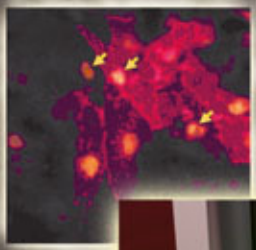
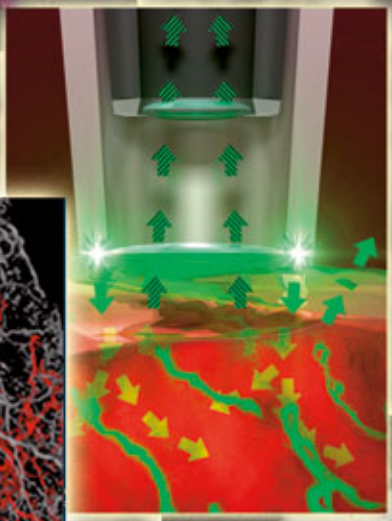
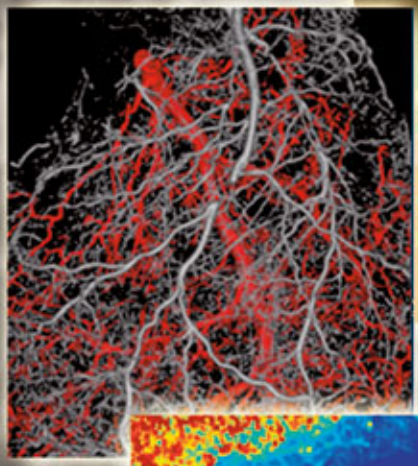
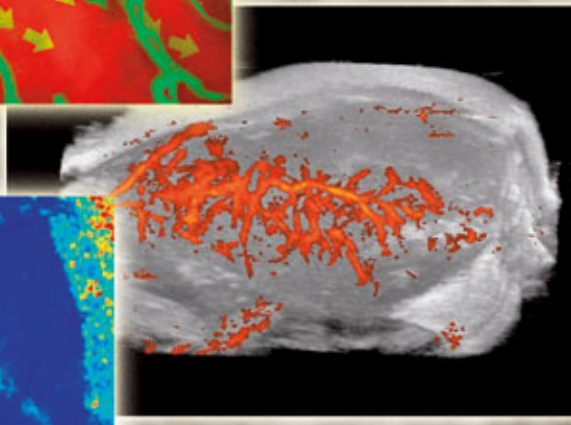
Microcirculation Imaging

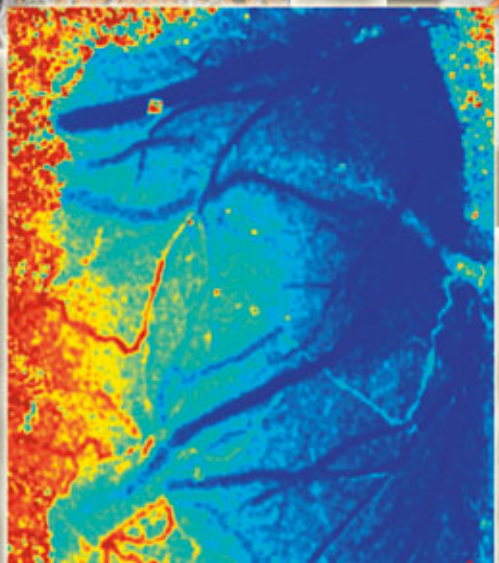
Edited by Martin J. Leahy











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Microcirculation Imaging

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Cover
1. Live imaging of the developing vasculature in Tg(Flk1-myr::mCherry) X Tg(Flk1-H2B::EYFP) mice using confocal microscopy (for more details, see Fig. 2c in Chapter 12 by Irina V. Larina and Mary E. Dickinson). 2. The working principles of sidestream dark-field (SDF) imaging (for more details, see Fig. 2 in Chapter 2 by M.J. Milstein *et al.*). 3. Three-dimensional image of an experimental tumor (KHT) growing in a mouse (for more details, see Fig. 6 in Chapter 13 by Stuart Foster). 4. Blood flow changes during stroke: relative cerebral blood flow 10 min after occlusion of the middle cerebral artery in a rat (for more details, see Fig. 12 in Chapter 8 by Bryers *et al.*, with kind permission by SPIE and A.K. Dunn, University of Texas). 5. Micro-CT image data (20 µm isotropic voxels) of the vascular bed of a rat heart, which was filled with a contrast agent (Microfil) and was imaged *in situ*. For more details, see Fig. 15 in Chapter 14 by Timothy L. Kline and Erik L. Ritman).

