BLOOD FLOW AND RETINAL OXIMETRY

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SUMMARY

The survey of methods for assessing ocular hemodynamics in glaucoma and AMD presented here is not complete, but it does cover those which are likely to be encountered in the literature. A fundamental problem in getting to grips with the ocular blood flow literature is the difficulty in comparing the results of similar studies employing different assessment techniques. As is evident from the discussion above, each technique evaluates a portion of the ocular circulation in a distinct way. Some of the methods overlap in respect of the tissues they can be used to examine; others are directed at entirely different parts of the ocular vasculature.

If the current pace of refinement of newly established technologies for evaluating ocular blood flow is maintained, they will soon be ready for deployment in the clinic. The ultimate beneficiaries of work in this area will not be researchers, but patients.

INTRODUCTION

The purpose of this paper is to provide an overview of the various ocular blood flow assessment technologies that appear in the literature. None of the techniques have reached clinical use; however the medical literature characterizing ocular blood flow in health and disease utilizes these technologies as the basis for current understanding. Therefore, if the clinical practitioner is to be an educated consumer of the medical literature, a basic understanding of these techniques is vital. The methods that will be discussed are scanning laser oph-

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thalmoscopic angiography with fluorescein and indocyanine green dye, confocal scanning laser Doppler flowmetry, color Doppler imaging, laser speckle flowmetry and non-invasive oximetry.

Scanning Laser Ophthalmoscopic Angiography

The scanning laser ophthalmoscope (SLO) increases the capabilities of quantitative angiography through an increase in image contrast and temporal resolution. The incandescent light source used in photographic and video techniques is replaced by a low power scanning argon laser beam providing excellent penetration of lens and corneal opacities. Since the laser beam only illuminates a single spot on the retina at any moment, overall retinal illumination is reduced and contrast is improved. The excellent optics and pure laser light sources of the SLO are currently most often used in spectral retinal analysis and microperimetry.[1-3]

The SLO is free from many of the deficiencies of the longer established techniques of photographic and video angiography. Through the application of confocal optics, light reflected from the fundus exits the eye, passing through an aperture at the exterior principal focus of the lens before reaching a solid-state detector.[4-6] This detector generates a voltage proportional to the intensity of incoming light. The detector voltage level, measured in real time, creates a standard video signal. Scattered light and light reflected from sources outside the focal plane cannot enter the confocal aperture. The aperture is fully open in angiography mode. The signal is generally passed through a video timer and then an S-VHS video recorder. The resulting images have the high temporal resolution of those obtained with standard video angiography, but spatial resolution and contrast are superior.

The SLO can be used for fluorescein or indocyanine green angiography.[7-9] Excitation provided by a 488nm argon blue laser and a 530nm barrier filter are used for fluorescein angiography.[10,11] A 790nm infrared diode laser with an 830nm barrier filter is used for indocyanine green angiography.[12]

Scanning-laser ophthalmoscopic fluorescein angiography

Macro and micro retinal hemodynamics are quantified by arteriovenous passage (AVP) time and capillary transit velocity (CTV)

