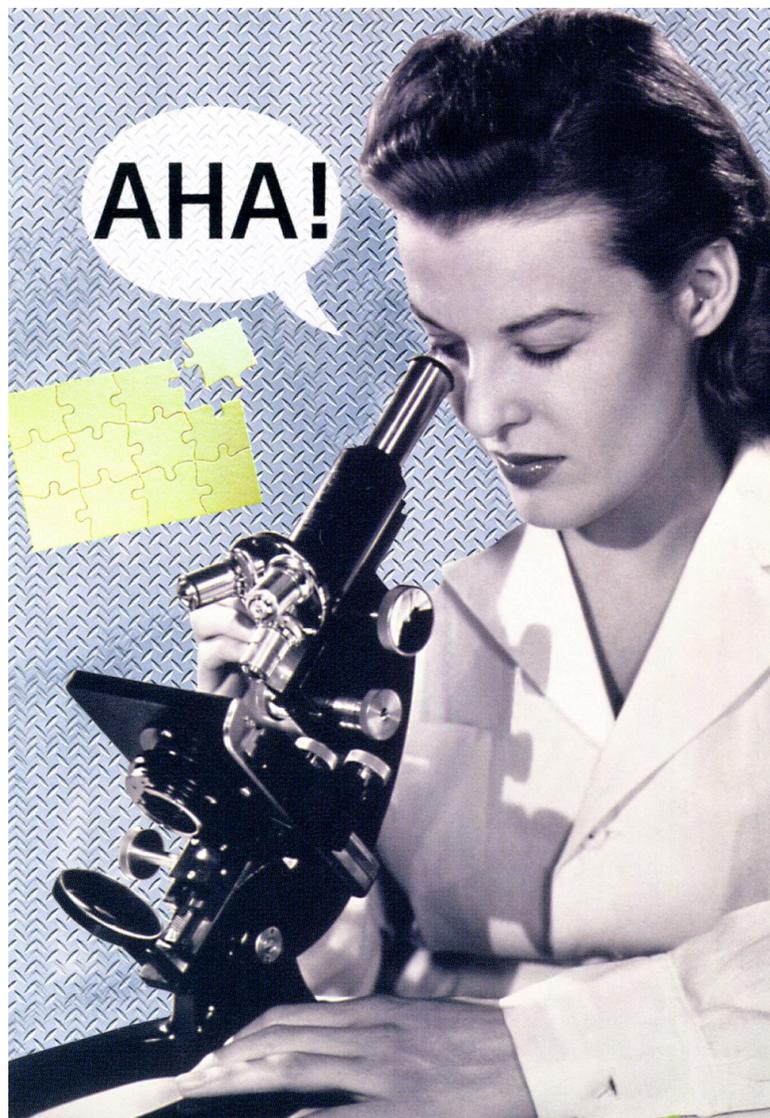


SYPHILIS AND THE MICROSCOPE, A CENTURY-LONG RELATIONSHIP

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INTRODUCTION

Recently, while reviewing some antique microscopes and old books, we were reminded of the fact that the century-old story of the microscopic identification of the agent of syphilis deserves to be remembered, as it was a turning point in the fight against the disease (Kohl and Winzer, 2005). That discovery was only possible through the ingenious application of specialized microscope instrumentation.

THE DISEASE

A new plague afflicted Europe in the early 1500s. Its geographic origin was unclear, its treatment ineffective, its prognosis serious or fatal. It was syphilis. The geographic origin of the disease was hotly debated. Did it come from America? La Enfermedad Indiana; from England? Morbus Anglicus; from France? Morbus Gallicus. Perhaps from Italy? Neapolitan Pox. Even mythology played a role. According to a poem by Girolamo Fracastoro (1530) a shepherd by the name of "Syphilus" was punished by the god Apollo with the disease and became the first patient. Regardless of its origin, war, commerce, and exploration secured the fast and world-wide distribution of the disease. One can better understand the horror created by the arrival of the syphilis epidemic by comparing it with the history of the AIDS epidemic in the late 20th century (Pappas, 1993; Sedano, 2005). Both diseases are transmissible, mostly by sexual contact. Both were "new diseases." Both were considered to be of "foreign origin." Both were incurable and eventually fatal.

