ISSN: 2996-0940
Research Article
Science Set Journal of Ophthalmology and Eye Care

# Evaluation of Imaging in Patients with Posterior Uveitis and Comparative Analysis of Fluorescein and Indocyanine Green Angiography with Spectralis® HRA and Optos

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**Submitted:** 13 November 2023 **Accepted:** 20 November 2023 **Published:** 30 November 2023

https://doi.org/10.63620/MKSSJOEC.2022.1005

***Citation:*** *Massamba, N., Rigo, S., Paris, A., Fardeau, C., Toutee, A., Soubrane, G., & Bodaghi, B. (2023). Evaluation of Imaging in Patients with Posterior Uveitis and Comparative Analysis of Fluorescein and Indocyanine Green Angiography with Spectralis® HRA and Optos. Sci Set J of Ophthalmology & Eye Care, 2(1), 01-07.*

***Abstract***

***Purpose:*** *Imaging patients with posterior uveitis and comparison of Fluorescein and Indocyanine green angiography with Heidelberg Spectralis®, as well as assessment and comparison with Optos.*

***Methods:*** *This is a single-center, observational case series study of thirty consecutive individuals with posterior uveitis (18 females and 12 males). 50 eyes received ultra-widefield fluorescein angiography using the Optos® panoramic P200Tx imaging system and the Heidelberg Spectralis® HRA+OCT system's noncontact ultra-widefield module. The photos were captured as a single, unguided shot focused on the macula. The overall area as well as the area inside each of the four visible quadrants were computed and compared between the two imaging modalities. Each quadrant per eye was also reviewed by three masked reviewers to determine which modality photographed the retinal vasculature more peripherally.*

***Results:*** *The majority of patients (60.0%) were male, 60% of the fifty eyes were right, and 40% were left. Both Fluorescein Fundus Angiography (FFA) and Indocyanine Green angiography (ICGA) groups had their Optos and HRA acquisition. With the exception of subretinal fluid separation ($p=0.843>0.05$), all other factors for FFA are statistically significant. Except for the Stromal granuloma late phase and CNV, there is no statistically significant difference between the other ICGA variables (Stromal granuloma intermediate phase, Atrophy and Scar). As a consequence, there is no statistically significant difference in any of these factors between the Optos and HRA for ICGA groups.*

***Conclusions:*** *HRA could diagnose uveitis damage in ICGA and FFA with a higher degree of sensitivity than Optos.*

**Keywords:** Fluorescein Angiography, Heidelberg Spectralis® HRA+OCT, Indocyanine Green Angiography, Optos® Panoramic P200Tx Imaging System, Peripheral and Occlusive Vasculitis, Posterior Uveitis, Vasculitis.

## Introduction

Over the last century, photographic imaging of the fundus has advanced dramatically. The Carl Zeiss Company created the first fundus camera in 1926, offering a 20° and subsequently 30° view of the posterior pole [1]. Traditional angiography images the central retina from 30° to 55°. The peripheral retina image is difficult to produce, and the quality suffers as a result of the eye's optical aberrations. To get around the constraints, we use a 7 or 9-field montage. Fluorescein Fundus Angiography (FFA) images are often utilised to reveal peripheral retinal findings and may reach up to 96° under optimal conditions [2]. Montage FFA pictures, on the other hand, need highly competent personnel, compliant patients, and is unable to get simultaneous images of the posterior pole and the periphery [3]. The imaging angles greater than the usual reference 30° and a wide field 55° are considered ultra-widefield (UWF) [4]. Several contact imaging techniques have been developed to provide a broader field view of the retina. The Pomerantzeff camera, Retcam (Clarity Medi-

cal Systems, Inc., Pleasanton, CA, USA), Panoret 1000TM camera (Medibell Medical Vision Technologies, Haifa, Israel), and Staurenghi lens (Ocular Staurenghi 230 scanning laser ophthalmoscope (SLO) Retina Lens; Ocular Instruments Inc., Bellevue, WA, USA) are examples [5]. Fundus imaging and technological advancements now enable clinicians to record up to 200° of the retina in a single capture [6].