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Contents

Preface

This book brings together the main techniques used for imaging the small blood vessels, which supply nutritional oxygen and remove waste products from the cells of the body. The rapid development of new techniques to image the microcirculation (vessels $<100\,\mu m$ in diameter) in two and three dimensions was the main driver for publishing this book. The macrocirculation of the cardiovascular system enjoys a special place in medicine and is well catered for with imaging modalities that are ever present in our hospitals. X-ray CT, ultrasound, MRI, and PET all play an important role in diagnosis and treatment of the disorders of the large vessels. However, there is growing realization that some of the diseases that most threaten the quality and quantity of life in the developed world, such as diabetes and cancer, have their origins in the microcirculation. Therefore, new techniques with appropriate resolution were required to image these smaller vessels, and these largely depend on the rapid developments in photonics.

It is impossible to present all techniques that have been applied to microcirculation in this book. The editor is grateful to the (unknown) reviewers of the original proposal for their suggestion to supplement the biophotonics techniques well known to him with MRI and high-frequency ultrasound. The result is a more thorough covering of the field, although I am open to further suggestions for additions in future editions. Researchers, practitioners, and professionals in the fields of diabetes, cancer, wound healing, biomedical optics, and biophotonics, as well as professionals in other disciplines, such as laser physics and technology, fibre optics, spectroscopy, and biology, will find the book a useful resource. Graduate and undergraduate students studying biomedical physics and engineering, biomedical optics and biophotonics, and medical science would benefit greatly from consulting this reference.

Several Irish and international grants supported this project, particularly the National Biophotonics & Imaging Platform Ireland, funded by the Irish Government under the national development plan (NDP) 2007–2013 HEA PRTLI IV. I greatly appreciate the cooperation, contributions, patience, and support of all the contributors, my colleagues from the School of Physics at NUI Galway, the Department of Physics at the University of Limerick, the Royal College of Surgeons, and the

National Biophotonics and Imaging Platform. Last, but not least, I would like to thank my family for their support and understanding during my work on this book.

NUI, Galway
March 2012

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1
A Historical Perspective of Imaging of the Skin and Its Gradual Uptake for Clinical Studies, Inclusive of Personal Reminiscences of Early Days of Microcirculation Societies

Terence J. Ryan and Martin J. Leahy

Modern microscopy of microcirculation was conceived by the observers of especially the seventeenth century, but gestation was in the hands of the national and continental Microcirculatory Societies that began in the decade 1954–1964. They were a meeting point between the laboratory investigator of microcirculation and the clinician. Zweifach [1] reviewing historical aspects of microcirculation research wrote ''The usage of the term 'microcirculation' in an organic context is of comparatively recent vintage, first appearing consistently in the literature during the 1950s.'' Global collaboration with a strong clinical input began with World Congresses of Microcirculation first held in Toronto in 1975, then in San Diego in 1979, next in Oxford in 1984, and every four to five years since then. As an Oxford clinician specializing in care of the skin, T. J. R. was roped in at the deep end of nonclinical pursuit of microcirculation on several occasions. However, this began by a chance observation that if one pushes tissue fluid away by indenting the skin with a steel probe, one gets much better visualization of the skin capillary bed [2, 3]. It has long been known that one sees at the surface of the skin only what its optical properties allow. Excised epidermis from white skin placed over a printed page is transparent enough to read through it. Melanin of pigmented skin prevents such visualization. The redness of blood provides *in vivo* pinkness, but blue blood in veins is a consequence of blue light being scattered more than red. This does not stop the practised clinician from easily recognizing the condition when black skin is flushed. Newton [4] discussed the decomposition of white light, and Doppler [5] made known that the effect of movement toward or away from the observer influenced the color observed. For several centuries, any observation of complex surfaces reflecting light was clarified by applying transparent oils to that surface.

