

Review

Laser Doppler imaging: a developing technique for application in the rheumatic diseases

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Microcirculatory abnormalities contribute to the pathogenesis and pathophysiology of many of the rheumatic diseases. This is best recognized in systemic sclerosis (SSc), in which structural microvessel disease can be well demonstrated using the technique of capillary microscopy [1], and more recently by video and digital capillaroscopy [2, 3]. However, in many other conditions the microvasculature is more subtly involved. By the 'microvasculature' we mean the arterioles, the capillaries and the venules. Any inflammatory state is associated with profound microvascular perturbation. For example, in rheumatoid arthritis the synovial microvasculature undergoes major change with formation of new blood vessels (angiogenesis) in the hypertrophied synovium and with lymphocyte trafficking through high endothelial venules. These high endothelial venules are lined by specialized endothelial cells whose formation has been induced during the inflammatory process. [4].

In the study of disease, we must be concerned not only with understanding basic pathophysiology but also with the measurement of disease progression. If we cannot measure the disease, then we cannot assess its progression or responsiveness to treatment. In addition, the ability to measure disease processes can give us indirect insights into pathophysiology by allowing us to assess response to therapeutic interventions which are known to have specific mechanisms of action.

Can we measure microvascular disease/involvement by disease, and apply this to the study of rheumatological disorders? As already mentioned, we can examine nailfold capillary structure in certain connective tissue diseases, such as SSc and dermatomyositis, using nailfold microscopy and video capillaroscopy, and one aspect of capillary function (permeability) can be examined by fluoroscopy [5, 6], which is, however, invasive in that it requires an intravenous dye injection. In this review we shall discuss the relatively new technique of laser Doppler imaging (otherwise termed 'scanning laser Doppler'), which gives a direct measure of microcirculatory flow. We believe laser Doppler imaging affords significant potential in the study of microcirculatory involvement of the rheumatic diseases and it is non-invasive.

Background to laser Doppler blood flow monitoring

The observed wavelength of electromagnetic radiation is affected by relative motion between the source and observer. This phenomenon (also applicable to sound waves, as in the technique

of Doppler ultrasound) is known as the Doppler effect. When low-level laser light, of a few milliwatts, is directed onto the skin's surface a fraction of the light penetrates the skin and interacts with both static tissue and moving cells (primarily red blood cells). The penetration depth of light is dependent upon the tissue morphology, absorption and the wavelength used [7, 8]. The light that is reflected or randomly scattered from the static tissue remains unchanged in wavelength. In contrast the light that is scattered from the moving blood cells undergoes a small change in wavelength, proportional to the speed of the erythrocytes, due to the Doppler effect. Backscattered light from the tissue, incident on a detector, is processed to provide a signal that is proportional to the speed and density of the moving cells [9].

Stern [10] was the first to exploit the Doppler effect to monitor blood flow. He collected the backscattered light, Doppler-broadened according to the internal motion of circulation, on a photodetector. He demonstrated the difference in perfusion of a fingertip under normal flow conditions and those of brachial occlusion using a helium–neon (HeNe, 633 nm) laser. It was also noted that, after administration of ethanol, the vasodilatory effects were observed as an increase in blood flow at the fingertip, which increased over a 15-min period.

From these initial observations came the method of laser Doppler flowmetry (LDF): fibre-delivered, single-point perfusion monitoring. LDF has been adapted and improved to remove many of the preliminary problems and has been widely used both in research and as a clinical tool over the past 20 yr in the measurement of cutaneous microcirculatory flow. The technique with which most clinicians are familiar is the single-probe technique. Single-probe laser Doppler has been used extensively by rheumatologists to quantify blood flow in studies of Raynaud's phenomenon [11–14]. The principles underlying this technique are demonstrated in Fig. 1a: the laser light is delivered to the tissue surface via an optical fibre and the backscattered and reflected light is collected by a second (or several) fibre(s). Delivery and collection fibres are housed in a single probe.

