velocity v_c as given by equation 6.

$$v_c = \frac{\lambda}{2\pi\tau_c} \quad (6)$$

The blood flow can be calculated if the area of illumination is known. The flow of blood in a vessel is related to velocity by the equation 7 where F = blood flow, v_c = mean velocity, and A = cross-sectional area of the blood vessel.

$$F = v_c \times A \quad (7)$$

When an object moves, the speckle pattern also changes. For small movements the speckles move with the object and remain correlated [59]. For larger motions the speckles decorrelate and the speckle pattern changes completely. The decorrelation occurs as light is scattered from a large number of individual scatterers that are moving in a fluid. The moving speckle pattern contains information about the object's motion [59]. One of the most important applications of this theory is to study the fluctuations caused by the flow of blood. The frequency spectrum of the fluctuations is dependent on the velocity of blood.

The state of the art in this technique begins with a single exposure speckle photography using a 50mW He-Ne laser source for imaging the human retinal blood flow [60, 61] carried out by J.D. Briers group in the early 1980s. The basic underlying principle was that the speckle pattern in an area where flow occurs will be blurred to an extent which will depend on the velocity of flow and on the exposure time of the photograph whereas in an area of no flow the speckle pattern is fully developed. The velocity distribution in the flow area is mapped as a variation in contrast of the speckle pattern. A high pass optical filtering technique was adopted to convert the contrast variations into intensity variations and differences in the speckle contrast between blood vessels and surrounding areas could be visualized. The disadvantage of this method is that it was a two stage process that precluded real time operation. As a clinical technique the method was not viable [61].

A modified method of monitoring blood flow using optical fibres and photomultiplier was proposed and in such an attempt, a He-Ne laser was used to illuminate the fingertip of human subjects [62]. The intensity fluctuations were observed at a certain point and was detected through a pinhole by a photomultiplier, then amplified and fed to a spectrum analyzer. The signal was plotted for normal flow and reduced flow by placing an inflated cuff on the upper arm. The analysis of the power spectral distribution (PSD) of the speckle pattern gave useful information on skin blood flow [62]. Experiments were also conducted to study the use of optical fibres with variation in diameter for illumination and to obtain the dynamic speckle patterns for velocity determination [63, 64]. The same group also investigated influence of illumination fiber movement, object movement and diameter of the detecting aperture on the time dependent behavior of the speckle pattern [63, 64].

In a contemporary study, the speckle signal from the skin of human subjects was detected using a CCD image sensor and a colour graphic display of the 2D microcirculation map was developed [65]. A He-Ne laser line spot was used to illuminate the skin surface and the output pulses from the CCD image sensor was digitized using an analog to digital converter and stored in a static RAM and the data was transferred to a host microcomputer. The difference in data between successive scans was calculated and integrated for each pixel point and the blood flow map was obtained. When a rubber band was placed on one of the fingers there was a corresponding decrease in the blood flow [65].

Blood flow evaluations have also been carried out in the gastric mucous membrane of anaesthetized albino rabbit using bio-speckles (speckles obtained from living objects) [66]. The power spectral distributions (frequency in kHz versus power spectrum in dB) were obtained for normal, reduced and enhanced blood flow. A relative increase of the lower and higher frequency components was observed in the power spectral distribution in response to reduced and enhanced blood flow [66].

A quasi real-time digital version of the single exposure speckle photography technique was developed [67]. A CCD camera and a frame grabber card were used to obtain the image of the hand the experimental setup of which is as shown in figure 7 and the processing time was about 4 minutes. The drawback of the technique was the need to

reduce the processing time and to introduce the option to vary the exposure time so that the velocity range being measured can be varied.

