

Laser Doppler Imaging of Skin Microcirculation

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Laser Doppler imaging (LDI), a new technique which allows measurement of skin blood perfusion at a distance from the skin surface, was assessed methodologically in healthy volunteers.

Each skin LDI value was based on virtually real-time measurements obtained from a number of discrete measuring sites. In scans made along the circumference of the lower arm, valid figures for LDI (as distinct from no output at all) were obtained in 8/8 measurements at 0° inclination, and in 16/16 measurements at 7°, 14°, 22°, 30° and 38°, respectively. Beyond this inclination a numerical output was obtained in only 9/16 of measurements at an inclination of 48°, in 7/16 at 69°, and in no more than 1/16 at 90°. Values obtained at angles of inclination greater than 38° fell within the relatively narrow range of values obtained at lesser angles of inclination. The findings are of interest since measuring sites of clinical importance may not be flat. Variability of measurement (coefficient of variation in per cent) was studied in the lower leg by performing LDI and conventional laser Doppler flowmetry (LDF) concomitantly. The coefficient of variation for measurements in one subject at rest was 13% for LDI vs. 19% for LDF, the corresponding interindividual coefficient of variation values being 25% vs. 28%. In response to heating, finger pulp perfusion increased by 55% as measured by LDI ($p=0.0051$) and by 44% ($p=0.0756$) as measured by LDF. In summary, the findings contribute to the validation of LDI for skin perfusion measurement. **Key words:** skin perfusion; angle of measurement; biological zero value; reproducibility; A-V shunt.

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Laser Doppler flowmetry (LDF) (1, 2) is used extensively for non-invasive measurement of microcirculatory changes in human skin. The technique is based on use of laser light as a means of penetrating the tissue and for gauging the skin perfusion by means of the Doppler shift of the reflected light. This flowmetry, accomplished with a fine, fiberoptic conductor, is particularly suitable for determining temporal changes in perfusion induced by physiological or pharmacological stimuli (3–5). Laser Doppler perfusion imaging (LDI) is a recent development based on LDF (6), which eliminates the need of a probe affixed to the skin and allows measurement from a distance of a much larger skin area. A map of skin perfusion based on several thousand measuring sites is produced in minutes. Based on measurements at a predetermined number of these sites, a mean perfusion value is obtained for skin blood flow within the corresponding skin area. This technique also appears to be useful for determining spatial changes in skin microcirculation (6, 7).

In this study, methodological aspects of LDI were assessed by application of the method to skin perfusion measurements in healthy volunteers. We determined the LDI biological zero

value during arterial occlusion, and intra- and inter-individual variability in measurements. We studied the effect of measuring with LDI on an inclined skin surface, a situation difficult to avoid in clinical practice. Finally, we compared LDI with LDF in measurements of digital pulp skin perfusion before and during thermal dilatation.

MATERIAL AND METHODS

Subjects

The study was undertaken in consenting volunteers after approval by the Central Oxford Research Ethics Committee and in accordance with the declaration of Helsinki. Fifteen healthy volunteers, 12 women and 3 men (mean age 35 years, range 16–59 years), all non-smokers, were studied. Tea and coffee were not allowed beginning 2 h prior to experimentation. Measurements were made on the lower leg, the upper arm or the hand. Prior to measurements on the leg, the subject rested in the supine position for 20 min; measurements on the arm or the hand were made with the subject seated comfortably. The measuring site was kept approximately at heart level. Conversation was avoided. The room was darkened during measurement and the ambient temperature was 20–23°C.

Laser Doppler perfusion measurement in skin

Briefly, a narrow, monochromatic light beam emitted from a laser light source was directed at the skin. After partial reflection in the epidermis, the remaining incident fraction of photons penetrated through the dermis to a depth determined by the optical properties of the tissues (8). The light was reflected in static and/or moving scatterers, i.e. surfaces of tissue and blood cells, and was frequency-shifted upon reflection from moving blood cells owing to the Doppler effect (9). On emission from the skin surface, the backscatter of frequency-shifted light was received as input in the photodetector and then converted to an electrical signal. Perfusion was expressed as the product of mean velocity and the concentration of (red) blood cells in the measuring volume, as distinct from volume flow through defined vessels (1, 2). A relationship to volume flow was established for LDF (10), though the slope of the relationship may vary between different sites and individuals (11). A significant fraction of the measured value was non-flow-related, being produced by tiny movements within the tissue (12, 13).

