

# Fluorescein Angiography

## *Insight and Serendipity a Half Century Ago*

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**I**t has been 50 years since fluorescein angiography was developed as a clinical procedure by 2 medical students at Indiana University. The story of its discovery and the recognition of its value to ophthalmology involve a combination of insight and serendipity. Fluorescein had been in use clinically for more than half a century, but it took a pulmonary medicine laboratory to provide the stimulus for the development of flash and barrier filters that would make vascular photography practical. The first article was rejected by the ophthalmology literature, but several clinics heard about it and soon documented the enormous diagnostic value of the procedure.

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Many articles<sup>1-6</sup> have been written about the history of fluorescein angiography and its discovery by 2 medical students at Indiana University in 1960. Of the 2 protagonists, David Alvis has written several articles<sup>1,4,5</sup> about the events that took place, whereas the junior student (but first author) Harold Novotny has rarely made a public comment.<sup>3</sup> The story is an interesting one in terms of the circumstances that allowed for the discovery and in terms of the interaction between the 2 individuals. We have had the opportunity to speak with both Alvis and Novotny and to revisit the story. The history of what happened is well known; we will recount why and how the test became practical for the field of ophthalmology. This is not a review of how angiography evolved over 50 years but a commemorative story of the challenges that were faced to discover and disseminate new medical information.

Fluorescein dye was introduced into the field of ophthalmology in 1882 by Paul Ehrlich, who injected it intravenously in rabbits to observe the dynamics of aqueous humor.<sup>7</sup> Many others, including Seidel,<sup>8</sup> Franceschetti,<sup>9</sup> and Goldmann,<sup>10</sup> used the

dye in various ways to observe different aspects of anterior segment and retinal tissue, but the concept of following the retinal circulation clinically was not realized.

The first insights (although unknown to the eventual developers of the technique) came from one of the great scholars of retinal vascular disease, Edward Maumenee, while head of the Division of Ophthalmology at Stanford University School of Medicine (1948-1955), which at that time was still located in San Francisco, California. In that era, it was difficult to distinguish choroidal hemangiomas from melanomas, and Maumenee was looking for ways to highlight the differences. According to Howard Schatz,<sup>3</sup> Maumenee said, "I recalled an article of Dr. Sorsby that I ran across when I was a resident at the Wilmer Institute. He used various dye substances to attempt to outline holes in the retina in patients with retinal detachments.<sup>11</sup> I also recalled the work of Goldmann<sup>10</sup> and others who had used fluorescein to study the circulation time in the anterior segment of the eye. Since the Goldmann slit lamp had a cobalt blue filter on it, I thought that I could inject fluorescein intravenously, and by using a contact lens and cobalt blue filter with the slit lamp I would be able to determine whether the patient had a vascular lesion or a solid

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melanoma.” This work (not published until several years later with Dr Maclean)<sup>12</sup> showed the value of the dye in recognizing hemangiomas and evaluating treatment, and included a notation about “the arteries filling first and then the veins.” But Maumenee did not develop the tools for monitoring the circulation photographically.

One of Maumenee’s staff, Milton Flocks (working with a visiting fellow from China, Peter Chao, and a doctor filling time before the starting date of his ophthalmology residency, John Miller) took the next step, in using the dye to study circulation time in the cat. Initially, in 1958, they watched the passage of trypan blue with an ophthalmoscope and a stopwatch, measuring a transit time of roughly 2 seconds.<sup>13</sup> They noted that more innocuous dyes such as fluorescein might allow for the clinical application of the technique. In the next report in 1959,<sup>14</sup> they attached a motion picture camera to a Zeiss fundus camera in which the carbon arc light was passed through a cobalt blue filter. They injected fluorescein intravenously into cats and succeeded in getting images (although the lighting was tricky) that confirmed a circulation time near 2 seconds. However, they failed in 2 attempts to do the procedure on humans because of “insufficient light.” Miller recalled that they did get some successful human images, including one that appeared to show extensive dye leakage in age-related macular degeneration. But they could not get serial images that would measure circulation time, which was their goal. Had they succeeded, or continued the work, the birth of clinical angiography might have been at Stanford. Maumenee, and later Flocks, had clearly recognized the potential clinical value of fluorescein, but Flocks struggled with the technical problems of cinematography in an era of slower films. He did not attempt still photography, which probably seemed a poor solution for following the rapid process of blood flow. He used a blue filter for the flash and put a UV filter (Wratten 2B) in the camera path. But the UV filter did not block visible blue light, and he apparently did not look for

barrier filters that would enhance the separation between flash and fluorescence in the retina.