Following this, a new non-invasive, fully digital and real time technique for monitoring blood flow termed as LASer Speckle Contrast Analysis (LASCA) based on the first order spatial statistics of time integrated speckle was developed [68]. It was a fully digital real time version of the single exposure speckle photography technique. The experimental setup used included a CCD camera, frame grabber card and specially developed software which was used to compute the speckle contrast of capillary blood flow. The contrast variation was then converted to velocity. Experiments were conducted to study the effect of temperature on blood flow. Human subjects were asked to immerse one hand in cold water (3 to 10°C for 30s) and the other in warm water (41 to 48°C for 2min). LASCA was used to calculate the speckle contrast variations in an area on the back of the hand. For all subjects the contrast measured on the warm hand was lower than that measured on the cold hand. The changes in speckle contrast after conversion to blood velocity showed an increase of velocity of about 10% to 50% [68].

The LASCA technique has also been used to produce 2D velocity maps of blood flow in capillaries [69]. The processed image of 3 fingers of a subject was obtained at different exposure times. Higher velocity values obtained using the faster exposure times suggested that the blood flow is being measured from deeper blood vessels in the finger [69]. Trials were also done using red laser to grab a series of images which could then be post-processed to produce a video clip of the changes in blood flow. Although similar experiments carried out using a green laser (514nm) couldn't give substantial information regarding the blood flow parameters, the use of green laser for illumination may provide a method for removing the artifacts due to gross body movements [70]. The effects due to body movements could also be corrected by placing an opaque scatterer attached to the patient's skin [69] or by applying suitable algorithms [71].

Various computer algorithms used for the LASCA method to monitor the capillary blood flow and velocity has been described [71, 72]. Using one such algorithm the processing time was brought down to few seconds from several minutes.

Spatial statistics of time-integrated speckle was used to obtain the image of the hand illuminated by laser light [73]. The method of linking the intensity fluctuations to the velocity was described in this paper.

Modifications were introduced in the existing laser speckle imaging (LSI) method, and based on the temporal statistics a time-integrated speckle was developed [74]. Cerebral blood flow map under different temperatures was measured on the rat cortex. The spatial resolution of the modified LSI method was five times higher compared to LASCA method and more blood vessels appeared clearly in the image. When the temperature was increased from 35 to 50°C the blood flow map obtained showed an increase in blood perfusion. The disadvantage of this method was that it was an invasive method and the temporal resolution was also much lower when compared to LASCA method but however, this method could be used to measure relatively slow changes of blood flow [74].

Blood velocity measurements were made on the elevated flaps of the skin of juvenile pigs using LASCA/MS multiple scattering method [75]. The readings obtained were compared to those obtained using laser Doppler velocimetry technique and there was a good correlation between the two. The same principle was also used to study the retinal blood velocity in rabbits using a modified fundus camera in which the pulsed light source was replaced using a CW He-Ne laser [76].

The cortical vascular blood flow pattern of rats which is associated with the functional response in cerebral cortex was obtained using temporal LASCA method [77]. A centerline extraction method based on intensity ridge tracking was used to segment the blood vessels from the acquired raw speckle image and the blood vessel patterns could be clearly seen. Although the technique is an invasive method, the vascular blood flow pattern obtained could be used to study the spatiotemporal activities of the somatosensory centre and in the diagnosis of the focal stroke or ischemia (inadequate blood supply (circulation) to a local area due to blockage of the blood vessels to the area) [77].

A simplified LASCA imaging analysis method which improved the computation time was developed [78]. The method was applied to image blood flow in the retina of three rats under normal and physiologic challenge conditions. The method may be applied for the early detection of retinal diseases.

Another group which was motivated by the usefulness of laser Doppler perfusion images and the advantages of full-field illumination using a laser speckle imaging approach went in for designing a new hybrid technique which they called as laser speckle perfusion imaging (LSPI) [79]. They designed an in vitro blood flow model to mimic a range of tissue optical characteristics. The model simulated a range of red blood cell concentrations and velocities in a matrix with absorptive and scattering properties similar to tissues of interest. The results obtained validated the use of the technique for measuring tissue perfusion [79].

