FLUORESCEIN ANGIOGRAPHY

AUTHOR

Luigi Bilotto: Brien Holden Vision Institute

PEER REVIEWER

James Loughman: Dublin Institute of Technology

INTRODUCTION

This chapter includes a review of:

- Definition
- Purpose
- Indications
- Contra-indications (relative)
- Technique principle
- Procedure
- Factors affecting visualization
- Interpretation
- Terminology
- Interpretation steps of Fluorescein Angiography
- Indocyanine green angiography

DEFINITION

Fluorescein Angiography (FA) is a technique used to dynamically evaluate vascular conditions of the eye by photographing Sodium Fluorescein (NaFl) dye in the vasculature following intravenous (IV) injection. FA allows the visualisation of the normal passage of blood (dye) from the arterial to the venous systems in both the retinal and choroidal circulations of the eye. Pathological changes on blood vessels which affect the vascular circulation and the inner or outer blood-retinal barrier can also be viewed with FA.

PURPOSE

- Advanced understanding of ocular vasculopathies reveals that earlier disease diagnosis and treatment yields better clinical results.
- Progress in laser therapeutics permits very early intervention in disease conditions.
- FA permits earlier and more effective detection of disease processes that may be otherwise subclinical and not observable ophthalmoscopically. FA enables viewing retinal details as small as 5 microns compared to direct ophthalmoscopy which permits viewing of details > 72 microns even with 15X magnification.

INDICATIONS

1. Suspected or possible Sub-Retinal Neovascular Membranes (SRNVM)
2. Proliferative vascular changes (e.g. diabetes mellitus)
3. Suspected “leaking” problems of the retina or choroid (e.g. macular oedema))
4. Unexplained visual loss
5. Unidentified Fundus Observation (Tumours, Drusen, etc.)
6. Suspected diseases of the RPE/Bruch Membrane complex (e.g. dystrophies).

OCULAR DISEASE CONDITIONS INDICATING THE NEED FOR FA

- Acute posterior multifocal placoid pigment epitheliopathy (APMPPE)
- Angiomatosis retinae
- Anterior ischemic optic neuropathy
- Behcet’s disease
- Branch retinal vein occlusion
- Cavernous haemangioma of the retina
- Choroidal rupture (when developing choroidal neovascularisation)
- Coat’s disease
- Cystoid maculopathy (Irvine-Gass syndrome)
- Diabetic retinopathy
- Eales disease
- Fuch’s spot
- Hemicentral retinal vein occlusion
- Idiopathic central serous choroidopathy
- Iris neovascularisation
- Maculopathy of angioid streaks
- Malignant choroidal melanoma
- Preretinal macular fibrosis
- Presumed ocular histoplasmosis (macular changes)
- Proliferative peripheral retinal disease
- Retinal capillary haemangioma
- Retinal microaneurism
- Retinal pigment epithelial dystrophies (central)
- Retinal pigment epithelial detachment
- Retinal tumours
- Sensory retinal detachments
- Tumours of iris and ciliary body.

CONTRA-INDICATIONS

- Frail Health
- Cardiac/Bronchial Disease
- Renal Diseases
- Hypersensitive Status
- Asthma (poorly controlled)
- Severe diabetes mellitus
- Hypertension
- Hypersensitivity to NaFl
- Pregnancy / Lactating mother.

TECHNIQUE PRINCIPLE

Sodium Fluorescein (NaFl) is a pharmacologically inactive substance which fluoresces when stimulated by an exciting light source. The substance may be available in solution concentrations of 5%, 10% and 25%. Absorbing light maximally at 485nm (blue), it emits maximally (peak transmission) at 520nm (green). In the bloodstream, when IV injected or orally ingested, it can be visualised at the ocular level by several methods:

- **Direct** with a BIO equipped with special filters
- **Video-based** system equipped with special filters permitting live recording of the Fluorescein flow
- **Photography** on standard 35mm negatives or positives viewed on a lighted box.

Photography is the usual method of performing FA. The procedure requires a special fundus camera which includes a high intensity flash with rapid recycle time delay (1 - 1,5 sec) to allow “rapid fire” serial photographs and a timer to register interval from injection time to picture time. A 36 exposure black and white film (200-400 ASA or Kodak Tri-X film) is usually used.

