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Abbe's Theory and its Introduction in Spain: The Use of Instruments for Scientific Demonstrations

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Abstract: The theory of image formation in a microscope proposed by Ernst Abbe changed the scientific approach to microscopy. Though the theory had many detractors, his new approach led to a technological revolution in the design and construction of high-quality microscopes. It paved the way for new discoveries in the fields of biology and medicine. Joaquín María de Castellarnau, was a contemporary connoisseur of Abbe's ideas who decided to disseminate them in Spain through various publications and training courses. In his lectures, he used various devices for practical demonstrations that allowed some concepts of the new theory to be better understood. At the Museo Nacional de Ciencias Naturales in Madrid (MNCN–CSIC), one such original and unusual instrument designed by Abbe and used by Castellarnau has been preserved in perfect condition. Castellarnau used this instrument for various experiments that helped clarify the most complex points of Abbe's theory. In this work, we explore how the context in which science developed in Spain favoured practical activities to demonstrate new scientific theories, such as Abbe's in the early twentieth century.

Keywords: Ernst Abbe; Joaquín Mª de Castellarnau; diffraction; microscope; demonstration apparatus

Introduction

In 1893, the optics company Zeiss presented item no. 21 in its catalogue, which was called *Apparat zur Demonstration des Zusammenhanges zwischen Beugungswirkung und Bild eines Objectes* (an apparatus for the demonstration of the relationship between diffraction effect and the image of an object). More than 20 years earlier, the German physicist Ernst Abbe had already developed studies from which he formulated the concepts and laws necessary to understand and improve the mechanism of image formation through a microscope: his conclusions are known as the Abbe theory or the theory of image formation in a microscope.

Many of the instruments that Abbe designed are still preserved in various collections around the world. The Consejo Superior de Investigaciones Científicas (CSIC), maintains many scientific instruments of historical interest. At the end of the last century, an ambitious project that resulted in the recovery and cataloguing of more than 700 instruments from the different CSIC centres was conducted.[1] This action was resumed in 2014 when the institution launched a programme to continue with the recovery of this instrumental heritage.[2] To date, the CSIC has catalogued, or is in the process of cataloguing, more than a thousand apparatus of varying scientific and historical importance.

The Historical Scientific Instruments Collection held at the Museo Nacional de Ciencias Naturales (MNCN) is one of greatest historical interest. This collection is comprised of more than 300 pieces of varied origins, includes a unique apparatus that played an important role in demonstrating the importance of the diffraction phenomenon in microscopic observation (figure 1).

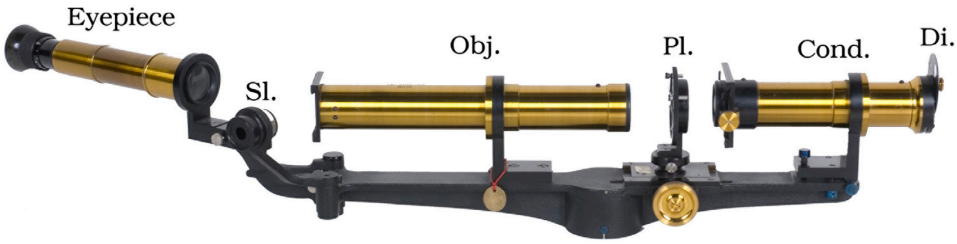

Figure 1. Main components of Abbe Demonstration Microscope owned by Castellarnau. Small lens (Sl.), Objective (Obj.), Stage (Pl.), Condenser (Cond.), Diaphragms (Di.). Museo Nacional de Ciencias Naturales, CSIC. Photography by MNCN Photography Service.

[1] Roberto Moreno, Ana Romero and Fernando Redrajo, "La Recuperación de la Instrumentación Científico-histórica del Consejo Superior de Investigaciones Científicas," *Arbor* 153, no. 603 (1996): 9-54, on 11.

[2] See "Plan de Identificación, Recuperación y Conservación de Instrumentos y Aparatos Científicos de Interés Histórico del CSIC" (Plan for the Identification, Recovery and Conservation of CSIC Scientific Instruments and Apparatus of Historical Interest), http://museovirtual.csic.es/instrumental-csic.html, accessed February 7, 2021.

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The study of these types of instruments in scientific collections has, traditionally, been associated with museum and heritage conservation: these pieces are considered historical sources with undeniable relevance in the teaching and dissemination of science.[3]

Scientific instruments are physical devices by which observations and data have been collected along the history of science. By keeping them operational we can bring back to life experimental setups that were crucial in the emergence of new scientific concepts and that established the limits of the study of reality in its time. As it will be shown, the instrument we are dealing with, used by the Forestry Engineer Joaquín María de Castellarnau (1848-1943) was aimed to didactically expose the principles of Abbe´s theory and how they set up the limits of optical microscopy.[4]

Abbe and the Theory of Image Formation in a Microscope

Ernst Abbe (1840-1905) began his university studies at the University of Jena, and then moved to the University of Göttingen, where he received his doctorate in 1861.[5] In Göttingen, he became acquainted with the theory and practice of precision measurements, the statistical control of errors in experimentation and design, and with the construction of scientific instruments. After completing his doctorate, Abbe focused on astronomy, becoming director of the Jena Observatory in 1877.[6] However, it was his relationship with Carl Zeiss (1816-1888) that was decisive for the development of optical instruments, an activity that led him to formulate his theory of image formation in a microscope.

Carl Zeiss founded a workshop for precision mechanics and optics in the city of Jena in 1846. One of his goals was to produce small, easily transportable microscopes of sufficient quality to aid in natural history studies. From his very beginning in the business world, Zeiss relied on the advice and collaboration of scientists who, due to their field of research, would end up being recipients of his products. Examples include the botanists Matthias Jakob Schleiden (1804-1881), who, together with Theodor Schwann (1810-1882), formulated the cell theory, and Leopold Dippel (1827-1914), an expert microscopist who became the director of The

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[3] José R. Bertomeu and Antonio García, "Introducción," in *Abriendo las Cajas Negras. Colección de Instrumentos Científicos de la Universidad de Valencia*, eds. José R. Bertomeu and Antonio García, 9-13 (Valencia: Universidad de Valencia, 2003), 11.

[4] Roberto Moreno, Ana Romero, and Fernando Redrajo, *La Recuperación de la Instrumentación Científico-histórica del CSIC: El Museo Nacional de Ciencias Naturales* (Madrid: Consejo Superior de Investigaciones Científicas, 1995), 97.

[5] Norbert Günther, "Ernst Abbe," in *Dictionary of Scientific Biography,* vol. 1, ed. Charles Coulston Gillispie, 6-9 (New York: Charles Scribner's Sons, 1970), 6.

[6] Barry Masters, "Ernst Abbe and the foundation of scientific microscopes," *Optics and Photonics News* 18, no. 2 (2007): 18-23, on 19.

Botanical Garden at the Technical University of Darmstadt.[7] He also surrounded himself with specialists in optics and mathematics such as the Germans Friedrich Körner (1778-1847) and Friedrich Wilhelm Barfuss (1809-1854).[8] In this way, Zeiss was trying to meet the needs of his future customers, mainly naturalists and physicians, by using the knowledge and skills of physicists and mathematicians.