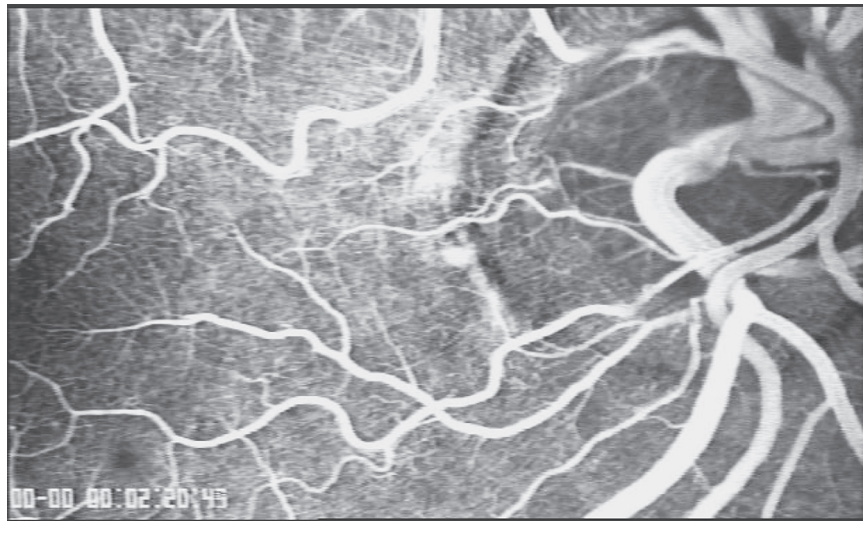

Figure 1. 20 degree fluorescein retinal angiogram using scanning laser ophthalmoscopy.

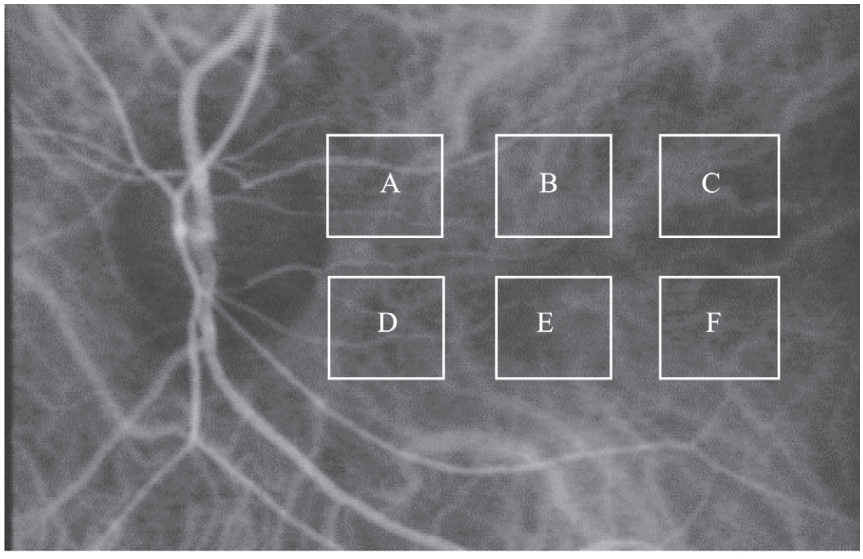

Figure 2. 40 degree indocyanine green choroidal angiogram using scanning laser ophthalmoscopy. Six locations on the image, each a 6° square, are identified for area dilution analysis: A and D for peripapillary choroid, and B, C, E, and F for macular area.

respectively.[4,6] (Figure 1) AVP time is analogous to mean circulation time. It is equal to the difference in the time of dye arrival in a retinal artery and corresponding vein.[4,6] The SLO's high temporal resolution permits visualization of hyper- and hypo-fluorescent segments in the perimacular and peripapillary capillary circulation. These dark and light segments are readily visible as they pass through the capillaries. Using an image analysis system and custom written software, it is possible to quantify CTV by measuring the distance they travel in a sequence of frames and dividing by the time taken to cover that distance.[11,13,14]

Scanning-laser ophthalmoscopic indocyanine green angiography

The bulk of ocular blood flow is supplied by the choroid, especially flow to the outer retinal layers and optic nerve. It is therefore important to have a method for the evaluation of the vasculature of this region. The application of ICG angiography and SLO has overcome some of the limitations of fluorescein angiography in the study of choroidal blood flow.[15] The near-infrared light used for scanning laser ICG angiography penetrates the pigmented layers of the fundus much more efficiently than the shorter-wavelength light used in fluorescein angiography.[8,12] ICG's high affinity for plasma proteins is also advantageous. Within the circulation approximately 98 % of the dye binds to plasma albumin or lipoprotein.[16,17] As a result, ICG diffuses slowly out of the fenestrated choriocapillaris; unlike fluorescein dye, which leaks very rapidly, severely impairing the delineation of choroidal detail. High-resolution ICG images can now be produced by scanning laser ophthalmoscopy.[8,12]