LDI. The laser Doppler imager (6) (Lisca Development AB, Linköping, Sweden) consists of a laser light source, a scanner, a photodetector and a processing unit connected to a computer and plotter. A data acquisition and analysis system generates, processes and displays images of tissue perfusion. By means of mirrors connected with stepper motors, the laser beam is moved sequentially step by step, over the tissue through a maximum of 4,096 measuring sites (about 0.04 cm²), covering a maximum area of approximately 144 cm² in about 5 min. Correction factors for the distance between the detector and the object and the angle between the detector and measuring site have proved satisfactory in *in vitro* experiments (6). Signals derived from each measuring site are processed and stored. Once all measuring sites have been scanned, a perfusion image of the underlying tissue can be generated. This image is colour-coded, each colour corresponding to a certain level of perfusion defined as a fraction of the maximum perfusion level of a specific image. Mean LDI values, expressed in volts (V) and based on a predetermined number of measuring sites, are retrieved automatically. A non-valid

LDI measurement was defined by the absence of a numerical output, i.e. the signals derived from a measuring site had been automatically excluded during processing. In the present study the stepper motors were set to move two steps at a time, and the lower cut-off frequency of the processor was set at 20 Hz. The "optical axis" of the LDI scanner was positioned perpendicular to the surface of the object to be scanned, the distance between the aperture and the object being 14 cm unless otherwise stated. Black arrow-heads (adhered to the skin) were used as markers for orientation purposes (i.e. for aligning the "optical axis" and for matching object and image). The numerical values elicited from the LDI scan were analysed, each LDI value being based on >1,000 individual measuring sites in series A, 10–30 sites in series B, >1,000 sites in series C and 25–88 sites in series D.

LDF. Technical and functional data for the laser Doppler flowmeter (PF3, Perimed AB, Sweden) have been reported elsewhere (1, 2, 14). The laser light is transmitted to and from the tissue in a conductor composed of flexible optical fibres, via a rigid probe fitted into a holder and affixed to the skin with adhesive tape during the experiments. A standard probe (PF 308, Perimed AB) was used.

The cut-off frequency of the flowmeter was set at 12 kHz and its time constant at 0.2 s. The flowmeter was connected to a plotter and a PC. Recordings were continued until a steady state level had been observed for at least 3 min. Special software (Perisoft, Perimed AB) was used for determining mean values, which were expressed in perfusion units (PU).

Experimental series

A. Instrumental vs. biological zero value, LDI. To obtain an instrumental zero value, 10 images were made of an immobilized, light grey, rigid plastic plate (11 × 11 cm). A minimum biological zero value was then obtained on locally "desanguinated" skin in 4 volunteers in the following way. A blood pressure cuff was loosely applied distal to the subject's knee. The foot was raised 50 cm above heart level for 5 min. An Esmarch bandage was tightly applied from the level of the toes to the lower edge of the cuff, and the cuff was inflated to a pressure twice the systolic arm pressure. The Esmarch bandage was removed and the limb lowered to its original position. One scan was made before cuff deflation (*n*: 4).

B. Effect of inclining skin surface, LDI. LDI scans were made of both of the subject's arms in the following manner. The (mean) arm circumference (distal to the elbow; circumference 22 cm, radius: 34 mm) was divided into four equally sized segments (*f*, *f'*, *f''*, *f'''* in Fig. 1A) by four black markers adhered to the skin for orientation purposes. Four scans were made of the arm, with the "optical axis" of the LDI scanner perpendicularly centred on the tip of each of the four markers in turn. Each of the four rectangular perfusion images thus comprised a 180° segment of the arm circumference. Each such image was divided into two sets of nine fractions (*f*_{1–9} and *f'*_{1–9} in Fig. 1B). The angle between the tangent (*T* in Fig. 1C) at the centre point of each fraction and the plane of the perfusion image (e.g. *f*₄ for angle *a*₄ in Fig. 1C) was 0° (*f*₁), 7°, 14°, 22°, 30°, 38°, 48°, 69° and 90° (*f*₉). Sixteen measurements were made at each angle except at 0°, where there were eight measurements.

