Fundamentals of Intravenous Fluorescein Angiography

For nearly four decades, ophthalmologists have relied on fluorescein angiography as an important tool in the understanding, diagnosis and treatment of retinal disorders. This diagnostic procedure utilizes a specialized fundus camera (Figure 1) to capture rapid-sequence photographs of the retinal vasculature following an intravenous injection of fluorescein sodium. Fluorescein angiography facilitates the in-vivo study of the retinal circulation and is particularly useful in the management of diabetic retinopathy and macular degeneration, two leading causes of blindness.

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Characteristics of Fluorescein

Fluorescein Sodium

Fluorescein sodium is a highly fluorescent chemical compound synthesized from the petroleum derivatives resorcinol and phthalic anhydride.[1] It absorbs blue light, with peak absorption and excitation occurring at wavelengths between 465–490nm. Fluorescence occurs at the yellow-green wavelengths of 520 to 530nm (Figure 2). These fluorescent properties have made fluorescein useful in a variety of industrial, scientific and medical applications since the dye was first synthesized by Von Baeyer in 1871. Although commonly referred to as fluorescein, the dye used in angiography is fluorescein sodium, the sodium salt of fluorescein. Another common misconception, often passed on to patients, is that it is a "vegetable dye" rather than a synthetic.

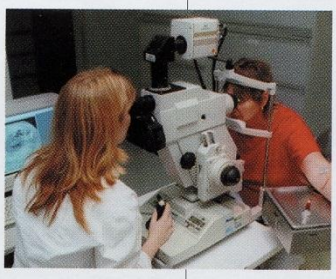


Figure 1 — Fundus camera equipped with a digital capture system for performing fluorescein angiography.

The normal adult dosage is 500mg injected intravenously. It is typically packaged in doses of 5 ml. of 10 percent or 2 ml. of 25 percent. Upon entering the circulation, approximately 80 percent of the dye molecules bind to serum protein. The remaining unbound or free fluorescein molecules fluoresce when excited with light of the appropriate wavelength. The dye is metabolized by the kidneys and is eliminated through the urine within 24 to 36 hours of administration. During this period of metabolism and elimination, fluorescein has the potential to interfere with clinical laboratory tests that use fluorescence as a diagnostic marker.[2] To avoid any false readings, it may be prudent to schedule clinical lab tests either before the angiogram, or postpone the test for a day or two to allow sufficient elimination of the dye. Side effects of intravenous fluorescein include discoloration of the urine for 24 to 36 hours and a slight yellow skin discoloration that fades within a few hours. Nursing mothers should be cautioned that fluorescein is also excreted in human milk.[3]

Complications and Adverse Reactions

Fluorescein is well tolerated by most patients, but angiography is an invasive procedure with an associated risk of complication or adverse reaction (Table 1). Adverse reactions occur in 5 to 10 percent of patients and can range from mild to severe. Transient nausea and occasional vomiting are the most common reactions and require no treatment. These mild reactions seem to be related to the volume of dye and rate of injection. A relatively slow rate of injection often reduces or eliminates this type of reaction. More severe

Table 1
Complications and Adverse Reactions

Extravasation of dye
Transient nausea
Vomiting
Pruritis
Urticaria
Bronchospasm
Laryngeal edema
Anaphylaxis
Hypotension
Syncope
Seizures
Myocardial infarction

reactions are rare, but include hives, laryngeal edema, bronchospasm, syncope, anaphylaxis, myocardial infarction and cardiac arrest.[4]

Although there are no known risks or adverse reactions associated with pregnancy, most practitioners will avoid performing fluorescein angiography in pregnant women, especially in their first trimester.[5-7]

Extravasation of fluorescein dye during the injection can be a serious complication of angiography. With a pH of 8 to 9.8, fluorescein infiltration can be quite painful. Sloughing of the skin, localized necrosis, subcutaneous granuloma, and toxic neuritis have been reported following extravasation of fluorescein. With proper injection technique, these complications can usually be avoided. Although life-threatening reactions during angiography are rare, the angiographic facility should be properly equipped and prepared to manage serious reactions to the procedure. It is generally recommended that a physician be present or available during angiography.

