# RETRO-TRANS-ILLUMINATION (*)

## Photomicrography (**)

AVELINO GOMES DA SILVA, M. D. (XXX) — São Paulo

In our first article on R.T.I. (7) we established the foundations of what seemed to us a new method of biomicroscopic examination. In a later publication (8), when referring to the technique of this method we admitted that same was already known to a few authors and regretted that the 2nd volume of BERLINER's (1) book only reached our hands after the publication of our **Preliminary Report.**

In spite of being working in a field that is not new as is seemed to us at first, we know little from the authors who made use of it. This was the reason why we are making an effort to continue to make public what we have been able to obtain through such a useful semiotic means as this. It is for this reason that we are now publishing the third article from a series. We consider these articles of interest to the practitioner oculists as well as to those doing research work. In view of the practical results that such a method of examination has given us, we commend DAVIDSON's (3) attitude when he praises DIAPUPILLARY TRANS-ILLUMINATION for the examination of the pathological findings fo the cornea. This method is in fact of unquestionable value in the study of the cornea by making possible the observation of its pathological details with great accuracy. But this does not exclude it for the study of deeper regions of the eye, especially the lens. We are so much convinced of it that, for quite some time now, we introduced this biomicroscopic method into the routine of our clinical examinations.

Among all the advantages that this method of examination provides us, none is so important as the possibility of photographing what is being examined. We believe this photographic process to be an original one and because we consider it so easy and simple cannot conceive that it was not foreseen. For the photography of details has always constituted one of the greatest problems for ophtalmologists. We can add to this photomicroghaphic process the absence of complicated and expensive equipment together with the brilliant results it can give us. We shall therefore be able to give evidence as to the value of R.T.I. and to admit to its useful participation in future ophtalmologic research.

---

(x) Research work under execution in the Institute of Trachoma and Visual Hygiene of São Paulo, Brazil.
(xx) Report to the Ophtalmologic Society of São Paulo on September 14, 1949.
(xxx) Oculist of above mentioned Institute.

Photomicrography through R.T.I. does not require any device or phographic trick. Just a slit lamp and an intermemiary simplified **Leitz** adaptable to any microscope are sufficient. The process is simples and elementary. It is always the same, whether it refers to photographing aspects of the external surface of the cornea or pathological findings more deeply situated, as for instance in the lens or in the anterior third of the vitreous.

For many months we have been devoting ourselves to this type of photography. We find it becomes more and more simple and compensating when compared with the complicated and old fashioned tec niques of some time ago. It must be said, for truth's sake, that this type of photography only shows good results when the ocular means are in such a condition as to be run through by the immergent and emergent ray. Furthermore it is necessary that the bodies have a relative opacity in order to be photographed, because if they are too transparent they do not produce enough contrast to impregnate the emulsion of the film. If they are too dense they do not give an accurate picture. Therefore, there is an optimum of light and an optimum of image in order that a picture 100% accurate is obtained. But this results merely from practice on the operator's part.

We use for our photographs **Haag-Streit's** slit lamp to which we adapted KOEPPE's mirror by **Bausch-Lomb** (fig. n. 1) in order to reduce the angle of incidence of light. POSER's slit lamp (Bausch-Lomb) dispenses with any adaptations because it is already equipped with an attachment permitting KOEPPE's mirror to be millimetrically moved forward or backward, thus greatly facilitating our task.

We use as luminous source only that of the slit lamp at its maximum intensity. This in order to reduce the time of exposure, which is an essential factor for the success of the procedure. We always use the slit wide open but of the conditions of the eye under examination are very good, the slit can be used at medium opening. This will of course always depend on the conditions existing at the moment. Practice will set the rules to be followed.

The microscope is the same corneal microscope of the apparatus being used. For system's sake we always used the small magnification it provides. Thus we can cover a much larger area to be photographed, wich gives us a better idea of the situation and also a greater accuracy of details to be studied. We use the maximum magnification of the microscope for photographing details only, whenever the conditions of the eye allow it.

