

Review Article

Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging

A report from the Standardization Group of the European Society of Contact Dermatitis* based upon the HIRELADO European community project

A. FULLERTON¹, M. STÜCKER², K.-P. WILHELM³, K. WÄRDELL⁴, C. ANDERSON⁵, T. FISCHER⁶, G. E. NILSSON⁴ AND J. SERUP¹

¹Department of Dermatological Research, Leo Pharmaceutical Products Ltd, Ballerup, DK-2750 Denmark,

²Dermatological Department of Ruhr Universität Bochum, D-44 791 Germany, ³proDERM Institute for Applied Derm. Res. GmbH, Schenefeld/Hamburg, D-22 869 Germany, ⁴Department of Biomedical Engineering, Linköping University, SE-581 85 Linköping, Sweden, ⁵Department of Dermatology, University Hospital, S-581 85 Linköping, Sweden, ⁶National Institute of Working Life, S-171 84 Solna, Sweden

This report reviews how to set up a laser Doppler perfusion imaging system intended for visualization of skin blood perfusion, capture images and evaluate the results obtained. A brief summary of related papers published in the literature within the areas of skin irritant and allergy patch testing, microdialysis and skin tumour circulation is presented, as well as early applications within other fields such as diabetology, wound healing and microvascular research.

Key words: bioengineering methods; cutaneous blood flow; laser Doppler perfusion imaging; microcirculation; measurement guidelines. © Blackwell Munksgaard, 2002.

Accepted for publication 8 January 2002

Section I

Laser Doppler measurement of cutaneous blood flow was introduced more than 2 decades ago following the development of laser technologies and fibre-optic systems (1–3). Various devices operating with a Helium-Neon laser as light source and a fibre-optic cable fixed in contact with the skin surface have come into use. Blood flow is measured under a single spot of skin defined by the incident and Doppler-shifted reflected light. The recorded *in situ* sample is sized about 1 mm (3), the exact dimensions being dependent on the tissue optical properties and the probe geometry (4). This sample mainly includes the superficial vascular

plexus. Relative perfusion values representing the total blood flow in capillaries and small sized vessels are recorded and the results are expressed in arbitrary units. The local site variation in blood flow recorded with such single spot devices is major, and the number of measurements required is determined by the desired precision in the cutaneous blood flow estimate (5). This is a logical consequence of the microanatomical resolution of single spot laser Doppler flowmetry.

Recently, laser Doppler perfusion imaging (LDPI) was introduced with systematic X–Y scanning of the laser beam and horizontal mapping of the cutaneous blood flow in a larger area of the skin (6–9). This emerging medical technology is a non-contact system with the laser beam position controlled in the X and Y directions by a system of mirrors. A digital image composed of numerous single-point recordings forms a two-dimensional flow map of the blood flow variability over an extended skin surface. This image is displayed on a computer monitor and can be stored, analysed, and printed out.

Cutaneous blood flow is a dynamically fluctuating biological variable, which possesses substantial

*Members of the standardization group on non-invasive methods: T. Agner (Copenhagen), E. Berardesca (Rome), A. Bircher (Basle), P.J. Coenraads (Groningen), T. Fischer (Stockholm), P.J. Frosch (Dortmund), J.-M. Lachapelle (Brussels), A. Lahti (Oulu), H.I. Maibach (San Francisco), J. Roed-Petersen (Copenhagen), J. Serup, chairman (Copenhagen), R.A. Tupker (Nieuwegein), P. van der Valk (Nijmegen), J.E. Wahlberg (Stockholm).

spatial heterogeneity, and it was soon understood that many parameters influence its magnitude and regulation. The European Society of Contact Dermatitis in 1994 published formal guidelines on laser Doppler flowmetry based upon experiences with the early single-point systems (10). These guidelines summarized individual-related, environmental-related and technique-related variables and established the arterial occlusion/reactive hyperaemia experiment as an aid to assess instrumental performance, especially the biological zero level and the dynamic range. Additionally, the European Society of Contact Dermatitis in 1996 published guidelines on colourimetric measurement of skin colour according to spectrophotometric and tri-stimulus principles (11). Blood flow and colour (redness) are different albeit overlapping manifestations of vascular functions.

The present guidelines on LDPI resulted from a multicentre European Union project named HIRELADO (High REsolution LAser DOPpler perfusion imaging in dermatology, project number IN 10178I) involving centres in Stockholm, Linköping, Copenhagen, Hamburg and Bochum. The guidelines address mainly technical aspects of LDPI, standard usage, application principles and interpretation of the images generated. Individual-related and environment-related variables are covered by the previous guidelines (10). The HIRELADO group used the Perimed/Lisca PIM Laser Doppler Perfusion Imager in this project. This technology was originally developed by two of the authors (Nilsson and Wårdell), who founded the company Lisca in the early 1990s. The present guidelines are, however, aimed to be generic and relevant also for LDPI instrumentation produced by other manufacturers.

In addition to the laser Doppler technique, many other methods for the assessment of tissue blood perfusion have been presented in the scientific literature. Of those methods, orthogonal polarization imaging (12), video-photometric capillaroscopy (13) and thermographic imaging (14), constitute technologies that create images directly reflecting or relating to the tissue blood perfusion. The two former technologies, however, create images that include only a small number of microvessels representing the local blood perfusion, while thermographic imaging techniques visualize the tissue surface temperature rather than the dynamics of the underlying blood perfusion.

Cutaneous blood flow

The cutaneous blood flow is mainly in the service of thermoregulation and an instrument for maintenance of a constant body core temperature under

the influence of different environmental conditions. Its net function is the controlled release of metabolic heat. This is mainly achieved through adjustments of vascular tone and skin perfusion. Peripheral vessels show vasomotion with rhythmic contractions (6–8/min), which may be important for vessel-to-tissue interactions and exchange of nutrients, gas and heat. Vascular tone is dependent on control by the autonomic nervous system, on cerebral functions including emotional stimuli, ocular stimuli and sounds, and on a variety of other factors such as orthostatic position relative to the heart level and gravity, nutrients, medicines, smoking, etc. The cutaneous blood flow can, under physiological conditions, be reduced to less than half of its basal resting value but increase 20-fold to reach maximal capacity. The vasculature is normally in a relatively constricted state. Thus, measurement of cutaneous blood flow requires understanding and control of many variables other than the instrumental factors.

The local blood perfusion of the skin may be increased in various skin disorders associated with inflammation and reduced in conditions with vasoconstriction and occlusion.