The incident laser light beam has a depth of penetration of approximately 1 mm, depending upon the wavelength and configuration of the equipment used. Therefore all elements of the dermis may be included, from superficial nutritional to deeper thermoregulatory vessels. For a given wavelength of light, the absorption spectrum of components of the tissue determines the interaction that occurs [7]. The penetration of light is predetermined by

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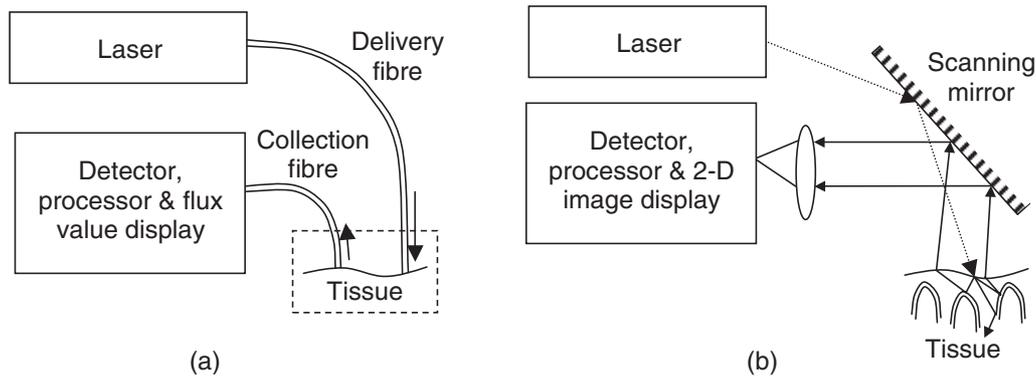


FIG. 1. (a) Schematic of LDF. (b) Schematic of LDI.

the path that the light takes through the skin and is limited by the absorption of the tissue. Towards the red end of the visible spectrum haemoglobin and water absorption are lower, hence the dominance of the HeNe laser in earlier LDF. For this same reason, near-infrared diode lasers (670, 780 and 810–850 nm) are also popular; these long wavelengths exhibit a deeper penetration depth and, due to the low absorption of melanin in the near infrared, show less dependence on skin colour.

The broadened signal gives information on the quantity 'flux', F , which is proportional to the product of the mean speed, \bar{v} , of the flow and the number concentration, N , of blood cells [15]:

$$F = k\bar{v}N$$

where k is an arbitrary constant. Flux is expressed in terms of arbitrary perfusion units, which do not give absolute values for the blood flow speed. Therefore, although it is not meaningful to compare absolute values of perfusion between individuals, intra- and inter-individual comparisons of dynamic responses (to standard stimuli) can be made. The LDF instrument can be calibrated in perfusion units by measuring the Brownian motion of a standard suspension of polystyrene microparticles in water.

Laser Doppler imaging (scanning laser Doppler)

LDF offers continuous perfusion measurement. However, cutaneous circulation is known to be heterogeneous, and examining a small area of perfusion does not necessarily give representative data for the surrounding perfusion [16]. This problem can be overcome by collection of blood flow data over a larger area.

A relatively recent application of laser Doppler is the development of laser Doppler imaging (LDI). This technique has two major advantages over the single-probe technique. (i) The first is that blood flow is measured over an area rather than at a single site, obviating some of the difficulties with site-to-site variability inherent in the signal-probe technique. Thus, reproducibility might be improved. (ii) Secondly, the laser beam is non-contact, as opposed to the single probe, which involves direct contact with the skin and which could, therefore, through this contact, influence blood flow via pressure and movement artefacts [17].

Figure 1b illustrates the principles underlying the LDI technique. The laser beam, approximately 1 mm in diameter, is scanned across tissue in two dimensions using a moving mirror. The scattered light signal is analysed to provide a two-dimensional image of blood flow. The bandwidth of the collected signal varies according to the scan speed (ms/pixel). A high-frequency cut-off (3–22 kHz) improves the signal-to-noise ratio and a low-frequency cut-off (20–250 Hz) eliminates movement artefacts. This low-frequency cut-off has some effect on the ability to measure low-speed blood flows; for low perfusion a slower scan rate can be used.

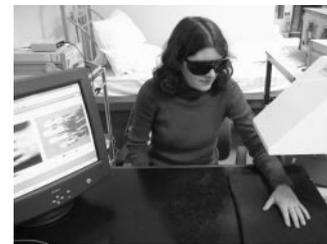


FIG. 2. LDI system in use. As the laser beam scans across the hand, the image appears on the monitor.

Both large (e.g. torso) and small (e.g. finger, hand) areas can be scanned, larger areas by increasing the imaging height. Figure 2 shows a laser Doppler imager in use.

Already a number of investigators have recognized the potential of the technique for clinical application and some examples of these clinical applications in specialities other than rheumatology are as follows.

Assessment of burns

Several groups have described the measurement of burn depth with LDI [18–20]. Brown *et al.* [21] demonstrated the use of LDI in the evaluation of the clinical management of vesicant burns and Jeng *et al.* [22] described LDI confirmation of clinical judgement in the requirement for excision of burns of indeterminate depth.