Although Zeiss represents a clear example of a then incipient relationship between academia and industry, and established an industrial organization from 1872 onwards, the production of microscopes still had a strong artisanal component that focused mainly on the manufacturing of lenses, for whose elaboration, a system of trial and error was still used.[9] Artisans polished different pieces of glass and combined them to test their optical utility, selecting the best ones.[10] This approach depended largely on the craftsmen's expertise, and excluded any theoretical planning of a calculated technique for making microscopes.[11]

The way of making microscopes changed soon after the meeting between Abbe and Zeiss in 1866, during which Abbe agreed to work in the Zeiss workshops as an independent contractor. As soon as this collaboration began, Abbe initiated an ambitious research programme aimed at improving the lens production process for microscope manufacturing.[12] Abbe questioned why theoretical calculations allowed telescopic lenses to be manufactured in a mathematical way, but not microscopic lenses. He also questioned why the theory that convincingly explained the operation of a microscope after its construction was not applicable to the design of its own production. According to Abbe, the reason was due to an incorrect theoretical description of the optical processes occurring in a microscope.[13]

Abbe performed a systematic study of the optical behaviour of the various lenses comprising a compound microscope. During his investigation, he analysed everything, from the lens manufacturing method to the material from which they were constructed, and of course, their thickness, shape and behaviour in the presence of light.[14] To this end, he developed new

7 Felix Auerbach, *The Zeiss Works and the Carl-Zeiss Stiftung in Jena; Their Scientific, Technical and Sociological Development and Importance* (London: Marshall, Brookes & Chalkley, 1904), 10.

8 Wolfgang Wimmer, "Carl Zeiss, Ernst Abbe, and Advances in the Light Microscope," *Microscopy Today* 25, no. 4 (2017): 50-4, on 52.

9 Auerbach, *The Zeiss Works*, 31.

10 Masters, "Ernst Abbe and the foundation," 20; Wimmer, "Carl Zeiss, Ernst Abbe," 53.

11 Ernst Abbe, "Beiträge zur Theorie des Mikroskops und der Mikroskopischen Wahrnehmung," *Archiv* für *Mikroskopische Anatomie* 9 (1873): 413-68, on 413.

12 Stuart M. Feffer, "Microscopes to Munitions: Ernst Abbe, Carl Zeiss, and the Transformation of Technical Optics, 1850–1914" (PhD Dissertation, University of California, Berkeley, 1994), 66; Masters, "Ernst Abbe and the foundation," 19.

13 Abbe, "Beiträge zur Theorie," 414.

14 Stuart M. Feffer, "Ernst Abbe, Carl Zeiss, and the Transformation of Microscopical Optics," in *Scientific Credibility and Technical Standards in 19th and Early 20th Century Germany and Britain*, ed.

measuring instruments, such as the focometer and the apertometer, and improved others, such as a type of spectrometer and the refractometer.[15] Abbe's work to improve the quality of microscopes led him to delve deeper into the physical foundations of their operation. After a little more than five years of research in the Zeiss workshops, Abbe succeeded in developing a new theoretical framework that explained image formation in a microscope. The result was his theory of image formation in the microscope, which he published in 1873 under the title *Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung* (Contributions to the theory of the microscope and microscopic perception).[16] His new theory contributed decisively to the development of new scientific theories and discoveries, and moreover, it resulted in a technological and industrial revolution. By the end of the nineteenth century, the main brands of microscopes and optical devices were offering microscopes that included Abbe's technical innovations (e.g., apochromatic lenses and condensers) in their commercial catalogues. Companies offering such microscopes included Ernst Leitz (Germany), Reichert (Austria) and Bausch & Lomb (USA).[17]

The primary original contribution resulting from Abbe's work is his consideration of the diffraction phenomenon that light undergoes when it illuminates an object observed through a microscope. As he demonstrated, the structure and surface details of the observed object cause the light that illuminates it to diffract into a group of beams that, after passing through the microscope objective, is separated at regular angular intervals according to wavelength, forming what is called a diffraction spectrum. This spectrum, similar to the Fraunhofer spectrum, is formed in the rear focal plane of an objective. In the case of the optical microscope, it manifests as bands, rectilinear or circular, surrounding a high-intensity point, corresponding to the undiffracted light beam situated in the centre, which, when separated at regular intervals, show a gradation according to the colour, or wavelength, of the light.[18] To illustrate his ideas, Abbe used a particular species of diatom, *Pleurosigma angulatum*, to show the diffraction that occurs in the fine structures of its shells. The species was well chosen. Depending on the observation conditions and the illumination techniques used by biologists at the time, the shell morphology was described in a wide variety of ways, which led to controversy. Some species of diatoms and other samples with regular structure were used as test objects to compare the quality of microscopic lenses.[19] Abbe concluded that the fine detail structure of *P. angulatum*

Jed Z. Buchwald, *Archimedes* 1, 23-66 (Dordrecht: Kluwer, 1996), 34.

[15] Abbe, "Beiträge zur Theorie," 415; Feffer, "Microscopes to Munitions," 76; Masters, "Ernst Abbe and the foundation," 20.

[16] Abbe, "Beiträge zur Theorie," 413-68.

[17] See, e.g., the following catalogues: Ernst Leitz, *Microscopes and Accessory Apparatus,* no. 36 (Leipzig: Fr. Richter, 1896); Bausch & Lomb, *Microscopes and Accessories*, cat. A (Rochester: The Genesse Press, 1900).

[18] Abbe, "Beiträge zur Theorie," 443-44.

[19] Jutta Schickore, *The Microscope and the Eye: A History of Reflections, 1740-1870* (Chicago: University

was far from being interpreted correctly because the diffraction phenomenon that produced the structure was not taken into account.[20] Abbe highlighted that, although the wave theory of light predicted and demonstrated diffraction phenomena, these had not been taken into account in microscope function.[21]

To demonstrate the effect that diffraction has on the quality of the image observed in a microscope, Abbe developed a series of experiments and instruments aimed at varying the quantity of diffracted light rays that enter the objective of an apparatus. This work led him to propose a new definition for the resolution of the optical microscope, that is, the capacity of a microscope to allow the observer to distinguish accurately between two separate objects, and not mistake them as one. Through various ingenious experiments in which he controlled the amount of diffracted light rays passing through the objective, he demonstrated that resolution depends solely on the angular aperture of the objective and the wavelength of the light used. In the case of the optical microscope, illumination with a short wavelength light, e.g., blue, provides greater observation resolution (compared with other wavelengths).[22] Some of his experiments included several steps. First, he successfully observed the diffraction spectrum caused by the sample by removing the eyepiece from the microscope. He then used diaphragms to control the amount of diffracted light rays reaching the objective. When placed behind the sample, they block the desired part of the diffraction spectrum. Abbe describes how the observation of the object is affected when different diffracted light rays are obstructed. He controlled this by continually observing which parts of the spectrum disappear when the diaphragms acted on the diffracted rays.[23] Another way he regulated the amount of diffracted light rays reaching the objective was to change the angular aperture of the objective. This parameter indicates the number of light rays that can enter a lens. Abbe did this by combining lenses of different apertures, thus varying the amount of diffracted light rays that could enter them.[24] Instead of using the concept of angular aperture, Abbe introduced the concept of numerical aperture, which relates the refractive index of the medium through which the diffracted light passes to the angular ratio of the diffracted rays to the axis of the microscope.[25]

In short, the ability to resolve the details of specimens observed under a microscope is related to the amount of diffracted light rays entering the objective, which, in turn, depends on the angular aperture of the objective.