We use as photographic camera a simplified but excellent intermediary made by **Leitz** (Micro-Ibso-Attachment)to which we adapted a common camera (fig. n. 2). We made this adaptation because the **Leica** camera which is normally adapted to the intermediary, provides very small pictures only, about 2,5 x 3 cm., in number of 36. Our adaptation besides supplying larger pictures, about 4 x 5 cm., uses n. 127 film, which is cheaper and gives 16 pictures. This facilitates the development process later. We are at present trying a new adaptation in order to make possible the uses of ns. 120 and 620 films. These are more easily found in our market and among them it will be easier to find films for colour photographs which we are planning to take.

We have been using as an ocular only that which accompanies the intermediary. i.e., a **10 x Zeiss** ocular. Other oculars of different values can be used provided they can be adapted to the intermediary. We have been able to measure with absolute accuracy the magnification obtained, by means of paper indicators of previously known sizes adherent to the patient's cornea. Such a magnification is of exactly 10 diameters, which is sufficient for us, the rest being done with calculated magnifications.

As a preliminary technique we focus the object to be photographed using the corneal microscope separately. After making sure that everything is in order we request, the patient not to move his eyes, and removing the microscope ocular, substitute for it the one which comes already attached to the intermediary. This done, we focus again using the side ocular existing in the intermediary. The former, being equiped with a reticule, facilitates to bring into focus the image to be photographed. The examination is a monocular onde and therefore quick and easy to perform. When examining through the side ocular of the intermediary, we find that the image observed is always less intensely illuminated than the one supplied by the corneal microscope. We wish to call attention to this fact because it might seem that with the preceding operations a deviation of the incidence of light has taken place. This might confuse the beginner, who will waste time trying to obtain a better focus, thus risking to lose good opportunities. The reduction of the luminous intensity supplied by the side ocular of the intermediary is caused by the prismo existing between the latter and the microscope ocular. As the prism automatically recedes the moment we release the catch of the intermediary, the image that reaches the film has the same luminousity as that observed through the microscope ocular.

After contless experiments with different films, we established a preference for the **Super XX** film, made by Kodak, which has been the one to offer better contrast. All similar films that we used, although they were good, did not satisfy us as the **Super XX,** and in fact we are indebted to it for excellent pictures. It must be remembered that we use light reflected by the retina, which is of a red-orange shade and of a very weak intensity. It is therefore necessary for the film to be very sensitive to ordinary as well as to red light, as is the Super XX.

We tried several times to obtain colour pictures but up to present moment failed for the reason that light reflected by the retina has not sufficient power to impregnate the films existing in our market. These require a longer exposure to light than the one we use. Perhaps with the use of special films as Kodakchrome we can see this wish of ours changed into reality. We are also studying the possibilities of adapting a movie camera for the dynamic study of circulation in the corneal, parenchymatous or this method of biomicroscopic examination.

After innumerous tests on the best time of exposure we reached the conclusion that same should always be of 1/5 of a second. With this we have obtained the best pictures. Whether of the cornea or of the deeper parts of the eye. If we extend the time of exposure to 1/2 of a second we always obtain a double image. This attracts our attention for it must be related to an imperceptible physiological trembling of the eyeball. In order to elucidate this point we are photographing very small strips of paper of previously known sizes, placed upon the patient's cornea. Our purpose is to determine the dislacement, direction and speed of this movement. With an exposure above 1/2 a second we have obtained nothing because the eye trembles, throwing the image out of focus, which in turn often results in several images. With a time of exposure inferior to 1/2 a second we also can also can obtain good pictures, the majority of which however offer little contrast, thus making impossible enlargements beyond a certain limit.

In our daily experiments with R.T.I. we have obtained a very to be put before us. Today we shall limit ourselves only to making as we go on studying the clinical cases of greater interest that happened large amount of good pictures. We intend to give publicity to them comments on certain pecularities of the subject which is absorbing us at present.

For better emphasizing the remarkable possibilities provided by photography through R.T.I. we shall now make a brief survery of what we have been able to study thanks to such an extraordinary semeiotic means as this.

**CÓRNEA:** — This ocular membrane is reached by R.T.I. in all its extension, from the zone nearer to the limbus up to the central area, where but a small portion might offer certain difficulties to examination. This however can be overcome through skill and practice. In the cornea there exist 3 layers which become well differentiated by pathological changes that can be registered by photography through R.T.I. Tey are external surface, parenchyma and internal surface.