At the same time, Swan and Bailey in the Department of Ophthalmology at the University of Oregon were developing a system for fundus cinematography.<sup>15,16</sup> They modified the optics from a fundus camera to use through a dissecting microscope and took color motion pictures of the fundus. The device was practical for clinical use but was not a simple modification of commercially available equipment. And they did not use dyes to follow the circulation. It is not clear whether the light would have been sufficient after interposing a blue filter. Nor do we know whether Flocks and Chao were aware of this work, which was not published until 1959.

Thus, the groundwork for fluorescein angiography was laid (ie, the recognition that fluorescein could be imaged entering and leaving vessels through a fundus camera, and that fundus cinematography was possible). What was needed to make a clinical test? A practical test would need equipment that was readily available, a means of recording the data in a manner that would allow clinical display and storage and, of course, a demonstration of the value in practice. As obvious as these steps may seem to us half a century later, they were not so obvious at the time when the technology was new and clinical applications were hypothetical. One may guess that these laboratories might have hit on a workable combination of techniques eventually; serendipitously, however, the right conditions appeared first in Indiana and outside the field of ophthalmology.

Harold Novotny was a junior medical student at Indiana University who had trained in college at Purdue to be a pharmacist and practiced briefly before entering the military. His knowledge of drugs and chemistry would later prove useful. Novotny decided to switch to medicine after his military service, and he met Professor John Hickam, the chair of medicine and an eminent pulmonologist, in his medical school classes on physical diagnosis. Hickam became a father figure to Novotny. Novotny needed money to

get through school, and although he already had 2 jobs, he asked Hickam about working in the laboratory as a third job. Hickam said “no,” but Novotny was persistent and was finally hired in his junior year to help with experiments on oxygen saturation of the blood. This topic was not of special interest to him, and he basically hired to do “scut work.” But once he started the project, it tweaked his imagination.

Hickam had acquired a new Zeiss fundus camera because he recognized that retinal circulation could be a means of visualizing the vasculature for his oxygenation saturation studies.<sup>17</sup> Novotny had a set of responsibilities in the laboratory that included learning how to use the new camera. He told Howard Schatz many years ago, “I had learned a great deal about the camera and equipment and so I started to think about passing some kind of dye through the system to examine the retina in order to determine saturation levels. . . . I was trying to decide how I was going to measure its concentration. I thought that if I could look at something in a highly specific way by observing fluorescence from it, it might be easier. I knew something about fluorescein and as I became involved in this project, I learned a great deal about it.”<sup>3</sup>

As Novotny was becoming familiar with Hickam’s camera, another medical student, David Alvis, joined the laboratory during the “quarter off” of his senior year. Together, by chance or by serendipity, they experienced a revelatory moment in October of 1959 that led to the investigation of fluorescein angiography. Alvis related the story to us: “One day Harold was looking through the camera, and he discovered the different colors in the crystalline lens. He asked me what I thought it was. Since my father was an ophthalmologist, and knew about my interest in eyes, he talked a lot about his various medications used in his general practice. He saw lots of industrial patients for injuries and used topical fluorescein frequently. For reasons unknown to me in 1959 and still unknown to be 51 years later, I told him that perhaps that was fluorescent light he saw. Harold was right on the ball, and he wondered

aloud if we could photograph fluorescein as it circulated in the retinal vessels that we were studying for Dr. Hickam . . . If Harold had not been so observant and I with the quick answer, there would not have been any [work on fluorescein angiography]" (e-mail communication, July 2010).

Novotny and Alvis were unaware of the work by Flocks and others on angiography, and they wanted to explore the idea. They ran it by Fred Wilson, the chair of ophthalmology, but he told them that he doubted the technique could work because lens fluorescence would interfere with recognition of the dye in the retinal circulation.

