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A LABORATORY and CLINICAL STUDY on VITREOUS FLUOROPHOTOMETRY

by

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ABSTRACT

The optical, electronic sensoring and data acquisition systems were assembled and the software developed for a vitreous fluorophotometer which was then calibrated and used to quantify the integrity of the blood-retinal barrier in a pilot study of diabetic retinopathy and multiple sclerosis compared to normal controls.

Breakdown of the blood-retinal barrier was quantified by measuring fluorescence in the vitreous at standard time intervals over one hour following intravenous injection of sodium fluorescein. The plasma dye concentration was measured throughout the procedure. Leakage was expressed as a penetration ratio of the average concentration at 3mm from the retina to the total plasma dye concentration.

The results from diabetic subjects showing well defined stages of retinopathy severity demonstrated the proper functioning of the instrument by showing values in approximate agreement with retinopathy severity, thus confirming the findings of previous observers.

Of 16 multiple sclerosis subjects, results showed no significant difference between activity categories or level of current activity. Abnormally high penetration ratio was associated with active periphlebitis. A new finding was the presence of abnormally high leakage in 2 subjects showing no ocular signs of disease. Subjects without or with inactive periphlebitis showed breakdown of the blood-retinal barrier comparable in severity to diabetic subjects showing no or mild retinopathy. The vitreous diffusion constant of the dye for normal controls and multiple sclerosis subjects was not significantly different from that in water.

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LAMENT

"Pangs of hunger, Pang?..."
No!
Pangs of loneliness.

Pangs of being different;
Pangs of behaving differently.
Pangs of being misunderstood.

Pangs of having different ideas;
Pangs of having different ideals.
Pangs of being misunderstood.

Pangs of being in a different world.
Pangs of wanting to be the same; yet,
Pangs of needing to be understood to be different.
Pangs of being misunderstood.

Pangs of always different interpretations.
Pangs of very different experiences;
Pangs of a different upbringing;
Pangs of being misunderstood.

Pangs of a small world; but,
We are of this one world.
It hurts.

My Father's hopes are my reality.

Thank you, my **FRIENDS.**

Art thou for something rare and profitable?
Wouldst thou see a truth within a fable?

From "A Pilgrim's Progress"
by John Bunyan.

I. INTRODUCTION

1.1 Vitreous Fluorophotometry

Vitreous Fluorophotometry (VF) is a clinical research technique first described by Maurice in 1963 [1]. Its objective is to provide a non-invasive, standardized, reproducible procedure for examining the integrity of the blood-retinal barrier. Initial uses included the investigation of diabetic retinopathy (DRP), a retinal vascular disease which can cause blindness. Several other retinal vascular diseases have subsequently been investigated.

VF is a method of sampling the vitreous close to the retina to assess the state of intactness of the tissue. In the procedure, a fluorescent dye called sodium fluorescein is injected intravenously into a subject and its entry into the vitreous is measured by projecting a beam of light into the vitreous compartment and monitoring the resulting fluorescence. A profile of the amount of fluorescence is established by scanning along the axis of the eye. By relating the intensity of fluorescence to the amount of dye present at each position along a scan, the total mass of fluorescein that has entered the vitreous is then a measure of the permeability of the blood-retinal barrier.

The basic components of the fluorophotometer are:

a) A source of focussed light to excite the dye.

b) A probe to detect the amount of fluorescence.

c) A photomultiplier/radiometer system to convert the signals.

d) A data acquisition system to store the converted signals.

The light source usually employed is a tungsten bulb that can be varied in intensity. Its output into the eye is directed through a focussing system of lenses, and a slit.

The probe consists of a fibre optic conduit that is placed at the focus of the slit-lamp microscope objective [2]. It is positioned at an angle to the beam so that it is focussed on a cross-section of the beam, detecting the fluorescence from the "intersecting" volume called the "diamond". (See Figure 1.)

The fibre optic probe conducts the collected light to a radiometric detection system which then outputs to a data acquisition system. Recording and/or data-storing device(s) then store the converted signals.

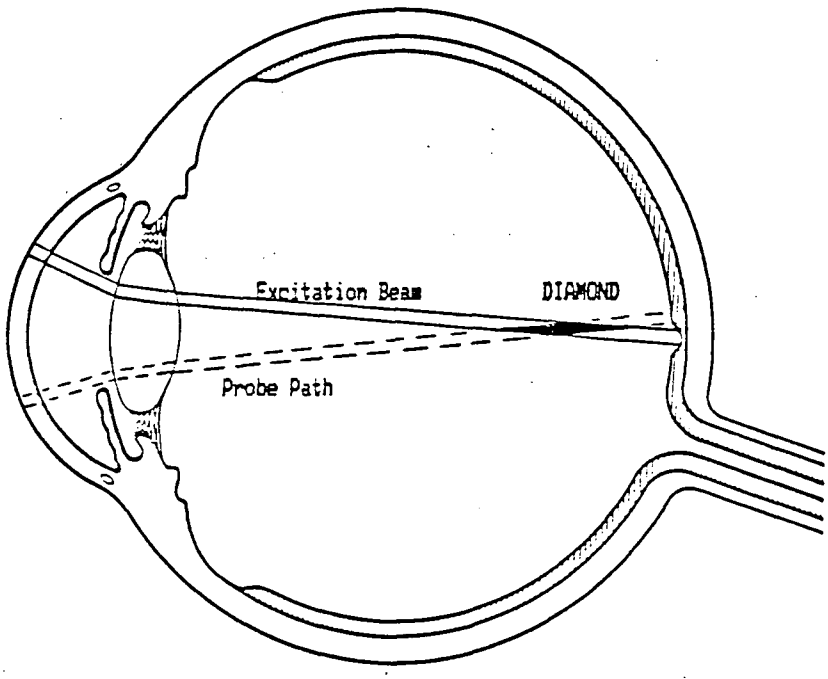


Figure 1. The "diamond" is the intersection of the beam and the probe.

1.2 Blood-Retinal Barrier

In the human eye, there are two barriers to the transfer of molecules into and out of the vitreous and aqueous media. These are the blood-aqueous barrier (BAB) and the blood-retinal barrier (BRB). The BRB is a situation of restricted permeability between the blood and the retina. It functions at the level of the retinal pigment epithelium (RPE) and the retinal vessels. Restricted permeability serves to maintain the regulated physical and chemical environment of the retinal neural tissues, i.e., homeostasis of the retina.

The RPE, considered the outer BRB [3], forms a uniform, continuous, single layer of cells united laterally by zonulae occludens. (See Appendix D.) The retinal vessels forming the inner BRB [3], are lined by a continuous layer of non-fenestrated endothelial cells which are joined near their luminal surfaces by zonulae occludens, a junctional complex in which there is complete fusion of the outer leaflet of neighbouring cell membranes. The integrity or "tightness" of the BRB may be compromised by disease processes which affect any of its components - the RPE, the retinal capillaries, arteries and veins.

In the present study, VF instrumentation and analysis techniques were developed and applied to an investigation of the BRB in multiple sclerosis (MS), an application that had not previously been investigated. The results were compared to normal controls and graded severities of DRP.

1.3 Applications

In VF studies of diabetes mellitus, researchers found abnormal leakage of dye even when there was no visible DRP [4,5]. This suggested that VF would be useful to detect the onset of the breakdown of the BRB. Diabetic persons with well established DRP showed large amounts of the dye in the vitreous. These results implied that VF could possibly be used to monitor the leakage component of subclinical retinopathy progression to severe stages.

The technique has since been used to study other retinal vascular diseases, for example, hypertension and pars planitis [6], and the effects of drugs such as sulindac which has recently been found to be effective in reversing abnormal early leakage in diabetes [7].

1.4 Multiple Sclerosis

The limited understanding of the disease processes in MS arises from the lack of good correlations between relapses and disease progression. This imposes difficulty in the development of an effective treatment.

The perivenular cell infiltrate in the cerebrum that has been described as an early event in the formation of an MS plaque [8], appears similar to the retinal perivenular infiltrate found in the eye. In laboratory studies of tissue preparations (immunoperoxidase), it was found that there was abnormal retinal venous permeability in areas with and without visible periphlebitis [9], and that such effects were much more frequent and extensive than previously considered. Engell and Andersen [10] estimated that almost all MS patients

would develop retinal periphlebitis at some point in their lifetime. If this is correct, it can be expected that following the onset of periphlebitis, there is breakdown of the BRB (to molecules much larger than fluorescein) which persists despite clinical and histopathologic resolution of the lesions.

Clinically, retinal periphlebitis in MS may affect one, several or all of the retinal veins and appears either as an active lesion with patches of fluffy white haziness surrounding the veins or as an inactive venous sclerosis when there is halo sheathing. The course is mild, asymptomatic and transient with activity lasting weeks, months or up to two years. Resolution leaves no sequelae or else replacement sclerosis. Inflammatory activity can be confirmed clinically by fluorescein angiography photography which shows leakage, whereas inactive venous sclerosis shows no leakage [11,12]. Photographic information, however, is limited by the sensitivity of the emulsion and would not show a subtle breakdown of the BRB that might persist after resolution of inflammation.

A significant proportion of MS patients with active periphlebitis also show abnormal brain scans when compared to those with inactive periphlebitis [13]. An explicit relationship between the activity at the two sites - the BRB and the blood-brain barrier - with regards to relapses and disease progression has not been investigated.

1.5 Aim

The objectives of this study are the following:

(1) To use VF as a sensitive system to study possible BRB changes in MS cases showing active and inactive periphlebitis with regard to quantitative differences in leakage. A parallel study on persons with diabetes is conducted as a control on the performance of the VF system.

(2) To document the frequency and severity of leakage in relation to the clinical grading of the certainty of the diagnosis, and in relation to the the standard clinical activity categories with the aim of establishing relationships between the ocular and central nervous system activities, particularly relapses and disease progression.

(3) To assess the utility of the procedure as a non-invasive technique in the diagnosis of MS, and as an indirect monitoring method of grading the central nervous system activity.

II. THEORY AND ALGORITHMS

In this chapter, the details of the VF technique are elaborated. The algorithms proposed by two groups of investigators were closely followed in this experiment in order to compare results. The methods of these two groups are detailed in the following sections.

The first section explains the "systematic errors" inherent in each VF scan. These errors arise from the limitations of the measuring instruments, as well as from the complex optical, biological system of the human eye. Thereafter, there is an explanation of the earlier, more basic models used in analysis.

The second set of sections discusses the two more elaborate methods of analysis that were used in this study. The algorithm to accommodate the "systematic errors" was proposed by J.G. Cunha-Vaz and co-workers and is referred to as "The C-V Group".** The state of the BRB is then embodied in a single number called the Penetration Ratio. "The L-A Group" described a more mathematically involved solution to find the Permeability and Diffusion coefficients. This algorithm is due to H. Lund-Andersen and co-workers.

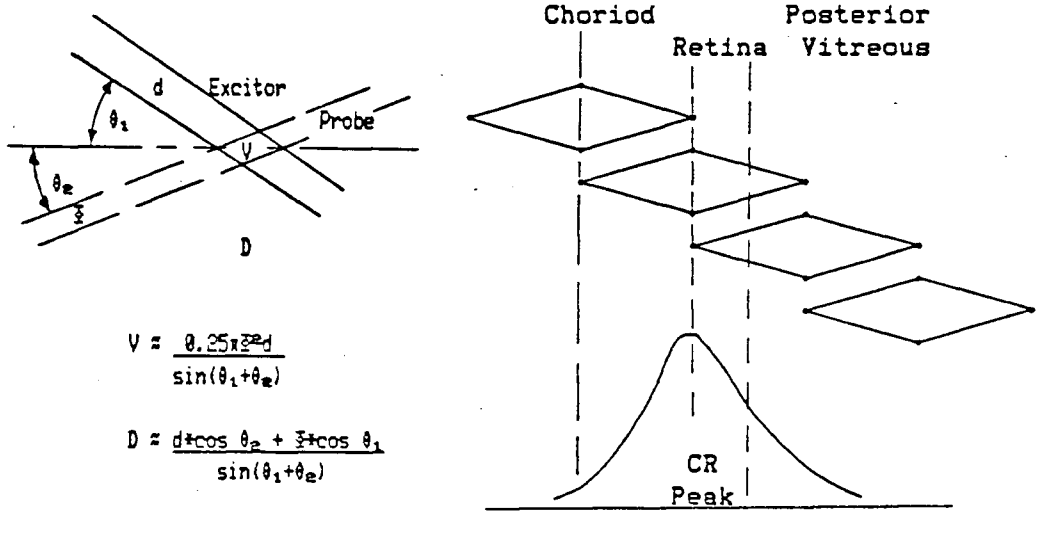
In the development of the VF system, alterations to the protocol and the algorithm necessarily occur because of differences in procedures and instrumentation. Some modifications to the methods of the two groups are discussed in Section 2.5.

** "... Group" refers to a general geographical distribution of the various investigators, and is also used to distinguish between those using one method of analysis and those using the other.

2.1 Systematic Errors

Figure 1 shows that the "diamond" is of finite dimensions. As it is moved across an interface from a compartment of high dye concentration into a compartment without dye, a non-zero signal is obtained from the empty compartment. One such interface is at the choriod-retina (CR) and the vitreous, where this non-zero signal effect is especially significant during the first few minutes after the introduction of fluorescein into the blood.

This "false" signal is due to the depth or length of the diamond as illustrated below. It persists for some distance into the dye-free compartment. This effect is sometimes called the "tailings" or "spread function" due to the associated peak because its strength depends on the peak signal at the interface [14].



a. Estimates of Axial Resolution and diamond volume.

b. The "tailings" due to the choroid-retinal peak [18].

Figure 2. Diamond dimensions and effects.

There are other sources of "false" signals, especially very close to the retina. One effect is halation [15]. When focussed on the retina, the edges of the slit are not distinct. This is due to the transparent depth of the retina (about 0.5mm), and to scattering. There is also a possible dependence on retinal pigmentation [16].

There may be signals from light reflected off the walls of the vitreous cavity although it is unlikely that, away from the retina, the reflected light intensity can be sufficiently high, or be in the direction of the probe. This is also apparent from the relative volumes of the diamond and the vitreal cavity.

As tailings are strongest near the retina, the solution is to consider data that are collected at a more remote point where the spread function is small. However, the retina and its associated CR peak must always be included in any scan as they constitute a reference zero-position by which displacement can be measured.

The distance from the retina within which data cannot be accepted depends on the dimensions of the diamond. The in vivo axial resolution (AR) is defined as the distance from the CR peak where the signal is a small fraction of the peak [14]. The fraction chosen must not be so small that the signal is at the noise level of the detector. A practical definition of AR is the ratio of the signal at a fixed distance from the retina to the CR peak signal [17].

Figure 2a estimates the AR and the volume of the diamond. They depend on the angle between the directions of the excitation beam and probe, the width of the slit, and the diameter of the probe. These parameters must be varied until an optimized AR is attained [18].

Reducing the slit width and/or using a smaller probe diameter need not necessarily result in better ARs, as a reduction of either or both reduces the amount of fluorescence detected. The noise of the detection system then limits the reduction that is possible. However, it is necessary to have signals distinctly above the noise levels so that the data can be analyzed with greater confidence. Also, a small slit width cannot be measured (i.e., calibrated) with precision. (Refer to Sections 3.2, 4.1 and Figure 17.)

Increasing the angle shown in Figure 2a improves the AR and reduces the volume of the diamond. However, this angle is limited by the diameter to which the pupil of the eye can be dilated. The cornea's curvature and its varying thickness also distort the finite beam and slit, thereby reducing the probe's focus on the beam. The formulae in Figure 2a are therefore approximations as the in vivo dimensions of the diamond cannot be measured directly.

The maximum dilation diameter of the pupil varies among subjects. When the pupil cannot be dilated to a minimum acceptable diameter, the procedure cannot be used. The power of the convex cornea curvature, however, can be offset by a plano-concave lens placed on the cornea. The plane surface of the lens provides a "window" to view the fundus. (See Figure 3.)

Figure 3 also shows the importance of AR. The angle between the beam and the probe in the vitreous varies during a scan. The AR changes with it. (See Figure 2a.) The AR is larger in the posterior vitreous than in the anterior segments. As the larger AR near the CR causes more severe tailings, its optimization is thus vital to the

instrument's performance.

Many biological substances (tissues) are known to be autofluorescent. They fluoresce at certain incident wavelengths. The crystalline lens and the cornea (as well as the retina) are autofluorescent. They give off false signals in their vicinity (i.e., tailings), as well as absorb part of the excitation beam which must pass through them.

The autofluorescence of the crystalline lens is unavoidable when the vitreous is to be scanned. It depends on the age of the subject as well as the disease process [19]. Cataracts (or opacities) in the lens also cause loss of input intensity, which limits the application of VF to eyes with clear media.

In the later scans, there is usually a high concentration of dye in the anterior chamber which can cause a loss of incident light due to absorption, or stray signals from scattering. However, the expected levels of fluorescein in the aqueous at 60 minutes after injection are sufficiently small that they do not attenuate the excitation beam appreciably. (See Appendix C.5.)

Other sources of error are related to the apparatus. In Section 3.1, where the properties of fluorescein are discussed, the problem of filter overlap will be mentioned. Problems in the radiometric system such as dark current noise due to random photon events in the photomultiplier tube (PMT) are minimized by constantly checking instrument zero adjustments.

All the above sources of variation are affected by the intensity of the excitation beam, which affects the amount of scattering

and therefore autofluorescence, tailings, and AR. Hence, optimization of the apparatus involves the adjustment of all parameters which contribute to the quality of the data acquisition.

2.2 Theory

The permeability of the BRB is related to the diffusion of the dye across it. Concentration differences and electric potentials are some of the forces driving the diffusion phenomenon. In the first two hours after the injection of the dye, passive diffusion governs its penetration through the BRB from the blood into the vitreous [20,21]. This means that Fick's Law, in which a concentration gradient is the driving force, can be applied. Hence, to measure the permeability of the BRB, the change in the blood-dye concentration over time must be known. The concentrations of dye on both sides of BRB may then be related by a proportionality constant which represents the permeability.

The simplest mathematical model is that of a plane retina [22]. The equivalent one-dimensional problem is

$$D * d^2c(r,t)/dr^2 = dc(r,t)/dt \quad (1)$$

where the concentration, c, is position-dependent and time-dependent. r is the distance from the middle of the vitreous. D is the diffusion coefficient which is assumed to tbe independent of c, and hence, may alternatively be called the diffusion constant.

The assumptions of such a model are that (a) fluorescein can only diffuse towards the middle of the vitreous after penetrating the

BRB plane, (i.e., a one-directional transport process); and, (b) the boundary conditions, which are:

$$dc(0,t)/dr = 0 \quad (2)$$

$$D * dc(a,t)/dr = P^I c^P(t) \quad (3)$$

where the retina is at a distance a from the mid-vitreous, and c^P is the concentration of fluorescein in the blood at time t. Like D, the diffusion constant, P^I, the "permeability coefficient" is independent of c, and is referred to as the permeability index [23].

The solution to Eq. 1, using Eqs. 2 and 3 is

$$P^I = \frac{\int_{r=0}^{r=a} c(r,t)\, dr}{\int_{\tau=0}^{\tau=t} c^P(\tau)\, d\tau} \quad (4)$$

P^I relates the total amount of dye in the blood available to penetrate the BRB at the post-injection (p.i.) time, t, to the total amount in the vitreous at t. Mathematically, the numerator is the area under the concentration profile (taken at t) between the mid-vitreous and the retina. The denominator is the area under the plasma-fluorescein concentration profile up to t.

Several problems arise in solving Eq. 4. The processes at work at the BRB are not simple. It has been established that "active transport phenomena" drive solutes against concentration gradients [20,21]. The forces driving these active transport processes are, in fact, greater than those for passive diffusion by about 31 times.

They come into effect after the first two hours p.i. Unless these outward active processes are to be studied the last scan is usually taken at approximately one hour p.i.

The time taken for the dye to reach the eye depends on the site of injection, e.g., the dye appears at the retina 10s sooner when it is injected into a carotid artery than when it is injected into a peripheral vein in the arm [24,25]. Injecting the dye slowly or quickly also affects the time of appearance at the BRB and the profile of the bolus which is attenuated even after a fast intravenous injection due to mixing with blood.

Venous blood samples are drawn throughout the procedure to obtain the plasma profile. The first sample is usually taken after several minutes p.i. and the number of samples required depends on the method of solving the integral, e.g. more samples are needed within the 60 minutes if the area is found using the trapezoidal rule. Assumptions must also be made about the profile between 0 and $t^{(1)}$, the first sampling time when the latter method is used [26].

Curve-fitting techniques may be used in solving the plasma integral. In pharmacokinetic studies [27], it has been found that the time-course of fluorescein in the blood is best approximated by a two-compartment model of mixing. This requires a mode of curve-fitting a sum of two negative exponentials to the data [28,29] which includes a fast and a slow decay in the levels of fluorescein in the blood. (Refer to Section 3.1.)

The integral in the numerator in Eq. 4 assumes in its lower limit, that dye penetrating the BAB and dye penetrating the BRB have

not mixed within one hour p.i. [27] Tailings of the CR peak restrict the upper limit of the integral as previously mentioned. However, it can be expected that most of the fluorescein in the vitreous is in the vicinity of the retina as will be explained.

Attenuation of the excitation source by fluorescein in the anterior chamber was briefly mentioned in the previous section. Because of its small volume, the dye quickly fills the anterior chamber by way of the iris and ciliary body. Assuming a uniform distribution (because of its small volume), the attenuation may be expressed in the form of the Beer-Lambert law:

$$(5) \qquad c(\text{measured}) = c(\text{true}) * \exp(- b * c^{A} * d) .$$

This assumes that only the concentration, c^{A}, in the aqueous chamber of depth, d, attenuates the excitation beam. The dye in the anterior segment of the vitreous (adjacent next to the posterior surface of the lens) and lens autofluorescence are not included. b is the attenuation (or extinction) coefficient [18]. This attenuation is less than 10% for $c^{A} < 1000$ ng.ml^{-1} at the one hour scan. (See Appendix C.5.)

Figure 3 below shows how rays are refracted at the interfaces in the eye. As the diamond is moved anteriorly along the optical axis, the angles of incidence change at the various surfaces so that AR is also position-dependent.

Figure 3 also demontrates that a 1-mm translation of the slit lamp does not correspondingly produce a 1-mm diamond displacement in the medium in which it is focussed. It is thus necessary to translate

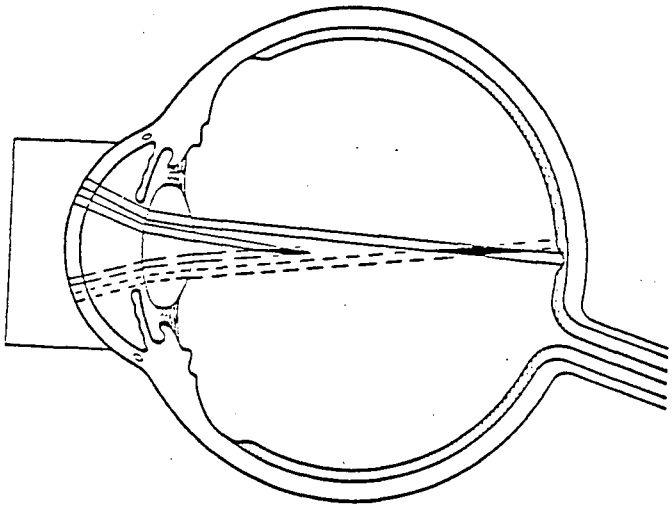


Figure 3. Diamond displacement with slit lamp translation.

slit lamp translations to displacements of the diamond since the latter are not directly measurable.

Slit lamp translations, d are related to diamond displacements, x, by [30]

$$x = F * d .$$

The "F-number" represents the effects of refraction at each interface. F itself depends on x. Table 1 and Figure 4 show the results for a model eye by Krogsaa, et al [30]. Note that F is different in the compartments because there are fewer interfaces to traverse as the diamond is moved towards the cornea. Also, F>1 in all cases demonstrating the power of the ocular system.

Compartment	Model Eye Distances in mm	Slit Lamp Movements in mm	F-number
Aqueous Chamber	3.60	2.49	1.45
Crystalline Lens	3.60	2.20	1.64
Vitreous Chamber	16.97	11.75	1.44

Table 1. Average F-numbers using a Gullstrands emmetropic model eye. [30]

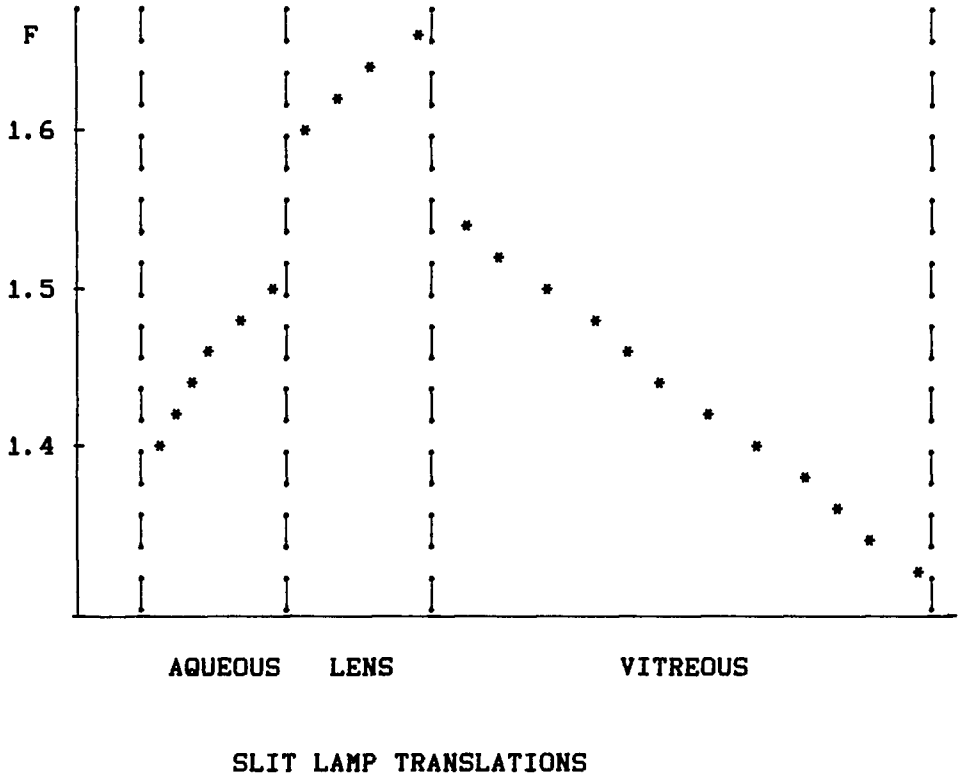


Figure 4. Variation of F with slit lamp movement. [30]

2.3 The C-V Group

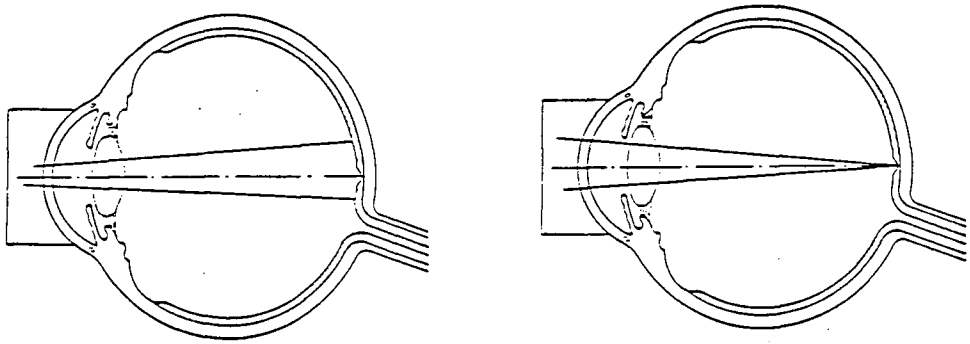
A simple way to quantify the permeability of the BRB is to measure the amount of fluorescein in the posterior vitreous segment. However, different instruments with different sensitivities and ARs, measure different strengths of CR tailings. Therefore, a number that quantifies the permeability must be independent of instrument differences.

To offset errors due to CR peak tailings, a background, pre-injection scan is subtracted from all other post-injection (p.i.) scans. Lens autofluorescence is also eliminated by this subtraction.

The subtraction is carried out by aligning "landmarks" such as the lens and CR peaks of each scan. This method, however, does not consider possible shifts in the CR peak position with time, i.e., the CR peak may not be the true position of the retina in the later scans.

As fluorescein is continuously removed from the blood, a point may be reached when the signal from the posterior vitreous is greater than that from the CR (in high leakage cases). That is, the CR peak appears in front of the retina. Also, due to different fixation or scanning axes, the distances between peaks may vary.

An alternative method is to align only the retinal position. The retina may be located visually at the start of each scan. This assumes that the starting points of each scan are at the same location on the retina. The macula, for example, may be used as such a starting landmark. An advantage of this method is that errors due to different alignments (shown in Figure 5b) are smaller near the CR.



a. Different starting points. b. Macula alignment.

Figure 5. Different scanning axes.

However, the data collected from the anterior half of the eye may no longer be analyzed with confidence.

The difficulty in this method is the accuracy with which the CR can be located due to halation, scattering, and the centring of a small slit on a larger probe.

In order to account for the possible effects of CR tailings in the later scans, a "bolus" scan is made within 3 to 5 minutes p.i. The dye is not expected to have penetrated the BRB (in most cases) within the first 10 minutes p.i. The bolus scan then provides the strength of the CR tailings at specified distances from the retina after the background has been subtracted.

The distance from the retina at which calculations are done is usually the 3-mm point. It is expected that the ARs of most instruments are smaller than this [31]. Thus, CR tailings should not be

very significant at this point. Any point where the tailings are not substantial can be chosen. However, to study leakage from the BRB, it is important to scan close to the retina. Therefore, 3mm from the latter is a suitable choice (after AR considerations).

The CR tailings are subtracted from later measurement scans** only if the former are significant. Ishimoto, et al [32], suggested a criterion for this bolus CR correction with a recommendation for implementation if

(6) (CR Peak value)/(3-mm Vitreous value) > 10 .

The above equation was based on the commercial Fluorotron® Master fluorophotometer. The condition may be different for other instruments.

The measurement scans are taken at specified time intervals before the outward transport processes become significant. After alignment and background subtraction, (and bolus CR correction, if necessary), the average value of the dye concentration around the 3-mm position is found [32]. The result is divided by the plasma integral up to the p.i. time of the scan. The final result is called the penetration ratio, PR3, of units, s^{-1}.

The advantage of using PR3 instead of P^1 in Eq. 4, is that the 3-mm value from the retina may be used for comparison between different patients and instruments. There are no problems of CR tailings, and it is not required to integrate very close to the CR.

** All scans other than the pre-injection and bolus scans are referred to as measurement scans.

The averaging is carried out between the 2-mm and the 4-mm points [32]. This reduces errors usually caused by random fluctuations in the data which can persist, even after subtraction, for a penetration ratio at the 3-mm point, PR3*. The averaging also diminishes the errors due to the alignment problems mentioned before.

2.4 The L-A Group

Much of the scanning and data-correction techniques used by this group are the same as that of the C-V Group (by virtue of similar instrumentation). However, instead of using PR3 or P^1, a more elaborate, mathematical model of the transport of fluorescein in the eye is constructed to estimate the vitreous diffusion constant, D, and the permeability constant, P, of the BRB [33].

The analysis involves solving the diffusion equation,

$$\nabla \cdot (D \nabla c) = \partial c / \partial t \ , \tag{7}$$

as a boundary-value problem directly. The difficulty in such an approach is that fluorescein is being continuously removed from circulation. This means that the transport problem is a "transient" one.

Several assumptions of the previously mentioned plane-retina model of the C-V group apply to this model. (1) Dye from the BAB and from the BRB have not mixed uniformly at 60 minutes p.i. (2) The retina is assumed to be spherical with radius of curvature a so that the diffusion is radially inward, towards the centre of the vitreous chamber [23]. Furthermore, if D is independent of c, then

Eq. 7 reduces to an r-dependence only:

$$(8) \qquad (D/r)\cdot\{\ 2\ \partial/\partial r + r\ \partial^2/\partial r^2\ \}\ c(r,t) = \partial c(r,t)/\partial t\ ,$$

$$\text{for } 0 \le r \le a\ .$$

Due to symmetry, the boundary condition is

$$(9) \qquad \partial c(0,t)/\partial r = 0\ .$$

The initial condition is

$$(10) \qquad c(r,0) = 0\ , \qquad \text{for} \qquad 0 \le r \le a\ .$$

Thus far, the model assumes that the concentrations on both sides of the BRB are related through a proportionality constant, P which represents the permeability of the entire BRB. P makes no reference to the location of breakdown within the BRB (inner or outer). Thus, at any time, t, the amount of dye available for diffusion towards the mid-vitreous, depends on the amount of dye c(a,t) that has already penetrated the BRB. But c(a,t) depends on $c^p(t)$ by assumption. The barrier condition is then

$$(11) \qquad -D\ \partial c(a,t)/\partial r = P\cdot\{\ c(a,t) - c^p(t)\ \}\ ,$$

$$(12) \qquad \{\ 1 + (D/P)\cdot\partial/\partial r\ \}\ c(a,t) = c^p(t)\ .$$

Using the method of Laplace transforms, the solution is

$$(13) \qquad c'(r,s) = c^p{}'(s)\cdot F'(r,s)\ ,$$

where

$$F'(r,s) = \frac{u(r)\,\sinh w(r,s)}{\Xi \sinh w(a,s) + \sqrt{s}\cosh w(a,s)} , \tag{14}$$

$$u(r) = aP/r\sqrt{D} , \quad \text{for} \quad 0 \le r \le a , \tag{15}$$

$$w(r,s) = r\sqrt{(s/D)} , \quad \text{for} \quad 0 \le r \le a , \tag{16}$$

$$\Xi = u(a) - \sqrt{D}/a . \tag{17}$$

s is the transform variable; prime implies the transformed functions.

Transforming back to t-space, a slowly-convergent series results for small t [33]. However, if Eq. 14 is expanded for large real part of s, and then inverted, the approximate solution to Eq. 7 is

$$c(r,t) \approx \int_{\tau=0}^{\tau=t} c^{o}(t-\tau)\, F(r,\tau)\, d\tau , \tag{18}$$

where

$$F(r,\tau) = u(r)\cdot\{ (\exp(-M(-1)^2) - \exp(-M(1)^2))/\sqrt{(\tau\pi)} - \Xi\cdot\exp(\Xi^2\tau)\cdot\{\exp(2g(\tau)M(-1))\cdot\operatorname{erfc}(g(\tau)+M(-1)) - \exp(2g(\tau)M(1))\cdot\operatorname{erfc}(g(\tau)+M(1))\} \} , \tag{19}$$

and

$$M(j) = M(r,\tau,j) = (a-jr)/(2\sqrt{(D\tau)}) , \quad j=-1,1 \tag{20}$$

$$g(\tau) = \Xi\sqrt{\tau} . \tag{21}$$

$$\operatorname{erfc}(x) = (2/\sqrt{\pi}) \int_{\mu=x}^{\mu=\infty} \exp(-\mu^2)\, d\mu \tag{22}$$

Eq. 22 is the complementary error function.

Eq. 18 may then be used as the theoretical solution in a non-linear curve-fitting calculation to the experimental data. The goodness of fit may be tested by

$$S^2 = \sum_{k=1}^{k=N} \{c^{o}(r(k),t) - c(r(k),t)\}^2\cdot\delta(k)^2 , \tag{23}$$

where

(24) $$\delta(k)^2 = \max (\Omega^2 , c^*(r(k),t)^2)$$

serves as a 'weighting factor', and,

(25) $$\Omega = \text{lowest concentration that can be detected .}$$

$c^*(r(k),t)$ is the concentration measured at $(a-r(k))$ mm from the retina, of the scan taken at time t. The index k represents the order of the data-points along the axis of a scan. Non-linear curve-fitting is then carried out by the Marquardt algorithm [34]. A search through the parameter space of P and D is done and the best fit is determined when S^2 is a minimum for one set of P and D values.

2.5 Other Methods

The algorithms used by other groups of investigators are usually variations of those discussed above. For example, Eq. 19 may be simplified for computational purposes [23]. Most modifications, however, arise owing to differences in instrumentation which necessitate differences in protocol and algorithm.

Different methods have been proposed for the bolus CR peak correction mentioned in Section 2.3. Since many investigators use the Fluorotron® Master, one method of correction suggested for this apparatus was to multiply the bolus profile (between 2 and 4 mm from the retina) by the ratio of the CR peaks of the measurement and the bolus scans [15]. The modified bolus profile was then subtracted from the measurement scan. This seems reasonable as tailings depend on the peaks causing them.

Bursell, et al [35] argued that this algorithm over-corrected

the errors due to tailings because the CR peak values included fluorescence from the vitreous because of the finite diamond. (See Figures 1 and 2.) The correcting ratio should in fact be slightly smaller than that between CR peaks. However, the variations of the dimensions of the diamond in vivo cannot be determined, and the appropriate correction factor is unknown.

It has been found that retinal blood flow increased by 40 to 70% in the transition from light to darkness [36]. Although this is not likely to affect most scans, except possibly the bolus and the plasma integral, some investigators use more intense sources of excitation such as xenon flash tubes to attain signal levels well above the dark current noise so that room lighting need only be dimmed and not completely turned off [37].

For instruments that scan continuously, i.e., collect data continuously along the scanning path, the signals from adjacent positions overlap because of the finite diamond. The methods used in "smoothing" the signals (integral and curve-fitting methods) are then important [38]. In other instruments that employ the "spot" method using "chopped" or flash excitation sources, data are collected at specific points along the scanning axis, e.g. at every 1mm interval. These two methods of data collection determined by characteristics such as AR also determine whether PR3 or PR3* is to be employed.

To compare the results obtained by various fluorophotometers, a set of instrument characteristics is used to describe each instrument's capabilities. This set of performance data includes parameters such as angle between the beam and the probe directions, AR,

filter overlap, lower limit of detection (LLoD), reproducibility (R) and error of measurement (EoM).

The in vivo LLoD may be defined as the lowest detected (or detectable) concentration plus twice its standard deviation [32]. Practically, it means that, for example, at the mid-vitreous of a background scan, the LLoD is the average value of the concentration detected in that region plus twice the standard deviation of that average.

The sensitivity of the detection system is defined as the ability of the detection system to differentiate changes in adjacent concentration volumes. It is invariably dependent on the diamond and the ambient concentration. (See Appendix C.6.)

R and EoM also have the same dependences. Their definitions are:

(26) $$EoM = \{ c(measured)/c(true) \} - 1 ;$$

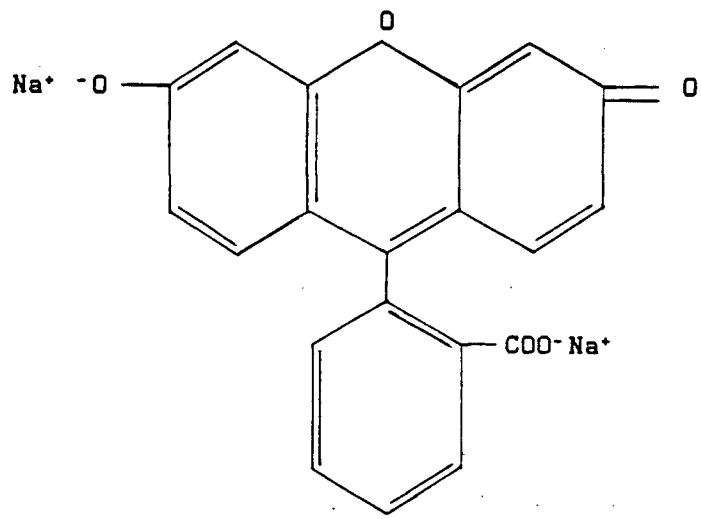
(27) $$R = \text{standard deviation of repeated measurements.}$$

The problem with these definitions is that the in vivo "true" concentrations cannot be determined.

III. THE APPARATUS

3.1 Sodium Fluorescein

Sodium fluorescein was first synthesized from resorcinol and phthalic anhydride in 1872 [39]. It is a very weak dibasic acid with a molecular weight of 376.27. Its solubility is increased as a sodium salt.



RESORCINOLPHTHALEIN SODIUM - $C_{20}H_{10}O_5Na_2$

Figure 6. Structural formula of Sodium Fluorescein [40].

Its suitability as an indicator in ophthalmological research is due to the fact that the peak excitation wavelength (490nm) is different from the peak emission wavelength (520nm). In addition, the de-excitation time is short: approximately 4ns. Hence, with a suitable combination of filters to separate the two wavelengths of light, the concentration may be deduced from the amount of fluorescence.

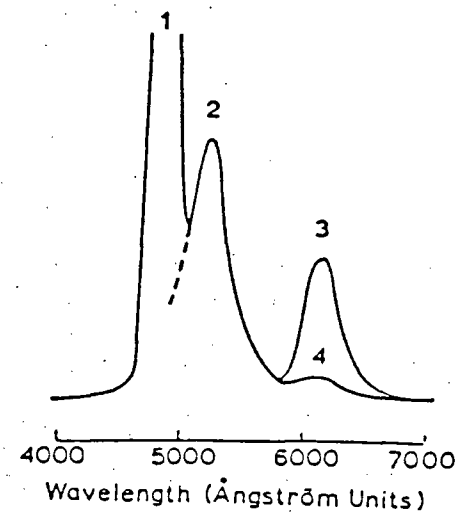


Figure 7. Excitation (1) and de-excitation (2,3,4) peaks of fluorescein in blood [25].

The choice of filter combinations, however, is made difficult by the fact that the ranges of excitation and emission wavelengths shift towards the red end of the spectrum when fluorescein is measured in blood compared to measurements in water solutions [41]. This effect may be caused by multiple scattering, absorption and autofluorescence of the tissues that are scanned. The optimum "cross-over" point for the filter combination should be about 525nm.

Fluorescein diffuses readily from the blood into all extracellular fluids except across the retina (BRB), and the brain (blood-brain barrier). As it is a weak acid, it does not bind with (or stain) normal vital tissues, and is highly fluorescent in alkaline media. In aqueous solution, about 80% of incident light is converted to fluorescent radiation [39,40]. However, the dye only returns approximately 26% fluorescence when dissolved in blood. This loss is due to binding to proteins (serum albumin) and red blood cell

membranes. Another effect is quenching by the haemoglobin. The absorption spectrum of haemoglobin is about identical to that of fluorescein. This can be demonstrated in severely anaemic patients where there is a stronger fluorescence as proportinately less of the dose is quenched by the haemoglobin. By means of equilibrium dialysis or ultra-filtration [40], it is estimated that between 50 to 84% of fluorescein is bound. It is however, the unbound fluorescein (17%) that diffuses across cellular membranes and the BRB if it is disrupted. This effect must be taken into account when analyzing plasma scans.

Fluorescein has low toxicity which is probably due to its inability to bind with vital tissues [39]. In animal experiments, lethal doses were at 2 to 3 grammes per kilogramme body weight. In this VF study and other investigations on human subjects, the dose administered is calculated at 14 $mg.kg^{-1}$ body weight using pharmaceutically prepared ampoules of 25% concentration (2.5 $mg.ml^{-1}$).

Fluorescein is well tolerated but there are occasional side effects such as transient nausea or vomiting immediately after injection. Yellowish tinting of the skin lasts for several hours after injection and the urine is yellow for about two days. Allergic reactions are rare. (See Appendix A.17.)

Other important dependences of its fluorescent property are on the pH, concentration and temperature. Only in an alkaline medium is its fluorescent property enhanced [39,40]. It was found that pH 7.4 is the level at which the dye fluoresces most efficiently [42]. This is approximately the pH of the cellular fluids of the body which

varies little. Importantly, the pH of the calibration sample solutions as well as the buffer for diluting plasma samples must be specially prepared.

The dependence on the ambient concentration is a result of scattering of the incident beam at the focus of the probe. At high concentrations, the excitation beam cannot penetrate the volume of dye. Attenuation of the incident beam causes loss of signal at the detector. In this study, the upper limit is about 0.01 $mg.ml^{-1}$.

3.2 The Hardware

This section describes the instrumentation and the modifications that were made to the instruments. Figure 8 shows the block diagram of the VF system assembled for this study. (Appendix B.2)

The principal component of the fluorophotometer is the slit lamp microscope. (Figure 9.) The built-in power supply (from mains) with specific intensity settings was replaced by a regulated d.c. supply because random variations in beam intensity were found to occur. These fluctuations were believed to arise from variations of the line voltage when the number of users increased (i.e., unregulated line).