**External surface of the cornea:** In this layer we have to consider in the first place the lachrymal film which spreads all over the cornea. This film presents normally an infinity of small air bubbles which appear in R.T.I. with a beautiful aspect, black-red or yellow coloured, depending on the incidence of light reflected by the retina. These formations are photographic thus making possible the study of the variations they show in patients suffering from diseases that alter the lachrymal secretion or in those who are making use of oily collyrium. In the latter besides the small air bubbles peculiar to the tear, an infinity of oil droplets appear and these are of a very interesting aspect and of great refringency.

Still in the external surface of the cornea the keratitis must be considered, which may or not show sufficient contrast for good photography. Ulcerations, foreign bodies and desiccation caused by air also can be present and we have been able to photograph their evolution in the different stages. Pterygium, etc., can also be found, and as to the pterygium, they are easy to distinguish when they cover a good part of the cornea and can offer splendid photographic effects. R.T.I. is therefore an excellent means of registering their details. Figure n. 3 shows an excellent photomicrography of anapex of pterygium, in which we are able to distinguish only the body of this formation bul also its interesting vascularization. Further more we can distinguish its invading extremity which actually resemble "pseudopodes". This photograph is equivalent to an X-ray plate of a pterygium, and according to it this pathological formation shows us an entirely new aspect.

Still in the external surface of the cornea we must finally consider the study of vascularization, whether or not trachomatous. This appears through R.T.I. under a new aspect and requires therefore more detailed considerations which are too lengthy to quote here. The

Photomicrography through R.T.I. can be achieved with complete sucess provided that the object to be examined is placed in such a manner as to be retro-illuminated by the rays of light reflected by the retina. Thus, everything within the illuminated pupillary area is able to offer sufficient contrast for a good impregnation of the film. Objects situated more to the periphery of the pupillary area are more easily focused for they always receive more light. Objects situated in the centre of the pupillary area are hardly perceptible and require special operations in order to be individualized. As the illuminated area is always hemi-pupillary we usually take two photographs, one of each side in order to obtain the entire pupillary area. However, its central region sometimes appears less intensely illuminated, which makes it rather difficult to study the objects situated in it. A situation in the sense of depth is no obstacle for a good picture, except for what is situated beyond the anterior third of the vitreous and therefore far aways from the reach of the corneal microscope.
superficial vascular networks of the cornea appear very clearly and separated in R.T.I. in such a manner as to make possible the photographing not only of their elements but also of the actual blood column circulating in them.

The normal vascularization of the limbus in certain cases, and the pathological vascularization in nearly all of the rest are a motive for beautiful and accurate pictures. In these we can easily distinguish the arteries and veins, and in the more perfect ones we can study the circulation, conglomerated into points or rods. These are intercepted by the photographic camera and appear very clearly in the area outlined in figure n. 4, which represents the rich and interesting superficial vascular network of a cornea in a patient with keratoconjuntivitis of the eczema type. Trachomatous vascularization supplies a great deal of subject to study because it can be observed under all its aspects, for it appears isolated as though it were a net hanging before a window illuminated by a red-orange light. We are thus able to study the position of the vessel, observing that the arteries are in a deeper layer, as already stated by MORETTI, tha they are lesser in number and have more straight paths and that they are divided only in the zone further from the pannus. The veins have a greater diameter, winding paths and are more numerous, occupying a more external layer. All this can be seen in picture n. 5 the aspect of which, we think is original with regard to the recording of the trachomatous pannus.

The photographic registration of the superficial vascularization of the cornea the way we do it is of real value. Its elements are photographed under physiological conditions, nothing existing that can be a cause of error, except the heat of the light running across the cornea, the minimum action of which sould however be overlooked.