1.1
Early History

George P. Fulton [6], Professor of Biology at the Boston University, writing on the historical perspective of the founding of the American Microcirculatory Society, lists Harvey and Lord Lister amongt the early influences on microcirculation, but it

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was interest in microscopy and improved microscopes in the seventeenth century that led to the first observations of blood flow by better imaging. The discoverers and forerunners of imaging were fascinated when they applied their new magnifying devices, and detecting transparency in some living tissues saw for the first time the movement of the content of the small capillaries. These early observations especially on red cells were well reviewed for the journal *Blood Cells* by Bessis and Delpechi [7]. As stated in that review, three men, Malpighi, Leeuwenhoek, and Swammerdam, made the most of the improvements in magnifying lenses in the early seventeenth century and noted red particles in blood capillaries in transparent tissues. Of these, van Leeuwenhoek of Delft (1632–1723) gained the most publicity by getting his observations published by the Royal Society of London [8]. Indeed, it was the tax collector, van Leeuwenhoek, who contributed one of the greatest innovations through his hobby by producing a very short focal length lens. This avoided chromatic aberrations, which plagued compound microscopes of the day, and yet produced sufficient magnification to reveal the structure of the blood cell (Figure 1.1) and its movement within organs and organisms if they were sufficiently transparent. It was this innovation that allowed Malpighi to confirm Harvey's theory that blood circulates from the arterial to the venous side via these small capillaries, and indeed, it can be considered the discovery of the microcirculation.

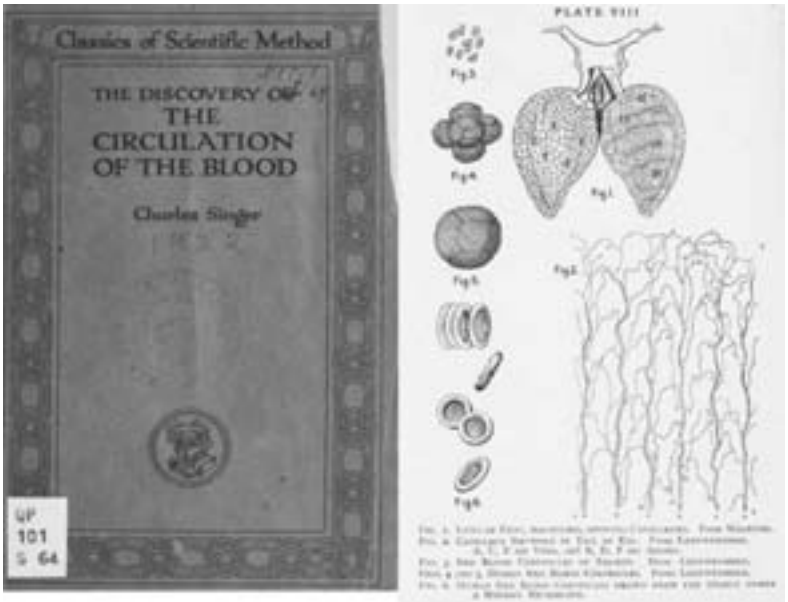

Figure 1.1 The discovery of the circulation of the blood, the shape of the red blood cell, and, most importantly, the microcirculation (*after The Discovery of the Circulation of the Blood*, Charles Singer, G. Bell and Sons Ltd., London, Ref. [9]).

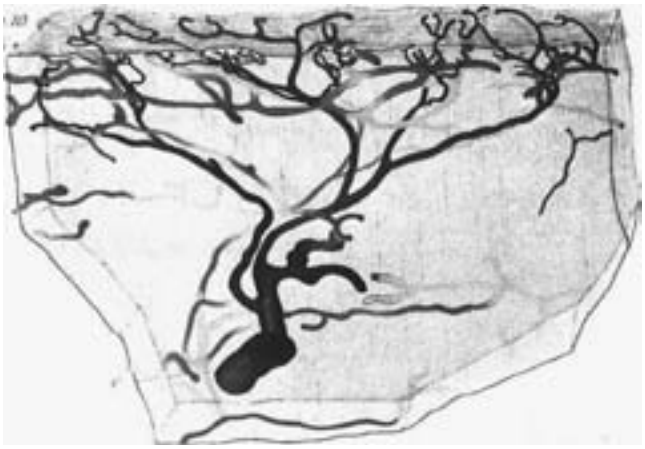

Figure 1.2 The late nineteenth century drawings of Spalteholz, illustrating the distinctive candelabra pattern of the blood supply of the skin.