Assessment of dermal inflammation

Dermatologists have drawn attention to the fact that, because of its accessibility, the skin provides the opportunity of studying mechanisms of inflammation. Harrison *et al.* [23] described the increase in cutaneous blood flow imaged during the tuberculin reaction. Clough [24] reported how LDI allows us to build upon pathophysiological studies (in this particular example investigating the role of nitric oxide in dermal inflammation) by including direct measurement of microvascular response.

Assessment of wound healing

Eichhorn *et al.* [25] used LDI to monitor the healing of flaps in the maxillofacial area, identifying necrotic areas, venous stasis and normal wound healing. Ljung *et al.* [26] examined postoperative wound healing.

Assessment of cutaneous ulceration

Responses to heating and iontophoresis have been assessed in both patients with arterial disease and patients with diabetes [27].

Dermal replacement therapy for foot ulceration has been monitored in diabetic patients [28] and diabetologists have applied LDI in the study of other aspects of disease, including autonomic responses [29].

Applications of laser Doppler imaging in rheumatology

Primary and secondary Raynaud's phenomenon

In rheumatology, an obvious clinical application is in quantitation of dermal blood flow in patients with primary and secondary Raynaud's phenomenon. To date a small number of cross-sectional studies have explored the potential of LDI to measure blood flow in patients with Raynaud's and SSc. Seifalian *et al.* [30] recommend the application of LDI in patients with vascular disease, including Raynaud's phenomenon, citing its advantages over single-point LDF, as a method to monitor perfusion in the hands of patients with SSc. We know that dermal microcirculatory flow is reduced, at least during vasospastic attacks, as evidenced by the classic colour changes which occur in the skin of the digits during these episodic attacks of ischaemia, which characterize the condition. Thus, in patients with Raynaud's there is reduction in flow at least in response to a dynamic challenge, and it is likely that in many patients with Raynaud's secondary to structural blood vessel disease, as occurs in SSc [31], there is reduced flow even under baseline conditions. Thus, blood flow studies need to consider both resting conditions and dynamic responses to a standardized challenge, usually a cold or warm stimulus. Picart *et al.* [32] have compared laser Doppler images in patients with primary Raynaud's phenomenon, patients with SSc and healthy control subjects, examining effects of a cold stress. They reported that patients with SSc demonstrated lower baseline perfusion than patients with primary Raynaud's and healthy controls, and that differences between groups became more marked during the cold exposure and rewarming periods. Bornmyr *et al.* [33] investigated the changes in finger perfusion due to local hot and cold provocation in patients with traumatic vasospastic disease and controls; the fingers were warmed to 40°C, cooled to 10°C then rewarmed. Although cooling induced a decrease in perfusion, changes in blood flow monitored by LDI were found to be insufficient to distinguish patients from controls.

A small pilot study to determine in the first instance whether flux patterns differed between patients with SSc, patients with primary Raynaud's phenomenon and healthy control subjects was reported by our own group [34]. Images were obtained of the dorsum of the hand at room temperatures of 23 and at 30°C, and it was found that two parameters using the imaging technique (the gradient in flux along the finger and the difference in flux between the fingers of the same hand) were able to pick up trends across the three subject groups. However, there were no trends across subject groups using single-site measurements. Figure 3 demonstrates three key points: (i) the inhomogeneity of the flux map (highlighting how single-site measurements are very site-dependent); (ii) the reduced fingertip flux in patients with SSc; and (iii) the asymmetry of the flux between fingers in patients with SSc, reflecting the variation between the fingers of the structural vascular disease.

One important issue is how LDI compares to thermography as a measurement tool, thermography being well established in the assessment of Raynaud's phenomenon [35, 36]. Seifalian *et al.* [37] made comparisons between thermography and LDI, identifying a lack of correlation between the two techniques. They concluded that influences such as the variation of heat transport in tissue by conduction, radiation and convection may account for the deficit between direct blood flow measurements and thermography. A comparison of the techniques of LDI and thermography was also made by Clark *et al.* [38], examining the difference

between the dorsum of the hand and the tip of the middle finger; measurements were made at both 23 and 30°C, for both hands. The study confirmed that LDI and thermography correlated poorly. In contrast to LDI, which measures blood flow on a microscopic scale, thermography indirectly measures tissue temperature, which other internal and external factors may influence and which is highly damped. Each procedure offers discrete, valuable information, which complements the information given by the other.

LDI has also been compared with arteriography in the evaluation of digital ischaemia [39]. Twenty-three patients were evaluated for upper limb ischaemia (four of whom had scleroderma, one dermatomyositis and one Raynaud's/sarcoidosis). LDI perfusion measurements corresponded well with upper extremity symptoms and function and the investigators suggested its use to assess the adequacy of nutritive microcirculation. LDI was not found to be a replacement but to provide complementary information to arteriography.

