

Filter Systems in Fluorescein Angiography

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In recent years fluorescein angiography has become an important tool in both clinical and experimental ophthalmology. Several methods of black-and-white fluorescein angiography are already well established, whereas further development is needed in color angiography and in angiographic techniques that combine the use of fluorescein with some other dye. The two purposes of this chapter are (1) to describe the principles applicable to selection of filters for conventional fluorescein angiography and (2) to discuss their use.

Because most problems related to fluorescein angiography derive from the close spectral relationship of absorption and emission curves of fluorescein, for reference these well-known curves are shown in Figure 1.

Fluorescein absorbs light energy maximally between 470 and 490 nm. The absorption of energy in longer wavelengths decreases rapidly, whereas in shorter wavelengths the curve tapers off more slowly. The excited fluorescein emits green fluorescent light of approximately 485 to 600 nm., the peak emission being at 520 to 530 nm.

The wavelengths useful for excitation of fluorescein are restricted by the absorption characteristics of the human eye. According to Geeraets [3] the absorption of light in ocular media is approximately 40 percent at 450 nm. and 90 percent at 400 nm., the increase between these points being linear. The absorption increases from these values in older age groups sometimes by an order of magnitude in seemingly clear media. Although fluorescein absorbs energy below 450 nm., the

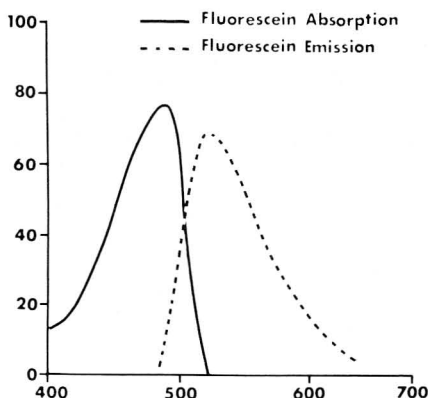


FIGURE 1. *Fluorescein absorption and emission. The dye absorbs energy maximally between 470 and 490 nm. The peak emission is at 520 to 530 nm. as measured on a Beckman DK recording spectrophotometer. (Beckman Instruments, Inc., Palo Alto, Calif.)*

increased absorption of the ocular media at these wavelengths makes this energy less useful in fluorescein angiography.

IDEAL FILTERS

It has been customary to consider ideal filters for fluorescein angiography in the following way:

The barrier filter should block all wavelengths shorter than 500 nm. and allow free passage to all wavelengths up to 700 nm. (Fig. 2), whereas the excitor filter should transmit light between 400 and 500 nm. and block all wavelengths longer than 500 nm. On closer scrutiny it may prove that this approach is oversimplified.

Barrier Filter

The ideal barrier filter should transmit all of the emitted fluorescent light. The fluorescent emission starts at 485 nm. and therefore, ideally, the barrier filter should pass the wavelengths above 485 to 490 nm.

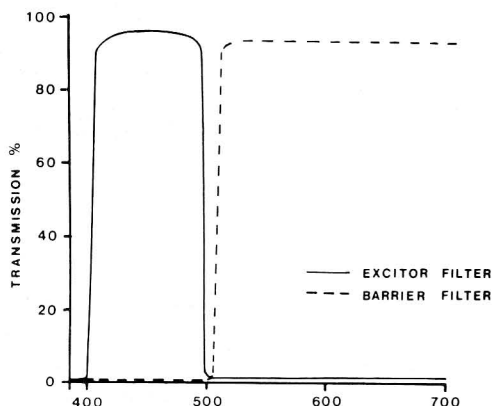


FIGURE 2. The "ideal" filters for fluorescein angiography.

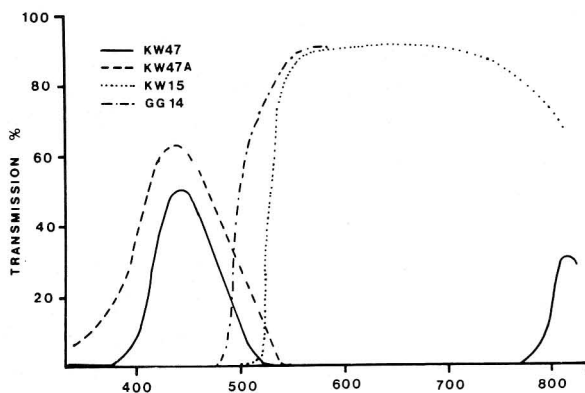


FIGURE 3. Examples of absorption filters for fluorescein angiography: Excitor filters, Kodak Wratten (KW) 47 and 47A; and barrier filters, Kodak Wratten (KW) 15 and Schott glass filter GG 14-3 mm.