It is a well known fact the zeal existing among researchers in order to make more noticeable the vessels that appear on the surface of the cornea of animals or man in cases of deficiency of vitamins. They are so important in the pathogenesis of the deficiency of vitamins that their search and registration are obligatory in the study of such diseases. In order to give evidence to existence of these vessels of the external surface of the cornea of animals and man, COCHRANE and assistants (2) invented an interesting photomicrographic method of great exactness. These authors, after the patient or animal dies, inject India ink into the ophtalmic artery and after dissecting the cornea submit it to the light of the slit lamp, photographing the vessels of the limbus by transparency. The vessels thus photographed appear well individualized, but their extremities being thinner, are not visible or are only partially so and in a divided manner, which by the way does not demerit this method of study. Now, COCHRANE and assistant do with the corpe what we are doing with the living, therefore with more probalities of sucess. We are also nearer truth since we photograph the vessels in normal conditions of life. This is also possible experimentably at least in the dog, as we shall demonstrate further on. This is by consequence a contribution R.T.I. makes to us, the value of which cannot be doubted by anyone.

**Corneal parenchyma:** — In this layer of the cornea only two peculiarities are of interest to photomicrography: infiltrations and vessels. Infiltrations into the parenchyma need be somewhat thick which is hte infiltrated cornea.

**Internal surface of the cornea:** Among the pathological findings subjecto being photographed through R.T.I. two are of great importance because they constitute subject of daily observation. We refer to the folds of Descemet's membrane and to the keratic precipitates common to all cases of iridocyclitis. The folds of Descemet's membrane easily photographed but require great practice on the operator's part, because they need a good incidence of light in order that they produce enough shadow to make them distinguishable. According to the incidence of light they can either disappear completely or become less distinguishable from the red-orange backgroud, thus not allowing good

pictures. Figure n. 7 reproduces a successful photomicrography of the folds of Decemet's membrane, made through R.T.I. It represents no in order to offer good contrast, since if they are obscure they are run through by emerging light and cannot therefore be in evidence. The parenchymatous vessels are as perfectly identifiable as those of the external surface of the cornea, but not so easily photographed. We can also study in a special manner each vessel individually, as well as study in a confortable and lenghty way its circulation. Fig. n. 6 shows a good aspect of a large vascular network of the corneal parenchyma in a patient suffering from parenchymatous keratitis in convalescing form. By comparison we can have an idea of the greater visibility of the superficial vessels as shown in the previous figures. This can be understood once one knows that the parenchymatous vessels are situated in a deeper layer, immerse in a less transparent tissue doubt a photographic aspect little known in ophtalmology.

The precipitates of the internal surface of the cornea are another subject for excellent pictures through this method of examination. In a recent publication (9) we even suggested the photographic method of such precipitates as a means for the study of the evolution of uveitis, allowing us to meausre the development of the disease by analogy to what is done with temperature, weight and pulsation charts and other succeeding clinical examinations. As we stated in the above mentioned article, the precipitates of the internal surface of the cornea can be studied in detail either as a whole or individually. We are therefore able to measure the increase or reduction in the number of precipitates, its morphologic aspect, and, what is more important, study in succeeding photographs the evolution of a group or even of a sole precipitate. We can thus obtain a chart of the development of the disease that originated it. Figure n. 8 shows a picture of a group of precipitates in the internal surface of the cornea, from a patient suffering from acute uveitis. In this as well as in many other patients we were able to study the evolution of the disease from its beginning till the complete disappearance of such pathological formations.

**IRIS:** This ocular formation is o flittle use to examination through R.T.I. in spite of BERLINER's (1) contrary opinion, because the iris should be distended in order to be examined. This is practically impossible since for the good performance of this examination it is necessary to have at least an average mydriasis. However, it is in fact possible to examine the atrophic regions, where the thick-

ness of the iris is diminished, the holes it might show and certain formations of the free edge of the popil. Thus we can photograph rather easily the remainings of the pupillary membrane and all the outstanding formations of the free edge of the pupil, such as small eventrations of the uvea, etc.

**LENS:** The lens, like the cornea, also presents for examination three distinct layers. The pathological finding in these can easily be photographed through R.T.I. We have accordindly the anterior capsule, the peculiar mass to the lens and the posterior capsule.