In order to continuously monitor the intensity, a photovoltaic cell was placed along the path of the beam before it was focussed through the slit and prism system. This cell, placed close to the bulb, did not block the beam's path. This method of monitoring lamp intensity, which is dependent on optical alignment, was compared with another method which monitors the intensity of the output of the slit

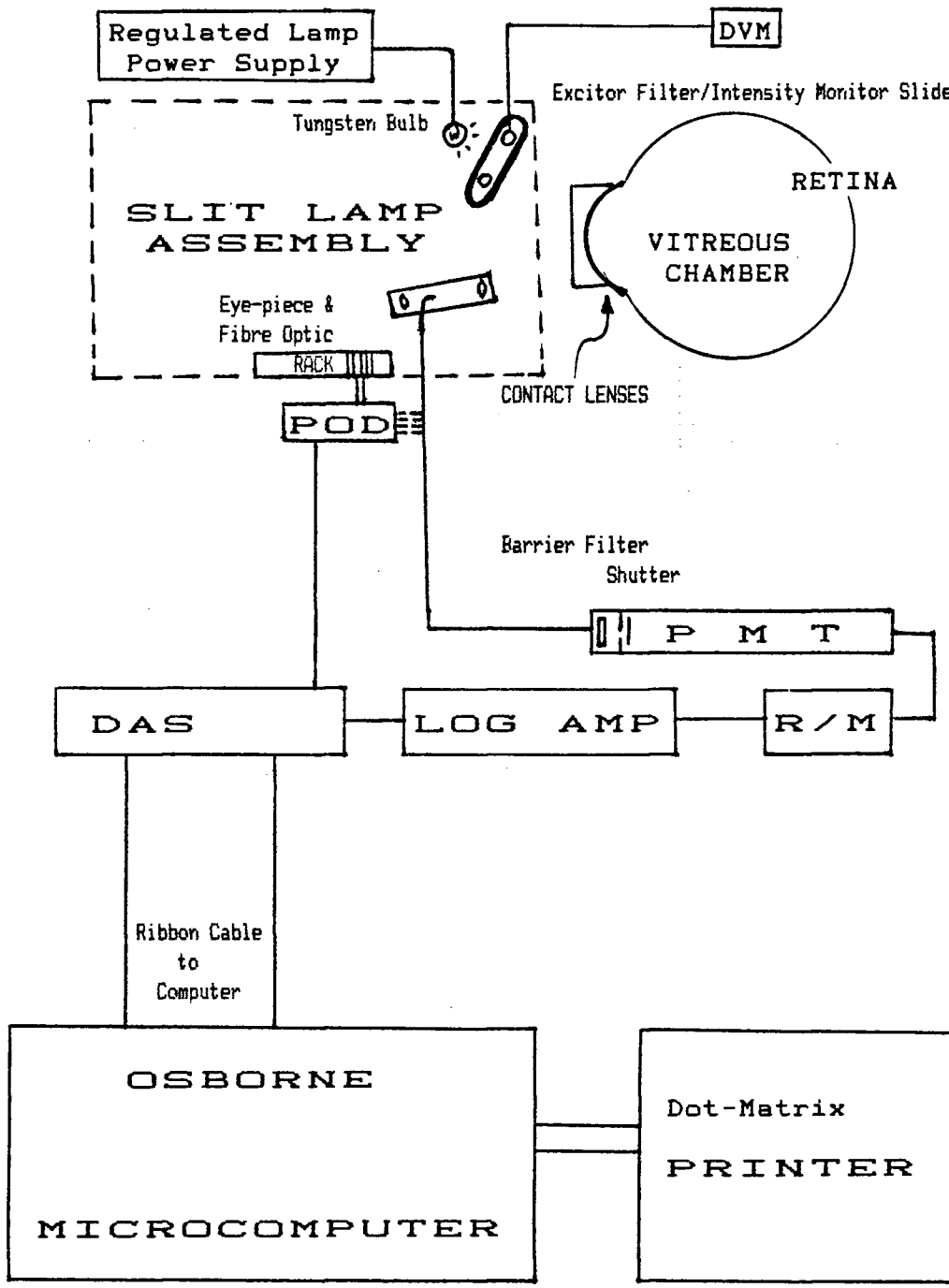
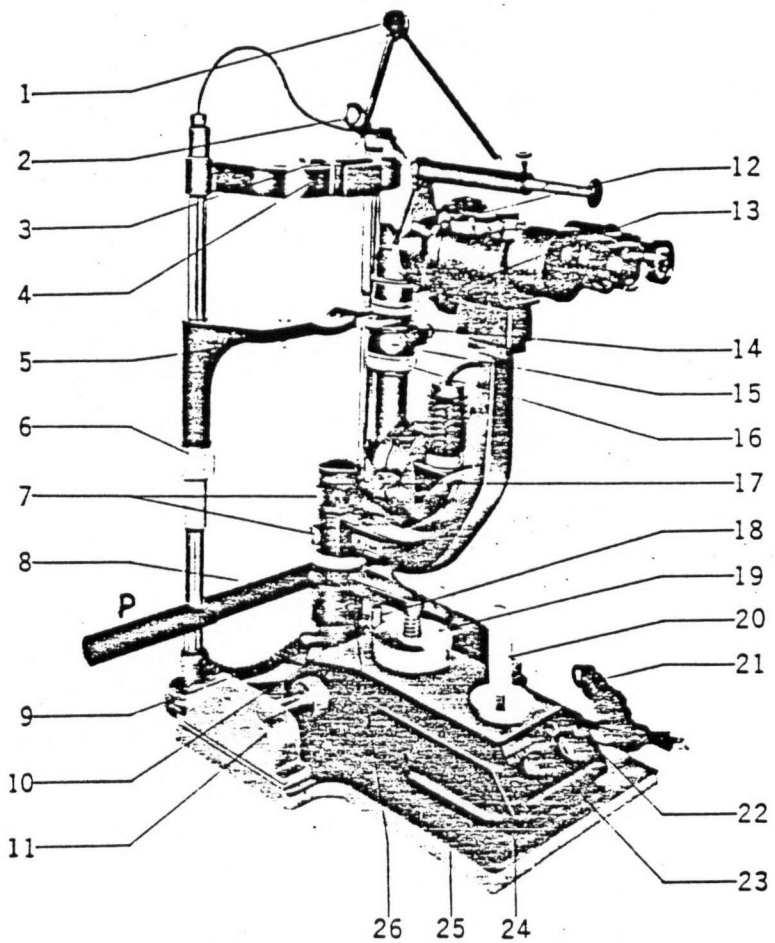


Figure 8. BLOCK Diagram of the VITREOUS FLUOROPHOTOMETER



1. Fixation lamp
2. Hruby lens
3. Hruby lens guide rail
4. Forehead rest
5. Chin rest
6. Knob for chin rest height adjustment
7. Arm clamping screws
8. Grip bar
9. Cord for fixation lamp
10. Socket for fixation lamp cord
11. Gear box cover
12. Zoom lever
13. Slit tilting ring
14. Filter slide
15. Slit rotation knob
16. Aperture diaphragm
17. Coaxial knob for slit control and rotation
18. Socket for slit lamp housing
19. Knob for height adjustment of microscope slit lamp assembly
20. Lever for cross-slide motion, coarse and fine, of the table
21. Power cord
22. Main switch and control for secondary voltage output
23. Pilot lamp
24. Cross-slide table
25. Base plate
26. Swivel arm connecting microscope and slit lamp assembly to cross-slide table

Figure 9. The Slit Lamp.
(From NIKON Zoom-Photo Slit Lamp Microscope Bench Type Instructions Manual)

illumination using a photocell.

Testing showed that the latter method was more sensitive to variations in beam intensity. At high intensities (i.e., running high currents through the filament), fluctuations were noted when used on the a.c. mains. These fluctuations were reduced (halved) when the regulated supply was installed.

It should be noted that these intensity fluctuations appear as variations in the signal about an average because the system is continuously exciting and detecting the fluorescence in overlapping volumes (because of the diamond). (Refer to Section 2.5.) Therefore, provided that the intensity of the bulb does not vary significantly from the same average value during each scan, the fluorescence fluctuations can be interpreted as deviations about an average concentration at any position in the scan.

It was also found that maintaining a constant, high intensity illumination was difficult because the high currents (and temperatures) cause the bulb intensity to fall continuously. Stable high intensities could only be attained after a "warming-up" period - usually about 30 minutes. However, excessive, long periods at high intensities caused a reduction in the lifetime of the bulb.

The configuration to monitor the intensity employs the unused side of the excitor filter holder-slide. A small solar cell was glued to a microscope cover-glass. The assembly was, in turn, glued to a washer that fitted into the slide. This method does not monitor the beam during a scan because the chip cuts off the beam when it is in operation.

Intensity checks are carried out immediately prior to scanning. The output of the solar cell is measured on an LED voltmeter. The intensity output is always adjusted to the value (on the voltmeter) at which calibrations were carried out.

Slit lamp translations are measured by constructing a potentiometer with a 10-turn rotary potentiometer/resistor. Fitted with a gear on its shaft, the pot is held by an arm attached to the grip bar of the slit lamp. (See Figures 8 and 9.) The assembly is referred to as the Pod.

The gear rests on a rack which is mechanically coupled to the body of the slit lamp. When the rack moves with the slit lamp during a scan, the Pod produces the analogue voltage signals which are read by the microcomputer. (See Figure 8.)

The most important modification to the slit lamp concerns the oculars or eye-pieces. One ocular is replaced by a special adaptor with a fibre optic conduit at the focal plane of the microscope objective. The fibre optic collects the fluorescence from the diamond and conducts the light to the Photo-Multiplier Tube (PMT).

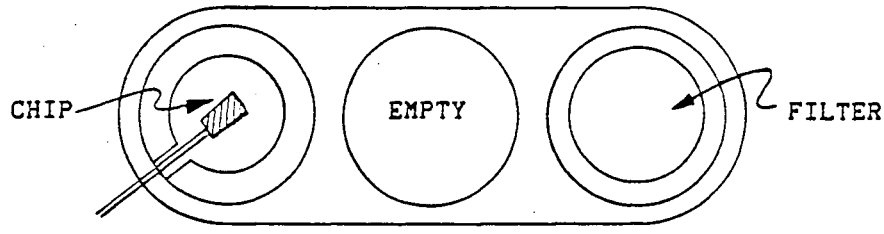


Figure 10. Excitor filter holder-slide and intensity monitor.

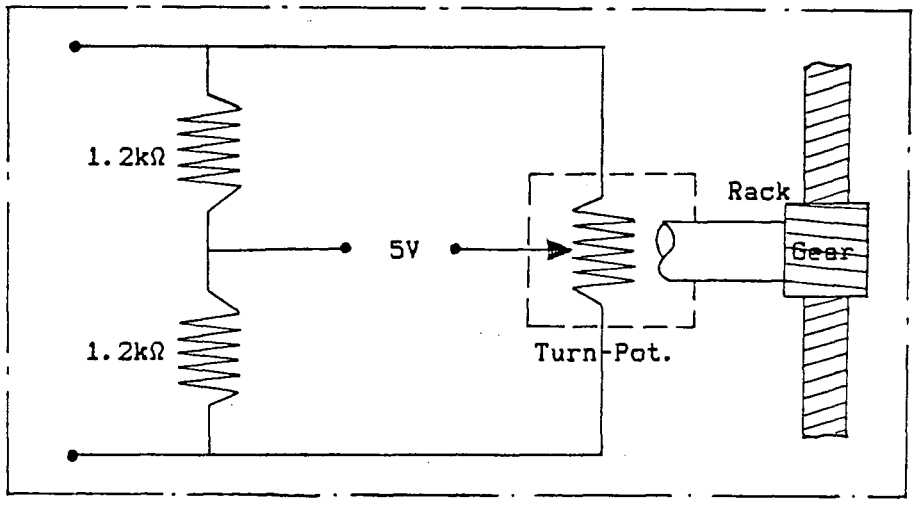


Figure 11. Pod assembly and circuit.

An electronic shutter and the barrier (green) filter are placed between the output of the fibre optic conduit and the PMT. (See Figures 8.) The PMT then relays to the radiometer (R/M) whose output voltage varies linearly with the amount of input fluorescent light.

The R/M has several exponent settings, including an AUTO-adjusting exponent option. The AUTO setting, which keeps outputs between 0 and +100 mV, was found to be unstable at the "cross-over" points where output voltages greater than +100 mV were scaled down. Hence, the R/M is set in the (most sensitive) 0-exponent range.

To offset high R/M outputs when high concentrations are scanned, a logarithmic amplifier (called LOG AMP in Figure 8) is used. The Log Amp was calibrated in conjunction with the Data Acquisition System (DAS). This is to ensure that the amplification of small input signals is such that its outputs remain logarithmic.

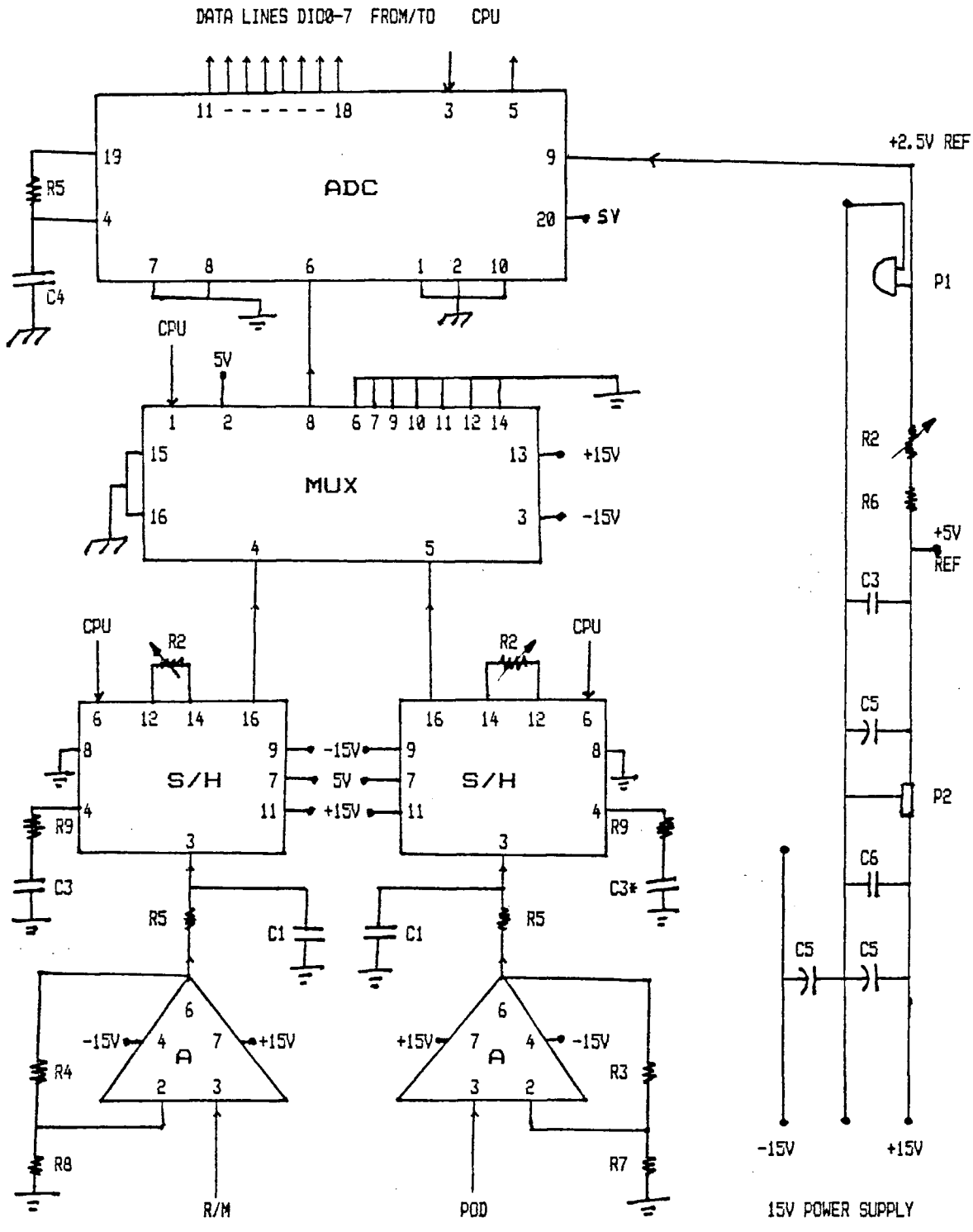


Figure 12. Pin diagram of the blue circuit board in the DAS.
C3* is 3 C2-capacitors in series.
Unused S/H pins are not shown.
Offset trimpots, R1, for A are not shown.
(See Appendix B.1 for part numbers.)

The outputs of the Pod and the Log Amp are connected to the DAS. Two circuit-boards comprise in the DAS. A 15-V power supply (white) board produces the necessary power for the components on the principal (blue) board. The latter circuit is shown in Figure 12. (Part numbers are given in Appendix B.1.)

The Pod and Log Amp outputs are connected to the inputs of two operational amplifiers (A) which are each followed by a sample-and-hold chip (S/H). The outputs from the latter are then connected to two channels of an 8-channel analogue multiplexer (MUX) which is an electronic switching device. The other six channels, that are not used are grounded. The MUX selects one output of the S/Hs after the other, passing the signal onto the next chip.

The next chip is an 8-bit Analogue-to-Digital Converter (ADC) which is the principal component of the DAS. Its purpose is to convert the analogue inputs, held steady by the S/Hs, to the digital format that the microcomputer understands. As it can only convert one input at the time, the S/Hs become necessary for maintaining those input voltages until the MUX selects them for the ADC.

The output of the ADC goes out on a ribbon cable to the microcomputer. The cable also carries the sequence of instructions from the computer to the various DAS components in order to properly organize the conversion of the signals.

Besides powering the As, the MUX and the S/Hs, the +/- 15-V supply also operates the shutter in the PMT. The latter opens when the voltage is changed from -15 to +15V, and closes when the polarity is reversed. The shutter movements are co-ordinated with the

activation of the DAS by a mechanical toggle switch called Switch A on the front face of the DAS box.

Lastly, the +5V logic level required in CMOS digital electronics [43] is obtained by connecting the +15-V line to a voltage regulator chip, (P2 in Figure 12). The +5V output also provides the voltage drop across the Pod circuit. It is also, in turn, connected to another voltage regulator-reference chip, P1 to produce the 2.5V required for the ADC reference.

The microcomputer is the 8-bit, 64-K Osborne 1 with CPM operating system. Its main advantages are its parallel and serial ports. The disadvantage of this machine is that it is configured with three memory banks. Bank #1 contains the usual transient programming area, while Bank #2 partially shadows it. All ports are accessible from Bank #2 only [44,45]. Bank #3 is video memory.

As the Osborne controls the DAS directly through the parallel port, considerations such as the transition time between bank had to be taken into account during programming in order to optimize the data acquisition. Although the ingenuity of the electronic design of this configuration did not go unappreciated, the time required to understand and work with it could have been put to better use.

The last component in the system is a dot-matrix printer that produces the hard-copy results. It is also accessed through the parallel port (by Centronics communication protocol). As no printout is ever required until all scanning has been completed, there is no competition for the port between the printer and the DAS.

3.3 Other Material

Two contact lenses are used during scanning. One is a hydrophilic soft contact lens which is fitted first. Its purpose is to alleviate the discomfort of the second lens without using repeated instillation of topical anaesthetic. The second lens (Luma® lens) is plano-concave to offset the power of the cornea. It is made of a mildly pliable plastic, and provides the "window" for viewing the fundus.

The soft lens is attached to the cornea by the surface tension of tears and the hard lens attached to the soft lens by the surface tension of viscous methyl-cellulose. Saline solution was tried but did not hold the Luma® lens in position. It also caused irritation in some subjects.

The two band-pass filters used are Spectrotech SE4 and SB5. Their transmission profiles and overlap are shown in Figures 13 and 14. Their cut-off wavelengths are in accordance with specifications for fluorescein in water. (Refer to Section 3.1.) The SE4 excitation filter passes wavelengths between 453 and 493 nm only. Similarly, only the main emission peaks between 509 and 612 nm are transmitted by the SB5 barrier filter.

The smaller (blue) SE4 is mounted in the holder-slide which also holds the intensity monitoring chip. It is inserted into the path of the beam before scanning. The larger green filter (SB5) is placed between the output of the fibre optic and the electronic shutter above the PMT. Its large aperture ensures that all signals from the conduit pass through it before activating the PMT.

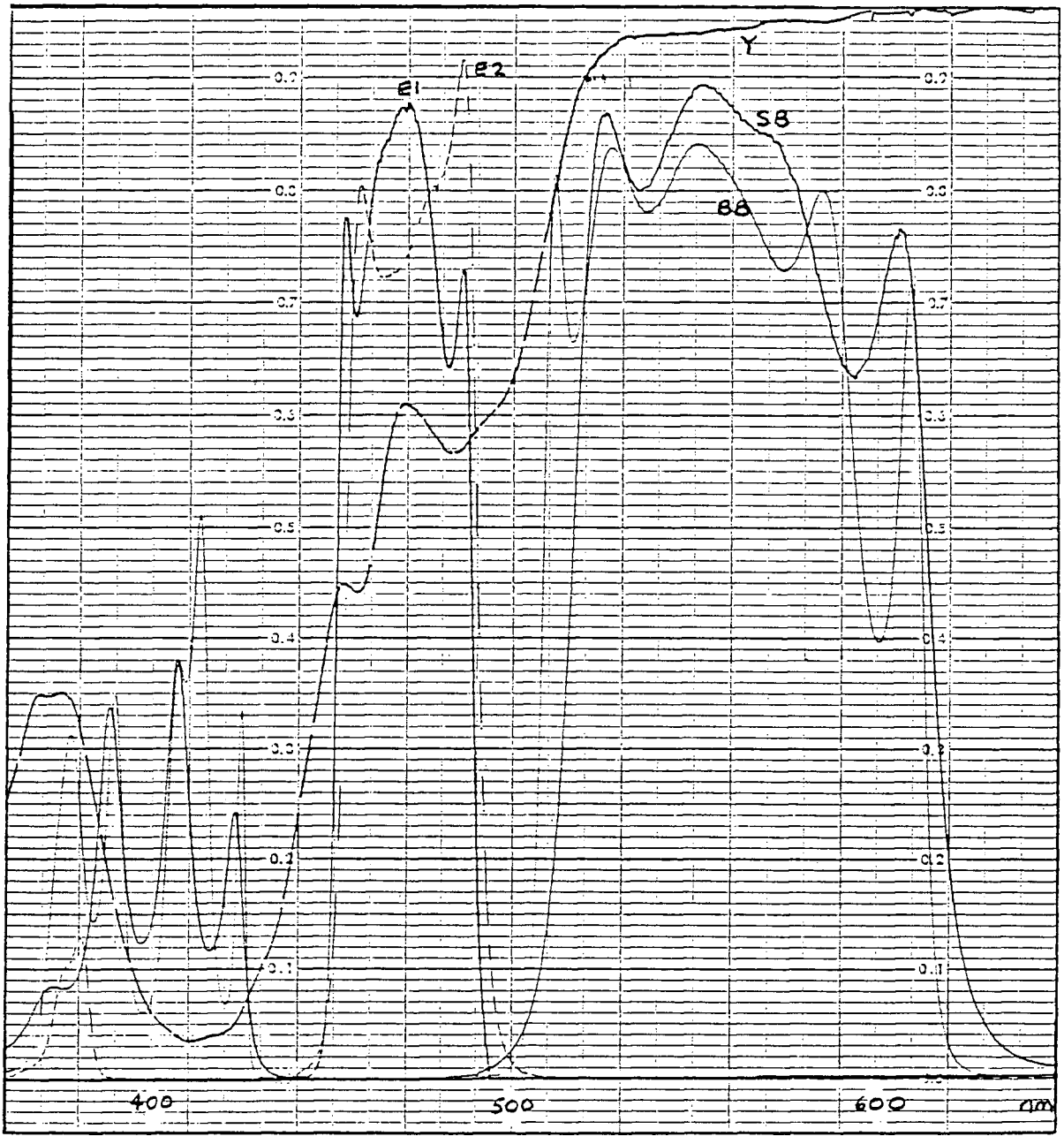


Figure 13. Filter transmission profiles.
E2 and BB were used.
Y is the yellow filter.

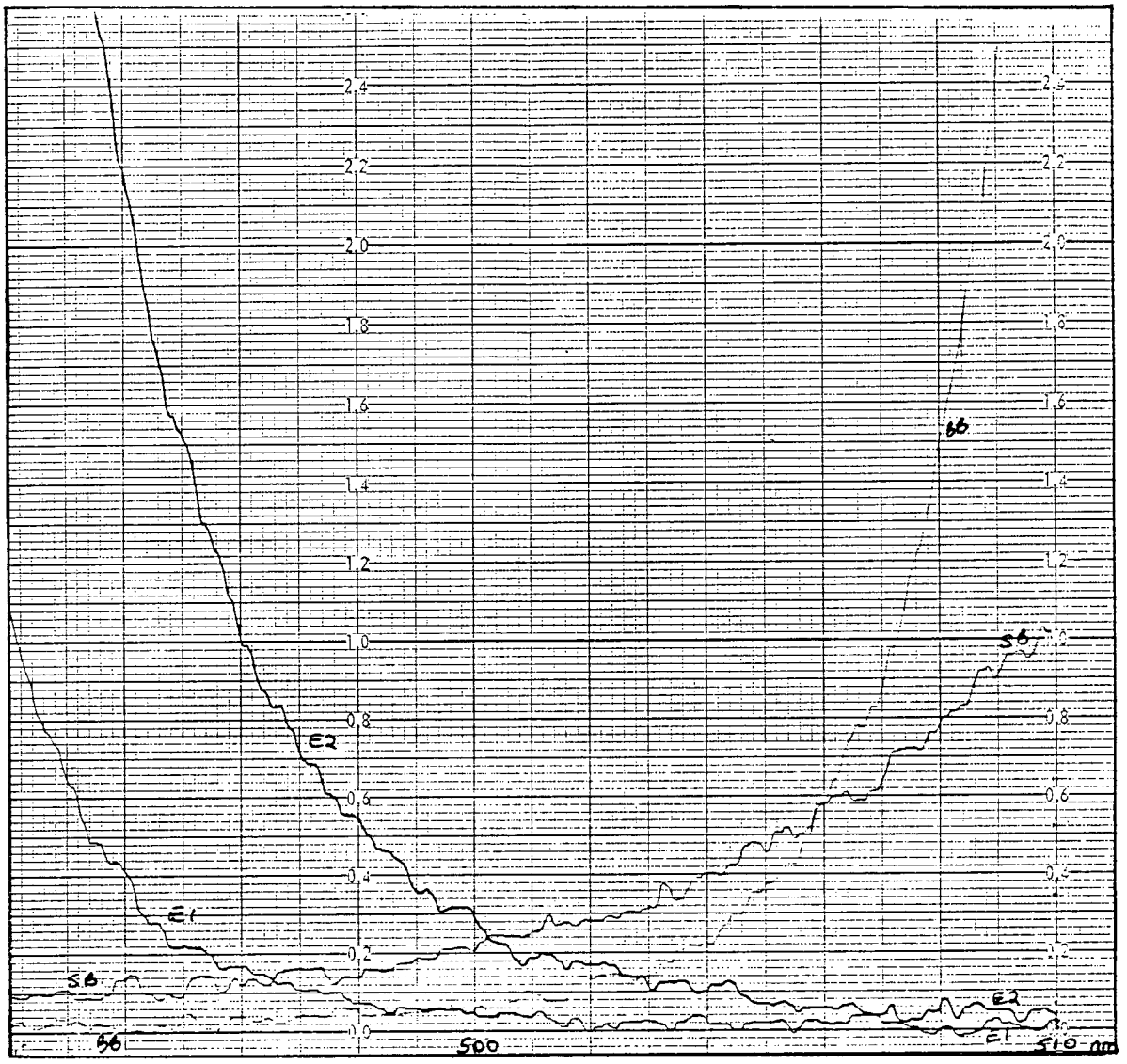


Figure 14. Filter Overlap.
E2 and BB were used.

A model eye was also constructed. Its purpose was to calibrate slit lamp translations. However, the calibrations were made with a more precise micrometer translation stage fixed on an optical bench. (Refer to Section 4.1.) The model eye is also used as a sample cell (for plasma scanning). A cross-sectional profile is shown in Appendix B.3.

3.4 The Software

Most programmes were written in Microsoft BASIC (MBASIC). Although a compiled version called CBASIC was available, MBASIC was chosen because it had many built-in functions for file and string manipulation. The one exception was the programme for the control of the DAS. Written in 8080 Assembly Language codes [46], its "listings" file is called DAS.PRN. Certain programming "habits" were developed because of restrictions (and economy) in the use of memory space. For example, many of the MBASIC statements written were concatenated, as is allowed by the language. "Free" variables were re-used wherever possible. (Refer to Appendix A for all programme listings.)

Specific subroutines are called from menu programmes. The first such menu, SCANMENU.BAS directs control to one of three subprogrammes for scanning and filing. These are VITSCAN.BAS and PLASCAN.BAS which are stored on the disk in Drive B, and a subroutine (in SCANMENU.BAS itself,) called SUBJECT DATA ENTRY. A subroutine, when called, is merged above SCANMENU.BAS in the memory bank. When a scan is ended, control is returned to the menu.

Prompts to operate the data acquisition were written into this

set of subroutines to enhance "user-friendliness". NO and YES responses are indicated by hitting the ESC and ANY (other) key respectively. This association is appropriate as the ESC key is located at the upper left corner of the keyboard and has little probability of being mistakenly activated, especially when lights are dimmed during scanning.

A version called DAS.ASM was first prepared using the specification sheets of the various electronic components in the DAS as guides [43]. It was then assembled by the 8080 two-pass assembler provided with the Osborne 1, producing the listings, DAS.PRN as shown in Appendix A.1. Note that the left four hexadecimal (hex) numbers denote the memory addresses (in Banks #1 and #2) of the machine language codes given by the next 2 to 6 hex digits. Entry and exit loops to Bank #2 are clearly marked.

The port status test is executed only on the first entry where, if necessary, the port-controlling, Peripheral Interface Adaptor, MC6821, is re-configured to suit the DAS.

The strategy of this subroutine is simple. After preparing the port, poll Switch A until the toggle is up. Tell the S/Hs to sample their inputs simultaneously, then hold them. Ascertain that the ADC is not busy. Next, order the MUX to switch on and the ADC to begin digitizing the pod-S/H output. When the conversion is done, store the result at a specific address (D1D2 hex) in memory. Make sure the ADC is ready. Now order the MUX and ADC to do the same for the R/M-Log Amp-S/H output. This time, store the answer at D1D4 hex. Test the interrupt status line (Switch A) and put the result at D1D0 hex. Go

back to the MBASIC calling subroutine.

When SCANMENU.BAS is loaded, it reserves the area above D1CF hex for the machine language code numbers (from DAS.PRN), and the abovementioned results after each call. The machine language codes are loaded only if one of the first two subprogrammes is called. They are placed into the reserved memory by the DATA-READ-POKE sequence of commands, starting at memory address D1D6 hex.

If VITSCAN.BAS is loaded, a checklist of the VF system is immediately displayed and the first call to DAS.PRN is immediately made; usually to measure the various intra-ocular distances. VITSCAN .BAS marks a position by sounding a "beep" when any key is depressed. When "landmarking" is ended, it displays the difference between positions corresponding to consecutive beeps, then asks whether to repeat landmarking or continue on to scanning.

Before scanning begins, a prompt to ascertain the eye to be scanned is given: ESC for the right eye; ANY (other) key for the left. Once answered, a set of "SCANNING INSTRUCTIONS" is displayed. Switch A is toggled up to initialize the DAS in a 3-second loop. (This delay time may be varied by software to suit the time constant of the DAS.)

The digitized outputs of the Pod and the Log Amp are displayed in (approximately) the first second of this delay loop, after which, the screen is blanked. The remaining time of the loop is for "dark adaptation" by the subject and the system to reduce the noise picked up by the PMT.

A "beep" sounds to mark the position of the retina (where every

scan must begin), as well as to cue the user to begin scanning. Control toggles back and forth between Banks #1 and #2. After each return from DAS.PRN, the abovementioned specified addresses are PEEKed. The data are transferred to elements of two 2x1600 arrays (depending on the eye being scanned). Switch A is tested to ascertain that the scan is to continue or to stop.

The maximum time available for scanning before the arrays are filled is approximately 25s. If they are used up, VITSCAN.BAS automatically exits from the scanning loop to flash "You are out of memory...". Alternatively, Switch A may be toggled down to end a scan.

Upon leaving the scanning loop, VITSCAN.BAS goes into a plotting and filing subroutine. Left-eye data are filed on the disk in the left drive; right-eye on the right. In this way, a one-diskette-per-subject-eye system of data storage is maintained. This flexiblity allows for both eyes of one subject to be tested, or, two subjects to be examined within the same period by assigning one diskette to one subject-eye. (As many subjects as time restrictions allow may be examined, but only the diskette in the LOGGED drive may be changed - a quirk of MBASIC.)

An interrupt is included to enable an "ABORT" during plotting and filing. It is activated by depressing any key. Two prompts to confirm the "ABORT" appear. An positive response produces another prompt to continue scanning. A negative reply returns control to the position within the plotting and filing subroutine where the interrupt was activated.

The temporary file that stores the data is renamed when the plotting and filing subroutine has been completed and it is confirmed that the data is to be saved. The filename is entered when the response is affirmative; otherwise, the temporary data file is overwritten or erased later. Files are named by the p.i. time. For example, the 3-minute p.i. bolus scan is named 3.DAT where the filetype, ".DAT", is automatically juxtaposed to the "3" entered.

The prompt to continue scanning appears next. If scanning is to be continued, there is a choice to begin again at SYSTEMS CHECKS or at SCANNING INSTRUCTIONS. If the scanning mode is to be terminated, control is returned to SCANMENU.BAS.

In the plasma scanning option, similar calls to DAS.PRN are made. No plotting loop is required in this case as the slit lamp is fixed in position. (See Section 4.3.) The arrays used are smaller: two arrays of 1000 elements. An averaging subroutine is immediately entered when the scanning loop is exited. A similar interrupt capability is also installed.

The results of the averaging and the standard deviations are displayed. The time of the blood sampling in minutes p.i. is then entered. Three 55-element arrays are used to hold the results of each plasma sample scanned. At the end of scanning, the arrays are filed in PLASMA.DAT in the appropriate subject-eye diskette.

The SUBJECT DATA ENTRY subroutine is usually called after VITSCAN.BAS and PLASCAN.BAS have been executed. This subroutine is used to enter pertinent subject information: name, age, eye that was scanned, date, lengths of intra-ocular distances recorded by both the

slit lamp and ultra-sound scans, volume of dye that was injected, comments, and observations. The last category is used to enter notes on a particular scan or the subject's medical history. All information entered is filed in SUBJECT.DAT in the appropriate diskette.

At the end of an examination period, there should be a SUBJECT .DAT file, a PLASMA.DAT file, and the scanning .DAT files on a subject-eye diskette. All other non-.DAT files are erased. For later analysis, a working copy of the data diskette is always made.

The second menu routine is BATCHRUN.BAS. It allows the execution of individual subprogrammes, or a specified sequence of subprogrammes. On activation, a menu shows all the subprogrammes with brief descriptions of their purposes. The order of the subprogrammes to be run is entered and confirmed. The file directory of the data diskette next appears, and the files to be analyzed are then entered.

After printer status and paper supply have been checked, execution is begun by CHAIN MERGEing the first subprogramme in the sequence. Control is then transferred to the task. On completion, the next subprogramme in the sequence is loaded over the first, and so on until the series is completed. The following describes the subprogrammes and their tasks.

REDUCE.BAS averages the raw data in each .DAT file, reducing the number of data-points from a possible maximum of 2x1600 per file to a possible averaged maximum of 3x256 - Pod output, averaged R/M output and its standard deviation. The algorithm, although slow because of memory space restrictions, is not difficult to follow. A flowchart is shown in Figure 15.

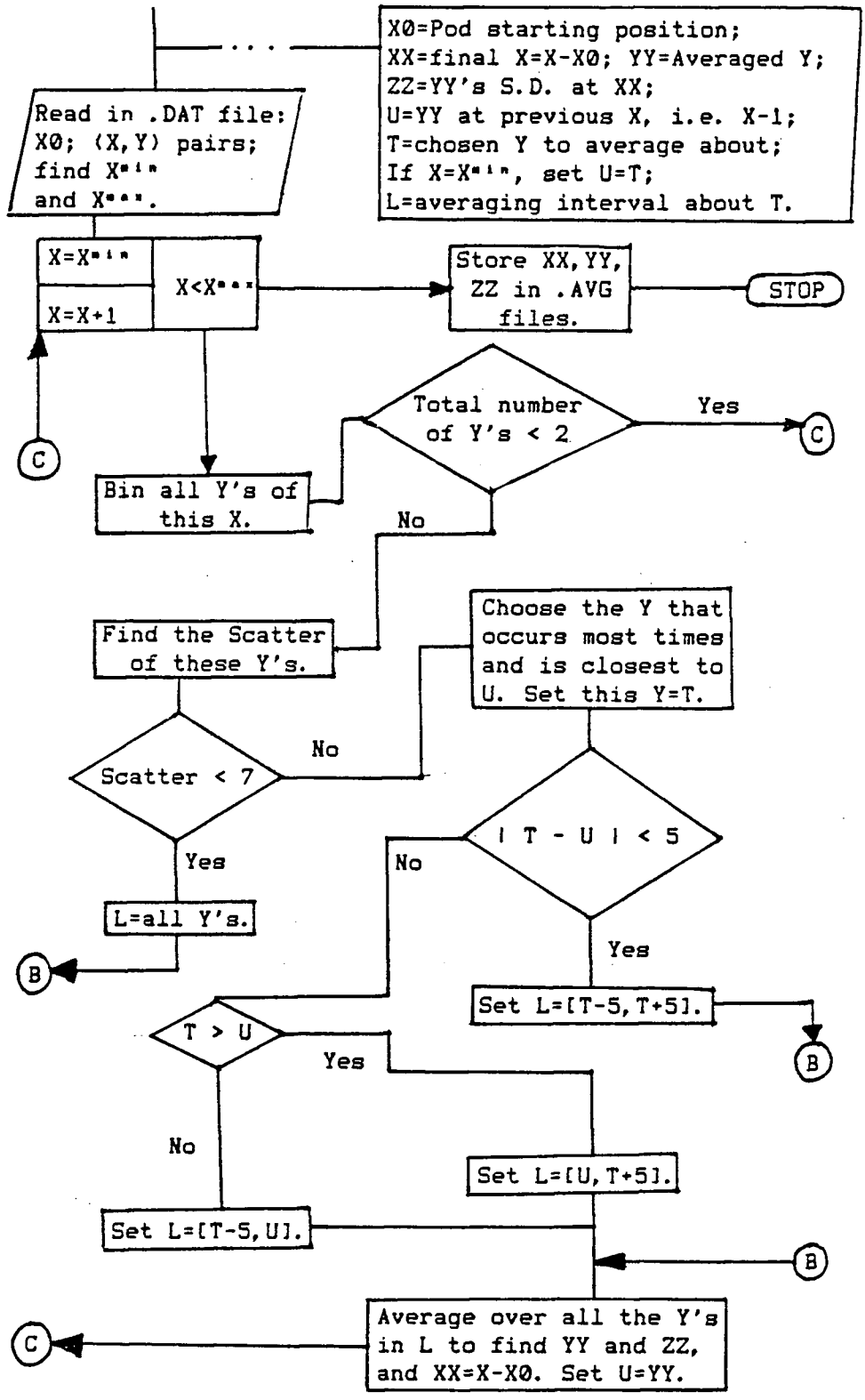


Figure 15. FLOWCHART for REDUCE.BAS.

Basically, REDUCE.BAS considers the scatter of the R/M outputs at each Pod output. It sets an interval within which all R/M outputs are included in the averaging. This interval is dependent on the result of the averaging of the Pod output before it. Hence, the averaging process is carried out point by point in ascending order of Pod output from the posterior vitreous to the cornea. The results are zeroed by the retinal position (at the "beep") and converted to concentration units before being stored in a .AVG file with the same p.i. time filename.

After a resultant .AVG file has been entered, the associated .DAT file is destroyed. This action frees diskette space for later files. This is the reason for creating a working copy of the original data diskette.

This technique of data reduction or "smoothing" was chosen over the usual methods such as curve-fitting because of the method of scanning. Also, as mentioned before, this VF system is a time-averaging DAS as opposed to the "spot" system [38]. It is "backward-biased", as it is dependent on the average of the previous Pod position because of the capacitive time constant (approximately 2.2s) in the electronics.

Several background scans are usually made. They are .DAT files identified as "0", "00", "000", etc. B/G.BAS contains a background-file-testing loop to ascertain that multiple pre-injection scans were made. If the loop finds several such scans, B/G.BAS takes these already averaged background scans, aligns (by the location of retinal position) and averages over all of them to produce one final 0.AVG

file. All other background .DAT and .AVG files are then deleted to free diskette space. If only one background scan was made, B/G.BAS returns to BATCHRUN.BAS to the next subprogramme in the series.

MINUS.BAS subtracts the final 0.AVG file from all other .AVG files. Two sets of results are produced by two types of alignment:

a) by matching the positions of the retina as they were marked on each scan (at the beep); or,

b) by searching out and matching the CR peaks.

Subtraction is then carried out after each alignment. The results by (a) and (b) are filed in .RET and .CRP files respectively. Again the filenames are the p.i. times of scan.

SUDATA.BAS prints out the subject and scanning data. A formatted output of the information in the SUBJECT.DAT file is produced. The F-numbers (in Section 2.2) are calculated and printed. The routine reads through every .AVG, .RET and .CRP file, compiling the concentrations at a set of specified points in each profile. These points are the retina, the CR, lens and corneal peaks, the minima and the centres of the vitreous and the aqueous chambers, and points (that are in the vitreous chamber only) which are 0, 3, 6, 9, 12, 15... mm from the retina. At the end of compilation, SUDATA.BAS calculates, when appropriate, the performance specifications: LLoD, AR and R.

BLOOD.BAS is the curve-fitting subprogramme that calculates the areas under the plasma profile. PLASMA.DAT is read, and the data are converted to concentration units with a constant that accounts for buffer dilution and the unbound fluorescein factor - 17%. (See

Section 3.1 and [47].)

To calculate this constant, it is assumed that after centrifuging, all the red blood cells have been removed to one end of the haematocrit but that the fluorescein is homogeneously distributed throughout the plasma. If $c^p(t)$ is the plasma fluorescein concentration at sampling time, t, and only 17% is available to penetrate the BRB at all times, the area under the plasma-fluorescein profile is given by

$$I(t) = 0.17 \int_{\tau=0}^{\tau=t} c^p(\tau)\, d\tau \qquad (28)$$

If X ml of each spun sample is diluted with Y ml of buffer, then the resultant concentration, $c^p(t)$, measured by the slit lamp is given by

$$c^p(t) = X * c^p(t) / (X + Y) \quad . \qquad (29)$$

Eq. 28 becomes

$$I(t) = 0.17 (1 + Y/X) \int_{\tau=0}^{\tau=t} c^p(\tau)\, d\tau \qquad (30)$$

The factor before the integral depends on the amount of free fluorescein (17%), and the volumes of plasma sample and the buffer used in the dilution process. Note that any amounts of X and Y may be used.

A set of four polynomials of order 2 is fitted to the data, i.e., (2+1)-parameter curve-fitting [34]. The polynomials are:

0) $y = A + B * x + C * x^2$;

1) $y = A + B * \log x + C * (\log x)^2$;

2) $y = A * \exp (B * x + C * x^2)$;

and, 3) $y = A + B / x + C / x^2$.

They were chosen to approximate the removal of the dye from the blood. The difficulty in producing a fast and efficient algorithm in a non-linear fit to the sum of two negative exponentials (expected of the fast and slow decays of the two-compartment model mentioned in Section 2.2 and [28]) forced such a method for estimating the plasma integral. It is also difficult to justify such a non-linear fit to a few data-points only. (Refer to Sections 4.2 and 4.3.)

Areas under the best fit are calulated as follows - (1) the integration lower limit is t=0.5 minutes p.i. (2) The upper limits are the measurement scans' p.i. times (given by the filenames). (3) The area from 0 to 0.5 minutes is approximated by the area of a right-angle triangle with base 0.5 and height equal to the value of the best fit at 0.5. (Refer to Section 2.2.) Plots with the fits superimposed on the raw data are produced. The goodness of fit is determined by the reduced chi-square of each fit.

At the end of the four fits, the one with the lowest reduced chi-square is chosen. Its code number (0-3) is entered in a PLASMA.FIT file, followed by the coefficients (A, B and C) and their calculated errors, the upper limits of each integration, the areas up to those limits, and their errors.

C/VAZ.BAS follows closely the algorithm of Section 2.3. This subprogramme uses .RET and .CRP files only. When .RET files are used, all profiles are aligned for subtraction by the positions of the retina as marked at the beginning of each scan. Similarly, .CRP

files are aligned by the CR peaks before any subtraction.

The bolus .RET file and a measurement .RET file are read simultaneously. The permeability indices, P^1 (of Section 2.2) and the penetration ratios, PR3 (of Section 2.3), as well as PR3* are calculated for each profile. The CR correction condition, the left-hand side of Eq. 6, is also calculated.

Two types of bolus correction are undertaken. The first involves subtraction of the bolus from the measurement scan without applying the CR bolus ratio. (Refer to Section 2.5.) A plot of the results between 0 and 11 mm from the retina is produced, followed by calculations of P^1, PR3 and PR3*. For the second type pf correction, the subtraction is repeated but with the bolus profile modified by the CR bolus ratio. A plot is produced, and the three parameters calculated. The procedure is carried out for the .CRP files with the same p.i. times.

If more than 10 non-negative points exist within the posterior half of the vitreous chamber after each subtraction, C/VAZ.BAS files them in .CV# files on the data diskette. The files are named with the same p.i. time filenames, but, with the following filetypes:

.CV1 "unaltered bolus subtraction of .RET files";

.CV2 "modified bolus subtraction of .RET files";

.CV3 "unaltered bolus subtraction of .CRP files";

.CV4 "modified bolus subtraction of .CRP files".

If many measurement scans are made, the data diskette can run out of storage space. To prevent this from halting the calculations, C/VAZ.BAS contains an error-trapping subroutine which automatically

files the .CV# files on the diskette which contains the MBASIC programmes in the other drive. By studying the plots produced by C/VAZ .BAS, some of the .CV# files can be deleted. The .CV# files on the programme diskette can then be transferred back onto the data diskette.

SLOPES.BAS checks the results of C/VAZ.BAS by curve-fitting at the .AVG-file level, using the same set of (2+1)-parameter functions as those in BLOOD.BAS. Only vitreous data are considered in the curve-fitting. A vitreous profile is divided into three sections: posterior, mid-, and anterior vitreous. The four functions are fitted to one section at a time. The fit with the lowest reduced chi-square is chosen to represent that section in all later calculations.

When all the best fits have been calculated for each section, SLOPES.BAS uses them to estimate the following:

a) the concentrations after background subtraction, c(x,t).

b) the penetration ratios, PR3*, before and after bolus subtraction.

c) the gradients, $c^{\bullet}(x,t)$.

d) the diffusion constants, D.