The cross-sectional studies described above suggest that LDI deserves further evaluation in patients with Raynaud's phenomenon, including studies of reproducibility and treatment responsiveness. So far there has been very little experience in the assessment of response to vasoactive treatments. However, we have demonstrated the potential of LDI in quantifying change in microcirculatory flow in response to vasodilators [40]. Patients with SSc and primary Raynaud's phenomenon and healthy control subjects all demonstrated a rise in perfusion after local administration of topical glyceryl trinitrate, in comparison with after placebo and with no treatment.

While the assessment of digital microcirculatory flow in patients with Raynaud's phenomenon is a very obvious application of LDI, there are many other potential applications in musculoskeletal disease, as described below.

Inflammatory joint disease

Ferrell *et al.* [41] used LDI to measure hyperaemia over proximal interphalangeal joints in eleven patients with rheumatoid arthritis using red and near infrared wavelengths. An overall increase in perfusion was observed in the joints of patients in comparison with healthy controls, which was more prominent at 850 nm (infrared) than at 633 nm (red). Measuring blood flow over the small joints of the hands, as in this study, might usefully quantify the degree of inflammation. In this context, it is unlikely that absolute flux recordings are likely to be of interest but rather the ratio of flux overlying the joint to flux over adjacent skin. Being able to quantify dermal blood flow in this way might be useful in measuring the treatment response, and this could also be true for soft tissue inflammation. For example, the efficacy of a non-steroidal anti-inflammatory gel or of a periarticular/intra-articular injection might be assessed by looking at the degree of hyperaemia overlying the affected site before and after application/injection.

In a later study with patients with rheumatoid arthritis, Ferrell *et al.* cited the advantages of LDI over alternative methods, such as magnetic resonance imaging and ultrasound, in identifying the early stages of synovitis and highlighted LDI's potential for the diagnosis and observation of disease progression [42]. Metacarpophalangeal joints were examined at both 633 and 835 nm, alongside ultrasound. Following the initial scans of six patients, the obvious inability of the red laser to image elevated perfusion at the joints led to the remaining patients' joints being imaged with only the near-infrared laser, indicating that the source of hyperaemia is below the upper layers of skin microcirculation (the near-infrared laser penetrates more deeply than the red). Of the patients examined during the study, six were observed to have a significant increase in perfusion at the affected joints when compared with healthy controls, allowing subjects to be divided into two groups, with high and low perfusion; this grouping corresponded to the

duration of disease, the higher perfusion relating to shorter duration. With regard to pain, those in the high-perfusion group had a linear correlation between increased blood flow and pain score, implying an inflammatory cause, whereas in the low-perfusion group pain scores were not significantly linked, implying mechanical damage as the source of pain. Areas of elevated perfusion as imaged with LDI corresponded significantly with areas of anechoica or hyperechoica identified by ultrasound, suggestive of synovitis. This study suggests that LDI could guide treatment decisions.

Ng and How [43] report a case study using LDI for examination of osteoarthritis in the proximal interphalangeal joints of 35 patients; the only identification of hyperaemia at this shorter wavelength (633 nm) was over a deformed joint in a single patient, which was associated with the pain and stiffness index. The authors suggest that longer wavelengths should be investigated further.

Thus, LDI offers potential in the assessment of peripheral joint disease, both with respect to assessment of inflammation and (although still to be explored) assessment of treatment response.

Complex regional pain syndrome

The pathogenesis of complex regional pain syndrome (CRPS, also known as 'reflex sympathetic dystrophy') is poorly understood; it is likely that both neurogenic inflammation and microvascular dysfunction contribute [44–47]. An investigation using transcutaneous electrical stimulation to provoke neurogenic inflammation in patients' affected limbs and in controls measured superficial blood flow with LDI during and after stimulation [48]. Axon reflex erythema was observed in both patients and in controls as an increase in perfusion (imaged with LDI), and was significantly increased in CRPS patients. Gorodkin *et al.* [49] used LDI to examine the response of CRPS patients and healthy controls to iontophoresis of vasoactive chemicals in order to assess their microvascular function. Comparisons were made between the affected and contralateral limbs for patients and also between the responses of patients and controls. No significant differences were identified between the perfusion increase in patients' limbs, or between patients and healthy controls. The study found that CRPS was not associated with impairment of microvascular endothelial function. However, it was concluded that this may reflect the diversity of the CRPS disease process. These studies demonstrate the ability of LDI to provide mechanistic insight into conditions associated with abnormalities in thermoregulation and/or perfusion.

