mediately alter exposure rather than 2 or 3 weeks later.

Data from Tables I and II indicate that there is a gradual, or progressive, loss of photoreceptors from the light-damaged retina during the 10 to 14 days subsequent to the exposure period. Quantification of photoreceptor damage at any single period prior to that time would represent the condition for that particular postexposure period but would not be a true indicator of the amount of total damage to the retina during previous light exposure. The present results therefore indicate that an evaluation of light-induced retinal damage should be made only after a postexposure stabilization period of 10 to 14 days.

It is possible that fluctuations in the ONL and retinal thicknesses related to factors other than photic influences may have occurred in the Wag/Rij rats at 2 and 28 days (Tables I and II, Figs. 1 and 2). These factors perhaps were genetic (as compared to the SD rats), due to increased edema at particular time periods after exposure, or due to slight, uncontrollable variations in histotechnique. There was a definite difference in the susceptibility of Wag/Rij animals to photic damage as compared to the SD rats, as evidenced by the final differences in the ONL and retinal thicknesses as well as the rate of clearance for damaged cells during the postexposure period. It is also interesting that the development of the genetic retinopathy in the Wag/Rij strain during the 56-day experimental period (LDn vs. LD2 = 22% reduction) was comparable to results extrapolated from the earlier histopathologic study of Lai et al.6

The dissimilarity in the susceptibility of the superior and inferior retinal hemispheres to photic damage in rats without genetic retinopathy was previously observed by Rapp and Williams,10 but the greater disparity in the response of the two hemispheres in Wag/Rij rats has not been reported.

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Key words: retina, photoreceptors, photodamage, hereditary retinopathy, rats

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Retinal blood-flow visualization by means of laser speckle photography. J. D. BRIERS AND A. F. FERCHER.

We present the preliminary findings of an investigation into the visualization of retinal blood-flow distribution by means of single-exposure laser-speckle photography. The technique relies on the speckle effect produced when laser light is scattered at a diffusing surface and on the fact that the speckle will be averaged out to some extent when the surface concerned is moving. Spatial filtering techniques are used to enhance the resulting variations in speckle contrast. The method is noncontacting and noninvasive, properties shared with the laser Doppler anemometry technique. Although it is less quantitative than the Doppler technique, it does have the advantage of giving an overall map of blood velocities instead of point measurements only, and we offer it as a complementary technique. (Invest Ophthalmol Vis Sci 22:255-259, 1982.)
"Laser speckle" is the name given to the granular pattern that is observed when a diffusing surface is illuminated with laser light. The phenomenon has already found at least one application in ophthalmology—in the measurement of eye refraction by the observation by the patient of a laser-illuminated moving surface. We present here a preliminary report on another possible ophthalmologic application of laser speckle.

**Background.** The noncontact, noninvasive measurement of retinal blood flow with lasers has already been investigated by several groups using laser Doppler anemometry or similar correlation techniques. However, these methods measure the velocity at only one point on the retina at a time, and many measurements must be made sequentially to build up an overall picture of retinal blood flow. It has been suggested that a method which gives an instantaneous "map" of blood velocity might be a useful additional tool. We have confronted this problem by the use of single-exposure laser speckle photography.

**Theory.** Laser speckle is an interference phenomenon that is observed when coherent light (such as laser light) is scattered from a diffusing surface. In general, a speckle pattern is a random pattern with properties that can only be described statistically. One of the most useful statistics is the standard deviation of the intensity distribution in the speckle pattern. It can be shown that for an "ideal" speckle pattern (using, for example, perfectly coherent light and an ideal diffuser), the standard deviation is equal to the mean intensity:

\[ \sigma = \langle I \rangle \]

In general, under nonideal conditions, \( \sigma \) is less than \( \langle I \rangle \), and in fact we can use the ratio \( \sigma / \langle I \rangle \) as a measure of the contrast of the speckle pattern:

\[ 0 \leq \frac{\sigma}{\langle I \rangle} \leq 1 \]

The basic argument on which we base our method for visualizing flow runs as follows: In a photograph taken in laser light, the speckle pattern in an area where flow is occurring will be blurred to an extent that will depend on the velocity of flow and on the exposure time of the photograph. This blurring will result in a reduction in the speckle contrast, i.e., in the ratio \( \sigma / \langle I \rangle \). The speckle pattern in an area of no flow, on the other hand, will remain of high contrast. The phenomenon is similar to that observed when botanical specimens are illuminated with laser light, which proves to be a limiting factor in the measurement of plant growth by means of holographic interferometry.