Treatment was empirical and erratic. Even four centuries after the disease had been recognized, in the threshold of the 20th century, Fournier (1902), a world authority in the subject, could say:

"At the hospital we deal with the accidents of the disease, nothing more. At the hospital, in the present state of affairs, *we do not treat syphilis.*" (emphasis, Fournier's; translation MdC).

Treatment, such as it was, had developed over centuries by painful trial and error, using substances that could poison the still unidentified causative agent more and the patient less. It was based on the use of arsenicals, iodates, and mercuric compounds. Yes, mercury in abundance, and recommended by leading authorities in the field (ex., Jakob, 1899; Fournier, 1902).

Alternative methods have been tried. Infecting the patient with the malaria parasite, was one of those heroic attempts (Wagner von Jauregg, 1946). The rationale, back in 1917, was that the high fever of the malaria attacks killed the sensitive *Treponema*. In the brain, the mild inflammation caused by malaria obliterated the far more malignant inflammation caused by syphilis, as shown by Sträussler and Koskinas in 1923-1926 (Triarhou, 2007). Once the patient was considered free from syphilis, then he or she, was treated with antimalarial drugs to get rid of the *Plasmodium* causative of malaria. The idea appears bizarre now, but it brought the 1927 Nobel Prize to its author. Such was the desperate need for an effective antisiphilis therapy!



Figure 1. Vienna's Professor von Wagner-Jauregg with his microscope.

The somber picture presented by Fournier in 1902 was to change drastically in the decades following the publication of his book. Two events made that change possible; the identification of the causing agent in 1905, and the general availability of penicillin, in the late 1940s. Fifty years after Fournier's book was published physicians could say with confidence that they could treat syphilis, and that they could cure it. Treatment and cure of the disease is beyond the scope of this article, but the role that the microscope played in the identification of the causative agent deserves our attention.

THE AGENT

In August 1884 an editorial in *Scientific American* noted, “The brilliant discoveries by Pasteur and by Koch are as much due to the perfected microscope as to any cause.” During the last quarter of the 19th century, the Continental type of microscopes developed by the “Dynasty” of Durkheim, Oberhäuser, Hartnack, Nachet, and Zeiss (Moe, 2004) allowed the French and German schools of bacteriology to find the agents of anthrax, cholera, gangrene, tuberculosis, diphtheria, and other infectious diseases (Lechevalier and Solotorovsky, 1974). Unfortunately, repeated efforts to find the agent of syphilis had failed.

Fritz Richard Schaudinn and Erich Hoffmann (1905) at the Institute of Tropical Medicine in Hamburg, first identified *Treponema* (Spirochete) *pallidum* as the causative agent of syphilis. In a study that combined effectiveness with elegant simplicity, they used dark-field to observe exudates from a syphilitic chancre (syphilis primary lesion). Figure 2, is an old photomicrograph that beautifully depicts *Treponema pallidum*, as seen under dark-field. Although there are other spirilar organisms occasionally living on human individuals, the identification of *Treponema pallidum*, is not difficult. As Gage (1925) noted, “The spirochaetes are so characteristic in form and movement that there should be no confusion.” Truly, nothing replaces the feeling of actually seeing the living bacterium. Observing this delicate, even elegant, screwdriver-like, luminescent object moving across the pitch-black field, “without haste or pause, like the stars,” is an unforgettable experience.

Serological tests for the diagnosis of syphilis, initially the Wasserman reaction, have existed since 1907 (Gastou and Girauld, 1910) and are now greatly perfected (Sparling, 1992). Microscopical stains, metal impregnation, and fluorescent histo-immunological techniques have been developed that show the *Treponema* in tissue sections or fixed smears. However, even at the beginning of the 21st

century, dark-field microscopy remains what it was a century ago, the most direct means of visualizing the living *Treponema* as obtained from the primary lesions of infected patients.