Furthermore, using the typical FFA technique, inspection and exact measurement of structures and lesions surrounding the fundus can only be accomplished after image reconstruction [7]. However, since blood circulation is a dynamic process and pictures are acquired with a time delay, there are certain restrictions in seeing the anatomy of the retinal and choroidal blood circulation. Previous research has demonstrated the significance of ultra wide-field imaging in determining the severity of retinal diseases, including diabetes, retinal venous occlusions, uveitis, retinal vasculitis, and retinopathy of prematurity [8-11, 6]. Optos® is currently being utilised consistently in several different therapeutic settings [6]. The Optos® device uses an ellipsoid mirror to capture more than 80% of the retina in a single shot [12]. Optos® Optomap® has various limitations, such as inferior and superior peripheral retina imaging that is less extensive than temporal and nasal retina imaging [13].

Furthermore, the distant temporal and nasal peripheral retinas have significant distortion and poor resolution. Heidelberg Engineering (Heidelberg, Germany) has developed a noncontact ultra-widefield angiography (UWA) module (HRA 2). The Heidelberg Spectralis® had a 25º and 35º field of view of the retina, with a 55º noncontact lens attachment for autofluorescence, fluorescein, and Indocyanine Green Angiography (ICGA) images. Using the Staurenghi contact lens with the Spectralis® provided a 150º field of vision in a single shot. The newly developed Heidelberg UWA module uses a replaceable noncontact lens to give high-contrast, undistorted, evenly lighted images to the peripheral retina. This UWA Heidelberg Spectralis® module can conduct both FFA and ICGA [12]. Imaging the retina's periphery has become more critical for assessing, categorising, diagnosing, and treating various retinal illnesses. After that, comparing the various imaging systems would be helpful, mainly because the quality of the pictures' algorithms can increase but not the quality of the pictures themselves. In this research, we sought to share our experience using a non-contact large field module of 55° from Heidelberg Spectralis®, as well as assess and compare it to Optos to determine the various features of inflammatory illnesses, such as vasculitis, papillitis, and intra-retinal infiltrates in the middle and late phases of FFA and ICGA.

## Methods

### Study Design and Period

A single-center, observational case series study was performed at the Department of Ophthalmology, Pitié-Salpêtrière Hospital, Sorbonne University, from May to December 2021.

### Patients

Thirty consecutive individuals with posterior uveitis were included in this study (18 females and 12 males). The study was carried out following the Helsinki Declaration for human subject's research and our local ethics committee. All patients underwent a comprehensive ophthalmologic examination, which included best-corrected visual acuity with standard Early Treatment Diabetic Retinopathy Study charts, fundus examination, colour fundus photographs, red-free, fundus autofluorescence, fluorescein fundus angiography (FFA) and Indocyanine Green Angiography (ICGA) on Heidelberg Spectralis HRA + OCT and 200Tx Optos ultra-widefield imaging system.

### Inclusion and Exclusion Criteria

Thirty adult patients (50 eyes) were chosen for the study. The following were the inclusion criteria: ≥18-year-old patient with posterior and intermediate uveitis. Exclusion criteria were retinal and choroidal detachment, vascular disease, Age-related Macular Degeneration, complicated image capture due to impaired eyesight, intolerance or poor cooperation with the FFA examination, and allergy to fluorescein sodium (for injection).

### Image Acquisition Technique

The images were taken one after the other with an average of 1-2 minutes between devices in the intermediate and late phases to ascertain our outcome.

### Fluorescein Fundus Angiography (FFA)

The overall area and area within four visualised quadrants (superior, inferior, nasal, and temporal) were calculated and compared for outcome evaluation. The fundus angiography images were taken using the Optos 200Tx ultra-widefield imaging system and the Heidelberg Spectralis HRA+OCT system. Before the exam, a specialised technician inquired about the patient's allergy to contrast media. The pupil was dilated for half an hour using tropicamide and phenylephrine eye drops. After that, 1% fluorescein sodium diluent was injected, and the patients were examined to ensure they were not allergic to the contrast media. Following that, a 5 ml 20% fluorescein sodium injection was administered into the elbow vein for 5 seconds. After capturing a 30-second image with a 55-degree super wide-angle front lens for the Spectralis, the patients were instructed to rotate their eyeballs in 9 directions in turn: the posterior pole, superior, superior temporal, temporal, infratemporal, inferior, superior nasal, nasal, and infra nasal approach, and one image was collected per second. After obtaining 60-second images of the posterior pole with the Optos 200Tx, patients were directed to rotate their eyeballs in four directions: superior, inferior, nasal, and temporal, and one image was acquired every second. For comparison, translucent, representative, and venous retinography images ranging from 30 seconds to 3 minutes were chosen. A skilled technician captured all of the angiographic images. Before quantitative analysis, images from the Optos 200Tx were turned into stereographic projection images using proprietary software available from the manufacturer (Doheny Eye Institute) [14].