Monitoring cerebral blood flow (CBF) is important for studying both normal and pathophysiological conditions of brain metabolism [80]. Many groups around the world have made use of LASCA for full field imaging of cerebral blood flow dynamics in animal models [80-85]. Monitoring blood flow dynamics in the in vivo rodent dorsal skin fold model using laser speckle imaging has been carried out [86]. A new image processing method for laser speckle, using adaptive

windowing laser speckle contrast analysis(aLASCA) that adaptively processes laser speckle images in the temporal and spatial direction has been developed [87]. A comparison of LASCA and aLASCA has been presented in this paper in terms of image quality and processing complexity [87]. The most recent application of LASCA was to conduct experiments on the retina of male Long-Evans rats in which laser speckle flowmetry(LSF) was used in conjunction with confocal microscopy to monitor light-evoked changes in blood flow in retinal vessels [88]. This dual imaging technique aided the group to stimulate retinal photoreceptors and measure vessel diameter with confocal microscopy while simultaneously monitoring blood flow with LSF. LASCA has also started giving promising results in analyzing real time changes in blood flow associated with different stages of skin diseases [89].

4. CONCLUSION

The measurement of blood flow provides vital and essential information for the diagnosis of various diseases due to any blood flow disorders. Taking into consideration this importance, various techniques has been introduced, developed and tested up all over the world for the measurement of various dynamic parameters of blood flow such as flow rate and flow velocity. This review is intended to provide a brief overview of these techniques so as to have a better understanding with respect to their principle and area of application. Knowledge about the current status of the existing blood flow measuring methods provided here is expected to assist interested readers to pursue further research in the field. These techniques are categorized into two major groups under this review, optical and non-optical methods of measurement. Although each method has its own pros and cons, in general the non-optical methods of measurement of blood flow suffer from the disadvantage of being invasive, an exception being the ultrasound techniques. Also, these techniques are more useful in measuring blood flow in major blood vessels having considerable diameter and cannot be primarily considered for capillary/microcirculation estimation required for tissue perfusion studies. Some research in these lines has been attempted previously considering the relations of heat distribution pattern generated on the tissue surface by an external source and the flow of the blood which are mentioned in the review. The estimation of perfusion from ultrasound spectrums by applying specific processing algorithms to differentiate between spatially correlated tissue motion and uncorrelated blood flow are being studied recently. But none of these attempts are purely experimental and real time.

One of the major advantages of blood flow measurement using optical imaging techniques is its non-invasive nature. A part of the non-invasiveness comes from the use of a low power laser source. Also, neither the source nor the detector comes in direct contact with the test surface. Of the two optical imaging techniques, although laser Doppler perfusion imagers/analyzers are readily available in the market, LASCA has the advantage of being a more compact solution with simple design based on a real time and whole field concept. Additionally it does not provide any radiation hazards.

LASCA technique is highly useful for the tissue perfusion studies due to its whole field nature. It can be made useful in disease diagnosis since perfusion is considered as an important parameter in understanding both normal and pathologic physiology. It is generally found that both blood flow and metabolism are significantly higher in tumor than in normal tissue. Further research in the field of laser speckle imaging can reveal the details regarding the disease development, offering a highly promising method for disease staging. In yet another work by the authors, LASCA is also being explored to analyze the static scatterer concentration in blood like body fluids [90]. In future, these results can be combined with the results on dynamic blood flow and the can provide a complete analysis of the static and dynamic parameters of body fluids such as blood. Also, LASCA can be integrated with endoscopes with appropriate design for the assessment of blood flow in body cavity structures. Future research in these areas will definitely open up new possibilities of disease diagnosis based on changes in the blood flow in the internal parts of body.