Figure 2.
Treponema pallidum seen in dark-field in a 1910 photograph by Gastou and Girauld. Besides the *Treponema*, three nucleated cells stand up on account of the granules inside their cytoplasm.



Why dark-field microscopy?

“Because *Treponema pallidum* is not stained readily by ordinary laboratory methods and is so similar to other spirochetes which inhabit the mouth and genitalia of non-syphilitic persons, it is essential that the organism be seen in the living state. Because the narrow width of *T. pallidum* the ordinary microscope does not permit sufficient resolution to visualize the organism.” (USDHEW, 1968).

And because,

“The dark-field examination is almost always positive in primary syphilis and in the moist mucosal lesions of secondary or congenital syphilis. [It is] The most definitive means of making a diagnosis.” (Sparling, 1992).

Letting aside the error of confusing resolution with visibility in the first of the just cited paragraphs, an error that was pandemic during most of the 20th century, the difficulty in visualizing *T. pallidum* under bright-field lies in part in the fact that while the organism is up to 50 μm long, it is only 0.15 μm thick (Davis et al., 1973; Sparling, 1992).

THE SYPHILIS MICROSCOPE

A microscope typical of those designed for the identification of *Treponema* in the clinical laboratory is shown in figure 3 (MdC Collection #375) by Spencer, Buffalo, USA, is a monocular serial #149707, with dark field condenser (“Syphilis microscope”). This is an instrument of the black and chrome era (circa 1938). The horseshoe base is 12 cm wide with parallel arms extending 17.5 cm. The pillars are continuous with the base and rise 7 cm to meet the lower end of the limb at the adjustable inclination joint. A tailpiece attached to the understage supports the dark field condenser-illuminator unit, which is focused by a rack-and-pinion controlled by a knob located on the left side. Two small chrome-finished knobs permit centering the condenser. A transformer provides current for the illuminator. A metal plate on this transformer notes that it is a Model 393, from the Spencer Lens Company; it transforms 115 volts current into 6.5v. The stage is square, 12.3 cm by side; it has a plain mechanical stage that permits scanning 75x50 cm slides. The mechanical stage can be removed and stage clips installed. The upper portion of the arm has the knobs for coarse and fine focus. The right side fine-focus knob has 2 μm graduations. The body tube is 14 cm long; the lower, wider part carries the inscription “Spencer Buffalo U.S.A.” and the serial number 149707.