Indiana University's Glaucoma Research and Diagnostic Center has developed a new analysis technique for the quantification of choroidal ICG angiography using the SLO.[18] The entire 40° ICG angiogram is divided into a number of small regions, and dye-dilution curves are created for each region. Six locations on the image, each a 6° square, are identified for analysis (Fig. 2).[18] The average brightness of the area contained in each box is computed for each frame of the angiogram (Fig.3). Area dye-dilution analysis identifies three parameters from the dye-dilution curves: 10% filling time, the slope of the curve, and maximum brightness (Fig. 3). The 10% filling time is the amount of time required to reach a brightness 10% above baseline. This

parameter describes rapidity of filling in the early choroidal filling phase. Slope of the filling curve is calculated by (i) noting the difference between the intensity at 10% filling and that at 90% filling, and (ii) dividing the difference by the number of frames during that time, where each frame represents a known time interval. This parameter represents the overall speed of blood flow as it enters the choroid.[18]

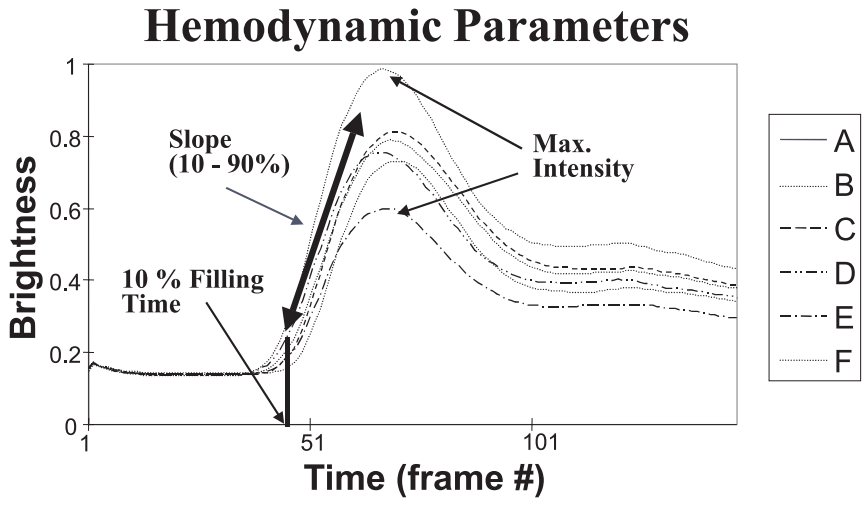

Figure 3. Hemodynamic parameters in area dilution analysis of indocyanine green choroidal angiography using scanning laser ophthalmoscopy. Area dye-dilution analysis identifies three parameters from the brightness maps: 10% filling time, the slope of each curve, and the maximum intensity of brightness. The 10% filling time is the amount of time required to reach a brightness 10% above baseline. The slope is the intensity difference between 10 % and 90% filling divided by the time interval between them.

Confocal Scanning Laser Doppler Flowmetry

The confocal scanning laser Doppler flowmeter (CSLDF), or Heidelberg retinal flowmeter (HRF) manufactured by Heidelberg Engineeering GmbH, Heidelberg, Germany, combines a laser Doppler flowmeter with a confocal scanning laser tomograph.[19-21] The instrument obtains images of a 2560 x 640 mm^2 x 400 mm deep region of the retina or optic nerve head with a measurement accuracy of 10 mm. A 790 nm laser scans every line of the target at a line sampling rate of 4000 Hz.[22] Upon completion of the scan, the HRF computer performs a fast Fourier transform to extract the Doppler frequency shift of reflected light, point by point. A frequency spectrum is calculated for each point of the scan. Each frequency location on the x axis of the spectrum represents a blood velocity, and the height of the spectrum at that point is a function of the number of blood cells giving rise to the intensity of emission observed at that point. Integrating the spectrum yields a value for total blood flow. On its default setting the instrument analyses a 10 pixel x 10 pixel (100 x 100 mm) region of tissue.[19,21,22]