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of Chicago Press, 2007), 105-06.

[20] Abbe, "Beiträge zur Theorie," 453.

[21] Ibid., 443.

[22] Ibid., 455-56.

[23] Ibid., 445.

[24] Ibid., 415 and 440-41.

[25] Ernst Abbe, "On the Estimation of Aperture in the Microscope," *Journal of the Royal Microscopical Society* 1, no. 3 (1881): 388-423, on 395.

In his experiments, Abbe also introduced illumination as a variable. He explains why oblique illumination, much appreciated by microscopists of the time, sometimes allows more details to be observed compared with central illumination. In the case of wide-angle lenses, oblique illumination allows more specimen details to be observed because more diffracted light rays penetrate the lens. By contrast, central illumination causes greater divergence of the diffracted rays, such that some will not enter the lens, leading to a consequent reduction in resolution.[26] He specifies that illumination must be oblique with respect to the axis of the microscope and not the specimen, as was the prevailing thought at the time.[27] That same year, Abbe introduced a new device to improve illumination, known as the Abbe condenser, which would become an indispensable part of microscopes.[28]

Although Abbe completed and clarified his theory in publications since 1873, it was clear that the advancement of microscope design also required the improvement of its lenses. For this reason, he sought ways to improve their optical quality, and in 1889, he even travelled to the Swiss Alps to learn about and obtain samples of a variety of fluorite, which has a high level of transparency and would be used to construct the first apochromatic lenses.[29] With this type of lens, chromatic aberration, among other factors, was considerably reduced.

Acceptance of Abbe's Theory and Demonstration Apparatuses

The theory that Abbe published in 1873 was not immediately accepted and had to endure criticism from, for example, a large part of the English scientific community. Within the Royal Microscopical Society (RMS), heated debates on Abbe's theory ensued for nearly half a century.[30] In this section, we focus briefly on the main controversies that Abbe's ideas caused in England, and how he designed and built various instruments to clarify, through demonstration experiments, the main aspects of his theory. We also describe Castellarnau's point of view in the debate.

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[26] Abbe, "Beiträge zur Theorie," 449.

[27] Ibid., 442.

[28] Ernst Abbe, "Ueber einen neuen Beleuchtungsapparat am Mikroskop," *Archiv* für *Mikroskopische Anatomie* 9, (1873): 469-80, on 475.

[29] Edmund v. Fellenberg, "Ueber den Flussspath von Oltschenalp und dessen technische Verwerthung," *Mittheilungen der Naturforschenden Gesellschaft in Bern*, no. 1215-1243 (1890): 202-19, on 206; Anonymous, "In memory of Ernst Abbe," *Innovation: The Magazine from Carl Zeiss,* no. 15 (2005): 4-11, on 11; David Cahan, "The Zeiss Werke and the Ultramicroscope: The Creation of a Scientific Instrument in Context," in *Scientific Credibility and Technical Standards in 19th and Early 20th Century Germany and Britain*, ed. Jed Z. Buchwald, Archimedes 1, 67–115 (Dordrecht: Kluwer, 1996), 76.

[30] Horst Köhler, "On Abbe´s Theory of Image Formation in the Microscope," *Optica Acta: International Journal of Optics* 28, no. 12 (1981): 1691-1701, on 1700; Savile Bradbury, "The Reception of Abbe´s Theory in England," *Proceedings of the Royal Microscopical Society* 31, no. 4 (1996): 293-301, on 296.

As previously mentioned, Abbe published his initial work in the *Archiv* für *Mikroskopische Anatomie*, a publication devoted to microscopic anatomical observations, mainly in the fields of medicine and biology. In line with Zeiss's commercial approach, Abbe was interested in making his ideas known to the community of researchers who used microscopes the most; thus, naturalists, botanists, physicians and geologists in England were among the first to be introduced to his theory.

In 1874, a transcription of Abbe's original publication, translated and read by Henry E. Fripp, was presented to the Bristol Microscopical Society. This lecture was published in 1876 under the title *A Contribution to the Theory of the Microscope, and the nature of Microscopic Vision* in the Proceedings of the Bristol Naturalists' Society.[31] For several years, this version was the most consulted Abbe text in England, but its incorrect translation posed several problems.[32]

Other factors hindered the assimilation of Abbe's theory in England, such as the lack of equations relating concepts and the absence of diagrams, particularly those that could clarify the path of the light rays and their interactions with the sample and with the different parts of the microscope.[33] Castellarnau, believed that Abbe did not include mathematical findings because he did not consider them necessary as they were "too well known and commonplace" among the microscopists of the Royal Microscopical Society.[34] It has also been suggested that Abbe was so busy developing new lenses that he did not have time to fulfil his promise. According to Auerbach, Abbe had already developed his theory by 1870 but did not publish it earlier because he had prioritized the development of new microscopes designed and built according to his premises, so that they lacked competition. Castellarnau interprets Auerbach as saying that Abbe had "kept the most important innovations, which, in practice, were deduced from the new theory, to himself and half secret, until they were of common construction in the Zeiss manufacturing process, and there was nothing to fear from the competition."[35]

By contrast, there are studies asserting that equations can be deduced from Abbe's original publication.[36] In fact, the equation of the diffraction limit, or minimum resolution distance, is

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[31] Ernst Abbe, "A Contribution to the Theory of the Microscope, and the Nature of Microscopic Vision," *Proceedings of the Bristol Naturalists Society* 1, no. 2 (1876): 200-61.

[32] Barry Masters, *Superresolution Optical Microscopy* (Cham: Springer Series in Optical Sciences, 2020), 93; Ernst Abbe, "A Contribution to the Theory of the Microscope, and the Nature of Microscopic Vision," *Proceedings of the Bristol Naturalists Society* 1, no. 2 (1876): 200-61, on 201.

[33] Joaquín María Castellarnau, "Visión microscópica. Notas Sobre las Condiciones de Verdad de la Imagen Microscópica y el Modo de Expresarlas," *Anales de la Sociedad Española de Historia Natural* no. 14 (1885): 257-352, on 262; Joaquín María Castellarnau, *Recuerdos de Mi Vida (1854-1941)*, 2nd ed. (Burgos: Imprenta Aldecoa, 1942), 110; Masters, *Superresolution*, 66.

[34] Castellarnau, "Visión Microscópica," 262.

[35] Auerbach, *The Zeiss Works*, 20; Castellarnau, *Recuerdos de Mi Vida*, 110.

[36] Köhler, "On Abbe's Theory of Image," 1691; Masters, *Superresolution,* 91.

deduced from the text when examining the limits of the resolving power for each lens.[37] And those mathematical demonstrations of his theory were developed in various later publications by Abbe, his followers, and other authors.[38]

Finally, reasons related to scientific and cultural tradition also appear to have played a role in the initial rejection of Abbe's ideas by an important part of the English scientific community. Most English microscopists had no interest in theory and their main focus was having proper equipment that improved the quality and magnification of their observations, so they could discuss the anatomical differences found in their specimens.