Soft tissue lesions

Ljung *et al.* [26] measured microcirculatory flow before and after elbow replacement in five patients with rheumatoid arthritis to monitor wound healing. Blood flow rose post-operatively and hence the authors suggest that surgery had not compromised blood flow.

The application of LDI may be helpful in monitoring soft tissue inflammatory lesions. A single case of epicondylitis has been described by Ferrell *et al.* [50] in which LDI was used in parallel with ultrasound and temperature measurement to observe the changes in oedema and blood flow following local injection of methylprednisolone. Hyperaemia present in the affected elbow was observed to decrease over an 11-day period and LDI was found to be more sensitive than tissue surface temperature measurements to changes in inflammation.

Psoriasis

Psoriatic plaques have also been studied by LDI and because psoriasis is of interest to rheumatologists, from both clinical

and research perspectives, we felt that this should be discussed. Speight *et al.* [51] used LDI to determine differences in blood flow within psoriatic plaques and to determine the location of their leading edge. A fourfold increase in perfusion was determined within the plaque area compared with surrounding tissue, and an annulus of 2–4 mm of increased blood flow was found around the periphery of the plaque, as measured by eye. Davison *et al.* [52] also found that a rim of increased flux extended beyond the clinically apparent edge of the plaque. Krogstad *et al.* [53] found that histamine was not responsible for the increase in perfusion observed in plaques by monitoring both blood flow (LDI) and histamine release (microdialysis) upon administration of topical anaesthesia. In a second study the same authors found that 24 h of capsaicin treatment lowered the perfusion within psoriatic lesions [54]. Stucker *et al.* [55] studied the inflammatory response of psoriasis patients to retinoids with LDI. They found that initial skin irritation could be reduced by adapting therapy according to the patient's skin type. Yosipovitch *et al.* [56] studied the blood flow of psoriatic plaques before and after barrier function of the skin was altered using tape stripping. They reported that the increase in blood flow within plaques correlated with a decrease in pain threshold with heat, in contrast to healthy skin, where increased perfusion correlated with an increased pain threshold with heat. This result indicates that the thermosensory response of patients with psoriasis is abnormal. Together, these studies demonstrate how LDI has been applied in patients with psoriasis to study the pathophysiology as well as the treatment response.

Cutaneous ulceration

Cutaneous ulcers deserve consideration. Patients with musculoskeletal disease are at increased risk of skin ulcers for a variety of reasons and their consequences can be devastating in, for example, the patient with rheumatoid arthritis and prosthetic joints, in whom the ulcer becomes infected. Gschwandtner *et al.* [57–60] have carried out several LDI studies on the microcirculatory characteristics of ulcers. An early randomized study was performed by the group to determine whether prostanoids, which have been shown to aid ulcer healing and reduce pain, increase the blood flow of ulcers. The effects of Iloprost, prostaglandin E₁ (alprostadiol) and saline were compared [57]. The group have also used LDI in parallel with capillary microscopy to image and compare ischaemic and venous ulcers, known to impair both the thermoregulatory and the nutritive microcirculation. The aim of the authors was to study factors including the relationship between laser Doppler flux and capillary density, both within the ulcer locality and the surrounding tissue [58, 59], and to examine these data and establish the genesis, development and healing of the ulcers [60]. LDI provided measurement of perfusion in the subpapillary layer in contrast to capillary microscopy observations, which imaged the superficial network. Blood flow within both kinds of ulcer was identified as low, and the pattern of microcirculation was similar in the two kinds of ulcer, implying comparable pathogenesis in this regard. Regions of granulation, representative of wound healing, had a higher blood flow than those with no granulation, particularly for venous ulcers, a consequence of a higher density of capillaries (as imaged by microscopy) in the granulated area. Ischaemic ulcers were found to have high nutritive blood flow and low subpapillary blood flow, whereas venous ulcers were found to have moderate nutritive and high subpapillary flow, associated with venous insufficiency in these regions. The authors concluded that the development of both types of ulcers is similar during healing, from granulation to scar formation, but that the surrounding skin microcirculation differs due to the underlying pathophysiology. Studies of vasculitic ulcers would be of interest.

Application of different wavelengths

LDI has, to date, largely used a wavelength of 633 nm. Since the scattering and absorption of light by blood and tissue is wavelength-dependent, using different wavelengths should allow different depths of tissue to be explored, thereby increasing the potential applications of the technique.