The values chosen for x are 3 and 9 mm from the retina, as well as 3mm from the posterior surface of the lens. PR3* is found at the 3-mm point from the retina and the lens without CR bolus correction. Alignment is by RET only.

D is approximated by converting the diffusion equation, Eq. 1, into a difference equation [34,48]. This approximation uses the calculations at the 3-mm and 9-mm points. It is

(31) $$c(9,t)/t = D * \{ c^0(9,t) - c^0(3,t) \}/(9 - 3) .$$

t is in minutes p.i. Solving for D,

(32) $$D = c(9,t) * t^{-1} * 10^{-3} / \{ c^0(9,t) - c^0(3,t) \} \ cm^2 s^{-1}.$$

The 10^{-3} factor appears when converting to centimetres and seconds. These values of PR3* and D may be used as initial estimates for LUND.BAS curve-fitting as well as checking the solutions of RET C/VAZ.BAS.

PLOT.BAS is an earlier version of SUDATA.BAS. It produces individual scaled-down plots of any .AVG, .RET or .CRP file. All the information gathered by SUDATA.BAS is also collected and printed below each plot. Comparison of data and plots by inspection is difficult.

DRAW.BAS is the plotting subprogramme that complements SUDATA .BAS. This subprogramme uses the first entered file to produce semi-log plots recorded data-point by data-point. All subsequent plots are scaled and superimposed on the first plot. Its principal advantage is that it can superimpose the profiles from different subjects as well as producing "fully stretched" profiles. Limited by the loss of clarity when too many files are superimposed, the routine provides a means for easy comparisons of the profiles from different subjects, and the evolution of fluorescein profile of one subject.

The last routine written was LUND.BAS. The algorithm is that stated in Section 2.4. The routine - largely translated from the FORTRAN subroutines in Bevington [34] - uses the Marquardt gradient-expansion method of non-linear curve-fitting. Although it is able to

read data from any file(-type), LUND.BAS is usually run on data from .CV# files.

The following are required at each run:

a) chosen file from which data are read.

b) interval within which data are to be fitted.

c) initial estimate of P, and its step increment.

d) initial estimate of D, and its step increment.

The chosen interval is usually between 1.5 and 6 mm from the retina. Initial P and D values depend on the expectations of the results for the particular subject i.e., the results of C/VAZ.BAS and SLOPES.BAS. Increments are usually 10% of the initial estimates.

The subprogramme tests the fit by calculating a reduced chi-square rather than the S^2 of Eq. 23 in Section 2.4. The weights are the S.D. of the data as calculated by REDUCE.BAS (or others), i.e., at each Pod position. It is also possible to use the LLoD (if known) in Eq. 24.

The reduced chi-square for the initial values entered is found. The subprogramme then goes on to find better fits by varying P and D by the increments entered. After each fit, a new reduced chi-square is found. If it is smaller, the subprogramme can be terminated. If not, it is used to determine the direction to vary D and P for the next fit. The search can only be terminated in three ways - (1) hardware interrupt (abort) or, (2) overflow errors (division-by-zero), (3) reduced chi-square less than 2. As poor initial estimates may result in slow convergence and long computing time, this subprogramme is only used in overnight runs.

IV. CALIBRATONS AND PROTOCOL

In this chapter, the calibrations of various components of the VF system and the preparation of subjects for scanning are described. The results of the former are given in Appendix C.

As the ADC is an 8-bit converter for a maximum input of 5V, one digit increment of its output 256 "Osborne/DAS" units - 0 to 255 - is a +19-mV change of input voltage. All calibrations must be in ranges that are expected during a scan. The gains at every stage in the DAS must be carefully adjusted so that the full range of the ADC can be used. In this way, the sensitivity of the VF system is optimized.

4.1 Instrument Preparation

The operational amplifier (A) receiving the Pod output, and the S/H were offset and zeroed. The gain was adjusted to approximately 12. This allowed a net displacement of the slit lamp of approximately 22 mm. The focus of the probe in the vitreous chamber however, was displaced more than this value because of the F-numbers (in Section 2.2).

The Pod (or slit lamp translation) was calibrated by placing the slit lamp microscope assembly on an optical bench on which a micrometer translation stage was fixed. The micrometer was used to displace the slit lamp in precise, 0.025-in increments. The result of each displacement was recorded from the computer monitor. A linear least-squares fit was carried out on the data collected. Only the slope of the fit is required as the zero position changes with each

scan. (See Appendix C.1.)

As the Log Amp and the DAS are both capable of amplifying any input signal their gains have to be adjusted in tandem allowing low pre-injection scan signals to be differentiated from noise but not allowing high concentration signals to be amplified to greater than the maximum 5V that the ADC can convert.

The first step was to ascertain the range over which the Log Amp remained logarithmic. At low voltage inputs, the Log Amp did not maintain its function, because it was performing close to "zero" at 1 mV (the logarithm of 0 was of course not defined). For input values up to 10V, the Log Amp performed satisfactorily.

Other conditions were:

a) R/M set at 0-exponent outputs a maximum signal of about 10V for a maximum concentration of about 100000 $ng.ml^{-1}$;

b) Log Amp output in the range of 0 to 5V for the ADC using 1 mV to 10 V as the input range from the R/M to the Log Amp.

The Log Amp was adjusted so that its output was logarithmic in the range of input voltages. (See Appendix C.2.) The operational amplifier to which the output of the Log Amp was connected, was then adjusted to a gain of 1.

Samples of various concentrations of sodium fluorescein were freshly made at each calibration because solutions lose their fluorescence efficiency over long storage periods. As the pH of the solvent affects its efficiency, the appropriate buffer solution (pH 7.4, discussed in Section 3.1) was also prepared.

A pH meter was calibrated at 20C and pH 7.0 using a standard pH

7.0 solution. The Sorensen's Phosphate buffer solution at pH 7.4 was then made up as follows -

a) Prepare a 0.907% weight by volume (or, 0.067 moles) solution of monobasic potassium phosphate in demineralized distilled water. - Solution "A".

b) Prepare a 2.39% weight by volume (or, 0.067 moles) solution of sodium phosphate in demineralized distilled water. - Solution "B".

c) To get 100 ml of a pH 7.4 buffer solution, for example, mix 19.7ml of A and 80.3ml of B, i.e., mix in A:B volume ratio of 1:4.076.

As an alternative, once the pH meter was calibrated, the pH 7.0 buffer concentrate, (the standard used to calibrate the pH meter) was used as a "base" to make the pH 7.4 buffer. This method was possible because the concentrate was made from sodium phosphate. Hence, by adding potassium phosphate to the solution at pH 7.0, a pH 7.4 buffer was obtained. This method was used to prepare the large volume of the pH 7.4 solution that was required for the sample concentrations needed for calibration, and also for diluting plasma samples from blood taken during scanning.

Sample concentrations were made by a dilution method.

a) Weigh an empty test-tube with seal in place.

b) Add an amount of sodium fluorescein powder, then weigh again.

c) Fill the test-tube with the pH 7.4 buffer and weigh again.

The first prepared sample was a "master solution" from which all other samples were derived. Its concentration was estimated by assuming that the mass and volume of sodium fluorescein were negligible compared to the mass and volume of added buffer. The

concentration of the master solution was then

(33) $$c^{ms} = \{ (b) - (a) \} / \{ (c) - (b) \} \quad ng.ml^{-1}$$

where (a), (b) and (c) are the results of the stages mentioned before. The mass of the dissolved salts was also assumed to be negligible.

The following procedure was used to make other samples:

a) Weigh an empty test-tube with its seal.

b) Draw a small volume of c^{ms} with a 1-ml tuberculin syringe, place it in the test-tube and weigh it.

c) Add buffer then weigh again.

The concentration of the new solution was

(34) $$c = c^{ms} * \{ (b) - (c) \} / \{ (c) - (a) \} \quad ng.ml^{-1} .$$

where (a), (b) and (c) are the results of the steps discussed immediately above. Other samples were made by varying the volumes of c^{ms} and the buffer in order to prepare concentrations between 5 and 9000 $ng.ml^{-1}$. Note that the error due to the mass of the fluorescein became less with greater dilution.

The prepared concentrations were placed in several sample cells (cuvettes) which were clamped in front of the slit lamp. Each cell was scanned through its 1-cm depth. This was carried out to study the effects of attenuation. (See Appendix C.5.) The peak in each concentration profile was used to find the R/M-Log Amp-DAS calibration curve. The reason for this was explained in Figure 2.

The R/M output increased linearly with the concentration that

is scanned but not the output of the Log Amp. Hence, the calibration equation was expected to be an exponential that translates the Osborne/DAS units to concentration values. The curve-fitting functions used were those in BLOOD.BAS. (Refer to Section 3.4.) The best fit was the one with minimum reduced chi-square. The mathematical solution is given in Appendix C.4.

Other slit lamp calibrations included slit dimensions, angle between excitation beam and probe, and the intensity of the beam. The slit size used was 2 X 0.1 mm. As the length did not affect the AR (in Figure 2), this magnitude was chosen to provide better visibility when focussing at the retina.

The width of the slit was defined by the rulings on a red blood cell counter (or hemacytometer). The vertical and horizontal lines formed a grid of 1 mm^2 with subdivisions of 1/400 mm^2. The slit was focussed on the grid. The microscope oculars were adjusted to the maximum (35X) magnification and the slit width adjusted to the desired size. The slit-adjustment knobs were then secured to prevent accidental reset.

Slit-lamp intensity, monitored by the solar-cell chip, was set at a chip output voltage of 141 +/- 1 mV which was the lamp "intensity" used to carry out the concentration calibrations. All scans had to be made at this LED voltmeter reading. The output had to be checked before and after each scan to ascertain that the excitation beam did not fluctuate significantly during scanning.

When the angle between the directions of the probe and the excitation beam was maximum, the AR was at a minimum. Using the

average depths and refractive indices of the model eye - Gullstrands schematic eye [30], this angle was found to be approximately 16°. (Other investigators' instruments are usually set at 14°. [31])

The entire slit lamp (objectives and bulb housing) was rotated, then locked at 8° from the translation axis of the slit lamp assembly. This latter adjustment could be changed for particular subject to overcome iris clipping or specular reflection off the plane surface of the Luma^R Lens onto the probe.

To test the suitability of all instrument adjustments, a scan of the model eye was carried out. Different concentrations were used in the various compartments. The profile is shown in Appendix C.7.

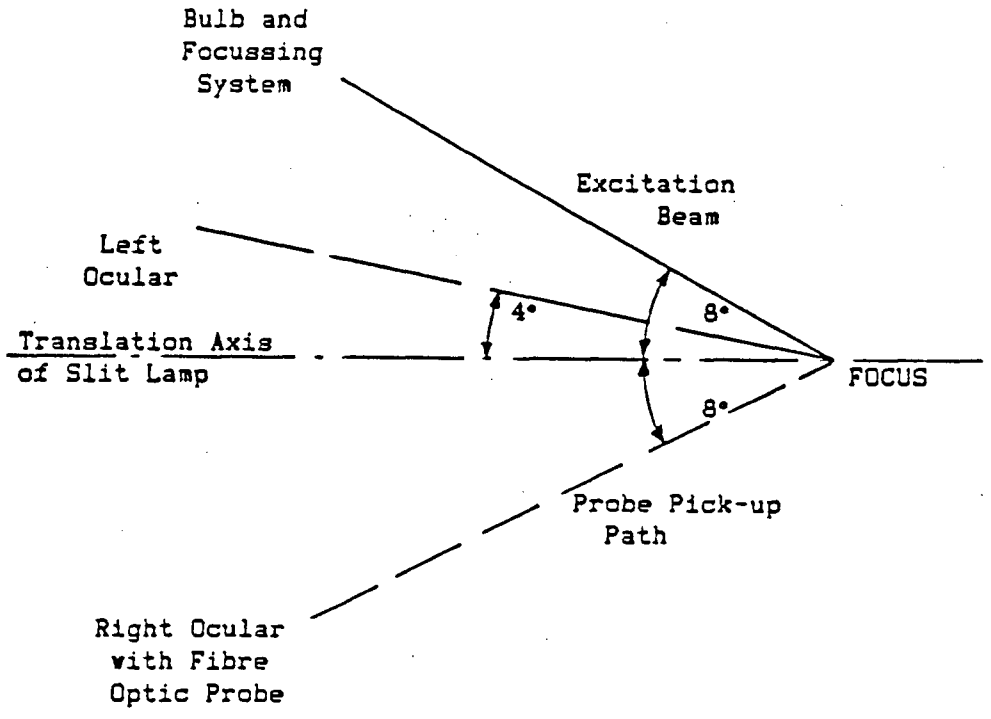


Figure 16. Overhead view of the angles at which the slit lamp was set for LEFT eye scans.

4.2 Subject Preparation

A subject is given a CONSENT FORM (shown in Appendix A.17) to read and sign. The attendant ophthalmologist answers additional questions the subject may have and countersigns the form. The age and the weight of the subject are noted and the latter used to calculate the amount of sodium fluorescein for injection (at 14 $mg.kg^{-1}$ body weight).

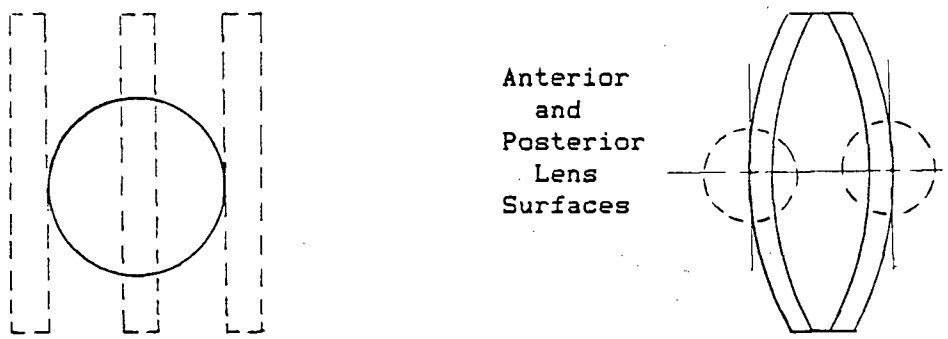
The pupils are dilated using drops of tropicamide 1% and phenylephrine 5%, repeated after 10 minutes. After a 30-minute waiting period for the drugs to take effect, the pupil diameter is measured. If it is 7 mm or larger, the scanning procedure is started. If the dilation is still inadequate for scanning after additional tropicamide and phenylephrine, the procedure is cancelled.

The subject's seated position is explained and tried. The slit lamp and chin rest are adjusted to the subject's height to minimize discomfort during the scanning. The sterilized soft-contact lens is then fitted to the left eye, then the sterilized Luma® Lens is mounted with a drop of methyl-cellulose. Each mounting is checked for trapped air bubbles. The subject then places his/her head in position on the slit lamp assembly with instruction to press the forehead against the headband.

The first scan for "landmarking" uses unfiltered light to localize the surface positions of the retina, the posterior and anterior lens, and posterior cornea (along the optic axis). The depth or thickness of each section is displayed and noted (in Osborne /DAS units) by the programme, VITSCAN.BAS. The measurements are

usually done three times to check reproducibility.

The retina is marked by two methods. One way is to centre the smaller, 0.1mm slit on the larger, 0.45mm diameter probe. However, the effects of scattering (halation) makes this method difficult. Another method consists of placing the left edge of the slit on the right edge of the probe; then the right edge of the slit on the left edge of the probe. The two results are later averaged to "define" the retinal position. Both methods are used within the three measurements.



(a) Retinal landmarking (b) Other interfaces

Figure 17. Localizing techniques.

Other surfaces are marked by the contrast at the interfaces. For example, at the posterior surface of the lens, a sharp demarcation is seen as there is autofluorescence and scattering from within the lens tissue, and reflection at the interface between the lens and the "dark" vitreous volume. (Refer to Section 2.1.) The smaller depth of the anterior chamber makes the measuring of that

compartment more difficult.

The pre-injection scan(s) are done immediately after the length measurements are completed. The probe is first focussed on the retina, and slit intensity is checked. The blue excitation filter is slid into the path of the beam; Switch A is toggled up. Scanning is started at the "beep", and at the end of each scan, the visual plot is studied for signs of clipping or subject movement. Up to four background scans can be made. (Refer to Section 3.4.)

When background scanning is completed, the Luma^R lens is removed and the subject brought to the photography room where the fluorescein is injected and photographs of both maculae and optic discs are taken. The injection is given by a registered nurse who also advises the subject of the dye's possible immediate effects (nausea), and delayed effects such as yellow-coloured urine. The dye is injected quickly. Two stopwatches are started when the syringe is half-emptied. The photography is usually completed 55 to 70 s after the injection.

The bolus scan is the critical scan as there is a maximum time interval within which it must be made. (Refer to the theory in Section 2.3.) The protocol requires a 3-minute p.i. bolus scan. However, events such as instrument intensity re-adjustments may delay this scan. A bolus is never accepted past 7 minutes p.i. Other "bolus" scans are taken to study the early changes in the levels of plasma fluorescein, and, therefore, CR bolus tailings. Plotting and filing usually take up to 90 s.

Immediately after bolus scanning and the removal of the Luma^R

lens, a blood sample is collected. A finger is pricked with a sterile lancet and approximately 0.1 ml of blood is collected in a heparinized capillary tube (haematocrit). The p.i. time is noted when half the tube is filled. Another sampling is done at about 15 minutes p.i.

The first measurement scan is taken around 30 minutes p.i. Other scans can also be taken for reproducibility studies. The third blood sample is taken after the Luma Lens has been removed. This procedure is repeated around 60 minutes p.i. The soft lens is also removed as scanning with the slit lamp is ended after the 1-hour measurement scan(s).

The last parts of the examination are monochromatic nerve-fibre photography and ultrasound measurements of intra-ocular distances. The latter is used to compare against the distances of the various segments of the eye measured by the slit lamp. The ultrasound results are also used to scale the profiles collected, and to calculate the F-numbers for the optics in this VF system.

START		Read and sign CONSENT Form. Answer questions.
-30	min.p.i.	Dilate pupils with tropicamide.
-5	min.p.i.	Mount lenses. Measure intra-ocular distances.
.		Background scanning.
0	min.p.i.	Injection and photography.
3-7	min.p.i.	Bolus scanning and blood sampling.
15	min.p.i.	Blood sampling.
28-33	min.p.i.	Measurement scanning and blood sampling.
55-65	min.p.i.	Measurement scanning and blood sampling.
.		Nerve-fibre photography; Ultrasound scanning.
.		Plasma-fluorescein scanning.

Figure 18. Flowchart of the scanning procedure.

4.3 Blood-Plasma Preparation

The four blood samples collected are centrifuged immediately after the ultra-sound measurements have been made. The speed is set at 2000 rpm for approximately 12 minutes. This adequately separates the red blood cells.

2 ml of pH 7.4 buffer solution are measured out by a volumetric pipette and placed in each of five (cuvette) dry sample cells. One cell is used as the background sample. 0.01 ml are drawn from each haematocrit using a 0.025-ml pipette and emptied into each cell. The constant outside the integral in Eq. 30 is then 34.17. The cells are then scanned by the slit lamp.

Each cell is placed so that no light is reflected off any of its surfaces onto the probe. The probe is positioned visually, in such a way that it is not focussed on air bubbles on the inner surfaces of the cells. The focus of the probe is slightly behind the inner front wall of each cell. This is due to possible attenuation of the incident beam owing to the high concentrations. Also, from Figure 2b, the probe must be totally included in the sample volume.

The first sample to be scanned is always the background cell. It is scanned once only. Samples with fluorescein can be scanned more than once by varying the position of the probe's focus slightly each time. Also, plasma fluorescein samples are usually scanned in chronological order of their p.i. times.

SUBJECT DATA ENTRY is usually executed last.

V. ANALYSIS AND DISCUSSION

5.1 Classifications

The procedure was completed on the following sample population:

TYPE	NUMBER	AGE RANGE
MALE Diabetic	10+2	21 - 64
FEMALE Diabetic	5	18 - 57
MALE Normal	4	27 - 38
MALE MS	3	32 - 39
FEMALE MS	13	20 - 59
TOTAL	35+2	

Table 2. Distribution of subjects.

Two male diabetic subjects were recalled at one and three months after their first scans to study the reproducibility. The two sets of results for these two subjects were averaged in the following analysis. Only three blood samples were taken from the first two male and the first two female diabetic subjects on whom the procedure was carried out.

Whenever possible each parameter studied was classified and tested on the basis of disease (MS or diabetes), age, and sex. Only left eyes were scanned because of the time restriction for the bolus scans i.e., a one-eye design [49].

Diabetics with non-proliferative retinopathy were separated into three groups according to the severity of capillary disease as

shown by fundus photography and fluorescein angiography. Group D(1) consisted of subjects with no or early DRP (0 to 5 microaneurysms); group D(2), subjects with mild to moderate non-proliferative DRP, and group D(3), subjects with severe non-proliferative DRP.

DISEASE STATES	NUMBER
D(1) - Diabetic with zero to early DRP (0 - 5 microaneurysms).	7M 1F
D(2) - Diabetic with mild to moderate DRP.	1M 2F
D(3) - Diabetic with severe DRP.	2M 2F
TOTAL DIABETIC SUBJECTS AVAILABLE	10M:5F
MS(1) : stable recovering from relapse	2M:3F 1M:2F
MS(2) : slowly progressive in relapse	0M:4F 0M:4F
TOTAL NUMBER of MS subjects AVAILABLE	3M:13F
MS(3) : benign relapsing-remitting	1M:1F 1M:6F
MS(4) : relapsing-progressive chronic progressive	1M:5F 0M:1F
TOTAL NUMBER of MS subjects AVAILABLE	3M:13F
MS(5) : no periphlebitis	2M:5F
MS(6) : active periphlebitis	0M:2F
MS(7) : inactive periphlebitis	1M:3F
TOTAL NUMBER of MS subjects AVAILABLE	3M:10F

Table 3. Detail subject classifications.

MS subjects were grouped for analysis in three ways. The first was by the activity of MS at the time of the scans. The subdivisions comprised MS(1) - subjects who were either stable or recovering from a relapse or had recovered completely from a recent relapse at the time of scanning and MS(2) - subjects who were either in slow progression or in relapse. The second was by the standard clinical activity categories of MS as reported in case records. The subjects were again subdivided into two sections - MS(3) and MS(4). MS(3) included those classified as benign (only one attack) or relapsing-remitting (almost complete recovery from each attack). MS(4) subjects were classified as relapsing-progressive, or chronic-progressive. The third grouping separated the subjects according to the absence or presence of either active or inactive retinal periphlebitis. These were called MS(5), MS(6) and MS(7) respectively. As not all subjects were examined for these states, the sample size for this grouping was reduced.

5.2 F-Numbers

The F-numbers in Section 2.2, were calculated by SUDATA.BAS for each subject. The average and standard deviation were found for each intra-ocular compartment and tested against the averages from Gullstrands emmetropic model eye in Table 1. The results were calculated using the data from 34 subjects only. Three subjects (from Table 3) were excluded because they did not have the ultra-sound scans. As F-numbers are characteristics of the system, the subjects were not separated into age, sex or disease states.

MEDIUM	AVERAGE	S.D.
Aqueous	1.735	0.359
Lens	1.511	0.157
Vitreous	1.245	0.089

Table 4. Average F-numbers and their S.D.

Since 34 measurements were made, the mean values in Table 4 were tested against those in Table 1 using a normal(0,1) distribution test. A P=1% level of significance was imposed. The results showed that the F-numbers from Table 1 were not applicable to this VF system: the F-numbers in the two tables were significantly different. Hence, the Luma® contact lens (which replaces the Goldmann contact lens in Lund-Andersen's calculations) changes the F-numbers significantly.

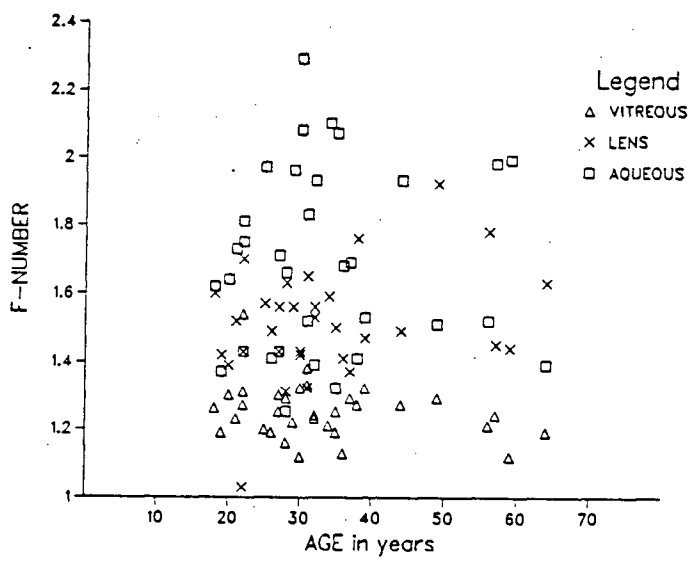


Figure 19. Plot of F-number results.

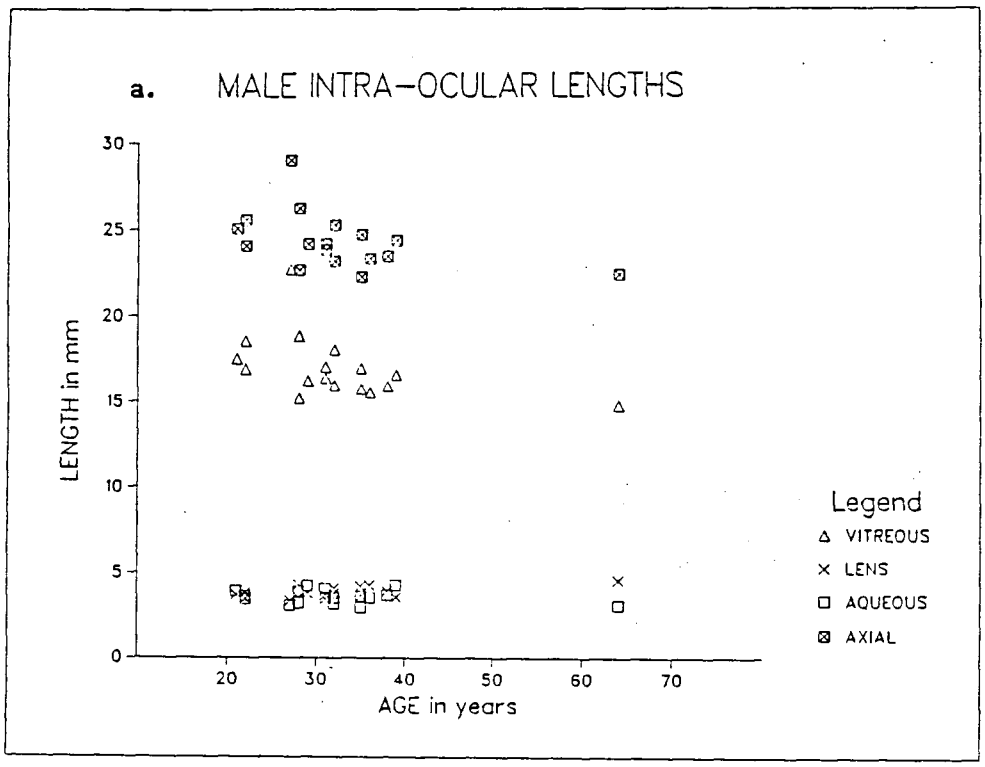
5.3 Intra-ocular Lengths

The averages for the vitreous and aqueous chamber depths, the lens thickness and the total axial length as measured by ultrasound for male and female subjects are shown below. The sample sizes were 17 in each group.

AGE RANGE	SEX	VITREOUS	LENS	AQUEOUS	AXIAL	NUMBER
16-20	F	15.76	3.38	4.45	23.59	3
21-30	M	17.96	3.67	3.60	25.23	7
	F	16,24	3.38	3.96	23.58	6
31-40	M	16.44	3.81	3.56	23.81	9
	F	15.94	3.96	3.42	23.31	2
41-50	F	16.13	3.84	3.48	23.46	2
51-60	F	14.45	4.32	3.22	21.99	2
61-65	M	14.78	4.54	3.06	22.38	1
TOTAL					23.74	34

Table 5. Average lengths of the intra-ocular media (in mm).

Although the sample sizes were small, and the various disease states were not taken into account, the above table suggests either that axial length decreases with age, or, that the lens thickens with age. [50] Linear least-squares fits were done on each treatment. The null hypothesis, H^o, in each case was that the slope of the straight line was zero. The alternative hypothesis, H^a, for the axial length test was that it decreased with age, (i.e. a negative slope); while for the lens, it thickened with age (i.e. a positive slope). The levels of significance (P) at which H^o would be rejected were found in a t(17-2) test. The results are shown in the Table 6.



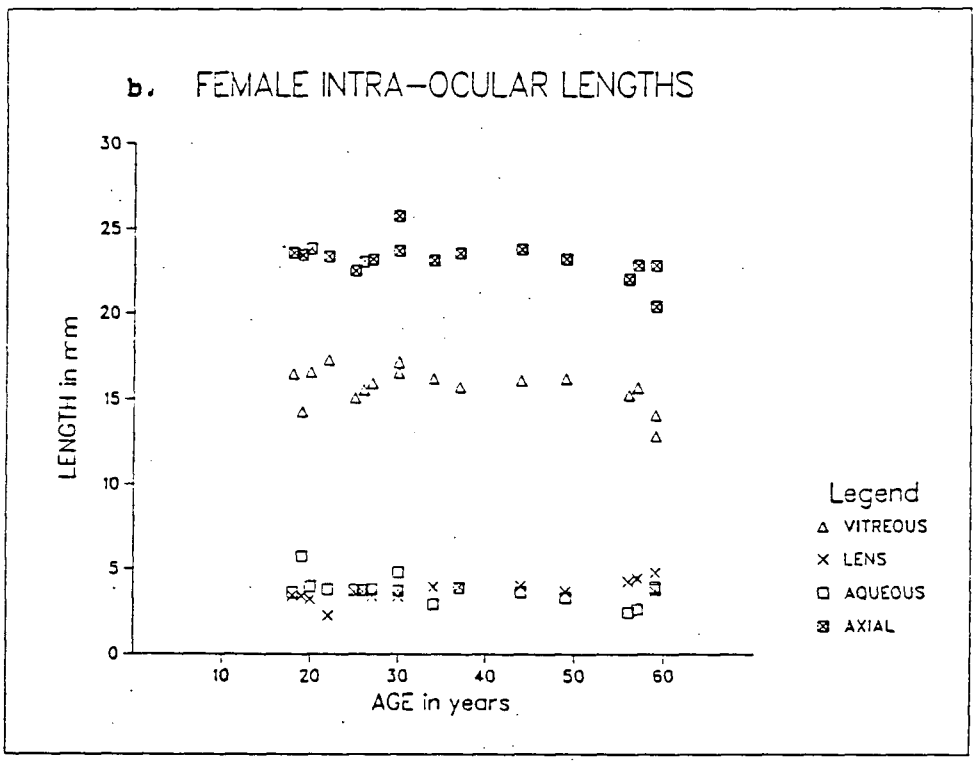


Figure 20. Intra-ocular Lengths.

TEST	MALE	FEMALE
Lens vs Age	< 1% 3.79 +/- 0.35	< 1% 3.72 +/- 0.55
Axial vs Age	< 5% 24.31 +/- 1.65	< 2.5% 23.16 +/- 1.05

Table 6. Significance levels (P) for intra-ocular lengths tests. Average +/- S.D. (mm) calculated from Table 5.

MALE	FEMALE
L = 26.79 - 0.07*A r = 0.45	L = 24.49 - 0.04*A r = 0.52
T = 3.11 + 0.02*A r = 0.59	T = 2.76 + 0.03*A r = 0.72

Table 7. Results of linear, least-squares fit of axial length (L), and lens thickness (T) in mm to the subject's age (A in yrs). r = linear correlation coefficient.

Hence, it would seem that the crystalline lens thickened with age. The spread of the data was significant when comparing case by case. This is observed in the low value of the linear correlation coefficients, r of the fits in Table 7.

5.4 Plasma Curve-fits

The four (2+1)-parameter fitting polynomials in BLOOD.BAS can be reduced to two. The fits to the 35+2 cases in Table 2 showed that most plasma profiles were (as expected) best described by the logarithmic or the exponential forms. The simple parabola was a poor fit for what was expected to be a fast exponential-decay-type behaviour of the two-compartment model. It produced a minimum between t=30 and t=60 minutes p.i. in almost all cases. The one that was accepted had a minimum that was situated in the neighbourhood of t=60 minutes p.i.

Fit #3, the second-order polynomial in 1/t, usually rose from negative values to a maximum in $0.5 < t < t^{(1)}$, the first sampling time. This resulted in negative areas upon integration. Such results were rejected even though the reduced chi-square might have been the smallest among the fits.

SUBJECT TYPE	FIT FUNCTION				TOTAL
	#0	#1	#2	#3	
Male Diabetic	0	6	4	2	12
Female Diabetic	0	3	1	1	5
Male Normal	0	3	1	0	4
Male MS	0	3	0	0	3
Female MS	1	7	3	2	13
TOTAL	1	22	9	5	37

Table 8. Plasma fits.

Testing the above table in a two-way classification without interaction at P=5% shows that the types of disease (rows) did not have significant effect on which function was the best fit. There was significant difference between the type of best-fit functions: function #1 was significantly the most probable result.

As mentioned before, the amount of dye in the blood rises from zero at t=0 (injection) to a maximum in less than one minute then begins to fall. The area depends on the fit which is defined by the number of samples collected. The more samples taken within the one-hour p.i. interval, the better defined the fit. However, with only four samples, (or less in 4 diabetic subjects), the area between t=0 and $t^{(1)}$ may have significant effect on the integration. Hence, the effect of the time at which the first blood sample was taken on the resulting fit was investigated. The treatments were the intervals containing $t^{(1)}$. The replications [51,52] were the fitting functions. Sex was not considered.

At P=10%, $t^{(1)}$ did not have a significant effect; the types of fit were significant (as shown before). There did not seem to be any interaction between the types of disease and $t^{(1)}$.

The above results imply that the curve-fitting in BLOOD.BAS may be shortened to save running and printing time: the first and the last function(s) may be omitted from consideration. Table 9 shows the distribution of the 35+2 fits in the $t^{(1)}$ intervals. Note that these intervals were arbitrarily chosen.

INTERVAL of $t^{(1)}$ in minutes p.i.	FIT FUNCTION				TOTAL
	#0	#1	#2	#3	
< 6	0 0 0	2M 3D 2N	0 3D 1N	0 2D 0	13
6 to < 10	0 0 0	8M 3D 1N	3M 2D 0	2M 0 0	19
> 10	1M 0 0	0 2D 0	0 2D 0	0 0 0	5
TOTAL	1	21	11	4	37

Table 9. Effect of the first blood sampling time, $t^{(1)}$.
M = MS; D = Diabetic; N = Normal

5.5 LLoD

TYPE	AVERAGE	S.D.	NUMBER
Male Diabetic	4.32	1.25	10+2
Female Diabetic	4.57	1.84	5
Male Normal	3.99	0.68	4
Male MS	4.62	3.19	3
Female MS	4.51	2.10	13
FINAL	4.41	1.72	35+2

Table 10. Average LLoDs and S.D.s. ($ng.ml^{-1}$)

The LLoD was calculated from the background scans (0.AVG files) in the interval between 8 and 10 mm from the retina. Specifically, the in vivo LLoD is defined in this study as the average concentration in the interval plus twice the root-mean-square value of the standard deviations of the data collected in that interval.

An analysis of variance at P=25% showed that the types of disease did not affect the averages in each treatment. This was expected because the pre-injection scans were dye-free. Hence, the average LLoD was equivalent to a concentration of 4.41 +/- 1.72 $ng.ml^{-1}$. This value compares favourably with the in vitro LLoD of approximately 5 $ng.ml^{-1}$ that was estimated during concentration calibrations, and as stated in [14].

5.6 Autofluorescence

Plots of lens autofluorescence vs age of subject, and duration of disease are shown on the next page. Least-squares straight-line fits were found for each disease category. t(n-2) tests on the slopes of the fits were performed on H^o: the slope was 0 in each case; and on H^a: the slope was positive in each case. Table 11 shows the results of the tests against age only.

These results imply that lens autofluorescence increased with age [19,35]. Also, the averages, intercepts and approximately equal slopes of the fits suggested that lens autofluorescence was higher and occurred earlier in the diabetic subjects than the normal and MS subjects. However, this trend was not clearly defined between the MS and the normal subjects as the slopes were different.

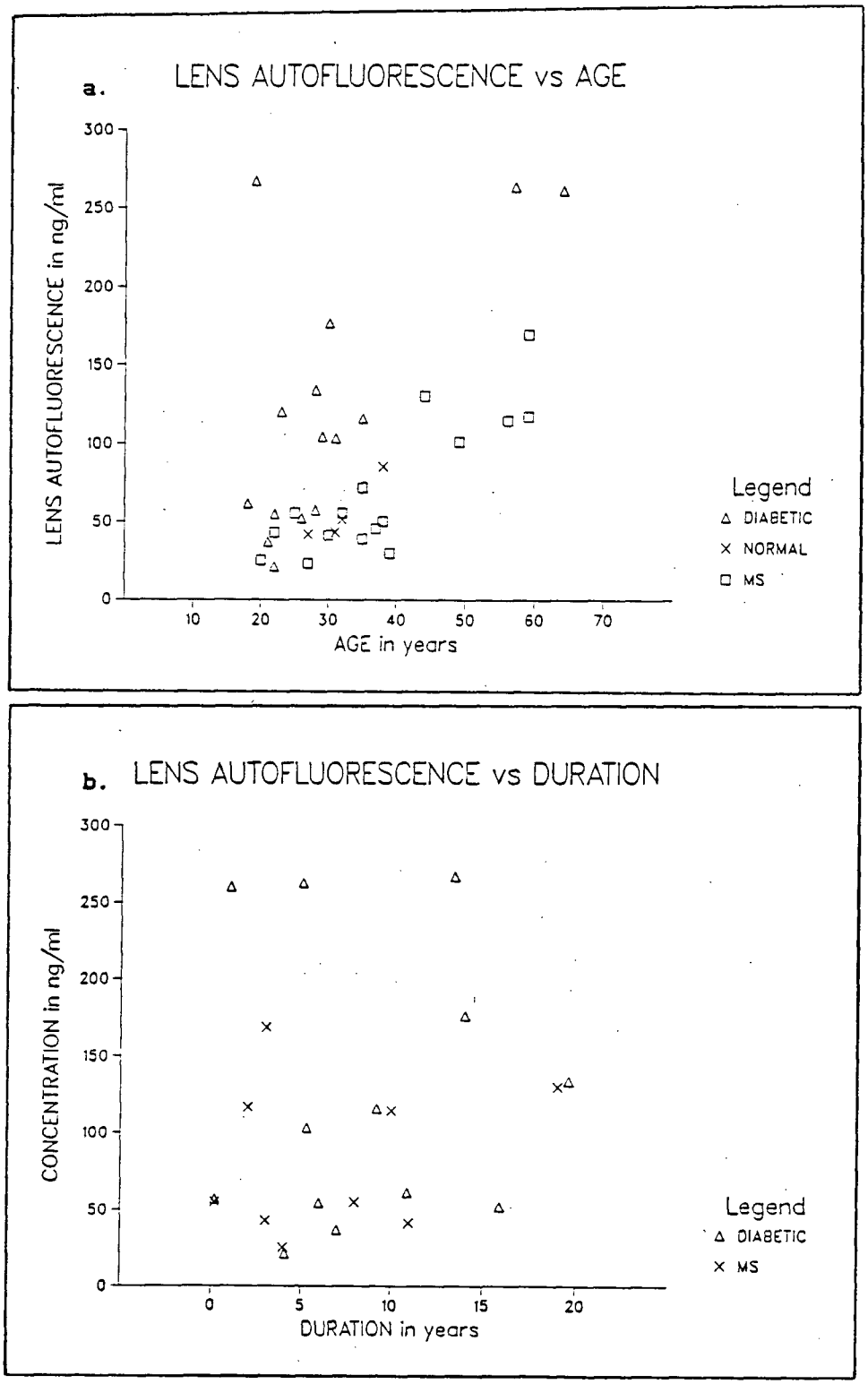


Figure 21. Autofluorescence.

	DIABETIC	NORMAL	MS
Sample size, n	15	4	16
Correlation, r	0.65	0.93	0.84
Average age (yrs)	30.2	32.0	37.9
Average reading	121.8	55.4	69.4
Standard Deviation	83.8	20.4	43.4
Slope ($ng.ml^{-1}.yr^{-1}$)	4.11	4.20	2.93
Intercept ($ng.ml^{-1}$)	-2.32	-78.0	-41.7
P(reject $H^{\bullet}$)	1%	5%	1%

Table 11. Lens autofluorescence, S.D. ($ng.ml^{-1}$) vs age.

The duration of diabetes did not have a significant effect on lens autofluorescence (P>25%), and was not included. Autofluorescence versus state of DRP was tested.

	D(1)	D(2)	D(3)
Number	8	3	4
Average	69.94	184.9	178.3
S.D.	35.36	73.7	104.0

Table 12. Autofluorescence, S.D. ($ng.ml^{-1}$) vs DRP states.

t-tests on differences showed that D(1) and D(2), D(1) and D(3) were significantly different (P=10%); while D(2) and D(3) were not (P>25%). Note that the S.D.s were sizeable fractions of the averages, i.e., there was a large variation from case to case. (An analysis of variance at P=2.5% showed that at least two of the means were not the same.)

One conclusion is that lens autofluorescence increased

significantly with progression from no DRP to severe DRP [19,35]. These results partly explain the low correlation coefficient, r=0.65, for the curve-fitting in Table 11. Diabetic subjects between the age of 18 and 27 years with severe DRP reduced the goodness of the straight-line fit. However, it has not been explicitly shown that the increase in lens autofluorescence is due to the thickening of the lens with age. This requires a much larger sample size of normal subjects spanning a large age range.

MS STATES	AVERAGE	S.D.	NUMBER
MS(1)	69.96	48.93	8
MS(2)	68.85	40.45	8
MS(3)	47.38	27.69	9
MS(4)	97.71	44.93	7
MS(5)	69.74	54.35	7
MS(6)	73.03	49.66	3
MS(7)	67.30	29.60	3

Table 13. Autofluorescence, S.D. ($ng.ml^{-1}$) vs MS states.

There was no significant difference between the means of MS(1) and MS(2), or that of MS(5), MS(6) and MS(7), (P>25%). However, the means of MS(3) and MS(4) were significantly different (P=2.5%). Note the large S.D.

Although the trend of increasing autofluorescence with age was seen in Table 11, the classification in terms of graded severity of recent clinical activity did not show trends similar to the cases with diabetes. The distribution of those older subjects among the MS

groups had "biased" the results. No trends were observed except that autofluorescence was higher than for normals.

The trends established thus far are in accord with other researchers' findings. However, comparing average readings, the values here are much lower than from [35]. One possible reason for this is that the instrument characteristics and calibrations were different. For example, the angle between beam and probe was different. However, it is more likely that alignment errors were the cause of the differences.

5.7 Profiles

The effects of CR tailings were studied in a D(1) subject. (Figure 22.) Some of the scanning problems discussed in Section 2.1 are demonstrated in these profiles.

The excitation beam in the 2-minute scan was probably partially clipped by the iris as suggested by the plateau shown within the first 2 mm from the retina. The tailings however, coincide with the 4-minute scan for distances greater than 2 mm from the retina.

The 4-minute scan peaked at about the position of the retina that was located visually. Note that the tailings at this time persisted well into the vitreous where, for this subject at this p.i. time, no dye was expected to have penetrated.

The 6-minute profile shows the difficulty in visually locating the retina. The peak is approximately 1 mm anterior to the retina. This particular bolus alignment error can be corrected by setting the zero at the CR peaks, but for later measurement scans the actual

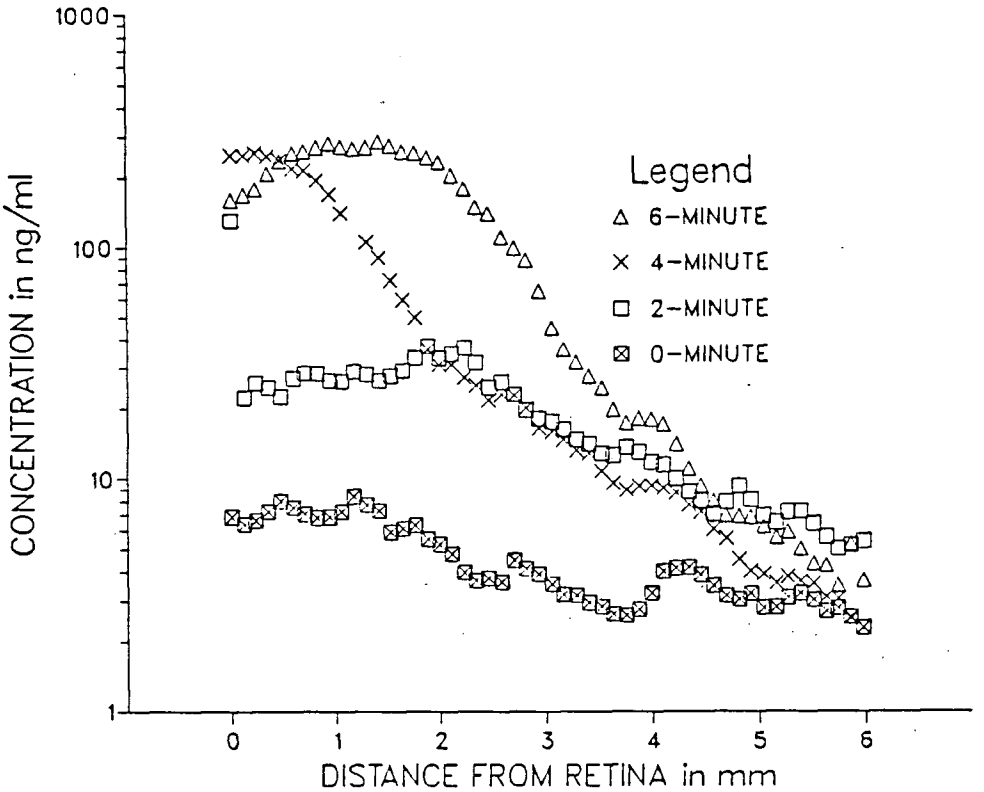


Figure 22. Bolus effects.

position of the retina by CR peaks can not be clearly defined in practice or in theory.