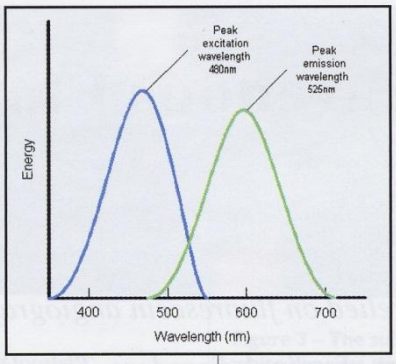


Figure 2 — Representative excitation and emission curves of fluorescein sodium. Excitation occurs at wavelengths between 465-490nm. Fluorescence occurs at wavelengths of 520-530nm.

Indications and Uses

The most common uses of fluorescein angiography are in retinal or choroidal vascular diseases such as diabetic retinopathy, macular degeneration, hypertensive retinopathy and vascular occlusions (Table 2). For the most part, these are clinical diagnoses. The angiogram is used to determine the extent of damage, to develop a treatment plan or to monitor the results of treatment. In diabetic retinopathy the angiogram is useful in identifying the extent of ischemia, the location of microaneurysms, the presence of neovascularization and the extent of macular edema (Figures 3a, 3b). In macular degeneration, angiography is useful in identifying the presence and location of subretinal neovascularization (Figures 4a, 4b). Post treatment angiograms also check the efficacy of laser treatment.

Other uses include degenerative and inflammatory conditions. Some of these conditions exhibit characteristic staining patterns, which can confirm the diagnosis. Stargardt's Disease is an example, exhibiting a silent choroid and a central bulls-eye staining pattern in the macula.

Angiography has long played a role in advancing the understanding of retinal vascular disorders and potential treatment modalities. A number of multicenter clinical trials utilize fluorescein angiography in investigating new treatment options in diabetic retinopathy and macular degeneration.

Photodynamic therapy (PDT) is a new treatment option for macular degeneration that received FDA approval in 2000. Pre-treatment angiograms are utilized to determine the location and size of the lesion to be treated. Digital imaging software routines will measure the actual size of the lesion and calculate the appropriate spot size of the PDT laser (Figure 5).

Table 2
Common Diagnostic Uses and Indications for Fluorescein Angiography

Diabetic retinopathy
Age related macular degeneration
Subretinal neovascular membrane from other causes (myopia, histoplasmosis, etc.)
Central retinal vein occlusion
Branch retinal vein occlusion
Central serous chorioretinopathy
Cystoid macular edema
Hypertensive retinopathy
Central retinal artery occlusion
Branch retinal artery occlusion
Retinal arterial macroaneurysms
Pattern dystrophies of the retinal pigment epithelium
Choroidal tumors
Chorioretinal inflammatory conditions
Hereditary retinal dystrophies

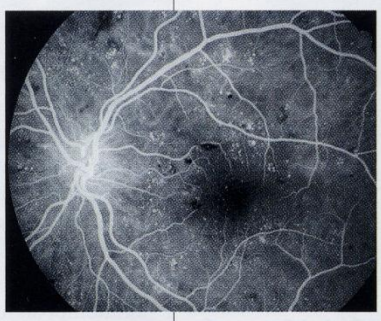
Equipment and Technique

Fundus Camera

Angiography requires the use of a specialized fundus camera equipped with a matched pair of exciter and barrier filters along with a fast recycling electronic flash tube that allows a capture rate of up to one frame per second. Narrow band-pass interference filters are utilized to allow maximum transmission of peak wavelengths, while minimizing any crossover of transmission curves. The exciter filter transmits blue-green light at 465-490nm, the peak excitation range of fluorescein. The barrier filter transmits a narrow band of yellow at fluorescein's peak emission range of 520-530nm. The barrier filter effectively blocks all visible wavelengths but the specific color of fluorescein. The fundus-illuminating beam is delivered axially, through the image forming optics and filters of the fundus camera. Fundus cameras equipped for angiography have a timer that records the

angiographic sequencing on each frame of the study.