van Leeuwenhoek, like several other observers, noted blood circulating in the capillaries of the louse intestine. He also noted the flexibility of the red cell, with shape change to facilitate, he supposed, their passing through small capillaries. Probably, however, he was observing clumped red cells, known as *rouleaux*, rather than single red cells. Furthermore, he was not an anatomist and could not even distinguish muscle tendon from nerve, until a critical anatomist, Swammerdam, on several visits, somewhat to his annoyance, took over his project.

Malpighi of Bologna (1628–1694) was a more casual observer, probably with less passion for red cells than for microscopes. He had observed these particles a few years earlier in fish gills, in dog and frog lungs, and in the hedgehog. Compared to van Leeuwenhoek, he was not an enthusiastic publisher of his findings, the importance of which was only recognized long after his death [10]. The only other significant technology of the time was the use by Leeuwenhoek of a fine glass capillary pipette to draw blood from the vessels of the louse, which could then be more easily manipulated under the microscope.

In the nineteenth century, anatomists studying cadavers provided clear images of the smallest blood vessels, and Spalteholz [11] provided some of the best imaging by injecting colored gelatin solutions into the arteries (Figure 1.2), showing clearly the candelabra pattern of the skin's blood supply.

The challenge was finding transparent tissues that could be placed under a microscope and a short-distance high-power lens that limited all but totally immobile tissues and thus except for the nail fold, excluded humans from observation. It was the window of glass, or the lighter and less breakable Perspex placed in the skin of the rabbit's ear or the cheek pouch of the hamster or in the dorsal skin of the mouse, that allowed long-term and frequent observations of life events and allowed greater understanding of the role of the skin's circulation. Sandison

[12, 13] and Clark and Clark [14, 15] in their series of publications from 1918 to 1942 first in the tadpole tail and then in the rabbit ear window provided some of the best ever descriptions of observations on visualizing the blood supply of the wounded skin. However, the hamster cheek pouch favored by Herman Berman in the United States and by Sanders and Shubik [16] in the United Kingdom had a vascular bed that could not be criticized for being a wound. It was the preparation that convinced one that the epidermis was the governing body of its capillary bed [17].

It was Starling [18] at the University College London (1896) who made the physiologist aware of pressure, both hydrostatic and oncotic, inside and outside of the capillary, thereby determining one of its most important functions, viz, control of permeability, and opening up the topic of ''*exchange*.'' August Krogh, Professor of Zoophysiology, in Copenhagen, by receiving the Nobel prize and authoring *Anatomy and Physiology of the Capillaries* (1922) [19] not only convinced the scientist and the clinician of the importance of microcirculation but also attracted a new generation of investigators, many of whom were clinicians. He was the teacher of those who refined for human studies modern microscopy and cinephotomicroscopy including Melvin Knisely, at the University of Chicago and the Medical University of South Carolina, who, by improved lighting using quartz conduction [20] and increasing the working distance of the high-power lenses demonstrated how blood flow could be best observed in the human by examining the conjunctival blood vessels. His special interest was the changes that occurred in malaria. The conjunctiva remains the most transparent organ, requiring no anesthesia and being immobilized by looking at a single point. It was the development of long-working-distance lenses that facilitated easy examination with which to astonish a new recruit and introduce the wonders of the microcirculation in the human. Knisely showed that in malaria, the blood became ''sludged'' [21].

1.2 The Microcirculatory Societies

The American Microcirculatory Society was founded at a 1954 meeting of anatomists in Galveston. Most presentations were on *in vivo* microscopy (Conference report 1954) [6, 22].