Can we deduce the velocity from the observed speckle contrast? A preliminary mathematical analysis has been carried out, which suggests that the change in speckle contrast from maximum (i.e., no blurring) to minimum (fully blurred) should correspond to a velocity range of between two and three orders of magnitude for a given exposure time. For example, an exposure time of 1/1000 sec should allow the mapping of velocities between 0.02 and 5 mm/sec. Changing the exposure time would, of course, change this range.

**Basic technique.** The experimental arrangement is illustrated in Fig. 1. The retina is illuminated with laser light from a helium-neon laser while the
Fig. 2. Examples of laser photographs of a human retina in vivo (50-mW helium-neon laser, Kodak 2415 film, 1/60 sec exposure; maximum retinal irradiance <2 mW/mm²; maximum radiant exposure <30 μJ/mm²).
Fig. 3. High-pass optical filtering of the photographs shown in Fig. 2.

eye is focused via the camera lens on the film plane of a camera. This latter condition is achieved by having an illuminated target, effectively located in the film plane, on which the subject focuses and fixates his eye. This ensures that an image of the retina is focused via the lenses of the eye and of the camera onto the film. At present we use half the pupil of the eye for the illuminating beam, and we photograph the retina through the other half of the pupil.
Retinal photographs taken by this technique do show differences in speckle contrast between blood vessels and surrounding areas. However, the differences are difficult to observe visually and would certainly be difficult to quantify, as can be seen from the examples presented in Fig. 2. Some method is needed to enhance these contrast variations, and for this reason we have added a second step to the technique.

Enhancement of the photographs. We use a standard high-pass optical filtering technique to convert the speckle contrast variations to intensity variations. This has a similar effect to that observed in dark-ground microscopy—areas with fine detail, in our case with speckle, are reproduced with a higher intensity than areas lacking such fine detail. Thus blood vessels in which flow is occurring will be reproduced darker than other areas, including blood vessels in which the flow is impeded.

Early results with this optical filtering technique are presented in Fig. 3. This shows the filtered versions of the photographs of Fig. 2, and the enhancement of the blood vessels is clearly evident. Other methods of optical filtering are being attempted to improve the final photographs.

Conclusions. We stress that this project is only at the feasibility testing stage. As stated above, exposures of the order of 1/1000 sec would be needed to map velocities greater than about 1 mm/sec. At present we are using a 50-mW helium-neon laser, and with the high-resolution film necessary for the recording of the speckle photographs we find that we need an exposure time of at least 1/60 sec. At these speeds the blood flow has completely blurred the speckle, so that all we see in our photographs is the difference between areas of flow and areas of no flow. In order to use exposure times short enough to resolve the velocities encountered in retinal blood vessels, we need more light. For this reason we are looking at the possibility of using a pulsed laser. We are also considering the advantages that may be gained by using a different laser wavelength.

The results so far are encouraging, in that we can differentiate between areas of flow and areas without flow (Fig. 3). However, more work needs to be carried out before we are in a position to quantify the information about blood-flow velocities. It will also be necessary to improve the quality of the photographs, and ways of achieving this are also being investigated.

In conclusion, it is unlikely that this technique will ever be able to compete with laser Doppler anemometry so far as precision is concerned, but we believe that the advantage of offering a "map" of blood velocities, even if it is only semiquantitative, makes the method attractive as an additional aid in ophthalmology.

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Key words: laser speckle, laser photography, retina, blood flow, flow velocity, velocity measurement

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Localization of substance P-like immunoreactivity in the anterior eye segment of squirrels: an immunohistochemical analysis. YOSHIKI SHIMIZU, YASUAKI KUWATA, MASAKATSU FUKUDA, ICCHIRO ISHIMOTO, SADAO SHIOSAKA, SHINOBU INAGAKI, HIROSHI TAKACI, MASAHITO SAKANAKA, EMIKO SENBA, YUKIKO KAWAI, KENICHI TAKATSUKI, AND MASAYA TOYAMA.

Localization of substance P (SP)-like immunoreactivity in the anterior eye segment of the squirrel was examined immunohistochemically. The present study demonstrates...