Some fundus cameras provide multiple magnification fields. A 30-degree field of view (with a magnification of approximately 2.5x on 35 mm film) is the most common field for documenting macular detail. Wide-angle cameras of 50 or 60 degrees are useful for documenting larger areas of the retina. Images are captured either with high-speed black-and-white 35mm panchromatic film or electronically, with a charge-coupled device (CCD) and computerized system for digital imaging. Film-based angiography requires either the use of a processing service, or access to a darkroom for processing films on-site.



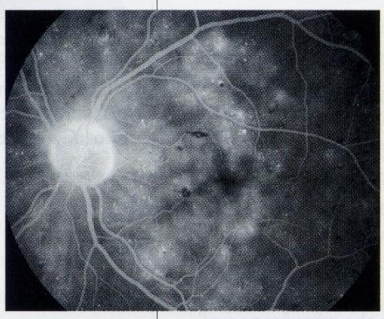


Figure 3A — Venous phase photograph of nonproliferative diabetic retinopathy demonstrating retinal hemorrhages and microaneurysms.

Figure 3B — Late phase photograph of the same eye showing macular edema.

Stereo Imaging

Some facilities produce stereo photographs during portions of the angiogram. This is achieved by shifting the fundus camera laterally between sequential photographs. The lateral shift causes the illuminating beam of the fundus camera to fall on opposite slopes of the cornea. The resulting cornea-induced parallax creates a hyperstereoscopic effect that is evident when the sequential pair of photographs is viewed together. Stereo pairs are particularly useful in identifying the histopathologic location of angiographic findings within or beneath the retina.

Digital Imaging

Commercial digital angiography imaging systems have been available for over fifteen years and continue to improve in quality each year. Although photographic film is still capable of capturing greater detail than current digital systems, digital imaging offers some distinct advantages over the more traditional film-based angiogram. Instant access to the electronic images increases efficiency and promotes better patient education by reviewing images on a monitor with the patient. Image enhancement and manipulation is easily achieved with imaging software. Lesions can be measured, or digital overlays used to identify changes in lesion size in serial photographs. Images can be stored on magnetic media like CD-ROMs and transmitted electronically to remote sites equipped with a computer for viewing. Digital systems also offer the additional advantage of shortening the learning curve for novice angiographers. Having instant feedback allows the angiographer to adjust exposure settings and camera alignment to correct any flaws in technique. Despite these advantages, the high initial cost of digital systems has prevented them from being employed universally. It is estimated that less than 50 percent of angiographic facilities are utilizing digital technology.

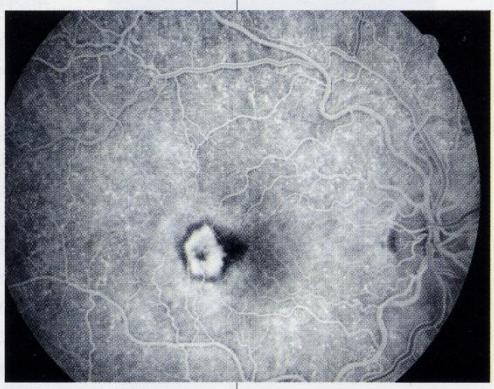


Figure 4 — Age-related macular degeneration with a juxtafoveal choroidal new vessel membrane.

Fluorescein angiography can also be recorded using a confocal scanning laser ophthalmoscope (SLO). Scanning laser imaging technology is quite different than that of the conventional fundus camera system. A laser beam of the appropriate wavelength scans across the fundus in a raster pattern to illuminate successive elements of the retina. The scan rate of the laser is synchronized at 30 frames per second, a rate that is compatible with video display. This imaging technique provides a continuous, real time representation of the flow dynamics of the retina and choroid. The SLO lessens the need for pupillary dilation and patients can easily tolerate the low light level of the laser. The major drawback of scanning laser technology is the high cost of the equipment.[8]

Sequencing

Proper sequencing of the angiographic series is essential in obtaining maximum diagnostic information. Color fundus photographs as well as black-and-white monochromatic green filter images are routinely taken as baseline views before administering the dye. The early transit phase is the most critical part of the angiogram and usually lasts less than a minute. Before injecting the dye, the illuminating beam of the fundus camera is centered within the dilated pupil. The angiographer then pre-focuses the camera on the appropriate area of interest. The dye is administered as a bolus injection, typically through a scalp vein needle into the

antecubital vein. The timer is started and photography commences. The arm-to-retina circulation time varies, but in a normal patient takes 10–12 seconds. The experienced angiographer anticipates the initial appearance of the dye and begins rapid sequence photography before the dye is visible. Images are routinely captured at a rate of one frame per second until maximum fluorescence occurs. During this dynamic early phase only one eye can be captured. In the seconds it takes to switch from one eye to the other, valuable information could easily be missed. After completion of the early phase, photographs of the fellow eye or other areas of interest in the retina can be taken.