In the 1960s, many discussions were held with the founders of the American Microcirculatory Society and with like-minded Europeans about collaboration and the setting up of Microcirculation Societies. The Europeans held preliminary conferences in Lund (1959), Hamburg (1960), Pavia (1962), and Jerusalem (1964). The range and the breadth of the field can be read in the Karger of Basel series *Biblio Anatomica* and became further apparent at the 4th European Conference in 1966 organized by the Cambridge anatomist Alexander Monro [23] at Cambridge with the Oxford experimental pathologist, a colleague of Lord Florey, Gordon Sanders as President. These early meetings suffered from the technologies of the time, as described by Alexander Monro [24] when writing about the meeting in Pavia.

"I am sure my Italian friends will forgive me when I say that the organisation of over 150 participants to give the 115 papers on the programme, met with some difficulties-not really anyone's fault- but just one of those things; and one which eventually resulted in all the Italians being asked to read their papers by title only: this must have been very disappointing for them but thanks to their self sacrifice the visitors were able to get home on time! Simultaneous translation into four languages had been arranged, but the opening session for the principle speakers started with a problem, for the slide projectionist was late and when he switched on the lamp, the wrong voltage had been connected and he had no spare: half an hour later the first American-sized slide was inserted but it did not fit, and we watched-fascinated-while progressive cracks in the glass were followed across the screen. The projector was taken out of service in order that it could be dismantled. No other slides were shown that morning. Those who had hoped to show their slides insisted that they should have equivalent time in the afternoon, but as I was the chairman at that session I insisted that we should endeavour to keep more closely to the published timetable. At last a compromise was found, but inevitably everything was late, and when the speakers read their papers faster than they had intended originally, the translators could not keep up in all languages, because some were not so concise as others. Fortunately tolerance and good humour prevailed and all the social events were superb. We started with a blessing from a Cardinal and finished with a banquet at which an enormous fish was displayed before we devoured it. I believe the dancing continued until the dawn, to a piano expertly played by Dr Krahl, from Maryland USA who had paid his way through medical school by use of this talent."

As a medical student in Oxford in the 1950s, one would attend Florey's formidable lectures and find that his first love was not penicillin but lymphatics. Something seen in his lectures but not seen in his well-known text [25] was a cine film made by Sanders, Florey, and Ebert, in 1940, which introduced slow motion to illustrate shape change of red cells in capillaries using the rabbit's ear chamber and in which they also clearly demonstrated capillary endothelial contraction. It was this film that inspired Alexander Monro to take up the field of blood vessel study. In the same laboratory, John Casley-Smith did his D. Phil on lymphatics with Florey as supervisor after World War II and John became the leader of the field of lymphatic study in Australia and later globally [26]. Sanders led the studies on microcirculation, and many learned the technologies from him.

On the other side of the road from William Dunn School of pathology was the post graduate centre where any visitor would be taken. The glasses of sherry were not small and the conversation was intense with persons such as the haematologist (and biographer of Florey and Fleming) Gwyn Macfarlane to listen to. Gwyn would choose an obscure topic like the origin of the term non-undeodorized cowcake given to his cattle and half an hour of discussion would follow. Gordon would have demonstrated

technologies before lunch and would snooze in the afternoon while his visitor practiced the technology. The day would end on the high table at Lincoln College. 40 years later one Japanese visitor would still recount the day in his lectures as one of the most challenging he had experienced: not yet proficient in English and wedged between a professor of Aviation and a professor of Saxon English for at least two hours.

Terence Ryan was invited by Gordon Sanders in 1965 into the world of microcirculation experts. Gordon was a heavy sherry and wine imbiber at lunch and dinner so observations were only done in the morning. Oddly some of his best dinners at his home were given for the American microcirculation experts, Edward Bloch and John Irwin both of whom were teetotal so Gordon and Terence Ryan often had whole bottles to consume.

The British Microcirculation Society (BMS) was founded in 1965 at a meeting of The Royal Microscopical Society, demonstrating again the importance of the subject of imaging but still tied to microscopes. Its history was recorded by Alexander Monro [24].

The committee meeting that created the British Microcirculation Society was, for someone as junior as Terence Ryan, rather hair raising. Lord Florey had consented to be a member but did not turn up. The mild Gordon Sanders could not control the pedantic Alexander Munro who argued every point of the newly developing rules of the society and Sir Henry Barcroft walked out in a huff. We all had to pay for our lunch which was expensive (Figure 1.3).