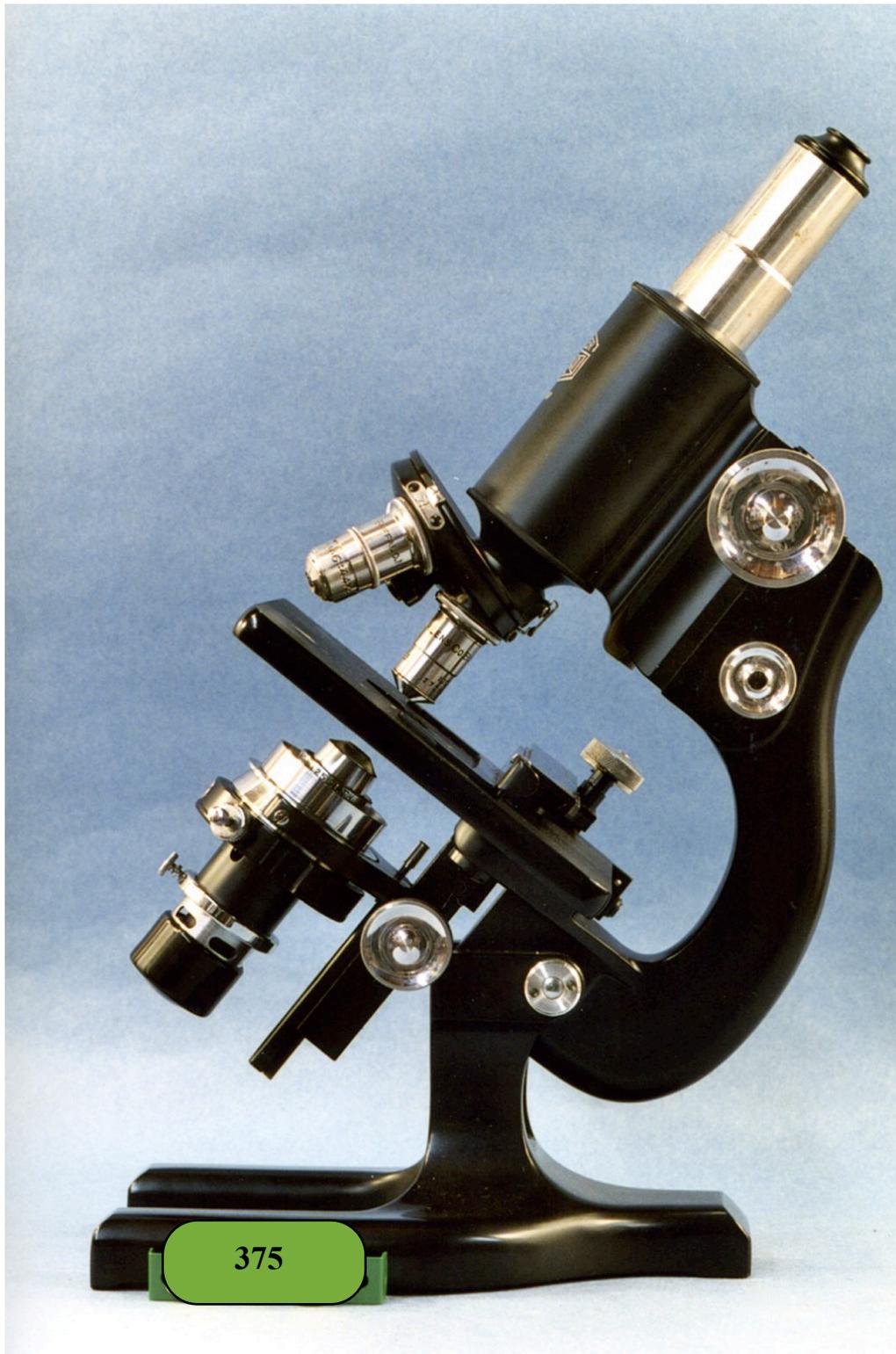


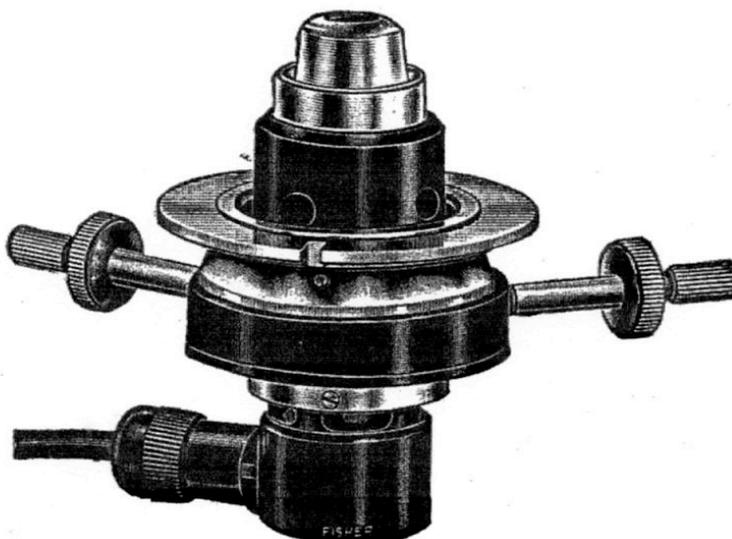
Figure 3. The Spencer microscope shown here is equipped for testing suspected syphilitic exudates. The special dark-field condenser has been lowered for illustration purposes; in practice it was used almost fully raised and oil-immersed.