### Indocyanine Green Angiography (ICGA)

Reconstitute one (1) 25 mg vial of Indocyanine Green for Injection USP under sterile conditions with one (1) 10 mL Sterile Water for Injection USP vial from the ICG for Injection Set. To dissolve the ICG vial, gently shake it. A 25 mg vial of ICG contains 2.5 mg of dye per mL of solution after reconstitution. Therefore, a 1.0 mL injection contains a 2.5 mg dosage of ICG. The diluted ICG (0.025 mg/mL) was delivered intravenously, and a single ICG dose was 0.25mg. During the mid-venous phase of ICGA,

we considered the intermediate time. The choroidal veins are scarcely visible between 4 and 15 minutes. And we considered the late-stage between 30-45 minutes.

### Analysing Uveitis Imaging in FFA and ICGA in the Intermediate and Late Phases in Optos vs HRA

We conducted a retrospective review of all included eyes. In the intermediate and late stages of FFA and ICGA, images in both devices were evaluated by two distinct readers. In the event of a disagreement, the advice of a third reader was sought. Only acute uveitis disease was studied in FFA for presence of vasculitis, papillitis, retinal lesions, subretinal detachment, and retro foveolar cystoid macular oedema. In FFA, vasculitis is defined as a breakdown in the blood-retinal barrier as seen by retinal vascular leakage. FFA demonstrates peripheral retinal nonperfusion and a region of hypofluorescence in occlusive vasculitis, the "petaloid" pattern of parafoveal hyperfluorescence in eyes with cystoid macular edema, hyperfluorescence of the optic disk and hyperfluorescent or hypofluorescent intraretinal infiltrates. Subretinal detachment in intermediate FFA uveitis disease displays numerous pinpoint leaking and late phase dye pooling. In the intermediate and late phases of ICGA, choroidal granulomas may be hypofluorescent, isofluorescent, or barely hyperfluorescent. The researchers also looked into choroidal neovascularization (CNV) and late-stage hypo-fluorescent atrophy.

### Statistical Analysis

The collected data were entered into an excel file and analyzed using SPSS 22.0. The frequency method is used to find the number of times the data item has to occur. Then Independent t-sample tests were used to compare the Optos and HRA for FFA and ICGA procedures.

## Results

### Demographic Characteristics of Patients

Table 1 represents the frequency of demographical variables for the FFA and ICGA procedure. Among the thirty patients, about 30.0% of the patient's age were under the age group of 21-30, 23.3% of the patients were under the age group 31-40, 20.0% of the patients were under the age group of 51-60, and only 6.7% and 3.3% of the patients were under the age group of 11-20 and above 60 years respectively and the mean age and SD for the patients included in the FFA group are 37.10 ± 13.63. When considering the Gender majority, 60.0% of the patients were Female and 40.0% of the patients were Male. Among the fifty eyes 60.0% of the eyes were Right and 40.0 % of the eyes were Left.