Even with LDF, only a few studies have been carried out to compare wavelengths. Duteil *et al.* [61] concluded that 458 nm (blue) investigated a more superficial blood flow than 633 nm when comparing blue and red wavelengths *in vitro* and *in vivo*, and Obeid *et al.* [62] attributed differences between 780, 633 and 543 nm (infrared, red and green wavelengths respectively) to differences in the absorption and scattering properties of the skin and, in the case of the green light, to the absorption of blood. Gush and King [63] showed that using a combination of red and green wavelengths allowed discrimination between capillary and arteriovenular flow and discussed the higher relevance of blood absorption and scattering over tissue absorption for imaging depth at these wavelengths. Therefore the combination of red and green wavelengths affords the possibility of distinguishing between deeper thermoregulatory and more superficial nutritional microvessels.

Two LDF studies have been carried out with near infrared (780 nm) and green (543 nm) wavelengths. Tulevski *et al.* [64] made simultaneous measurements in the feet of healthy controls and demonstrated that the green laser measured a lower perfusion. However, it was suggested that the lower perfusion was not entirely derived from capillary blood flow because changes in green laser flux did not parallel changes in blood flow as measured by capillary microscopy. A second study by the same group measured blood flow in patients with peripheral vascular disease [65]. Results with the green laser had distinctly different characteristics compared with the near infrared laser and again did not follow the capillary microscopy measurements.

Abbot *et al.* [66] explored the use of longer, near-infrared, wavelengths (780 and 830 nm) in comparison with red in LDI and suggested that this wavelength could have potential when a deeper skin penetration is required and in patients with deeply pigmented skin. The longer wavelength, when applied to the proximal interphalangeal joints in volleyball players, demonstrated increased blood flow in comparison with healthy controls [67]. Blood flow was higher when measured with the infrared wavelength than with the red, and the authors postulated that this meant that the longer wavelength was detecting flow in subcutaneous structures rather than the skin.

Our own group has carried out some preliminary clinical studies with red and green (532 nm) LDI, investigating patients with primary and secondary Raynaud's phenomenon [68] and healthy controls [69], so far reported only in abstract form; differences were found to exist between the two wavelengths. An example of a typical hyperaemic response, in a healthy control, returning to baseline following heat stimulus is shown in Fig. 4, imaged with both red and green wavelengths. From these initial images it can be seen that the area of response and the duration of the effect differ, as imaged by the two wavelengths. The two images are shown on different scales to optimize the contrast between the increased perfusion and baseline (first frame); however, the area and response of the flux in comparison with baseline remains the same, independent of the scale. During analysis the flux measurements are normalized with respect to baseline. Direct comparisons of flux cannot be made since the two wavelengths penetrate to different depths in the skin, and therefore images represent different blood flows (as shown by the difference in flux values for the equivalent baseline scans); this is one reason for dynamic testing.

Using different wavelengths may therefore expand the potential of the laser Doppler technique to rheumatologists by (i) allowing assessment of blood flow in deeper structures, such as entheses and the capsules of superficial joints, and (ii) allowing more detailed

assessment of the different skin microvessels than has been possible previously, with implications for the study of the pathophysiology of Raynaud's phenomenon and complex regional pain syndrome.

Cautionary notes

Unlike LDF, LDI is still primarily in the research arena. Since LDI provides arbitrary perfusion measurements, its strength as an investigative tool lies in comparative data such as baseline and stimulus or treatment response. It is therefore vital that measurements are taken under the same conditions where appropriate (e.g. same site studied, same ambient temperature, full acclimatization, caffeine-, nicotine- and vasodilator-free etc.), with the same LDI parameters (e.g. wavelength, scanning speed, scanning distance, DC values and image normalization) to ensure valid comparisons.

It may also be necessary to consider issues which are normally more relevant to LDF, such as the electrical and biological zeroes of the system, particularly if non-relative measurements or comparisons between wavelengths are being made. Although it would be ideal to assume that when the imager is incident on inert material, for example white plastic, the signal would fall to zero, this is not the case. Instead an offset flux value exists, known as the electrical zero of the system. This is the value of residual signal due to electrical components of the system, which includes 'dark' or 'shot' noise [70]. This value should be constant for a given single wavelength imager and therefore is not normally of concern. The biological zero, however, varies from person to person and represents the signal from static tissue with no blood flow; for example, the flux signal from a finger, occluded above systolic pressure. This signal is due to several factors, including cell motion within the bone and Brownian motion of cells [71].

When more than one wavelength is used, it must be realized that absolute comparisons cannot be made since the light penetrates to differing depths, may image different vessels and may have differing electrical and biological zeroes.