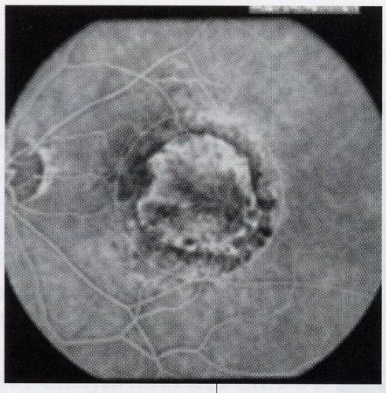


Figure 5 — Digital fluorescein image of a choroidal new vessel membrane outlined with an area measurement to calculate spot size for photodynamic therapy.

Over the next few minutes the appearance of the dye stabilizes and begins to slowly fade. The angiographer can capture appropriate views as necessary without the urgency needed during the early phase. Late phase photographs are taken as the dye dissipates, anywhere from 7 to 15 minutes after injection.

Many facilities develop disease specific protocols for sequencing and appropriate fields of view. For example, peripheral shots of the retina are routinely taken after the early transit in diabetic retinopathy, while the macula or posterior pole is the major area of interest in macular degeneration and peripheral views are not usually necessary. The angiographer often adjusts the specific protocol based on changes that may occur as the angiogram progresses.

In rare cases when venous access is severely compromised or the patient is known to be highly allergic to the dye, fluorescein can be administered orally. Due to the slow absorption rate, an early transit sequence is not possible. The resulting images are less than ideal, but may provide limited diagnostic information in situations where late phase photographs are helpful.

Quality Issues

When conditions are favorable, angiographic images can be quite dramatic and worthy of display or publication. Unfortunately not all angiograms will be this high in quality. It is important however, for each angiogram to be of adequate and consistent diagnostic quality. Quality results rely on a number of factors. The skill of the angiographer and the optical and mechanical quality of the instrumentation can have a direct effect on quality. There are a number of common factors that can adversely affect angiographic quality. They include: the presence of media opacities, inadequate pupillary dilation, poor fixation, inadequate patient cooperation and extravasation of the dye. Some of these factors are beyond the direct control of the angiographer, but every attempt should be made to minimize their detrimental effects.

Since fluorescein angiography is a dynamic process, successful results depend on complete preparation before the dye is injected. Many angiographers follow a specific protocol or checklist to ensure that everything is ready. Good communication between the ophthalmologist and angiographer is essential to ensure that maximum diagnostic information is obtained. The photographic timing sequence and the angiographer's ability to adapt to changing conditions are also important elements in producing quality angiographic results. Experience is invaluable, especially in managing the patient if complications occur during the critical early phase of the study.

The Role of the Angiographer

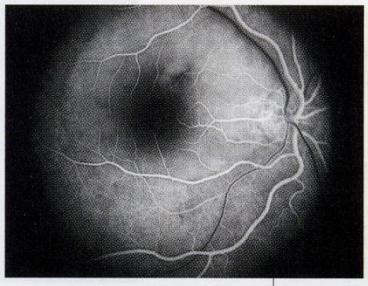
Some ophthalmologists perform their own angiography, but this is an exception rather than the rule. Most facilities employ a photographer or technician dedicated to performing ophthalmic photography procedures. Unfortunately, there is very little education available in fluorescein angiography. In the absence of formal education, certification plays an important role in developing competent practitioners in angiography. The Ophthalmic Photographers Society offers a voluntary certification program in fluorescein angiography that has established standards of competence in angiography. The Certified Retinal Angiographer (CRA) program was established in 1978. The program is accredited by the National Commission for Certifying Agencies and has certified over 600 individuals to date. Although certification is not mandatory, the CRA credential offers some assurance of competence and safety to both patient and physician.