7. REFERENCES

- [1]. Arthur C. Guyton and John E. Hall, "Textbook of Medical Physiology", Elsevier Saunders, Pennsylvania (2006).
- [2]. Clement Kleinstreuer, "Biofluid Dynamics – Principles and Selected Applications", CRC Press, Taylor & Francis, (2006).
- [3]. John G. Webster, "Medical Instrumentation-Application and Design", John Wiley and Sons, Singapore (2005).
- [4]. Dean L. Franklin, Techniques for measurement of blood flow through intact vessels, *Med. Electron. Biol. Engng.* **3**, 27-37 (1965).
- [5]. P.R. Hoskins, A review of the measurement of blood flow velocity and related quantities using Doppler ultrasound, *Journal of Engineering in Medicine*, **213**, 391-400 (1999).
- [6]. Peter Vennemann, Ralph Lindken, Jerry Westerweel, In vivo whole field blood velocity measurement techniques, *Exp. Fluids*, **42**, 495-511 (2007).
- [7]. A.V. Dokunin, A modification of the method of differential manometry for registration of the volume velocity of the blood flow, *Bulletin of Experimental Biology and Medicine*, **46**, 1414-1417 (1958).
- [8]. Janusz Sadowski, A simple venous outflow recorder, *Pflugers Archiv European Journal of Physiology*, **325**, 90-94 (1971).
- [9]. Hobe Schroder, An easy to build digital flow meter(Drop recorder), *Pflugers Archiv European Journal of Physiology*, **350**, 97-100 (1974).
- [10]. Percy Lindgren, An improved method for drop recording of arterial or venous blood flow, *Acta Physiologica Scandinavica*, **42**, 5-11 (1958).
- [11]. Hans R. Ulfendahl, Ingemar Vogeler, An automatic bubble flow meter, *Acta Physiologica Scandinavica*, **55**, 286-290 (1962).
- [12]. H. Ludt, H.D. Herrmann, A simple bubble flow meter with quasicontinuous registration, *Res. Exp. Med.* **168**, 15-21 (1976).
- [13]. Kenneth Zierler, Indicator dilution methods for measuring blood flow, volume and other properties of biological systems: A brief history and memoir, *Annals of Biomedical Engineering*, **28**, 836-848 (2000).
- [14]. John L. Stephenson, Theory of measurement of blood flow by dye dilution technique, *IRE Transactions on Medical Electronics*, 82-88 (1958).