Figures 23a-23g show the evolution of the dye profile in the posterior vitreous of subjects in various classifications. Note that the vertical log scales are different. The important points are as follows -

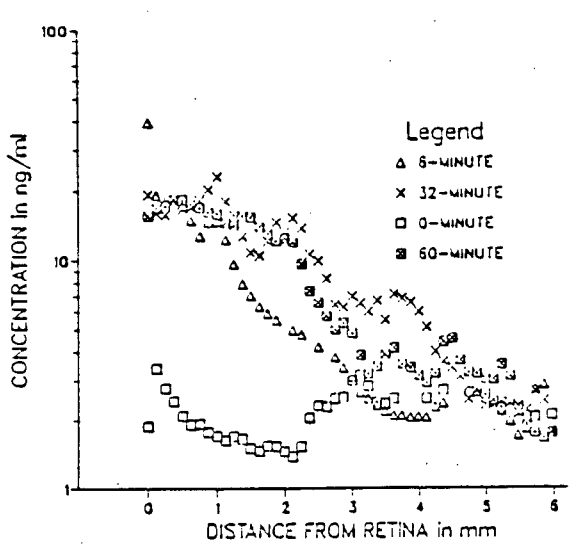
a) the prominance of the bolus profiles and CR peaks.

b) the change of slope with time about 3mm.

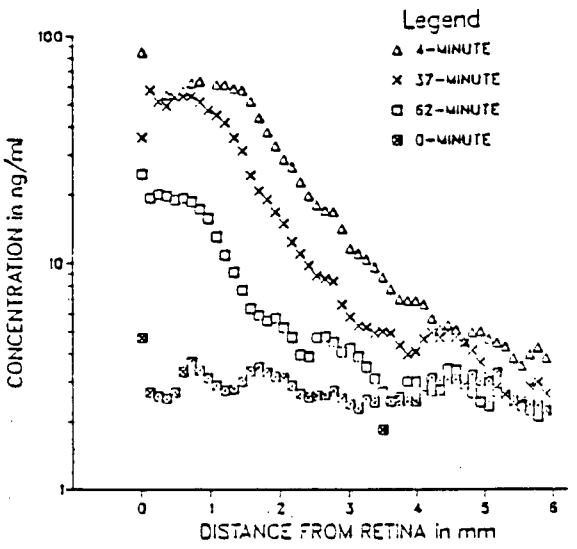
c) alignment and peak shifts anteriorly.

d) the difference in the concentrations farther from the retina.

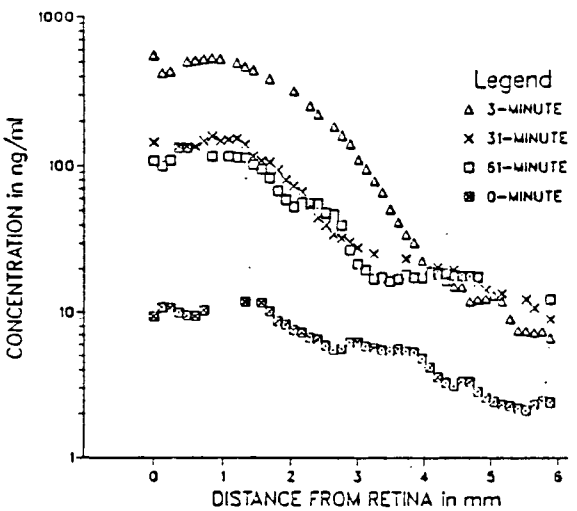
The profiles of the normal (Figure 23a) are "noisier" than



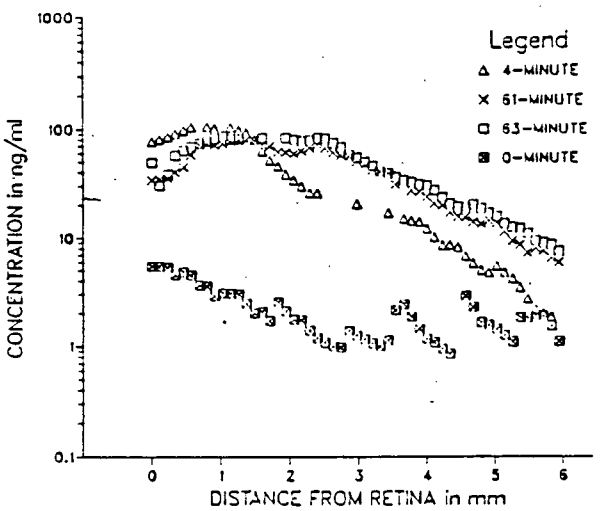
a. Normal.



b. D(1) - no DRP.

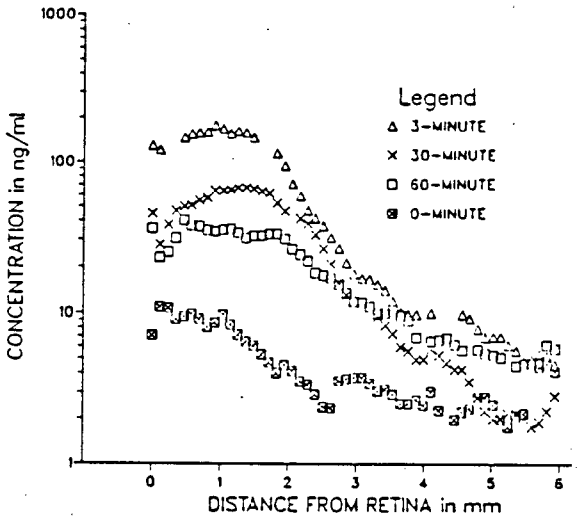


c. D(2) - moderate DRP.

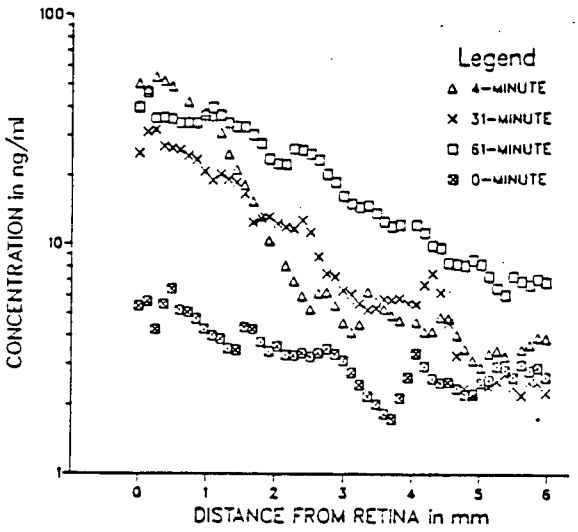


d. D(3) - severe DRP.

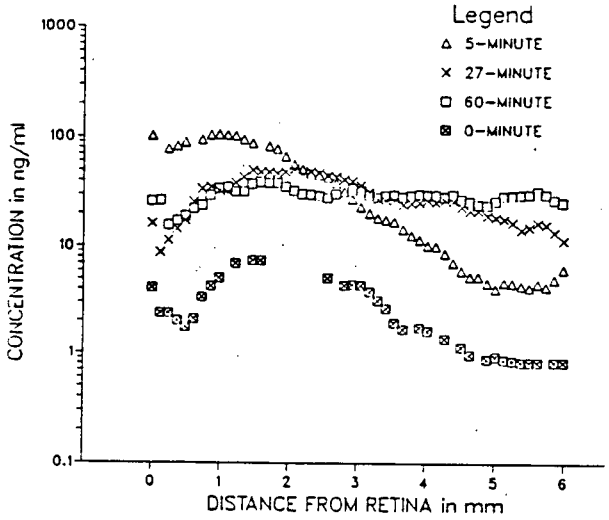
Figure 23. Sample profiles.



e. Stable MS.



f. Relapsing MS.



f. Liquefied vitreous.

Figure 23. Sample profiles (continued).

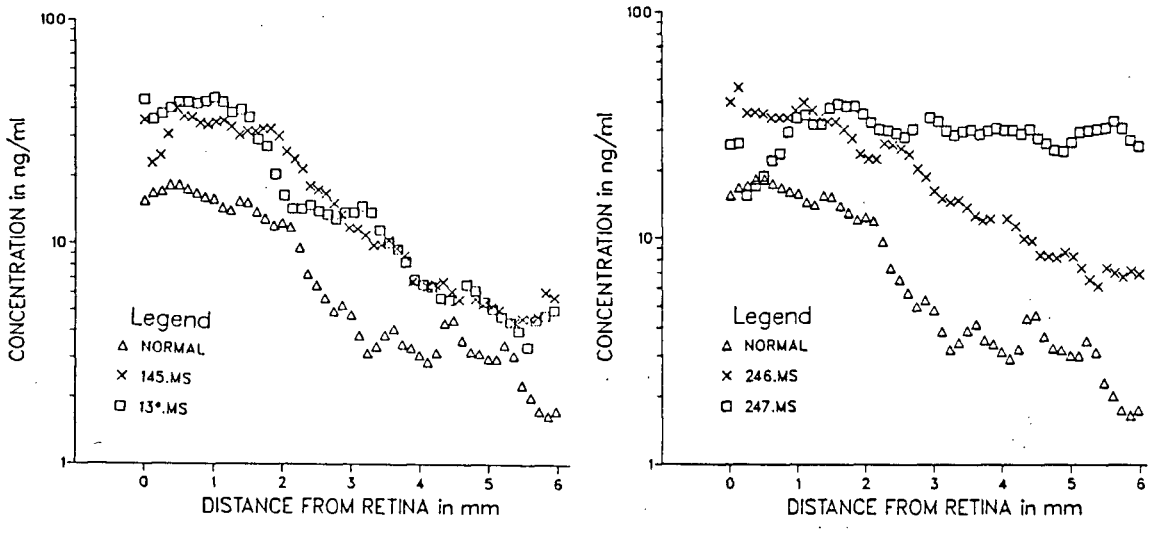
others as one might expect from the lower concentrations of dye. All profiles more or less coincide at 6mm unlike for others, especially the D(3) and the MS cases in Figures 23f,g.

The special case of the female, MS subject with the liquefied vitreous in Figure 23g is worth noting. The gradient of the 1-hour profile (at the posterior vitreous) is small compared to the bolus at various positions from the retina. The 60-minute CR peak is thus not well defined. However, as the profile about the 3-mm point was "flat", misalignment should not produce large errors in this special case. However, regardless of the state of the vitreous, Figure 23g shows that a large amount of dye had indeed entered the posterior vitreous through the BRB.

Figure 24a compares the 1-hour scans of two stable, MS subjects' profiles. The male, MS(4) profile is very similar in slope and magnitude to the MS(3), female subject. Both are elevated above the normal subject's profile. The plateau between 2 and 3 mm is probably due to movement by the subject during the scan [32]. The male subject had no periphlebitis; the female subject was not examined for this.

Figure 24b compares the 1-hour scans of two female, MS subjects, with active and inactive periphlebitis respectively. The latter subject was the person with the liquefied vitreous. Both belonged to the MS(2) and the MS(4) groups. Note that the MS profiles are clearly above the normal.

Figures 25a,b,c compare a male and a female diabetic person's 1-hour profiles in the D(1), D(2) and D(3) groups respectively. The magnitude of leakage (vertical axis) is progressively greater from



a. Relapsing-Remitting vs Relapsing-Progressive.

b. Relapsing-Progressive vs liquefied vitreous.

Figure 24. Comparison of MS profiles.
(Refer to Table 14 for number codes.)

D(1) to D(3). This demonstrates good correspondence with clinical grading of severity of DRP. The differences in the magnitude of leakage in Figure 25a of D(1) profiles may have been accentuated by poor control of diabetes in one case despite the presence of less than 5 microaneurysms. The leakage is similar to that of a normal subject when there is good control of diabetes.

Figure 26 compares the 1-hour scans of the following subjects:

a) a male subject in the MS(1), MS(4) and MS(5) groups.

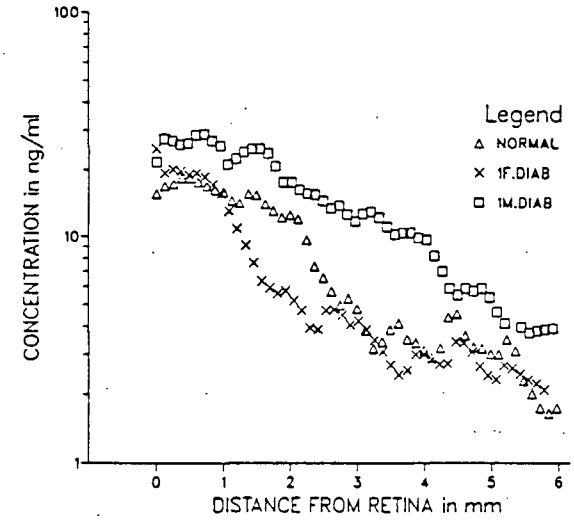
b) a female subject in the MS(2), MS(4) and MS(6) groups.

c) a female subject in the D(1) group.

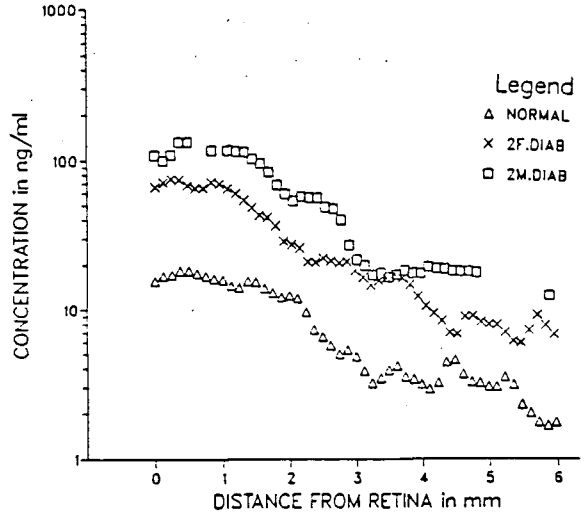
d) a female subject in the D(2) group.

e) a male subject in the D(3) group.

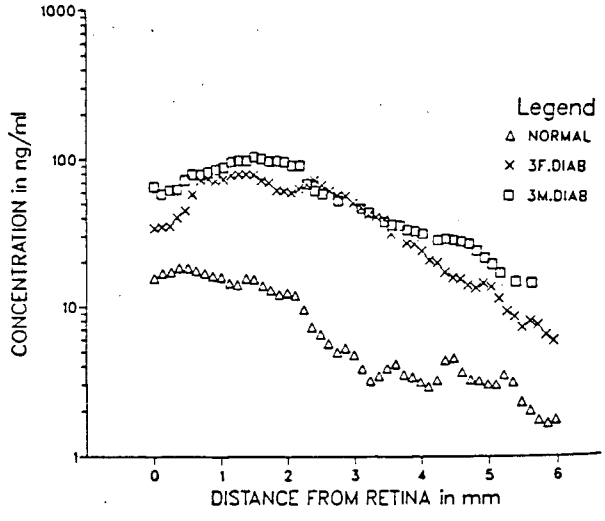
These illustrations of profiles are intended to demonstrate



a. Male vs Female D(1).



b. Male vs Female D(2).



c. Male vs Female D(3).

Figure 25. Comparison of diabetic subjects' profiles.

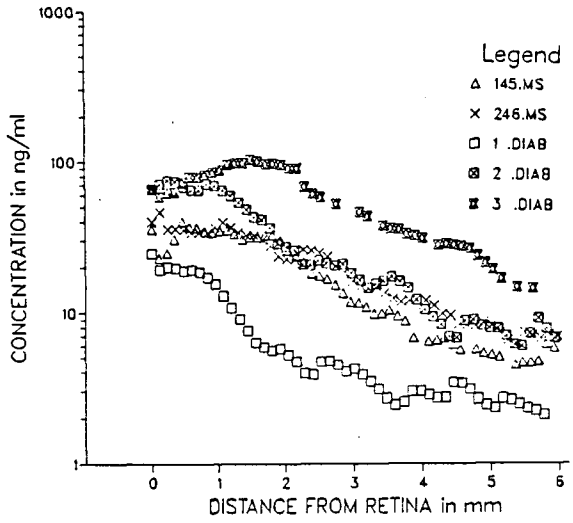


Figure 26. Overall comparison.
(Refer to Table 14 for number codes.)

different magnitudes of leakage within MS and diabetes. Profiles provide qualitative comparisons of the integrity of the BRB - such as visibly different gradients (at some point), or, that one lies above or below the other. In the calculations of PR3 or P^1, the division by the results of the plasma integral may produce quite different quantitative descriptions of the BRB.

5.8 Diffusion Constant

The diffusion constant, D was calculated by SLOPES.BAS. Table 14 gives the results of averaging over all measurement scans made. Note that D and all calculations pertaining to it are always given in units of $*10^{-6}$ $cm^2 s^{-1}$.

Least-squares fits to D = A + B*(age), and D = X + Y*(duration). were carried out on each disease group with H^o : slope = 0, and, H^A : slope < 0 or > 0, i.e., one-sided t-tests depending on the coefficient, B or Y of the fits.

AGE	DIABETIC	NORMAL	MS
18	4.44 (F1)		
19	0.85 (F3)		
20			4.42 (F235)
21	3.73 (M1)		
22	3.69 (M1)		1.35 (F23?)
	9.78 (M1)		
23	0.55 (M1)		
25			5.18 (F135)
26	2.94 (F3)		
27		7.38 (M)	17.13 (F135)
28	7.18 (M1)		
	2.54 (M3)		
29	5.22 (M1)		
30	1.84 (F2)		6.42 (F13?)
31	6.02 (M1)	6.72 (M)	
32		4.12 (M)	3.62 (M137)
35	3.58 (M2)		7.11 (M145)
			9.33 (F236)
37			5.22 (F247)
38		15.15 (M)	4.34 (F246)
39			11.49 (M135)
44			29.90*(F247)
49			8.72 (F247)
56			27.59 (F135)
57	2.69 (F2)		
59			18.68 (F145)
			13.13 (F24?)
64	2.31 (M3)		
AVERAGE	3.83	8.34	10.86
S.D.	2.44	4.75	8.49
NUMBER	10M:5F	4M:0F	3M:13F

Table 14. D averaged over all measurement scans.
CODES : (XA) for diabetics; (XBCD) for MS
where
X = Male or Female
A = 1,2,3 for D(1),D(2),D(3) respectively;
B = 1,2 for MS(1),MS(2) respeotively;
C = 3,4 for MS(3),MS(4) respectively;
and, D = 5,6,7 for MS(5),MS(6),MS(7) respectively.
D = ? means subject not examined.
* is the subject with liquefied vitreous.

	DIABETIC	NORMAL	MS	TOTAL
Sample Size	15	4	16	35
Coefficient, A	4.82	-16.06	-4.96	-1.08
Coefficient, B	-0.03	0.76	0.42	0.25
Correlation, r	0.18	0.73	0.62	0.46
P(reject H°)	> 25%	25%	1%	< 0.5%
Sample Size	13		9	
Coefficient, X	5.84		4.61	
Coefficient, Y	-0.22		1.14	
Correlation, r	0.53		0.63	
P(reject H°)	5%		5%	

Table 15. Tests of D vs age and duration.

	AVERAGE	+/- S.D.	NUMBER	P
D(1)	5.08	2.73	8	25%
D(2)	2.70	0.87	3	2.5%
D(3)	2.16	0.91	4	0.5%
Male Diabetic	4.46	2.67	10	10%
Female Diabetic	2.55	1.33	5	0.5%
DIABETIC AVERAGE	3.83	2.44	15	0.5%
Male Normal	8.34	4.75	4	25%
MS(1)	12.15	8.33	8	5%
MS(2)	9.58	9.01	8	25%
MS(3)	9.61	8.25	9	25%
MS(4)	12.47	9.17	7	10%
MS(5)	13.08	8.50	7	5%
MS(6)	6.84	2.50	2	> 25%
MS(7)	11.87	12.21	4	25%
Male MS	7.41	3.94	3	> 25%
Female MS	11.66	9.15	13	2.5%
MS AVERAGE	19.86	8.49	16	2.5%
FINAL AVERAGE	7.56	6.90	35	9%

Table 16. Diffusion constant, D by sex-disease states.

The results in Tables 14 and 15 show that D tended to increase with age, This result was dominated by the larger MS sample with numerically more older subjects. Also, the B-coefficient for diabetic subjects was negative but not significantly so. Others had also noted this trend but were unable to prove it statistically [53].

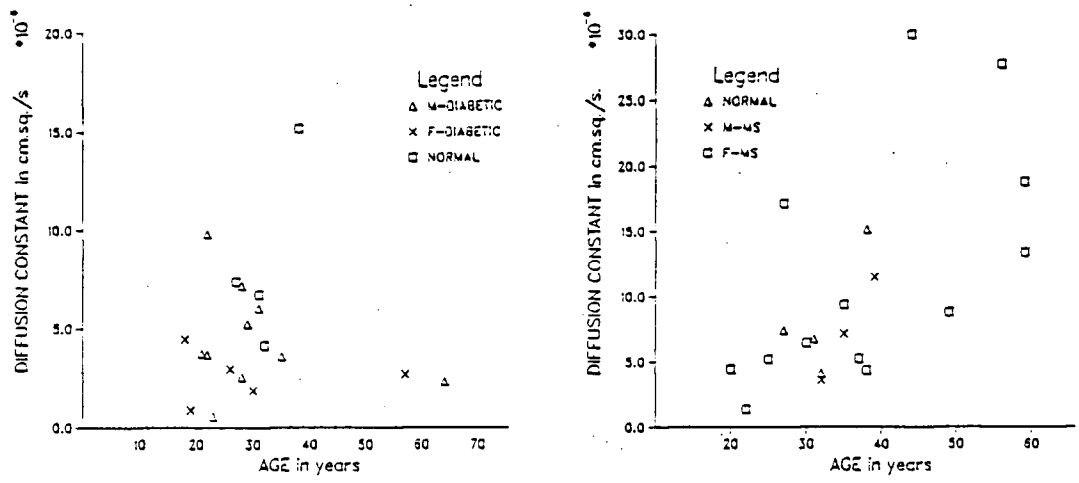
The tests of D against the duration of diabetes (Table 15) were inconclusive because the sample sizes were small, and the Y-coefficients for diabetes and MS were of opposite signs, showing opposite trends. This may be due to intra-group variation in severity. Larger samples of each disease state are needed to establish the existence of any trend. Note the significant scatter of data as the correlation coefficients, r, are not close to 1. (Figure 27.)

The extreme right column in Table 16 shows the results of the tests for

$$H^{*} \ : \text{ average D in each group} = D^{w} \ ,$$

where $D^{w}=6$ [20] is the diffusion constant of the dye in water. Despite the large spread of the data in each group, one notable result was that the D's for diabetics were significantly lower than D^{w}, for this sample. The reason for this is not known. Similarly, the explanation for the D(2) and the D(3) averages being half that of D(1) is not apparent. It should be noted that the D(1) sample was twice the size of D(2) or D(3).

In contrast, the average D for MS subjects was significantly higher than D^{w}. As stated before, the high values came from most of the older, MS subjects, notably, the female, MS subject with liquefied vitreous who had the highest value. This result may be due to



a. Diabetic subjects. b. MS subjects.

Figure 27. Diffusion ocnstant vs age.

the effect of mechanical mixing on the difference equation, Eq. 32.

Other tests on the results in Table 16 showed that the value of D for D(1) was significantly different from those of D(2) and D(3) (P<5%). There was no significant difference between D(2) and D(3) (P>25%).

Between MS(1) and MS(2), MS(3) and MS(4), and MS(5), MS(6) and MS(7), there was no difference (P>25%). These results were due to the large spread of data in each group with high values distributed throughout all groups.

The diffusion coefficient in the normal eye was found by others to be

a) 13.3 +/- 6.8 and 11.9 +/- 5.4 (Chahal, et al. [23]),

b) 13.2 +/- 4.3 (Ogura, et al. [53]),

c) 7.4 +/- 3.4 (Lund-Andersen, et al. [54]).

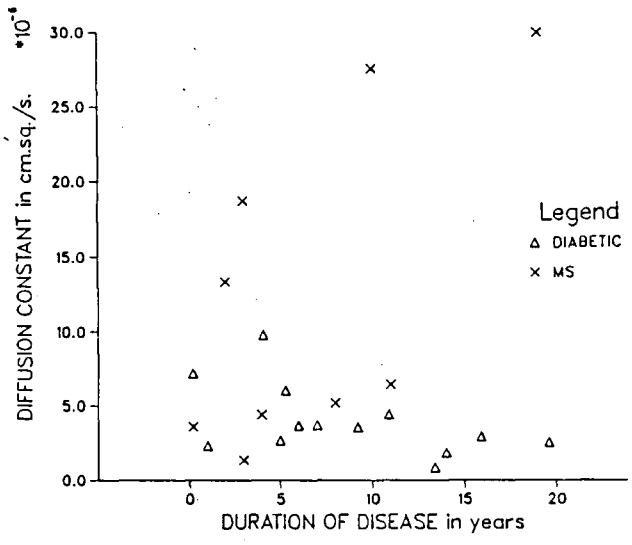


Figure 28. Diffusion constant vs duration.

For diabetic eyes, D = 9.6 +/- 2.0 [54]. Note that the large S.D.s allow for much overlap.

Comparing these results to those in Tables 15 and 16, for P=5%, there was no difference between the average found here and those derived by others for normals. Individual results in Table 15 were within the range in (c); but, somewhat lower than those values in (a) and (b).

Comparing the averages of any diabetic group, or, of individual cases, all were found to be significantly lower (P<0.5%) than the value stated by Lund-Andersen, et al [54].

Many of the D values for MS subjects were within the quoted values for normal and diabetic persons. The exception was the liquefied vitreous case for which D was higher than all others. D values were also elevated for several older MS subjects. No explanation in terms of clinical activity is known. (Refer to Table 14.)

The results of D in Table 14 were derived for profiles aligned by RET only. Misalignment might account for some of the extreme values. As the average over all measurement scans was used, this should alleviate the alignment errors. Also, no correlation was seen between D and the PR3 values studied below.

5.9 Penetration Ratio

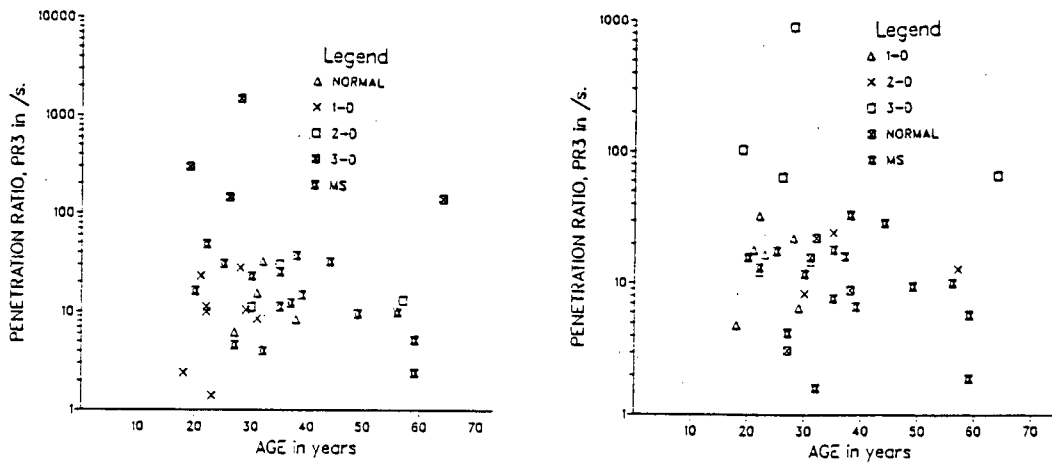
Recall that PR3 (in units of $*10^{-6}$ s^{-1}) was calculated in two methods of alignment by the programme, C/VAZ.BAS: by RET, and, by CRP. (Refer to Section 3.4.) The results are presented in Table 17. In some cases, PR3 was more than halved when the alignment was changed from RET to CRP; in others, it remained approximately the same. These changes are evidence of the difficulty of locating the retina by sight.

A case in point was the 22-year old, MS subject whose 1-hour, PR3 value was 48.2 by RET - the highest of all MS subjects; while, by CRP, it fell to 13.1. Her fluorescein profile at 1-hour p.i. showed that the CR peak was more than 2 mm from the located zero position. Although she was not examined for the presence or activity of peri-phlebitis, her profiles were not distinctly different from other MS subjects in the MS(2) and MS(3) groups, but, were when compared to the MS(6) category.

Another noteworthy result is that of the 32-year old normal who is the brother of the 35-year old, MS subject. His PR3 values were about 3 times that of other normals. His sister, who, at the time of scanning, was in relapse, had lower PR3 values than his (in either

AGE	DIABETIC		NORMAL		MS	
	RET	CRP	RET	CRP	RET	CRP
18	2.4 (F1)	4.8				
19	294.2 (F3)	103.1				
20					16.2 (F235)	15.7
21	29.5 (M1)	18.0				
22	11.2 (M1)	12.1			48.2 (F23?)	13.1
	10.0 (M1)	32.7				
23	1.4 (M1)	16.6				
25					30.6 (F135)	17.6
26	144.4 (F3)	63.8				
27			6.2 (M)	3.1	4.6 (F135)	4.2
28	1458.5 (M3)	886.8				
	27.5 (M1)	22.1				
29	10.4 (M1)	6.5				
30	11.1 (F2)	8.3			22.8 (F13?)	11.7
31	8.5 (M1)	14.6	15.4 (M)	15.8		
32			32.3 (M)	22.3	4.0 (M137)	1.6
35	30.0 (M2)	24.6			11.2 (M145)	7.7
					25.2 (F236)	18.2
37					12.2 (F247)	16.2
38			8.4 (M)	8.9	37.0 (F246)	33.5
39					14.7 (M135)	6.7
44					31.9*(F247)	*29.0
49					9.5 (F247)	9.5
56					9.9 (F135)	10.1
57	13.0 (F2)	12.9				
59					2.4 (F145)	1.9
					5.2 (F24?)	5.8
64	138.2 (M3)	66.1				
No.	10M:5F		4M:0F		3M:13F	

Table 17. PR3 averaged over all 55-70 minute scans, after background subtraction only.
CODES : (XA) for diabetics; (XBCD) for MS
where
X = Male or Female
A = 1,2,3 for D(1),D(2),D(3) respectively;
B = 1,2 for MS(1),MS(2) respectively;
C = 3,4 for MS(3),MS(4) respectively;
and, D = 5,6,7 for MS(5),MS(6),MS(7) respectively.
D = ? means subject not examined.
* is the subject with liquefied vitreous.



a. By RET. b. By CRP.

Figure 29. Penetration Ratio.

alignment)! This anomaly might have been due to improper instrument calibrations (settings) at that time; otherwise, it cannot be explained. This subject (and his sister) will have to be recalled for further testing. His reading was omitted from analysis. (Note that other data such as F-number calculations were still admissible.)

The results of statistical-inference testing were anticipated by ranking PR3 results in ascending order (by RET and by CRP separately) for MS subjects. The first noticeable point was that all MS(6) subjects had higher values. The case of (F247), the subject with the liquefied vitreous, also had a high PR3. All other MS classifications were distributed throughout the order with no obvious "clustering". This implied that there was no detected difference between MS(1) and MS(2), MS(3) and MS(4), MS(5) and MS(7).

The highest PR3 results calculated was that of a 22-year old, male, D(3) subject. Although he has severe DRP, his reading was about 10 times higher than other D(3) subjects which may be an intra-group variation. The 1-hour profile showed indisputable, elevated levels of dye in the vitreous. His PR3 value was also omitted from all testing.

The ordering of diabetic PR3 results showed that the results of the D(3) group were consistently the highest values. Cases with obviously high amounts of leakage were clearly detected (and detectable) by this fluorophotometer. This "justifies" the omission of the above D(3) case because the other D(3) results, taken individually or together, were already significantly higher than those of the other two diabetic groups.

GROUP	RET			CRP		
	MEAN	+/- S.D.	#	MEAN	+/- S.D.	#
D(1)	12.6	10.5	8	15.9	8.9	8
D(2)	18.0	10.4	3	15.3	8.4	3
D(3)	192.3	88.3	3	77.7	22.1	3
NORMAL	10.0	4.8	3	9.3	6.4	3
MS(1)	12.5	9.9	8	7.7	5.4	8
MS(2)	19.6	12.0	7	17.6	9.4	8
MS(3)	16.0	9.7	8	11.0	5.9	9
MS(4)	15.6	13.4	7	14.8	12.1	7
MS(5)	12.8	9.3	7	9.1	5.8	7
MS(6)	31.1	8.3	2	25.8	10.8	2
MS(7)	14.4	12.2	4	14.1	11.6	4

Table 18. PR3 Average +/- S.D. of the various groups.

The D(1) and D(2) classifications, like the MS(1), MS(2), and MS(3), MS(4) groups, did not distinctly separate out in either the RET or the CRP sorts. This could imply that intra-group and inter-group fluctuations were significant. Poor alignment and/or an algorithm not optimized for such calculations could also have prevented the appearance of any expected order.

PR3* results by SLOPES.BAS ordered in the same manner as those by RET. This was expected because SLOPES.BAS was written to approximate RET results by curve-fitting. (Refer to Section 3.4.) The order for the D(3) group was exactly the same but the PR3 values were about 10% greater than the PR3* values. On average, however, PR3* values were neither always greater than nor always lower than PR3 (by the sign test at P=5%). PR3* results from SLOPES.BAS could then be used to check C/VAZ.BAS's RET PR3 results. Either set of results could be consistently used to represent the penetration ratio when only alignment by RET was considered; computing time could be shortened by choosing to run one of the two programmes only.

It is observed from Tables 17 and 18, that most individual and average results of diabetic and MS subjects were higher than those of the normals, despite the large S.D.s. Although age-matching tests between the members of each group were not possible because of the small sample sizes, such trends demonstrated that differences between groups and indivduals existed (for this sample) and were detected!

Tests of the significance of the differences between the PR3 means between any two groups were carried out. Analysis of variance was used to test

H° : the means of any 2 groups were the same,

i.e. their difference was 0, against

H^{a} : the means of any 2 groups were different,

i.e. their difference was not 0.

The P(reject H°) are shown in the respective tables below. The entries in the upper triangles are for RET alignment. Those in the lower triangles are for CRP alignment.

		BY RET			
		NORM	D(1)	D(2)	D(3)
BY	NORM	****	> 25	> 25	2.5
	D(1)	> 25	****	> 25	< .5
CRP	D(2)	> 25	> 25	****	5
	D(3)	1	< .5	2.5	****

Table 19. Significance level (%) to reject H° between diabetic and normal groups.

In the above table, only the D(3) group was clearly and significantly different from all other groups. But, the results in D(1) and D(2) ranged from 1.4 to 30, and 6.5 to 32.7 for RET and CRP respectively. These indicate that breakdown of the BRB had already occurred and was detected in subjects without signs of DRP but were at the early stages [4,5].

The only significant difference for the groups in Table 20a below is that between MS(1) and MS(2) in CRP. In Table 20b, MS(6) is

significantly different from MS(5) and normal but not from MS(7). Between other groups, there is no significant difference. Table 18 shows the large S.D.s of these groups.

(a)

BY CRP	NORMAL	MS(1)	MS(2)	MS(3)	MS(4)
NORMAL	******	> 25	25	> 25	> 25
MS(1)	> 25	*****	25	*****	*****
MS(2)	25	2.5	*****	*****	*****
MS(3)	> 25	*****	*****	*****	> 25
MS(4)	> 25	*****	*****	> 25	*****

(b)

BY CRP	NORMAL	MS(5)	MS(6)	MS(7)
NORMAL	******	> 25	5	> 25
MS(5)	> 25	*****	5	> 25
MS(6)	25	2.5	*****	25
MS(7)	> 25	> 25	> 25	*****

Table 20. Significance level (%) to reject $H^{\bullet}$ between MS and normal groups.

One reason for the great dispersion of data in MS(7) is the placement of the case of the liquefied vitreous. Her classifications, place her into the respective categories, but it is not clear if her results should be included at all because of her unique case. When

MS(7) is tested without it, against MS(6) in RET, P falls to 2.5% from 25%. All other comparisons remain the same.

From the averages and S.D.s in Table 18, it is easy to see that between the two diseases, there is no significant difference between D(1)-D(2), MS(1)-MS(2) and MS(3)-MS(4) classifications when comparing within or between these groups. D(3) is, of course, very much greater then all other groups. The only set which is "internally" significantly distinct is the MS(5)-MS(6)-MS(7) set. Hence, it is tested against D(1) and D(2) only.

		MS(5)	MS(6)	MS(7)
RET	D(1)	> 25	10	> 25
	D(2)	> 25	25	> 25
CRP	D(1)	> 25	25	> 25
	D(2)	25	> 25	> 25

Table 21. Significance level (%) to reject H* between diabetic and MS states.

There is no significant difference (at P=5%) between D(1), D(2) and all MS groups. If these treatments and results are correct, there is no significant difference between an MS subject's PR3 and that of a diabetic with nil to moderate DRP, or a normal. However, individual variations in PR3 values (Table 17) should be noted.

The results are similar when the CR bolus corrections are applied, with and without the correcting peak-to-peak ratio. (See

Section 2.3.) It is hence not possible to study the effects of applying these corrections.

The results thus far indicate the problems that are inherent in the VF technique. Assuming no instrumental errors, the difficulties in the positioning of the retina and the alignment of profiles in analysis reduce the certainty of the PR3 results.

The above results and tests show that MS(1) and MS(2), and MS(3) and MS(4) cannot be differentiated. This is due to the large variations within each group. It does not mean that the VF technique is not applicable to MS as the subjects in MS(6), with active periphlebitis, were discernible from others of the MS(5) and MS(7) groups as was the case for diabetes where the severity of leakage also corresponded to the severity of DRP.

Comparing the results for normals in Table 17 to other investigators' results, which are from 3.5 to 5.3 [32], it is seen that the PR3 values calculated here are within or above this range. No comparisons are available for MS PR3. However, abnormal leakage was seen in some MS subjects other than the two with active periphlebitis. (Refer to Table 17.) These elevated PR3 values cannot be explained by retinal vasculature appearance (photographs and clinical examination). They imply that the VF system may be useful as a sensitive technique to detect subclinical activity. However, the present study only included a small sample of subjects and is not able to relate the clinical gradings of activity of MS or the current activity at the time of the procedure.

5.10 LUND.BAS Results

LUND.BAS was not tested on all subject. Firstly, the gradient-expansion algorithm is slow (on this computer), and is dependent on the initializing estimates of P and D. Convergence is slow if any of the input values are far from the "true" values. (Refer to Section 3.4 and [33,34].) Secondly, the conditions set to halt calculations when the reduced chi-square value begins to diverge from a minimum, or is less than 1, are not amply stringent in terms of convergence to a final solution set. The programme outputs the residues of the final, best fit but plot outputs to visually check the answers are not (yet) available.

Several outputs were returned on this subprogramme for three subjects tested. They were the 27- and 38-year old normals, and the 38-year old MS subject in relapse. (See Table 17.)

Table 22 shows the case-by-case results. The units of P are $*10^{-8}$ $cm.s^{-1}$. D remains in units of $*10^{-6}$ cm^2s^{-1}. Only data points that were between 1.5 mm from the retina and the mid-vitreous were accepted for curve-fitting to Eq. 18 (Section 2.4). The number of data-points that was accepted and fitted in each case is shown in the extreme right column. The results of P values from other investigators for normals and diabetic subjects are also included.

In comparison with published results, the ones obtained in this study are just within the range or less than those in the references. The diffusion constants, D, also follow the same trends when compared to those calculated in the previous section. Again, the large S.D.s of the quoted results should be noted.

INITIAL ESTIMATES		FINAL FITS		CHI-SQ.	#
P	D	P	D		
27-year old NORMAL: 60.RET					
10.0	6.6	7.9	2.7	4.78	15
7.0	2.0	20.8	1.6	3.62	15
27-year old NORMAL: 60.CRP					
10.0	6.6	2.8	5.9	1.13	16
27-year old NORMAL: 68.RET					
10.0	6.6	4.0	5.0	1.44	14
38-year old NORMAL: 64.CRP					
0.5	6.6	5.3	5.1	0.70	20
38-year old MS, F246 : 61.CV2					
10.0	10.0	8.1	14.4	0.63	**
38-year old MS, F246 : 61.CV4					
10.0	10.0	7.7	16.0	0.72	**

References: NORMAL P-values

a) 11.0 +/- 4.0 by Lund-Andersen, et al [54]

b) 30.0 +/- 8.3 by Ogura, et al [53]

c) 7.2 +/- 4.4 by Zeimer, et al [29]

d) 19.1 +/- 9.4 by Chahal, et al [23]

References: DIABETIC P-values

71.0 +/- 38.0 by Lund-Andersen, et al [54]

Table 22. Results of LUND.BAS.
** means unavailable.

If the algorithm of LUND.BAS is to be the adopted method by which different investigators compare P and D values, it is apparent that the computational conditions on the reduced chi-square in LUND.BAS must be more restrictive and selective. Double-checking with output plots must also be implemented.

5.11 Other Parameters

P^1, the permeability index was also calculated by C/VAZ.BAS. (Refer to Section 3.4.) The results were, however, not useful. They often turned out negative and were rejected. This failure was probably due to the algorithm itself. The necessity to integrate very close to the retina or to find an approximation when integrating in that region was machine-(AR-)dependent [32].

Another penetration ratio not metioned thus far is that of the BAB. This result was not investigated because the source of leakage was the iris and ciliary body. The diamond does not scan close to the source of leakage and the models used in the algorithms employed here are not applicable. Another point is that misalignment errors are greater farther away from the retina. (See Figure 5.) Hence, a "PR3" cannot be calculated for the BAB. These conclusions were borne out by SLOPES.BAS which includes such a calculation at 3 mm from the posterior surface of the lens. No correlations in any of the groups were found.

Two other performance parameters were calculated from subject data. The first was the in vivo reproducibility (R). The definition in Eq. 27 in Section 2.5 was changed as this parameter was calculated from two sources. One way to test R is to take scans within 3 minutes of one another; the averages about certain regions of each profile are found. R is then defined as:

$$R = 100 * | a(x,t^1) - a(x,t^2) | / \{ a(x,t^1) + a(x,t^2) \} \ \%,$$

where $a(x,t^1)$ is the average about x of the t^1-minute p.i. profile. R

is simply half the deviation from the average divided by the average. The alternative is to use the S.D. in the numerator, but as there are only two entries at each calculation, this was thought to be unnecessary. Note that small R-values imply good reproducibility.

The region about the mid-vitreous of measurement scans taken at t>55 minutes p.i. was selected. The 3-mm interval was not used because of the influence of tailings or large dye concentrations at later p.i. times, when there is leakage. Further, no mixing was expected at the mid-vitreous at these times.

Only CRP-aligned profiles were considered owing to the problems in locating the retina surface. However, the final R was the average from all scans made after 55 minutes p.i. for which another scan was made within 3 minutes from the first. R is a systems characteristic, and no tests were made against disease classifications. etc.

Taking all the above into consideration, the reproducibility was estimated in averaging over 25 cases, to be

$$R = 19.0 \pm 12.7\ \% .$$

The other method of estimating R is to replace a(x,t) in the above formula with the calculated values of D or PR3, or any calculated characteristics. The conditions that the numbers must be from t > 55 minutes p.i. and within 3 minutes of one another still hold. The number found by replacing with PR3 by CRP was

$$R = 15.8 \pm 14.2\ \% .$$

The last parameter considered was the axial resolution (AR) alluded to in Section 2.1. It was here defined as the ratio of the concentrations at 3mm to the CR peak of the bolus scan after the pre-

injection scan had been subtracted. This definition allowed only CRP-aligned profiles to be used. Also, diseased eyes were excluded owing to possible bolus effects. Note that not all bolus scans were made at exactly 3 minutes p.i. Depending on the subjects, bolus scans were made between 2 and 7 minutes p.i.

Averaged over the three normals,

$$AR = 0.032 \pm 0.026 .$$

VI. CONCLUSION

The performance of the assembled vitreous fluorophotometer was in close agreement with the data that describe the type of light detection system used in this study. Hardware and software designed to interface the light detection system with a microcomputer provided the signal conversion, data analysis and storage capabilities.

The inherent limitation of the optical system was the dependence on a plano-concave contact lens for scanning the vitreous chamber. Discomfort caused by this lens from sequential measurements was largely overcome by using a combination of a bandage soft contact lens and a plastic Luma® lens in place of the glass Goldmann lens used by other investigators. The effect of substituting the lenses was a different set of F-numbers which were re-calculated by measuring intraocular distances using ultrasound.

The most outstanding problem was locating the same reference points along each scan for the purpose of alignment, reduction of data and subtraction in the subsequent analysis. The method used to lessen the effects of alignment errors was to average about an interval along the profile instead of selecting a specific point for comparison. The effectiveness of this algorithm however could not be determined because precise in vivo data on vitreous concentration could not be obtained.

The correction algorithm for the choriod-retinal bolus tailing effects used in the Fluorotron® Master did not improve the separation between groups when applied to the fluorophotometer assembled for

this study.

The penetration ratios in the 15 diabetic subjects were found to increase progressively with the severity of retinopathy (3 gradings), in agreement with published reports. However, there was significant dispersion of results about the averages in each group.

In the sample of 16 multiple sclerosis subjects, the penetration ratios were not significantly different between the two groups that represented standard clinical activity categories or the two groups that represented current activity categories. The usefulness of vitreous fluorophotometry as a non-invasive test for monitoring central nervous system activity could not be ascertained because of the small sample sizes. Abnormal leakage was found in 4 of 15 cases with normal vitreous and either minimal or no evidence of retinal periphlebitis activity (2). The penetration ratios in active periphlebitis were elevated (3-4 times normal control). Abnormal leakage in the absence of active periphlebitis has not previously been recorded. An elevated penetration ratio was also found in one case with vitreous liquefaction.