C. Variability of measurement: LDI vs. LDF. These experiments were carried out on the lower leg with the subject supine, the centre of the measurement area being located 6–8 cm proximal to the medial malleolus. In one group of volunteers, the following sets of measurements were obtained: 1) in one subject, eight sets of measurements were made over a period of 1 h, the imager and probe being removed and repositioned between one set and the next; 2) in 3 subjects, eight sets of measurements were made at regular intervals over a period of 4 h, the subjects being allowed to move about freely between one set and the next; 3) in 3 subjects, one set of measurements was made daily over a period of 8 days; and 4) in another group of 8 volunteers, one set of measurements was performed.

D. Finger pulp response to heating; LDI vs. LDF. In a group of 10 volunteers, pulp skin perfusion was measured by LDI and LDF on the middle and ring fingers, respectively, the order alternating from

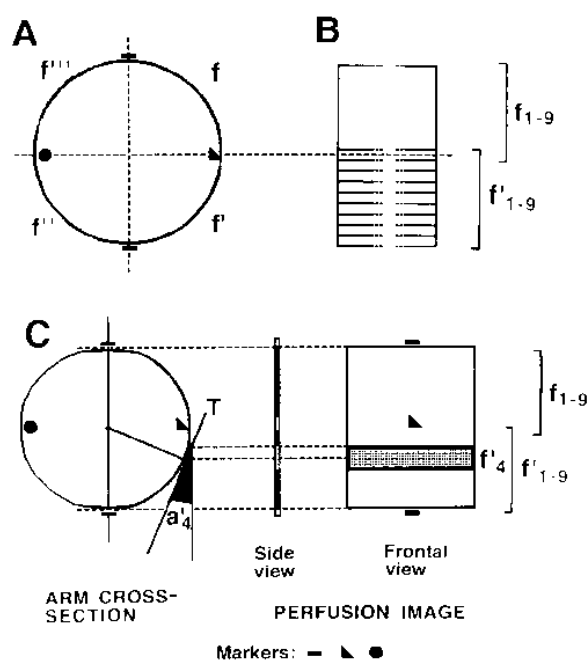


Fig. 1. Effect of angle of inclination - plan for experiment. For explanation, see text.

one subject to the next. The subject's arm was bared and placed in an empty container with the elbow flexed. The container was filled with water at 42°C, and the arm and hand were immersed except for the said digits. The water temperature was maintained thermostatically. Measurements were made before immersion and at 5 and 10 min after immersion. LDF was recorded continuously.

Calculations and statistical analysis

The mean instrumental zero value was subtracted from all in vivo LDI values. In series D, the perfusion value during heating constitutes the mean of measurements made at 5 and 10 min. Owing to the skewness in the distributions of the LDI values and the relatively small number of observations, all mean values were logarithmically transformed and given as geometric means (gm) (gm - SD, gm + SD) (15). The coefficient of variation (CV) was determined as the SD in per cent of the arithmetic mean value. Statistical differences were assessed with the Wilcoxon matched pairs rank-sum test. The relationship between variables was expressed with the Spearman *r*_s coefficient. A *p*-value of 0.05 or less was considered significant.

RESULTS

The mean LDI instrumental zero value was $0.90 \pm 0.01V$ (SEM), and the biological zero value was $1.05 \pm 0.02V$.

Valid figures for LDI (as distinct from no output at all) were obtained in 8/8 measurements at 0° inclination, and in 16/16 measurements at 7°, 14°, 22°, 30° and 38°, respectively. Beyond this inclination valid figures were obtained in only 9/16 of measurements at an inclination of 48°, in 7/16 at 69°, and in no more than 1/16 at 90°. Valid figures obtained at angles of inclination greater than 38° fell within the relatively narrow range of values obtained at lesser angles of inclination (i.e. 1.09–1.56 V, see Fig. 2).