The camera is adapted to contain two special filters. The excitation filter is a blue filter of wavelength 465-490 nm [Baird Atomic B4 470 or Kodak Wratten 47]. Placed between the illumination system of the camera and the retina, it serves to excite and raise the NaFl electrons to a higher energy level causing them to fluoresce. The barrier filter is a yellow-green filter of wavelength 525-630nm [Iford 109 Delta Chromatic 3 or Kodak Wratten g15]. Placed between the light reflected from the retina and the camera film plane, it blocks the emitted light except the light from the excited NaFl (λ ~ 525nm) to allow the viewing and imaging of fluorescing tissues and blood.

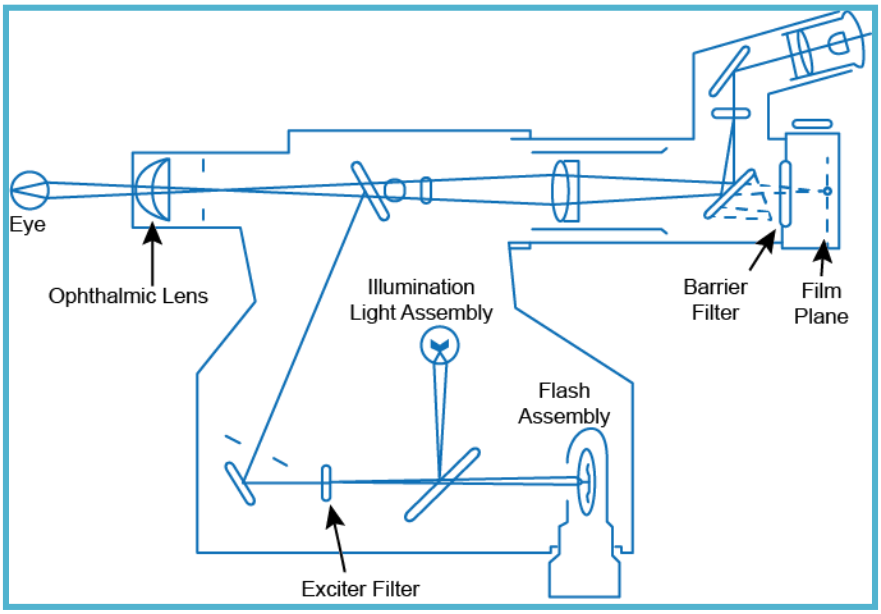

Figure 15.1: *Optics of the camera used in FA*

PROCEDURE

INTRA-VENOUS

- Have a crash cart (cardiopulmonary resuscitation unit) and emergency protocol on site
- Educate patient and obtain consent signature
- Maximum pupillary dilation required
- Seat patient comfortably and appropriately at fundus camera
- Direct patient fixation, which must be maintained throughout the procedure
- Obtain colour fundus photos (stereo pairs preferable)
- Obtain baseline red-free (green or Wratten 57) photos on black/white film through system filters
- Check for pseudofluorescence as a result of poor filter quality
- Ensure that proper filters and flashes are in place
- Set appropriate IV line on antecubital vein or hand vein and maintain with heparinised saline.
- Set 5ml NaFl 10% (preferred dosage) ready in a syringe for injection (or 10ml of 5% or 3ml of 25%)
- Locate and focus area of interest in the fundus
- Inject bolus of NaFl regularly over 10-15sec.
- Start photo shoot 10-15 sec later (time for NaFl to reach ocular circulation)
- Shoot rapid sequence photos first minute: 1 per 1-2 sec. first 15 sec.\ 1 per 10-15 sec. next 45 sec.
- Next 10-20 minutes shoot intermittently at regular time intervals (e.g. 1 per minute)
- Shoot photos of fellow eye after primary eye is done
- Shoot stereo pairs when possible

SIDE-EFFECTS

The procedure is relatively safe with a clinical dose of 100 X less than the lethal dose of NaFl but complications arise in ~ 5% of IV procedures (Table 1). Occurring during the first 30 sec. of the procedure, these are usually minor and transient. However cardiac arrest and even death can occur.

Common (in 5% of procedures within 30 sec)	Less Common
Nausea	Cardiac arrest
Warm flush	Respiratory reactions
Vomiting	Acute pulmonary oedema
Urticaria and pruritus (hives and itching)	Seizures
Skin discolouration (yellowing for ~1 hour)	Extravasation
Urine discolouration (yellow-orange for ~24 hours)	
Syncope	

Table 15.1: *Complications of IV procedures*

ORAL FLUOROGRAPHY

Oral Fluorography provides similar results but is not as good or as clear as IV FA. As such, it is not so valuable for the observation of fine details but it is especially useful when expected late dye leakage is of interest. With minimal to no side-effects, it is much safer than IVFA and may be used for children and patients with cardiovascular compromise. The procedure includes similar observation elements but with the following technical changes:

- Patient must fast 8 hours before exam
- NaFl is given orally: 15ml of 10% NaFl with citrus solution over crushed ice
- Dentures must be removed to avoid staining
- Red-free photos are taken before ingestion
- Photo shoot starts 15-30 min. after ingestion - must look for 1st sign of fluorescence in the fundus
- Late phases are ~ 1 hour after ingestion.