By contrast, continental microscopists, especially those from Germany, tended to use the microscope in more applied areas, such as pathology and cytology, which led them to develop new staining techniques and to analyse the role of illumination in their observations.[39] The disinterest shown by many English microscopists for the theory underlying microscopic observation was recognized by Frank Crisp (1843-1919), then secretary of the RMS, and a great supporter of Abbe's ideas.[40] Among other possible cultural reasons for the rejection of Abbe's theory in England, one of the most renowned Spanish microscopists of the time, Domingo de Orueta y Duarte (1862-1926), suggested that it was due to the defence of the English scientific and industrial tradition against the onslaught of the ideas of a German, that were, moreover, supported by the increasingly better quality of Zeiss microscopes.[41] Domingo de Orueta, who is considered a disciple of Castellarnau, was a mining engineer, geologist, businessman and industrialist. He was also an expert in microphotography. He became acquainted with Professor Abbe while in Jena and ultimately collaborated with the Zeiss company in the design of several microphotography setups.[42] Given the aforementioned reasons, we conclude that, after the publication of Fripp's translation, the new theory did not have very many followers in England. However, following Abbe's decisive trip to London, and the publication of new articles, this situation gradually changed as he managed to convince more English microscopists of the veracity of his ideas.

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[37] In current notation: $d = \lambda / 2 \cdot n \cdot \sin\theta$, where d is the resolution of the lens or diffraction limit; λ, is the wavelength of the light; n, is the refractive index of the medium in which the light is transmitted and θ, is half the angle of the light cone entering the lens. Abbe, "Beiträge zur Theorie," 456.

[38] Köhler, "On Abbe's Theory of Image," 1692.

[39] Bradbury, "The Reception of Abbe´s Theory," 296.

[40] Frank Crisp, "On the Present Condition of Microscopy in England," *Journal of the Royal Microscopical Society* 1 (1878): 121-32, on 121.

[41] Domigo de Orueta, "Las Obras Sobre Visión Microscópica de D. Joaquín María de Castellarnau y Lleopart, Inspector General del Cuerpo de Ingenieros de Montes," *Boletín de la Real Sociedad Española de Historia Natural* 12, no. 5 (1912): 289-97, on 289-90.

[42] José Manuel Sanchis, "Domingo de Orueta y Duarte," *Hastial. Revista Digital de Patrimonio Minero ibérico* 1 (2011): 1-33, on 15; Orueta, "Las Obras Sobre Visión," 292.

In 1876, Abbe travelled to London to attend a major international exhibition of scientific instruments. This exhibition, held at the South Kensington Museum, is considered the first international exhibition devoted exclusively to scientific instrumentation. Upon its completion, a large number of loaned equipment became foundational pieces for the Science Museum in London.[43] His purpose was primarily commercial, as he wanted to show the new microscopes and measuring devices that had been built at the Zeiss factory in Jena according to his design, and also, to test instruments from other companies.[44] During his stay in the English capital, Abbe was able to meet with several microscopists including John Ware Stephenson (1819-1901), treasurer of the RMS. At this meeting, the attendees were able to hear from Abbe himself, and see him experimentally demonstrate some of the ideas he described in his 1873 publication. Stephenson was grateful and enthusiastic, and on January 3, 1877, he reported to the RMS his observations from his meeting with Abbe.[45]

The German professor used a microscope and several specially designed gratings and diaphragms to demonstrate the effect of diffraction on microscopic observation.[46] Abbe used glass slides on which he had glued coverslips, with different patterns of lines like grids, that had been drawn by making scratches on a silver film.[47] The process of making the plates changed over time, but from 1878 onward they became known as "Abbe diffraction plates" (*Diffractionsplatte nach Abbe*). He also used a set of diaphragms that, when inserted into a particular type of objective, could be rotated to block parts of the observed diffraction spectrum. This kit, known as the "Abbe diffraction apparatus," was developed by Abbe in 1876 and represents the first instrument designed to demonstrate his theory. The plates showed up in the Zeiss archives from 1878 onwards, and since 1889, they appeared in successive Zeiss commercial catalogues.[48] Following this private demonstration, Stephenson published a short paper in which he describes, in detail, five Abbe's experiments and the devices he used.[49] As we have seen before, Abbe had already described many of these experiments in his 1873 treatise; however, following Stephenson's publication, the clarifications that Abbe presented in that meeting related to illumination, and

43 Robert Bud, "Responding to Stories: the 1876 Loan Collection of Scientific Apparatus and the Science Museum," *Science Museum Group Journal* 1 (2014), http://dx.doi.org/10.15180/140104.

44 Masters, *Superresolution*, 95.

45 John W. Stephenson, "Observations on Professor Abbe's Experiments Illustrating His Theory of Microscopic Vision," *The Monthly Microscopical Journal: Transactions of the Royal Microscopical Society and Record of Histological Research at home and Abroad* 17 (1877): 82–8.

46 Feffer, "Microscopes to Munitions," 140-42.

47 Abbe, "Beiträge zur Theorie," 434; Leopold Dippel, *Das Mikroskop und seine Anwendung* (Braunschweig: Zweite Auflage Friedrich Vieweg und Sohn, 1882), 340.

48 See, e.g., the following catalogues: Carl Zeiss, *Microscopes and Microscopical Accessories*, no. 28 (Jena: Hermann Pohle, 1889), 20; Carl Zeiss, *Microscopes and Accessories for the Microscope*, no. 35 (Jena, 1913), 93.

49 Stephenson, "Observations on Professor Abbe's," 82, 88.

the practical demonstrations with the Abbe diffraction apparatus, made it easier to understand and, therefore, accept the new theory (figure 2).

Figure 2. Abbe Demonstration Microscope with various accessories. Among others various original Zeiss diffraction plates and others prepared by Castellarnau. This special microscope improved the demonstration possibilities of Abbe's diffraction apparatus. Museo Nacional de Ciencias Naturales, CSIC. Photography by Esteban Moreno Gómez.

Castellarnau and the Introduction of Abbe's Ideas in Spain

"Does the microscope give a true image of objects?" With this question, Castellarnau began the publication with which he introduced in Spain the theory of image formation in a microscope that Abbe had proposed 12 years earlier.[50] Both the bibliography on Castellarnau and his own writings reveal a forest engineer who delved into many different scientific fields, with plant histology and microscopy standing out above all others. Some of his research is considered pioneering in Spain, such as his ornithological studies and his micrographs of different conifer species.[51] As was common at the time, Castellarnau had to complete his university studies

[50] Castellarnau, "Visión Microscópica," 257.