The CSLDF has been shown to measure blood flow in an artificial capillary tube accurately ($r=0.97$, $p<0.0001$), and operators have obtained coefficients of reliability near 0.85 for acutely repeated volume, velocity, and flow measurements from 10 pixel x 10 pixel sampling areas.[23,24] Over a longer time, reproducibility from these small sampling boxes is inadequate; the coefficients of variation of measurements taken each week for four weeks has been shown to average 30% of the mean.[22] Furthermore, perfusion of the conventional / default 10 pixel by 10 pixel area for data collection may not be representative of blood flow in the retina as a whole. To overcome these limitations the Glaucoma Research and Diagnostic Center of Indiana University has developed a pixel-by-

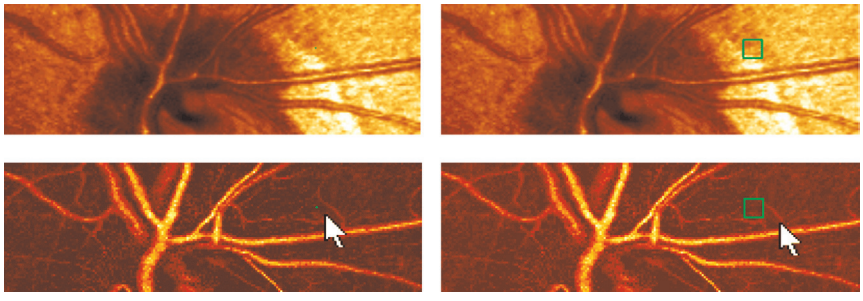

Figure 4. Confocal scanning laser Doppler flowmetry (Heidelberg Retinal Flowmeter) of optic nerve head and peripapillary retina. The left arrow indicates a 1 pixel x 1 pixel measurement window, which collects flow values from the entire retina except for large vessels, for new pixel-by-pixel analysis. The right arrow indicates a 10 pixel x 10 pixel measurement window used for conventional analysis.

pixel analysis method in which individual qualifying pixels from the entire 256 pixel x 64 pixel image are examined (fig. 4).[22] Large vessels, peripapillary atrophic regions, and image areas interrupted by movement saccades are avoided. In processing the data the total number of pixels in the image is determined, an average flow value is calculated, and a histogram of flow data is produced. Flow, volume, and velocity data at the 25th, 50th, 75th, and 90th percentiles are the derived characteristics which are used for analysis. The percentage occurrence of zero flow pixels within the image is also calculated. Broadening the analysis to include every qualifying pixel within the entire image improves test/retest reliability, reducing the coefficient of variation for repeated weekly measurements to 15% for selected portions of the flow histogram.

Ocular Pulse Measurement

In 1962 Eisenlohr and Langham published the first study of the relationship between the observable pulsatile change in IOP during the cardiac cycle and the resulting changes in ocular volume.[25] On the basis of his observations of the volume - pressure relationship, Langham developed the ocular blood flow (OBF) device, which calculates the real-time change in ocular volume from real-time measurement of IOP.[26-29] Diastolic flow is the steady flow delivered during diastole which accounts for perhaps two-thirds of total ocular flow. If pulsation in IOP is due to blood surging into the eye during systole, then it might be possible to measure an unknown percentage of total ocular blood flow in terms of IOP fluctuation.[29]