The results of the variability of measurement experiments for LDI and LDF are presented in Table I.

Table I. Intra- and inter-individual variability in measurements Both LDI and LDF measurements were obtained at a defined site on the lower leg (see text)

Measurements	CV (%) LDI (LDF)
At rest during 1 h ($n=1$)	13 (19)
At intervals during 4 h ($n=3$)	8(20), 28(10), 13(17)
Daily ($n=3$)	34(20), 34(23), 27(15)
$n=8$	25(28)

LDI and LDF data for the finger pulp perfusion are presented in Table II.

DISCUSSION

LDF has previously been used for assessing spatial changes in skin microcirculation (7, 16), for instance by manually moving an LDF probe along a straight track at the turn of a screw and taking measurements at predetermined intervals (17). Using this methodology, blood flow "profile" were determined for standardized 5-mm suction de-epithelialized wounds and burns in humans (17–19). Topographic LDF maps of the cutaneous circulation have been generated using a grid with holes for positioning the LDF probe (20). The accomplishment of automatic, virtually real-time measurements from a distance in almost real-time LDI brings this technology a major step forward.

The digital pulp skin is unique in being richly supplied with arteriovenous anastomoses located in the reticular dermis (21, 22). Although possibly beneath the level of effective light penetration and maximal sensitivity of either LDF or LDI, shunt flow should contribute significantly to the Doppler signal (4). There may be as many as approximately 150 anastomoses

per cm^2 in the pulp, and the vessels are arranged chiefly in small groups (23). By immersing the arm in water at 42° , vasoconstrictor tone is diminished and the digital blood flow increases markedly, chiefly due to opening of the shunts. An uneven pattern of dermal blood flow during warming may result, with small "islands" of more pronounced hyperaemia corresponding to the shunts. We expected the shunt to open gradually and with varying degrees of vessel tone loss in response to the relatively mild thermal stimulus used, and chose to take our measurements 5 min and 10 min after immersion. The pooled values may represent an underestimation of the maximal changes that were in fact induced.

Methodologically, the LDF biological zero skin perfusion value (12, 13) is determined on an extremity during arterial occlusion produced by a blood pressure cuff. Owing to its magnitude, this zero value constitutes a significant fraction of the total LDF value only when skin perfusion is low, and it is then usually deducted from the total value. It has become clear that this zero value is variable and may be subject to changes in blood volume, haematocrit and temperature, and locally by hyperaemia and oedema, i.e. shifts may be unrelated to perfusion (24). Practically, we determined the mean instrumental zero value by repeated measurements on a static surface with scattering properties similar to that of Caucasian skin and deducted it from the total LDI value. In the present experiments, where relative values were compared, we thus accepted that a certain fraction of the value obtained would be unrelated to actual perfusion. That this non-flow-related fraction may have been underestimated is suggested by the finding that the value measured on the skin following compressive desanguination exceeded the value measured on the static scatterer by 16.7%. The relatively large non-flow-related fraction produces another experimental effect even if it is deducted: the distribution of the LDI values in a particular series of measurements tends to become positively skewed, since one loses valid values as the (flow-related) perfusion value decreases towards zero. It is suggested that the magnitude of the instrumental zero might be minimized relative to the perfusion-related value in order to overcome this inconvenience.

In assessing the effect of differences in values due to skin inclination, we assumed skin perfusion to be similar within all segments of the arm circumference, an assumption justified by the relatively narrow range of mean LDI values. LDI values were obtained from all portions of the sloping skin surface until the angle of inclination exceeded 38° . Beyond this inclination it became increasingly difficult to obtain valid measurements. All values recorded beyond 38° fell within the same relatively narrow range of values obtained at lesser angles of inclination. The findings indicate that the correction factors for angles and distances that have previously proved satisfactory in *in vitro* experiments (6) are also satisfactory *in vivo*.