The light of a laser Doppler flowmeter penetrates the skin variably to a depth of about 0.6 mm (15). The gradually decreasing sensitivity with depth in the dermis allows the sampling volume to be probed in a non-uniform manner, with an average measurement depth of about 0.3 mm (16). The vasculature within the sampling volume consists of vessels of variable size, function, tension, tonicity and microanatomical orientation relative to the incident and reflected laser light (Fig. 1).

Rete vessels and vessels around adnexae are special and differ from the superficial plexus. Regional differences are major and the head, palms and soles represent hyperperfused zones. Due to the in-sample and between-sample heterogeneity in biological structure and function of the microvasculature, it is ambiguous to express the cutaneous blood flow in absolute terms, for example as $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ tissue. Instead, emphasis should be put on careful and detailed description of experimental conditions, instrument validation and setting, selection and preconditioning of study subjects, the measurement site selected, environment and laboratory room control and experimental design. Of further importance are adequately selected and explained comparative situations, etc., in order to demonstrate significant relative flow changes and regional flow distributions, as results of experiments which may be repeated and the results reproduced at another study centre using another LDPI device. In practice, LDPI technology is well suited for comparisons of the average cu-

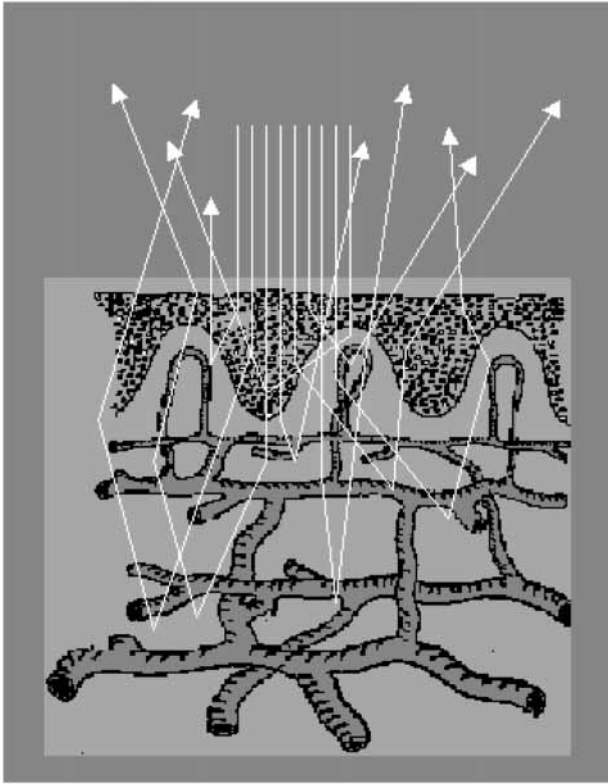


Fig. 1. Schematic diagram of the superficial cutaneous microvasculature and the photon pathways in tissue.

taneous blood flow recorded at two separate skin areas within the same image. Furthermore, provocative experiments may be employed where the resting-state blood flow before a stimulus can be used as a reference for the increase or reduction in flow instituted by the stimulus. In either case, the multitude of measurement points made available by the use of LDPI (as opposed to single-point laser Doppler flowmetry) overcomes the problem with cutaneous blood flow heterogeneity, and allows for adequate statistical testing of possible differences in blood flow between selected skin areas within the same image or at comparative experimental situations.

LDPI technology

LDPI is a non-invasive technology that creates two-dimensional flow-maps of tissue perfusion with no need for physical contact with the tissue or use of dyes and tracer elements. The technology was first commercialized in the early 1990s. In one embodiment of LDPI technology (7), a laser beam scans the tissue step-wise by means of a computer-controlled mirror system housed in a camera-like scanner head, which is positioned about 10–30 cm above the tissue surface (Fig. 2). This brand of LDPI is commercially available from Perimed/Lis-

Scanner head

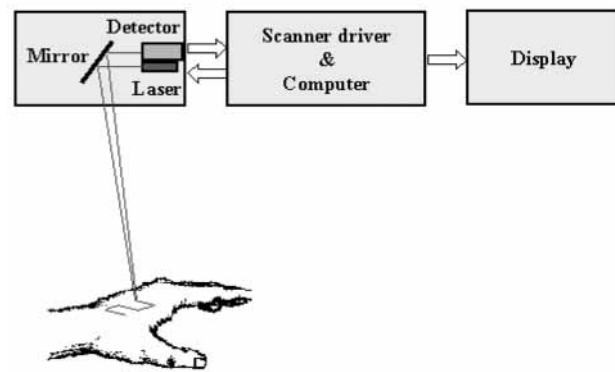


Fig. 2. Split drawing of the LDPI scanner head showing laser, mirrors, beam path, object, scanning pattern and detector.

ca AB, Sweden. Two versions (standard and high resolution) of the PIM laser Doppler perfusion imager were used by partners in the HIREALDO project to evaluate the generic performances of this medical technology. Other commercial versions of LDPI technology utilize a continuously moving laser beam (8) and different ways of processing the Doppler signal (9).

In the LDPI technology used by the HIREALDO group, a low power (1 mW) laser beam, generated by a solid-state laser operating at the wavelength 670 nm, is directed through a mirror system to the skin, where it forms a light spot with a size of about 1 mm². As the beam is successively moved in discrete steps over the skin surface, the back-scattered light is collected by a photo-detector positioned inside the scanner head. At each measurement site the beam is arrested for a short period of time, during which the back-scattered light, partly Doppler-shifted by the moving blood cells in the superficial microvascular network, is detected. On the surface of the detector, frequency-shifted and non-frequency-shifted light is mixed to form a photo-current. The frequency content of the fluctuating portion of this photo-current is related to the blood cell average speed, while the magnitude is determined primarily by the number of moving blood cells within the scattering volume. After processing, a signal that scales linearly with tissue perfusion is formed that is proportional to the product of the blood cell speed and concentration. This signal is stored in the computer memory and the laser beam is automatically moved to the next measurement site, where the procedure is repeated. When all measurement sites have been sampled, the stored values are displayed as a colour-coded perfusion image in parallel with a black-and-white photo image of the object. This perfusion image can be further analysed by the integrated system software as well as exported to other packages.