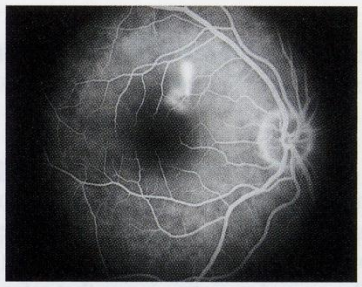
The responsibility for injecting the dye sometimes falls to the angiographer or a technician. In some practice settings this makes sense. There are however, some legal

issues associated with unlicensed personnel performing fluorescein injections. It is generally recommended that angiographic facilities check their current state or local laws regarding the credentialing requirements of personnel performing intravenous injections.[9,10]

Interpretation

Fluorescein angiography records the dynamic interaction of fluorescein with both normal and abnormal anatomic structures of the ocular fundus. A thorough understanding of the circulation phases and appearance of the dye in a normal eye is essential for interpretation of abnormalities. In a normal eye, the retinal blood vessels and the retinal pigment epithelium (RPE) both act as barriers to fluorescein leakage within the retina. The tight junctions of the endothelial cells in normal retinal capillaries make them impermeable to fluorescein leakage. The choriocapillaris is fenestrated and leaks dye freely into the extravascular space within the choroid. The tight cellular junctions of the healthy retinal pigment epithelium provide a blood/retinal barrier preventing the normal choroidal leakage from penetrating the retinal tissues.





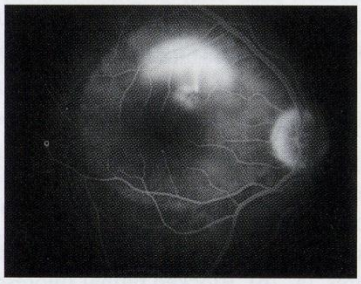


Figure 6A (top left) — Early arteriovenous phase photograph in a patient with idiopathic central serous chorioretinopathy. The veins exhibit early laminar flow along the vessel walls.

Figure 6B (top right) — Venous phase photograph demonstrating maximum vessel fluorescence and a classic 'smokestack' area of hyperfluorescence.

Figure 6C (bottom) — Late phase photograph demonstrating reduced fluorescence of the retinal vasculature. Late hyperfluorescence is a result of pooling of dye within a serous detachment.

Phases of the Normal Angiogram

Early Phase

The early phase of the angiogram can be broken down into distinct circulation phases that are useful for interpreting the results:

- *Choroidal flush.* In a normal patient, the dye appears first in the choroid in 10–12 seconds. The major choroidal vessels are impermeable to fluorescein, but the choriocapillaris leaks fluorescein dye freely into the extravascular space. There is usually little detail in the choroidal flush as the retinal pigment epithelium (RPE) acts as an irregular filter that partially obscures the view of the choroid. If a cilioretinal artery is present, this fills along with the choroidal flush as both are fed by the short posterior ciliary artery.

- *Arterial phase.* The retinal arterioles typically fill a second or two after the choroid.

- *Arteriovenous phase.* Complete filling of the retinal capillary bed follows the arterial phase and the retinal veins begin to exhibit laminar filling (Figure 6a).

- *Venous phase.* Complete filling of the veins occurs over the next ten seconds with maximum vessel fluorescence occurring within 30-35 seconds after injection (Figure 6b).

Table 3
Abnormal Angiographic Findings

Hypofluorescence
Filling defect
Blocking defect

Hyperfluoresence
Autofluorescence
Psuedofluorescence
Transmission or 'window' defect
Leakage
Pooling
Staining

Mid Phase

Also known as the recirculation phase, this occurs about 2 to 4 minutes after injection. The veins and arteries remain roughly equal in brightness. The intensity of fluorescence diminishes slowly during this phase as much of the fluorescein is removed from the bloodstream on the first pass through the kidneys.

Late Phase

The late or elimination phase demonstrates the gradual elimination of dye from the retinal and choroidal vasculature. Staining of the optic disc is a normal finding. Any other areas of

late hyperfluorescence suggest the presence of an abnormality (Figure 6c).