Almost all subjects in the diabetes sample, irrespective of retinopathy severity, showed vitreous diffusion constants significantly less than the diffusion constant of sodium fluorescein in water. In the multiple sclerosis sample and controls, the diffusion constants in the vitreous and in water were not significantly different. The diffusion constant in the vitreous was 2-4 times greater than the value for water in 4 older multiple sclerosis subjects and 5 times greater in the case with vitreous liquefaction.

APPENDIX A

COMPUTER PROGRAMMES

A.1 DAS.PRN

```
                        ; This programme, DAS configures the PIA in a
                        ;  triggering mode of the Data Acquisition System.
                        ;  The control lines are needed for INTR, WR, MUX,
                        ;  switch A and S/H. RD and CS are held low, i.e.
                        ;  the output of the ADC is always enabled.
                        ; This routine, as entered in the MBASIC DATA
                        ;  statements, is separated by a semi-colon and a
                        ;  number as seen below. E.g. - ;#20
                        ;
                        ; This version was written in July 1985 by PANG
                        ;                Kian Tiong.
                        ;
                        ;       PA0-7 to be inputs. - line #1-8
                        ;       CA1 = not used. - line #19
                        ;       CA2 = not used. - line #17
                        ;     =====>> CRA word = 0010_1110
                        ;
                        ;       PB0 = 1 to set PA0-7 as inputs.
                        ;       PB1 = 1 for PB6,7 to be inputs.
                        ;       PB2 = 0 for PB3,5 to be outputs.
                        ;       PB3 = ? - WR control: - line #9
                        ;                 0 = start conversion
                        ;                 1 = reset and wait
                        ;       PB4 = ? - MUX address: - line #21
                        ;                 0 = pod
                        ;                 1 = radiometer
                        ;       PB5 = ? - S/H address: - line #11
                        ;                 0 = sample
                        ;                 1 = hold
                        ;       PB6 = ? - Switch A interrupt input - line #15
                        ;                 0 = continue reading
                        ;                 1 = wait to start or stop
                        ;       PB7 = ? - INTR interrupt input - line #13
                        ;                 0 = INTR hi
                        ;                 1 = INTR lo
                        ;=====>> DRB word: 00??_?011
                        ;
EF08 =                  flagpos         equ     0ef08h  ;ram/rom jump vector
2901 =                  cra     equ     02901h  ;control register A
2903 =                  crb     equ     02903h  ;control register B
2900 =                  dra     equ     02900h  ;data/direction register A
2902 =                  drb     equ     02902h  ;data/direction register B
EFDE =                  status equ      0efdeh  ;PIA status
                        ;
D1D6                            org     0d1d6h  ;starting address
                        ;
                        ; Configuring the PIA begins.
                        ;#1
D1D6 AF                 mode:   xra     a
D1D7 3ADEEF                     lda     status
D1DA FE02                       cpi     2
D1DC C8                         rz              ;if already in input mode
                        ;#2
D1DD 3E2A                       mvi     a,02ah  ;0010_1010
D1DF 320129                     sta     cra
D1E2 3E00                       mvi     a,0
                        ;#3
D1E4 320029                     sta     dra
D1E7 3E2E                       mvi     a,02eh  ;0010_1110
D1E9 320129                     sta     cra     ;port A is input
                        ;#4
D1EC 3E00                       mvi     a,0
D1EE 320329                     sta     crb
D1F1 3E3F                       mvi     a,03fh  ;0011_1111
                        ;#5
D1F3 320229                     sta     drb
D1F6 3E36                       mvi     a,036h  ;0011_0110
D1F8 320329                     sta     crb     ;port B is set
                        ;#6
```

```
D1FB 3E02           mvi     a,2
D1FD 32DEEF         sta     status
D200 C9             ret
                    ;
                    ; Reading and interrupt status: INTR
                    ;#7
D201 3A0229     input: lda     drb
D204 17             ral
D205 D201D2         jnc     input   ;if INTR hi
                    ;#8
D208 3A0229     high:  lda     drb
D20B 17             ral
D20C DA08D2         jc      high    ;if INTR lo
                    ;#9
D20F 3A0029         lda     dra
D212 2F             cma
D213 77             mov     M,a
                    ;#10
D214 3E2B           mvi     a,02bh  ;0010_1011
D216 320229         sta     drb
D219 C9             ret
                    ;
                    ; First entry point from BASIC programme.
                    ;#11
D21A F3         entry: di
D21B D300           out     0
D21D 3E00           mvi     a,0
D21F 3208EF         sta     flagpos ;in Bank 2
                    ;#12
D222 32D0D1         sta     0d1d0h  ;stop code
D225 CDD6D1         call    mode    ;test PIA status
                    ;#13
D228 3A0229     start: lda     drb
D22B 17             ral
D22C 17             ral
D22D DA28D2         jc      start   ;poll Switch A
                    ;#14
D230 C33BD2         jmp     read
                    ;
                    ; Subsequent entries from BASIC routine.
                    ;#15
D233 F3             di
D234 D300           out     0
D236 3E00           mvi     a,0
D238 3208EF         sta     flagpos ;in Bank 2
                    ;#16
D23B 3E1B       read:  mvi     a,01bh  ;0001_1011
D23D 320229         sta     drb
                    ;
                    ; Reading radiometer
                    ;#17
D240 21D4D1         lxi     h,0d1d4h
D243 3E33           mvi     a,033h  ;0011_0011
                    ;#18
D245 320229         sta     drb
D248 CD01D2         call    input
                    ;
                    ; Reading pod.
                    ;#19
D24B 21D2D1         lxi     h,0d1d2h
D24E 3E23           mvi     a,023h  ;0010_0011
                    ;#20
D250 320229         sta     drb
D253 CD01D2         call    input
                    ;
                    ; Check for stop acquisition
                    ;#21
D256 3A0229         lda     drb
D259 17             ral
D25A 17             ral
D25B D263D2         jnc     goback
                    ;#22
D25E 3EF0           mvi     a,0f0h  ;stop code
D260 32D0D1         sta     0d1d0h
                    ;#23
D263 D301       goback:        out     1
D265 3E01           mvi     a,1
D267 3208EF         sta     flagpos
D26A FB             ei              ;back in Bank 1
D26B C9             ret             ;to BASIC routine
```

A.2 SCANMENU.BAS

```
100 WIDTH 52 : CLEAR,&HD1CF : REM Updated 200186
110 DEFSTR A
120 DEFINT I-N
130 A="**********" : PRINT A : PRINT "SCAN MENU" : PRINT A : PRINT
140 RESET : PRINT "(0) Run VITREOUS SCANNING programme" : PRINT
150 PRINT "(1) Run PLASMA SCANNING programme" : PRINT
160 PRINT "(2) Run SUBJECT DATA ENTRY programme" : PRINT
170 PRINT : PRINT "Enter ANY OTHER number to EXIT." : PRINT
180 PRINT "Which programme do you wish to run "; : INPUT II
190 IF (II>2) THEN END ELSE PRINT
200 IF (II=2) GOTO 520
210 PRINT "Loading assembly-language subroutine, DAS."
220 DATA &HAF,&H3A,&HDE,&HEF,&HFE,2,&HC8
230 DATA &H3E,&H2A,&H32,1,&H29,&H3E,0
240 DATA &H32,0,&H29,&H3E,&H2E,&H32,1,&H29
250 DATA &H3E,3,&H32,3,&H29,&H3E,&H3F
260 DATA &H32,2,&H29,&H3E,&H36,&H32,3,&H29
270 DATA &H3E,2,&H32,&HDE,&HEF,&HC9
280 DATA &H3A,2,&H29,&H17,&HD2,1,&HD2
290 DATA &H3A,2,&H29,&H17,&HDA,8,&HD2
300 DATA &H3A,0,&H29,&H2F,&H77
310 DATA &H3E,&H28,&H32,2,&H29,&HC9
320 DATA &HF3,&HD3,0,&H3E,0,&H32,8,&HEF
330 DATA &H32,&HD0,&HD1,&HCD,&HD6,&HD1
340 DATA &H3A,2,&H29,&H17,&H17,&HDA,&H28,&HD2
350 DATA &HC3,&H3B,&HD2
360 DATA &HF3,&HD3,0,&H3E,0,&H32,8,&HEF
370 DATA &H3E,&H1B,&H32,2,&H29
380 DATA &H21,&HD4,&HD1,&H3E,&H33
390 DATA &H32,2,&H29,&HCD,1,&HD2
400 DATA &H21,&HD2,&HD1,&H3E,&H23
410 DATA &H32,2,&H29,&HCD,1,&HD2
420 DATA &H3A,2,&H29,&H17,&H17,&HD2,&H63,&HD2
430 DATA &H3E,&HF0,&H32,&HD0,&HD1
440 DATA &HD3,1,&H3E,1,&H32,8,&HEF,&HFB,&HC9,0,0,0
450 FOR I=1 TO 151
460   READ J : K=&HD1D5+I : POKE K,J
470   NEXT I
480 PRINT : PRINT "=> DAS has been successfully loaded!" : PRINT
490 IF (II=1) GOTO 510
500   CHAIN MERGE "B:VITSCAN",1010,ALL
510   CHAIN MERGE "B:PLASCAN",1010,ALL
520 DIM AA(22),X(8)
530 A="**********" : PRINT : PRINT A
540 PRINT "SUBJECT DATA" : PRINT A : PRINT
550 PRINT "Enter the following --->" : PRINT
560   LINE INPUT "Subject's NAME -> ";AA(0) : PRINT
570   LINE INPUT "Subject's AGE --> ";AA(1) : PRINT
580   LINE INPUT "     Scan EYE --> ";AA(2) : PRINT
590   LINE INPUT "     Scan DATE -> ";AA(3) : PRINT : PRINT
600 FOR I=0 TO 5 STEP 3
610   PRINT "Enter the ";
620   IF (I<>0) GOTO 640
630     PRINT "LENGTHS noted (in Osb/DAS units) --->" : GOTO 660
640   PRINT "ULTRA-SOUND scan results (in mm) --->" : PRINT
650   PRINT TAB(10);"If NO ultra-sound taken, enter 0."
660   PRINT : PRINT "VITREOUS ---> "; : INPUT X(I)
670   PRINT : PRINT "    LENS ---> "; : INPUT X(I+1)
680   PRINT : PRINT " AQUEOUS ---> "; : INPUT X(I+2)
690   PRINT : PRINT : PRINT : PRINT
700   NEXT I
710 PRINT "Amount of FLUORESCEIN injected was ";
720   INPUT X(6) : PRINT : PRINT : PRINT : PRINT
730 PRINT "Enter any REMARKS, COMMENTS or OBSERVATIONS -->" : PRINT
740   PRINT TAB(10);"Press RETURN to exit." : PRINT : I=3
750 LINE INPUT "Enter -> ";A
760   IF (A="") GOTO 780
770     I=I+1 : AA(I)=A : GOTO 750
780 PRINT : PRINT : PRINT "The above is to be filed in"
790   PRINT : PRINT TAB(10);"A for the  LEFT Drive"
800   PRINT TAB(10);"B for the RIGHT Drive ";
810   A=INPUT$(1) : PRINT : PRINT : KEY=0
820 IF (A="A" OR A="a" OR A="B" OR A="b") THEN KEY=1
830 IF (KEY=0) GOTO 980
840   IF A="b" THEN A="B"
850   IF A="a" THEN A="A"
```

```
860   OPEN "O",#1,A+":SUBJECT.DAT" : J=0 : M=3 : N=0
870   FOR K=J TO M
880     IF N=1 THEN PRINT #1,X(K) ELSE PRINT #1,AA(K)
890     NEXT K
900   N=N+1
910   ON N GOTO 920,930,940
920     M=6 : GOTO 870
930     J=4 : M=I : GOTO 870
940   PRINT : PRINT "SUBJECT.DAT has been completed."
950   ERASE AA,X
960   PRINT : PRINT : PRINT : PRINT "Do NOT forget to COPY onto a run
          Diskette before BATCHRUN !!"
970   PRINT : PRINT : GOTO 130
980 PRINT : PRINT : PRINT "BAD Entry !!!  Please try again."
990 PRINT : PRINT : GOTO 780
```

A.3 VITSCAN.BAS

```
1000 REM Updated 040286
1010 DIM IX(1600),IY(1600),ML(9)
1020 A="****************" : PRINT : PRINT : PRINT A
1030   PRINT "VITREOUS SCANNING" : PRINT A : PRINT : PRINT
1040 PRINT "SYSTEMS CHECKS" : PRINT "=============="
1050   PRINT "(1) All equipment ON." : PRINT "(2) All cables connected."
1060   PRINT "(3) Switch A LOW." : PRINT "(4) VOLTMETER/Intensity detector ON."
1070   PRINT "(5) Intensity = 141 +/- 1" : PRINT "(6) HV = 739"
1080   PRINT "(7) R/M exp = 0" : PRINT "(8) R/M-DAS output = 0"
1090   PRINT "(9) Switch A HIGH to begin." : PRINT
1100 K=&HD21A : IP=&HD233 : PRINT : PRINT "LANDMARK SCANNING"
1110   PRINT : CALL K : A=INKEY$ : JM=-1
1120 CALL IP : PRINT "Intensity =";PEEK(&HD1D4);
1130   K=PEEK(&HD1D2) : PRINT TAB(30);"Pod =";K
1140   IF (INKEY$="") GOTO 1160
1150     JM=JM+1 : IX(JM)=K : PRINT CHR$(7);
1160   IF (PEEK(&HD1D0)<>240) GOTO 1120
1170 IF JM<1 THEN GOTO 1250 ELSE PRINT
1180   IF JM>8 THEN JM=8
1190   FOR K=1 TO JM
1200     I=K-1 : ML(I)=IX(K)-IX(I)
1210     PRINT TAB(10);"Mark";K;"- Mark";I;"=";ML(I)
1220     NEXT K
1230   PRINT : PRINT "Total LENGTH =";IX(JM)-IX(0) : PRINT
1240   PRINT "IMPORTANT: WRITE down these numbers." : PRINT
1250 PRINT : PRINT "LANDMARKing will NOT be Repeated, OK?" : GOSUB 1730
1260   IF (A=CHR$(27)) GOTO 1100
1270 A=INKEY$ : PRINT : PRINT "Which EYE is to be scanned?" : PRINT
1280   PRINT "     Enter :   ESC  for the  RIGHT eye,"
1290   PRINT TAB(16);"ANY  for the  LEFT  eye. "; : A=INPUT$(1) : PRINT : PRINT
1300     IF A=CHR$(27) THEN JJ=1 ELSE JJ=0
1310     IF JJ=0 THEN PRINT "LEFT"; ELSE PRINT "RIGHT";
1320     PRINT " Eye will be scanned." : PRINT : PRINT
1330 PRINT "SCANNING INSTRUCTIONS" : PRINT "====================="
1340   PRINT "(1) Scan STEADILY (from the RETINA each time)." : PRINT
1350   PRINT "(2) Wait for the BEEP before starting." : PRINT
1360   PRINT "(3) Switch A to HIGH to BEGIN scanning." : PRINT
1370 K=&HD21A : IP=&HD233 : CALL K
1380 FOR I=0 TO 120
1390   CALL IP : IM=PEEK(&HD1D2)
1400   IF I<50 THEN PRINT "Pod =";IM,"Retina =";PEEK(&HD1D4)
1410   IF I=50 THEN PRINT CHR$(26)
1420   NEXT I
1430 I=-1 : PRINT CHR$(7);
1440 IF I=1599 THEN GOTO 1470 ELSE I=I+1
1450   CALL IP : IX(I)=PEEK(&HD1D2) : IY(I)=PEEK(&HD1D4)
1460   IF (PEEK(&HD1D0)<>240) GOTO 1440 ELSE GOTO 1480
1470 PRINT "You are out of MEMORY.   Switch A to LOW." : PRINT
1480   N=I : GOSUB 1770 : PRINT : PRINT
1490 IF (KQ<>0) GOTO 1590
1500 PRINT "Is the scan to be SAVEd?" : GOSUB 1730 : PRINT
```

```
1510   IF A=CHR$(27) THEN PRINT "NO !" ELSE PRINT "YES !"
1520   PRINT : PRINT "Please CONFIRM with the SAME answer." : GOSUB 1730 : PRINT
1530   IF A=CHR$(27) THEN GOTO 1590 ELSE PRINT
1540     PRINT "Enter the P.I. Scan TIME" : PRINT TAB(20);"for the ";
1550     IF JJ=0 THEN PRINT "LEFT"; ELSE PRINT "RIGHT";
1560     LINE INPUT " eye. ";A : PRINT : PRINT
1570     NAME AA+"TEM.DAT" AS AA+A+".DAT" : PRINT
1580     PRINT "===>> The file has been successfully RENAMEd."
1590 PRINT : PRINT "Is SCANNING to be CONTINUEd?" : GOSUB 1730
1600   IF (A=CHR$(27)) GOTO 1630
1610   PRINT "SYSTEMS will NOT be checked, OK?" : GOSUB 1730
1620     IF (A<>CHR$(27)) GOTO 1270 ELSE GOTO 1040
1630 PRINT : PRINT : PRINT "The files in Drive A are :"
1640   PRINT : FILES "A:*.*" : PRINT : PRINT
1650 PRINT "The files in Drive B are :" : PRINT
1660   FILES "B:*.*" : PRINT : PRINT
1670 PRINT "Do you wish to return to the SCAN MENU?" : GOSUB 1730
1680   IF (A=CHR$(27)) GOTO 1590
1690     ERASE IX,IY,ML
1700     ON ERROR GOTO 0
1710     CLOSE : GOTO 130
1720 REM Prompt options display.
1730 A=INKEY$ : PRINT : PRINT TAB(5);"Enter : ANY KEY  for  YES"
1740   PRINT TAB(17);"ESC  for  NO "; : A=INPUT$(1)
1750   PRINT : PRINT : PRINT : RETURN
1760 REM Subroutine for video-plotting.
1770 WIDTH 127
1780 K=155 : W=0 : Q=201/(K-W) : IR=IX(N) : LL=IX(0)-10 : KQ=0
1790   IF LL<0 THEN LL=0
1800   IF (IR-LL)<50 THEN U=11 ELSE U=125!/(IR-LL)
1810 PRINT CHR$(26);CHR$(27)+")";CHR$(27)+"="+CHR$(54)+CHR$(36);
1820   PRINT "    Press ";CHR$(27)+"(";"ANY";CHR$(27)+")";
1830   PRINT " KEY to ABORT this SCAN at any time." : A=INKEY$
1840 FOR I=0 TO 20
1850   PRINT CHR$(27)+"="+CHR$(32+I)+CHR$(32);
1860   IF (I=0 OR I=5 OR I=10 OR I=15 OR I=20) THEN PRINT "+"; ELSE PRINT "|";
1870   NEXT I
1880 FOR I=0 TO 125
1890   PRINT CHR$(27)+"="+CHR$(53)+CHR$(33+I);
1900   IF (INKEY$<>"") GOTO 2180
1910     IF RIGHT$(STR$(I),1)="0" THEN PRINT "+"; ELSE PRINT "-";
1920   NEXT I
1930 IF JJ=0 THEN AA="A:" ELSE AA="B:"
1940 OPEN "O",#3,AA+"TEM.DAT" : PRINT #3,IM : A=INKEY$
1950   PRINT CHR$(27)+"="+CHR$(54)+CHR$(32);LL; : M=0
1960   PRINT CHR$(27)+"="+CHR$(54)+CHR$(150);IR;
1970 FOR I=0 TO N
1980   IF (INKEY$<>"") GOTO 2210
1990   IF (IY(I)=128 OR IY(I)=127) GOTO 2050
2000     IP=CINT((IX(I)-LL)*U) : K=CINT((IY(I)-W)*Q)
2010     PRINT #3,IX(I) : PRINT #3,IY(I) : M=M+1
2020     IF (IP>124 OR K>20) GOTO 2050
2030     IF (IP<0 OR K<0) GOTO 2050
2040       PRINT CHR$(27)+"="+CHR$(52-K)+CHR$(33+IP);"*"
2050   NEXT I
2060 IP=IM-LL : K=CINT(IP*U) : PRINT CHR$(27)+"(";
2070  PRINT CHR$(27)+"="+CHR$(34)+CHR$(35);M;"pairs were entered."
2080   PRINT CHR$(27)+"="+CHR$(53)+CHR$(33+K);"R";
2090 IF (JM<0) GOTO 2150
2100   FOR I=0 TO JM
2110     IP=IP+ML(I) : K=CINT(IP*U)
2120     IF (K<0 OR K>124) GOTO 2140
2130       PRINT CHR$(27)+"="+CHR$(53)+CHR$(33+K);"!";
2140     NEXT I
2150 A=INKEY$ : PRINT CHR$(27)+"="+CHR$(54)+CHR$(40);
2160   PRINT "Press ANY key to return to PROMPT mode."; : A=INPUT$(1)
2170 CLOSE
2180   PRINT : PRINT : PRINT CHR$(27)+"(" : PRINT
2190 WIDTH 52
2200 RETURN
2210 PRINT CHR$(27)+"="+CHR$(35)+CHR$(35);
2220 PRINT "Please CONFIRM that you want this scan ABORTED!!"
2230 GOSUB 1730 : PRINT : ATT=INKEY$ : ATT=INKEY$
2240 IF (A=CHR$(27)) GOTO 1990
2250   KQ=1 : GOTO 2170
```

A.4 PLASCAN.BAS

```
1000 REM Updated 180186
1010 DIM IX(1200),IY(1200),L(256),V(256),S(256),X(55),Y(55),Z(55)
1020 A="***************" : PRINT : PRINT A : KEY=-1
1030   PRINT "PLASMA SCANNING" : PRINT A : PRINT : PRINT
1040 PRINT : PRINT "SCANNING INSTRUCTIONS" : PRINT "====================="
1050   PRINT "(1) Aim PROBE with WHITE light." : PRINT
1060   PRINT "(2) Adjust cell for NO REFLECTION." : PRINT
1070   PRINT "(3) Move PROBE until just behind glass surface." : PRINT
1080   PRINT "(4) Fix the POD position." : PRINT
1090   PRINT "(5) Switch A to HIGH to BEGIN scanning."
1100 K=&HD21A : IP=&HD233 : CALL K : PRINT CHR$(26);
1110 FOR I=0 TO 99
1120   CALL IP : K=PEEK(&HD1D2) : K=PEEK(&HD1D4)
1130   NEXT I
1140 I=-1 : PRINT CHR$(7);
1150 IF I=1199 THEN GOTO 1180 ELSE I=I+1
1160   CALL IP : IX(I)=PEEK(&HD1D2) : IY(I)=PEEK(&HD1D4)
1170   IF (PEEK(&HD1D0)<>240) GOTO 1150 ELSE GOTO 1190
1180 PRINT "You are out of MEMORY.   Switch A to LOW." : PRINT
1190 NN=I : PRINT "AVERAGING begins." : PRINT : PRINT
1200   PRINT TAB(5);"Press ANY key to interrupt." : PRINT : PRINT
1210 FOR J=0 TO 255
1220   L(J)=0 : V(J)=0! : S(J)=0! : A=INKEY$
1230   NEXT J
1240 FOR J=0 TO NN
1250   I=IX(J) : MM=IY(J)
1260   IF (INKEY$<>"") GOTO 1450
1270   IF (MM=127 OR MM=128) GOTO 1290
1280     L(I)=L(I)+1 : V(I)=V(I)+MM : S(I)=S(I)+MM^2
1290   NEXT J
1300 J=-1 : JM=0 : KS=0
1310 FOR I=0 TO 255
1320   MM=L(I) : KS=KS+MM
1330   IF (INKEY$<>"") GOTO 1450
1340   IF (MM<2) GOTO 1390
1350     J=J+1 : V(J)=V(I)/MM : L(J)=I
1360     S(J)=(S(I)-MM*V(J)^2)/(MM-1)
1370     IF (MM<JM) GOTO 1390
1380       JM=MM : M=J
1390   NEXT I
1400 KEY=KEY+1 : Y(KEY)=V(M) : Z(KEY)=SQR(S(M))
1410   PRINT : PRINT "Average =";V(M);"+/-";Z(KEY) : PRINT
1420   PRINT TAB(10);"for";JM;"out of";KS;"points." : PRINT
1430 PRINT : PRINT "What is the Sample TIME (in min. P.I.) ";
1440   INPUT X(KEY) : PRINT : PRINT : GOTO 1460
1450 PRINT TAB(5);"Averaging interrupted !!" : PRINT : PRINT
1460 PRINT : PRINT "Is SCANNING to be CONTINUEd?" : GOSUB 1720
1470   IF (A<>CHR$(27)) GOTO 1040
1480 PRINT : PRINT "Was the scan for (0) BOTH  eyes ?"
1490   PRINT TAB(18);"(1) LEFT  only ?" : PRINT TAB(18);"(2) RIGHT only ";
1500   INPUT KM : PRINT : PRINT
1510   IF KM=2 THEN J=1 ELSE J=0
1520 A=":PLASMA.DAT" : PRINT "The PLASMA data are:" : PRINT
1530   IF J=0 THEN OPEN "O",#2,"A"+A ELSE OPEN "O",#2,"B"+A
1540   FOR I=0 TO KEY
1550     PRINT #2,X(I) : PRINT #2,Y(I) : PRINT #2,Z(I)
1560     PRINT I+1;") ";X(I),Y(I);" +/- ";Z(I)
1570     NEXT I
1580   CLOSE : J=J+1 : PRINT : PRINT
1590 IF (KM=0 AND J=1) GOTO 1530
1600   PRINT "Plasma averages have been filed." : PRINT
1610 PRINT "The files in Drive A are :" : PRINT : FILES "A:*.*"
1620   PRINT : PRINT : PRINT "The files in Drive B are :"
1630   PRINT : FILES "B:*.*" : PRINT : PRINT : PRINT
1640 PRINT "Do you have another set of samples to do?" : GOSUB 1720
1650   IF A=CHR$(27) THEN GOTO 1690 ELSE RESET
1660 PRINT : PRINT "Enter ANY key after changing LOGGED diskette."
1670   A=INPUT$(1) : RESET : PRINT : PRINT
1680   GOTO 1020
1690 ERASE IX,IY,L,V,S,X,Y,Z
1700   PRINT : PRINT : PRINT : PRINT
1710   CLOSE : GOTO 130
1720 A=INKEY$ : PRINT : PRINT TAB(5);"Enter : ANY KEY  for  YES"
1730 PRINT TAB(17);"ESC  for  NO "; : A=INPUT$(1) : PRINT : PRINT : PRINT
1740 RETURN
```

A.5 BATCHRUN.BAS

```
100 WIDTH 52 : REM Updated 120386
110 DEFINT I-N
120 DEFSTR A
130 DIM AN(13),ITASK(11)
140 GOTO 170
150 GOTO 680
160 RESET
170 PRINT CHR$(26);"Are you using a COPY of the original data?"
180 A="**************" : PRINT : PRINT A
190 PRINT "PROGRAMME MENU" : PRINT A : PRINT
200   PRINT "(0) Run REDUCE -  Rav data averaging"
210   PRINT "(1) Run B/G    -  Background averaging"
220   PRINT "(2) Run MINUS  -  Background subtraction"
230   PRINT "(3) Run SUDATA -  Reprod./LLoD/AxRes"
240   PRINT "(4) Run BLOOD  -  Plasma data integration"
250   PRINT "(5) Run C/VAZ  -  Cunha-Vaz's algorithm"
260   PRINT "(6) Run SLOPES -  Curve-fitting method"
270   PRINT "(7) Run PLOT   -  Coarse plotting"
280   PRINT "(8) Run DRAW   -  Super-impose plotting"
290   PRINT "(9) Run LUND   -  Lund-Andersen's algorithm"
300 FOR I=0 TO 10
310   ITASK(I)=-99
320   NEXT I
330 PRINT : PRINT "Enter the SEQUENCE to run ->" : PRINT
340   PRINT "Runs ALWAYS begins with the left to right." : PRINT
350   PRINT TAB(16);"Yes = ANY key  :  No = ESC"
360   PRINT : JTASK=-99 : A=INKEY$ : A="" : NTASK=-99
370 PRINT "0   1   2   3   4   5   6   7   8   9"
380 FOR I=0 TO 9
390   A=INPUT$(1) : PRINT "    ";
400   IF A=CHR$(27) THEN GOTO 420 ELSE ITASK(I)=55
410     IF I<7 THEN JTASK=55 ELSE NTASK=55
420   NEXT I
430 IF (JTASK>0 OR NTASK>0) GOTO 490
440   PRINT : PRINT : PRINT "NO task assigned!" : PRINT
450   PRINT "Do you want to continue? (Y) ";
460   A=INPUT$(1) : PRINT : PRINT
470   IF (A="N" OR A="n") THEN LPRINT CHR$(27);CHR$(79); ELSE GOTO 160
480 END
490 PRINT
500 FOR I=0 TO 9
510   IF ITASK(I)>0 THEN A="Y" ELSE A="N"
520   PRINT A;"   ";
530   NEXT I
540 PRINT : PRINT : PRINT TAB(15);"Please CONFIRM !  (Y)  "; : A=INPUT$(1)
550   IF (A="N" OR A="n") GOTO 170
560 IF (JTASK<0 AND NTASK=55) GOTO 640
570 PRINT : PRINT : PRINT "Files in Drive B are:"
580   PRINT : FILES "B:*.*" : PRINT : PRINT : PRINT
590   PRINT TAB(5);"Enter in CHRONOLOGICAL, ASCENDING order."
600   PRINT : PRINT TAB(10);"Enter RETURN to exit." : PRINT : MQ=-1
610 LINE INPUT "Enter --> ";A
620   IF (A="") GOTO 640
630     MQ=MQ+1 : AN(MQ)=A : GOTO 610
640 PRINT : PRINT "Check PRINTER/PAPER." : PRINT
650   LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);
660   LPRINT CHR$(18);CHR$(27);CHR$(73);CHR$(1);
670   LPRINT CHR$(27);CHR$(78);CHR$(6); : LTASK=0
680 NERR=0 : PSCALE=.095919
690 FOR KTASK=LTASK TO 9
700   IF ITASK(KTASK)<0 THEN GOTO 820 ELSE LTASK=KTASK+1
710     ON KTASK GOTO 730,740,750,760,770,780,790,800,810,820
720   CHAIN MERGE "A:REDUCE",1010,ALL
730   CHAIN MERGE "A:B/G",1010,ALL
740   CHAIN MERGE "A:MINUS",1010,ALL
750   CHAIN MERGE "A:SUDATA",1010,ALL
760   CHAIN MERGE "A:BLOOD",1010,ALL
770   CHAIN MERGE "A:C/VAZ",1010,ALL
780   CHAIN MERGE "A:SLOPES",1010,ALL
790   CHAIN MERGE "A:PLOT",1010,ALL
800   CHAIN MERGE "A:DRAW",1010,ALL
810   CHAIN MERGE "A:LUND",1010,ALL
820   NEXT KTASK
830 PRINT : PRINT : GOTO 450
```

A.6 REDUCE.BAS

```
1000 REM Updated 210186
1010 A="++++++" : PRINT A : PRINT "REDUCE" : PRINT A
1020 DIM I(256),IX(4000),IY(4000),IS(256),Y(256),W(256)
1030 DEF FNCONC(X)=EXP(-109.339+SQR(11915.9+X/.114441))
1040 ON ERROR GOTO 1790
1050 A="++++++++++++++++++++++++++++++++++++++++++++++++++++++++"
1060 FOR LT=0 TO MQ
1070   FOR K=0 TO 255
1080     I(K)=0 : IS(K)=0
1090     NEXT K
1100   JCO=255 : JCR=0 : OPEN "I",#1,"B:"+AN(LT)+".DAT" : INPUT #1,N : K=-1
1110   IF EOF(1)<>0 THEN GOTO 1160 ELSE K=K+1
1120     INPUT #1,J : INPUT #1,IY(K) : I(J)=I(J)+1
1130     IF J>JCR THEN JCR=J
1140     IF J<JCO THEN JCO=J
1150     IX(K)=J : GOTO 1110
1160   CLOSE #1 : JVL=0 : IMA=K : NUM=-1 : JMV=0
1170   FOR K=JCO TO JCR
1180     IF (I(K)<2) GOTO 1630
1190       PRINT "For X =";K;", there are";I(K);"points." : PRINT
1200       IMAX=0 : IMIN=255 : J=-1 : JLE=I(K) : U=0! : V=0! : KP=0
1210       FOR KK=JMV TO IMA
1220         IF (KP=1 OR IX(KK)<=K) GOTO 1240
1230           KP=1 : IMV=KK
1240         IF IX(KK)<>K THEN GOTO 1290 ELSE JAL=IY(KK)
1250           IF J=(JLE-1) THEN GOTO 1300 ELSE J=J+1
1260             IS(JAL)=IS(JAL)+1 : U=U+JAL : V=V+JAL^2
1270             IF JAL>IMAX THEN IMAX=JAL
1280             IF JAL<IMIN THEN IMIN=JAL
1290         NEXT KK
1300       IF KP=1 THEN JMV=IMV ELSE JMV=KK
1310       KK=IMAX-IMIN : PRINT "MAX =";IMAX,"MIN =";IMIN,"MAX-MIN =";KK
1320       IF KK<7 THEN GOTO 1530 ELSE JLE=0
1330         IF JVL=0 THEN P=U/(J+1)
1340       FOR KK=IMIN TO IMAX
1350         IF (IS(KK)=0) GOTO 1430
1360           PRINT "    For Y =";KK;",";TAB(18);"there are";IS(KK);"points."
1370           IF (IS(KK)<JLE) GOTO 1430
1380             IF IS(KK)>JLE THEN KJ=255
1390             ICO=ABS(CINT(KK-P))
1400             IF (ICO>KJ) GOTO 1420
1410               KJ=ICO : IMV=KK
1420             JLE=IS(KK)
1430         NEXT KK
1440       KK=IMV : PRINT : PRINT "Estimate used is";KK : V=0! : U=0! : JLE=0
1450       ICO=KK+5 : ICR=KK-5 : KK=CINT(KK-P)
1460       IF KK>5 THEN ICR=CINT(P)
1470       IF KK<-5 THEN ICO=CINT(P)
1480       FOR KK=IMIN TO IMAX
1490         IF (KK>ICO OR KK<ICR) GOTO 1510
1500           JLE=JLE+IS(KK) : V=V+IS(KK)*KK^2 : U=U+KK*IS(KK)
1510         NEXT KK
1520       PRINT : PRINT "Upper Bound =";ICO,"Lower Bound =";ICR
1530       U=U/JLE : PRINT : PRINT TAB(10);"No. of points used =";JLE;"of";I(K)
1540       FOR KK=IMIN TO IMAX
1550         IS(KK)=0
1560         NEXT KK
1570       IF JLE<2 THEN GOTO 1630 ELSE V=(V-JLE*U^2)/(JLE-1)
1580         IF V=0! THEN V=1!
1590         IF V<0! THEN V=ABS(V)
1600         NUM=NUM+1 : V=SQR(V) : I(NUM)=K : Y(NUM)=U : W(NUM)=V
1610         PRINT : PRINT "(";AN(LT);") Ans :";K,U;"+/-";V
1620         JVL=1 : P=U : PRINT A
1630     NEXT K
1640   OPEN "O",#2,"B:"+AN(LT)+".AVG" : PRINT "Filing ";AN(LT);".AVG"
1650   FOR K=0 TO NUM
1660     KK=I(K)-N : U=FNCONC(Y(K)) : V=FNCONC(W(K))
1670     PRINT #2,KK : PRINT #2,U : PRINT #2,V
1680     NEXT K
1690   CLOSE : AA="B:"+AN(LT)+".DAT"
1700   NAME AA AS "B:TEM.TEM"
1710   PRINT : PRINT AN(LT);".DAT has been renamed."
1720   KILL "B:TEM.TEM"
1730   PRINT : PRINT AN(LT);".DAT has been erased." : PRINT A
1740   NEXT LT
1750 ERASE I,IX,IY,IS,Y,W
```

```
1760   ON ERROR GOTO 0
1770   CLOSE : GOTO 150
1780 REM Error subroutine to print positions where errors occur.
1790 IF NERR>10 THEN END ELSE NERR=NERR+1
1800   LPRINT "REDUCE Error Code #";ERR;"in Loop #";LT;"at Line #";ERL
1810   RESUME NEXT
```

A.7 B/G.BAS

```
1000 REM Updated 210186
1010 DIM X(3,256),Y(3,256),Z(3,256),L(4),M(256),C(256),D(256)
1020 PRINT "+++" : PRINT "B/G" : PRINT "+++" : PRINT : NN=-1
1030 FOR I=0 TO MQ
1040   IF VAL(AN(I))=0 THEN NN=NN+1
1050   NEXT I
1060 IF (NN=0) GOTO 1570
1070 FOR I=0 TO 255
1080   M(I)=0 : C(I)=0! : D(I)=0!
1090   NEXT I
1100 ON ERROR GOTO 1610 : XMIN=255!
1110 FOR N=0 TO NN
1120   ON N GOTO 1140,1150
1130     A="0" : GOTO 1160
1140     A="00" : GOTO 1160
1150     A="000"
1160   OPEN "I",#1,"B:"+A+".AVG" : I=-1
1170   PRINT : PRINT TAB(10);"Reading ";A;".AVG" : PRINT
1180   I=I+1 : INPUT #1,X(N,I) : INPUT #1,Y(N,I) : INPUT #1,Z(N,I)
1190     IF X(N,I)<XMIN THEN XMIN=X(N,I)
1200     IF (EOF(1)=0) GOTO 1180
1210   CLOSE : L(N)=I
1220   NEXT N
1230 PRINT : XMIN=ABS(XMIN) : PRINT TAB(10);"Min =";XMIN : PRINT
1240 FOR I=0 TO N-1
1250   FOR J=0 TO L(I)
1260     K=CINT(X(I,J)+XMIN) : C(K)=C(K)+Y(I,J)
1270     D(K)=D(K)+Z(I,J)^2 : M(K)=M(K)+1
1280     NEXT J
1290   NEXT I
1300 K=-1
1310 FOR I=0 TO 255
1320   IF M(I)=0 THEN GOTO 1350 ELSE K=K+1
1330     IF M(I)=1 THEN J=2 ELSE J=M(I)
1340     C(K)=C(I)/M(I) : D(K)=SQR(D(I)/(J-1)) : M(K)=I-XMIN
1350   NEXT I
1360 OPEN "O",#2,"B:B/G.DAT"
1370 FOR I=0 TO K
1380   PRINT #2,M(I) : PRINT #2,C(I) : PRINT #2,D(I)
1390   NEXT I
1400 CLOSE
1410 KILL "B:0.AVG"
1420 NAME "B:B/G.DAT" AS "B:0.AVG"
1430 FOR I=1 TO NN
1440   ON I GOTO 1450,1470,1490
1450     KILL "B:00.AVG"
1460     GOTO 1500
1470     KILL "B:000.AVG"
1480     GOTO 1500
1490     KILL "B:0000.AVG"
1500   NEXT I
1510 N=0 : AN(N)="0"
1520 FOR I=1 TO MQ
1530   IF (VAL(AN(I))=0) GOTO 1550
1540     N=N+1 : AN(N)=AN(I)
1550   NEXT I
1560 MQ=N
1570 ERASE X,Y,Z,L,M,C,D
1580   PRINT "B/G - average completed and filed." : PRINT
1590   ON ERROR GOTO 0
1600   CLOSE : GOTO 150
1610 IF (ERL=1160 AND ERR=53) THEN RESUME 1230
1620   IF NERR>10 THEN END ELSE NERR=NERR+1
1630   LPRINT "B/G ERROR Code #";ERR;" in Line #";ERL
1640   RESUME NEXT
```

A.8 MINUS.BAS

```
1000 REM Updated 210186
1010 DIM X(2,256),Y(2,256),Z(2,256),M(2),W(2)
1020 A="*****" : PRINT A : PRINT "MINUS" : PRINT A : PRINT
1030 ON ERROR GOTO 1350
1040 FOR LT=0 TO MQ
1050   IF LT=0 THEN I=0 ELSE I=1
1060   OPEN "I",#1,"B:"+AN(LT)+".AVG" : K=-1 : U=0!
1070   K=K+1 : INPUT #1,X(I,K) : INPUT #1,Y(I,K)
1080   INPUT #1,P : Z(I,K)=P^2
1090   IF (X(I,K)>20 OR Y(I,K)<U) GOTO 1110
1100     U=Y(I,K) : W(I)=X(I,K)
1110   IF EOF(1)<>0 THEN CLOSE ELSE GOTO 1070
1120   M(I)=K
1130   IF (LT=0) GOTO 1300
1140     J=0 : ICR=0 : ICO=0
1150     IF J=0 THEN A=".RET" ELSE A=".CRP"
1160     OPEN "O",#2,"B:"+AN(LT)+A : JCO=-1 : JAL=0 : JVL=X(0,M(0))-ICO
1170     FOR K=0 TO M(I)
1180       JMV=X(I,K)-ICR
1190       IF (JMV>JVL) GOTO 1270
1200         FOR JCR=JAL TO M(0)
1210           JLE=X(0,JCR)-ICO
1220           IF JMV<>JLE THEN GOTO 1250 ELSE JCO=JCO+1
1230             U=Y(I,K)-Y(0,JCR) : V=SQR(Z(I,K)+Z(0,JCR)) : P=JMV
1240             PRINT #2,P : PRINT #2,U : PRINT #2,V : JAL=JCR+1 : GOTO 1260
1250           NEXT JCR
1260       NEXT K
1270     PRINT : PRINT TAB(10);AN(LT)+A;" has";JCO+1;"data-sets."
1280     CLOSE : J=J+1 : ICR=W(I) : ICO=W(0)
1290     IF (J=1) GOTO 1150
1300   NEXT LT
1310 ERASE X,Y,Z,M,W
1320   ON ERROR GOTO 0 : PRINT : PRINT
1330   CLOSE : GOTO 150
1340 REM Error subroutine to print positions where errors occur.
1350 IF NERR>10 THEN END ELSE NERR=NERR+1
1360   LPRINT "MINUS ERROR Code #";ERR;"in Loop #";LT;"at Line =";ERL
1370   RESUME NEXT
```