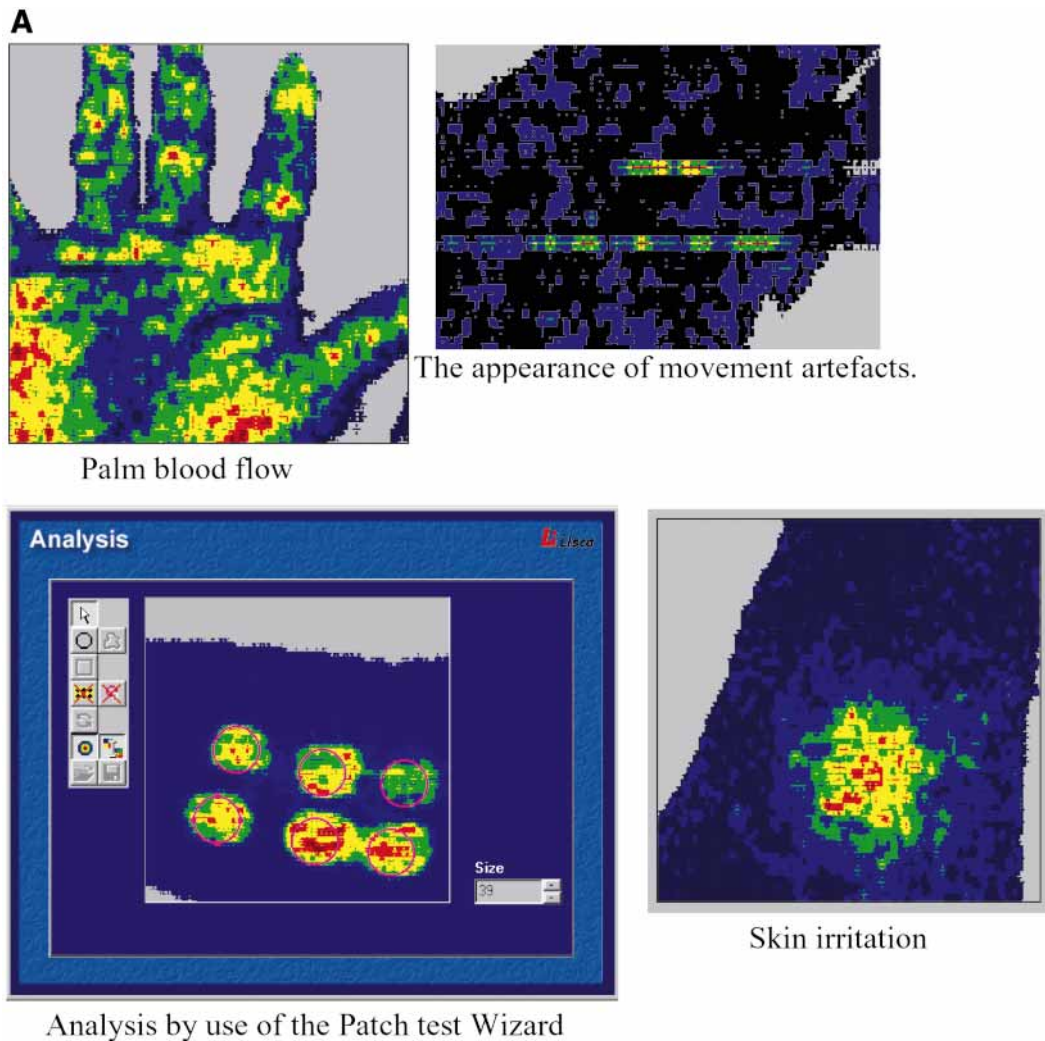


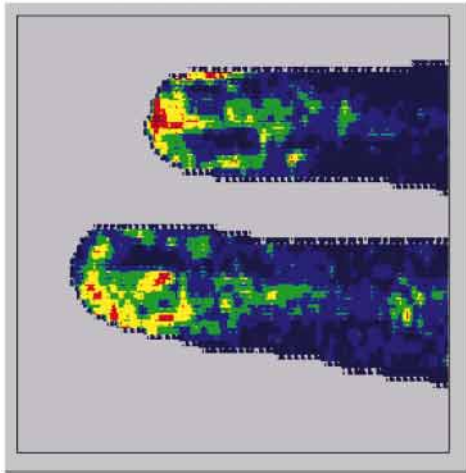
Fig. 3. Image examples.

By use of the system setting software, images can be recorded one at a time or as a sequence of images. The size and resolution of the images as well as the speed by which they can be captured are set separately by the user. The individual images can be displayed in *Relative* or *User defined colour scale*. Using *Relative colour scale*, the colour scale is stretched between the smallest and the largest perfusion value. This setting is useful for visualizing the spatial heterogeneity in tissue perfusion within a single image. By use of the *User defined colour scale*, the user sets the end-point perfusion values of the colour scale. This setting is useful when spatial differences in low perfusion areas are to be visualized. In addition, a black-and-white photo of the object based on the back-scattered laser intensity can be generated. Various examples of perfusion images are shown in Fig. 3.

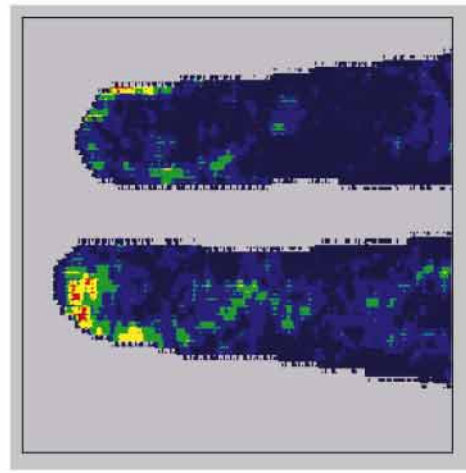
When the LDPI system is set to *Monitor* mode, which enables the recording of tissue perfusion in

the time domain, the laser beam can be moved to a certain site on the tissue surface where it will record the tissue perfusion continuously in a single or small number of adjacent sites. The result is displayed as a time trace on the computer monitor. The possibility of probing the tissue perfusion at adjacent sites in a cyclic pattern enables the time traces to be displayed as average perfusion values. This feature yields a higher reproducibility at repeated measurements and suppresses the influence of variation in tissue perfusion at adjacent sites, which is one of the main limitations of fibre-optics-based laser Doppler perfusion monitors (Fig. 4).