**Table 1: Demographic Variables for the FA and ICGA Procedures**

| Variables | | FA | ICGA |
|---|---|---|---|
| | | N (%) | |
| Age | 11-20 | 2 (6.7) | 2 (6.5) |
| | 21-30 | 9 (30.0) | 10 (32.3) |
| | 31-40 | 7 (23.3) | 7 (22.6) |
| | 41-50 | 5 (16.7) | 4 (12.9) |
| | 51-60 | 6 (20.0) | 7 (22.6) |
| | Above 60 | 1 (3.3) | 1 (3.2) |
| Gender | Female | 18 (60.0) | 18 (58.1) |
| | Male | 12 (40.0) | 13 (41.9) |
| Eye | Left | 20 (40.0) | 20 (40.0) |
| | Right | 30 (60.0) | 30 (60.0) |
| Diagnosis | ARN | 4 (8.0) | 4 (8.0) |
| | Sarcoidosis | 10 (20.0) | 10 (20.0) |
| | Behcet | 3 (6.0) | 4 (8.0) |
| | Birdshot | 8 (16.0) | 8 (16.0) |
| | Cat Scratch Disease | 1 (2.0) | 1 (2.0) |
| | Indertermine | 2 (4.0) | 2 (4.0) |
| | Irvan | 1 (2.0) | 1 (2.0) |
| | Sympathetic Ophthalmopsthie | 1 (2.0) | 1 (2.0) |
| | Uveitis Pars Planitis | 2 (4.0) | 2 (4.0) |
| | Scleritis | 2 (4.0) | 2 (4.0) |
| | Lyme Disease | 4 (8.0) | 4 (8.0) |
| | Syphilis | 1 (2.0) | 1 (2.0) |
| | Tuberculosis | 5 (10.0) | 4(8.0) |
| | | 6 (12.0) | 6 (12.0) |

About 20.0 % of the patients were diagnosed with Sarcoidosis, 16.0% of the patients were diagnosed with Birdshot, 12% of the patients were diagnosed with VKH and 8 % of the patients were diagnosed with Lyme Disease as well as ARN, 10% of the patients were diagnosed with Tuberculosis, 6% of the patients were diagnosed with Behcet, 4 % of the patients were diagnosed with

ARN, Indeterminate Uveitis, Pars Planitis, Scleritis, and finally only 2% of the patients were diagnosed with the Cat Scratch Disease, IRVAN, Sympathetic Ophthalmopathy and Syphilis.

Among the thirty patients in ICGA procedure, the majority of 32.3% of the patients were under the age group of 21-30, 22.6 % of the patients were under the age group of 31-40 as well as 51-60, 12.9 % of the patients were under the age group of 41-50, 6.5% and 3.2% of the patients were under the age group of 11-20 and Above 60 respectively. The mean age and SD for the patients included in the ICGA group are 37.13 ± 13.98. In gender, 58.1% of the patients were Female and 41.9% of the patients were Male. Among the fifty eyes 60% of the eyes were Right and 40% of the eyes were Left.

Majority of 20.0 % of the patients were diagnosed with Sarcoidosis, 16.0% of the patients were diagnosed with Birdshot, 12% of the patients were diagnosed with VKH and 8 % of the patients were diagnosed with Lyme Disease, ARN, Tuberculosis and Behcet, 4 % of the patients were diagnosed with Indeterminate Uveitis, Pars Planitis and Scleritis and finally only 2% of the patients were diagnosed with the Cat Scratch Disease, IRVAN, Sympathetic Ophthalmopathy and Syphilis.

Table 2 represents the descriptive statistics and independent t-sample test for both FFA and ICGA. Mean and SD for the Optos and HRA were calculated for both FFA and ICGA group. The Independent t-sample test is used to compare both Optos and HRA with seven variables for FFA group. Among these, except Sub retinal fluid detachment (p=0.843>0.05) all the other variables is statistically significant, hence p-value is less than 0.05 for Optic disk (p=0.000<0.05), Vasculitis (p=0.001<0.05), Peripheral vasculitis (p=0.000<0.05), Retinal Foci (p=0.000<0.05), Occlusive vasculitis (p=0.000<0.05) and CME (p=0.000<0.05). Hence, there is a statistically significant difference among all these variables between the Optos and HRA for FFA group.