Fortunately, Novotny and Alvis were not easily discouraged, and they decided to see just what properties the dye would have in the blood. Hickam was interested in the project that the 2 students proposed (to explore dyes as a means of photographing the circulation) but saw it as a sideline of his studies on oxygen saturation. He would offer occasional advice, but Novotny and Alvis had to do the work after hours when Novotny's primary laboratory assignments had been finished and the equipment was free. Novotny knew enough about the pharmacology to realize that they must find out the spectral properties of the dye, and he had the idea that one might be able to enhance the recognition of fluorescence in vessels by pairing 2 filters. They took a sample of Alvis's blood that had been injected with fluorescein to Eli Lilly and Co, which had spectrophotometers, to determine the absorption and emission curves. They learned that the dye absorbed maximally at about 490 nm and emitted with a peak at 520 nm, and they looked for filters that would enhance photographic separation. Perhaps fortuitously, the standard Kodak Wratten filters 47 and 58 had peaks in the blue and green, with relatively little overlap at 500 nm.

The next step was to insert these filters into the fundus camera, which almost killed the project. While Hickam was on a trip, Novotny removed the side panel of the camera to explore where to place the filters. When Hickam returned, he was

furiously that his expensive instrument had been violated. Novotny hastened to show him what the filtered camera could do and reassured him that it would not be hard to put it all back together. Fortunately, Hickam recognized the potential power of angiography, and he decided to let the experiments continue (and to continue paying the expenses). Novotny estimates that the whole project cost about \$2000, which was not insignificant in those days. Hickam told him to get whatever he needed, as long as it did not cost more than \$500. In fact, his policy was that, if you need 10 vials of fluorescein, buy 100, because you did not want to run out in the middle of the experiment. Hickam continued to use fluorescein angiography years after Novotny and Alvis had left, for studies on retinal circulation and oxygenation.<sup>18</sup>

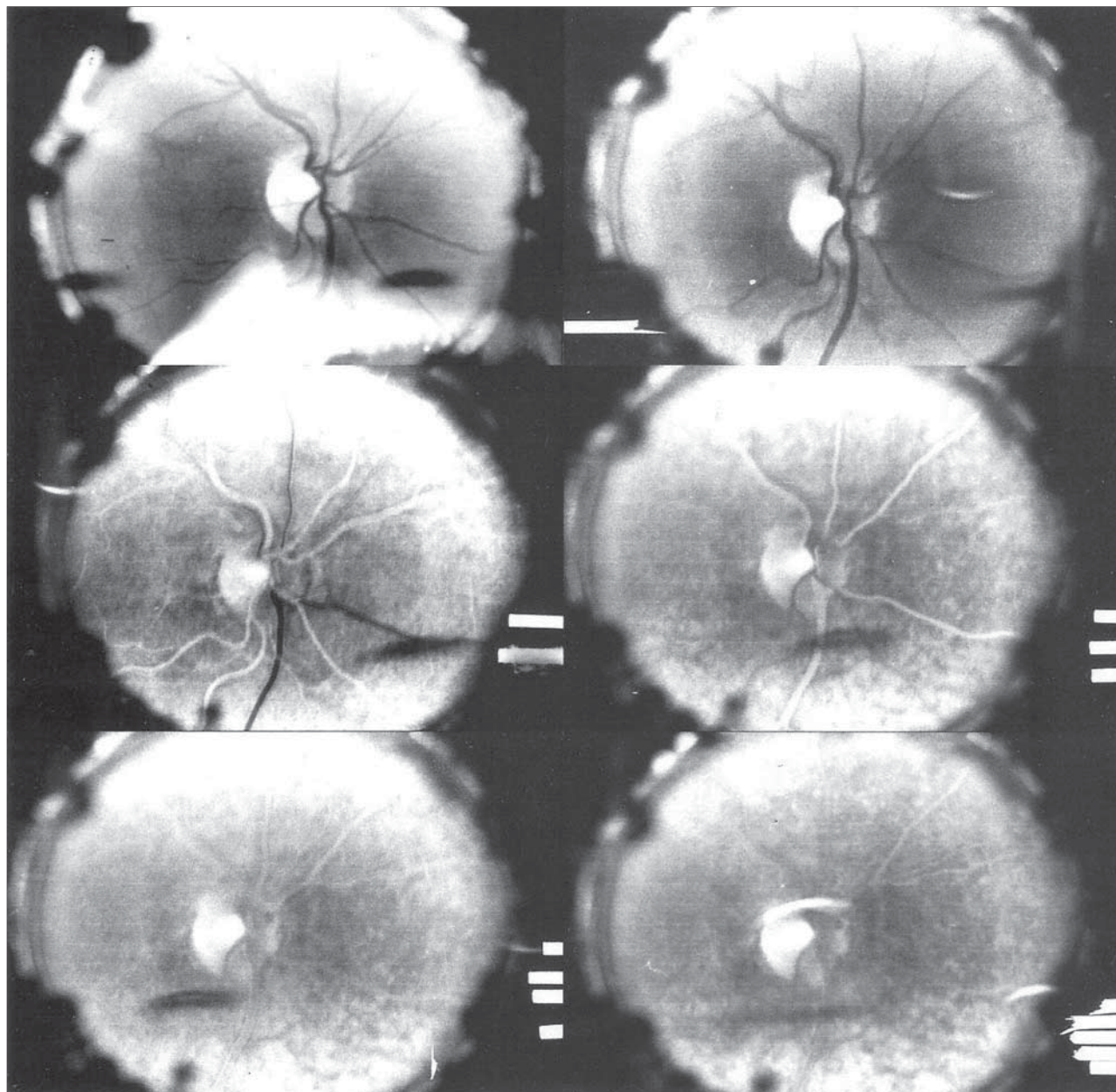
With the filters in place and with the flash system of the early Zeiss cameras, the brightness of vascular fluorescence was dim. Novotny and Alvis sought the fastest 35-mm film they could find and learned that Ansco Super Hypan film could be force developed to reach the equivalent of a 2400 ASA rating. It was decided to try the procedure on Alvis first because Novotny was most familiar with the camera. Flashes could only be produced every 12 seconds, which they hoped would be fast enough to recognize an arterial filling phase and venous return. Perhaps fortuitously, or because they had prepared well, they were successful on the first attempt (**Figure**), and fluorescein angiography was born.