By use of the *Imaging* and the *Monitor* mode, both the spatial heterogeneity and the temporal variability of tissue perfusion can be investigated. The *Image* mode is most useful when perfusion in different tissue areas in an image is to be compared, while *Monitor* mode is to be employed in a provocative experiment in which tissue perfusion is

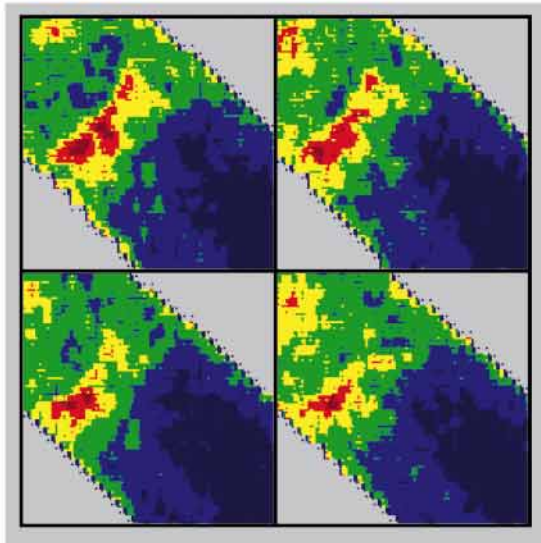
B

Before smoking

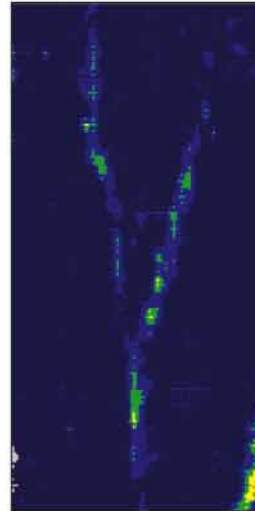


After smoking

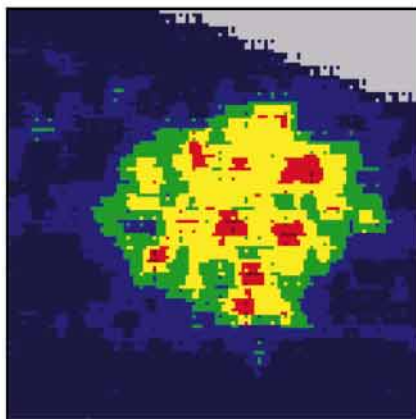
Effect on blood flow from smoking a cigarette



Sequentially recorded images



Veins on forearm skin



Histamine injection

Fig. 3. Image examples.

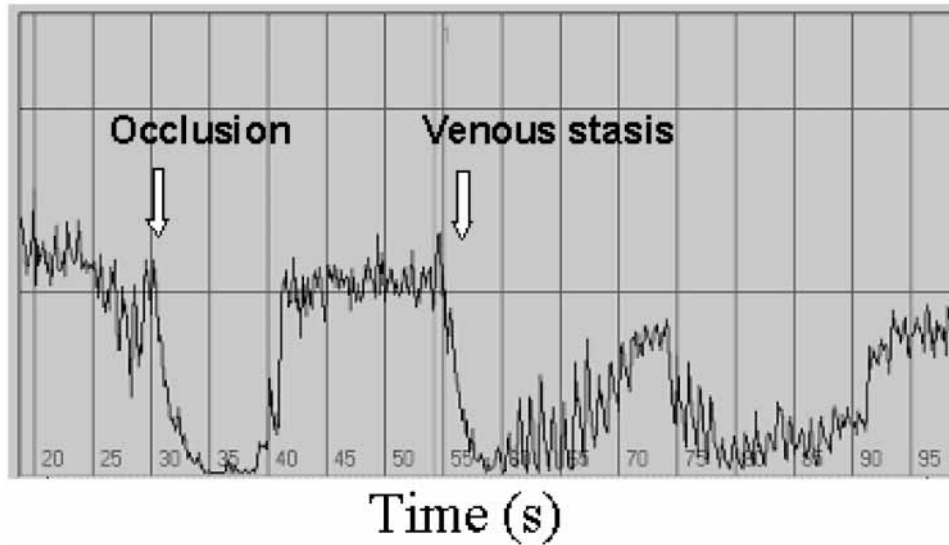


Fig. 4. A time trace of cutaneous blood flow showing the effect of occlusion (left) and venous stasis (right) on the recorded signal.

expected to change over time. If both temporal and spatial variability are of concern, the LDPI system should be set-up to record a series of images in *Repeated* mode.

Calibration

Laser Doppler perfusion imaging systems process the Doppler signal in a way similar to that of laser Doppler perfusion monitors. The output signal is linearly related to the product of the blood cell speed and concentration within the scattering volume. Like other equipment with a linear response, a two-point calibration procedure is sufficient. As the 'no-flow' reference point the zero value is generally selected. This 'no-flow' reference point value is obtained by calculating the average value of an image created by use of a diffusely scattering solid material, such as a piece of white cardboard or a flat, white plastic disk. The 'high-flow' reference point can best be generated by imaging an object with known, stable and uniform internal motion.

By use of standardized calibration boxes and uniformly selected target values for the 'high-flow' reference point, the variation in *interinstrumental* sensitivity and *intrainstrumental* drift can be kept to a minimum. Standardization of the calibration boxes is, however, a complex task because their reproducibility, long-term stability and precision need to be significantly better than the instrument that is to be calibrated. The currently available calibration methods for laser Doppler equipment, based on recording of the mobility of microspheres in random motion, therefore need further improvement and evaluation.

The possibility of maintaining a low variability

of results obtained by different users (*interuser variability*) is mainly dependent on careful design of the individual experiment, control of environmental factors and test subject/patient selection, as well as instrumental set-up. If these factors are carefully considered and established protocols are followed, the *interuser variability* can generally be kept low, because the image capturing process is inherently user-independent. For the same reasons, the *interlaboratory variability* can be kept low provided that the precautions are carefully considered.

Image capturing

Preparing for the capturing of perfusion images includes assessment of both environmental and subject-related factors. The main factors and variables that influence cutaneous blood perfusion are (from major to minor impact): temperature, anatomical site, physical and mental activity, food and drug intake, temporal (day-to-day), temporal (diurnal), posture, age, sex, menstrual cycle and race. Since these aspects have been dealt with in previous guidelines (10), this section will focus on how to set up the system and choose the adequate image parameters for best system performance.

Marking and identifying areas of interest

In order to be able to identify an area of interest in the individual images, small ink dots or tape of a colour that effectively absorbs the laser light may be placed as markers on the skin surface coinciding with, for example, the corners of the area to be investigated. Since these identifiers tend to absorb the laser light they will show up against the

background colour in the perfusion image and the associated photo image. The size of such markers should be no smaller than about 1 mm^2 . The markers should be applied at least 15 min before image capturing starts in order to avoid unintentional local alteration in skin perfusion. It is furthermore important that the skin sites to be investigated are left uncovered during the acclimatizing period prior to image capturing, because of the influence of the ambient temperature on the tissue blood perfusion.