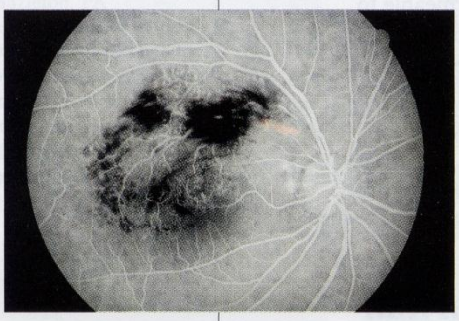
The Abnormal Angiogram

Interpretation of the abnormal angiogram relies on the identification of areas that exhibit hypofluorescence or hyperfluorescence (Table 3). These are descriptive terms that refer to the time specific, relative brightness of fluorescence in comparison with a normal study.

Hypofluorescence

Hypofluorescence is defined as an absence or reduction of normal fluorescence. Hypofluorescence is the result of either blocked fluorescence or vascular filling defects (Figures 7a, 7b). Blocked retinal fluorescence is most commonly caused by superficial or intraretinal hemorrhages. Choroidal fluorescence can be blocked by the anterior deposition of abnormal materials such as blood, lipid exudates, lipofuscin, xanthophyll or melanin. It is important to differentiate this type of choroidal hypofluorescence from the normal attenuating effect of the retinal pigment epithelium. It is often possible to determine the histologic location of the blocking structure based on the visibility of blood vessels. Preretinal hemorrhages, for example would block the visibility of both the retinal and choroidal vasculature while subretinal blood would only obscure the choroidal circulation.

Hypofluorescence can also be the result of vascular filling defects. Inadequate circulation will cause the affected vessels to appear darker than expected. Vascular occlusions can cause a delay or decrease in perfusion that appears hypofluorescent. Capillary nonperfusion is a common finding in diabetic retinopathy. Filling defects can also occur in the optic nerve head as a result of atrophy or ischemia. It is important to understand the relationship between hypofluorescence due to filling defects and the specific phase of the angiogram. In many vascular occlusions, hypofluorescence is a temporary finding until delayed filling of the affected vessel occurs.



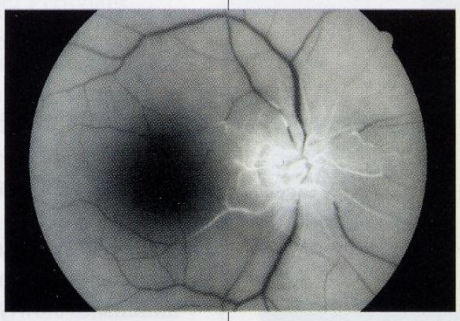


Figure 7A (top) — Hypofluorescence: Blocking defect from superficial retinal hemorrhages due to a branch retinal vein occlusion.

Figure 7B (bottom) — Hypofluorescence: Vascular filling defect in a patient with an acute central retinal artery occlusion.

Hyperfluorescence

Autofluorescence and psuedofluorescence are terms to describe the appearance of apparent hyperfluorescence in the absence of fluorescein. **Autofluorescence** refers to recordable hyperfluorescence that is believed to occur naturally in certain pathologic entities such as optic nerve drusen and astrocytic hamartomas. Some, but not all drusen appear to fluoresce under blue light. Some controversy exists over whether this is actual fluorescence or reflectance.[11] These structures are highly reflective in the same spectral range of fluorescence and could actually be exhibiting pseudofluorescence.

Pseudofluorescence occurs as a result of crossover in the spectral transmission curves of the exciter and barrier filters. If too much crossover is present, reflectance from bright fundus structures will not be fully blocked by the barrier filter. Crossover can be the result of mismatched or aging filters. Modern interference filters rarely exhibit significant crossover unless they have deteriorated. Control photographs are routinely taken before injection of fluorescein to detect the possible presence of pseudofluorescence.

Transmission defect. Depending on the density of retinal pigmentation, background fluorescence from the choroid can be visible as hyperfluorescence in the angiogram. A "window defect" is an area of hyper-fluorescence that occurs when there is an absence of pigmentation due to damage of the pigment epithelium. The absence of pigment allows a view of the underlying choriocapillaris (Figure 8). Window defects remain uniform in size throughout the angiogram. Their brightness rises and falls with the choroidal fluorescence. It is important to differentiate hyperfluorescence due to transmission defects from leakage.