[51] Celso Arévalo, *Castellarnau, Biólogo* (Segovia: Universidad Popular Segoviana, 1934), 7; Juan Manuel Moreno, "Apuntes Para una Biografía de Joaquín María de Castellarnau y Lleopart," in *Ciencia y Memoria del Guadarrama en Joaquín María de Castellarnau* (Madrid: Dirección General de Promoción

without the use of a microscope. In 1875, after acquiring his first microscope, he was finally able to satisfy his curiosity and see at first hand the tissues and cells of living beings that, until then, he had only known through the engravings found in histology books made by foreign researchers.[52] Castellarnau's interest in microscopic techniques led him to be the first in Spain to use it to study the internal structures of woody plant species. His skills as an accomplished microscopist are demonstrated in his original micrographic research on the Spanish fir tree in which he provides, in addition to thorough histological descriptions, a detailed commentary on the microscope and the illumination techniques used, and on sample preparation and staining.[53] For his study of the Spanish fir, Castellarnau used a microscope from the French firm Nachet, which allowed the use of polarized light (this instrument is preserved in the MNCN, Nº. MNCN-ICH-004). During his assignment as a Royal Engineer in Segovia, in which he managed the Valsaín pine forest, Castellarnau spent his free time performing microscopic studies on woody plant species and broadening his knowledge of microscopic techniques.[54]

According to his memoirs, Castellarnau first became aware of Abbe's theory around 1880. He likely learned of the theory from some bibliographic reference, as he subscribed to the *Journal de Micrographie* of the French Academy of Medicine. Like many others, the first text he obtained was the English translation by Fripp. Over the following three years, Castellarnau read everything he could about the new theory and kept abreast of the intense discussions it was causing in London. He subscribed to the journal of the RMS, which gave him access to new explanatory articles published by Abbe, and also by his detractors and defenders. As soon as he could, he acquired an "Abbe diffraction apparatus" and enthusiastically reproduced, in his laboratory in San Ildefonso (Segovia), all the experiments he could to understand the role of diffraction in image formation in a microscope.[55]

When studying Abbe's ideas in depth, Castellarnau, who had extended his micrographic research to conifers, decided that he needed to master "the most modern procedures for the microscopic examination of animals for their dissection and conservation."

Consequently, he requested a commission to visit the laboratories of the Zoological Station in Naples. Both the Ministry of Agriculture and the Intendancy of the Royal House, institutions

y Disciplina Ambiental, 2003), 15-9. See also a biographical overview of Castellarnau in the database of the Real Academia de la Historia: Vicente Casals, "Joaquín Castellarnau y Lleopart," http://dbe.rah.es/biografias/19184/joaquin-castellarnau-y-lleopart, accessed January 4, 2020.

52 Castellarnau, *Recuerdos de Mi Vida*, 92-5, 102.

53 Joaquín María Castellarnau, "Estudio Micrográfico del Tallo del Pinsapo," *Anales de la Sociedad Española de Historia Natural* 9 (1880): 401-64, on 403-4.

54 Castellarnau, *Recuerdos de Mi Vida*, 47-8.

55 Ibid., 110-12.

on which he depended, granted him permission to spend there the winter months of 1883.[56] This was the first time he was able to interact directly with foreign microscopists.[57] He arrived with just a microscope and states that the "naturalist who arrives in Naples with the right to occupy a table need not bring any scientific material other than his microscope."[58]

His stay in Italy was enriching. He interacted with prominent European biologists, mainly from Germany and Italy, and more importantly, he was able to learn first-hand about modern microscopic techniques and equipment. From his stay in Naples, Castellarnau highlights his acquisition of knowledge and skills to prepare samples. For instance, he describes, in detail, the technique of kerosene embedding and the use of the Zeiss-type horizontal mount for microphotographs.[59] It may be surprising that a botanist would want to visit a Zoological Station, but Castellarnau needed expertise on the microscopic examination techniques applied to the morphological studies carried out at the Station. His experience covered techniques aimed at medical and physiological studies.[60]

In Naples, he was able to use equipment from Zeiss, which was available there at great discount due to the friendship between its director, Anton Dohrn (1840-1909), and Abbe. Through this relationship, the station benefited financially, and Zeiss was able to present its products to a large community of potential customers.[61] Castellarnau, who, at this point, had been studying and experimenting with Abbe's theory for about three years, recalls in his memoirs how surprised he was that, although Abbe's name was well known among German naturalists, the physical foundation on which his theory was based was ignored by nearly all the German naturalists he had met in Naples. On his return to Spain, he resigned his position as an engineer of the Royal Household and, after taking up residence in Segovia, decided to write a treatise in which Abbe's ideas would be clearly and concisely detailed. Castellarnau published *Visión Microscópica: notas sobre las condiciones de verdad de la imagen microscópica y el modo de expresarlas* (Microscopic Vision: notes on the true condition of the microscopic image and how to express them), the

56 Joaquín María Castellarnau, *La Estación Zoológica de Nápoles, y Sus Procedimientos Para el Examen Microscópico*: *Memoria Presentada al Ilmo. Sr. Director General de Agricultura, Industria y Comercio* (Madrid: Ministerio de Fomento, 1885), VI-VII.

57 Castellarnau, *Recuerdos de Mi Vida*, 48-9.

58 Castellarnau, *La Estación Zoológica de Nápoles,* 20.

59 Ibid., 203-7.

60 Vicente Casals, "Ciencia y Burocracia. El Caso de Joaquín María de Castellarnau y Lleopart," in *Actas del V Congreso de la Sociedad Española del Historia de las Ciencias y las Técnicas*, eds. Manuel Valera Candel and Carlos López Fernández, 660-73 (Murcia: Sociedad Española del Historia de las Ciencias y las Técnicas, 1991), 664.

61 Christiane Groeben, "The Stazione Zoologica Anton Dohrn as a Place for the Circulation of Scientific Ideas: Vision and Management," in *Information for Responsible Fisheries: Libraries as Mediators: Proceedings of the 31st Annual Conference: Rome, Italy,* eds. Kristen L Anderson and Cecile Thiery, 291-99 (FL: International Association of Aquatic and Marine Science Libraries and Information Centers, 2006), 295.

first extensive text in Spanish on Abbe's theory, in the 1885 Annals of the Spanish Society of Natural History.[62]

In *Visión Microscópica*, Castellarnau displays all his knowledge of microscopy. The text, aimed at microscopists, had few illustrations but was written using accessible language. The essential mathematical concepts, origins and physical bases of Abbe's theory were laid out, as well as the first polemics. "The new theory—the true one—on microscopic vision is still very little known . . . it has emerged victorious in such terms that no one today dares to dispute its triumph."[63] He proposed experiments to demonstrate the theory, using both diffraction plates and natural objects, and developed main concepts introduced by Abbe, such as numerical aperture and its relationship with magnification. A very important aspect of Castellarnau's treatise was his intention to make it instructive by proposing a great number of practical examples of real value to a microscopist, from the use of objectives and immersion liquids to the types of glass, and their refraction, to specific observations for the use of oblique illumination. For example, he developed tables showing how to calculate the magnification of objectives according to their angular aperture. This type of table would later be provided by microscope manufacturers to facilitate the work of microscopists. The calculations also serve as an example of the "useful limits of magnification and aperture."[64] The book contained three figures and a plate, the latter being the most informative contribution of the treatise as it illustrates how to position Abbe diffraction plates and diaphragms in order to block various parts of the diffraction spectrum. He also described how to observe the diffraction spectrum of different samples, including the diatom *Pleurosigma angulatum*, according to the aperture of the objective and the type of illumination.[65] With this book, Castellarnau proved to be the foremost expert on microscopy in Spain at the time and one of the first defenders of Abbe's theories on the periphery of continental European science. To do justice to Castellarnau's complete and pioneering work, we quote Frank Crisp: "The present work is extremely well put together; indeed, it is quite unique in the completeness of its treatment of the question." Crisp, who was very critical of the attitude of many English microscopists towards the new theory, further says, with some derision: "If there now remained in this country any microscopists who seriously questioned . . . or the Abbe diffraction theory, a translation of the author's treatise would, we feel sure, have been of benefit to English readers." Crisp did not hide his astonishment that such an elaborate work was written in Spanish. He identified Castellarnau as the most advanced of the Spanish microscopists and recognizes the originality of his work as a good synthesis of Abbe's ideas. Although in the original text the opinion was expressed in the plural, it is attributed to Crisp