A.9 SUDATA.BAS

```
1000 REM Updated 070386
1010 DIM XN(3),B(3),C(3)
1020 A="************" : AA="SUBJECT DATA" : PRINT
1030   PRINT A : PRINT AA : PRINT A : PRINT
1040   LPRINT SPC(27)CHR$(14);AA : LPRINT : LPRINT
1050 OPEN "I",#1,"B:SUBJECT.DAT" : LPRINT : LPRINT
1060   INPUT #1,AN : LPRINT SPC(30)"Name : ";AN : LPRINT
1070   INPUT #1,A : LPRINT SPC(30)" Age : ";A : LPRINT
1080   INPUT #1,A : LPRINT SPC(30)" Eye : ";A : LPRINT
1090   INPUT #1,A : LPRINT SPC(30)"Date : ";A : LPRINT : LPRINT
1100 INPUT #1,XN(0) : INPUT #1,XN(1) : INPUT #1,XN(2)
1110   INPUT #1,B(0) : INPUT #1,B(1) : INPUT #1,B(2)
1120   C(0)=B(0)/XN(0) : C(1)=B(1)/XN(1) : C(2)=B(2)/XN(2)
1130 INPUT #1,QQ : QQ=250!+QQ : LPRINT SPC(18)"FLUORESCEIN injected";
1140    GOSUB 1370 : LPRINT : LPRINT
1150 LPRINT SPC(10)"Scaling by Lund-Andersen's F-numbers :"
1160   QQ=.13812*XN(0) : GOSUB 1330 : GOSUB 1390 : PP=QQ
1170   QQ=.15731*XN(1) : GOSUB 1340 : GOSUB 1390 : PP=PP+QQ
1180   QQ=.13908*XN(2) : GOSUB 1350 : GOSUB 1390 : QQ=PP+QQ
1190   GOSUB 1360 : GOSUB 1390 : LPRINT : LPRINT
1200 LPRINT SPC(10)"Ultra-sound results :"
1210   QQ=B(0) : GOSUB 1330 : GOSUB 1390 : PP=QQ
1220   QQ=B(1) : GOSUB 1340 : GOSUB 1390 : PP=PP+QQ
1230   QQ=B(2) : GOSUB 1350 : GOSUB 1390 : QQ=PP+QQ
1240   GOSUB 1360 : GOSUB 1390 : LPRINT : LPRINT
1250 LPRINT SPC(10)"Recalculated F-numbers :"
```

```
1260   QQ=C(0)/PSCALE : GOSUB 1330 : LPRINT
1270   QQ=C(1)/PSCALE : GOSUB 1340 : LPRINT
1280   QQ=C(2)/PSCALE : GOSUB 1350 : LPRINT : LPRINT : LPRINT
1290 LPRINT SPC(10)"REMARKS & COMMENTS :";CHR$(27);CHR$(58)
1300 IF (EOF(1)<>0) GOTO 1320
1310   INPUT #1,A : LPRINT SPC(20)A : GOTO 1300
1320 CLOSE : LPRINT CHR$(18);CHR$(12); : GOTO 1400
1330 LPRINT SPC(22)"Vitreous Chamber"; : GOTO 1370
1340   LPRINT SPC(34)"Lens"; : GOTO 1370
1350   LPRINT SPC(23)"Aqueous Chamber"; : GOTO 1370
1360   LPRINT SPC(41)"---------" : LPRINT SPC(26)"Axial Length";
1370 LPRINT " ";CHR$(247);" ";
1380   LPRINT USING "####.###";QQ; : RETURN
1390 LPRINT " mm" : RETURN
1400 DEF FNSL(J,P)=C(J+1)*(P-XN(J))+B(J)
1410 ON ERROR GOTO 2770
1420 XN(1)=XN(1)+XN(0) : XN(2)=XN(2)+XN(1)
1430   B(1)=B(1)+B(0) : B(2)=B(2)+B(1)
1440   XMV=XN(0)/2 : XMA=(XN(1)+XN(2))/2 : XL=XMV-10!
1450   YL=XMV+10! : PRINT : PRINT : KEY=0 : I=MQ+1 : KZ=0
1460 DIM SX(20,I),SY(20,I),SZ(20,I)
1470 LPRINT : LPRINT : LPRINT : LPRINT : LPRINT
1480 ON KEY GOTO 1500,1510,2560
1500   AF=".RET" : GOTO 1520
1510   AF=".CRP"
1520 FOR I=KZ TO MQ
1530   SY(0,I)=-10! : SY(2,I)=1E+22 : SY(3,I)=-10! : IM=9
1540   SY(4,I)=SY(2,I) : SY(6,I)=-10! : ZP=255! : YP=ZP
1550   PP=0! : PT=0! : PD=0! : PZ=0! : QT=0! : QD=0! : QZ=0!
1560   JJ=-1 : PRINT TAB(10);"Reading  ";AN(I);AF;" .....";
1570   OPEN "I",#3,"B:"+AN(I)+AF
1580   INPUT #3,PX : INPUT #3,Q : INPUT #3,R
1590     JJ=JJ+1 : WP=ABS(PX-XMV) : VP=ABS(XMA-PX)
1600     IF (PX<XL OR PX>YL) GOTO 1620
1610       PT=PT+1! : PD=PD+Q : PZ=PZ+R^2
1620     IF (WP>YP) GOTO 1640
1630       SX(1,I)=PX+C(0) : SY(1,I)=Q : SZ(1,I)=R : YP=WP
1640     IF (VP>ZP) GOTO 1660
1650       SX(5,I)=FNSL(1,PX) : SY(5,I)=Q : SZ(5,I)=R : ZP=VP
1660     IF (PX>XMV OR Q<SY(0,I)) GOTO 1680
1670       SX(0,I)=PX+C(0) : SY(0,I)=Q : SZ(0,I)=R
1680     IF (PX<0! OR PX>XN(0) OR Q>SY(2,I)) GOTO 1700
1690       SX(2,I)=PX+C(0) : SY(2,I)=Q : SZ(2,I)=R
1700     IF (PX<XMV OR PX>XN(1) OR Q<SY(3,I)) GOTO 1730
1710       IF PX<=XN(0) THEN SX(3,I)=PX+C(0) ELSE SX(3,I)=FNSL(0,PX)
1720       SY(3,I)=Q : SZ(3,I)=R
1730     IF (PX<XN(1) OR PX>XN(2) OR Q>SY(4,I)) GOTO 1750
1740       SX(4,I)=FNSL(1,PX) : SY(4,I)=Q : SZ(4,I)=R
1750     IF (PX<XMA OR Q<SY(6,I)) GOTO 1770
1760       SX(6,I)=FNSL(1,PX) : SY(6,I)=Q : SZ(6,I)=R
1770     IF PX>XN(0) THEN GOTO 1830 ELSE QQ=C(0)*PX
1780       IF (QQ>3.5 OR QQ<2.5) GOTO 1800
1790         QT=QT+1! : QD=QD+Q : QZ=QZ+R^2
1800       IF (QQ<PP) GOTO 1830
1810         IM=IM+1 : SX(IM,I)=QQ : SY(IM,I)=Q
1820         SZ(IM,I)=R : PP=PP+3!
1830     IF (EOF(3)=0) GOTO 1580
1840   CLOSE : PRINT " done !" : PRINT
1850   IF (PT=0!) GOTO 1900
1860     SX(9,I)=XMV+C(0) : SY(9,I)=PD/PT : SZ(9,I)=PT
1870     IF (PZ<=0!) GOTO 1910
1880       SZ(7,I)=SQR(PZ/PT) : SX(7,I)=PT
1890       SY(7,I)=2!*ABS(SZ(7,I)) : GOTO 1920
1900     SX(9,I)=0! : SY(9,I)=0! : SZ(9,I)=0!
1910     SX(7,I)=0! : SY(7,I)=0! : SZ(7,I)=0!
1920   IF (QT=0!) GOTO 1940
1930     SZ(8,I)=QT : SY(8,I)=QD/QT : SX(8,I)=3! : GOTO 1950
1940   SX(8,I)=0! : SY(8,I)=0! : SZ(8,I)=0!
1950   NEXT I
1960 LPRINT CHR$(18);SPC(20)"Name : ";AM;"  [ SUBJECT DATA -";
       KEY+2;"]"
1970   LPRINT : LPRINT SPC(10)"(";KEY+1;") For ";CHR$(14);
         AF;CHR$(20);
1980   LPRINT " files :";CHR$(27);CHR$(58) : LPRINT
1990 LPRINT SPC(10)"The Choroid-Retinal Peak"; : J=0 : GOSUB 2590
```

```
2000   LPRINT "Mid-Vitreous"; : J=1 : GOSUB 2590
2010   LPRINT "Vitreous Minimum"; : J=2 : GOSUB 2590
2020   IF KEY=0 THEN LPRINT "(Autofluorescence) ";
2030     LPRINT "Lens Peak"; : J=3 : GOSUB 2590
2040   LPRINT "Aqueous Minimum"; : J=4 : GOSUB 2590
2050   LPRINT "Mid-Aqueous"; : J=5 : GOSUB 2590
2060   LPRINT "Corneal Peak"; : J=6 : GOSUB 2590
2070   LPRINT "values closest to the 3-, 6-, 9-mm , etc., are :"
2080     GOSUB 2710
2090     FOR J=10 TO IM
2100       GOSUB 2600
2110       NEXT J
2120   LPRINT CHR$(27);CHR$(58);
2130 IF (KEY=0) GOTO 2260
2140   LPRINT SPC(10)"The Axial Resolution-Ratio are :"
2150     GOSUB 2800 : LPRINT SPC(10)"At 3 mm";
2160     LPRINT SPC(20)"About 3 mm";CHR$(27);CHR$(45);CHR$(0)
2170     FOR I=KZ TO MQ
2180       LPRINT SPC(25); : PD=VAL(AN(I)) : LPRINT USING "###.#";PD;
2190       PD=SY(11,I)/SY(0,I) : PT=SY(8,I)/SY(0,I)
2200       LPRINT SPC(24); : LPRINT USING "###.###";PD;
2210       LPRINT SPC(21); : LPRINT USING "###.###";PT;
2220       IF I<MQ THEN LPRINT ELSE LPRINT CHR$(13);
2230       NEXT I
2240     LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);SPC(72);
2250     LPRINT CHR$(27);CHR$(45);CHR$(0);CHR$(27);CHR$(58)
2260 LPRINT SPC(10)"The Lower Limit of Detection (or Sensitivity) ->"
2270   LPRINT SPC(15)"Average about Mid-Vitreous + 2SD";
2280   LPRINT CHR$(27);CHR$(83);CHR$(1);"rms";CHR$(27);CHR$(84);
         ", are  :"
2290   GOSUB 2800 : LPRINT "Fluorescein Equivalent (ng/ml)";
2300   LPRINT SPC(14)"Number";CHR$(27);CHR$(45);CHR$(0)
2310 FOR I=KZ TO MQ
2320   LPRINT SPC(25); : PT=VAL(AN(I)) : LPRINT USING "###.#";PT;
2330   LPRINT SPC(25); : PT=SY(7,I)+SY(1,KZ) : LPRINT USING
         "####.###";PT;
2340   LPRINT SPC(23); : LPRINT USING "####.###";SX(7,I);
2350   IF I<MQ THEN LPRINT ELSE LPRINT CHR$(13);
2360   NEXT I
2370 LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);SPC(75);
2380 LPRINT CHR$(27);CHR$(45);CHR$(0);CHR$(27);CHR$(58)
2390 LPRINT SPC(10)"The Reproducibility Percentages are :"
2400 GOSUB 2800 : LPRINT SPC(10)"Percent";SPC(10)"About";
2410 LPRINT CHR$(27)CHR$(45);CHR$(0) : JJ=0
2420   FOR J=8 TO 9
2430     FOR I=2 TO MQ
2440       IF (SY(J,I-1)=0!) GOTO 2520
2450         PD=VAL(AN(I)) : PT=VAL(AN(I-1))
2460         IF (PD-PT)>4! THEN GOTO 2520 ELSE JJ=JJ+1
2470           LPRINT SPC(21); : LPRINT USING "###.#";PT;
2480           LPRINT " ~"; : LPRINT USING "###.#";PD;
2490           PD=100!*(SY(J,I)-SY(J,I-1))/SY(J,I-1)
2500           LPRINT SPC(22); : LPRINT USING "####.##";PD;
2510           LPRINT SPC(9); : LPRINT USING "###.#";SX(J,I-1)
2520       NEXT I
2530   IF (J=9 AND JJ=0) THEN LPRINT SPC(35)"No Value can
         be calculated!"
2540   NEXT J
2550 KEY=KEY+1 : KZ=1 : GOTO 1470
2560 ERASE XN,B,C,SX,SY,SZ
2570   LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);CHR$(18);
2580   CLOSE : ON ERROR GOTO 0 : GOTO 150
2590 LPRINT " values are :" : KW=0 : GOSUB 2710
2600 FOR I=KZ TO MQ
2610   LPRINT SPC(25); : PD=VAL(AN(I)) : LPRINT USING "###.#";PD;
2620   LPRINT SPC(20); : LPRINT USING "####.###";SY(J,I);
2630   LPRINT " +/-"; : LPRINT USING "###.###";SZ(J,I);
2640   LPRINT SPC(22); :  LPRINT USING "###.###";SX(J,I);
2650   IF I<>MQ THEN LPRINT
2660   NEXT I
2670 LPRINT CHR$(13);SPC(20)CHR$(27);CHR$(45);CHR$(1);
2680   LPRINT SPC(90)CHR$(27);CHR$(45);CHR$(0)
2690   IF J<10 THEN LPRINT CHR$(27);CHR$(58);SPC(10)"The ";
2700   RETURN
```

```
2710 GOSUB 2800 : LPRINT "Concentration +/- S.E. (ng/ml)";
2720   ON KW GOTO 2740
2730     LPRINT SPC(10)"Distance from Retina (mm)"; : GOTO 2750
2740     LPRINT SPC(10)"Number of points entered";
2750   LPRINT CHR$(27);CHR$(45);CHR$(0)
2760   RETURN
2770 LPRINT "INFO ERROR Code #";ERR;"at Line #";ERL;
2780 IF NERR>10 THEN RESUME 2560 ELSE NERR=NERR+1
2790 RESUME NEXT
2800 LPRINT CHR$(15);SPC(20)CHR$(27);CHR$(45);CHR$(1);
2810   LPRINT "P.I. Time (min)";SPC(10);
2820   RETURN
```

A.10 BLOOD.BAS

```
1000 REM Updated Z00186
1010 A="*****" : PRINT A : PRINT "BLOOD" : PRINT A : PRINT
1020 DIM AF(6),IX(135),IY(135),T(56),Y(56),W(56),X(5)
1030 DIM Z(5),FR(11),C(4,8),HS(4,10),FQ(4,10)
1040 DEF FNCONC(X)=34.17*EXP(-109.339+SQR(11915.9+X/.114441))
1050 DEF FNIP(X)=CINT(.018315*(LOG(X)+109.339)^2-232.586)
1060 ON ERROR GOTO 3620 : M=-1
1070 OPEN "I",#3,"B:SUBJECT.DAT" : INPUT #3,AF(5)
1080 PRINT TAB(10);"Name : ";AF(5) : CLOSE : PRINT : PRINT
1090 FOR K=1 TO MQ
1100   HP=VAL(AN(K))
1110   IF (HP=0!) GOTO 1130
1120     M=M+1 : FR(M)=HP
1130   NEXT K
1140 OPEN "I",#2,"B:PLASMA.DAT" : NUM=-1 : HP=0! : INPUT #2,GU
1150 INPUT #2,GU : B=FNCONC(GU) : INPUT #2,GV : D=FNCONC(GV)
1160 NUM=NUM+1 : INPUT #2,T(NUM) : INPUT #2,GU : INPUT #2,GV
1170   IF T(NUM)>HP THEN HP=T(NUM)
1180   Y(NUM)=FNCONC(GU)-B : W(NUM)=SQR((FNCONC(GV))^2+D^2)
1190   IF EOF(2)<>0 THEN CLOSE ELSE GOTO 1160
1200   IF (HP>100!) GOTO 1220
1210   GU=1! : IY(134)=10 : KC=99 : GOTO 1230
1220 GU=2!/3! : IY(134)=15 : KC=132
1230 FOR K=0 TO KC
1240   IX(K)=1+CINT(K*GU)
1250   NEXT K
1260 FOR K=0 TO 3
1270   C(K,6)=0! : C(K,7)=99
1280   NEXT K
1290 DEF FNFP(J,T)=C(J,0)+C(J,2)*T+C(J,4)*T^2
1300 DEF FNFQ(J,T)=C(J,1)*(T-.25)^2
1310 IX(133)=CINT(FR(M)) : KX=0 : KY=0 : J=0 : GOSUB 3160
1320 AF(J)="A + B*t + C*t^2" : GOSUB 3030 : KK=0 : IY(0)=0
         : GOSUB 3110
1330 FOR K=0 TO NUM
1340   GU=T(K) : GOSUB 3500 : KK=KK+1
1350   NEXT K
1360 GOSUB 2960 : E=1!/48! : D=C(J,4)*E-C(J,0)/4!
1370 GV=C(J,2)/2! : GU=C(J,4)/3! : HP=C(J,3)/4!
1380 FOR K=0 TO M
1390   F=FR(K) : HS(J,K)=D+F*(C(J,0)+GV+F+GU*F^2)
1400   FQ(J,K)=FNFQ(J,F)+HP*F^4+C(J,5)*(E+F^3/3!)^2+(FNFP(J,F))^2/4!
1410   GOSUB 3570
1420   IF HS(J,K)<=0! THEN C(J,7)=HS(J,K)
1430   NEXT K
1440 LPRINT CHR$(12)
1450 FOR K=1 TO KC
1460   HP=FNFP(J,K) : GOSUB 2370
1470   NEXT K
1480 GOSUB 2490 : KX=2 : KY=0 : J=1
         : AF(J)="A + B*log(t) + C*[log(t)]^2"
1490 GOSUB 3160 : GU=C(J,0)-C(J,2) : GOSUB 3030 : KK=0 : GOSUB 3110
1500 FOR K=0 TO NUM
1510   GU=LOG(T(K)) : GOSUB 3500 : KK=KK+1
1520   NEXT K
1530 GOSUB 2960 : E=LOG(2!) : F=E+2! : H=F^2 : HP=F/4! : D=H/4!
1540 B=C(J,2)*HP-C(J,4)*D-C(J,0)/4!
1550 FOR K=0 TO M
1560   E=FR(K) : GU=LOG(E)-1! : HS(J,K)=B+(FNFP(J,GU)+C(J,4))*E
1570   FQ(J,K)=FNFQ(J,E)+C(J,3)*(HP+E*GU)^2+C(J,5)*(E*(GU^2+1!)-D)^2
```

```
1580   GU=GU+1! : FQ(J,K)=FQ(J,K)+(FNFP(J,GU))^2/4! : GOSUB 3570
1590   IF HS(J,K)<=0! THEN C(J,7)=HS(J,K)
1600   NEXT K
1610 LPRINT CHR$(12)
1620 FOR K=1 TO KC
1630   GU=LOG(K) : HP=FNFP(J,GU) : GOSUB 2370
1640   NEXT K
1650 GOSUB 2490 : KX=0 : KY=1 : J=2 : AF(J)="A*exp(B*t+C*t^2)"
1660 GOSUB 3160 : KK=0 : C(J,0)=EXP(C(J,0)) : C(J,1)=C(J,1)*C(J,0)^2
1670 B=.5*C(J,0) : GOSUB 3030 : GOSUB 3110
1680 DEF FNFE(J,T)=B*EXP(C(J,2)*T+C(J,4)*T^2)
1690 FOR K=0 TO NUM
1700   KK=KK+1 : DW=2!*FNFE(J,T(K))-Y(K) : GOSUB 3510
1710   NEXT K
1720 GOSUB 2960 : E=FNFE(J,1!) : H=E/2! : G=FNFE(J,.5) : GU=G+H
1730 GV=C(J,1)*(GU/C(J,0))^2+C(J,3)*(H+G/2!)^2+C(J,5)*(H+G/4!)^2
1740 KY=2 : G=C(J,1)/C(J,0)^2 : H=2!*C(J,4)
1750 D=(G+C(J,3)+C(J,5)*(C(J,2)+H)^2)*E^2
1760 FOR K=0 TO M
1770   L=CINT(FR(K)) : HS(J,K)=GU : FQ(J,K)=GV
1780   FOR N=KY TO L
1790     F=FNFE(J,N) : HS(J,K)=HS(J,K)+E+F : HP=N^2
1800     CO=(G+C(J,3)*HP+C(J,5)*HP^2+(C(J,2)+H*N)^2/4!)*F^2
1810     FQ(J,K)=FQ(J,K)+D+CO : D=CO : E=F
1820     NEXT N
1830   GOSUB 3570 : KY=L+1 : GU=HS(J,K) : GV=FQ(J,K)
1840   IF GU<=0! THEN C(J,7)=GU
1850   NEXT K
1860 LPRINT CHR$(12)
1870 FOR K=1 TO KC
1880   HP=2!*FNFE(J,K) : GOSUB 2370
1890   NEXT K
1900 GOSUB 2490 : KX=1 : KY=0 : J=3 : AF(J)="A + B/t + C/t^2"
       : GOSUB 3160
1910 GU=C(J,0)+2!*C(J,2)+3!*C(J,4) : KK=0 : GOSUB 3030 : GOSUB 3110
1920 FOR K=0 TO NUM
1930   KK=KK+1 : GU=1!/T(K) : GOSUB 3500
1940   NEXT K
1950 GOSUB 2960 : B=LOG(2!)+.5 : HP=C(J,2)+B-C(J,0)/4!+3!*C(J,4)
       : PRINT
1960 FOR K=0 TO M
1970   E=FR(K) : GU=LOG(E) : GV=1!/E
         : HS(J,K)=HP+C(J,0)+E-C(J,2)+GU-C(J,4)+GV
1980   FQ(J,K)=FNFQ(J,E)+C(J,3)*(GU+B)^2+C(J,5)*(3!-GV)^2
1990   FQ(J,K)=FQ(J,K)+.25*(FNFP(J,GV))^2 : GOSUB 3570
2000   IF HS(J,K)<=0! THEN C(J,7)=HS(J,K)
2010   NEXT K
2020 LPRINT CHR$(12)
2030 FOR K=1 TO KC
2040   GU=1!/K : HP=FNFP(J,GU) : GOSUB 2370
2050   NEXT K
2060 GOSUB 2490 : GU=1D+22 : LPRINT CHR$(18) : PRINT : PRINT
2070 PRINT "The Reduced CHI^2 of the FITs are;" : PRINT
2080 FOR J=0 TO 3
2090   PRINT : PRINT "(";J+1;")",C(J,6);"     ";SGN(C(J,7))
2100   IF (C(J,7)<=0! OR C(J,6)=>GU) GOTO 2120
2110     GU=C(J,6) : K=J
2120   NEXT J
2130 PRINT : PRINT "The BEST fit was " : PRINT
       : PRINT "  f = ";AF(K) : PRINT
2140 LPRINT SPC(20)"NAME : ";AF(5);"  (Plasma Integration)" : LPRINT
2150 LPRINT : LPRINT : LPRINT SPC(10)CHR$(27);CHR$(45);CHR$(1);
       CHR$(14);
2160 LPRINT "PLASMA FLUORESCEIN ";CHR$(20);"Results";
       CHR$(27);CHR$(45);CHR$(0)
2170 LPRINT : LPRINT : LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);
       "Time (min)";
2180 LPRINT SPC(10)"Concentration (ng/ml)";CHR$(27);CHR$(45);CHR$(0)
2190 LPRINT CHR$(27);CHR$(58); : KP=11
2200 FOR J=0 TO NUM
2210   DW=T(J) : BW=Y(J) : CW=W(J) : LPRINT SPC(27); : GOSUB 2420
       : LPRINT
2220   NEXT J
2230 LPRINT : LPRINT : LPRINT SPC(10)CHR$(14);"The BEST fit was  =>"
2240 LPRINT : LPRINT SPC(12)CHR$(14);"f = ";AF(K);"."
2250 OPEN "O",#3,"B:PLASMA.FIT" : PRINT #3,K
2260 FOR J=0 TO 5 STEP 2
2270   PRINT #3,C(K,J) : GU=SQR(ABS(C(K,J+1))) : PRINT #3,GU
2280   NEXT J
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```
2290 FOR J=0 TO M
2300   PRINT #3,FR(J) : PRINT #3,HS(K,J) : PRINT #3,FQ(K,J)
2310   NEXT J
2320 ERASE AF,IX,IY,T,X,Y,Z,W,FR,C,HS,FQ
2330 ON ERROR GOTO 0 : LPRINT CHR$(27);CHR$(58);
2340 CLOSE
2350 KILL "B:PLASMA.DAT"
2360 GOTO 150
2370 IF HP>100! THEN GOTO 2400 ELSE IY(K)=0
2380   IF (K<IX(133) AND HP<0!) THEN C(J,7)=HP
2390   RETURN
2400 IY(K)=FNIP(HP)
2410 RETURN
2420 LPRINT USING "###.##";DW;
2430 LPRINT SPC(KP);
2450 LPRINT " +/-";
2460 LPRINT USING "##.#####^^^^";CW;
2470 RETURN
2480 REM Subroutine for printer-plotting.
2490 GOSUB 3030 : LPRINT CHR$(15);CHR$(27);CHR$(49);
2500 LPRINT SPC(20)"Vertical (LOG) Scale = X 10 ng/ml / 10 div.";
2510 LPRINT SPC(10)"Horizontal Scale =";IY(134);"min / 10 div."
2520 FOR K=0 TO 50
2530   KY=50-K : HP=KY/5! : N=0 : L=-1 : LPRINT CHR$(10);
         CHR$(13);CHR$(9);
2540   IF ((HP-FIX(HP))=0) GOTO 2560
2550     LPRINT CHR$(9);CHR$(124); : GOTO 2600
2560   IF (K=15 OR K=25 OR K=35 OR K=45 OR K=5) GOTO 2590
2570     IF K=0 THEN LPRINT "ng/ml   +"; ELSE LPRINT CHR$(9);"+";
2580     GOTO 2600
2590   LPRINT "  10";CHR$(27);CHR$(83);CHR$(0);2*KY\10;
         CHR$(27);CHR$(84);" +";
2600   FOR KZ=0 TO NUM
2610     IF FNIP(Y(KZ))=KY THEN L=KZ
2620     NEXT KZ
2630   IF (L=-1) GOTO 2710
2640     FOR KZ=0 TO NUM
2650       IF FNIP(Y(KZ))<>KY THEN GOTO 2690 ELSE KX=1+CINT(T(KZ))
2660         IF KX=0 THEN KX=1 ELSE KX=KX-N
2670         IF KX<>0 THEN LPRINT SPC(KX-1)"o";
2680         N=CINT(T(KZ))+1
2690       NEXT KZ
2700     N=0 : LPRINT CHR$(13);CHR$(9);CHR$(9);
2710   FOR KZ=0 TO KC
2720     IF IY(KZ)<>KY THEN GOTO 2760 ELSE KX=IX(KZ)
2730       IF KX=0 THEN KX=1 ELSE KX=KX-N
2740       IF KX<>0 THEN LPRINT SPC(KX-1)CHR$(249);
2750       N=IX(KZ)
2760     NEXT KZ
2770   NEXT K
2780 LPRINT CHR$(10);CHR$(13);CHR$(9);CHR$(9);" ";
2790 FOR K=0 TO 9
2800   LPRINT "+";
2810   FOR N=0 TO 8
2820     LPRINT "-";
2830     NEXT N
2840   NEXT K
2850 LPRINT "+" : KY=2*IY(134) : LPRINT CHR$(9);CHR$(9);0;
2860 FOR K=1 TO 4
2870   LPRINT SPC(16)KY*K;
2880   NEXT K
2890 LPRINT SPC(17)"min";CHR$(27);CHR$(58);
       CHR$(27);CHR$(65);CHR$(12);
2900 LPRINT CHR$(27);CHR$(50) : LPRINT : LPRINT SPC(41)"CHI^2 = ";
2910 CW=C(J,6) : GOSUB 2460 : LPRINT
2920 IF KK>3 THEN C(J,6)=C(J,6)/(KK-3)
2930 LPRINT SPC(33)"Reduced CHI^2 = "; : CW=C(J,6)
2940 GOSUB 2460 : LPRINT CHR$(12)
2950 RETURN
2960 LPRINT CHR$(18) : LPRINT : LPRINT SPC(10)CHR$(27);
       CHR$(45);CHR$(1);
2970 LPRINT "RESULTS of INTEGRATION";CHR$(27);CHR$(45);CHR$(0)
2980 LPRINT CHR$(27);CHR$(58) : LPRINT SPC(15)CHR$(27);
       CHR$(45);CHR$(1);
2990 LPRINT "For 0 < t < T  min.     AREA +/- S.E. (min-ng/ml)";
3000 LPRINT SPC(6)"Percent (%)";CHR$(27);CHR$(45);CHR$(0)
3010 RETURN
3020 REM Subroutine for printing curve-fitting coefficients.
3030 LPRINT CHR$(18);CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);
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3040 LPRINT SPC(20)"NAME : ";AF(5);"  (Plasma Integration)" : LPRINT
3050 LPRINT : LPRINT SPC(10)"(";RIGHT$(STR$(J+1),1);
        ") Fitting to f = ";AF(J)
3060 LPRINT SPC(25)"A = "; : BW=C(J,0) : CW=SQR(ABS(C(J,1)))
        : GOSUB 2440
3070 LPRINT : LPRINT SPC(25)"B = "; : BW=C(J,2) : CW=SQR(ABS(C(J,3)))
3080 GOSUB 2440 : LPRINT : LPRINT SPC(25)"C = "; : BW=C(J,4)
3090 CW=SQR(ABS(C(J,5))) : GOSUB 2440 : LPRINT : LPRINT : LPRINT
3100 RETURN
3110 LPRINT CHR$(27);CHR$(58) : LPRINT SPC(20)CHR$(27);
        CHR$(45);CHR$(1);
3120 LPRINT "Time (min)  Data (ng/ml)  (Fit-Data)   Percent (%)";
3130 LPRINT CHR$(27);CHR$(45);CHR$(0);CHR$(15)
3140 RETURN
3150 REM Subroutine for curve-fitting to order 2.
3160 FOR K=0 TO 4
3170   X(K)=0! : Z(K)=0!
3180   NEXT K
3190 FOR K=0 TO NUM
3200   ON KX GOTO 3220,3230
3210     HP=T(K) : GOTO 3240
3220     HP=1!/T(K) : GOTO 3240
3230     HP=LOG(T(K))
3240   ON KY GOTO 3260
3250     GV=Y(K) : GU=1!/W(K)^2 : GOTO 3280
3260     IF (Y(K)<=0!) GOTO 3350
3270       GV=LOG(Y(K)) : GU=(Y(K)/W(K))^2
3280   Z(4)=GU : Z(3)=GV*GU
3290   FOR N=0 TO 4
3300     X(N)=X(N)-Z(4) : Z(4)=Z(4)*HP
3310     NEXT N
3320   FOR N=0 TO 2
3330     Z(N)=Z(N)-Z(3) : Z(3)=Z(3)*HP
3340     NEXT N
3350   NEXT K
3360 HP=X(1)/X(0) : GU=X(2)/X(1) : GV=X(3)/X(2) : B=X(4)/X(3)
        : G=GU-GV
3370 D=1!/GV-1!/GU : E=1!/HP-1!/GU : Z(3)=G*D-(B-GV)*E
3380 F=Z(0)/X(1)-Z(1)/X(2)
        : C(J,4)=(F*D-(Z(2)/X(3)-Z(1)/X(2))*E)/Z(3)
3390 C(J,0)=(F-C(J,4)*G)/E : C(J,2)=Z(0)/X(1)-C(J,0)/HP-C(J,4)*GU
        : F=B-GU
3400 H=(B*(GU-HP)/GU+HP-GV-G*HP/GV)^2 : CO=B-GV
3410 C(J,1)=CO^2/X(0)-2!*HP^2*CO*F/X(2)
3420 C(J,1)=C(J,1)-(HP*F)^2/X(2)-2!*HP*G*CO/(X(0)*GV)+(HP*G)^2*B/X(3)
3430 C(J,1)=(C(J,1)-2!*HP^2*F*G/X(2))/H : CO=X(4)/X(2)-X(2)/X(0)
        : G=GV-HP
3440 C(J,3)=(F/GU)^2/X(0)-CO^2/(X(3)*GV)-B*G^2/X(3)-2!*
        F*CO*HP/(GU*X(3))
3450 C(J,3)=(C(J,3)-2!*G*F*HP/X(3)-2!*CO*G/X(3))/H : F=GV-GU
        : CO=GU-HP
3460 C(J,5)=(F*X(1)/X(3))^2/X(0)+G^2/(X(3)*GV)+B*(CO/GU)^2/X(3)
3470 C(J,5)=(C(J,5)-2!*(G-CO)*F*HP*X(1)/X(3)^2-2!*G*CO/(GU*X(3)))/H
3480 RETURN
3490 REM Subroutine for printing deviations.
3500 DW=FNFP(J,GU)-Y(K)
3510 LPRINT SPC(33); : LPRINT USING "###.##";T(K); : LPRINT SPC(8);
3520 CW=Y(K) : GOSUB 2460 : LPRINT SPC(8); : CW=DW : GOSUB 2460
3530 LPRINT SPC(7); : CW=100!*DW/Y(K) : GOSUB 2460
3540 LPRINT : C(J,6)=C(J,6)+(DW/W(K))^2
3550 RETURN
3560 REM Subroutine for printing result of integration of fit.
3570 FQ(J,K)=SQR(ABS(FQ(J,K))) : LPRINT SPC(18)"T = "; : KP=8
        : DW=FR(K)
3580 BW=HS(J,K) : CW=FQ(J,K) : GOSUB 2420 : LPRINT CHR$(15);" =>   ";
3590 CW=100!*FQ(J,K)/HS(J,K) : GOSUB 2460 : LPRINT CHR$(27);CHR$(58)
3600 RETURN
3610 REM Error subroutine
3620 IF NERR>10 THEN END ELSE NERR=NERR+1
3630 LPRINT "BLOOD ERROR Code #";ERR;"at Line #";ERL;
3640 RESUME NEXT
```

A.11 C/VAZ.BAS

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1000 REM Updated 120386
1010 DIM AE(2),N(2),R(256),S(256),U(256),T(10),Q(6),ZH(2)
1020 DIM X(2,256),Y(2,256),Z(2,256),W(2),TG(2),O(2),P(2),US(3)
1030 A="*****" : PRINT : PRINT A : PRINT "C/VAZ" : PRINT A
1040 ON ERROR GOTO 3460
1050 OPEN "I",#3,"B:SUBJECT.DAT" : INPUT #3,AW
        : PRINT TAB(10);"Name : ";AW
1060   INPUT #3,A : INPUT #3,A : INPUT #3,A
1070   INPUT #3,Q(0) : INPUT #3,Q(1) : INPUT #3,Q(2)
1080   INPUT #3,US(0) : INPUT #3,US(1) : INPUT #3,US(2)
1090   INPUT #3,WW : Q(5)=250!*WW : SS=US(0)/Q(0)
1100 CLOSE : NC=1 : Q(3)=(US(0)+US(1)+US(2))/2!
1110 KP=0 : NC=NC+1 : J=-1 : KC=0
1120 AE(0)=AN(1) : OO=VAL(AE(0)) : AE(1)=AN(NC) : PP=VAL(AE(1))
1130   OPEN "I",#2,"B:PLASMA.FIT" : INPUT #2,I
1140   FOR I=0 TO 5
1150     INPUT #2,CV
1160     NEXT I
1170   INPUT #2,YY : INPUT #2,CW : INPUT #2,CV
1180   IF (YY=OO OR YY=PP) THEN J=J+1 ELSE GOTO 1170
1190     O(J)=60!*CW : CV=60!*CV : P(J)=CV^2
1200     IF J=1 THEN CLOSE ELSE GOTO 1170
1210 LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);
1220   GOSUB 1910 : PP=Q(3) : PRINT
1230   LPRINT SPC(8)"Centre of Retinal Curvature";
1240   GOSUB 1950 : LPRINT : PRINT
1250   LPRINT SPC(19)"FLUORESCEIN Used =";Q(5);" mg ."
1260   LPRINT : LPRINT : LPRINT
1270 LPRINT SPC(5)CHR$(14);"(";RIGHT$(STR$(KC+1),1);
        ") ALIGNMENT by ";
1280   IF KC=0 THEN LPRINT "RETINA" ELSE LPRINT "C/R PEAK"
1290   IF KC=0 THEN AP=".RET" ELSE AP=".CRP"
1300 FOR I=0 TO 1
1310   LPRINT : LPRINT : A="B:"+AE(I)+AP : OPEN "I",#1,A
1320   L=-1 : TT=0! : RR=1E-22 : XX=RR : PRINT TAB(10);
          "Reading ";A : PRINT
1330   L=L+1 : INPUT #1,R(L) : X(I,L)=R(L) : INPUT #1,S(L)
1340     Y(I,L)=S(L) : INPUT #1,YY : U(L)=YY^2 : Z(I,L)=U(L)
1350     OO=SS*R(L) : QQ=ABS(OO-3!) : PP=ABS(OO)
1360     IF (QQ>RR) GOTO 1380
1370       RR=QQ : WW=S(L)
1380     IF (PP>XX) GOTO 1400
1390       XX=PP : W(I)=S(L) : TG(I)=U(L)
1400     IF (OO>3! OR S(L)<TT) GOTO 1420
1410       TT=S(L) : ZH(I)=R(L)
1420     IF (EOF(1)=0) GOTO 1330
1430   CLOSE : LPRINT CHR$(18) : LPRINT SPC(20)"File : ";
1440   LPRINT CHR$(14);AE(I)+AP;CHR$(20);" has";L+1;"data-sets."
1450   LPRINT : N(I)=L : KEY=0 : GOSUB 2810
1460   NEXT I
1470 QQ=W(0)/WW : LPRINT CHR$(18) : LPRINT SPC(5)CHR$(14);
1480   LPRINT "C/R CORRECTION CONDITION";CHR$(20);" =";
1490   GOSUB 2020 : PP=1! : QQ=0! : WW=1! : GOTO 1570
1500 WW=W(1)/W(0) : XX=TG(1)/W(1)^2+TG(0)/W(0)^2 : TT=0!
1510 FOR K=0 TO N(0)
1520   YY=Y(0,K) : Y(0,K)=YY*WW
1530   IF (SS*X(0,K)>3! OR Y(0,K)<TT) GOTO 1550
1540     TT=Y(0,K) : ZH(0)=X(0,K)
1550   NEXT K
1560   QQ=WW*SQR(XX) : PP=WW
1570 GOSUB 1910 : LPRINT SPC(6)CHR$(14);
1580   LPRINT "For a C/R RATIO";CHR$(20);" =";
1590   GOSUB 2000 : LPRINT CHR$(18)
1600   IF KC=0 THEN GOSUB 2100 ELSE GOSUB 2050
1610 LPRINT SPC(10)CHR$(14);AE(1);CHR$(20);"-minute minus ";
1620   LPRINT CHR$(14);AE(0);CHR$(20);"-minute scan leaves";
1630   LPRINT L+1;"data-sets." : LPRINT
1640 I=1 : GOSUB 2210 : J5=0
1650 FOR K=0 TO L
1660   IF (R(K)>Q(3)) GOTO 1690
1670   IF (R(K)>0! AND S(K)>0!) THEN J5=J5+1
1680   NEXT K
1690 IF (J5>11) GOTO 1720
1700   LPRINT CHR$(18);SPC(16)"Only";J5;
          "data-sets => no file was created."
1710   GOTO 1840
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1720 IF WW=1! THEN K=0 ELSE K=1
1730   KEY=2*KC+1 : AA=".CV"+RIGHT$(STR$(K+KEY),1)
1740   A="B:"+AE(I)+AA
1750   OPEN "O",#3,A
1760 FOR K=0 TO L
1770   IF (R(K)>Q(3)) GOTO 1810
1780     IF (R(K)<0! OR S(K)<=0! OR U(K)<=0!) GOTO 1800
1790       QQ=SQR(U(K)) : PRINT #3,R(K) : PRINT #3,S(K) : PRINT #3,QQ
1800   NEXT K
1810 CLOSE : LPRINT CHR$(18);SPC(10)JS;"data-sets were saved in ";
1820   LPRINT CHR$(14);A : LPRINT : LPRINT : LPRINT
1830   LPRINT SPC(25)"Deleted : Yes [  ]   No [  ]"
1840   IF WW=1! THEN GOTO 1500 ELSE KC=KC+1
1850   IF (KC=1) GOTO 1210
1860 IF (NC<MQ) GOTO 1110
1870   ERASE AE,N,R,S,U,T,Q,ZH,X,Y,Z,W,TG,O,P,US
1880   LPRINT CHR$(27);CHR$(58);CHR$(27);CHR$(65);CHR$(12);
1890   ON ERROR GOTO 0 : LPRINT CHR$(27);CHR$(50)
1900   CLOSE : GOTO 150
1910 KP=KP+1
1920   LPRINT CHR$(12);CHR$(18);SPC(25)"NAME : ";AW;
         "  [ C/VAZ -";KP;"]"
1930   LPRINT : LPRINT
1940   RETURN
1950 LPRINT " ";CHR$(247);" ";
1960   LPRINT USING "##.######";PP;
1970   LPRINT " mm from the RETINA."
1980   RETURN
1990 LPRINT SPC(40); : LPRINT USING "##.#####";QQ; : LPRINT SPC(20);
2000   IF PP=-2.2E+22 THEN GOSUB 3360 ELSE LPRINT USING
         "##.#####^^^^";PP;
2010   LPRINT " +/-";
2020   IF QQ=-2.2E+22 THEN GOSUB 3360 ELSE LPRINT USING
         "##.#####^^^^";QQ
2030   RETURN
2040 REM Subroutine to (re-order and) subtract data-sets.
2050 FOR J=0 TO 1
2060   FOR K=0 TO N(J)
2070     X(J,K)=X(J,K)-ZH(J)
2080     NEXT K
2090   NEXT J
2100 L=-1 : M=0
2110 FOR K=0 TO N(1)
2120   IF (X(1,K)>X(0,N(0))) GOTO 2190
2130     FOR J=M TO N(0)
2140       IF X(1,K)<>X(0,J) THEN GOTO 2170 ELSE L=L+1
2150       S(L)=Y(1,K)-Y(0,J) : R(L)=X(1,K) : U(L)=Z(1,K)+Z(0,J)
2160       M=J+1 : GOTO 2180
2170       NEXT J
2180   NEXT K
2190 RETURN
2200 REM Subroutine for printer-plot.
2210 LPRINT CHR$(15) : LPRINT CHR$(27);CHR$(49);
2220   LPRINT SPC(25)"Vertical (LOG) Scale = X 10 / 10 div.";
2230   LPRINT SPC(10)"Horizontal Scale = 1 mm / 10 div.";
2240 PJ=0! : RR=Q(0)+Q(1) : QQ=RR+Q(2)
2250 FOR K=0 TO L
2260   IF (R(K)>Q(0)) GOTO 2280
2270     R(K)=SS*R(K) : YY=R(K) : GOTO 2330
2280   IF (R(K)>RR) GOTO 2300
2290     R(K)=US(1)*(R(K)-Q(0))/Q(1)+YY : QQ=R(K) : GOTO 2340
2300   IF (R(K)>QQ) GOTO 2320
2310     R(K)=US(2)*(R(K)-RR)/Q(2)+QQ : TT=R(K) : GOTO 2340
2320   R(K)=PSCALE*(R(K)-QQ)+TT
2330   IF (R(K)<Q(3) AND S(K)>PJ) THEN PJ=S(K)
2340   NEXT K
2350 JY=0
2360   IF PJ>.8364 THEN JY=CINT(.018315*(LOG(PJ)+109.339)^2-212.586)
2370 FOR K=0 TO 30
2380   J=30-K : YY=J/5! : M=0 : LPRINT CHR$(10);CHR$(13);CHR$(9);
2390   IF ((YY-FIX(YY))=0!) GOTO 2410
2400     LPRINT CHR$(9);CHR$(124); : GOTO 2460
2410   IF (K=5 OR K=15 OR K=25) GOTO 2440
2420     IF K=0 THEN LPRINT "ng/ml   +"; ELSE LPRINT CHR$(9);"+";
2430     GOTO 2460
2440   LPRINT "  10";CHR$(27);CHR$(83);CHR$(0);INT(J/10);
2450     LPRINT CHR$(27);CHR$(84);" +";
2460   IF (J>JY) GOTO 2570
```

```
2470   FOR J5=0 TO L
2480     KW=0
2490       IF S(J5)>.8364 THEN
               KW=CINT(.018315*(LOG(S(J5))+109.339)^2-212.586)
2500     IF KW<>J THEN GOTO 2560 ELSE KL=CINT(101*R(J5))+1
2510       IF (KL>110 OR KL<0) GOTO 2560
2520         IF KL=0 THEN KR=1 ELSE KR=KL-M
2530         IF (KR=0) GOTO 2550
2540           LPRINT SPC(KR-1)CHR$(249);
2550         M=KL
2560     NEXT J5
2570   NEXT K
2580 OO=1E+22 : RR=0! : QQ=OO : TT=RR : PP=11! : YY=0! : LPRINT
2590 FOR K=0 TO L
2600   IF (R(K)>PP) GOTO 2670
2610   IF (R(K)<YY) GOTO 2660
2620     IF S(K)>RR THEN RR=S(K)
2630     IF S(K)<OO THEN OO=S(K)
2640     IF R(K)>TT THEN TT=R(K)
2650     IF R(K)<QQ THEN QQ=R(K)
2660   NEXT K
2670 PP=0! : LPRINT CHR$(9);CHR$(9);" ";
2680 FOR K=0 TO 110
2690   IF (K/10<>PP) GOTO 2710
2700     LPRINT "+"; : PP=PP+1! : GOTO 2720
2710   LPRINT "-";
2720   NEXT K
2730 LPRINT CHR$(9);CHR$(9);" R";SPC(19)"2";SPC(19)"4";
2740   LPRINT SPC(19)"6";SPC(19)"8";SPC(18)"10";SPC(8)"mm" : KEY=1
2750 LPRINT : PP=QQ : LPRINT SPC(51)"LEFTMOST point"; : GOSUB 1950
2760   LPRINT : PP=TT : LPRINT SPC(50)"RIGHTMOST point"; : GOSUB 1950
2770 LPRINT : PP=RR : LPRINT SPC(44)"MAXIMUM"; : GOSUB 3420
2780   LPRINT : PP=OO : LPRINT SPC(44)"MINIMUM";
2790   GOSUB 3420 : LPRINT : LPRINT : LPRINT
2800 LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);CHR$(18)
2810 KW=0 : KR=0 : TT=1E-22 : GOSUB 3380
2820   KK=-99 : KQ=KK : J5=KK : KT=KK : KL=KT
2830 FOR K=0 TO L
2840   IF KEY=1 THEN OO=R(K) ELSE OO=SS*R(K)
2850   IF OO>6.5 THEN GOTO 3010 ELSE QQ=ABS(31-OO)
2860     IF (QQ>TT) GOTO 2880
2870       TT=QQ : KT=K
2880     IF (S(K)<=0!) GOTO 3000
2890       IF OO<2.5 THEN GOTO 2970 ELSE KW=KW+1
2900         IF KW=1 THEN J5=K ELSE KL=K
2910         PP=(Q(3)-OO)^2 : T(3)=S(K)*PP
                   : XX=1.59293E-03*OO^2+.0381543
2920         PP=4!*(Q(4)+XX)/PP : T(5)=(U(K)/S(K)^2-PP)*T(3)^2
2930         IF (KW=1) GOTO 2960
2940           PP=T(2)+T(3) : QQ=OO-T(4) : RR=PP*QQ/2! : T(0)=T(0)+RR
2950           T(1)=T(1)+((T(5)+T(6))/PP^2+(T(9)+XX)/QQ^2)*RR^2
2960           T(4)=OO : T(6)=T(5) : T(2)=T(3) : T(9)=XX
2970       IF (OO<2! OR OO>4!) GOTO 3000
2980         T(7)=T(7)+S(K) : T(8)=T(8)+U(K) : KR=KR+1
2990         IF KR=1 THEN KK=K ELSE KQ=K
3000   NEXT K
3010 FOR K=2 TO 6
3020   T(K)=-9.9
3030   NEXT K
3040 IF KEY=1 THEN PP=1! ELSE PP=SS
3050   IF J5=>0 THEN T(2)=R(J5)*PP
3060   IF KL=>0 THEN T(3)=R(KL)*PP
3070   IF KK=>0 THEN T(4)=R(KK)*PP
3080   IF KQ=>0 THEN T(5)=R(KQ)*PP
3090   IF KT=>0 THEN T(6)=R(KT)*PP
3100 LPRINT SPC(10)CHR$(27);CHR$(45);CHR$(1);"PERMEABILITY ";
3110   LPRINT "COEFFICIENT & PENETRATION RATIO";
3120   LPRINT CHR$(27);CHR$(45);CHR$(0);CHR$(27);CHR$(58)
3130 LPRINT : YY=P(I)/O(I)^2 : LPRINT SPC(15)"Mean PERMEABILITY ";
3140   LPRINT "COEFFICIENT (cm"; : J5=1 : GOSUB 3300 : QQ=-2.2E+22
3150   LPRINT SPC(30)"is   "; : PP=.32*T(0)/(O(I)*Q(3)^2)
3160   IF (T(0)<>0!) THEN QQ=PP*SQR(T(1)/T(0)^2+YY*4!*Q(4)/Q(3)^2)
3170   GOSUB 2000 : LPRINT : LPRINT SPC(15)"Mean PENETRATION
         RATIO (";
```