Ambient light level

The ambient light level must be kept to a minimum in order to avoid interference between ambient light and the laser beam. Although images can readily be recorded under different light conditions, the influence of ambient light may alter the magnitude of the actual perfusion values to a degree dependent on the light source employed (light bulbs or fluorescent light) and its intensity. Interference from ambient lighting frequently manifests itself as a regular speckled pattern appearing in the photo image. The user is encouraged to record test images of the skin site under study with the light on and off and to calculate the average values of identical areas of interest, in order to find out whether the influence of ambient light can be tolerated in the actual situation.

Distance to tissue

The distance between the lower edge of the scanner head and the skin can range from about 10 to 30 cm with the system used by the HIRELADO group. A shorter distance may saturate the detector unit and a longer distance results in impaired signal-to-noise ratio and thereby reduced image quality. The recommended distance is somewhat dependent on the colour of the tissue, but a target distance of about 15–17 cm will generally work well for most tissues. If there is a tendency for detector saturation, this will show up as uniform white areas in the photo image, while too low a detected light level will generally result in areas clearly belonging to the object being classified as background.

To compare the perfusion values within areas of interest in two images it is important to use the same distance for the image capturing procedures. This can be achieved by use of a laser pen fixed to the scanner head, setting its beam to cross the LDPI laser beam at about a 45° angle at the target distance. In consecutive measurements the same distance is easily maintained by letting the two laser beams coincide to form a single spot on the tissue surface. The additional laser beam must be

switched off during the image capturing procedure. The optimal working distance can be assessed by recording test images at different distances.

Image mode

If a single image of a tissue area with essentially stationary perfusion is to be captured, the *Image* mode may be set to *Single*. When a slow change in perfusion over time is expected, with accompanying involvement of the extent of the area over which the perfusion is influenced, *Image* mode should be set to *Repeated*. This setting enables the automatic recording of many images over an extended period of time. If more rapid changes in tissue perfusion following, e.g. a vasoactive stimuli is expected, *Sequential* mode displaying a number of small subimages within the same image window may be the best choice. When a time trace recording of tissue perfusion in a single or small number of measurement sites is of primary interest, the best choice of setting is probably the *Monitor* mode. In this mode, the system allows for averaging the recorded tissue perfusion within an area, the extent of which is set by the user, thereby eliminating the main shortcoming of the single-point laser Doppler flowmeter.

Image size and resolution

The size of the image should be set by use of the *Image size* control (determining the number of measurement sites in the image) or by the *Resolution* control (determining the zoom-in and zoom-out effect making the overall tissue area covered smaller or larger at a given number of total measurement sites), rather than by changing the distance between scanner head and tissue. A full format image is composed of 64×64 perfusion values and is recorded in a matter of minutes, the exact time dependent on the *Measurement speed* (see below). The optimal choice of *Image size* and *Resolution* depends on the application. The resolution limit is further determined by the laser-beam diameter and the distance between two consecutive measurement sites. With a laser-beam diameter of 0.6 mm the smallest objects that can be resolved are of the dimension 1.2 mm. LDPI technologies that employ focused laser beams in order to improve further the resolution, and systems that can generate larger images for flow mapping of, for example, the entire upper part of the body are available on the market.

Measurement speed

In the system used by the HIRELADO group, the beam is kept still in relation to the tissue for a cer-

tain time period (about 50 ms in Standard Speed setting, compatible with a lower frequency cut-off frequency of 20 Hz) at each measurement site. With this setting about 4 min is required to capture a full format image including altogether 4096 perfusion values. To be able to obtain overview images at higher speed, the capturing time can be reduced to about 90 s, albeit at the expense of image quality (higher noise levels). It is recommended that the Standard Speed setting be used if image integrity is of primary importance. The overall image capturing time can best be kept to a minimum by setting the *Image size* to the smallest possible area covering the tissue region of interest.

Background threshold

The *Background threshold* determines at what total light intensity the actual measurement point should be classified as belonging to the background. In order to maintain the best background discrimination, it is therefore recommended that the object be placed on a light absorbing background, such as a green cloth. By adjusting the *Background threshold* level to comply with the tissue colour, distance to tissue and ambient light level, adequate background discrimination is generally possible to achieve in most situations.

Colour scale setting

During the capturing procedure, the *Colour scale* should preferably be set to *Relative colour scale*, because this will calculate an individual colour scale, stretched between the lowest and highest perfusion value, in each image. The Colour scale can easily be altered in the image analysis process and made identical for a given set of images, whereas the recorded perfusion values that form the basis of average value calculations within selected areas of interest cannot be altered by the user.

Mark area

The *Mark area* control makes the laser beam move quickly along the boundaries of the image to be captured. This feature is most useful in determining the appropriate size of the image and in positioning the scanner head over the tissue.

Capturing an image

During image capturing it is important that the test subjects/patients, after having been informed that no movement is allowed during the image capturing procedure, rest in a comfortable position in order not to influence the tissue blood perfusion to

be investigated. It is furthermore of fundamental importance that all conversation with the test subject/patient is abandoned during the image capturing process. The skin must not be touched immediately prior to or during image capturing, since the slightest mechanical stimuli may affect the micro-circulation. Slow movements, such as thoracic breathing movements, do normally not interfere with the Doppler signal. It is strongly recommended that the image is stored immediately after completion of the image capturing process.

If the result of a provocation is to be quantitatively assessed, it must be borne in mind that the numerical values of the perfusion calculated may differ if the area of interest is in the centre or at the periphery of the image, and if the skin surface is perpendicular to the beam whether it is in its central position or not. Recording test images with the area of interest in the centre and at the periphery and comparing the average perfusion values of these areas can easily test the influence of these factors.

Evaluation and interpretation

In this section, some generally applicable basic aspects of image evaluation and interpretation are addressed. Evaluation and interpretation in some specific applications of particular importance are dealt with separately in the next section.

When different images are to be compared they must be displayed in the same colour scale. This is achieved by first identifying the highest single perfusion value in any of the set of images to be compared or any other arbitrary value of choice, and then using this value as the maximum perfusion in the *User defined colour scale* for each of the images. The minimum value can in a similar way be set to the lowest perfusion value in any of the images or, alternatively, to zero.

By selecting a *Region of Interest* (ROI) in a perfusion image, the statistics (including mean value, standard deviation and number of measurement sites) of the ROI can be calculated. This information forms the basis for comparing the perfusion of two areas (in the same or different images) from a statistical point of view. The large number of measurement sites generally makes it possible to demonstrate statistically (at a reasonable level of significance) a difference in average perfusion, given that this difference exists in reality. The images displaying the detailed spatial variability in tissue perfusion can thus conveniently be transferred to numbers and figures of statistical significance, in contrast to the situation with single-point laser Doppler monitoring.