FACTORS AFFECTING VISUALISATION

- Clarity of media
- Dilation level
- Quality of camera
- Skill of examiner
- Patient cooperation
- NaFl concentration reaching the eye (depends most on injection quality)
- Quality of film processing
- Quality of filters (need regular changes) which can cause pseudofluorescence.

INTERPRETATION

The interpretation of FA is based on basic anatomical and physiological principles. Good knowledge of these is crucial to the understanding of FA.

Inner blood-retinal barrier - Healthy retinal vessels are impermeable to NaFl and are not leaky.

Fenestrated choriocapillaris - Healthy choriocapillaris is a sponge-like tissue whose vessels are fenestrated (porous). Hence blood and NaFl freely leak from it.

Outer blood-retinal barrier - Healthy RPE-Bruch complex with the tight “zonula occluden” junctions between RPE cells keeps blood (and NaFl) of the choroid and choriocapillaris away from the retina.

RPE filter - the RPE acts like an optical filter. The “choroidal glow” that results from the NaFl freely leaking within it only shows through partially, giving a typical **ground-glass appearance**.

Macular hypofluorescence - Dense cuboidal RPE and xantophyll mask the “choroidal glow” across healthy maculae.

TERMINOLOGY

Hyperfluorescence indicates more glow (NaFl) than would be normally expected (e.g. leakage, pooling, RPE faults...)

Hypofluorescence indicates less glow (NaFl) than would by normally expected (e.g. non-perfusion, blockage,...)

Autofluorescence refers to the ability of certain retinal components to fluoresce naturally without the presence of NaFl. The pre-injection as well as the black and white control photo through the camera filters allows the differentiation of autofluorescent elements (e.g. ONH drusen, myelinated NF,...)

Pseudofluorescence results from the overlap of the transmission curves of exciter and barrier filters that may result in apparent fluorescence. The pre-injection as well as the black and white control photo through camera filters also allows the detection of pseudofluorescence.

Transit refers to the time of the first passage of fluorescing blood in the eye (arm-retina cycle). Although it varies with the dynamics of blood circulation factors such as cardiac output, blood volume, etc., it is usually from 10-20 sec.

Recirculation Phase refers to subsequent passages of fluorescing blood in the eye. Recirculation phases are less intense.

Phases refer to specific physiological intervals in the FA cycle. Phases occur quite rapidly and may overlap with one another.

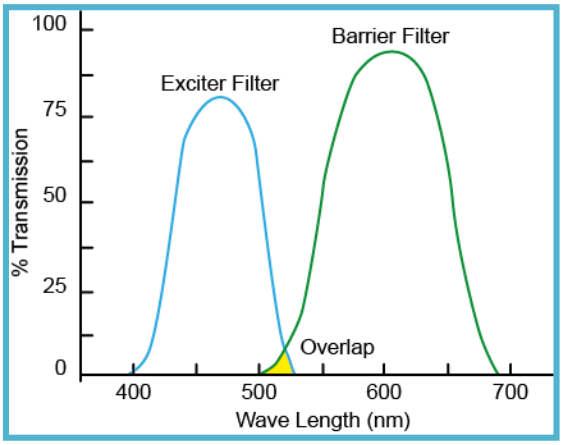

Figure 15.2: *Phases of the FA cycle*

Adapted from Eskridge JB, et al, Clinical Procedures in Optometry, Philadelphia, PA: J.B.Lippincott Company, 1991.

1. Choroidal Flush (7-10 sec. after injection)

The choroidal flush corresponds to the rapid "patchy" filling of the choroidal system (long and short ciliary arteries) with fluorescing blood (Fig. 15.3). Because of the fenestrated vessels of the choroid, the NaFl quickly leaks in the choroidal swamp and the choroidal glow becomes somewhat uniform. The RPE partly masks the choroidal flush to give it a "ground-glass" appearance; this stage occurs first as the choroidal circulatory route is slightly shorter than the retinal route. The Cilio-retinal artery fills at this stage because it originates from the choroidal vasculature.