**ANTERIOR CAPSULE OF THE LENS:** This anatomic ofrmation is easy to find when one guides oneself by the edge of the pupil to focalization. On this surface a few minimum ondulations can be demonstrated, and we actually have the picture of a small projection well individualized, as well as rests of the pupillary membrane adherent to it. Below this membrane are frequently encountered small vacuoles of the lens nearly always elongated or pear-shaped. These are more frequently localized in the periphery of the lens and appear more commonly in patients having incipient cataract. These formations, like the folds of Descemet's membrane require an excellent incidence of light in order to serve as subject fit for photography. Our figure n. 9 reproduces a good aspecto of some anterior sub-capsules vacuoles, taken from the lens of a patient suffering from complicated incipient cataract. In it can also be seen a few synechiae from the iris to the lens, some of which are broken uf due to the action of the mydriatic.

MASS OF THE LENS: In this layer, besides the already mentioned vacuoles, which also can be situated more deeply, we have been able to photograph various aspects of incipient cataract and to study in detail the delicate aspects that these pathological formations show. We have also been able to accompany with the photographic camera the development of opacity. This enables us to know how the lens is taken up by the pathological elements which invade it. Still in the mass of the lens it is possible to photograph the "Y" shaped anterior and posterior sutures, especially when they are very thick and opaque as in certain cases of congenital cataracts.

**POSTERIOR CAPSULE OF THE LENS:** This layer, when normal, is of difficult distinction through R.T.I. This membrane, however, when having incipient opacity shows us beautiful photographic aspects of its "chagrin", appearing us a delicate and complicated network of microscopic elements. Still in this layer it is

possible, with reference to patients showing a permanency of Cloquet's canal, to photograph its anterior portion, in spite of the that some is difficult to put in evidence because of its situation in a more central zone.

**VITREOUS**: Beyond any doubt, only the anterior third of this ocular formation is photographic through R.T.I. Furthermore, this is only possible when it has already suffered pathological changes. In its normal condition the vitreous is practically thansparent to this method of examination. In the pathologic vitreous it is possible to photograph some tsring shaped, head shaped and strip shaped formations, which are darker or lighter in accordance of the degree of the attack suffered. If the opacities from the vitreous are too thick they hamper the good penetration of immergent and emergent rays and consequently also the obtention of good pictures. We therefore admit that with regard to the vitreous, photomicrography through R.T.I. is not a choice method, but we hope that through improving the thecni que used we shall be able to obtain better results.

We have examined experimentally through R.T.I. laboratory animals, photographing several findings that seemed interesting to us. For these studies we used Rezende and Celeste's prism (5) which we adapted to Gullstrand's (6) slit lamp, making also use of the modification to the mechanical part or that slit lamp as proposed by REZENDE (4) for biomicroscopical examination in dogs.

With a certain facility we have been able to carry out R.T.I. in the dog, the rabbit and the rat. In the dog as in man it is possible to study the entire corneal surface, including a small part of the limbus, which is impossible to do inthe rat and the rabbit due to anatomical reasons. While dog's lens, iris and cornea are similar to man's, in the rat and rabbit the cornea is extremely curved, the iris is also curved and the lens is practically globular. This produces a very large peri-limbic zone of the cornea, which cannot be illuminated by rays reflected by the retina. This is due to the large screen constituted by the iris, fact that makes impossible the examination of the edge of the cornea, unless a surgical iridodyalisis is previously performed.

In the albino rat, however, due to complete absence of the iris pigment, not only the edge of the iris can be studied but also the normal vascular notwork itself. The pupil need not be dilated and photography can be performed without difficulty. Figure n. 10 shows

a beautiful vascular network of the iris of an albino rat, photographed thanks to R.T.I. in which, as one can see, the pupil was in miosis

The photography of the vascular network of the iris of albino rats through this method of examination, can bring up new discoveries in the experimental field of miotics and midryatic as well as in that of vaso constrictors and vaso dilators, which we have been able to submit to research work, in a few attempts,together with physicians Sergio Aranha Pereira and José Papaterra Limongi, of the Pharmacology Department of the Faculty of Medicine. To perform this it is sufficient to provide an elementary processo for keeping motionless the animal, which by the way must be alive and sane, and a mechanical system for keeping the eyelids open. The latter we obtained by using minute steel wire speculum similar to Graefe's.

Conditions for good photography through R.T.I. in laboratory animals are the same as described for photography in man. Results are always very satisfactory. especially when we work on anesthetized animals with their reflexe actions consequenttly abolished.