There are numerous pitfalls in interpreting areas

with deviating perfusion values within an image, many of which can easily be revealed and identified.

If the test subject/patient moves during the image capturing procedure, a distinct horizontal strip generally shows up in the perfusion image. This movement artefact is caused by general movement of the tissue, which the LDPI system cannot easily separate from the specific movements of blood cells. Movement artefacts can easily be recognized, however, since they generally occupy only a single (or a multitude of) horizontal line(s) in the image.

If the tissue surface is covered with a liquid film, direct surface reflections may reach the detector surface, generally resulting in an isolated spot of altered (in most cases reduced) perfusion values. This artefact can generally also be readily identified, since the corresponding photo image is plagued with a corresponding white spot, indicating locally intense light reflection in the liquid film.

Since the perfusion image is captured over a time period sometimes of several minutes, it must be assumed that the tissue perfusion during this time interval is stationary. If not, the temporal variability in tissue perfusion appearing on a shorter time scale may show up falsely as spatial differences in perfusion. These artefacts are generally more difficult to identify, but regular patterns in perfusion with intervals corresponding to respiratory cycles, vasomotion and even the pulse may be generated by temporal rather than spatial variations in tissue perfusion.

Summary of practical guidelines

Prior to image capturing

Subject-related factors

- See 'Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry' for an overview on how to prepare a laser Doppler-based recording session.
- Pay special attention to:
Subject should sit/lie in a comfortable position.
Acclimatization for 20–30 min with test sites left uncovered.

Environmental factors

- Keep ambient light level low.
- Avoid 'noisy' environments.
- Ambient temperature influences skin blood perfusion.

Measurement-related factors

- Carefully study the instrument manual for different set-up alternatives.

- Perform the calibration check on a daily basis.
- Select the optimal scanner head – tissue distance.
- Mark the area with ink spots or tape.
- Handle the skin carefully – avoid pressure on and scratches of the skin area under study.
- Stationary blood perfusion: if applicable, include a normal skin reference area in the image for comparison.
- Non-stationary blood perfusion: record a series of small images to demonstrate temporal perfusion changes.

During image capturing

- Avoid unnecessary communication with the test subject/patient.
- Avoid unnecessary activities in the examination room.
- Do not touch the instrument (or the tissue) during the recording procedure.
- Watch out for unintentional subject/patient movements.

After image capturing

- Insert comments in the Note Pad accompanying each image.
- Inspect images for movement artefacts (horizontal stripes).
- Save the image.
- Select the Region of Interest and calculate average values.
- To compare different images colour-wise they need to be set to the same colour scale.
- Save the postprocessed images.
- If applicable, convert images to TIFF-file format and combine them in, for example, a Word™ document for overview presentation and documentation.

Section II

Present use and future potential

LDPI technology has so far been used in dermatology, diabetology, pharmacology, wound healing and numerous other applications. Of particular interest for the research groups involved in the HIR-ELADO project are the use of LDPI in skin allergy and irritant patch testing, microdialysis and skin tumour blood perfusion evaluation. These applications are described in further detail below.

Skin allergy and irritant patch testing

A major dermatological application of LDPI is in the evaluation of irritant and allergic skin reactions, in which a high sensitivity and reproducibil-

Table 1. Papers reporting skin irritant and allergy patch testing of various substances

Topic
allergy (Kathon CG) (18)
allergy (nickel sulphate) (19)
allergy (corticosteroid) (20)
allergy (budesonide) (21)
principle (17, 22, 23)
allergy (diesel oil) (24)
allergy (sodium dodecyl sulphate) (25)
irritant (D vitamins) (26)
irritation (calcipotriol) (27)
irritation (methyl nicotinate) (28)
allergy (gold) (29)
atopic dermatitis (30)
image analysis (31)

ity have been demonstrated (see Table 1). Such investigations are best performed by use of an LDPI system with relatively high resolution. In such images the increased blood perfusion is caused by the hyperperfusion in inflamed and vasodilated skin. LDPI does not, however, directly distinguish between allergic and irritant reactions.

The gold standard for evaluation of skin tests is visual scoring. LDPI evaluation of epicutaneous and intracutaneous tests constitutes an objective and practically useful method for the quantification of such test reactions. LDPI values of visually positive tests allow a calculation of a lower limit of positive LDPI tests. Visually negative tests usually have low LDPI values that differ significantly from those obtained from positive readings. There is generally a high correlation between visual scoring and LDPI evaluation. The LDPI values tend to increase slightly before the visual reactions can be noticed and the high LDPI values last for a somewhat shorter time period than the positive visual reactions. Thus, reactions associated with high LDPI values, which are visually negative, as a rule later become visually positive. Visually positive test reactions with LDPI values below the calculated positive limit are as a rule late test readings of previously visually and LDPI-positive reactions. The interesting 'grey zone' of questionable reactions with LDPI readings between the negative and the positive LDPI limit needs to be further analysed.

Repeated and comparative reading of tests necessitates the use of control areas to overcome long-term temporal variations in vascular reactions and the influence of temperature. The use of two control patches with vehicle alone, constitutes an efficient way to increase the accuracy of the test evaluation.

The optimal size of a skin test image is somewhat dependent on the application and on the sub-

stance to be tested. Normally, the size of the skin area investigated should equal the size of the patch, plus 1–2 cm around its edges. In most cases this coincides with a square with the side equal to three times the diameter of the patch. With very strong reactions, an increased blood perfusion may be found in an area of six times the size of the patch. If possible, the area of the central patch size should include at least 100 measurement sites. This complies with a standard Finn Chamber of 9 mm diameter and a laser spot size of about 1 mm².

If the test patches are placed on the back, the test subject/patient should lie comfortably in the prone position. When the patches are placed on the forearm, the test subject/patient should sit comfortably in a chair with the test area at heart level. In the latter case, the patches should not be placed directly over any major vein, because the blood flow in those vessels will contribute to the recorded Doppler signal.

One method of evaluating a test is to find the mean increase in perfusion of the central area of the test reaction equal to the size of the patch. For intracutaneous tests, an area size of 1 cm² is satisfactory. A more exact but also more time-consuming method is to evaluate the increase in blood perfusion of the test area through a 'volume integration' of a larger area. With this method the total area of increased blood perfusion has to be compared with the control area(s). For this purpose it is necessary to evaluate an area of about 3 × 3 cm. An extremely strong reaction may, however, cover an area of 6 × 6 cm or more.