```
3180   GOSUB 3300 : LPRINT SPC(30)"is   ";
3190   IF (KR=0) GOTO 3210
3200     PP=T(7)/(Q(I)*KR) : QQ=PP*SQR(T(8)/T(7)^2+YY) : GOTO 3220
3210   PP=-2.2E-22 : QQ=PP
3220   GOSUB 2000 : LPRINT
3230   LPRINT SPC(15)"PENETRATION RATIO (/s), defined at";
3240   IF (KT<0) GOTO 3270
3250     J5=6 : GOSUB 3330 : PP=S(KT)/Q(I) : LPRINT SPC(30)"is   ";
3260     QQ=ABS(PP)*SQR(YY+U(KT)/S(KT)^2) : GOSUB 2000 : GOTO 3280
3270   GOSUB 3360 : GOSUB 3340 : GOSUB 3360 : LPRINT "+/-";
         : GOSUB 3360
3280   LPRINT : LPRINT : LPRINT : LPRINT
3290   RETURN
3300 LPRINT "/s), defined from"; : J5=J5+1
3310   IF T(J5)<0! THEN GOSUB 3360 ELSE LPRINT T(J5);
3320   LPRINT "to"; : J5=J5+1
3330   IF T(J5)<0! THEN GOSUB 3360 ELSE LPRINT T(J5);
3340   LPRINT "mm ,"
3350   RETURN
3360 LPRINT " **.***** ";
3370   RETURN
3380 FOR K=0 TO 9
3390   T(K)=0!
3400   NEXT K
3410   RETURN
3420 LPRINT " concentration ";CHR$(247);" ";
3430   LPRINT USING "##.#####^^^^";PP; : LPRINT " ng/ml ."
3440   RETURN
3450 REM Subroutine for error printing.
3460 IF (ERR<>61) GOTO 3490
3470   CLOSE : A="A:"+AE(I)+AA
3480   RESUME 1750
3490 IF NERR>10 THEN RESUME 1870 ELSE NERR=NERR+1
3500   LPRINT "C/VAZ ERROR Code #";ERR;"in Line #";ERL
3510   RESUME NEXT
```

A.12 SLOPES.BAS

```
1000 REM Updated 090386
1010 DIM T(5),W(5),B(4),E(4),P(13),S(13),U(13),C(13,3),
       D(13,3),Q(13,4),N(13,3)
1020 DIM X(200),Y(200),Z(200),Q(13,9),R(13,9),G(13,6),
       F(13,6),H(13,3),V(4,6)
1030 A="******" : PRINT : PRINT A : PRINT "SLOPES" : PRINT A : PRINT
1040 ON ERROR GOTO 2510 : NERR=0
1050 OPEN "I",#3,"B:SUBJECT.DAT" : INPUT #3,AE
1060   PRINT TAB(10);"Name : ";AE : PRINT
1070   INPUT #3,A : INPUT #3,A : INPUT #3,A
1080   INPUT #3,QQ : INPUT #3,SS : INPUT #3,PP
1090   INPUT #3,PP : INPUT #3,RR
1100 CLOSE : B(3)=RR/3!+PP : B(0)=PP/3! : SC=PP/QQ
1110   B(1)=2!*B(0) : B(2)=PP-3! : SL=RR/SS
1120 FOR I=0 TO MQ
1130   P(I)=VAL(AN(I))
1140   NEXT I
1150 OPEN "I",#2,"B:PLASMA.FIT" : INPUT #2,I : J=1
1160   FOR I=0 TO 5
1170     INPUT #2,VV
1180     NEXT I
1190   INPUT #2,VV : INPUT #2,SS : INPUT #2,PP
1200     IF (VV<>P(J)) GOTO 1220
1210       S(J)=60!*SS : U(J)=60!*PP : J=J+1
1220     IF EOF(2)<>0 THEN CLOSE ELSE GOTO 1190
1230 FOR I=0 TO MQ
1240   A="B:"+AN(I)+".AVG" : OPEN "I",#2,A : PRINT
1250   JJ=-1 : BB=0! : CC=0! : SS=0! : SG=3!*B(0) : SD=SG/SC
1260   IF (EOF(2)<>0 OR JJ=199 OR SS=>B(3)) GOTO 1350
```

```
1270     INPUT #2,PP : INPUT #2,QQ : INPUT #2,RR
1280     IF PP>SD THEN SS=SG+(PP-SD)*SL ELSE SS=SC*PP
1290     IF (SS<=0!) GOTO 1260
1300       JJ=JJ+1 : X(JJ)=SS : Y(JJ)=QQ : Z(JJ)=RR
1310       IF (SS>B(0) OR QQ<BB) GOTO 1330
1320         E(0)=SS : BB=QQ
1330       IF (SS<B(1) OR QQ<CC) GOTO 1260
1340         CC=QQ : E(3)=SS : GOTO 1260
1350   CLOSE : PRINT "(";I+1;")";JJ+1;"data-sets were used from ";A
1360   PP=E(3)-E(0) : E(1)=PP/3! : E(2)=2!*E(1) : O(I,3)=JJ+1
1370   E(1)=E(0)+E(1) : E(2)=E(0)+E(2) : M=0
1380   FOR J=0 TO 2
1390     PRINT TAB(10);"*** ";
1400     IF J=1 THEN PRINT "MID-";
1410     IF J<=1 THEN PRINT "POSTERIOR "; ELSE PRINT "ANTERIOR ";
1420     PRINT "VITREOUS ***" : WW=1E+12 : II=0
1430     FOR KK=0 TO 3
1440       GOSUB 2540 : ZZ=0! : YY=0!
1450       FOR K=M TO JJ
1460         IF (X(K)>E(J+1)) GOTO 1500
1470           IF X(K)<E(J) THEN GOTO 1490 ELSE PP=X(K)
1480             GOSUB 2930 : ZZ=ZZ+1! : YY=YY+((Y(K)-BB)/Z(K))^2
1490         NEXT K
1500       IF ZZ>2! THEN YY=YY/(ZZ-2!)
1510       PRINT "Fit";KK;"=> Reduced CHI^2 =";YY
1520       IF (YY>WW) GOTO 1540
1530         WW=YY : KC=KK : XX=ZZ
1540       NEXT KK
1550     M=K : II=1 : O(I,J)=XX : H(I,J)=WW : KK=KC : N(I,J)=KK
1560     FOR K=0 TO 2
1570       NN=3*J+K : L=2*K : Q(I,NN)=V(KC,L) : R(I,NN)=V(KC,L+1)
1580       NEXT K
1590     PRINT TAB(10);"Best for fit =";KC : PRINT
1600     IF J=2 THEN PP=B(2) ELSE PP=6!*J+3!
1610     GOSUB 2930 : C(I,J)=BB : D(I,J)=DD : G(I,J)=GG
1620     F(I,J)=HH : L=J+3 : G(I,L)=FF : F(I,L)=EE
1630     NEXT J
1640   NEXT I
1650 GOSUB 2240 : LPRINT SPC(25)CHR$(14);"REDUCED CHI^2"
1660 LPRINT CHR$(15) : GOSUB 2300
1670 FOR I=0 TO MQ
1680   PP=P(I) : GOSUB 2430 : LPRINT "    ";
1690   FOR J=0 TO 2
1700     QQ=H(I,J) : GOSUB 2490 : LPRINT " -";INT(O(I,J));
           "-";N(I,J);SPC(4);
1710     NEXT J
1720   LPRINT " ";INT(O(I,3))
1730   NEXT I
1740 LPRINT CHR$(18) : LPRINT SPC(25)CHR$(14)"CONCENTRATION";
1750   LPRINT CHR$(20);" - [ ng.ml"; : A="-1"
1760   GOSUB 2270 : LPRINT " ]" : II=0 : GOSUB 2350
1770 FOR I=0 TO MQ
1780   PP=P(I) : GOSUB 2430
1790   FOR J=0 TO 2
1800     IF (I=0) GOTO 1820
1810       C(I,J)=C(I,J)-C(0,J) : D(I,J)=D(I,J)+D(0,J)
1820     PP=C(I,J) : QQ=SQR(ABS(D(I,J))) : GOSUB 2460
1830     NEXT J
1840   LPRINT
1850   NEXT I
1860 II=0 : L=1 : XX=0! : YY=0!
1870   LPRINT CHR$(18) : LPRINT SPC(20)CHR$(14);"PENETRATION RATIO";
1880   LPRINT CHR$(20);" - [ s"; : A="-1"
1890   GOSUB 2270 : LPRINT " ]" : GOSUB 2350
1900 FOR I=L TO MQ
1910   PP=P(I) : GOSUB 2430 : BB=(U(I)/S(I))^2 : K=0
1920   IF (II=0) GOTO 1940
1930     XX=C(1,K) : YY=D(1,K)
1940   OO=C(I,K)-XX : PP=OO/S(I)
1950   QQ=ABS(PP)*SQR((D(I,K)+YY)/OO^2+BB) : GOSUB 2460
1960   IF (K=2) GOTO 1980
1970     LPRINT SPC(28); : K=2 : GOTO 1940
1980   LPRINT
```

```
1990   NEXT I
2000 II=II+1 : L=2
2010 IF (II=1) GOTO 1870
2020 LPRINT CHR$(18) : LPRINT SPC(27)CHR$(14);"GRADIENT";
2030   LPRINT CHR$(20);" - [ ng.ml"; : A="-1" : GOSUB 2270
2040   LPRINT ".mm"; : GOSUB 2270 : LPRINT " ]" : GOSUB 2380
2050 FOR I=0 TO MQ
2060   PP=P(I) : GOSUB 2430
2070   FOR J=0 TO 2
2080     PP=G(I,J) : QQ=SQR(ABS(F(I,J))) : GOSUB 2460
2090     NEXT J
2100   LPRINT
2110   NEXT I
2120 LPRINT CHR$(18) : LPRINT SPC(15)CHR$(14);"DIFFUSION CONSTANT";
2130   LPRINT CHR$(20);" - [ cm"; : A="2" : GOSUB 2270
2140   LPRINT "s"; : A="-1" : GOSUB 2270 : LPRINT " ]" : GOSUB 2380
2150 FOR I=1 TO MQ
2160   PP=P(I) : GOSUB 2430 : QQ=G(I,1)-G(I,0)
2170   LPRINT SPC(28); : PP=C(I,1)/(1000*PP*QQ)
2180   QQ=D(I,1)/C(I,1)^2+.25/P(I)^2+(F(I,1)+F(I,0))/QQ^2
2190   QQ=PP*SQR(ABS(QQ)) : GOSUB 2460 : LPRINT
2200   NEXT I
2210 ERASE T,W,B,E,P,S,U,C,D,O,N,X,Y,Z,Q,R,G,F,H,V
2220   ON ERROR GOTO 0 : LPRINT CHR$(12);CHR$(27);CHR$(58);
2230   CLOSE : GOTO 150
2240 LPRINT CHR$(12);CHR$(18);CHR$(27);CHR$(65);CHR$(12);
       CHR$(27);CHR$(50);
2250   LPRINT SPC(20)"NAME : ";AE;"  [ SLOPES - RETina ]"
       : LPRINT : LPRINT
2260   RETURN
2270 LPRINT CHR$(27);CHR$(83);CHR$(0);
2280   LPRINT A;CHR$(27);CHR$(84);
2290   RETURN
2300 LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);"TIME (min)";
2310   LPRINT SPC(5)"POSTERIOR VITREOUS";SPC(9)"MID-VITREOUS";
2320   LPRINT SPC(9)"ANTERIOR VITREOUS";SPC(7)"Points";
2330   LPRINT CHR$(27);CHR$(45);CHR$(0)
2340   RETURN
2350 LPRINT CHR$(27);CHR$(58);SPC(35)"after ";
2360   IF II=0 THEN LPRINT "BACKGROUND"; ELSE LPRINT "SOLUS";
2370   LPRINT " subtraction"
2380   LPRINT CHR$(15) : LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);
       "TIME (min)";
2390   LPRINT SPC(5)"POSTERIOR VITREOUS (3 mm/R)";
2400   LPRINT SPC(5)"MID-VITREOUS (9 mm/R)";
2410   LPRINT SPC(5)"ANTERIOR VITREOUS (3 mm/L)";
       CHR$(27);CHR$(45);CHR$(0)
2420   RETURN
2430 LPRINT SPC(22);
2440   LPRINT USING " ##.##";PP; : LPRINT SPC(4);
2450   RETURN
2460 LPRINT SPC(5);
2470   LPRINT USING "##.###^^^^";PP;
2480   LPRINT " +/-";
2490   LPRINT USING "##.###^^^^";QQ;
2500   RETURN
2510 IF NERR>10 THEN RESUME 2210 ELSE NERR=NERR+1
2520   LPRINT "SLOPES ERROR Code #";ERR;"in Line #";ERL
2530   RESUME NEXT
2540 FOR K=0 TO 4
2550   T(K)=0! : W(K)=0!
2560   NEXT K
2570 FOR K=M TO JJ
2580   IF (X(K)>E(J+1)) GOTO 2730
2590     IF (X(K)<E(J) OR Y(K)<=0!) GOTO 2720
2600       ON KK GOTO 2620,2630,2640
2610       BB=X(K) : CC=Y(K) : DD=Z(K)^2 : GOTO 2650
2620       BB=LOG(X(K)) : CC=Y(K) : DD=Z(K)^2 : GOTO 2650
2630       BB=X(K) : CC=LOG(Y(K)) : DD=(Z(K)/Y(K))^2 : GOTO 2650
2640       BB=X(K) : CC=1!/Y(K) : DD=(Z(K)/Y(K)^2)^2
2650       W(4)=1!/DD : W(3)=W(4)*CC
2660       FOR L=0 TO 4
2670         T(L)=T(L)+W(4) : W(4)=W(4)*BB
2680         NEXT L
2690       FOR L=0 TO 2
2700         W(L)=W(L)+W(3) : W(3)=W(3)*BB
2710         NEXT L
2720     NEXT K
```

```
2730 BB=T(1)/T(0) : CC=T(2)/T(1) : DD=T(3)/T(2) : EE=T(4)/T(3)
2740   FF=CC-DD : GG=1!/DD-1!/CC : HH=1!/BB-1!/CC
2750   OO=FF*GG-HH*(EE-DD) : PP=W(0)/T(1)-W(1)/T(2)
2760   QQ=(PP*GG-(W(2)/T(3)-W(1)/T(2))*HH)/W(3) : V(KK,4)=QQ
2770   RR=(PP-QQ*FF)/HH : V(KK,0)=RR : SS=W(0)/T(1)-RR/BB-QQ*CC
2780   V(KK,2)=SS : PP=EE-CC : TT=(EE*(CC-BB)/CC+BB-DD-FF*BB/DD)^2
2790   UU=EE-DD : QQ=UU^2/T(0)-2!*UU*PP*BB^2/T(2)
2800   QQ=QQ+(BB*PP)^2/T(2)-2!*BB*FF*UU/(T(0)*DD)+(BB*FF)^2*EE/T(3)
2810   QQ=(QQ+2!*BB^2*PP*FF/T(2))/TT : V(KK,1)=QQ
2820   UU=T(4)/T(2)-T(2)/T(0) : FF=DD-BB
2830   RR=(PP/CC)^2/T(0)+UU^2/(T(3)*DD)+EE*FF^2/T(3)
2840   RR=RR-2!*PP*UU*BB/(CC*T(3))+2!*FF*PP*BB/T(3)
2850   RR=(RR-2!*UU*FF/T(3))/TT : V(KK,3)=RR
2860   PP=DD-CC : UU=CC-BB : SS=(PP*T(1)/T(3))^2/T(0)
2870   SS=SS+FF^2/(T(3)*DD)+EE*(UU/CC)^2/T(3)
2880   SS=SS-2!*(FF-UU)*PP*BB*T(1)/T(3)^2
2890   SS=(SS-2!*FF*UU/(CC*T(3)))/TT : V(KK,5)=SS
2900 IF KK<>2 THEN RETURN
2910   V(KK,3)=EXP(V(KK,0)) : V(KK,1)=V(KK,1)*V(KK,0)^2
2920   RETURN
2930 ON KK GOTO 2950,3080,2940
2940   CC=PP : EE=1! : FF=2!*CC : GOTO 2960
2950   CC=LOG(PP) : EE=1!/PP : FF=2!*CC*EE
2960   BB=V(KK,0)+V(KK,2)*CC+V(KK,4)*CC^2
2970   IF KK=3 THEN BB=1!/BB
2980   IF II=0 THEN RETURN
2990   DD=V(KK,1)+V(KK,3)*CC^2+V(KK,5)*CC^4
3000   GG=V(KK,2)*EE+V(KK,4)*FF : HH=V(KK,3)*EE^2+V(KK,5)*FF^2
3010 ON KK GOTO 3030,3070,3040
3020   EE=4!*V(KK,5) : RETURN
3030   FF=EE*(-GG+FF) : EE=EE^2*(HH+4!*V(KK,5)*EE^2) : RETURN
3040   CC=BB^4 : DD=DD*CC : GG=-GG*BB^2 : HH=HH*CC+DD*(2!*GG/BB)^2
3050   CC=GG/BB : FF=2!*(GG*CC-V(KK,4)*BB^2) : EE=16!*HH*CC^2
3060   EE=EE+DD(4!*CC^4+(4!*BB*V(KK,4))^2)+4!*V(KK,5)*BB^4
3070 RETURN
3080 CC=PP : BB=V(KK,0)*EXP(V(KK,2)*CC+V(KK,4)*CC^2)
3090   IF II=0 THEN RETURN
3100 DD=V(KK,1)*(BB/V(KK,0))^2+V(KK,3)*(CC*BB)^2+V(KK,5)*(BB*CC^2)^2
3110   FF=V(KK,2)+2!*V(KK,4)*CC : GG=BB*FF
3120   HH=DD*FF^2+(V(KK,3)+4!*V(KK,5)*CC^2)*BB^2 : EE=HH*FF^2
3130   FF=FF*GG+2!*V(KK,4)*BB : EE=EE+V(KK,3)*GG^2
3140   EE=EE+4!*V(KK,5)*((GG*CC)^2+BB^2)+4!*DD*V(KK,4)^2
3150 RETURN
```

A.13 PLOT.BAS

```
1000 REM Updated 210186
1010 DIM L(256),M(256),N(3),SX(15),SY(15),SZ(15),B(3)
1020 A="**********" : PRINT : PRINT A : PRINT "PLOT MENU" : PRINT A
1030   PRINT : PRINT TAB(10);"The files in Drive B are:"
         : FILES"B:*.*"
1040   PRINT : PRINT : PRINT TAB(10);"FILE TYPE" : PRINT TAB(10);A
1050   PRINT "(0) .AVG files -> Averaged raw data"
1060   PRINT "(1) .RET files -> Retina-aligned, b/g-subtracted"
1070   PRINT "(2) .CRP files -> CRPeak-aligned, b/g-subtracted"
1080   PRINT : PRINT : PRINT "Which type do you wish to plot ";
1090   INPUT I : PRINT : PRINT : PRINT "Choose from:"
1100 IF (I<3) GOTO 1130
1110   PRINT : PRINT : PRINT "BAD ENTRY! Please try again."
1120   PRINT : PRINT : PRINT : GOTO 1020
1130 ON I GOTO 1150,1160
1140   AF="*.AVG" : KZ=0 : FILES "B:*.AVG" : GOTO 1170
1150   AF="*.RET" : KZ=1 : FILES "B:*.RET" : GOTO 1170
1160   AF="*.CRP" : KZ=1 : FILES "B:*.CRP"
1170 PRINT : PRINT : MQ=-1 : PRINT "PRESS RETURN TO EXIT." : PRINT
1180 LINE INPUT "Filename -> ";A
1190 IF (A="") GOTO 1210
1200   MQ=MQ+1 : AN(MQ)=A : GOTO 1180
```

```
1210 OPEN "I",#1,"B:SUBJECT.DAT"
1220   INPUT #1,AM : INPUT #1,A : INPUT #1,A : INPUT #1,A
1230   INPUT #1,N(0) : INPUT #1,N(1) : INPUT #1,N(2)
1240   INPUT #1,B(0) : INPUT #1,B(1) : INPUT #1,B(2)
1250 CLOSE : N(1)=N(1)+N(0) : N(2)=N(2)+N(1) : MV=N(0)\2
1260   MA=(N(1)+N(2))\2 : PRINT : PRINT : PRINT
1270 FOR I=0 TO 2
1280   B(I)=B(I)/N(I)
1290   NEXT I
1300 FOR I=KZ TO MQ
1310   SY(2)=-10! : SY(3)=1E+22 : SY(4)=-10! : SY(5)=SY(3)
         : SY(6)=-10!
1320   JJ=-1 : PRINT TAB(10);"Reading    ";AN(I)
1330   OPEN "I",#3,"B:"+AN(I)+AF : PP=0! : IM=6
1340   INPUT #3,P : INPUT #3,Q : INPUT #3,R : K=CINT(P) : JJ=JJ+1
1350     IF (K<>MV) GOTO 1370
1360       SX(0)=P : SY(0)=Q : SZ(0)=R
1370     IF (K<>MA) GOTO 1390
1380       SX(1)=P : SY(1)=Q : SZ(1)=R
1390     IF (K>MV OR Q<SY(2)) GOTO 1410
1400       SX(2)=P : SY(2)=Q : SZ(2)=R
1410     IF (K<0 OR K>N(0) OR Q>SY(3)) GOTO 1430
1420       SX(3)=P : SY(3)=Q : SZ(3)=R
1430     IF (K<MV OR K>MA OR Q<SY(4)) GOTO 1450
1440       SX(4)=P : SY(4)=Q : SZ(4)=R
1450     IF (K<N(1) OR K>N(2) OR Q>SY(5)) GOTO 1470
1460       SX(5)=P : SY(5)=Q : SZ(5)=R
1470     IF (K<MA OR Q<SY(6)) GOTO 1490
1480       SX(6)=P : SY(6)=Q : SZ(6)=R
1490     IF K>N(0) THEN GOTO 1530 ELSE QQ=B(0)*P
1500       IF (QQ<PP) GOTO 1520
1510         IM=IM+1 : SX(IM)=QQ : SY(IM)=Q : SZ(IM)=R : PP=PP+3!
1520       P=3.333*B(0)*P+11! : X=P : GOTO 1580
1530     IF (K>N(1)) GOTO 1550
1540       P=3.333*B(1)*(P-N(0))+X : Y=P : GOTO 1580
1550     IF (K>N(2)) GOTO 1570
1560       P=3.333*B(2)*(P-N(1))+Y : Z=P : GOTO 1580
1570     P=.32*(P-N(2))+Z
1580     L(JJ)=CINT(P)
1590     IF Q<=.8364 THEN M(JJ)=0 ELSE
               M(JJ)=CINT(.018315*(LOG(Q)+109.339)^2-212.586)
1600     IF EOF(3)<>0 THEN CLOSE ELSE GOTO 1340
1610   LPRINT CHR$(18);SPC(20)"NAME : ";AM;"  [ PLOT ]" : LPRINT
1620   LPRINT : LPRINT : LPRINT SPC(20)CHR$(14);AN(I);CHR$(20);
1630   LPRINT "-minute PROFILE has";JJ+1;"data-sets,"
1640   LPRINT SPC(30)"stored in ";AN(I)+AF;" ." : LPRINT
1650   LPRINT CHR$(15) : LPRINT CHR$(27);CHR$(49)
1660   LPRINT SPC(25)"Vertical (LOG) Scale = X 10 ng/ml per 10 div.";
1670   LPRINT SPC(7)"Horizontal Scale = 3 mm per 10 div."
1680   FOR J=0 TO 40
1690     KK=40-J : P=KK/5! : LL=0 : LPRINT CHR$(10);CHR$(13);CHR$(9);
1700     IF ((P-FIX(P))=0!) GOTO 1720
1710       LPRINT CHR$(9);CHR$(124); : GOTO 1770
1720     IF (KK=5 OR KK=15 OR KK=25 OR KK=35) GOTO 1750
1730       IF J=0 THEN LPRINT "ng/ml   +"; ELSE LPRINT CHR$(9);"+";
1740       GOTO 1770
1750     LPRINT "  10";CHR$(27);CHR$(83);CHR$(0);KK\10;
1760     LPRINT CHR$(27);CHR$(84);" +";
1770     FOR K=0 TO JJ
1780       IF (M(K)<>KK OR L(K)>110) GOTO 1820
1790         IF L(K)=0 THEN MM=1 ELSE MM=L(K)-LL
1800         IF MM=0 THEN GOTO 1810 ELSE LPRINT SPC(MM-1)CHR$(249);
1810           LL=L(K)
1820       NEXT K
1830     NEXT J
1840   LPRINT : LPRINT CHR$(9);CHR$(9);" "; : LL=0
1850   MM=CINT(.3333*B(0)*N(0))+11
1860   FOR J=0 TO 10
1870     IF (10*J)=MM THEN GOSUB 2380 ELSE LPRINT "+";
1880     FOR K=0 TO 8
1890       KK=10*J+K+1
1900       IF KK=MM THEN GOSUB 2380 ELSE LPRINT "-";
1910       NEXT K
1920     NEXT J
1930   IF MM=110 THEN LPRINT "+" ELSE LPRINT "+"
1940   LPRINT CHR$(9);CHR$(9);"-3";SPC(9)"R";SPC(19)"6";SPC(18);"12";
1950   LPRINT SPC(18)"18";SPC(18)"24";SPC(18)"mm" : LPRINT : LPRINT
```

```
1960   LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50)
1970   LPRINT SPC(20)CHR$(27);CHR$(45);CHR$(1);"Landmark";
1980   LPRINT SPC(20)"Concentration +/- S.E. (ng/ml)";
1990   LPRINT SPC(5)"Distance from RETINA (mm)";
          CHR$(27);CHR$(45);CHR$(0)
2000   LPRINT SPC(20)"Choroid-Retinal Peak";SPC(9); : X=B(0)*SX(2)
          : Y=SY(2)
2010   Z=SZ(2) : GOSUB 2250 : LPRINT "Mid-Vitreous";SPC(17);
          : X=B(0)*SX(0)
2020   Y=SY(0) : Z=SZ(0) : GOSUB 2250 : X=B(0)*SX(3) : Y=SY(3)
          : Z=SZ(3)
2030   LPRINT "Vitreous Low";SPC(17); : GOSUB 2250 : P=B(0)*N(0)
          : Y=SY(4)
2040   LPRINT "Lens Peak";SPC(20); : X=B(1)*(SX(4)-N(0))+P : Z=SZ(4)
2050   GOSUB 2250 : LPRINT "Anterior Low";SPC(17);
          : P=P+B(1)*(N(1)-N(0))
2060   X=B(2)*(SX(5)-N(1))+P : Y=SY(5) : Z=SZ(5) : GOSUB 2250
          : Y=SY(1)
2070   LPRINT "Mid-Aqueous";SPC(18); : X=B(2)*(SX(1)-N(1))+P
          : Z=SZ(1)
2080   GOSUB 2250 : LPRINT "Anterior Peak";SPC(16);
          : X=B(2)*(SX(6)-N(1))+P
2090   Y=SY(6) : Z=SZ(6) : GOSUB 2250 : LPRINT
2100   LPRINT SPC(30)CHR$(27);CHR$(45);CHR$(1);
          "DISTANCE from RETINA (mm)";
2110   LPRINT SPC(18)"READING +/- S.E. (ng/ml)";
          CHR$(27);CHR$(45);CHR$(0)
2120   FOR J=7 TO IM
2130     X=SX(J) : Y=SY(J) : Z=SZ(J) : GOSUB 2260
2140     NEXT J
2150   LPRINT CHR$(27);CHR$(58);CHR$(12)
2160   NEXT I
2170 PRINT : PRINT "Enter ESC to exit from plot ," : PRINT
2180   PRINT "     ANY OTHER key to continue."; : A=INPUT$(1) : PRINT
2190 IF (A<>CHR$(27)) GOTO 1020
2200   ERASE L,M,N,SX,SY,SZ,B
2210   LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);CHR$(18);
2220   CLOSE : GOTO 150
2230 LPRINT SPC(60)"The data are stored in ";
2240 RETURN
2250 KK=0 : GOTO 2330
2260   KK=1 : LPRINT SPC(40); : GOTO 2280
2270   LPRINT SPC(10);
2280   LPRINT USING "##.#####";X;
2290 IF (KK=1) GOTO 2320
2300   LPRINT : LPRINT SPC(20);
2310   RETURN
2320 LPRINT SPC(20);
2330   LPRINT USING "##.#####^^^^";Y;
2340   LPRINT " +/-";
2350   LPRINT USING "##.#####^^^^";Z;
2360 IF KK=1 THEN LPRINT ELSE GOTO 2270
2370   RETURN
2380 LPRINT "^"; : LL=LL+1
2390   MM=CINT(.3333*B(LL)*(N(LL)-N(LL-1)))+MM
2400   RETURN
```

A.14 DRAW.BAS

```
1000 REM Updated 250186
1010 DIM X(256),Y(256),N(4),Z(4)
1020 A="++++" : PRINT A : PRINT "DRAW" : PRINT A : II=0
1030 ON ERROR GOTO 1970
1040 LPRINT CHR$(15);CHR$(27);CHR$(49);CHR$(27);CHR$(79); : MQ=11
1060 FOR J=0 TO MQ
1070   OPEN "I",#1,"B:SUBJECT.DAT" : INPUT #1,AAA
1080     PRINT : PRINT TAB(15);"Name : ";AAA : PRINT : PRINT
1090     INPUT #1,A : INPUT #1,A : INPUT #1,A
1100     INPUT #1,KK : INPUT #1,MM : INPUT #1,LL : INPUT #1,EE
1110   CLOSE : PRINT "The files in Drive B are:" : PRINT
```

```
1120   IF (J=0) GOTO 1150
1130     Z(0)=N(0)/KK : Z(1)=(N(1)-N(0))/MM
1140     Z(2)=(N(2)-N(1))/LL : GOTO 1170
1150   N(0)=KK : N(1)=MM+KK : DD=KK/EE : JF=21+DD+20
1160     N(2)=LL+N(1) : N(3)=KK\2+15 : JG=41+DD+20
1170   LY=-1 : MM=MM+KK : LL=LL+MM
1180   FILES "B:*.*" : PRINT : PRINT
1190     PRINT TAB(5);"Enter . to change Diskette." : PRINT
1200     PRINT TAB(5);"Enter RETURN to exit." : PRINT : PRINT
1210   LINE INPUT "Enter the COMPLETE Filename.  ";AN(J)
1220     A=RIGHT$(AN(J),4) : PRINT : PRINT
1230   IF (AN(J)="") GOTO 1750
1240     IF (AN(J)<>".") GOTO 1260
1250       J=J-1 : PRINT : GOTO 1720
1260   IF (A=".AVG" OR A=".RET" OR A=".CRP") GOTO 1280
1270     PRINT "INCOMPLETE Entry. Please try again." : GOTO 1180
1280   OPEN "I",#2,"B:"+AN(J) : NN=-1
1290   NN=NN+1 : INPUT #2,X(NN) : INPUT #2,Y(NN) : INPUT #2,ZZ
1300     IF X(NN)<-20! THEN NN=NN-1
1310     IF (NN<0 OR J=0) THEN GOTO 1350 ELSE ZZ=X(NN)
1320       IF (ZZ<=LL AND ZZ>MM) THEN X(NN)=(ZZ-MM)+Z(2)+N(1)
1330       IF (ZZ<=MM AND ZZ>KK) THEN X(NN)=(ZZ-KK)+Z(1)+N(0)
1340       IF (ZZ<=KK) THEN X(NN)=ZZ+Z(0)
1350     IF EOF(2)<>0 THEN CLOSE ELSE GOTO 1290
1360   IF (J<>0) GOTO 1380
1370     LPRINT "+"; : GOSUB 1860 : LPRINT
1380   MC=0 : L=0 : M=0 : A=RIGHT$(STR$(J),1)
1390   IF N(2)>NN THEN KK=N(2)+10 ELSE KK=NN+10
1400   IF (KK>210) THEN KK=KK+10 ELSE KK=210
1410   FOR K=0 TO KK
1420     IF (J<>0) GOTO 1530
1430     IF ((N(M)+20)<>K) GOTO 1460
1440       IF (M=0 OR M=1) THEN LPRINT "L"; ELSE LPRINT "C";
1450       M=M+1 : GOTO 1510
1460     IF (K<>JF) GOTO 1480
1470       LPRINT "2"; : GOTO 1510
1480     IF (K<>JG) GOTO 1500
1490       LPRINT "4"; : GOTO 1510
1500     IF K<>20 THEN GOTO 1520 ELSE LPRINT "R";
1510       GOSUB 1860 : GOTO 1530
1520 IF RIGHT$(STR$(K),1)="0" THEN LPRINT "+"; ELSE LPRINT CHR$(124);
1530     LPRINT CHR$(13);" ";
1540     IF (K<>(N(3)+II) OR MC=1) GOTO 1560
1550       GOSUB 1830 : II=II+2
1560     FOR JJ=L TO NN
1570       LL=CINT(X(JJ)+20!)-K
1580       IF (LL<0) GOTO 1640
1590       IF (LL>0) GOTO 1660
1600         IF Y(JJ)>0! THEN ZZ=LOG(Y(JJ)) ELSE ZZ=0!
1610         MM=CINT(4.47+25+ZZ+.114+ZZ^2)
1620         IF MM<0 THEN MM=0
1630         IF MM<130 THEN LPRINT SPC(MM)A;
1640       L=L+1 : LPRINT CHR$(13);" ";
1650       NEXT JJ
1660     L=JJ : LPRINT
1670     NEXT K
1680   IF J=MQ THEN GOTO 1750 ELSE PRINT
1690     PRINT TAB(10);"PAUSE";J;"for PAPER Adjustment"
1700     PRINT TAB(12);"(Enter ESC to exit.)    "; : A=INPUT$(1)
1710     IF A=CHR$(27) THEN GOTO 1750 ELSE RESET
1720     PRINT : PRINT TAB(15);"INSERT NEW Diskette."; : A=INPUT$(1)
1730     PRINT : PRINT : PRINT : PRINT : PRINT : LPRINT : RESET
1740   NEXT J
1750 LPRINT CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);
1760 LPRINT CHR$(27);CHR$(58) : PRINT : PRINT :  A=INKEY$
1770 PRINT TAB(10);"Start a NEW plot? (N) "; : A=INPUT$(1)
1780 IF (A="Y" OR A="y") THEN PRINT ELSE GOTO 1800
1790   PRINT : PRINT : PRINT : J=0 : GOTO 1040
1800 ON ERROR GOTO 0
1810 ERASE X,Y,N,Z
1820 CLOSE : GOTO 150
1830 LPRINT SPC(80)"[";RIGHT$(STR$(J),1);"] => ";AN(J);
1840 LPRINT " Profile for ";AAA;CHR$(13);" "; : MC=1
1850 RETURN
1860 FOR LV=1 TO 120
1870   IF RIGHT$(STR$(LV),1)="0" THEN LPRINT "+"; ELSE LPRINT "-";
1880   NEXT LV
1890 LY=LY+1
```

```
1900 ON LY GOTO 1920,1930,1940,1950,1950,1960
1910   LPRINT " LOG"; : RETURN
1920   LPRINT " RETINA"; : RETURN
1930   LPRINT " 2 mm"; : RETURN
1940   LPRINT " 4 mm"; : RETURN
1950   LPRINT " LENS"; : RETURN
1960   LPRINT " CORNEA"; : RETURN
1970   IF (ERL<>1280) GOTO 2010
1980   PRINT : PRINT CHR$(7);TAB(10);"This file does not exist."
1990   PRINT : PRINT TAB(12);"Try again. " : PRINT
2000   RESUME 1180
2010 PRINT CHR$(7);"ERROR Code #";ERR;"in Line #";ERL
2020 RESUME NEXT
```

A.15 LUND.BAS

```
1000 REM Updated 180286
1010 DIM X(100),Y(100),Z(100),Q(100),P(2),Q(2),R(2)
1020 DIM S(2,2),T(2),U(2),V(2,2),W(2),PB(3),PLO(3)
1030 A="****" : PRINT A : PRINT "LUND" : PRINT A
1040 KP=0 : ASQ=CHR$(27)+CHR$(83)+CHR$(0)+"2"+CHR$(27)+CHR$(84)
1050 ON ERROR GOTO 3270 : NERR=0
1060 OPEN "I",#1,"B:SUBJECT.DAT" : INPUT #1,AA
1070   INPUT #1,A : INPUT #1,A : INPUT #1,A
1080   INPUT #1,X(0) : INPUT #1,Y(0) : INPUT #1,X(2)
1090   INPUT #1,Y(0) : INPUT #1,Y(1) : INPUT #1,Y(2)
1100 CLOSE : RAD=(Y(0)+Y(1)+Y(2))/2! : SC=Y(0)/X(0)
1110 OPEN "I",#2,"B:PLASMA.FIT" : INPUT #2,KK
1120   FOR I=0 TO 5
1130     INPUT #2,P0 : J=I\2
1140     IF (I=0 OR I=2 OR I=4) THEN PB(J)=P0
1150     NEXT I
1160   CLOSE
1170 PRINT "The files in Drive B are :"
1180   FILES "B:*.*" : PRINT : PRINT
1190   PRINT TAB(5);"Enter RETURN to exit." : PRINT
1200   PRINT TAB(5);"Enter . to CHANGE diskette." : PRINT : PRINT
1210 LINE INPUT "Enter the COMPLETE ????????.??? : ";AE : PRINT
1220   IF AE<>"." THEN GOTO 1250 ELSE PRINT
1230     RESET : PRINT "Replace diskette NOW; enter
               ANY key when ready.";
1240     A=INPUT$(1) : PRINT : RESET : GOTO 1060
1250 IF (AE<>"") GOTO 1290
1260   ERASE Q,P,Q,R,S,T,U,V,W,X,Y,Z,PB,PLO
1270   ON ERROR GOTO 0 : LPRINT CHR$(12);CHR$(27);CHR$(58);
1280   CLOSE : GOTO 150
1290 GOSUB 2340 : PRINT : PRINT "Enter the LEFT limit "; : INPUT PL
1300   PRINT "Enter the RIGHT limit "; : INPUT RP : PRINT : PRINT
1310   IF (RP<=PL OR RP>RAD) THEN RP=RAD
1320   LPRINT SPC(10)"Analyzing file : ";CHR$(14);AE;CHR$(20);
1330   LPRINT " within [";PL;",";RP;"] mm" : LPRINT
1340 A="INITIAL ESTIMATES of" : PRINT "Enter the ";A
1350   LPRINT SPC(18)A : A="Permeability Coefficient P (cm/s) ="
1360   PRINT A; : INPUT P(0) : LPRINT SPC(20)A;P(0)
1370   A="Increment of P to be used (cm/s) =" : PRINT A;
1380   INPUT R(0) : LPRINT SPC(21)A;R(0) : LPRINT SPC(22)"and"
1390   A="Diffusion coefficient D (cm"+ASQ+"/s) ="
1400   PRINT "Diffusion coefiicient D (cm^2/s) =";
1410   INPUT P(1) : LPRINT SPC(22)A;P(1)
1420   A="Increment of D to be used (cm"+ASQ+"/s) ="
1430   PRINT "Increment of D to be used (cm^2/s) =";
1440   INPUT R(1) : LPRINT SPC(20)A;R(1) : LPRINT : PRINT
1450 FOR I=0 TO 1
1460   IF I=0 THEN P0=600! ELSE P0=6000!
1470   P(I)=P(I)+P0 : R(I)=R(I)+P0
1480   NEXT I
```

```
1490 PRINT : II=-1 : PP=.001 : AAA=RIGHT$(AE,3)
1500   IF LEFT$(AAA,2)<>"CV" THEN P0=SC ELSE P0=1!
1510 OPEN "I",#3,"B:"+AE
1520 IF II>99 THEN GOTO 1560 ELSE II=II+1
1530   INPUT #3,X(II) : X(II)=P0*X(II) : INPUT #3,Y(II)
         : INPUT #3,Z(II)
1540   IF X(II)<0! THEN II=II-1
1550   IF (EOF(3)=0) GOTO 1520
1560 CLOSE : J=II : II=-1
1570 FOR K=0 TO J
1580   IF (X(K)=>RP) GOTO 1620
1590   IF (Y(K)<=0! OR X(K)<PL) GOTO 1620
1600     II=II+1 : X(II)=RAD-X(K) : Y(II)=Y(K) : Z(II)=Z(K)^2
1610     PRINT II+1;") ";X(II);TAB(18);Y(II);"+/-";Z(K)
1620   NEXT K
1630 PRINT : PRINT TAB(10);II+1;"points were entered." : PRINT
1640 PRINT : LPRINT SPC(30)II+1;"points were read."
1650 FOR J=0 TO 1
1660   T(J)=0!
1670   FOR K=0 TO J
1680     S(J,K)=0!
1690     NEXT K
1700   NEXT J
1710 OO=0! : J=LEN(AE)-4 : TT=VAL(LEFT$(AE,J))
1720 FOR I=0 TO II
1730   GOSUB 2450 : O(I)=WW : Z1=Y(I)-WW : OO=OO+Z1^2/Z(I)
1740   FOR J=0 TO 1
1750     U1=P(J) : P(J)=U1+R(J) : GOSUB 2450 : U2=WW : P(J)=U1-R(J)
1760     GOSUB 2450 : U(J)=.5*(U2-WW)/R(J) : P(J)=U1
1770     NEXT J
1780   FOR J=0 TO 1
1790     T(J)=T(J)+Z1*U(J)/Z(I)
1800     FOR K=0 TO J
1810       S(J,K)=S(J,K)+U(J)*U(K)/Z(I)
1820       NEXT K
1830     NEXT J
1840   NEXT I
1850 OO=OO/(II-2) : LPRINT SPC(30)"=> FIRST CHI";ASQ;" =";OO
1860 FOR J=0 TO 1
1870   FOR K=0 TO J
1880     S(K,J)=S(J,K)
1890     NEXT K
1900   NEXT J
1910 FOR J=0 TO 1
1920   FOR K=0 TO 1
1930     V(J,K)=S(J,K)/SQR(S(J,J)*S(K,K))
1940     NEXT K
1950   V(J,J)=1!+PP
1960   NEXT J
1970 GOSUB 2790
1980 FOR J=0 TO 1
1990   W(J)=P(J)
2000   FOR K=0 TO 1
2010     W(J)=W(J)+T(K)*V(J,K)/SQR(S(J,J)*S(K,K))
2020     NEXT K
2030   NEXT J
2040 GOSUB 2400 : SS=0! : LPRINT
2050 LPRINT SPC(14)CHR$(27);CHR$(45);CHR$(1);"S/No.";
2060 LPRINT SPC(8)"Reading";SPC(11)"Fit #1";SPC(11)"Fit #2";
2070 LPRINT CHR$(27);CHR$(45);CHR$(0)
2080 FOR I=0 TO II
2090   LPRINT SPC(15); : LPRINT USING "##";I;
2100   LPRINT SPC(10); : LPRINT USING "##.####";Y(I);
2110   LPRINT SPC(10); : LPRINT USING "##.####";O(I);
2120   GOSUB 2450 : LPRINT SPC(10); : LPRINT USING "##.####";WW
2130   O(I)=WW : SS=SS+(Y(I)-WW)^2/Z(I)
2140   NEXT I
2150 SS=SS/(II-2) : GOSUB 2400 : LPRINT : PLO(KP)=SS
2160 LPRINT SPC(30)"=> SECOND CHI";ASQ;" =";SS
2170 IF (OO=>SS OR KP=2) GOTO 2190
2180   PP=10!*PP : KP=KP+1 : GOTO 1910
2190 FOR J=0 TO 1
2200   P(J)=W(J) : Q(J)=SQR(V(J,J)/S(J,J))
2210   NEXT J
2220 PP=PP/10! : P1=P(0)/600! : P2=Q(0)/600! : LPRINT
2230   LPRINT SPC(12)"The BEST Fit Values are:"
2240   LPRINT SPC(13)"PERMEABILITY COEFFICIENT =";
2250   LPRINT USING "##.###^^^^";P1; : LPRINT " +/-";
2260   LPRINT USING "##.###^^^^";P2; : LPRINT "cm/s ."
```