One can only speculate on how long this work might have taken in our present era of institutional review board oversight, but in 1959, that was not an issue. Novotny and Alvis took pictures of their own eyes and recruited girlfriends and associates to be subjects. They accumulated normative data, and both Hickam and Wilson seemed very pleased with the results. Because Hickam was interested in vascular disease and was familiar with the complications of diabetes and hypertension, he encouraged them to see what the new technique might show in these disorders. They

walked up and down the wards, found out who had these diseases, and asked, "Would you like your eyes tested?" (H. Novotny, oral communication, June 2010).

Discoveries in medicine cannot be translated into practice until they are communicated. The Czech physiologist Purkinje described ophthalmoscopic observation of the fundus in 1823, but his monograph was published in Latin by Breslau University and never communicated to Europe at large.<sup>19,20</sup> The English inventor Charles Babbage designed an ophthalmoscope around 1847, and he showed it to an eminent ophthalmologist, Thomas Wharton Jones. However, Jones could not get a good view through the instrument (possibly because he was myopic),<sup>21,22</sup> and so it was put aside. We recognize Hermann von Helmholtz as the inventor of the ophthalmoscope not only because he built a working model in 1850 but because he demonstrated it and publicized it in 1851.<sup>23</sup> Within a few years, physicians were describing retinal disorders and other inventors were improving the device.

Both Hickam and Wilson recognized the potential value of fluorescein angiography and urged Novotny and Alvis to write a paper on the new technique; Hickam declined to be a coauthor because he felt that he did not really contribute to the development of the technique. Wilson read a first draft, and Hickam read multiple drafts of the manuscript. He gave one especially important piece of advice: "Make it so anyone can do it." He wanted the paper to describe every detail of the filters, camera, film, time sequence, etc, so that it would be a cookbook for doing the test, and any reader could duplicate the findings. Novotny remembers that the paper kept coming back to him because one or another detail was still unclear to Hickam. When the manuscript was finally finished, it was sent to the *American Journal of Ophthalmology*. It was a remarkable first paper: it described the technique, virtually as we do it today; it showed arterial and venous phases separated by laminar flow; and it showed many of the key findings in macular disease such as capillary neovascularization, leakage, and diffuse edema.