[62] Castellarnau, "Visión Microscópica," 257-352.
[63] Ibid., 259.
[64] Ibid., 324.
[65] Ibid., 272-75, 303, Lam. VI.

as he was the editor of the publication.[66] However, the best statement, and the most important for Castellarnau, was the one made by Abbe, who, in a personal interview with Orueta, went so far as to say: "But, in none of these books will you find such a complete and clear exposition of the subject as in one, published, in point of fact, by a Spaniard, by your compatriot, D. Joaquin María de Castellarnau."[67]

Having firmly established his authority of Abbe's new theory in Spain, Castellarnau continued to conduct micrographic research on woody plants over the following years, which he combined with other optical research. In Segovia, he had a laboratory for microphotography where he was visited by prominent researchers, some who came to exchange opinions and others who came to seek help with interpreting samples under the microscope. His laboratory was equipped with a Zeiss microphotographic instrument, with a horizontal and vertical camera arrangement, and a research microscope, also from Zeiss. Both pieces are preserved in the collection at the MNCN.[68]

The interest of the Spanish scientific and educational community in micrographic techniques had been evident years earlier. Although since 1877 the use of the microscope had already been introduced in teaching, it was still an instrument seldomly used even at university levels. This was reported by Castellarnau as well as by other scientists.[69] Interest in the renewal of experimental disciplines in Spain was clear. One of the most relevant geologists of the time, Francisco Quiroga, stated in 1881 that "a naturalist without a microscope is like a chemist without a balance, and an astronomer without a telescope."[70]

In support of this movement, the Royal Spanish Society of Natural History sent, in 1885, a series of proposals aimed at reforming Natural Sciences studies to the Ministry of Development.[71] One of the suggestions was to replace the histology course taught by the Faculty of Medicine with lessons in micrographic technique.[72] Also in 1885, one of the admission tests for an

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66 Frank Crisp, "Castellarnau y de Lleopart, J.M.-The Aperture Question," *Journal of the Royal Microscopical Society* 6, no. 2 (1886): 335-36.

67 Orueta, "Las Obras Sobre Visión," 292.

68 Arévalo, *Castellarnau, Biólogo,* 20; Castellarnau, *Recuerdos de Mi Vida*, 80; Moreno, Romero and Redrajo, *La Recuperación de la Instrumentación,* 33.

69 Luis Alfredo Baratas, *Introducción y Desarrollo de la Biología Experimental en España entre 1868 y 1936* (Madrid: Consejo Superior de Investigaciones Científicas, 1997), 58; Castellarnau, *Recuerdos de Mi Vida*, 43; José Ángel García Rodríguez, José González Núñez and José Prieto Prieto, *Santiago Ramón y Cajal, Bacteriólogo* (Madrid: Ars Medica, 2006), 210.

70 Francisco Quiroga, "La Biología Ante el Microscopio," *Boletín de la Institución Libre de Enseñanza* 5 (1881): 133-35.

71 Antonio Machado Núñez, "Exposición de Bases Para la Reforma de la Enseñanza de las Ciencias Naturales," *Anales de la Sociedad Española de Historia Natural (Actas)* 15 (1886): 3-13.

72 Baratas, *Introducción y Desarrollo*, 89.

assistantship at the medical school included the description and handling of the microscope.[73] Moreover, budgets started to include financing to promote the use of microscopes as a fundamental tool for both teaching and research. An example of such investment was the acquisition of microscopes for the micrography laboratory of the Estación de Biología Marítima de Santander that had been recently created (1886).[74]

Continuing these reforms, in 1900, the Ministry of Public Instruction and Fine Arts introduced Natural Sciences graduates to the subject "Micrographic Technique and Plant and Animal Histology." In addition, in 1910, the Junta para la Ampliación de Estudios e Investigaciones Científicas (JAE), created in 1907, organized the first extension courses, all of which had a common factor: the use of the microscope. The courses scheduled, and their teachers, were: Phylogenic evolution of the nervous system (Ramón y Cajal); Formation of the microscopic image (Castellarnau); Marine zoology. Coelenterata: anatomical, biological and taxonomic description of this group (Rioja); Research and practical teaching on microscopic photography (Orueta); Diatom preparation course (Caballero) and Diatom systematic study (Azpeitia).[75] Twenty-five years after publishing his synthesis of Abbe's work, Castellarnau was asked by the JAE to give a course on the formation of the microscopic image. Designed as an extension of the Histology and Microscopic Technique classes at the Faculties of Medicine, Science and Pharmacy, the course began on 27 March 1911 at the MNCN.[76]

The courses were designed such that "the work is one of cooperation between teacher and students, and has, as its immediate aim, the practical preparation of the latter in a speciality." In line with this ideology, the JAE also considered it necessary for Spanish naturalists and microscopists to be familiar with Abbe's theories in a "complete, elementary and practical way" and "the investigator needs to know what to expect with regard to the degree of truth offered by the image he observes, all the more so since it is in his power to increase or decrease it, as it depends on the circumstances under which the observation is made."[77]

At this time, the JAE was chaired by Santiago Ramón y Cajal (1852-1934), Nobel Prize in Medicine shared with Camilo Golgi (1843-1926) in 1906, for his microscopic studies of the structure of the nervous system. Cajal, who used and recommended Zeiss microscopes, had followed Castallarnau's efforts to spread Abbe's ideas in Spain. Castellarnau proposed a

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73 Josep L. Barona, *La Doctrina y el Laboratorio. Fisiología y Experimentación en la Sociedad Española del Siglo XIX* (Madrid: Consejo Superior de Investigaciones Científicas, 1992), 104-5.
74 Baratas, *Introducción y Desarrollo*, 70.
75 Ministerio de Instrucción Pública del Gobierno de España, *Memoria Correspondiente a los Años 1910 y 1911* (Madrid: Junta para Ampliación de Estudios e Investigaciones Científicas, 1912), 164-70.
76 "Noticias Profesionales," *Revista Clínica de Madrid*, 15 February 1911, 1; "Investigaciones Científicas," *El Imparcial*, 25 March 1911, 8.
77 Ministerio de Instrucción, *Memoria*, 164.

very complete theoretical–practical course. From the beginning, and without neglecting the necessary physical foundations and mathematical derivations, he used instruments to carry out numerous experimental demonstrations. He used Abbe's diffraction apparatus with its plates, as well as various ordinary microscopes, which he had dismantled and modified for his own needs for various experiments. Having learned that Abbe had used a "special apparatus" to demonstrate his theory in a lecture at the University of Halle, Castellarnau managed to import this instrument from Germany through the trading company Viuda de Aramburu for use in his classes.[78] Specifically, it was sent to Spain on 27 February 1911, as confirmed to the authors by the Zeiss archive service. As we will see below, this instrument allowed him to teach diverse demonstrations of Abbe's theory to his students.