It was not a happy occasion; the proceeding committee meeting being one of discord! The costs to individuals was carefully allocated and is seen in the handwriting of its first President Gordon Sanders, a close colleague of Howard Florey in Oxford and well known for his meticulous studies of blood supply in the Rabbits Ear Chamber and Hamster cheek Pouch.

The various continental and national societies were always international in outlook. Thus the committee that organized the European Society for Microcirculation in 1988 had Su Chien, United States, and M. Tsuchiya, Japan, as advisory members.

Hemorheology had as its main concern the fact that the diameter of the red cell was more than the width of the capillary lumen, and shape changes had to be understood if rapid flow were to occur, especially when red cells and the even larger white cells were grouped together.

Sadly, the tall and gangly Knisely allowed his enthusiasm to make him long winded so that his late-in-life guest lectures and after-dinner speeches to the new microcirculatory societies went on far too long. The topic of ''sludging'' and the appearance of rouleaux formation of red cells helped to introduce the subject of blood rheology to the Microcirculation Societies, which were inaugurated when rheology was much debated, and the experts on this were the burly figure of Copley contrasting with the asthenic Oxford figure, Scott Blair, editor of *Biorheology*,

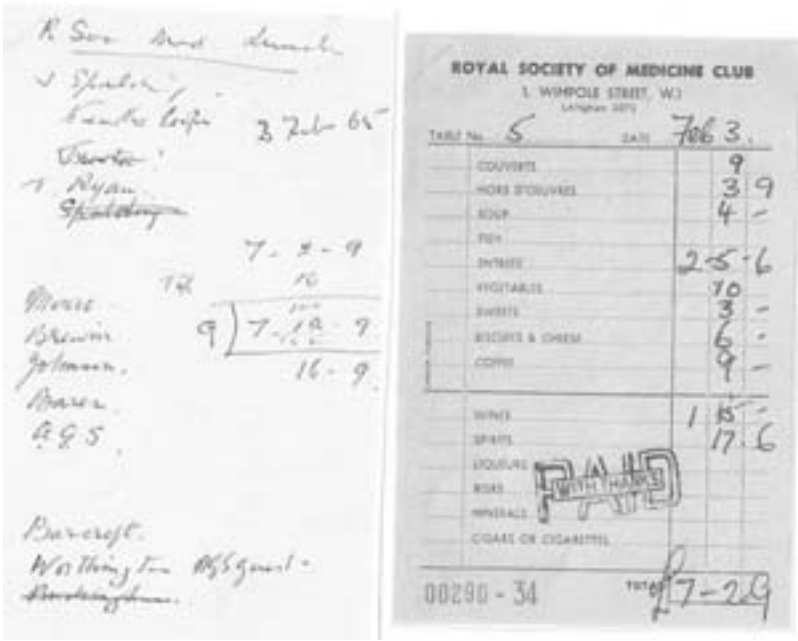

Figure 1.3 The lunch bill for the first meeting of the new committee of the British Microcirculation.

in which were published the early proceedings of the BMS edited by Terence Ryan. There was also a certain amount of modeling of red cells to show how the geometrical and elastic properties of one red cell during flow would affect the same properties of the immediately following cell. Such modeling by engineers (viz Lighthill of Imperial College London) [27] so that red cells looked like a series of square boxes lost some listener's respect for modeling because they seemed to have no visual resemblance to the real thing. The study of the vascular tree in different organs, and the circulation through it, raised increasing concern about the clinical relevance of statements made by those who had never observed the microcirculation *in vivo*. Each new observation raised new questions, many of which could only be answered by better imaging. Alexander Monro gave several talks to the BMS during its first 25 years about early measurements of red cell velocity, first in the eel by Leeuwenhoek in 1689 and then in the frog by Stephen Hales in 1733. He himself had rheology and microscopy as interests. In developing cine techniques for measuring individual red cell flow and shape change, Alexander Monro in 1969 [28] put much effort into using a narrow collimated beam with energy ranging from 0.23 to 200 J from the Strobex model 135 manufactured by Chadwick-Helmuth Co Inc. The further development of blood flow measurement depended on velocity techniques such as dual slit devices, allowing measurement of the time taken to move between two points. Advances in the sensitivity of computerized measurement improved accuracy and the recording of measurement. Later reviews [29] reveal a much