Another factor to be considered is skin colour. As previously discussed, darker skins are more difficult for light to penetrate due to absorption by chromophores, and near-infrared wavelengths tend to be used to overcome this problem. However, when heat or vasodilatory stimuli are used it is important to consider that Caucasian skin in general becomes pinker with increased blood flow and that this may affect the DC signal which is used to normalize the flux scan.

Finally, temporal variations in blood flow over short periods (days to weeks [72]) and over longer periods (due to seasonal changes [73]) may need to be taken into account, as does the effect of temperature variation, physical and mental activity and consuming certain vasoactive substances (for example caffeine), which have been shown to have significant effects on the laser Doppler technique [72].

Future developments

LDI will continue to evolve as laser and photodetection technology develops; simultaneous scanning with two wavelengths is currently possible. As measurements become less time-consuming and more cost-efficient, it is anticipated that there will be a movement of LDI from research into clinical diagnosis.

In addition to further examination of previously mentioned rheumatological conditions, investigation of LDI into conditions which have previously only been studied with LDF is warranted. These include systemic lupus erythematosus (LDF studies suggest that although nutritive perfusion is impaired, as observed by capillaroscopy, overall blood flow is not reduced but rather redistributed via non-nutritive blood flow [74]), Sjögren's syndrome (in

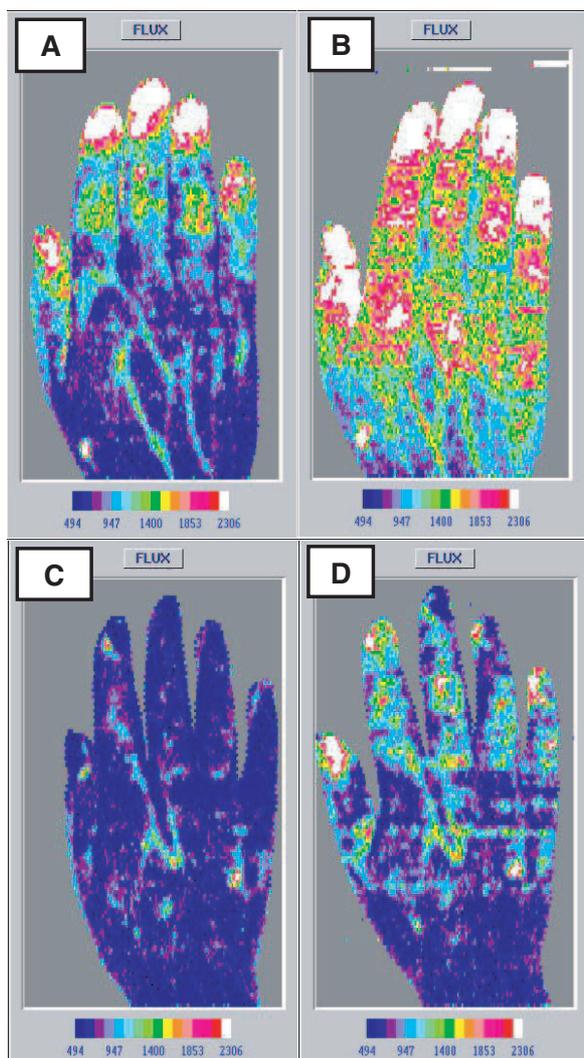


FIG. 3. Smoothed images of the perfusion of hands initially at 23°C (left column), warmed to 30°C (right column). (a and b) Healthy control. (c and d) Patient with SSc. The flux scale is displayed below each image (white, high perfusion; blue, low perfusion). All images are shown at the same scale for comparison.

which LDF studies point to cholinergic dysfunction [75]) and fibromyalgia (to further investigate the suggested relationship between fibromyalgia and temporary hypoxia [76]).

A further direction for future development is optical Doppler tomography (ODT), a non-contact, non-invasive, *in vivo* imaging modality which allows the simultaneous observation of spatial characteristics of tissue structure and blood perfusion dynamics, giving depth-resolved blood flow [77]. The technique is based on a combination of LDF and optical coherence tomography (OCT) [78, 79] which is widely used in research for an array of biomedical applications. Preliminary imaging with OCT has been carried out on normal and osteoarthritic cartilage specimens *in vitro*, highlighting specific structures, such as fibrillations and fibrosis. Herrmann *et al.* [80] suggest that future directions could include integration of OCT into arthroscopes for *in vivo* investigations.