- [15]. M.O. Olowo Okoron, Blood flow measurement to the duodenum, jejunum, ileum and caecum of conscious goats, *Vet. Sci. Commun.* **2**, 145-149 (1978).
- [16]. Kenneth E. Jochim, The development of the electromagnetic blood flowmeter, *IRE Transactions on Biomedical Electronics*, **9**, 228-235 (1962).
- [17]. D.G. Wyatt, The electromagnetic blood flowmeter, *J. Phys. E: Sci. Instrum.* **1**, 1146-1152 (1968).
- [18]. B. I. Bashchenko, V.B. Zakharzhevskii, Method using thermistors to measure blood flow velocity, *Biomedical Engineering*, **4**, 252-254 (1970).
- [19]. K. Golenhofen, R. Felix, Local heat clearance probes with alternative heating and their application in the measuring of human muscle blood flow, *Pflugers Archiv European Journal of Physiology*, **331**, 145-152 (1972).
- [20]. P. K. Paulsen, The hot film anemometer-a method for blood velocity determination, *Eur. Surg. Res.* **12**, 140-148 (1980).
- [21]. Ya.B. Maksimovich, L.F. Nurik, V.G. Solovlev, Method of determining the velocity of the blood flow in small laboratory animals, *Bulletin of Experimental Biology and Medicine*, **72**, 1457-1458 (1971).
- [22]. V.V. Suvorov, Measurement of the circulation time, *Bulletin of Experimental Biology and Medicine*, **52**, 1334-1335 (1962).
- [23]. K. Kirk Shung, "Diagnostic Ultrasound: Imaging and Blood Flow Measurements", CRC Press, Taylor & Francis Group, (2006).
- [24]. J. Yamaguchi, Azran Azhim, Y. Hirao, Y. Kinouchi, H. Yamaguchi, K. Yoshizaki, A change of blood flow during strenuous physical exercises using cycle ergometer, *Proc. IEEE Engineering in Medicine and Biology 27th Annual Conference*, 6615-6618 (2005).
- [25]. A. Azran, Y. Hirao, Y. Kinouchi, H. Yamaguchi, K. Yoshizaki, Variations of the maximum blood flow velocity in the carotid, brachial and femoral arteries in a passive postural changes by a Doppler ultrasound method, *Proc. IEEE EMBS*, 3708-3711 (2004).
- [26]. Y. Hirao, K. Tamukai, Y. Kinouchi, H. Yamaguchi, Synchronized measurements of blood flow velocity distributions in carotid, brachial and femoral arteries and ECG in human during exercise, *Proc. BMES/EMBS*, 224 (1999).
- [27]. David L. Raunig, Martin D. Fox, Doppler ultrasound determination of capillary blood flow, *Proc. IEEE*, 1-2 (1997).
- [28]. E. Leidig, Pulsed Doppler ultrasound blood flow measurements in the superior mesenteric artery of the Newborn, *Pediatr. Radiol.* **19**, 169-172 (1989).
- [29]. M. I. Qamar, A. E. Read, R. Skidmore, J.M. Evans, P.N.T. Wells, Transcutaneous Doppler ultrasound measurement of superior mesenteric artery blood flow in man, *Gut*, **27**, 100-105 (1986).
- [30]. www.neurocarecenter.com/tests/tcd.html
- [31]. Anthony R. Lupetin, Donalee A. Davis, Irwin Beckman, Nilima Dash, Transcranial Doppler sonography Part 1: Principles, technique and normal appearances, *Radiographics*, **15**, 179-191 (1995).
- [32]. Anthony R. Lupetin, Donalee A. Davis, Irwin Beckman, Nilima Dash, Transcranial Doppler sonography Part 2: Evaluation of intracranial and extracranial abnormalities and procedural monitoring, *Radiographics*, **15**, 193-209 (1995).
- [33]. H. Bode, Intracranial blood flow velocities during seizures and generalized epileptic discharges, *Eur. J. Pediatr.* **151**, 706-709 (1992).
- [34]. Ilmar A. Hein, 3-D Blood flow velocity estimation with a triple beam lens –Experimental results, IEEE Ultrasonics Symposium, 1471-1476 (1995).
- [35]. Ilmar A. Hein, Multidirectional ultrasonic blood flow measurement with a triple beam lens, IEEE Ultrasonics Symposium, 1065-1069 (1993).
- [36]. Donald W. McRobbie, Elizabeth A. Moore, Martin J. Graves, Martin R. Prince, "MRI-From picture to proton", Cambridge University Press, Cambridge (2007).