At the end of the course, the JAE published a systematic summary of his classes, Castellarnau's second treatise on the subject. Printed with the title *Teoría General de la formación de la imagen en el microscopio* (General Theory of Image Formation in the Microscope). It was a publication consisting of more than 400 pages, in which Castellarnau presented his knowledge of the subject in an orderly fashion. In this work, unlike in *Visión Microscópica* (Microscopic Vision) (1885), he provided more than a hundred diagrams and figures and made much greater use of mathematical equations. Structured in three parts, he devotes the first to explaining the basics of geometrical optics and of dioptrics in the microscope. The remaining parts of the treatise are devoted exclusively to developing Abbe's theory and all concepts necessary for its comprehension. For Orueta, who attended these lectures as a guest, this book "is, without a doubt, the fundamental work of a master."[79] A few years later, in 1918, Castellarnau was invited to give a series of lectures at the Residencia de Estudiantes, a major cultural centre in Madrid, for which he would, once again, use the demonstration apparatus mentioned above and discussed in detail in the following section.[80]

The Demonstration Apparatus Used by Castellarnau

Naturalists and microscopists gradually assimilated Abbe's theory, as it established the resolution limit of microscopes, with deep implications in the accuracy of observations: that is, the correspondence between the real object and the observed image. Abbe developed a theoretical framework to explain how light diffraction affects the observation of certain objects (plates with engraved lines and some diatoms) and then extended this phenomenon to other objects of increasing complexity, when observed through a microscope. However, his ideas were not

[78] Castellarnau, *Recuerdos de Mi Vida*, 117.

[79] Orueta, "Las Obras Sobre Visión," 295.

[80] Joaquín María Castellarnau, *La Imagen Óptica Telescopio y Microscopio. Conferencias Dadas en la Residencia de Estudiantes los días 23, 25, 27 y 30 de Abril de 1918* (Madrid: Publicaciones de la Residencia de Estudiantes, 1919), 79.

accepted until the scientific community, especially the naturalists who used the microscope, checked the quality of the Abbe-Zeiss images.[81]

Another factor that helped to better understand Abbe's postulates were the practical demonstrations. We have already seen the importance of these experiments with Stephenson, but for many years Abbe continued to need specially modified instruments for the demonstration of his theory.[82]

In his will, Castellarnau bequeathed to the MNCN all his microscopes and apparatus, and his books, notes and drafts. The instrumental part of the Castellarnau bequest was catalogued and mostly restored as part of a project to recover the historical instrumentation of the CSIC.[83] One of the pieces bestowed by Castellarnau is a special microscope, actually a compound microscope arranged horizontally, which he had used to complete a series of experiments that served to support Abbe's theory of image formation in a microscope. This piece, catalogued under the name "difractoscopio," was restored by Roberto Moreno and Fernando Redrajo in 1996; however, it was still missing the base. In 2015, after a new restoration, Jorge Pina designed and had a cast iron support built for the piece to provide it with the stability needed for its use. The first known description of this apparatus dates from 1893: it was published in a Zeiss catalogue of optical measuring instruments. Under catalogue number 21 and the name "Apparatus for demonstrating the relationship between the diffraction effect and the image of an object," Siegfried Czapski (1861-1907) details the characteristics of the apparatus and describes various experiments that can be performed with its accessories (figure 3).[84]

This model is the one acquired by Castellarnau. The company later changed the dimensions of the apparatus to improve its handling and presented it under the name "Abbe Demonstration Microscope."[85]

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[81] Ian Hacking, *Representing and Intervening: Introductory Topics in the Philosophy of Natural Science* (Cambridge: Cambridge University Press, 1983), 312-13

[82] Feffer, "Microscopes to Munitions," 140-42

[83] Moreno, Romero and Redrajo, *La Recuperación de la Instrumentación,* 33-41.

[84] Siegfried Czapski, "Apparat zur Demonstration des Zusammenhanges zwischen Beugungswirkung und Bild eines Objectes," in *Optische Messinstrumente,* 40–3 (Jena: Carl Zeiss Optische Werkstätte, 1893), 40.

[85] Carl Pulfrich, "Neue form des abbeschen demonstrations-mikroskops und über einige mit ihm angestellte neue versuche," in *Zeitschrift* für *wissenschaftliche Mikroskopie und für Mikroskopische Technik,* eds. Ernst Küster, Paul Schieflferdecker, and Raphael Eduard Liesegang, 264-69 (Leipzig: S. Hirzel, 1921), 265.

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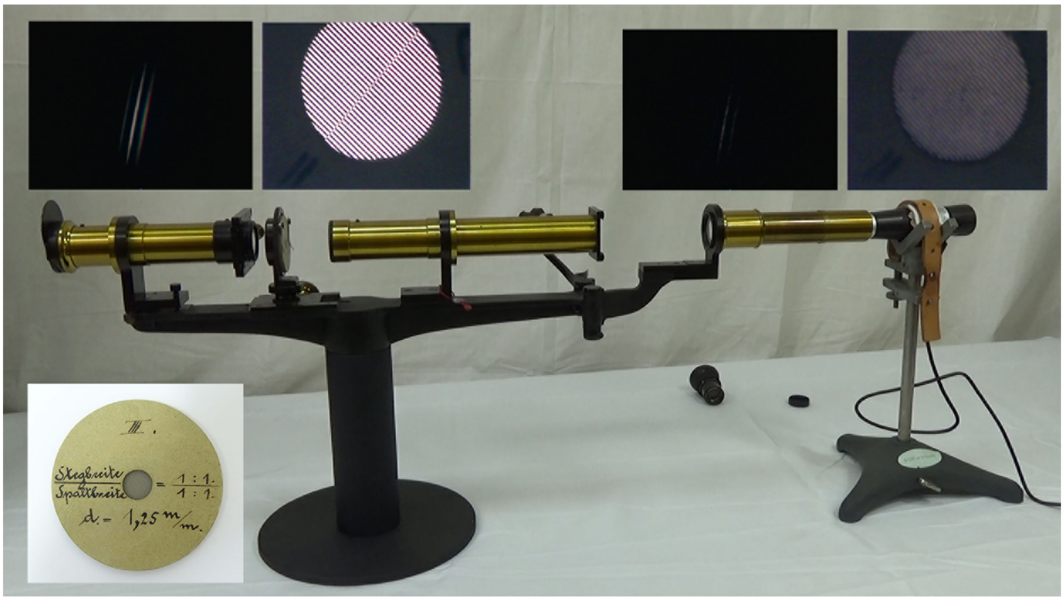

Figure 3. Experimental setup for the reproduction of experiments with the instrument. Observation of grid III (lower left). In the images of the upper part, it can be seen how the details of the grating structure are observed after allowing the passage of first-order and zero-order spectra (left). After occluding the first-order spectrum (right) with the diaphragm, the image of the structure loses sharpness and relevant details. Laboratorio de Recuperación de Instrumentos Históricos, CSIC Photography by Esteban Moreno Gómez.