**Figure.** The first fluorescein angiogram, taken in November 1959, of the right eye of David Alvis. He writes that “there was a lot of trouble with unwanted light in all of the pictures. We broke up wooden Q-tip sticks to act as markers so that we could be certain of the proper sequence of the pictures. Two pre-injection pictures are noted with just the one bit of Q-tip. We shot the pictures as rapidly as we could” (e-mail communication, July 2010; photograph courtesy of David Alvis).

Here begins another saga of serendipity. In terms of dating the discovery, Novotny made the first public presentation of the material at the Midwest meeting of the Association for Research in Ophthalmology on April 23, 1960, from which an abstract appeared in the July issue of *American Journal of Ophthalmology*.<sup>24</sup> As far as we know, there was little notice paid. It was also published as a technical report by the US Air Force School of Aviation Medicine, in September 1960.<sup>25</sup> This reflected the fact that much of Hick-

am’s research was funded by grants from the School of Aviation Medicine. But technical publications like this were not widely read. When the authors received a rejection letter from the *American Journal of Ophthalmology*, fluorescein angiography might have died.

The reviewers commented that the idea was not new because Chao et al<sup>14</sup> had already demonstrated circulation in the cat with fluorescein, and Swan and Bailey<sup>15</sup> had demonstrated retinal cinematography. Furthermore, the photographic method

of observation seemed too slow to be objective clinically. Of course, Novotny and Alvis had not been aware of the prior publications, and this was long before the era of PubMed and quick searches of the literature. Alvis relates that, in 1968, he met the editor of the journal *American Journal of Ophthalmology*, Derrick Vail, and Vail apologized graciously for his failure to publish the technique.<sup>1</sup> He had missed the opportunity to introduce one of the major advances in ophthalmology. Indeed, he commented in 1981 during

a discussion at the American Ophthalmological Society about who should receive the Howe medal: “. . . give it to Novotny and Alvis—I should have accepted their paper on fluorescein for the *American Journal of Ophthalmology*.”<sup>26(p28)</sup>

Fortunately, Hickam believed the technique had clinical value, and he was a good friend of the editor of the journal *Circulation*. With the added references, the paper was promptly accepted for publication in that journal and appeared in the July 1961 issue.<sup>27</sup> Of course, ophthalmologists do not normally read *Circulation*, which leads to the final part of our story.

The ophthalmology department at Indiana University had the initial opportunity to demonstrate the value of angiography because the technology was “in house.” But the opportunity seems to have been missed. Wilson was involved peripherally with the work and was himself a retina specialist who studied the biochemistry of subretinal fluid in the laboratory.<sup>28</sup> However, he was busy clinically with surgery and administrative responsibilities. Wilson is said to have asked for the equipment to be duplicated in the ophthalmology department, but he only had an old carbon-arc camera, and there is no record as to whether he added the filters. Novotny had one more year of work with Hickam before his internship, and so he began to explore with the Zeiss fundus camera whether the flash cycle could be shortened and whether an intense continuous light source could be developed that would allow cineangiography. But these were side projects to his assigned work, and nothing more was published. Alvis soon left for his internship training, and there are no further papers from Indiana University in that era, on either the investigation or clinical applications of angiography. So how did the word get out? Alvis was accepted into the ophthalmology residency at Wayne State University in Detroit, Michigan, but was soon drafted into military service. He had asked his chair, A. D. Ruedemann Sr, for support to continue the study of angiography, but Ruedemann’s initial reaction was reportedly one of disinterest.<sup>5</sup> After Alvis

returned to residency and was finishing up in 1966, papers on angiography were appearing from other institutions. A. D. Ruedemann Jr asked Alvis why he had not pursued the topic.