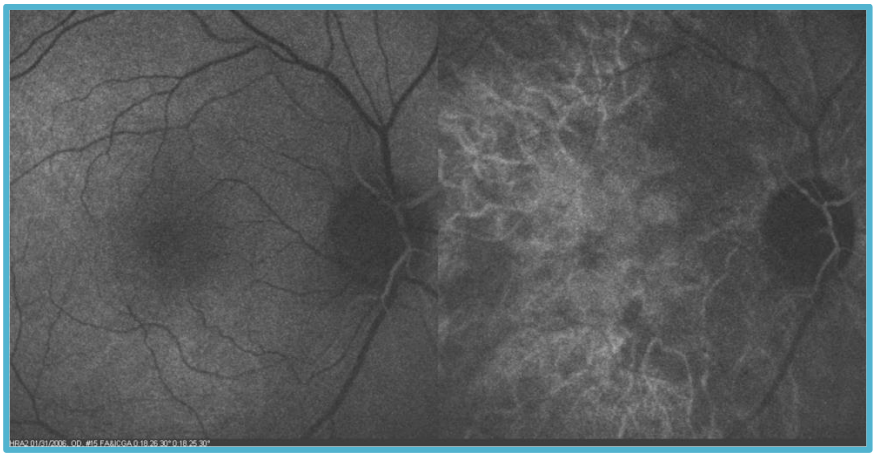

Figure 15.3: *Choroidal phase of FA cycle*

Photo Courtesy of Priyashangu Chandra: LV Prasad Eye Institute.

2. Arterial Phase (11-12sec. after injection)

The arterial phase corresponds to the filling of the Central Retinal Artery and retinal arteries. **Laminar flow** becomes observable (Fig. 15.4 and 15.5): blood flow dynamics make the arterial core glow before the arterial walls. This is followed by complete filling of the arteries (Fig. 15.6).

Figure 15.4: *Black indicates non-fluorescence; white area indicates fluorescence*

Figure 15.5: *Early arterial phase of FA cycle showing laminar flow*

Photo Courtesy of LV Prasad Eye Institute.

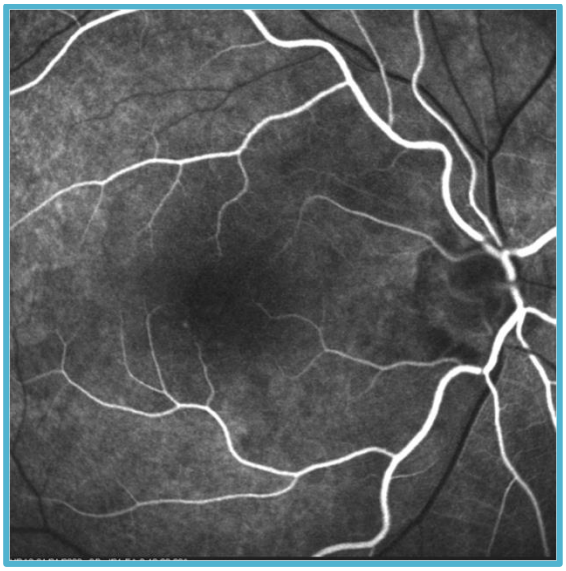

Figure 15.6: *Arterial phase of FA cycle*

Photo Courtesy of LV Prasad Eye Institute.

3. Arterio-Venous (A-V) Phase (13-15sec. after injection)

The A-V phase corresponds to the filling of capillaries in the interval between arterial and venous circulation (Fig. 15.7).

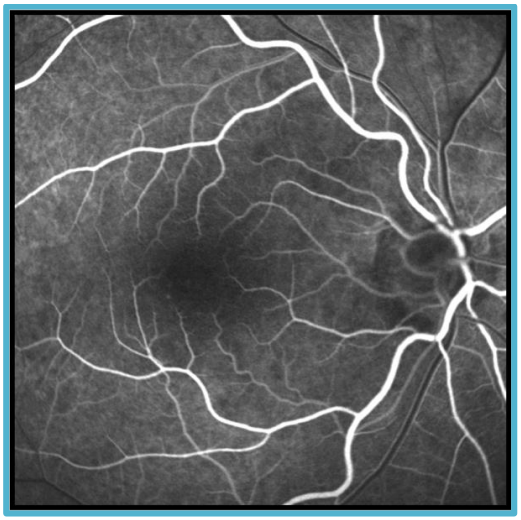

Figure 15.7: *Arteriovenous phase of FA cycle*

Photo Courtesy of LV Prasad Eye Institute

4. Venous Phase (16-20sec. after injection)

The venous phase corresponds to the filling of retinal veins. The laminar flow is initially observable but this time the venous wall glows before the venous core (Fig. 15.8 and 15.9). Laminar flow ceases at ~ 18-20sec. in the late venous phase when the veins are fully filled (Fig. 15.10).