- [37]. Dominik Weishaupt, Victor D.Kochli, Borut Marincek, "How does MRI work? – An introduction to the physics and function of magnetic resonance imaging", Springer-Verlag, Berlin Heidelberg (2003).
- [38]. Victoria L.Morgan, Robert J.Roselli, Christine H.Lorenz, Normal three-dimensional pulmonary artery flow determined by phase contrast magnetic resonance imaging, *Annals of Biomedical Engineering*, **26**, 557-566 (1998).
- [39]. S.J. Kim, D.H. Lee, J.H. Kim, J.H. Park, B.G. Min, Assessment of blood flow velocity profiles in heart ventricles and aorta with phase contrast magnetic resonance imaging, *Proc. IEEE*, **20**, 480-483 (1998).
- [40]. Hsiao-Ming Wu, Carl K. Hoh, Denis B. Buxton, William G. Kuhle, Heinrich R. Schelbert, Yong Choi, Randall A. Hawkins, Michael E. Phelps, Sung-Cheng Huang, Quantification of myocardial blood flow using dynamic nitrogen-13-ammonia PET studies and factor analysis of dynamic structures, *The Journal of Nuclear Medicine*, **36**, 2087-2093 (1995).
- [41]. Alan J. Fischman, Hongbing Hsu, Edward A. Carter, Yong M. Yu, Ronald G. Tompkins, J. Luis Guerrero, Vernon R. Young, Nathaniel M. Alpert, Regional measurement of canine skeletal muscle blood flow by positron emission tomography with H₂¹⁵O, *J Appl. Physiol.* **92**, 1709-1716 (2002).
- [42]. K.L. Leenders, PET: Blood flow and oxygen consumption in brain tumors, *Journal of Neuro-Oncology*, **22**, 269-273 (1994).
- [43]. John Allen, Photoplethysmography and its application in clinical physiological measurement, *Physiol. Meas.* **28**, R1-R39 (2007).
- [44]. B.Y. Lee, F.S. Trainor, D. Kavner, J.A. Crisologo, W.W. Shaw, J.L. Madden, Assessment of the healing potentials of ulcers of the skin by photoplethysmography, *Surg. Gynecol. Obstet.* **148**, 233-239 (1979).
- [45]. A. Murray, D. Marjanovic, Optical assessment of recovery of tissue blood supply after removal of externally applied pressure, *Med. Biol. Eng. Comput.* **35**, 425-427 (1997).
- [46]. Thay Q. Lee, Steven L. Barnett, Stewart L. Shanfield, Sanford H. Anzel, Potential application of photoplethysmography technique in evaluating microcirculatory status of STAMP patients:Preliminary report, *Journal of Rehabilitation Research and Development*, **27**, 363-368 (1990).
- [47]. J. Giltvedt, A. Sira, P. Helme, Pulsed multifrequency photoplethysmograph, *Med. Biol. Eng. Comput.* **22**, 212-215 (1984).
- [48]. Charles Riva, Benjamin Ross, George G. Benedek, Laser Doppler measurements of blood flow in capillary tubes and retinal arteries, *Invest. Ophthalmol.* **11**, 936-944 (1972).
- [49]. Charles E. Riva, Juan E. Grunwald, Stephen H. Sinclair, Laser Doppler measurement of relative blood velocity in the human optic nerve head, *Invest. Ophthalmol. Vis. Sci.* **22**, 241-248 (1982).
- [50]. T. Tamura, A. Okamoto, N. Kobayashi, K. Yokoyama, M. Hasegawa, T. Togawa, Evaluation of cochlear blood flow measurement by laser Doppler flowmetry, *Proc. IEEE*, 1185-1186 (1989).
- [51]. Antti Korpela, Pertti Aarnio, Ari Harjula, Evaluation of the bronchial mucosal blood flow by laser Doppler flowmeter, *International Journal of Angiology*, **4**, 110-112 (1995).
- [52]. T.J.H. Essex, P.O. Byrne, A laser Doppler scanner for imaging blood flow in skin, *J.Biomed. Eng.* **13**, 189-194 (1991).
- [53]. Klaus Dorschel, Gerhard Muller, Velocity resolved laser Doppler blood flow measurements in skin, *Flow Meas. Instrum.* **7**, 257-264 (1996).
- [54]. Eric Logean, Martial H. Geiser, Charles E. Riva, Laser Doppler instrument to investigate retinal neural activity induced changes in optic nerve head blood flow, *Optics and Lasers in Engineering*, **43**, 591-602 (2005).