Detailed recommendations, based on more than 25,000 allergic patch test assessments, on how to standardize the conditions for this particular application were compiled by Bjarnasson *et al.* in 1999 (17).

Table 1 includes some of the early publications in the field of allergy and irritant patch testing using the LDPI technology.

Microdialysis

Microvascular aspects of skin microdialysis are best studied by use of a very high resolution LDPI system (based on a focused laser beam to improve the resolution) that operates at a distance of 7 cm, giving a total field view of only 1.5 × 1.5 cm (32–34). This device is well suited for localization of the microdialysis probe in such perfusion experiments (35, 36) and helps to interpret the results obtained with this probe. In addition, it is possible to investigate the settling time of blood perfusion caused by the insertion of the probe and, when applicable, to use this information to trigger the sampling of substances by use of the microdialysis

probe, in order to avoid influences of increased skin blood perfusion on the results.

Skin tumour blood perfusion

Studies on skin tumours and other skin lesions constitute promising new applications for LDPI investigations. Port wine stains have been evaluated before and after laser treatment (37). The tumour circulation in association with photodynamic therapy of non-melanoma skin tumours has been followed over the treatment period (38). A recently published method to distinguish moles from melanomas with a high-resolution scanner (39) constitutes a promising approach for future applications in the differential diagnosis of skin tumours. In addition to simply recording the average perfusion within the tumour boundary or extension of the hyper-perfused tumour area, more advanced image processing methods may be employed to further refine the opportunity for tumour differential diagnosis offered by LDPI technology. These image-processing methods include feature extraction, analysis of boundary irregularities and measures of intratumour blood perfusion heterogeneity.

Other applications

In addition to the applications above, which have been addressed within the framework of the HIR-ELADO project, LDPI technology has been successfully applied in a number of other fields including: diabetes neuropathy (cold provocation) (40–42), endothelial cell malfunction in diabetes (iontophoresis of acetylcholine) (43, 44), leg ulcers and wound healing (drug influences) (45–47), angiogenesis and growth factor research (animal models) (48, 49), psoriasis (50, 51), scleroderma and Raynaud's disease (52, 53) and flap surveillance (54, 55).

Acknowledgement

The present work was supported by the European Commission DG XIII/D – Innovation programme (contract IN10178D).

References

1. Stern M D. *In vivo* evaluation of microcirculation by coherent light scattering. *Nature* 1975; 254: 56–58.
2. Holloway G A Jr, Watkins D W. Laser Doppler measurement of cutaneous blood flow. *J Invest Dermatol* 1977; 69: 306–309.
3. Nilsson G E, Tenland T, Öberg P Å. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng* 1980; 27: 12–19.
4. Leahy M J, de Mul F F, Nilsson G E, Maniewski R. Principles and practice of the laser-Doppler perfusion technique. *Technol Health Care* 1999; 7: 143–162.
5. Line P D, Mowinkel P, Lien B, Kvernebo K. Repeated measurement variation and precision of laser Doppler flowmetry measurements. *Microvasc Res* 1992; 43: 285–293.
6. Nilsson G E, Jakobsson A, Wårdell K. Imaging of tissue blood flow by coherent light scattering. In: *11th Ann EMBS conference*. Seattle: IEEE, 1989.
7. Wårdell K, Jakobsson A, Nilsson G E. Laser Doppler perfusion imaging by dynamic light scattering. *IEEE Trans Biomed Eng* 1993; 40: 309–316.
8. Essex T J, Byrne P O. A laser Doppler scanner for imaging blood flow in skin. *J Biomed Eng* 1991; 13: 189–194.
9. Fujii H, Nohira K, Yamamoto Y, Ikawa H, Ohura T. Evaluation of blood flow by laser speckle image sensing. *Applied Optics* 1987; 26: 5321–5325.
10. Bircher A, de Boer E M, Agner T, Wahlberg J E, Serup J. Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1994; 30: 65–72.
11. Fullerton A, Fischer T, Lahti A, Wilhelm K P, Takiwaki H, Serup J. Guidelines for measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1996; 35: 1–10.
12. Groner W, Winkelmann J W, Harris A G et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nature Med* 1999; 5: 1209–1212.
13. Bollinger A, Butti P, Barras J P, Trachsler H, Siegenthaler W. Red blood cell velocity in nailfold capillaries of man measured by a television microscopy technique. *Microvasc Res* 1974; 61.
14. Harrison D K, Abbot N C, Beck J S, McCollum P T. Laser Doppler perfusion imaging compared with light-guide laser Doppler flowmetry, dynamic thermographic imaging and tissue spectrophotometry for investigating blood flow in human skin. *Adv Exp Med Biol* 1994; 345: 853–859.
15. Andersson R R, Parrish J A. The optics of human skin. *J Invest Dermatol* 1981; 77: 13–19.
16. Jakobsson A, Nilsson G E. Prediction of sampling depth and photon pathlength in laser Doppler flowmetry. *Med Biol Engineering Computing* 1993; 31: 301–307.
17. Bjarnason B, Flosadottir E, Fischer T. Objective non-invasive assessment of patch tests with the laser Doppler perfusion scanning technique. *Contact Dermatitis* 1999; 40: 251–260.
18. Bjarnason B, Fischer T. Visual and laser Doppler perfusion scanning assessments of Kathon CG patch test reactions. *Am J Contact Dermatitis* 1998; 9: 224–230.
19. Bjarnason B, Fischer T. Objective assessment of nickel sulfate patch test reactions with laser Doppler perfusion imaging. *Contact Dermatitis* 1998; 39: 112–118.
20. Bjarnason B, Flosadottir E, Fischer T. Reactivity at edges of corticosteroid patch tests may be an indicator of a strong positive test response. *Dermatology* 1999; 199: 130–134.
21. Bjarnason B, Flosadottir E, Fischer T. Assessment of budesonide patch tests. *Contact Dermatitis* 1999; 41: 211–217.
22. Fullerton A, Serup J. Laser Doppler image scanning for assessment of skin irritation. In: Elsner P, Maibach H (eds.) *Irritant Dermatitis. New Clinical and Experimental Aspects. Current Problems in Dermatology*. Basel: Karger, 1995: 159–168.
23. Stücker M, Auer T, Hoffman K, Altmeyer P. Two-dimensional blood flow determinations in allergic reactions using laser Doppler scanning. *Contact Dermatitis* 1995; 33: 299–303.