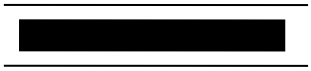

Figure 8. Black indicates non-fluorescence; white area indicates fluorescence

Figure 15.9: Early venous phase of FA cycle showing laminar flow

Photo Courtesy of LV Prasad Eye Institute.

Figure 15.10: *Late venous phase of FA cycle*

Photo Courtesy of LV Prasad Eye Institute.

5. Late Phase (~ 10minutes post-injection)

The late phase is the recirculation stages when the arteries and veins are empty of NaFl. The choroidal flush is minimised and the ONH hyperfluoresces because NaFl adheres to the scleral rim and nervous tissue (this glow is constant throughout the procedure and does not diffuse if the tissue is normal) (Fig. 15.11).

Figure 15.11: *Phases of the FA cycle*

Photo Courtesy of LV Prasad Eye Institute.

Leakage and **Pooling** become more apparent as NaFl diffuses into surrounding tissues. Leakage occurs with the extravasation or escape of fluorescing blood and indicates retinal vascular abnormality. Pooling represents accumulation of fluid and generally indicates a break in the RPE-Bruch barrier.

Note: Some authors prefer to use “Arterio-Venous Phase” to speak of stages 2,3,4 jointly.

INTERPRETATION STEPS OF FLUORESCEIN ANGIOGRAPHY

1. Study the colour photographs
2. Study the red-free photos (see everything)
3. Note the time, phase, location at all times
4. Look for hyper and hypofluorescence
5. Study frame by frame changes in size and intensity.

E.g. At 17 seconds, late choroidal filling phase, the OD shows 1 spot of hyperfluorescence in the inferior paramacular area. It is observed to percolate upward increasing in size and intensity throughout the test.

INTERPRETATION OF FLUORESCEIN ANGIOGRAPHY

Broadly two things need to be considered:

1. Hypofluoresce versus hyperfluoresce
2. Timing or delay in fluorescence.

Hypofluorescence	Hyperfluorescence →	Angiographic Features
Vascular filling defect	**Transmission defects** ⇐ atrophy of RPE revealing choroidal fluorescence	
Vascular occlusion Emboli Arteriosclerosis Vascular nonperfusion	Window defects Age-related macular degeneration pigment changes Retino-choroidal dystrophies Choroidal Rupture	⇐ Hyperfluorescence increases and decreases in phase with the FA
Blocked fluorescence	**Things that leak** ⇐ breakdown of inner retinal barrier	
Haemorrhages Exudates Cotton wool spots Glial tissue Retinal Pigment Masses	Microaneurisms Neovascularization Intra-retinal microvascular abnormalities Disruption of vessel integrity e.g. wet ARMD	⇐ Early intense leakage increasing throughout the FA
	Things that pool ⇐ breakdown of outer retinal barrier	
	Cystoid Macular Oedema Idiopathic Central Serous Choroidopathy Serous detachment of the RPE Papilloedema	⇐ Slowly increasing hyperfluorescence throughout the FA and after
	Things that stain ⇐ uptake of NaFl into tissues	
	Optic nerve head drusen Retinal Drusen Optic nerve head scleral rim and tissues Sclera Vessel Walls	⇐ Slow staining that does not diffuse or increase in size throughout the FA

Table 15.2: *Interpretation of FA*

INDOCYANINE GREEN ANGIOGRAPHY (ICG)

Indocyanine Green (ICG) is a relatively new procedure. ICG is advantageous over FA in that the ICG dye fluoresces near the infrared spectrum with peak absorption at 805nm and peak fluorescence at 835nm. This enhances the fluorescence through exudates, haemorrhages and normal ocular pigment (RPE). Being 98% protein-bound in the blood, the dye does not readily leak through the fenestrations of the choriocapillaris or neo-vessels. Therefore it does not obscure the underlying choroidal vasculature.

An improved visualization of the choroid is obtained with ICG because it permits a longer and uninterrupted view of the choroidal circulation. As such, it is primarily used to expose or define sub-retinal neo-vascular membranes (SRNVM) when they are suspected but not seen with NaFl angiography. ICG can however be used for all the same clinical applications as NaFl.

The only contra-indication to ICG is an allergy to shell fish or to iodine.