The ocular tube is of a fixed length; there is no drawtube. The unsigned Huygenian ocular has the typical Spencer conical top and it carries the inscription "10x". The triple nosepiece holds a 16 mm 10x NA 0.25, a 4 mm 44x NA 0.66, and a 1.8 mm 95x objectives. The objectives are signed and numbered. The box for the dark field illuminator contains the funnel-shaped diaphragms that are to be fitted inside the objectives. It also contains the original condenser, a dark field diaphragm, a blue filter, and the mirror. The original wooden carrying box is 22.5 cm wide, 22.5 cm deep, and 36.7 cm tall. It has its original key and it contains the metal cases for the three objectives, a spare 6x ocular, and the instructions for the use of the AO Spencer Dark field Illuminator (printed in 1937). The microscope was acquired July 1995, and it is in excellent condition both mechanically and optically.

Comments: The illuminator in this microscope and almost every feature in the stand are identical to those of the Spencer Dark Field Microscope NO. 32M (Spencer Scientific Instruments, 1939, American Instrument Division, Buffalo, NY, pp. 28-29). However the Spencer Dark Field microscopes, either in their mono- or binocular versions, were fitted with a single, oil immersion objective. The revolving, triple objective, nosepiece, was a feature of the high-end versions of the Bacteria- or Mold-counting microscopes (Spencer Scientific Instruments, 1939, pp. 35-39).

"Syphilis microscopes" were produced by all major makers and marketed for many years. At such a relatively late date as 1959, well into "the penicillin era," Catalog 59, Modern Laboratory Appliances, of the Fisher Scientific Company, lists at p. 640 the AO Dark-Field Illuminator (figure 4). This is the same condenser that was listed in the 1939 AO Spencer Catalog and it was adaptable to the late AO microscopes as well. The Fisher Catalog notes that this illuminator can be operated using either 6v of battery current, or house current via a transformer. The illuminator sold for \$105.00 and the transformer for \$18.00.

Figure 4 >>>



12-430

12-430. MICROSCOPE DARK-FIELD ILLUMINATOR, with Electric Light, AO Spencer. This substage dark-field illuminator is similar to No. 12-425 except that it has a built-in electric lamp, adjustable by centering screws.

It is supplied in a special case with objective funnel stop and a 6 volt electric lamp, which can be operated from a suitable battery or from a No. 12-431 transformer. Specify whether fork or sleeve type mounting is desired.

Without battery or transformer, Each, \$105.00

12-431. TRANSFORMER, to adapt 6 volt bulb of No. 12-430 Illuminator to 115 volt a-cord-c. **Each, \$18.00**

Naturally, not all clinical services had either the financial resources or the patient volume that would justify owning a microscope specifically devoted to the diagnosis of syphilis. There were for them more economical alternatives. The least expensive was the central stop disk, as shown in figure 5. This is a simple, black-finished, circular metal piece held at the center of the optical path. These stops were inexpensive and as easy to insert or to remove as a filter. They were in fact designed to fit the filter carrier placed below the standard condenser. For low magnifications the patch stops are very effective and simple to use, but as the magnification increases their performance deteriorates.

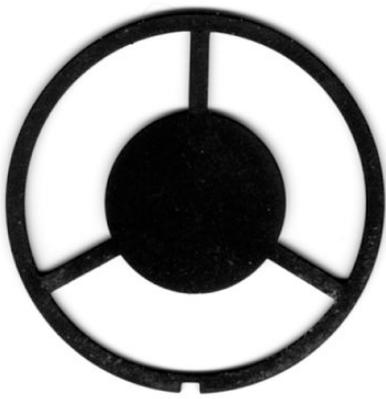


Figure 5. Digital scan of a dark-field stop patch.