24. Fischer T, Bjarnason B. Sensitizing and irritant properties of 3 environmental classes of diesel oil and their indicator dyes. *Contact Dermatitis* 1996; 34: 309–315.
25. Fischer T, Dahlen A, Bjarnason B. Influence of patch-test application tape on reactions to sodium dodecyl sulfate. *Contact Dermatitis* 1999; 40: 32–37.
26. Fullerton A, Serup J. Topical D-vitamins. multiparametric comparison of the irritant potential of calcipotriol, tacalcitol and calcitriol in a hairless guinea pig model. *Contact Dermatitis* 1997; 36: 184–190.
27. Fullerton A, Benfeldt E, Petersen J R, Jensen S B, Serup J. The calcipotriol dose–irritation relationship: 48 hour occlusive testing in healthy volunteers using Finn Chambers. *Br J Dermatol* 1998; 138: 259–265.
28. Issachar N, Gall Y, Borrel M T, Poelman M C. Correlation between percutaneous penetration of methyl nicotinate and sensitive skin, using laser Doppler imaging. *Contact Dermatitis* 1998; 39: 182–186.
29. Moller H, Bjorkner B, Bruze M, Lundqvist K, Wollmer P. Laser Doppler perfusion imaging for the documentation of flare-up in contact allergy to gold. *Contact Dermatitis* 1999; 41: 131–135.
30. Stucker M, Heese A, Hoffmann K, el Gammal S, Altmeyer P. Quantification of vascular dysregulation in atopic dermatitis using laser Doppler perfusion imaging. *Skin Res Technol* 1998; 4: 9–13.
31. Wårdell K, Andersson T, Anderson C. Analysis of laser Doppler perfusion images of experimental irritant skin reactions. *Skin Res Technol* 1996; 2: 149–157.
32. Anderson C, Andersson T, Wårdell K. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. *J Invest Dermatol* 1994; 102: 807–811.
33. Andersson T, Wårdell K, Anderson C. Human in vivo cutaneous microdialysis: estimation of histamine release in cold urticaria. *Acta Derm-Venereol* 1995; 75: 343–347.
34. Krogstad A L, Jansson P-A, Gisslén P, Lönnroth P. Microdialysis methodology for assessment of dermal fluid in humans. *Br J Dermatol* 1996; 134: 1005–1012.
35. Lindén M, Wårdell K, Andersson T, Anderson C. High resolution laser Doppler perfusion imaging for the investigation of blood circulatory changes after microdialysis probe insertion. *Skin Res Technol* 1997; 3: 227–232.
36. Groth L, Serup J. Cutaneous microdialysis in man. effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. *Acta Derm-Venereol* 1998; 78: 5–9.
37. Troilius A M, Ljunggren B. Evaluation of port wine stains by laser Doppler perfusion imaging and reflectance photometry before and after pulsed dye laser treatment. *Acta Derm-Venereol* 1996; 76: 291–294.
38. Wang I, Andersson-Engels S, Nilsson G E, Wårdell K, Svanberg K. Superficial blood flow following photodynamic therapy of malignant non-melanoma skin tumours measured by laser Doppler perfusion imaging. *Br J Dermatol* 1997; 136: 184–189.
39. Stücker M, Horstmann I, Nuchel C, Rochling A, Hoffmann K, Altmeyer P. Blood flow compared in benign melanocytic naevi, malignant melanomas and basal cell carcinomas. *Clin Exp Dermatol* 1999; 24: 107–111.
40. Arora S, Smakowski P, Frykberg R G, Simeone L R, Freeman R, LoGerfo F W, Veves A. Differences in foot and forearm skin microcirculation in diabetic patients with and without neuropathy. *Diabetes Care* 1998; 21: 1339–1344.
41. Bornmyr S, Castenfors J, Svensson H, Wroblewski M, Sundkvist G, Wollmer P. Detection of autonomic sympathetic dysfunction in diabetic patients. A study using laser Doppler imaging. *Diabetes Care* 1999; 22: 593–597.
42. Bornmyr S, Svensson H, Lilja B, Sundkvist G. Cutaneous vasomotor responses in young type I diabetic patients. *J Diabetes Complications* 1997; 11: 21–26.
43. Morris S J, Shore A C, Tooke J E. Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. *Diabetologia* 1995; 38: 1337–1344.
44. Veves A C M, Akbari J, Primavera V M et al. Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes* 1998; 47: 457–463.
45. Gschwandtner M E, Ambrózy E, Böhler K et al. Reproducibility of laser Doppler imager flux measurements within ischemic or venous ulcers and adjacent skin. *Skin Res Technol* 1998; 4: 222–227.
46. Gschwandtner M E, Ambrozy E, Schneider B, Fasching S, Willfort A, Ehringer H. Laser Doppler imaging and capillary microscopy in ischemic ulcers. *Atherosclerosis* 1999; 142: 225–232.
47. Mayrovitz H N, Smith J. Heel-skin microvascular blood perfusion responses to sustained pressure loading and unloading. *Microcirculation* 1998; 5: 227–233.
48. Couffinhal T, Silver M, Zheng L P, Kearney M, Witzembichler B, Isner J M. Mouse model of angiogenesis. *Am J Pathol* 1998; 152: 1667–1679.
49. Rivard A, Fabre J E, Silver M et al. Age-dependent impairment of angiogenesis. *Circulation* 1999; 99: 111–120.
50. Auer T, Bacharach-Buhles M, el Gammal S et al. The hyperperfusion of the psoriatic plaque correlates histologically with dilatation of vessels. *Acta Derm Venereol (Stockh)* 1994; 186 (Suppl): 30–32.
51. Krogstad A L, Lonnroth P, Larson G, Wallin B G. Capsaicin treatment induces histamine release and perfusion changes in psoriatic skin. *Br J Dermatol* 1999; 141: 87–93.
52. Aghassi D, Monoson T, Braverman I. Reproducible measurements to quantify cutaneous involvement in scleroderma. *Arch Dermatol* 1995; 131: 1160–1166.
53. Stücker M, Jeske M, Hoffmann K, Altmeyer P. Laser-Doppler-Scanning bei progressiver systemischer Sklerodermi. *Phlebologie* 1995; 24: 9–14.
54. Arnold F, He C F, Jia C Y, Cherry G W. Perfusion imaging of skin island flap blood flow by a scanning laser-Doppler technique. *Br J Plastic Surg* 1995; 48: 280–287.
55. Eichhorn W, Auer T, Voy E D, Hoffmann K. Laser Doppler imaging of axial and random pattern flaps in the maxillo-facial area. A preliminary report. *J Cranio-Maxillo-Facial Surg* 1994; 22: 301–306.

Address:
J. Serup
Gentoftegade 49
DK-2820 Gentofte
Denmark