OCT provides imaging with high spatial resolution, of about 10 μm , over a range of penetration depths, from the superficial to the deep dermis, as chosen by the operator. As the beam from a broadband light source (non-laser) is focused onto and scanned

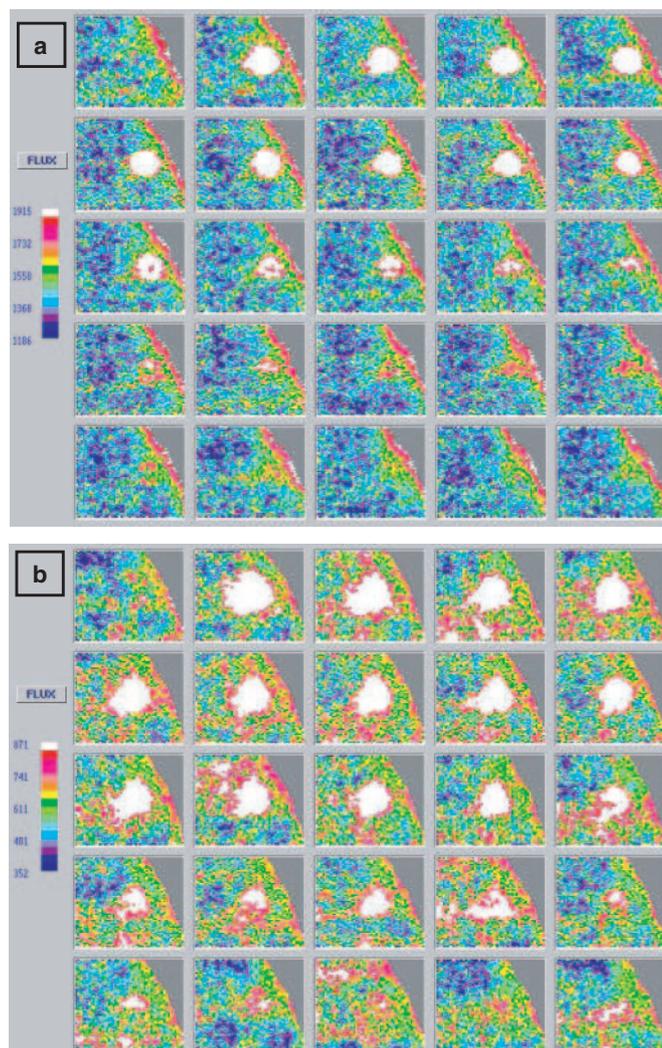


FIG. 4. Two sequences of images of the dorsum of the hand (timeline moving from left to right, top to bottom). Baseline (top left-hand corner) is followed by hyperaemia, induced by localized heating in a healthy control, which decreases back to baseline. Each frame is approximately 1 min in duration. As imaged by (a) 532 nm (green) and (b) 633 nm (red) wavelengths. The heat stimulus was provided by a circular heating pad at 34°C 2 cm in diameter. The pad temperature was increased to 40°C in increments of 2°C every minute and then remained at this final temperature for 3 min; total treatment time was 6 min. To optimize the contrast, different scales are used for each sequence and the images have been smoothed. The increase (with respect to baseline) and duration of hyperaemia are greater for perfusion imaged by the red wavelength than for that imaged by the green. The percentage increase of the peak flux from baseline for the above scans is 570% for 633 nm and 180% for 532 nm.

across the surface of the skin, a video-like image of the layer of tissue is formed in real time. This can then be repeated at various depths to provide a three-dimensional image of tissue structure providing *in vivo* histology. An alternative to scanning the laser is whole- or wide-field imaging, whereby the total area of interest is illuminated by a collimated beam, rather than scanned. Analysis is carried out, again in real time, but for simultaneous measurements across the whole area [81–83]. The combination of OCT with LDF in ODT provides an imaging system with spatial and blood flow velocity information superimposed, which is non-contact and highly sensitive.

ODT has many potential applications across a range of biomedical fields, several of which have already been explored with preliminary research [84–87], but there are several which are particularly of interest in the area of rheumatology. The method allows high-resolution observation of skin microcirculation over a continuous range of depths, facilitating, for example, efficacy studies of pharmacological intervention with vasoactive compounds and providing three-dimensional, real-time, determination of drug penetration and distribution kinetics.

Conclusions

While LDI has already contributed significantly to the measurement of cutaneous perfusion in rheumatic diseases and to an understanding of their pathogenesis, the full potential of the technique is still to be exploited. So far most studies have been cross-sectional; longitudinal studies assessing disease progression and response to vasoactive treatments will provide further insight into pathophysiology and treatment effectiveness.

As techniques and equipment continue to improve, so will the information gained, as with, for example, simultaneous dual-wavelength imaging, which removes the uncertainty of reproducibility when comparing the same stimuli. The relatively new techniques of OCT and ODT will further improve the study of rheumatic diseases, allowing real-time imaging of tissue and blood flow at much higher resolution.

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