**Table 2: Descriptive Statistics and Independent T-Sample Test for FA and ICGA Procedures**

| Variables | Optos (n=50) | HRA (n=50) | p-value |
|---|---|---|---|
| | Mean ± SD | | |
| **Fluorescein Fundus Angiography (FFA)** | | | |
| Optic Disk | 0.40 ± 0.495 | 0.90 ± 0.303 | 0.000** |
| Vasculitis | 0.28 ± 0.454 | 0.60 ± 0.495 | 0.001** |
| Peripheral Vasculitis | 0.38 ± 0.490 | 0.02 ± 0.141 | 0.000** |
| Retina Foci | 0.10 ± 0.303 | 0.50 ± 0.505 | 0.000** |
| Occlusive Vasculitis | 0.30 ± 0.463 | 0.02 ± 0.141 | 0.000** |
| CME | 0.02 ± 0.141 | 0.56 ± 0.501 | 0.000** |
| Subretinal Fluid Detachment | 0.48 ± 0.505 | 0.50 ± 0.505 | 0.843 |
| **Indocyanine Green Angiography (ICGA)** | | | |
| Stromal Granuloma Intermediate Phase | 0.40 ± 0.495 | 0.42 ± 0.499 | 0.841 |
| Stromal Granuloma Late Phase | 0.28 ± 0.454 | 0.58 ± 0.499 | 0.002** |
| CNV | 0.32 ± 0.471 | 0.10 ± 0.303 | 0.007** |
| Atrophy | 0.14 ± 0.351 | 0.12 ± 0.328 | 0.769 |
| Scar | 0.10 ± 0.306 | 0.06 ± 0.242 | 0.466 |

The Independent t-sample test is used to compare both Optos and HRA with five variables for ICGA group. Among these, except Stromal granuloma late phase (p=0.002<0.001) and CNV (p=0.007<0.001), all the other variables have no significant difference among the variables, hence p-value is greater than 0.05 for Stromal granuloma intermediate phase (p=0.841>0.05) (Figure 1), Atrophy (p=0.769>0.05 and Scar (p=0.466>0.05). Hence, there is no statistically significant difference among all these variables between the Optos and HRA for ICGA group.

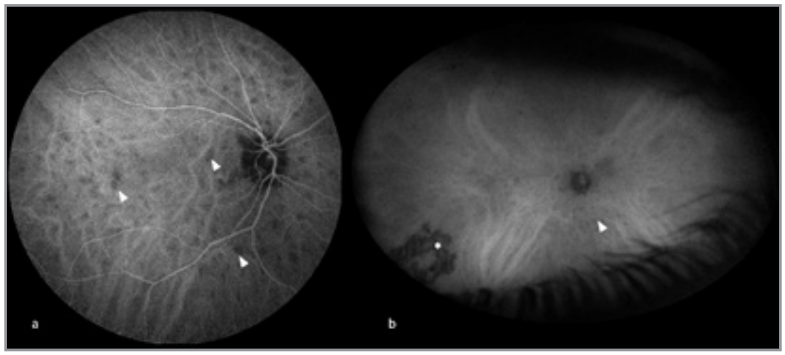


**Figure 1:** Right eye ICGA in the intermediate phase of a 43yo female with Birdshot retinochoroiditis. HRA (a) shows more stromal granulomas (white arrowheads) than Optos (b). The only benefit of the Optos is the visualization of peripheral laser retinopexy (white star).

## Discussion

Imaging the peripheral retina has become critical for diagnosing, classifying, and managing various retinal illnesses [15-17]. Noncontact ultra-widefield fluorescein angiography with the Optos® Optomap® and Heidelberg Spectralis® imaging systems captures both the posterior pole and peripheral retinal abnormalities in a single, nonsteered shot [12]. We report the first direct comparison of FFA and ICGA with Spectralis® HRA and Optos. Uveitis is an inflammatory eye disorder that may affect the retinal vessels. Retinal vasculitis is a kind of posterior uveitis characterised by retinal vascular nonperfusion, retinal haemorrhages, vascular sheathing, overlaying vitritis, and fluorescein leakage in the form of clinical vessel wall alterations detected by ophthalmoscopy and/or fundus FFA (Figure 2) [18]. The ultra-widefield Heidelberg Spectralis module can more peripherally film the superior and inferior retinas than the Optos. However, the Optos is more effective in the temporal and nasal areas and has a bigger total retinal picture (Figure 3). Despite imaging a wider total retinal surface area, Optos® Optomap® looks distorted, particularly in the distant temporal and nasal periphery, owing to the use of an ellipsoid mirror to scan the retina [12]. The Heidelberg Spectralis® HRA2 noncontact lens, on the other hand, could generate undistorted and crisp flat pictures of the central and peripheral retina in a single image. Despite advances in imaging technology, binocular indirect ophthalmoscopy is still required for peripheral retinal exams with scleral indentation. In the present study, Optos and HRA were calculated for both FFA and ICGA groups with seven variables (Optic disk, Vasculitis, Peripheral vasculitis, Retinal Foci, Occlusive vasculitis, Sub retinal fluid detachment and CME) for FFA and five variables for ICGA (Table 2). Except for Sub retinal fluid separation ($p=0.843>0.05$), all other variables were statistically significant for FFA. Except for the Stromal granuloma late phase (Figure 4) and CNV, there is no significant difference among the five variables (Stromal granuloma intermediate phase, Atrophy and Scar, Stromal granuloma late phase, and CNV) for ICGA. As a result, there is no statistically significant difference between the Optos and HRA for ICGA groups in any of these variables.