The Langham OBF consists of a modified pneumotonometer interfaced with a microcomputer which records the ocular pulse.[30] It monitors the rhythmic change in IOP during the cardiac cycle, which fluctuates within a range of up to 2mmHg in a sinusoidal fashion. In OBF examination procedure the tonometer is placed on the cornea for several seconds. The pneumotonometer sends an analog signal to the computer, where it is digitized and recorded. The amplitude of the IOP pulse wave is used to calculate the change in ocular volume, using the relationship described by Silver *et al.*, 1989.[29] Recently the OBF system (OBF Labs Ltd., Malmesbury, UK) has been introduced. This is similar to the Langham ocular blood flow system, and has rapidly gained popularity for use in ocular blood flow studies because it is fast and straightforward in operation, easy to use, relatively inexpensive, and offers acceptable reproducibility.[31,32]

Despite their attractiveness both systems are beset by limitations. POBF values are not obtained through direct measurement of ocular blood flow, but derived mathematically by estimating ocular pulse volume change on the basis of a preset equation relating ocular volume to IOP. This formula incorporates a model of the cardiac cycle and a standard value for scleral rigidity.[33] POBF measurements are therefore affected by individual differences of scleral rigidity, ocular volume, heart rate, systemic blood pressure, and IOP. This being the case, myopic eyes, which have less scleral rigidity and larger ocular volume, may yield lower POBF measurements than normal or hyperopic eyes. Understanding these limitations is essential to proper study design and data interpretation. POBF may be more useful for studying intra-individual blood flow changes (eg. before and after medication) than for making inter-individual comparisons (eg. glaucoma patients versus normal subjects).

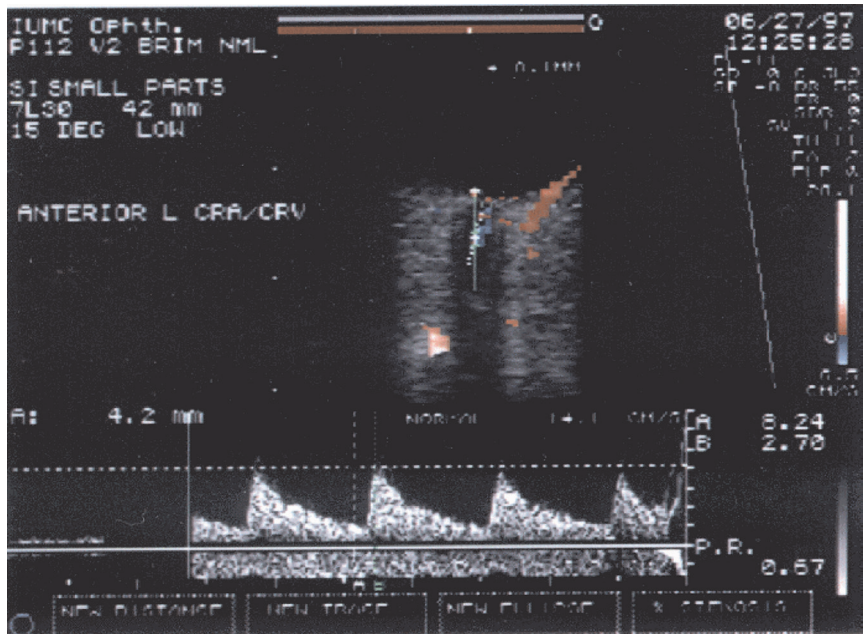

Figure 5: Color Doppler image of the central retinal artery and vein taken with a 7.5MHz linear probe (Siemens Quantum 2000 system). The Doppler-shifted spectrum (time velocity curve) is displayed at the bottom of the image. Red and blue pixels represent blood movement toward and away from the transducer respectively.

Color Doppler Ultrasound Imaging

A-scan ultrasound is commonly used to measure the eye's axial length, whilst B-scan ultrasound has been used to produce grayscale images of ocular structures for some years. Color Doppler imaging (CDI) is an ultrasound technique that combines B-scan grayscale imaging of anatomical detail, color representation of blood flow as measured by Doppler shift, and pulsed-Doppler measurement of blood flow velocities.[34-37] As with the other Doppler-based methods, blood flow velocity is determined by the shift in the frequency of the returning sound waves. The motion of blood through the vessels is represented by the superimposition of color upon the familiar B-scan grayscale image of the eye's structure. Most units code red-to-white for motion toward the probe and blue-to-white for motion away from the probe.