Among the absorption filters, Kodak Wratten filters, 12 and 15*; Corning Glass filter 3-69†; and Schott glass filter GG 14-3 mm.‡ have transmission characteristics close to ideal for a barrier filter. Kodak Wratten 15 and Schott GG 14-3 mm. filters are illustrated in Figure 3. The

* Eastman Kodak Co., Rochester, N.Y.

† Corning Glass Works, Corning, N.Y.

‡ Schott Bros. Co., West Salem, Ohio.

TABLE 1. *Transmission of barrier filters at 490–530 nm.*

nm.	GG 14	3-69	KW 12	KW 15
490	15	1		
500	50	6	1.5	
510	65	28	17	1.0
520	75	53	55	19
530	85	70	77	56

transmission curve for Kodak Wratten 12 is similar to that for 15 except that it is shifted toward the blue range. The transmissions of these filters at the range of 490 to 530 nm. are set forth in Table 1.

The Schott glass filter GG 14-3 mm. comes closest to the theoretical ideal filter, having a reasonably high transmission already at 500 nm. and a very high transmission at the peak emission range of 520 to 530 nm. However, this filter has not become widely accepted because its transmission overlaps with that of most excitor filters. The barrier filter most often used, Kodak Wratten 15, has less than 50 percent transmission at the peak emission range.

The barrier filter also can be an interference filter, useful in combination with a matched interference filter for excitation (Fig. 4). The matching of the transmission curves is essential because there are a number of small accessory peaks of transmission in both filters, and they should not overlap. An interference filter can be made wide enough to transmit between 500 and 600 nm., but interference filters that transmit broader bands cannot be manufactured easily. In clinical work there is not any notable difference in the transmissions of the Kodak Wratten 15 filter and a good interference filter.

Excitor Filter

In selecting an excitor filter the deciding factors are the absorption curve of fluorescein (Fig. 1) and the energy output of the lamp used in the fundus camera. Thus the excitor filter should transmit in the range of 400 to 500 nm., and it should transmit enough energy for sufficient exposure of the film.

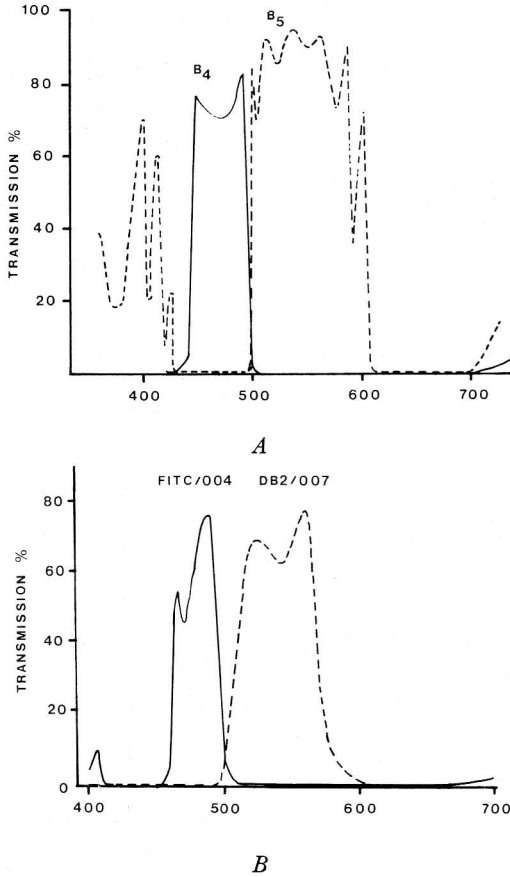


FIGURE 4. Examples of interference filters for fluorescein angiography. (A) A well-matched interference filter pair (Baird-Atomic). (B) A similar pair made by Barr & Stroud.