In his 1911 treatise, Castellarnau devotes the last part to the description of this instrument. He also proposes several experiments, with both the Abbe diffraction apparatus and the Abbe demonstration microscope.[86] The good state of conservation of the instrument, as well as its complementary preparations, have allowed us to reproduce some of the experiments carried out by Castellarnau when demonstrating Abbe's theory. As we have seen, to demonstrate his theory, on his trip to London, Abbe used a microscope with the eyepiece removed to observe the diffraction spectrum of the sample. This method allowed various observations to be made using only a microscope and some preparations. However, the "special microscope" used by Castellarnau had a number of advantages. First, the comfort of the observer, since the horizontal arrangement allowed the illumination and the instrument to be kept in a fixed position while the different observations were being made. Also, the possibility of switching with a smooth movement from the small lens to the eyepiece, facilitating the observation of the diffraction spectrum itself or the observed image. Second, the robustness of the instrument prevents movements and tremors, thus favoring observation. And finally, with a light source

[86] Joaquín María Castellarnau, *Teoría de la Formación de la Imagen en el Microscopio* (Madrid: Junta de Ampliación de Estudios, 1911), 321-22.

intense enough, the image can be projected on a screen, allowing it to be observed by several people. In the reproduction of the experiments, we have used a 20-watt halogen lamp instead of the oil lamp of Castellarnau. Several original preparations have been used, some from Zeiss and others built by Castellarnau himself. In order to photograph the sample and the diffraction spectrum, two digital cameras were placed at appropriate positions.

The five original preparations are gratings reproduced photographically, whose different structures have been designed to offer different diffraction patterns.[87] The condenser is provided with two diaphragms located on its outer edge: the first, a plate with a slit of adjustable width, and the second, a wheel with circular holes of different diameters. The purpose of both is to regulate the size of the light cone illuminating the object, placed on a rotating stage with a focusing mechanism. At the other side of the condenser, a lens illuminates the sample with beams of parallel rays that can be moved by means of an adjustment screw to provide central or oblique illumination of the sample.

After focusing the grating, its structure is observed through the eyepiece. If we wish to see the diffraction spectrum, we switch the eyepiece for a small lens, similar to a magnifying glass, that is positioned at a suitable distance from the focal plane of the objective.

Once we see the details of a grating's structure, we can use the small lens to observe the corresponding diffraction pattern. Depending on the grid, we will see a spectrum formed by a central maximum and several lateral lines of different order, in a circular or linear arrangement. The most relevant function of this instrument is to verify that limiting with the diaphragms the different parts of the spectrum produces a change in the observed image of the grid.

The role of the diaphragms in the "special microscope" is analogous to the angular aperture of the objective in a microscope: a wide-open diaphragm corresponds to an objective with a wide angular opening that consequently receives a wide cone of diffracted light.

Some gratings were designed to show how significant details of their structure are lost upon the interception of certain particular orders of the spectrum. Preparation no. 5, for example, reproduces the structure of the valves of *Pleurosigma angulatum* through a network of lines that intersect at 60 degrees. Depending on the part of the spectrum intercepted, we can see the fading of some rows and the consequent modification of the image.

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[87] Czapski, "Apparat zur Demonstration," 41.

Conclusions

Mario Bunge states that "scientific research begins at the very place where ordinary experience and knowledge cease to solve problems," and also that "the coherent systematization of well-founded and contrastable statements is achieved by means of theories, and these are the nucleus of science, rather than of common knowledge, an accumulation of loosely linked pieces of information."[88] In the case that we have studied here, both assertions are confirmed. The development of testable theories and their demonstration leads to the development of science itself, and at the same time, of the technology needed to further advance science.

At the end of the nineteenth century, scientific research was in a very advanced process of specialization. The enlightened science of the previous century led to the professionalization of science,[89] and specialization meant that the scientist had to sacrifice the acquisition of knowledge on many subjects in order to deepen his knowledge of a few. For this reason, it is not surprising that microscopists in general, and English microscopists in particular, did not have sufficient physical and mathematical knowledge to understand Abbe's theory when he published it, something that they were somewhat reluctant to admit. It is for this same reason that the German zoologists, whom Castellarnau met at the Zoological Station in Naples, knew little of the new theory.[90]

The case of Abbe's theory in Europe is similar to what happened in relation to the introduction of cell theory in Spain: in this case, the delay in its acceptance had to do with the low level of microscopic studies of morphology in comparison with other countries. The field of microscopy had major developments in the first half of the nineteenth century in Europe. In Spain, however, scientific investigation was practically non-existent during that period.[91]

Physicists and naturalists with physical and mathematical training were the first to incorporate Abbe's ideas. This was the case with Castellarnau, a naturalist who applied his training as an engineer to his interests in the natural sciences. An understanding of mathematics and technical knowledge in general were significant skills in the development of his career. Over time, there was also a process of acceptance by way of facts, and many microscopists did not need to understand Abbe's theory to accept its legitimacy, as it was enough for them to see the technological improvement of Zeiss microscopes following the application of his ideas.

[88] Mario Bunge, *La Investigación Científica. Su Estrategia y su Filosofía* (Barcelona: Ariel, 1969), 3-4.
[89] Gribbin, *Historia de la Ciencia*, 297.
[90] Castellarnau, *Recuerdos de Mi Vida*, 113.
[91] Alberto Gomis, "La Aceptación de la Teoría Celular por los Naturalistas Españoles," in *Actas II Congreso de la Sociedad Española de Historia de las Ciencias*: *La Ciencia y la Técnica en España Entre 1850 y 1936*, ed. Mariano Hormigón Blánquez, 133-50 (Jaca: Sociedad Española de Historia de las Ciencias, 1982), 146.

As a consequence of the professionalization of science, the number of researchers working in it increased considerably, and links were created with industry, and between industry and governments. In this context, the use of "demonstration apparatuses" was of great interest. In particular, the ones designed by Abbe allowed him to test his ideas, convince the scientific community of his theory and, at the same time, improve the quality and precision of microscopes and other optical instruments manufactured by Zeiss, providing an added value that would increase their marketability.

Castellarnau, due to his diverse scientific activities, and surely influenced by his basic training as an engineer, transformed his initial fondness for microscopy into a true specialization. He was able to apply it in his field in a pioneering way, and to study and disseminate its physical foundations in light of the new theory. As an advocate of the theory of image formation in a microscope, he remained aware of the discussions and clarifications of the new theory that were to follow over subsequent decades. He also used the demonstration experiments whenever he had the opportunity to disseminate the theory. Like other contemporary Spanish microscopists, he was constantly updating his instrumental equipment, with Zeiss being his favourite brand.[92]

We would be remiss not to add that, according to many specialists, the study and conservation of the historical scientific apparatus makes it possible to investigate the history of science from different points of view: the instrument as a tool designed and built to carry out a specific investigation; the new generation of instruments, as a product of research, that will provide the society with a new technology; and those aimed at teaching or disseminating new scientific ideas. It should be noted that although Abbe's various demonstration apparatuses were intended only for popularization purposes, the instruments we are studying were the result of extensive scientific research. Although used in educational activities, they were also part of the business development of the companies that marketed them.

We end with a reflection by Castellarnau himself, from one of his last publications on microscopy: "In our relations with external objects, do we always accept as true the image that is painted on the retina? . . . And as for having microscopic vision, a limit beyond which we are not able to see anything, what in this world does not have it?."[93]

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[92] Ramón y Cajal, Orueta, Quiroga and, of course, Castellarnau used this brand of microscope and recommended it in their publications. See Santiago Ramón Cajal, *Manual de Histología Normal y Técnica Micrográfica*, 2nd ed. (Valencia: Pascual Aguilar, 1893), 7-13.
[93] Castellarnau, *La Imagen Óptica,* 181.

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Competing interests

The authors declare that no competing interests exist.