To achieve a good dark field image with a high power objective requires, besides using a diaphragm to reduce the numerical aperture of the objective, a very precise adjustment of the illumination source and of the condenser. All major makers were aware of this situation and introduced on-the-stage condensers (figures 6 a and b, and 7), also called superstage dark-field condensers (Gage, 1925). These are an economic alternative to the complex dark-field illuminator shown in figure 4. They do not require that a microscope be exclusively devoted to dark field work, they allow the use of oil-immersion optics, and they provide good high-magnification images. To be used they are placed on the stage of a conventional light microscope, in the place reserved for the glass slide. The standard bright field condenser of the microscope has to be removed, then the specimen slide is placed on this on-the-stage condenser that has its glass surface oiled with immersion oil. The rest of the manipulation is the usual for dark-field work. The one difficulty is that the slide has to be moved without changing the position of the condenser, otherwise this becomes de-centered and the illumination is lost. To alleviate this difficulty, the Spencer unit has the two lateral cutouts shown in figure 6. They provide some extra room for the operator's thumbs to push the slide without disturbing the position of the condenser. A more effective means of dealing with the problem was introduced by Leitz (figure 7).



Figure 6. The upper surface of a Spencer on-stage dark-field condenser. The black, central area is the glass surface that allows light to reach the specimen. It is surrounded by a flat metal surface, and then by a groove that collects any overflow of immersion oil. Here, an optical illusion makes this groove to appear as a risen circle.

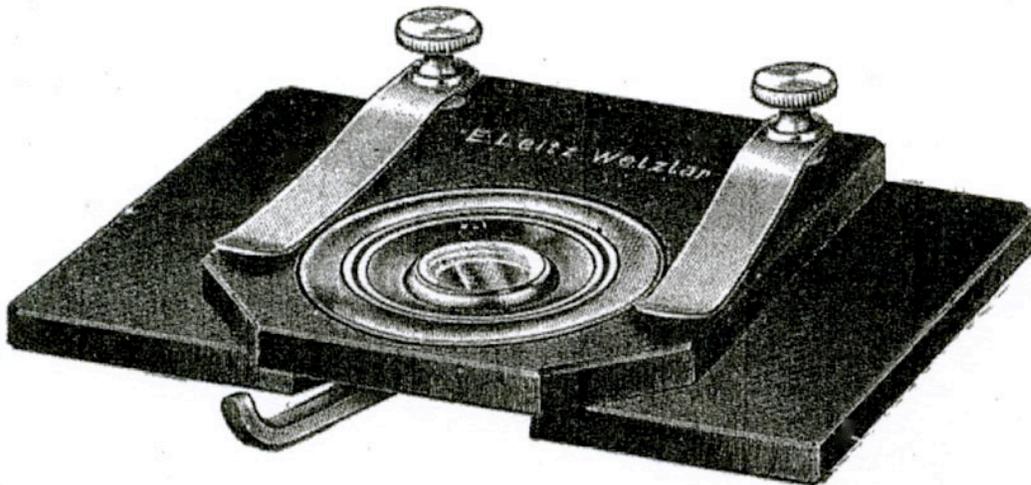


Figure 7. A circa 1907 Leitz stage condenser showing the stage clips that allowed the specimen to be displaced without changing the position of the condenser relative to the microscope stage.

CLOSING COMMENTS

For almost five centuries syphilis was a scourge of humankind. Hopes for its total eradication were raised in the 1950s as finally a safe and highly effective treatment became available. Those hopes unfortunately, were premature. In November 2005, the Center for

Disease Control and Prevention reported that syphilis had increased for a fourth consecutive year in the USA. It is then a sad reality that serological tests (Lukehart and Holmes, 1998) and microscopy will still have to be used in diagnosing the disease. Dark-field microscopy in particular will continue to provide a direct means of visualizing the living *Treponema*.

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