In order to demonstrate the relationship between the output of the lamp and the transmission characteristics of the excitor filter, we have chosen for description the excitor filter of a prototype cine camera for fluorescein angiography [5]. The lamp used in this camera is a high-pressure mercury arc lamp that has one of its peak emissions at the 435-nm. spectral line, but, by virtue of the high pressure in the bulb, the emission covers the entire excitation region for fluorescein (Fig. 5). The 435-nm. mercury line was chosen for excitation, al-

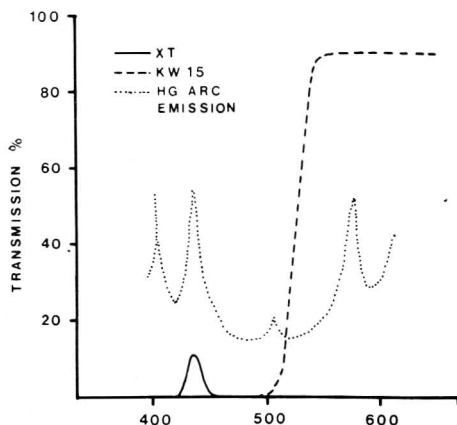


FIGURE 5. Emission spectrum of high-pressure mercury lamp (Hg arc) in a prototype camera for fluorescein cine angiography and filters used with this light source: XT: excitor filter; KW 15: Kodak Wratten 15 filter. The transmission of the excitor filter is low because emission is greater than required.

though the use of this wavelength means a loss in the excitation efficiency by a factor of about 3. Despite this disadvantage, the 435-nm. light is better accepted by the patient, since the visual sensitivity of the eye at 435 nm. is only about one-twentieth of that at the peak excitation wavelength (480 nm.).

Figure 5 shows the transmission curve of the excitor filter that is used with this lamp in the prototype camera. The transmission peak of this filter was reduced to only 10 percent of the available energy, because the bulb emits more energy than is necessary for sufficient excitation of fluorescein. Thus, in selecting the excitor filter, it must be remembered that there is not one ideal filter but one for each type of fundus camera. The more available light there is, the narrower the transmission band can be made, with a lower total amount of light transmitted.

In all commercially available fundus cameras the energy output is quite limited. Of this limited output, only a fraction enters the eye; in a typical fundus camera, energy level at the eye is about 0.1 joule of a total emission from source of 500 joules (watt-seconds). For such cameras it has been a problem to find excitor filters that would transmit sufficient energy without overlapping the transmission of the bar-

rier filter. The excitor filters most widely used in clinical fluorescein angiography are the absorption filters Kodak Wratten 47 and 47A and Schott BG 12 (0.7 and 1.0 mm.) and interference filters of the type Baird Atomic B4-4700.*

The use of absorption excitor filters is necessary in fundus cameras with a low flash-intensity output. The overall transmission of energy is enhanced by the addition of reflected blue light to the green fluorescent light to give sufficient total energy for correct film exposure. Use of filters with overlap in transmission does not necessarily mean that the quality of angiograms is inferior to those taken with a filter pair with no overlap; this has been demonstrated very clearly in the textbook of Shikano and Shimizu [8]. However, absorption filter pairs are unsatisfactory in fluorographic studies of the optic disc and pale fundus lesions. The transmission of the combined filters in the blue range means that some of the reflected blue light reaches the film. White areas strongly reflect the blue light and thus are already visible in the preinjection picture. The appearance of the dye in such areas causes fluorescence and additional density of film exposure, but this is of no advantage in black-and-white angiograms.

The use of absorption filters has a special place in the study of the macula [7, 9]; the macular area is often underexposed in films taken with a filter pair having no transmission overlap. In the macular area the combined screening of choroidal fluorescence and absence of central retinal vessels causes a dense umbral zone. When the absorption filters with overlapping transmission are used, the additional reflected blue light increases film exposure in the macular area as compared with its surroundings, which gives slightly better visibility of the small fluorescent foci in some macular lesions.

The low cost of the absorption filters compared with that of other types of filters makes them suitable for general use. The fact that the whitish lesions cannot be studied adequately can be compensated for by the use of fluoroscopy in which the green fluorescence can be differentiated from the reflected blue light. A simple fundus camera with absorption filters combined with fluoroscopy through a three-mirror lens is adequate for many diagnostic situations.

Interference excitation filters [4] were introduced because they have

* Baird Atomic Inc., Bedford, Mass. 01730.

no transmission in common with either the usual barrier filters or a matched interference barrier filter. Therefore, no blue light reaches the camera, but the transmitted energy is sufficient to expose the film if high flash intensities are available. The filter combination Baird-Atomic B4-B5 (Fig. 4A) and other similar filters of other manufacturers, e.g., Barr & Stroud FITC/004* (Fig. 4B) and Balzers K-2,† transmit so little energy because of the narrow bandpass that film underexposure is sometimes a problem. This underexposure also has been considered to be related to variations in the peak emission wavelength of a blood fluorescein mixture or to variance in the wavelength that most effectively excites fluorescence. In the clinical situation such variation is uncommon, and it is most probably caused by hemodilution of fluorescein in conditions of increased pulmonary circulation. It also may occur in some healthy young adults following deep inspiration prior to injection of the dye; this phenomenon sometimes is seen in angiographic practice when, in spite of a good injection, no dye appears in the ocular fundus at the expected time, and only during the second transit of the dye does a very faint fluorescence become visible. It is our experience that if the dye is injected while the patient exhales slowly, and fluorescence as seen through the camera eyepiece is bright, the film exposure is adequate.

