A new method for visualizing red blood cell content in the microcirculation

Martin J. Leahy, Jim O’Doherty, Gert E. Nilsson, Joakim Henricson, Chris Anderson, and Folke Sjöberg

A low-cost, noninvasive means of imaging red blood cells shows promise in applications aimed at assessing tissue viability.

Although clinicians continue to employ user-dependent observational methods to assess tissue microcirculation, newer imaging technologies offer improved accuracy. Laser Doppler perfusion imaging (LDPI) to assess burn depth, for example, provides accuracy of 97%, compared with 60–80% for other methods. Wound healing and skin graft monitoring, skin allergy patch testing, and similar assessments could likewise benefit from alternatives to visual inspection.

However, clinical acceptance of LDPI has been hampered due to susceptibility to movement artifacts, signal interference with ambient light, and difficulty registering decreases in blood perfusion at subnormal levels, which can occur with vasoconstriction or blanching.

As an alternative to LDPI, we have employed polarization spectroscopy (PS). This method performs subsurface imaging by accepting light that has been backscattered from deeper tissue layers, while rejecting polarization-maintaining light reflected from the skin surface. PS has proved useful in noninvasive investigation of many conditions associated with microcirculation. This form of subsurface imaging may be described as subepidermal.

Used in conjunction with reflectance spectroscopy, standard digital photography, and algorithmic calculation, PS has enabled us to develop, in collaboration with the Swedish firm Wheels-Bridge AB, which specializes in tissue viability, a cost-effective and portable technique. After a subepidermal photo of the subject area is captured, its 8bit red, green, and blue (RGB) representation is further processed by imaging software. An algorithm is applied that takes into account the higher absorption of green light by red blood cells (RBCs) and also the relatively low absorption of red and green light by surrounding background tissue. Each pixel is represented on a tissue viability index (TiViindex), defined as a global expression of the capacity of the tissue to react to stimuli by altering microvascular perfusion. The processed image is a pseudo-color-coded map in which red and blue represent high and low RBC content, respectively. An example is shown in Figure 1.

The algorithm is designed so that the processed image does not depend on total light intensity. Image acquisition is instantaneous, thereby eliminating the motion artifact problem or potential for misinterpreting temporal microvasculature alterations as spatial heterogeneity, which can happen with LDPI. Acquisition is full-field (wide angle) and does not require scanning or rastering of laser beams.

Although the research on PS is relatively well established, use of an RGB camera and specifically mapping RBC concentration represent novel uses in this context. Ex vivo experiments involving the imaging system and whole blood at various concentrations...
Figure 2. The comparative effects of iontophoresis of acetylcholine are shown in the bottom two quadrants.

Figure 3. The before and after effect of methyl nicotinate.

tions compare nicely with computer simulations of our theory, which describes a linear relationship between the output signal, or TiVi $\text{index}$, and increasing RBC concentration. Monte Carlo computer simulations of polarized light propagation through tissue provide an approximate penetration depth of photons into the simulated medium. When the polarization state is not taken into account, the average sampling depth of photons is approximately 350 $\mu$m in the red region of the spectrum. This sampling depth increases to 490 $\mu$m when only photons emerging from the subepidermal region are considered.

We have also worked with dermatologists at the University Hospital, Linköping, Sweden, to design specific tests that further assess our technology. Tests already carried out include iontophoresis of acetylcholine diluted in physiological saline (10mg/ml), and comparison of vasodilatation by methyl nicotinate (50mmol) with vasoconstriction produced by the topical corticosteroid clobetasol propionate (0.5mg/ml). The blood vessels undergo dilation or constriction after interaction with the receptors, giving rise to an increase or decrease in tissue/blood concentration. Images are acquired every 5s over 10min. Figure 2 shows the effects before (upper two quadrants) and after (lower two quadrants) iontophoresis. It is also possible to examine the area affected over time and the strength of the reaction for every image. This test may prove useful for the study of peripheral neuropathy in diabetics, a group known to have reduced or even no response to acetylcholine. The spatial heterogeneity of the reactions can both be shown in images and demonstrated statistically.

Future directions involve development of a ‘movie mode’ in which real-time online imaging will capture frames at a resolution of $256 \times 256$ pixels. We have already developed a system at 25 frames per second (fps), but it currently processes images offline only. Examples of video-mode TiVi imaging can be seen in Figures 4 and 5. We are hoping to develop substantially faster image acquisition and image resolution greater than current LDPI technology can provide (frame speed of $50 \times 64$ pixels in 5s).

Software is under development to capture TiVi images in real time from external devices at a frame rate of 5 frames per second using $256 \times 256$ pixels. High-speed FireWire technology in combination with a dedicated color filter for each region of the visible color spectrum should make this possible. Future work will involve testing of real-time physiological situations in which TiVi images will be beneficial. Study of reactive hyperemia in patients with neuropathy, known to have a weak response to this maneuver, is one example.

This research is supported in part by VINNOVA, the Swedish agency for Innovation Systems, and by IRCSET, the Irish Research Council for Science Engineering and Technology.
Martin J. Leahy
Physics
University of Limerick
Limerick, Ireland
http://www.ul.ie/~leahym/
http://www.ul.ie/~physics

Martin Leahy completed a DPhil in biomedical engineering in 1995 at the University of Oxford, Faculty of Clinical Medicine. In 1991 he and a colleague established Oxford Optronix Ltd. From 1995 he carried out instrumentation research at the University of Oxford. He joined the University of Limerick in 2000, where he is leading a number of projects in biomedical optics and is course director in applied physics. He was elected to fellowship of the Royal Academy of Medicine in Ireland in 2001.

Jim O’Doherty
Department of Physics
University of Limerick
Limerick, Ireland
http://www.ul.ie/~physics

Jim O’Doherty received a BSc in applied physics from the University of Limerick in 2003. He is currently completing a PhD in physics at the University of Limerick. His doctoral work is focused on developing the tissue viability system, and his interests include image processing, computational modeling, and imaging instrumentation.

Gert E. Nilsson
Department of Biomedical Engineering
Linköping University
WheelsBridge AB
Linköping, Sweden
http://www.wheelsbridge.se

Gert Nilsson is professor of biomedical instrumentation, Linköping University, Sweden and inventor of several technologies for skin assessment, including the Evaporimeter, Laser Doppler flowmeter, and Laser Doppler imager. He is the founder of WheelsBridge AB, the firm that has brought to market the technology presented in this article.

Joakim Henricson and Folke Sjöberg
Institute for Biomedicine and Surgery
University Hospital
Linköping, Sweden
http://www.hu.liu.se/ibk

Joakim Henricson received his degree in medical biology from the University of Linköping, Sweden, in 2003. He currently holds a postdoctoral position with the Department of Medicine and Care at the University of Linköping. He is working with iontophoresis and imaging instrumentation for assessment of skin microvasculature function.

Folke Sjöberg received his MD from Linköping University School of Medicine in 1983 and a PhD in 1990. He became full professor in 2000. Specializing in critical care and burn intensive care, his research interests include burn care, anesthesia, intensive care, laser doppler perfusion monitoring methodology, iontophoresis, and vascular dysfunction.

Chris Anderson
Department of Dermatology
Institute for Biomedicine and Surgery
University Hospital
Linköping, Sweden
http://www.hu.liu.se/ibk

Chris Anderson has research interests that encompass skin inflammation and skin barrier function. He uses microdialysis and bioengineering techniques in models of skin disease.

References
5. S. J. Morris and A. C. Shore, Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: possible mechanism, J. Physiol. 496.2, pp. 531–42, 1996.