The word did begin to spread. Dollery et al,<sup>29</sup> from Hammersmith Hospital in London, England, published the first ophthalmologic paper on fluorescein angiography in 1962 showing the characteristics of retinal circulation and the findings in a variety of diseases from the study of 60 patients. They adopted Novotny and Alvis’s technique, and Novotny remembers getting a reprint request from Dollery. But we do not know how they first heard of the work. Within another year, they had published several additional reports in which they accelerated the frequency of photographs to 2.5 seconds and illustrated a variety of clinical applications. These papers documented the great power of this new procedure and helped initiate a long series of technologic improvements in camera brightness, flash frequency, digitization, etc, that brought angiography to its present state of sophistication.

In the United States, Hickham helped to spread the word. He had formerly been on the faculty at Duke University in Durham, North Carolina, and returned there in 1960 to give a talk on the new technique of fluorescein angiography. He gave detailed instructions for the procedure to a senior neurologist, Albert Heyman, and to a younger protégé who was chief of neurology at a Veterans Administration hospital, Noble David. David was searching for academic identity at the time and latched onto the new procedure.<sup>30</sup> He outfitted a Bausch & Lomb fundus camera with the proper filters and was soon taking angiograms with the help of the hospital photographer, Leonard Hart, and an eager young assistant named Johnny Justice.<sup>6</sup> Working with Hart, David developed technology for taking photographs every 4 seconds, and he published the second set of fluorescein angiographic images in 1961.<sup>31</sup> This was in a neurology article devoted mostly to angioscopy for the recognition of carotid insufficiency, and it referred to the published ab-

stract<sup>24</sup> of Novotny and Alvis’s work for methodology. But no one else at Duke University seemed very interested in the technique. Two years later, J. Lawton Smith and Edward Norton convinced David to move to the new Bascom Palmer Eye Institute in Miami, Florida, and to bring his technology for fluorescein angiography. Not long after, Johnny Justice also moved to Miami to become the chief photographer. His high-quality images (and continued technological improvements) convinced Norton and his younger retina specialist J. Donald Gass (who was to become the leading scholar of macular disease in the latter part of the 20th century) of the enormous potential of the procedure. In 1964, the Miami group began a series of seminal papers<sup>32,33</sup> that showed the broad and indisputable value of fluorescein angiography in the practical diagnosis of macular and retinovascular disease. It is not irrelevant to note that ophthalmic lasers were also developed in the 1960s, and the argon laser was being applied effectively to macular lesions by 1970.<sup>34,35</sup> Fluorescein angiography provided a map for the photo-coagulation of vascular lesions, and conversely the therapeutic benefits of the laser treatment inspired improvements and dissemination of angiographic equipment.

In looking back on these events, both Novotny and Alvis are a bit wistful: proud of what they initiated but cognizant of what more they might have done if they had continued the investigations themselves. Novotny was already thinking of how to speed up the flashes, but he was busy with 3 jobs and trying to sort out his interests for a career. He did a rotating internship and finally chose psychiatry. He built a practice in child and adolescent psychiatry in Palo Alto, California, and served for many years on the clinical faculty of the Stanford University School of Medicine. His career at Stanford was highly successful, and on balance he followed his interests and has no regrets. Alvis practiced general ophthalmology in Indianapolis, Indiana, and had a frayed print of the original angiogram taken of his own eye on the wall of his office. Sometimes a patient would ask

Alvis about the significance of the print, and he would tell the story. He had an angiogram done many years later just for comparison,<sup>4</sup> but he never performed one clinically in all his years of practice.

Fluorescein angiography in its early years was not a simple test: it required modification of a fundus camera, injection of a dye, and photographic processing that might delay the review of data for a day or more. Why did it succeed? Two persistent students solved the problem of highlighting the vasculature with barrier filters. An insightful mentor pushed them to write clear methodology and knew how to get a rejected paper published. Clinicians in England and at Duke University learned of the technique and began to improve it, and the retina service of the Bascom Palmer Eye Institute showed that it could be practical in a busy clinic. Skill, insight, luck, serendipity? Perhaps all these things, of which we have been the beneficiaries for 50 years. The use of fluorescein angiography is diminishing nowadays, as newer imaging modalities provide more direct evaluation of macular thickness and cellular damage. But angiography remains critical for the assessment of vascular integrity and leakage, and only time will tell if it fades from practice or becomes rejuvenated with new dyes and photographic techniques.

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