Occasionally the coating characteristics of any given interference filter pair introduce unwanted bands of transmission that may be common to both filters. Therefore, it is essential to purchase interference filters as an individually matched pair.

When interference excitor filters are compared, the transmission curves of individual filters sometimes are seen to have quite dissimilar shapes, and it is advisable to request a spectrophotometric curve from the manufacturer before accepting delivery.

The excitor filter described by Allen and Frazier [1] (Fig. 6) was developed to be as close to the ideal excitor filter as possible. This filter is a combination of three filters: Leitz KP490,‡ Kodak Wratten 2B, and Schott BG 38. The total transmission of this excitor filter is not measured by the authors, but it can be calculated as being about

* Barr & Stroud, Glasgow, Scotland.

† Balzers, Liechtenstein.

‡ Leitz Inc., N.Y., N.Y.

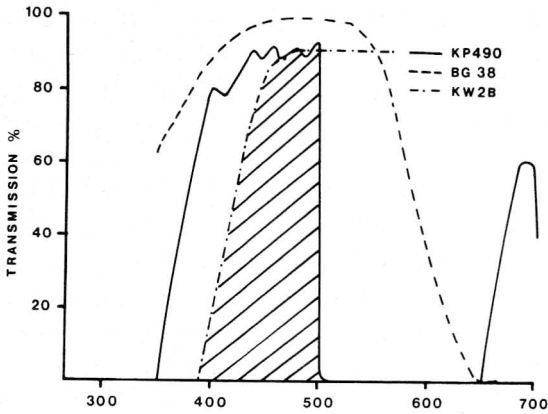


FIGURE 6. Combination excitor filter based on data of Allen and Frazier [7]. Final transmission is a product of the transmissions of the Schott KP 490 filter and the Kodak Wratten (KW) 2B. Shaded area shows the wavelengths of transmission common to these two filters and is not the transmission curve.

75 to 80 percent. Allen and Frazier have used slow films with normal development (Panatomic-X,* 32 ASA) and still obtained good photographic results. The transmission curve of this combination filter has no additional peaks above 500 nm. and, therefore, an absorption filter like Kodak Wratten 15 can be used as a barrier. As there is no overlap between the transmission of excitor and barrier filters, the problem of "pseudo-fluorescence" is avoided as effectively as with narrow-band interference filters.

This new excitor filter allows free passage to a broader band in blue as compared with the narrow bandpass interference filters. At the same time, the amount of light energy absorbed by the ocular media, and to which the retina is exposed, is increased. Blue light of shorter wavelengths is subjectively less disturbing than that at 270 to 480 nm. For example, in the normal macula a continuous exposure to the blue light (435 nm.) of the prototype cine camera, for 30 seconds, caused an after-image lasting 2 to 3 minutes and a slight depression in the sensitivity to blue for 20 minutes, detectable by profile perimetry and the Farnsworth-Munsell 100-hue test. Either of these tests, or the disap-

* Eastman Kodak Co., Rochester, N.Y.

pearance time of the after-image and the reduction in visual acuity, could be used to monitor the effect of a new excitor filter on the eye.

The filter combination of Allen and Frazier can be modified to cut off transmission in the range of 390 to 430 nm. by inserting a Kodak Wratten filter, either 2A or 2E. The resultant filter has transmission characteristics closely similar to those of a good B4-4700 filter. Since the film density is proportional to the logarithm of the exposure, a small exposure difference of anything less than a factor of 2 makes an insignificant change in density. Because such filters make it possible to use slower film, they can be used in selected cases requiring high resolution. However, in routine work a low light intensity combined with faster film and forced development is still the safest and most convenient method.

FILTERS FOR COLOR FLUORESCEIN ANGIOGRAPHY

Originally color fluorescein angiography was used because it provided a means of detecting "pseudofluorescence." The technique had limited use, however, mainly because of the loss of resolution, as compared with black-and-white films.

The advent of the argon laser for photocoagulation of macular lesions introduced a need for high-quality color angiography that can be used as a guide to treatment of abnormal vessels close to the macula. Neovascularizations at the disc also can be studied by color fluorescein angiography; thus the feeder vessel can be located for treatment. The color angiogram reveals feeder as well as vessels not filled with dye.