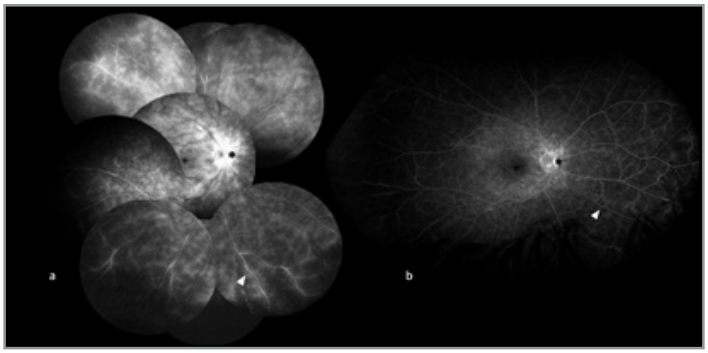

**Figure 2:** Fundus fluorescein angiography of a 61 yo black female with sarcoidosis showing hyper fluorescence of the optic nerve, peripheral phlebitis, and retinal venous leakage in the intermediate phase of fluorescein angiography shown on HRA (a) and Optos(b). The vasculitis (white arrowhead) and papillitis (black star) are better visualized in HRA than optos.

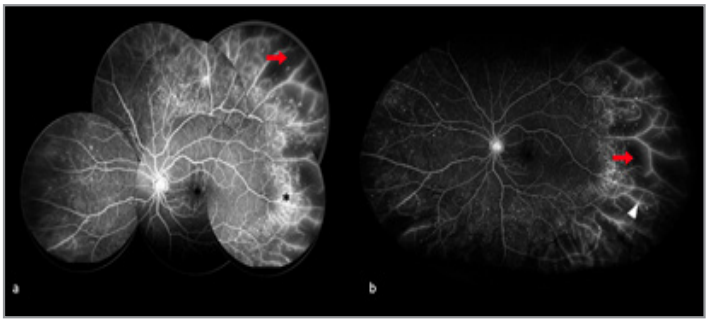

**Figure 3:** Follow-up fluorescein angiography of 68yo female undergoing sarcoidosis uveitis, on her left eye. HRA fluorescein angiography (a) shows more microvascular involvement (dark star) and a small area of the earlier stage of peripheral ischemia (red arrow), while Optos (b) shows it better and shows the vascular staining (white arrowhead). In both devices vitreous degeneration (synchesis) can be observed.

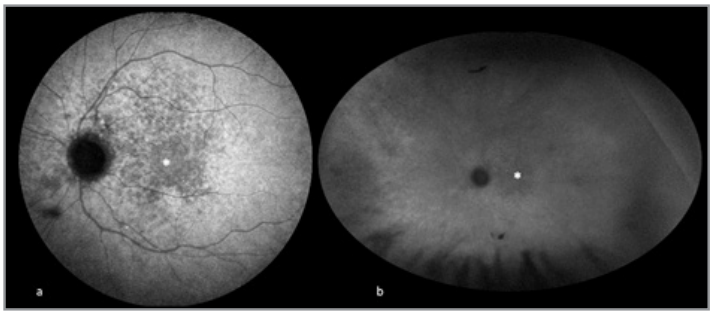

**Figure 4:** Left eye of a 49 yo male with indeterminate uveitis. ICGA shows multiple numerous hypo fluorescent lesions better visualized in the late phase in HRA (a) than Optos (b) (white star).