- [55]. N. Vongsavan, B. Matthews, Some aspects of the use of laser Doppler flowmeters for recording tissue blood flow, *Experimental Physiology*, **78**, 1-14 (1993).
- [56]. J. W. Goodman, Statistical properties of laser speckle patterns [In] Laser speckle and related phenomena [Ed] J.C. Dainty, Springer-Verlag, Berlin Heidelberg, New York (1975).
- [57]. J.W. Goodman, Some fundamental properties of speckle, *J. Opt. Soc. Am.* **66**, 1145-1150 (1976).
- [58]. J.D. Briers, Laser speckle contrast imaging for measuring blood flow, Proceedings of the Symposium on Photonics Technologies for 7th Framework Program, 328-332 (2006).
- [59]. J.D. Briers, Laser speckle contrast imaging for measuring blood flow, *Optica Applicata*, **37**, 139-152 (2007).
- [60]. A.F. Fercher, J.D. Briers, Flow visualization by means of single-exposure speckle photography, *Optics Communications*, **37**, 326-330 (1981).
- [61]. J.D. Briers, A.F. Fercher, Retinal blood-flow visualization by means of laser speckle photography, *Invest. Ophthalmol. Vis. Sci.* **22**, 255-59 (1982).
- [62]. Hitoshi Fujii, Toshimitsu Asakura, Kunihiro Nohira, Yoshihisa Shintomi, Takehiko Ohura, Blood flow observed by time varying laser speckle, *Optics Letters*, **10**, 104-106 (1985).
- [63]. B. Ruth, Velocity measurement by the laser speckle method using optical fibres, *Optics and Laser Technology*, **19**, 83-90 (1987).
- [64]. B. Ruth, Non-contact blood flow determination using a laser speckle method, *Optics and Laser Technology*, **20**, 309-316 (1988).
- [65]. Hitoshi Fujii, Kunihiro Nohira, Yuhei Yamamoto, Hiroharu Ikawa, Takehiko Ohura, Evaluation of blood flow by laser speckle image sensing – Part I, *Applied Optics*, **26**, 5321-5325 (1987).
- [66]. Y. Aizu, T. Asakura, Bio-speckle phenomena and their application to the evaluation of blood flow, *Optics and Laser Technology*, **23**, 205-219 (1991).
- [67]. J.D. Briers, S. Webster, Quasi real-time digital version of single-exposure speckle photography for full-field monitoring of velocity or flow fields, *Optics Communications*, **116**, 36-42 (1995).
- [68]. J.D. Briers, S. Webster, Laser speckle contrast analysis (LASCA): A non-scanning, full field technique for monitoring capillary blood flow, *Journal of Biomedical Optics*, **1**, 174-179 (1996).
- [69]. Glenn Richards, J.D. Briers, Laser speckle contrast analysis (LASCA): A technique for measuring capillary blood flow using the first order statistics of laser speckle patterns, The Institution of Electrical Engineers, (1997).
- [70]. Glenn Richards, J.D. Briers, Capillary blood flow monitoring using laser speckle contrast analysis (LASCA):improving the dynamic range, *Proc. SPIE*, **2981**, 160-171 (1997).
- [71]. J.D. Briers, Xiao Wei He, Laser speckle contrast analysis (LASCA) for blood flow visualization: improved image processing, *Proc. SPIE*, **3252**, 26-33 (1998).
- [72]. Xiao Wei He, J.D. Briers, Laser Speckle Contrast Analysis(LASCA):a real time solution for monitoring capillary blood flow and velocity, *Proc. SPIE*, **3337**, 98-107 (1998).
- [73]. J.D. Briers, Glenn Richards, Xiao Wei He, Capillary blood flow monitoring using laser speckle contrast analysis (LASCA), *Journal of Biomedical Optics*, **4**, 164-175 (1999).
- [74]. Haiying Cheng, Qingming Luo, Shaoqun Zeng, Shangbin Chen, Jian Cen, Hui Gong, Modified laser speckle imaging method with improved spatial resolution, *Journal of Biomedical Optics*, **8**, 559-564 (2003).