Color fluorescein angiograms are useful in circinate retinopathy for placement of photocoagulation, since they are directly comparable with the ophthalmoscopic appearance. As more private practitioners start to use fluorescein angiography as an adjunct to their office procedures, color Polaroid photography is becoming a practical and readily available fluorographic method.

Many of the absorption barrier filters used in black-and-white angiography are suitable for color fluorescein angiography. However, the excitor filter has to have a transmission range of from 400 to 500 nm., with some additional transmission in the red part of the spectrum

above 600 nm. The filter used by Allen and Frazier is suitable for color fluorescein angiography when the Schott BG 38 filter is removed. Interference filters such as those manufactured by Baird-Atomic and Barr & Stroud can be constructed to transmit red wavelengths and, in fact, the original Baird-Atomic B4 filters did have transmission of visible red.

In color fluorescein angiograms there are sources of possible error in interpretation because of the unusual color balance. A good example of this is seen in choroidal melanoma, where the brown pigment of the melanoma may become a blood-red color because of the absence of reflected green light. A regular color transparency taken at the time of color fluorescein angiography will help to identify and interpret bizarre color appearances.

THE ARGON LASER AS LIGHT SOURCE FOR FLUORESCEIN ANGIOGRAPHY

Advantages

The argon-ion laser has a continuous power output of several watts. One of the stronger lasing lines is at 488 nm. This wavelength is at the peak of the fluorescein absorption curve, and the laser energy in this line is separated easily from the other lines. Thus it might appear that the argon laser would make a suitable light source for fluorescein angiography. Filtering would be reduced to picking a suitable barrier filter. This can be done easily because of the lack of energy in the source at extraneous wavelengths. The energy can be transferred optically from point to point with little loss because of the coherence properties of the laser.

Disadvantages

Although the spectral value of the light emission is optimal, it is difficult to achieve safe control of the energy output from the laser source. There are other disadvantages: the cost of a laser installation is very high; it requires high electrical power and usually water cooling; and

an articulated arm would be required to direct the laser beam from a stationary laser to a movable fundus camera. The articulated arm is costly and delicate, and the alignment is very critical. With suitable engineering and adequate money, these disadvantages can be overcome. However, there is another, more subtle, disadvantage: Every piece of dust and every small imperfection in an optical system produces its own diffraction pattern, which is propagated through the system. Although this is of little importance with incoherent illumination, coherent laser beams show up each such artefact as a bright diffraction ring or interference fringe in the final image. Thus the argon laser is both bulky and complex to manage, in addition to the many problems arising from the coherence of the light beam.

FLUORESCEIN ANGIOGRAPHY COMBINED WITH SIMULTANEOUS INDOCYANINE GREEN ANGIOGRAPHY

The technique of infrared absorption angiography, using intravenously injected indocyanine green, has been reported by Hochheimer [6]. Infrared absorption angiography is especially suitable for study of the choroidal circulation, because retinal pigment epithelium and macular pigment freely transmit infrared light. Fluorescein angiography can be combined with indocyanine green angiography, although this requires several modifications to the Zeiss fundus camera and two separate camera backs to record fluorography and indocyanine angiography [2].

Filters used in this combined angiography include the Kodak Wratten 47, in front of the flashtube, because this filter transmits both blue light for excitation of fluorescein (400 to 500 nm.) and also infrared light (above 700 nm.). The infrared light and the emitted fluorescent light are passed through the Kodak Wratten 15 filter, filtering out the blue light, following which a dichroic beam-splitter reflects the fluorescent light to the camera recording the fluorescein angiogram. The same dichroic beam-splitter transmits the infrared light, from which a 15-nm.-wide band at 770 nm. is extracted by an additional interference filter and used for absorption angiography. Simultaneous angiograms by these two techniques will be very useful in the study of the

choroid. There is also an emission band from indocyanine green at 845 nm., which might be applied to the study of choroidal lesions.

These techniques appear complicated, but in practice the angiograms are taken in the same way as regular fluorescein angiograms, although the work of interpretation is more complex. Techniques of this type are providing additional information, and might become the next step in clinical angiography.

SUMMARY

At the present time the range of available filters is sufficient for existing methods of fluorescein angiography. By applying knowledge of the basic principles of filter systems, it is possible to construct a combination filter or to design interference filters as required for any particular purpose. However, the techniques involving several dyes or several emission bands require further study before their value in clinical work can be fully assessed.

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