```
2270   P1=P(1)/6000! : P2=Q(1)/6000!
2280   LPRINT SPC(16)"DIFFUSION COEFFICIENT =";
2290   LPRINT USING "##.###^^^^";P1; : LPRINT " +/-";
2300   LPRINT USING "##.###^^^^";P2; : LPRINT "cm";ASQ;"/s ."
2310   LPRINT SPC(20)"with Reduced CHI";ASQ;" =";SS;CHR$(12)
2320 IF (SS>2!) GOTO 1910 ELSE GOTO 1170
2330 REM Subroutine to print page title.
2340 LPRINT CHR$(18);CHR$(27);CHR$(65);CHR$(12);CHR$(27);CHR$(50);
2350   LPRINT SPC(25)"NAME : ";AA;" [ LUND ]"
2360   LPRINT : LPRINT SPC(15)"Radius of retinal curvature ";
          CHR$(247);" ";
2370   LPRINT USING "##.#####";RAD; : LPRINT " mm ." : LPRINT
          : LPRINT
2380   RETURN
2390 REM Subroutine for exchanging curve-fitting parameters, P(I).
2400 FOR J=0 TO 1
2410   SWAP P(J),W(J)
2420   NEXT J
2430   RETURN
2440 REM Subroutine for calculating function, O(I).
2450 P0=SQR(P(1)) : P1=P(0)/P0-P0/RAD : P6=P(0)*RAD/(X(I)*P0)
2460   P2=.56419*P6 : P3=P6*P1 : P5=.5/P0 : P4=P5*(RAD-X(I))
2470   P5=P5*(RAD*X(I)) : P6=P3*EXP(2!*P1*P4) : P7=P3*EXP(2!*P1*P5)
2480   WW=0! : T2=0! : N=INT(TT)
2490 FOR JI=1 TO N
2500   TU=JI : GOSUB 2590 : T1=1!/SQR(TU)
2510   QF=P2*T1*(EXP(-P4^2/TU)-EXP(-P5^2/TU))
2520   QD=EXP(TU*P1^2) : P3=P1/T1 : Q1=P3*T1*P4
2530   GOSUB 2710 : QF=QF-P6*QE*QD : Q1=P3*T1*P5
2540   GOSUB 2710 : QF=QF+P7*QE*QD : QF=TP*QF
2550   WW=WW+(T2+QF)/2! : T2=QF
2560   NEXT JI
2570 RETURN
2580 REM Subroutine for calculating plasma value, TP.
2590 T1=TT-TU
2600   IF T1=0! THEN TP=0! ELSE GOTO 2620
2610   RETURN
2620 ON KK GOTO 2640,2680,2650
2630   GOTO 2660
2640   T1=LOG(T1) : GOTO 2660
2650   T1=1!/T1
2660 TP=PB(0)+PB(1)*T1+PB(2)*T1^2
2670   RETURN
2680 TP=PB(0)*EXP(PB(1)*T1+PB(2)*T1^2)
2690   RETURN
2700 REM Subroutine for calculating error function, QE.
2710 Q1=Q1/50! : QE=0! : Q2=1! : Q4=Q1/2!
2720 FOR K=1 TO 50
2730   SK=K : P0=(SK*Q1)^2 : P0=EXP(-P0)
2740   QE=QE+Q4*(Q2+P0) : Q2=P0
2750   NEXT K
2760 QE=1!-1.12838*QE
2770   RETURN
2780 REM Subroutine to inverting matrix, V(I,J), and find det., QQ
2790 DIM IA(2),JA(2)
2800 QQ=1!
2810 FOR K=0 TO 1
2820   Q1=0!
2830   FOR I=K TO 1
2840     FOR  J=K TO 1
2850       IF (ABS(Q1)>ABS(V(I,J))) GOTO 2870
2860         Q1=V(I,J) : IA(K)=I : JA(K)=J
2870       NEXT J
2880     NEXT I
2890   IF (Q1<>0!) GOTO 2910
2900     QQ=0! : GOTO 3250
2910   IF (IA(K)<K) GOTO 2830
2920   IF (IA(K)=K) GOTO 2960
2930     FOR J=0 TO 1
2940       S1=V(K,J) : V(K,J)=V(IA(K),J) : V(IA(K),J)=-S1
2950       NEXT J
2960   IF (JA(K)<K) GOTO 2830
2970   IF (JA(K)=K) GOTO 3010
2980     FOR I=0 TO 1
2990       S1=V(I,K) : V(I,K)=V(I,JA(K)) : V(I,JA(K))=-S1
3000       NEXT I
3010   FOR I=0 TO 1
```

```
3020     IF I<>K THEN V(I,K)=-V(I,K)/Q1
3030     NEXT I
3040   FOR I=0 TO 1
3050     FOR J=0 TO 1
3060       IF (I<>K AND J<>K) THEN V(I,J)=V(I,J)+V(I,K)*V(K,J)
3070       NEXT J
3080     NEXT I
3090   FOR J=0 TO 1
3100     IF J<>K THEN V(K,J)=V(K,J)/Q1
3110     NEXT J
3120   V(K,K)=1!/Q1 : QQ=QQ*Q1
3130   NEXT K
3140 FOR J=0 TO 1
3150   K=1-J
3160   IF (IA(K)<=K) GOTO 3200
3170     FOR I=0 TO 1
3180       S1=V(I,K) : V(I,K)=-V(I,IA(K)) : V(I,IA(K))=S1
3190       NEXT I
3200   IF (JA(K)<=K) GOTO 3240
3210     FOR I=0 TO 1
3220       S1=V(K,I) : V(K,I)=-V(JA(K),I) : V(JA(K),I)=S1
3230       NEXT I
3240   NEXT J
3250 ERASE IA,JA
3260   RETURN
3270 IF NERR>10 THEN RESUME 1260 ELSE NERR=NERR+1
3280   PRINT CHR$(7);"ERROR Code #";ERR;"in Line #";ERL
3290   RESUME NEXT
```

A.16 File Formats

a) Filenames : ??.DAT

Created by : VITSCAN.BAS

Format : (1) Pod "zero" position at beep (in DAS units).
(2) Pod position (in DAS units).
(3) R/M-Log Amp reading (in DAS units).
(4) Repeat (2),(3),.....

b) Filenames : ??.AVG ; ??.RET ; ??.CRP

Created by : REDUCE.BAS

Format : (1) Retina-zeroed Pod position (in DAS units).
(2) Averaged Log Amp output (in $ng.ml^{-1}$).
(3) Standard deviation of (2) (in $ng.ml^{-1}$).
(4) Repeat (1),(2),(3),.....

c) Filename : SUBJECT.DAT

Created by : SCANMENU.BAS

Format : (1) Subject's name.
(2) Subject's age.
(3) Eye scanned.
(4) Date of scan.
(5) Vitreal length (in DAS units).
(6) Lens thickness (in DAS units).
(7) Aqueous depth (in DAS units).
(8) Ultra-sound vitreal length (in mm).
(9) Ultra-sound lens thickness (in mm).
(10) Ultra-sound aqueous depth (in mm).
(11) Fluorescein injected (in ml).
(12) Comments and observations.
(13) Repeat if necessary, (12).

d) Filename : PLASMA.DAT

Created by : PLASCAN.BAS

Format : (1) Background sample time (set = 0).
(2) Background average (in DAS units).
(3) Background standard deviation (in DAS units).
(4) Blood sampling time (in minutes p.i.).
(5) Average fluorescence reading (in DAS units).
(6) Standard deviation (in DAS units).
(7) Repeat (4),(5),(6),.....

e) Filename : PLASMA.FIT

Created by : BLOOD.BAS

Format : (1) Best-fit function's code number.
(2) A coefficient.
(3) Error of A.
(4) B coefficient.
(5) Error of B.
(6) C coefficient.
(7) Error of C.
(8) Measurement p.i. time (in minutes p.i.).
(9) Area up to (8) (in $ng.ml^{-1}.min$).
(10) Error of (9) (in $ng.ml^{-1}.min$).
(11) Repeat (8),(9),(10),.....

f) Filenames : ??.CV?

Created by : LUND.BAS

Format : (1) Position from Retina (in mm).
(2) Average concentration (in $ng.ml^{-1}$).
(3) Standard deviation of (2) (in $ng.ml^{-1}$).
(4) Repeat (1),(2),(3),.....

A.17 Sample CONSENT FORM

THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER, B.C., CANADA
V5Z 3N9

FACULTY OF MEDICINE
DEPARTMENT OF OPHTHALMOLOGY
2550 WILLOW STREET
TELEPHONE 875-5441 LOCAL 2451

CONSENT FORM

The Development of a Vitreous Fluorophotometer for Studying Alterations in the Blood Retinal Barrier

Dr. I.S. Begg; Dr. T. Cox; Dr. D.A. Balzarini; Mr Pang K.T.

A study is being carried out to measure the abnormal leakage from retinal blood vessels which indicate cell damage in the early stages of disease prior to visible retinal changes. In most patients and normal subjects, the measurements will be carried out following fluorescein angiography (photography) which is a customary diagnostic procedure frequently used in clinical practice to visualize damage to the retinal structure, and to indicate disease severity which is useful in prognosis and in planning treatment. The measurement of fluorescein leakage is made in a follow-up procedure called VITREOUS FLUOROPHOTOMETRY at several time intervals following the angiography. The vitreous cavity of the eye is scanned by the light beam (of an adapted clinical microscope,) which is directed into the eye through a contact lens. The scan of an eye takes about 30 seconds with minimal discomfort. After each scan, a finger-prick blood sample is taken to measure plasma fluorescein. There are no side effects related to these measurements. The injected fluorescein dye colours the skin slightly yellow for about 4 hours, and urine for about 24 hours. About 4% of patients experience brief spells of nausea. Serious allergic reactions are rare and have not been encountered in over some 20000 fluorescein angiograms done in the Department of Ophthalmology, UBC.

The results of the tests and the personal medical records will be kept confidential by using a code number for each patient.

The entire procedure, with repeated scanning, takes about 90 minutes.

The test may only be carried out with your signed consent and the understanding that you may decline to participate, or withdraw at any time during measurement without jeopardizing any routine medical treatment.

CONSENT for the procedure and acknowledgement of receipt of a copy of the consent form.

Signature of Patient	Date	Signature of Witness

APPENDIX B

MATERIAL USED

B.1 Electronics

TYPE	QTY	REMARKS
Capacitors (in nF)		(Code - Colour & Shape)
1000	2	C1 - red box
30	3	C2 - yellow cylinder
10	2	C3 - grey/yellow box
0.15	1	C4 - violet-white
10000	3	C5 - blue/yellow bulb
100	1	C6 - orange
Analogue-to-Digital Converter		
ADC0804LCN	1	ADC - black 20-pin
Analogue Multiplexer		
IH6108	1	MUX - black 16-pin
Operational Amplifiers		
TLD81ACP	2	OP - black 8-pin
Sample-and-Hold		
IH5111IDE	2	S/H - violet-gold 16-pin
Voltage Regulators		
LM336 2.5 V	1	P1 - black half-cylinder
LM340T5 5.0 V	1	P2 - black w/a heat sink
Resistors (in kOhms)		
10	2	R1 - grey trim-pot
20	3	R2 - grey trim-pot
50	1	R3 - grey trim-pot
100	1	R4 - grey trim-pot
10	3	R5 - cylinder
1.8	1	R6 - cylinder
1	1	R7 - cylinder
0.18	1	R8 - cylinder
0.1	2	R9 - cylinder

Table 23. Electronic components of blue circuit board of the DAS. (See Figure 12.)

B.2 Equipment

1. LOGARITHMIC AMPLIFIER Model E97 (built by the Electronic Shop, Department of Physics).

2. 15-V 100-mA POWER SUPPLY (designed by the Electronic Shop, Department of Physics).

3. GAMMA SCIENTIFIC DIGITAL RADIOMETER Model DR-2.

4. GAMMA SCIENTIFIC PHOTOMULTIPLIER DETECTOR Model D-47A.

5. GAMMA SCIENTIFIC SCANNING PHOTOMETRIC MICROSCOPE EYEPIECE Model 700-10-30X (Left ocular).

6. GAMMA SCIENTIFIC SCANNING PHOTOMETRIC MICROSCOPE EYEPIECE Model 700-10-34A (Right ocular with fibre optic).

7. Modified NIKON ZOOM-PHOTO SLIT LAMP MICROSCOPE.

8. 2-V VOLTMETER (built by Stephen CLARK to monitor the lamp intensity).

9. A MODEL EYE (built to required specifications by the Machine Shop, Department of Physics).

10. SPECTROTECH FILTERS:
    a. SE4 - excitor filter.
    b. SB5 - barrier filter.

11. KEPCO POWER SUPPLY Model RMK 09-S (for slit lamp).

12. OSBORNE 1 64K Microcomputer.

13. OKIDATA MICROLINE 192 Dot Matrix Printer.

14. HAYES SMARTMODEM 300 (for communicating with UBCNet).

15. FISHER ACCUMET Expanded Scale Research pH METER Model 320 (for preparing and measuring buffer pH).

16. IEC CENTRIFUGE Model CENTRA-4 (for spinning blood samples).

17. HAMILTON MICROLITER #702 Micropipette (for measuring out plasma samples).

18. SONOMETRIC Ultrasonic Digital Biometric Ruler Model DBR 400 (for measuring intra-ocular lengths).

19. CONTACT LENSES:
    a. Plano PERMALENSR Hydrophilic Contact Lens (soft lens).
    b. COOPER VISION Plano-concave Hard Plastic Lens.

20. RED TIP HEPARINIZED MICRO-HEMATOCRIT CAPILLARY TUBES (for collecting blood samples).

21. FUNDUSCEIN Fluorescein Sodium 25% Ampoules (for intravenous injections).

22. METHOCEL 2% Sterile (water-based, highly viscous methyl-cellulose for holding the hard lens in place, and, to provide the optical continuity between interfaces).

23. CYCOLGYL® 1% (Cyclopentolate Hydrochloride for dilating the pupil, and anaesthetizing the cornea).

24. M & B Fluorescein Sodium powder (for making calibration solutions).

25. MONOPAN 200 g Balance (for preparing calibration solutions).

B.3 Model Eye

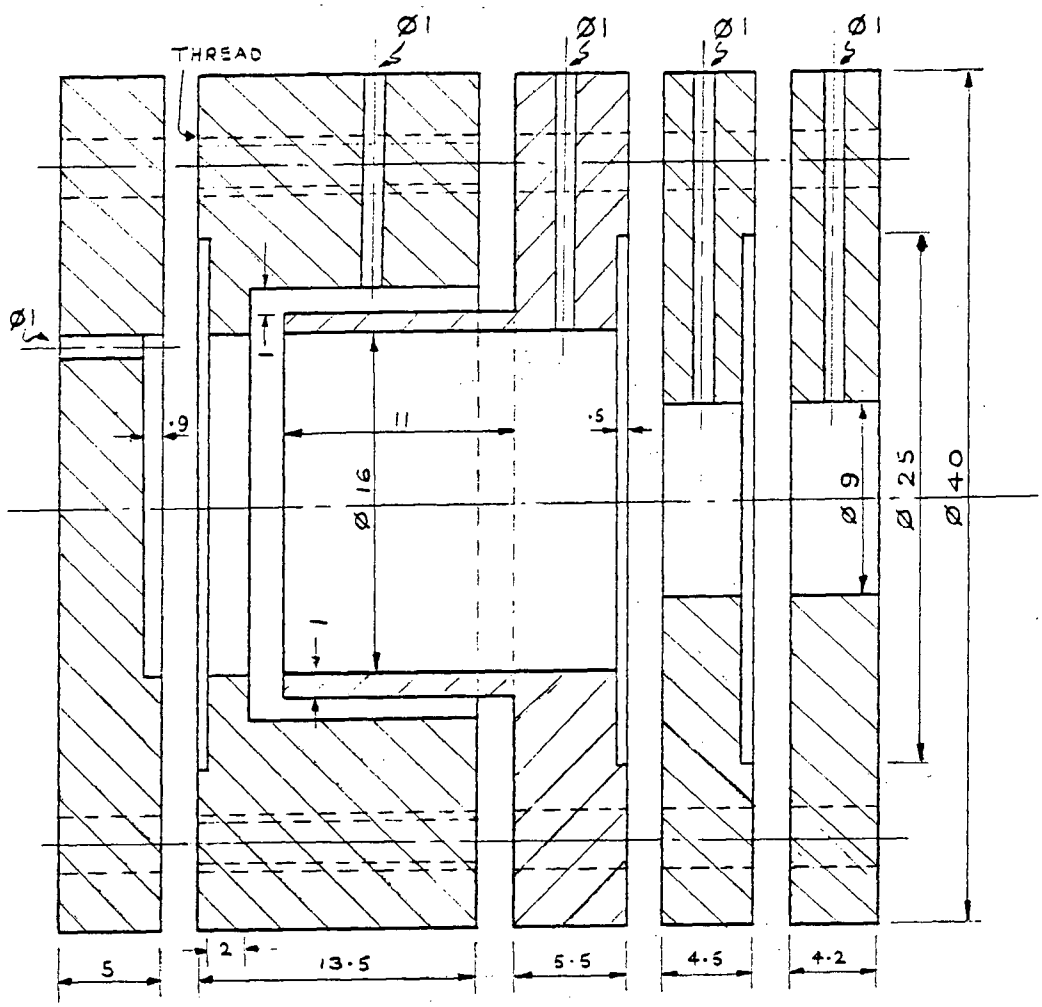

Figure 30. Cross-section of the model eye.

The cross-section of the model eye is shown below. The middle component is made of lucite (or plexiglass). All other components are of aluminium. All dimensions are in mm. The assembly is held together by four bolts (horizontal dash lines).

APPENDIX C

CALIBRATION RESULTS

C.1 Pod-DAS

The least-squares fit was to the straight line,

$$Y = A + B * X$$

by setting X = Osborne/DAS units, and

Y = translation in mm .

The gradient of the fit was found to be

0.095919 +/- 0.000070 mm per DAS unit.

The correlation coefficient was 0.999.

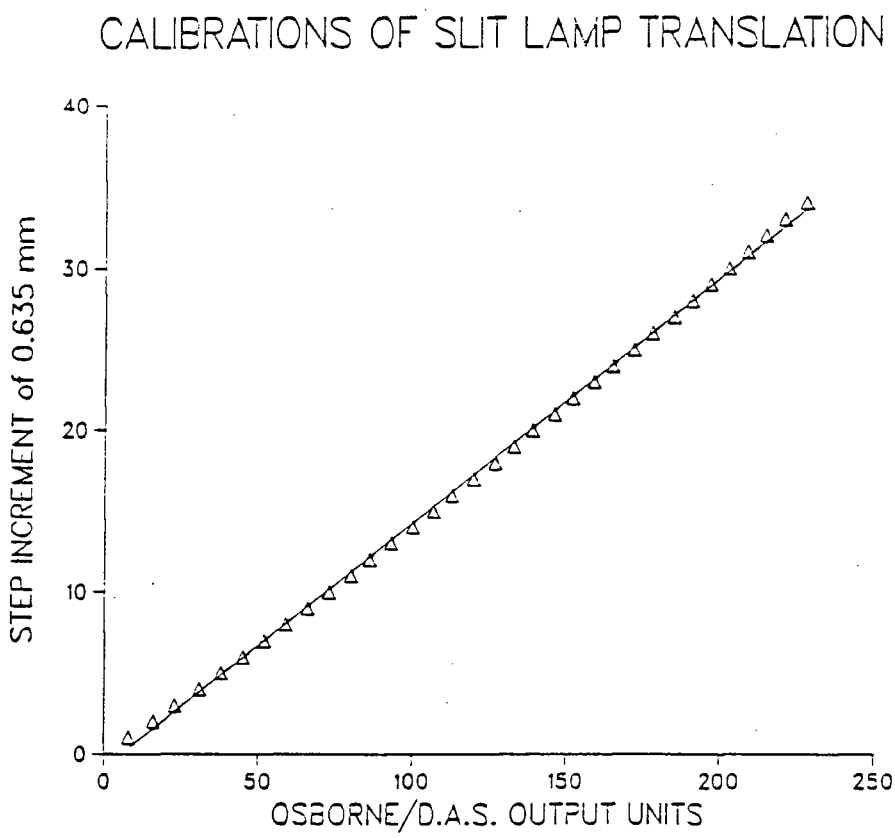

Figure 31. Calibration curve of the Pod-DAS.

DISPLACEMENT	OUTPUT/DIFFERENCE	
0	8	
1	16	8
2	23	7
3	31	8
4	38	7
5	45	7
6	52	7
7	59	7
8	66	7
9	73	7
10	80	7
11	86	6
12	93	7
13	100	7
14	107	7
15	113	6
16	120	7
17	127	7
18	133	6
19	139	6
20	146	7
21	152	6
22	159	7
23	165	6
24	172	7
25	178	6
26	185	7
27	191	6
29	197	6
30	203	6
31	209	6
32	215	6
33	221	6
34	228	7

Table 24. Results of Pod calibrations.

The data under the DISPLACEMENT column are multiples of 0.635 mm, i.e. the pod was advanced 1/40th of an inch at a time.

C.2 Logarithmic Amplifer

The least-squares fit was to a logarithmic function,

$$Y = A + B * \log X$$

where input voltages, X were in mV and output voltages, Y were in V. The correlation coefficient of this fit was 0.997.

INPUT VOLTAGE (mV)	OUTPUT VOLTAGE (V)
1.2	0.40
1.5	0.50
2.1	0.65
2.4	0.70
2.5	0.73
4.5	1.00
5.7	1.11
7.9	1.25
11.2	1.40
17.5	1.61
23.4	1.73
32.2	1.87
46.8	2.04
57.5	2.12
70.0	2.22
99.8	2.37
109.0	2.42
180.0	2.63
224.0	2.73
343.0	2.79
400.0	3.99
489.0	3.08
590.0	3.16
778.0	3.28
878.0	3.33
1000.0	3.38
1245.0	3.50
1574.0	3.60
1923.0	3.68
2230.0	3.75
3142.0	3.91
4085.0	4.02
5091.0	4.12
6000.0	4.20
7000.0	4.27
9000.0	4.38

Table 25. Results of Log Amp test.

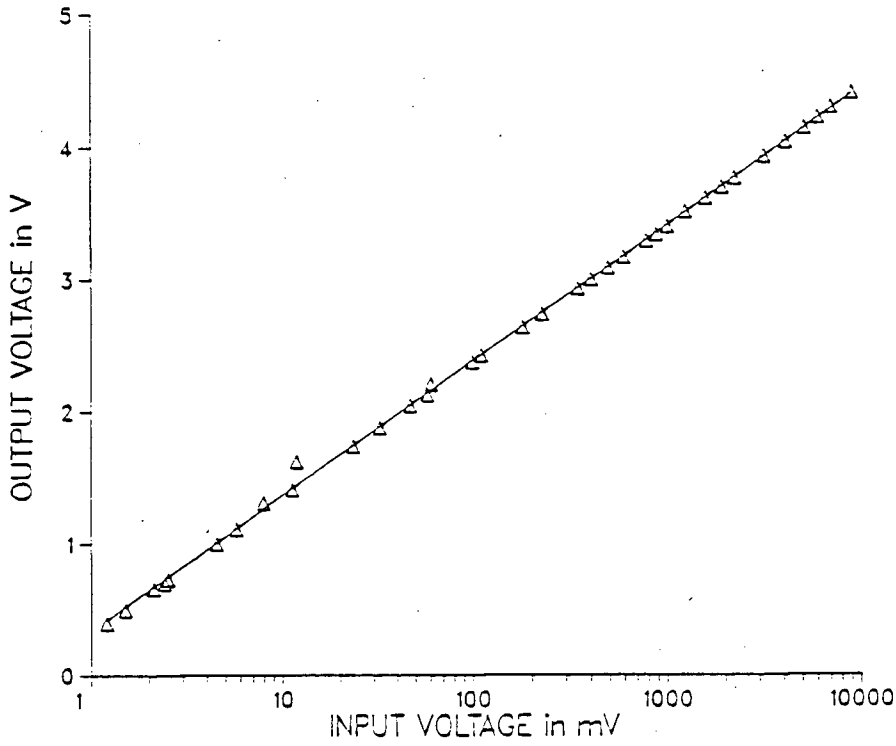

Figure 32. Performance of the Log Amp.

C.3 pH Dependence

Water		Buffer	
X	Y	X	Y
6.7	35.5	12.4	70
47	82	39	100
67	97	47.5	103
78	112	69.5	115
105	105	84	118
115	105	103	126
195	120	142	143
630	152	410	154
1830	172	870	179
3250	202	1220	178
3400	193	4850	219
8900	205	9258	228

Table 26. Water and buffer sample differences.

To test the pH dependence mentioned in Section 3.1, two sets of sample solutions were prepared. The first set was made with demineralized distilled water; the second set was made with the pH 7.4 Sorensen's buffer solution. (See Section 4.1.) Note that the buffer set was not the final calibration set given below in Appendix C.4.

The results of linear least-squares fit, of concentrations, X ($ng.ml^{-1}$) to DAS outputs, Y (Osborne/DAS units), were:

$$Y = -7.354 + 24.449 * \ln X \quad \text{for water samples,}$$

$$Y = 13.347 + 23.897 * \ln X \quad \text{for buffer sample.}$$

The correlation coefficients were both 0.99. The slopes of the two fits were not significantly different for a t(12-2) test (P = 5%). The "intercepts" were significantly different (P = 0.5%) Hence, buffer samples produced higher outputs than water.

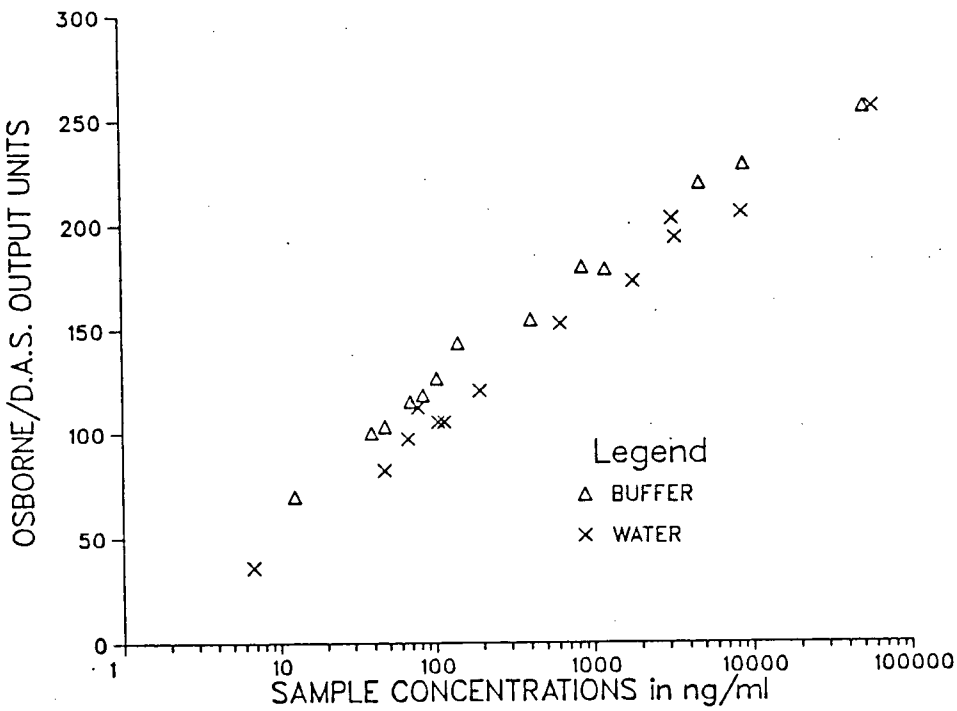

Figure 33. pH dependence.

C.4 R/M-Log Amp-DAS

Fits to two functions were done. They were:

a) $X = A + B * \log Y + C * (\log Y)^2$, and,

b) $Y = A * \exp(B * X + C * X^2)$,

where X = Osborne/DAS averaged result, and,

Y = sample concentration in $ng.ml^{-1}$.

Fit (a) was inverted, and then compared to fit (b) at every Osborne/DAS output. Fit (a) was chosen because it produced smaller deviations (especially at the lower concentrations). The reduced chi-square was 2.765. The coefficients were A = 4.47039 +/- 1.65113 , B = 25.0257 +/- 0.73117 , and, C = 0.11444 +/- 0.06722 .

The resulting equation on inverting fit (a), was

$$Y = \exp(AA + \sqrt{X/CC + BB}) ,$$

where AA = -109.339 , BB = 11915.9 , and, CC = 0.11444 .

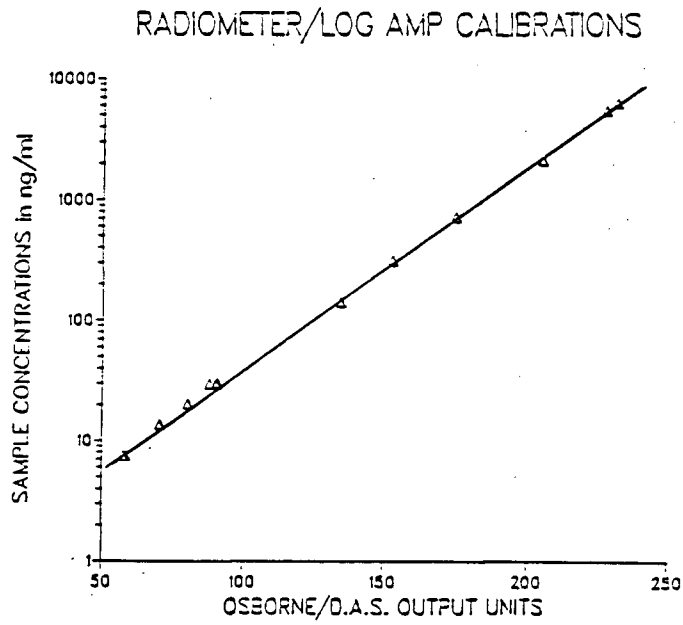

Figure 34. Calibration curve for R/M-Log Amp-DAS.

X	Y	Fit-Y	%
6357.47	231.34	1.07	0.46
5487.33	227.51	0.92	0.40
2143.54	204.83	-1.67	-0.82
719.50	174.66	-0.61	-0.35
313.79	152.80	-0.68	-0.45
143.01	134.50	-3.01	-2.24
30.41	90.26	1.00	1.11
30.41	90.58	0.68	0.75
29.87	87.95	2.85	3.24
29.87	90.09	0.71	0.79
20.42	80.17	0.84	1.05
20.42	80.25	0.76	0.94
13.78	70.08	0.81	1.16
13.78	70.37	0.53	0.75
7.52	58.24	-2.81	-4.82
7.52	57.32	-1.89	-3.30

Table 27. Calibration results of the R/M-Log Amp-DAS.

Note that several scans were made at the lower concentrations. This was to "weight" the lower part of the calibration curve during curve-fitting. The reason for doing this was to "improve" the calibration curve in this region so that PMT and R/M noise during the calibration procedure would not adversely affect the curve-fitting process.

C.5 Attenuation

The effects of the attenuation was studied during the calibrations of the R/M-Log Amp-DAS. Scans through the sample cells holding various sample concentrations were made.

Figure 36 shows two samples where attenuation effects were seen. The peak was the position when the entire diamond was in the solution. Attenuation effects were studied by re-zeroing the translation axis of each scan at the peak; then estimating the (negative) slope (if it existed).

Refraction caused the displacement of the slit lamp to be different from the diamond, like the F-numbers (in Figure 3). The conversion is given below.

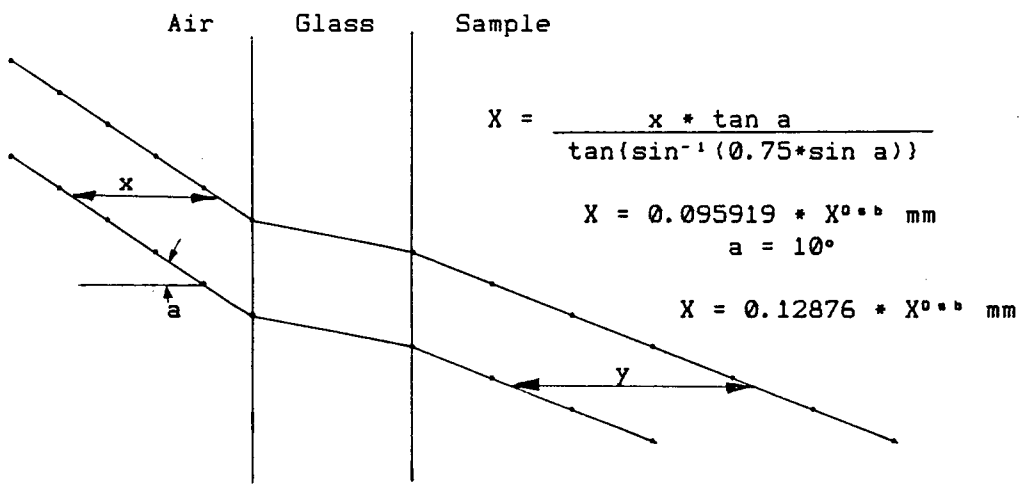

Figure 35. Refraction at sample cell surfaces.

Consider a profile where significant attenuation was observed. The negative, concentration-dependent slope, B(c), in the semi-log plot is found (from Eq. 5, Section 2.2) to be

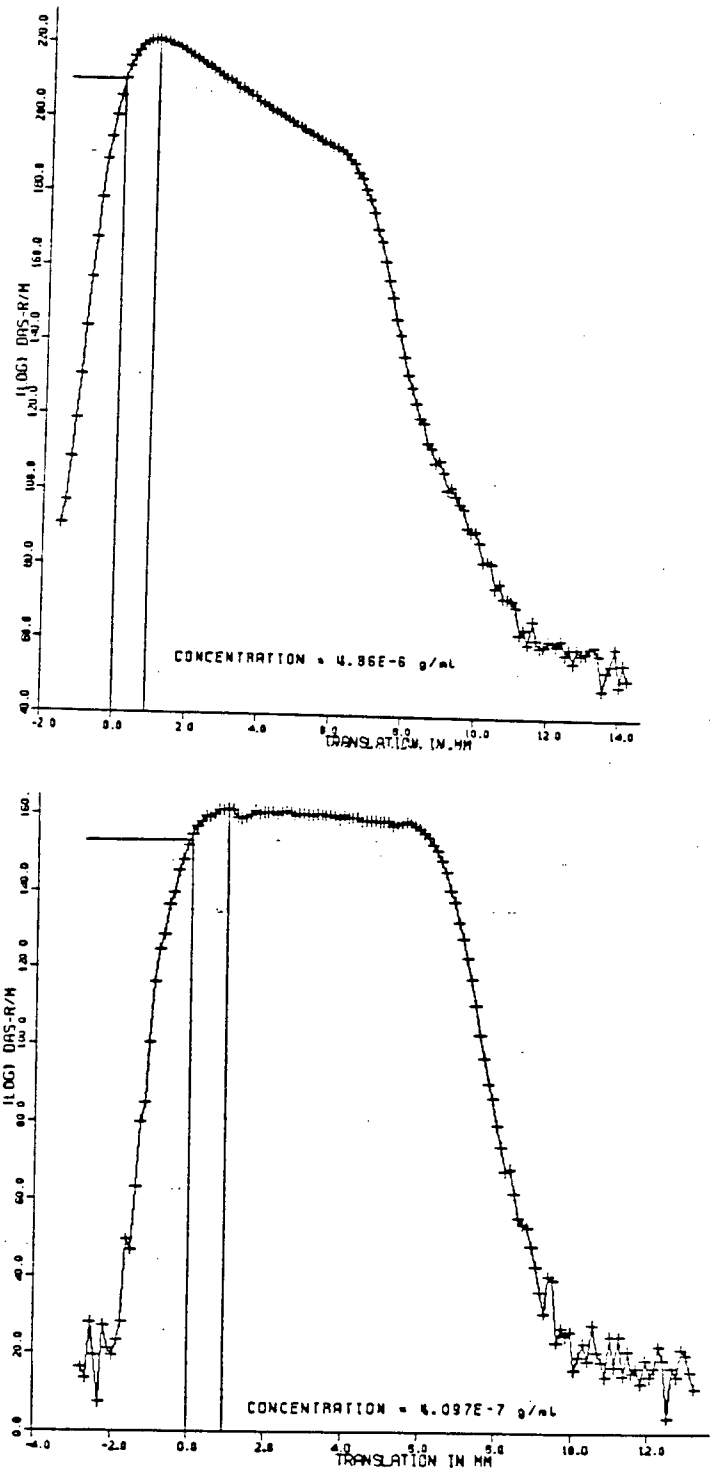

Figure 36. Attenuation in sample solutions.

$$c = A(c) - B(c) * X ,$$

where c and X are the concentration and translation respectively. A(c) is a "constant". B(c) represents the decrease in log-concentration per unit increase in the distance the probe focus "penetrates". When B(c) is small, there is little attenuation. Hence, an estimate of the concentration at which B(c) is small approximates the lower limit above which attenuation should be taken into consideration. The table below gives the results of the straight-line fits to the profiles and their respective correlation coefficients.

c $ng.ml^{-1}$	B(c) mm^{-1}	r
9493	0.289	0.994
6357	0.240	0.999
5487	0.182	0.999
2143	0.055	0.982

Table 28. Concentration and gradients of attenuated samples.

A least-squares fit to

$$B(c) = AA + BB * c$$

was made. The results were

$$AA = 1.08*10^{-3} \text{ and } BB = 3.25*10^{-5} ; r = 0.97 .$$

Hence, for B(c) = 0, c = -33 $ng.ml^{-1}$!

From Eq. 5, for a mean aqueous chamber depth of d(=X) = 3.5mm,

at c^A = 1000 $ng.ml^{-1}$, $B(c^A)$ = 0.034,

attenuation < 12% ;

and, at c^A = 100 $ng.ml^{-1}$, $B(c^A)$ = 0.0043,

attenuation < 2% .

C.6 Performance Data

CHARACTERISTIC/PARAMETER		UNITS
Slit width	0.1	mm
Slit height	2	mm
Probe diameter	0.45	mm
Beam-Probe angle	16	°
Lamp intensity monitor	141(1)	mV
Filter overlap at 502.9nm	0.5	%
Max. slit lamp displacement	24.5	mm
Max. in vitro concentration	6000	$ng.ml^{-1}$
AR in vitro	3	mm
AR ratio in vivo on Normals	0.032	
LLoD in vivo	4.4	$ng.ml^{-1}$
Sensitivity at 20 $ng.ml^{-1}$	0.77	$ng.ml^{-1}Osb^{-1}$
Sensitivity at 2000 $ng.ml^{-1}$	85.16	$ng.ml^{-1}Osb^{-1}$
EoM at 20 $ng.ml^{-1}$	-0.03	
EoM at 2000 $ng.ml^{-1}$	0.06	
R in vivo	19	%
No. of data-points in 10 s	800	pairs

Table 29. Performance characteristics of the VF system. Sensitivity and EoM are in vitro estimates using the calibrations in Appendix C.4.
Note: Sensitivity is defined as gradient of calibration curve at given concentration.

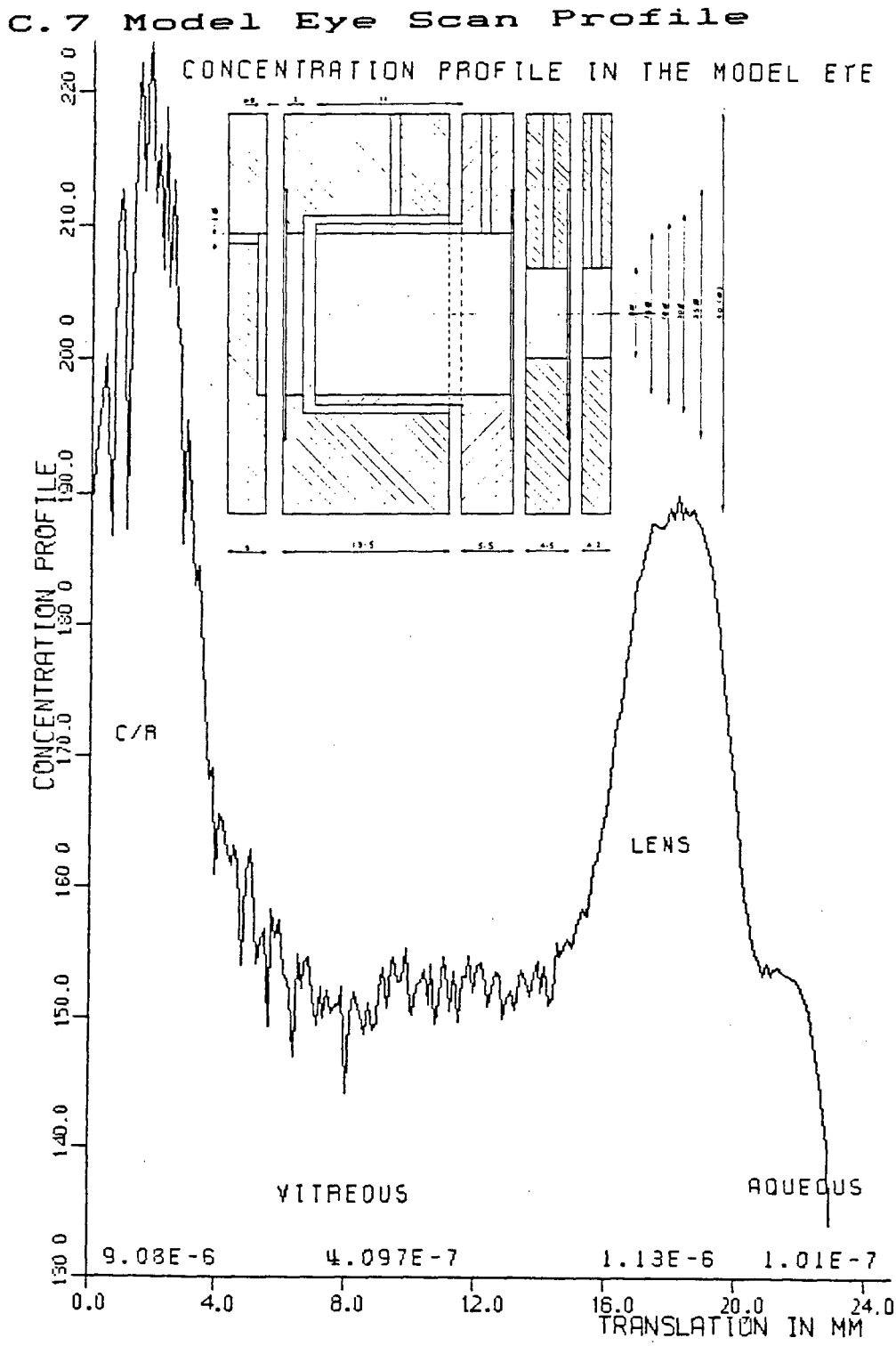

Figure 37. Model eye sample profile.

APPENDIX D

GLOSSARY

BLOOD-RETINAL BARRIER - The barrier that separates the retinal neural tissues from the blood.

BOLUS - A concentrated mass of pharmaceutical preparartion given intravenously for diagnostic purposes; a mass of scattering material.

CHOROID - The network of small blood vessels immediately behind the retina.

DIABETIC RETINOPATHY - A non-inflammatory disease of the retina due to diabetes which can lead to blindness.

ENDOTHELIUM - The layer of epithelial cells that lines the cavities of the heart and of the blood and lymph vessels, and the serous cavities of the body.

EPITHELIUM - The covering of the internal and external surfaces of the body, including the lining of vessels and other small cavities. It consists of cells joined by small amounts of cementing substances.

EMMETROPIC - When rays entering the eye parallel to the optic axis are brought to focus exactly at the retina.

FENESTRATED - Pierced with one or more openings.

FUNDUS - That part of the back of the eye furthest from the pupil.

HAEMOGLOBIN - The red pigment of the blood carried by the red blood cells; is composed of globin (a protein) and haem (an iron compound); is the means of oxygen transport.

HAEMACYTOMETER - Instrument for counting blood corpuscles.

HEMATOCRIT - Volume % of erythrocytes in whole blood. Originally applied to the apparatus of measurement.

Many of the above descriptions were summarized from "The Penguin Medical Encyclopedia" by Peter Wingate (U.K. 1976), or, from the "Dorland's Illustrated Medical Dictionary", 26th ed., published by W.B. Saunders Co., (Toronto, 1981).

HOMEOSTASIS - The primary function of most organs; the processes of maintaining constant physical and chemical conditions within the body despite external changes.

HYPERTENSION - High blood pressure secondary to specific disease, or, essential.

INFILTRATE - To penetrate the interstices of a tissue. Material deposited by infiltration.

JUNCTIONAL COMPLEX - The intercellular arrangement between the adjacent columnar epithelial cells.

LESION - Any pathological or traumatic discontinuity of tissue or loss of function of a part.

LUMINAL SURFACES - Surfaces of the cavity or channel within a tube or tubular organ.

MACULA - Usually the retinal macula. In general, any area that is distinguishable from its surrounding by colour, etc.

MICROANEURYSM - Bulge at the weak point in the wall of an artery.

MULTIPLE SCLEROSIS - A chronic central nervous system disease in which the nerve fibres lose their protective myelin sheaths and their ability to conduct impulses.

OPTIC DISC - The intraocular portion of the optic nerve formed by fibres converging from the retina and appearing as a pink to white disc.

PERIPHLEBITIS - Inflammation of the tissues around a vein, or of the external coat of a vein.

PLASMA - Sticky, pale amber liquid with faint, sickly smell; the medium in which vital substances are transported to all body tissues; a solution in water of salts, proteins, glucose, etc.

SEQUELA - Any lesion or affection following or caused by an attack of disease.

ZONULAE OCCLUDENS - That portion of the junctional complex of columnar epithelial cells, just beneath the free surface, where the intercellular space is obliterated. It extends completely around the cell perimeter. Also called "tight junctions".

APPENDIX E

ABBREVIATIONS USED

A	Operational Amplifier; Figure 12.
ADC	Analogue-to-Digital Converter; Figure 12.
AR	Axial Resolution.
AVG	Averaged data (by RET); filetype.
BAB	Blood-Aqueous Barrier.
BRB	Blood-Retinal Barrier.
b	attenuation or extinction coefficient; Eq. 5.
CR	Choroid-Retina(l).
CRP	Alignment by CR peaks; filetype.
c	concentration ($ng.ml^{-1}$); superscripts for various uses.
D	Diffusion constant; superscript, W means in water.
DAS	Data Acquisition System.
DAT	(Raw) Data (filetype).
DRP	Diabetic Retinopathy.
DVM	Digital VoltMeter; Figure 8.
d	Usually depth, or distance, or displacement.
EoM	Error of Measurement.
H^o	Null Hypothesis.
H^a	Alternative Hypothesis.
hex	hexadecimal (base-16) number(ing).
LLoD	Lower Limit of Detection.
Log Amp	Logarithmic Amplifier.
MS	Multiple Sclerosis.
MUX	MUltipleXer (analogue electronic switch); Figure 12.
P	Probability of Type I error - rejecting a true H^o; Permeability constant; permeability index if with superscript I.
PMT	PhotoMultiplier Tube.
PR3	Penetration Ratio averaged about 3mm from retina.
PR3*	Penetration Ratio at 3mm from retina.
p.i.	post-injection.

R	Reproducibility; Eq. 27.
RET	Alignment by visually pin-pointed RETinae; filetype.
RPE	Retinal Pigment Epithelium.
R/M	RadioMeter.
S.D.	Standard Deviation.
S/H	Sample-and-Hold electronic chip; Figure 12.
$t^{(1)}$	First blood sampling time.
VF	Vitreous Fluorophotometry (Fluorophotometric).

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