The color Doppler image (figure 5) enables the operator to identify the target vessel and position the sampling window for pulsed-Doppler recording. Measurements are of Doppler-shifted sound frequencies reflected by the tissue within the sampling window. The CDI unit plots flow velocity data against time, and the peak and trough of the wave are identified by the operator. From these, the computer calculates peak

systolic velocity (PSV) and end diastolic velocity (EDV). In addition, Pourcelot's resistive index – a measure of downstream vascular resistance – can be calculated according to the formula given below:[38]

$$RI = \frac{PSV - EDV}{PSV}$$

Values of this index range from zero to one, with higher values indicating higher distal vascular resistance.

In vitro studies have established the validity of Doppler ultrasound measures of flow velocity.[39] Reproducibility has also been studied.[40] The best reproducibility is found in the ophthalmic artery, where percentage coefficients of variation range from 4% for resistive index to 11% for peak systolic velocity.

Laser Speckle Flowmetry

When the fundus is illuminated by a laser source, blood within the retinal vasculature creates a speckle pattern. This pattern shifts temporally proportionally to the rate of flow within the vessels.[41-43] This phenomenon is that basis of a new instrument for the non-invasive assessment of blood flow in the retinal microcirculation.[43-45] An argon laser spot shone on the retina produces a speckle pattern, which is detected by a CCD. The differences between rapidly acquired images is quantified to calculate the rate of "blur", or change between images.[43] Laser speckle flowmetry has been validated in an animal model. In pigmented rabbits, normalized blur was found to be linearly correlated with blood flow as measured with microspheres.[46]

Oximetry

Reduced retinal blood flow implies ischemia, which in turn implies retinal hypoxia, which may lead to metabolic stress and neural damage. Measurement of hemoglobin oxygenation within retinal arteries and veins would provide a more direct assessment of the metabolic status of the eye.[47,48] Measuring the oxygenated hemoglobin as a percentage of total hemoglobin using optical spectral techniques is not a new concept.[49,50] Beach used a two-wavelength approach (569nm and 600nm) and confirmed that optical density ratios (ODRs) have a negative linear correlation with hemoglobin oxygenation in retinal vessels.[47] We have constructed a similar system utilizing newly available optical components, an isosbestic wavelength of 586nm, and an oxygen sensitive wavelength of 605nm. At 605 nm, hemoglobin absorbs approximately four times as much light as oxyhemoglobin. Thus, the use of the 605nm wavelength may provide a more sensitive measurement device than the Beach system.[23] The oximeter consists of a modified Topcon 50VT fundus camera. The top Polaroid mount of the fundus camera is fitted with a 1:4 image splitter and four dichroic filters (5nm bandwidth) centered at 542nm, 558nm, 586nm (isosbestic), and 605nm (figures 6 and 7). A single xenon flash image is split into four frames. The light in each band was quantified using a CoolSnap 12 bit scientific digital camera. A series of pixel samples is averaged from a 5 x

5 pixel region of the vessel ima-ge and an adjacent tissue image (Figure 7). Using custom written software, the user places a measurement window on a magnified vessel image. A second sample window is automatically placed in the adjacent tissue. The mean pixel value within each window was computed and stored. Optical density (OD) was calculated as:

OD = ln (pixel average in tissue ÷ pixel average in vessel)

The ratio of OD's from oxygen sensitive and insensitive images (ODR) is a linear function of oxygen saturation and was the primary outcome parameter.[47]

All images are analyzed in 16 bit format. Before all subject imaging sessions, a dark image is obtained with no background illumination in the fundus camera, no flash, no room lighting, and with the lens cap on the fundus camera. The dark image, therefore, quantified CCD noise within the scientific camera such that it consisted only of data that could be read from the CCD when it had not been exposed to light. The dark image is subtracted, pixel by pixel, from all images for that session before any analysis occurred. OD sampling is performed only on images after removing camera noise. Analysis of the images after noise removal is done by customized software which then automatically obtained pixel samples from vessels.