The CV for the LDF technique corroborates previous findings (25). LDI and LDF were characterized by similar ranges of CV values for measurements on the lower leg, i.e. the averaging of perfusion in a larger skin area on the lower leg by LDI did not result in a lower CV. In the finger tip, the mean increase in perfusion during heating was 55% for LDI, reflecting the opening of arteriovenous communications in the pulp (4) (see Table II). The LDF data demonstrated much

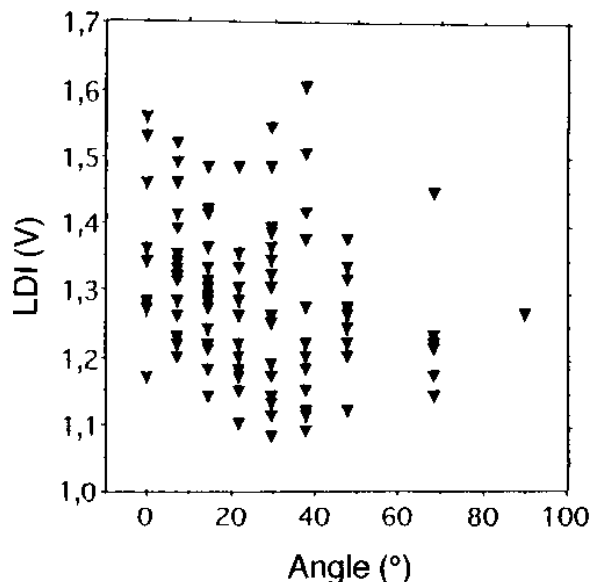


Fig. 2. Effect of angle of inclination. Increase in the angle was accompanied by an increase in the number of measurements where signal processing did not produce a numerical output. $n:8$ at 0° , otherwise $n:16$ at all inclination angles.

Table II. Effect of warming (the arm) on finger tip perfusion in healthy volunteers (n: 10)
LDI and LDF were performed concomitantly. *Geometric mean (gm)(gm - SD) - (gm + SD)

Volunteer	LDI			LDF		
	Before warming (PU)	During warming (PU)	Change (%)	Before warming (PU)	During warming (PU)	Change (%)
1	2.27	2.84	25	25	20	-20
2	1.86	3.04	63	22	24	9
3	2.69	3.08	14	12	24	70
4	2.33	3.20	31	17	17	0
5	2.57	3.52	38	8.0	27	238
6	2.00	2.63	32	16	17	9
7	0.36	0.85	136	5.0	10	100
8	2.35	5.51	134	19	40	110
9	2.11	2.45	16	27	24	-11
10	1.00	2.12	112	9.5	16	65
	1.7*	2.7	55	14.3	20.6	44
	0.9-3.2	1.7-4.3	16-106	8.3-24.6	14.2-29.9	-10-131
	$p=0.0051$			$p=0.0756$		
	$p=0.386$ Spearman $r_s=0.565$, $p=0.08$					

greater variability, and the mean increase for LDF of 44% was not statistically confirmed. The results for the 2 volunteers with deviant, distinctly greater increases in LDF suggest that the LDF probe may have been positioned corresponding to "islands" of markedly increased perfusion. The actual decreases observed in volunteers 1 and 9 suggest that here the LDF probe may have been positioned over parts of the vascular bed not responding to the heat stimulus at all (26).

To sum up, our findings contribute to the validation of LDI for measuring skin perfusion. In scans made around the circumference of the arm we found that the mean LDI values remained within a narrow range until the inclination of the skin surface exceeded 38°. This is an important finding, since measuring sites of clinical interest may not be flat. We found the variability of measurements on the lower leg to be in the same range for LDI as for LDF. In finger pulp skin, the LDI responded to opening of arteriovenous shunts due to a thermal stimulus. The findings suggest that LDI may yield more representative mean values for finger pulp perfusion than LDF. The LDI value contained a considerable non-perfusion-related fraction, which might advantageously be reduced by appropriate technical adjustment of the scanner by the manufacturer. Methodologically, it appears that LDI may constitute an improvement over LDF, particularly in the clinical setting.

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