Leakage is hyperfluorescence due to the active focal leakage of fluorescein into retinal tissues or the vitreous cavity. Any leakage of fluorescein from a retinal vessel or within the retinal tissues indicates an abnormality. It is

often the result of a breakdown or lack of tight vascular junctions in abnormal blood vessels. Retinal neovascularization can occur in any retinal condition that causes ischemia, but most notably in diabetic retinopathy. Retinal new vessels leak progressively throughout the angiogram.

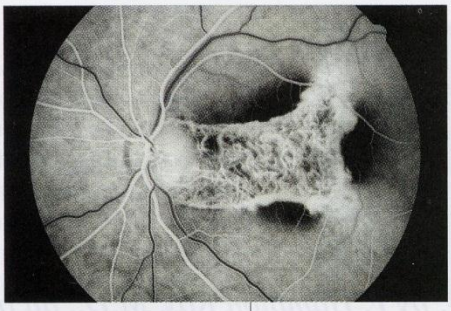
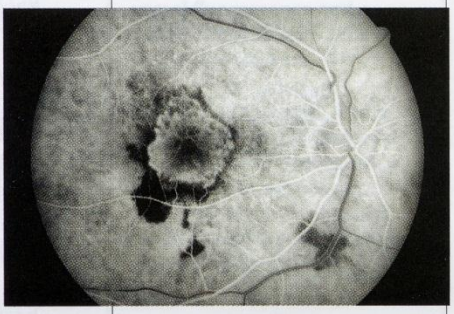


Figure 8 — Hyperfluorescence: The major choroidal vessels appear prominently as an area of hyperfluorescence through a 'window defect' in the retinal pigment epithelium.

Choroidal, or subretinal neovascularization will also leak fluorescein. These blood vessels originate in the choriocapillaris and can be found in any condition that causes breaks in Bruch's membrane. Choroidal new vessels are a common finding in age related macular degeneration and can be sight threatening. Fluorescein angiography is valuable in identifying the location and extent of choroidal neovascular membranes (Figures 9a, 9b).

Capillary microaneurysms, retinal telangiectasias, arterial macroanuerysm, papilledema and some vascularized tumors will also exhibit leakage. Leakage can lead to late staining or pooling of dye.

Staining refers to late hyperfluorescence that is the result of leakage which diffuses into certain tissues. Perivascular staining in vein occlusions and diabetic macular edema are common examples. Drusen and chorioretinal scars can also exhibit staining. Normal staining can occur in the optic nerve and sclera as a result of normal choroidal leakage. Scleral staining is usually only visible when there is a reduction or absence of the pigment epithelium and the sclera can be seen clinically.



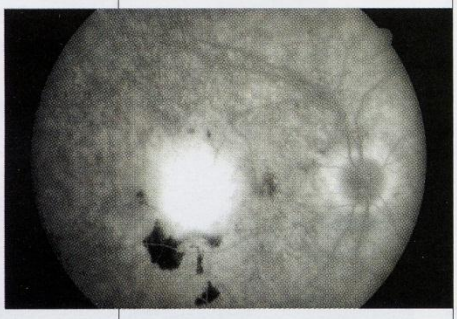


Figure 9A (top) — Hyperfluorescence: Leakage from a choroidal new vessel membrane in a patient with macular degeneration.

Figure 9B (bottom) — Hyperfluorescence: Late phase photograph of the same eye demonstrates continued leakage.

Pooling is the accumulation of dye within a distinct anatomic space. Pooling can occur in serous detachments of the sensory retina or the retinal pigment epithelium due to a breakdown of the blood/retinal barrier. Central serous chorioretinopathy is a condition that often demonstrates the pooling of fluorescein leakage.

Summary

Fluorescein angiography has revolutionized the understanding, diagnosis and treatment of retinal vascular disorders. For nearly forty years, ophthalmologists have used fluorescein angiography as a guide for laser treatment, benefiting many thousands of patients. This important diagnostic tool continues to evolve with new advances in digital imaging techniques. As new treatment modalities are developed, fluorescein angiography will continue to play an important role in the management of retinal conditions.

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