The working group on Standardization of Uveitis Nomenclature has proposed fundus pictures or fluorescein angiograms as evidence for documenting structural uveitis problems in epidemiological studies, clinical trials, and clinical reports [18]. FFA imaging has proved especially effective in the setting of uveitis, whether active or chronic, owing to the investigation of the optic disk, vein, artery, and presence of serous retinal detachment, CME, intraretinal infiltrates, CNV. ICGA can also be required in posterior uveitis to assess the presence of stromal granulomas [19]. Our investigation examined images of FFA and ICGA obtained with Optos and HRA in the middle and late stages of posterior uveitis or panuveitis. Modern systems include quick picture capture and processing times and the ability to transmit photographic data to other units or electronic health records. Wide-field imaging is critical in detecting, treating, and tracking retinal diseases. They often extend to the periphery or have major circulatory alterations that may be recognised and defined using wide-field imaging [20]. The same operator took the photos, which were then processed and reviewed by two separate observers who assessed the existence of various parameters. In the event of a disagreement between the two pupils, a senior third-grader was assigned to read the photographs. The acquisition time between the two additional devices was less than one minute. However, comparing the FFA, we found that the occlusive and peripheral vasculitis were more precise in Optos vs. HRA because the Optos® system uses an ellipsoid mirror to produce images at approximately 200 degrees. This allows simultaneous assessment of the peripheral and central retina without significant eye steering. These findings result from confocality, which allows selecting the depth of the image and defining the layer with the best quality.

HRA and Optos are both Scanning Laser Ophthalmoscopes (SLO). However, HRA is a confocal SLO. It allows us to choose the depth of the picture we wish to capture—this aids in removing noise created by the higher layers from the deeper levels. When evaluating a picture of the deeper levels captured with Optos, the reflection and noise caused by all of the top layers are unavoidable. Furthermore, HRA employs "Automatic Real-time" (ART) technology, which may benefit from a 20-image addition to minimise artefacts and boost contrasts, leading to a more transparent, brighter, and sharper picture. In a single acquisition, we revealed the relevance of Optos in FFA in occlusive and peripheral vasculitis. To observe the peripheral retinal, the patient must gaze at the nine spots with the HRA. Due to the lower contrast circulating in the blood, data demonstrated statistically significant results in ICGA pictures utilising HRA vs. Optos in the late stage. As a result, the sensor has a more difficult time acquiring the picture. The ICGA photos result from the Optos California ICG (Optomap) images' low resolution, which ranges from 14 to 20 µm. In high-resolution mode, HRA photos with the 55° lens are 10 µm, providing up to double the resolution.

## Study limitation

The limited sample size of our group and the involvement of only one clinical location are two limitations of our research. In assessing retinal disorders, notably in 11 uveitis, the usefulness of ultra-wide-field imaging has been shown in previous research and published literature. However, to the best of our knowledge, this is the first research to evaluate and compare both of these devices, including the selection of a patient population that was very well matched.

## Conclusion

In conclusion, HRA could diagnose uveitis damage in ICGA and FFA with a higher degree of sensitivity than Optos. In this study, we compared the sensitivity of the Optos assay to that of the HRA as it relates to detecting several clinical indicators in FFA and ICGA. Our study demonstrated that FFA imaging of vasculitis, retinal foci and optic disk hyperfluorescence was more precise using HRA than Optos. During the middle and late angiography stages of the ICGA imaging process, the difference between Optos and HRA becomes readily apparent. In the later phases of ICGA, Optos photos were unrecognisable due to the increasing noise levels and poorer resolution of the raw photographs used. Scanning Laser Ophthalmoscopes (SLO) and the ART can eliminate artefacts and boost contrasts, resulting in a more precise, clearer, and sharper picture and a high-resolution mode of 10 µm as opposed to 14-20 µm for Optos. Despite this, the Optos® technology creates pictures using an ellipsoid mirror to achieve an angle of roughly 200º.

## Summary Statement

We compared and demonstrated that HRA imaging was more precise and sensitive than Optos in patients with posterior uveitis, using FFA and ICGA. Nevertheless, we affirm that Optos is not as bad as it appeared, but its use would be more adapted in certain cases.

## Author's Role

Study design, data collection, data analysis, manuscript preparation: NM, AP, and BB Study design, NM, AP, AT, CF, SR: Data collection, data analysis, manuscript preparation and revision, NM, BB, manuscript revision, BB and GS


## Acknowledgments

The authors acknowledge Professor Gisele Soubrane for her in-depth knowledge in describing imaging.

## Financial Disclosures

No relevant financial disclosure with this work.

## Funding

None


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