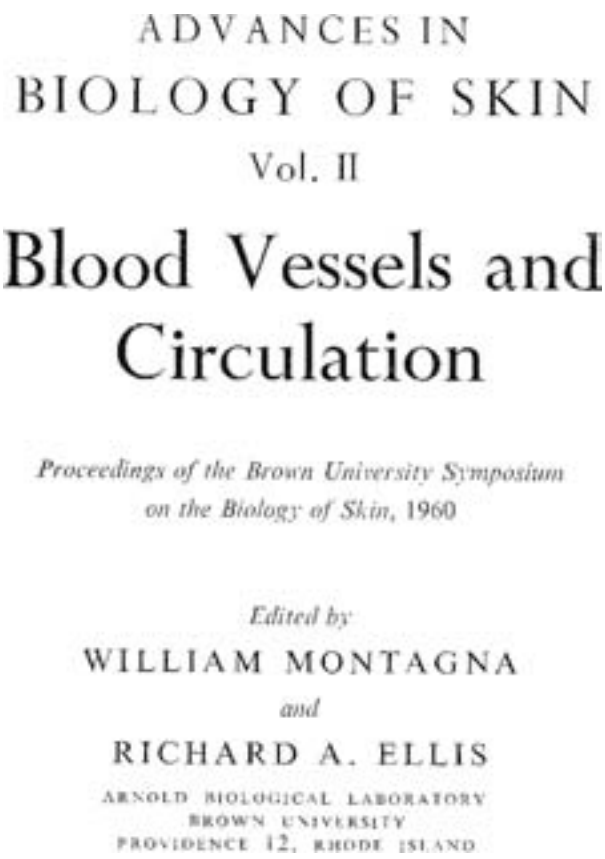

Figure 1.4 The Brown University Symposium on the Biology of the Skin in 1960 was a landmark for identifying the importance of the blood vessels and circulation of the skin.

altered and complex field, and few of the early observations that filled the early meetings of the 1960s have much relevance.

For those interested in the skin, the most influential event was the 1960 Brown University Symposium on the Biology of the Skin, the publication of which was *Advances in the Biology of the Skin, Blood Vessels and Circulation* (Figure 1.4) [30].

Therein was discussed injection of skin with India ink and alkaline phosphatase reaction studies by Winkelmann and his team of the Mayo Clinic as well as by Richard Ellis of Brown University. R. L. de C. H. Saunders reviewed the recent development of X-ray projection microscopy of the skin. George Odland from Seattle reviewed the recent advances in ultrastructure that had been led by Palade [31] and of the skin by Hibbs *et al*. [32]. This publication included an excellent review of capillary microscopy in normal and diseased skin by Michal J. Davis and James Lawler. The skin's capillaries had first been examined as a special interest using reflected light by Lombard in 1912 [33], and Gilje in 1953 [34] was

the first to effectively examine diseases such as psoriasis. Davis and Lorincz [35] showed that removal by stripping with Sellotape of the keratin layer overlying the vascular bed greatly increased the visibility of the capillaries and subpapillary vessels. Alrick B. Hertzman described the convective transfer of heat to the surface whence it is lost by radiative, convective, conductive, and evaporative pathways, with which developed our understanding of the correlation between blood flow and temperature. Grahame Weddell introduced the topic of the innervations of cutaneous blood vessels.

Grahame Weddell was later to become Professor of anatomy in Oxford. Because of his interest in cutaneous nerves he became the centre of attention of the Leprosy World of investigators. He lived in one of Europe's oldest leprosy hospitals. Immigrants from Africa and Asia were housed in the department of dermatology in Oxford so that fresh material could be available. Grahame was a jovial and generous to his junior collaborators but would be in bed by 9 p.m.! He spoke about himself as being worthy of "no more than B+", but he became the centre of a World Class centre for Leprosy research after his retirement from Anatomy and this was based in the Department of Dermatology which at that time had graduated from a septic ward in the old infirmary to the isolation hospital outside the city boundaries. Graduation to nearer the centre of Oxford would occur in 1992 well after Leprosy was shown no longer to be a public health problem. The role of the microcirculation in this disease was shown to account for the localisation of the causative organism in the nose and its dissemination from the nasal cavity. Several studies showed that cooling, hypoxia and impaired fibrinolysis were characteristics of nasal septal blood supply all encouraging the M Leprae to thrive at that site in endothelium [36].