- [75]. L.W. Winchester, N.Y. Chou, Blood velocity measurements using laser speckle imaging, *Proc. IEEE, Engineering in Medicine and Biology*, 1252-1255 (2004).
- [76]. Nee-Yin Chou, L.W. Winchester, Retinal blood velocity measurements using laser speckle imaging, *Proc. IEEE, Engineering in Medicine and Biology*, 1716-1719 (2005).
- [77]. Nan Li, Shanbao Tong, Deliang Ye, Hyunchool Shin, Nitish V. Thakor, Cortical vascular blood flow pattern by laser speckle imaging, *Proc. IEEE, Engineering in Medicine and Biology*, 3328-3331 (2005).
- [78]. Haiying Cheng, Timothy Q Duong, Simplified laser speckle imaging analysis method and its application to retinal blood flow imaging, *Optics Letters*, **32**, 2188- 2190 (2007).
- [79]. Kevin R. Forrester, J. Tulip, C. Leonard, C.Stewart, Robert C. Bray, A laser speckle imaging technique for measuring tissue perfusion, *IEEE Transactions on Biomedical Engineering*, **51**, 2074-2084 (2004).
- [80]. Andrew K. Dunn, Hayrunnisa Bolay, Michael A. Moskowitz, David A. Boas, Dynamic imaging of cerebral blood flow using laser speckle, *Journal of Cerebral Blood Flow and Metabolism*, **21**, 195-201 (2001).
- [81]. Shuai Yuan, Anna Devor, David A. Boas, Andrew K. Dunn, Determination of optimal exposure time for imaging of blood flow changes with laser speckle contrast imaging, *Applied Optics*, **44**, 1823-1830 (2005).
- [82]. William Lau, Shanbao Tong, Nitish V. Thakor, Spatiotemporal characteristics of low-frequency functional activation measured by laser speckle imaging, *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, **13**, 179-185 (2005).
- [83]. Pencheng Li, Songlin Ni, Li Zhang, Shaoqun Zeng, Qingming Luo, Imaging cerebral blood flow through the intact rat skull with temporal laser speckle imaging, *Optics Letters*, **31**, 1824-1826 (2006).
- [84]. K. Murari, N. Li, A. Rege, X. Jia, N. Thakor, Contrast-enhanced imaging of cerebral vasculature with laser speckle, *Applied Optics*, **46**, 5340-5346 (2007).
- [85]. P. Zakharov, A.C. Volker, M.T. Wyss, F. Haiss, N.Calcinaghi, C.Zunzunegui, A.Buck, F. Scheffold, B. Weber, Dynamic laser speckle imaging of cerebral blood flow, *Optics Express*, **17**, 13904-13917 (2009).
- [86]. Bernard Choi, Nicole M. Kang, J. Stuart Nelson, Laser speckle imaging for monitoring blood flow dynamics in the in vivo rodent dorsal skin fold model, *Microvascular Research*, **68**, 143-146 (2004).
- [87]. H.Y. Jin, N.V. Thakor, H.C. Shin, Laser speckle contrast analysis using adaptive window, *Chwee Tech Lim, James C.H. Goh (Eds.): ICBME 2008 Proceedings*, **23**, 444-447 (2009).
- [88]. Anja I. Srien, Zeb L. Kurth-Nelson and Eric A. Newman, Imaging retinal blood flow with laser speckle Flowmetry, *Frontiers in Neuroenergetics*, **2**, 1- 10 (2010).
- [89]. A.K. Jayanthi, N. Sujatha, M.Ramasubba Reddy, Laser speckle contrast imaging based blood flow analysis in normals and in patients with skin diseases, *International Conference on Computer, Electrical, Systems, Science and Engineering-ICCESS2010*, **69**, 80-82 (2010).
- [90]. A.K. Jayanthi, N. Sujatha, M.Ramasubba Reddy, Laser speckle contrast imaging for analysing static scatterer concentration in phantom body fluids, *International Conference on Optics and Photonics-ICOP 2009*, 26 (2009).