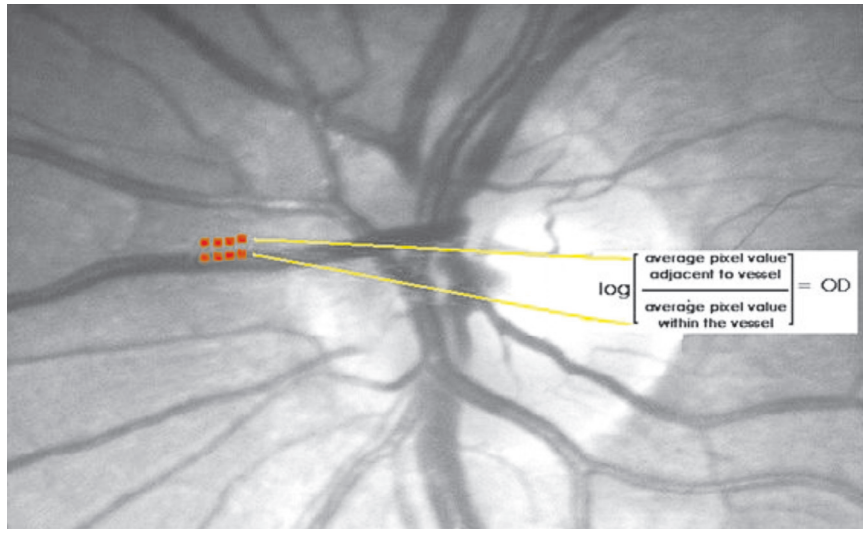

Figure 7. Optical density was defined as the natural log (ln) or the ratio of the reflectance of tissue and the reflectance of blood within the vessel.

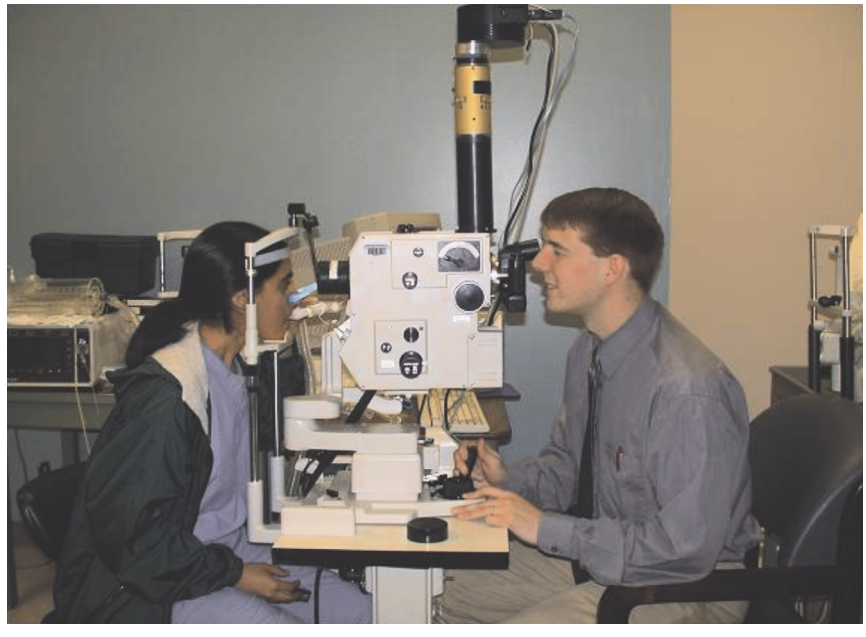

Figure 6. The oximeter was mounted on the Polaroid port of a Topcon 50VT camera. The black cylinder attached to the camera is a 2X lens. The gold cylinder immediately above is the image splitter and bandpass filters. The black digital camera on top is used to quantify light at the various frequencies

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