The increasing awareness of the importance of the blood supply of clinicians in Europe was slow to gel. Clinical investigation of the circulation of the skin by Sir Thomas Lewis [37] in the 1920s, a pupil of Starling, led to some detailed reports in *Clinical Science*, which remain a wonderful read. He described very thoroughly the axon flare, the white blanch reaction, reactive hyperemia, and other changes in blood flow. Besides being a good scientist and a clinician, he wrote beautiful English. He recognized that there were different responses to injury of the vascular tree within the skin at different levels within the vascular tree. It was his young colleague Sir George Pickering with whom Terence Ryan worked in 1958 when, as Regius Professor of Medicine, he was completing his book on "high blood pressure." He insisted that whatever one wrote should be in good basic English and he shortened the title of Ryan's thesis to "The blood supply of the skin" from something with seventeenth century length. A brief chapter on the history of imaging using capillary microscopy [38] was part of the first major publication for dermatologists, being a supplement of the *British Journal of Dermatology* in 1969, edited by Terence Ryan, followed by Volume 2 of the series *Physiology and Pathophysiology* of the skin [39], and a review of the lymphatic system [40] and

Microvascular Injury [41]. The emphasis of this book was on the clinical need for observations of skin blood supply, and the title was chosen to encourage the field of microvascular research to read an essentially clinical book.

The clinical relevance of microcirculation studies were frequently debated, and it was often stated (by, for example, Ben Zweifach (personal communication after reading *Microvascular Injury*)) that the best basic science was being generated in the United States but its applied clinical relevance was a feature of European work. Ben Zweifach was the doyen of microcirculation for longer than any other significant scientist. First working with chambers, they demonstrated preferential channels through the capillary bed in the early 1930s [42], and he continued publishing for 50 years. A scientist rather than a clinician, he hosted the second World Congress in San Diego.

TJR remembers there was a stall in the exhibition hall of a Chinese memory enhancing drug which Ben Zweifach and his wife with Mrs Anne Ryan set aside evidence and decided after only a little discussion to give it a go; seemingly 20 years later still effective especially in Ben's case who was much the elder of the three! The site of this 2nd World congress was shared with three other conferences. The normality of dress of the microcirculationists was distinctive in La Jolla in comparison with that of the bulging at the waist of a Fat Boys congress, the grouping and glamorous arm waving of the Chair Leaders Congress and the bearded gentlemen in shorts attending an Anthropology Congress.

After the successful first meeting of the BMS with the Royal Microscopical Society, it was decided to have a meeting of the BMS in Oxford. Terence Ryan, as local organizer, along with the orthopedic surgeon Joseph Trueta, who came to Oxford fleeing Spain after the Civil War and had been the first to study angiography [43], to image the kidney to delineate renal vasospastic responses after crush injury of the legs, relevant to the bombing injuries in London and Coventry during World War II. He was keen to demonstrate imaging of blood supply in bone while Terence Ryan along with the plastic surgeon Tom Patterson imaged the skin. Tom used vital dyes to produce bright green or blue pigs in the study of skin grafts. These colorful pigs were one of the most popular exhibits of early meetings. They became the animals whose skin seemed most like that of humans for several studies by several investigators in Oxford.

In 1967, at a meeting at the Royal Postgraduate Medical School [44], the cardiovascular group demonstrated one of the first instruments to measure forward and reverse flow using a 3 mm diameter nylon tube on which was mounted an electromagnetic velocity-sensing element, and Eva Kohner showed how useful fluorescence photography was for the study of diabetes, allowing the capillary bed of the retina to be studied and the natural history of retinopathy and response to treatment to be assessed. The full potential of high-quality fluorescein angiography and high-speed cine fluorescence photography was demonstrated at several of the