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Review

Microscopic examination of urine sediment: Phase contrast versus bright field

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ABSTRACT

Background: Today, phase contrast microscopy (PCM) is the recommended technique for manual urinary sediment (U-sed) examination. In fact, compared to bright field microscopy (BFM), it allows a better identification of most U-sed particles.
Methods: The main contributions, both as original papers in medical journals and as monographs on PCM applied to U-sed examination, published in the period 1950–1982 (which was chosen because it includes the results of the most important investigations on the subject) were identified and analysed. Moreover, a brief analysis on the use of PCM in U-sed examination today was carried out.
Results: After the discovery of PCM by the Dutch physicist Frits Zernike in the 1930s, several contributions were published, most of which are forgotten today. All of them demonstrated the advantages of PCM over BFM in identifying the U-sed particles, especially casts, renal tubular epithelial cells, atypical urothelial cells associated with urothelial carcinoma, and erythrocytes (which for the 1st time were classified as dysmorphic - of glomerular origin - and isomorphic - of non glomerular origin). The analysis of six recent monographs on U-sed or urinalysis, written in English and with an international distribution, demonstrated that only in two of them the U-sed particles were mostly shown by PCM.
Conclusion: Several papers and monographs, published since the early 1950s, have demonstrated the advantages of PCM over BFM for U-sed examination. In spite of this, PCM is not as widely used as it should be.

1. Introduction

Urinary sediment (U-sed) examination is an integral part of urinalysis and is used worldwide as a diagnostic tool for the diseases of the urinary tract [1,2]. Today, in most clinical laboratories in the developed world, it is performed mainly by automated U-sed analyzers, especially as a screening test [3]. However, when an "advanced level" of U-sed examination is needed, manual microscopy is indicated. For this latter, some international and national urinalysis guidelines recommend the use of phase contrast microscopy (PCM) [4,5], while other important documents such as CLSI guidelines state that "bright field microscopy is generally sufficient" even though "the use of phase optics enhances the identification of microscopic sediment elements." [6].

The aims of this paper are: to review the main contributions on PCM for the examination of U-sed which occurred in the period 1950–1982 (which includes the results of the most important investigations on the subject); to comment on the use of this technique today; to describe its advantages over BFM.

2. The discovery of PCM and its early diffusion

PCM was discovered by the Dutch physicist Frits Zernike (1888–1966), while working in the optical laboratory of the Department of Physics at Groningen University, The Netherlands. The discovery originated from Zernike's interest in optics, more precisely in diffraction gratings, on which he started working in about 1920 [7]. This opened the way to a number of observations and experiments, which gradually led to the discovery of PCM in the early 1930s. This was first described by Zernike in a paper published in 1935, in a German journal [8]. However, already three years before Zernicke had proposed his discovery to the firm of Carl Zeiss in Jena, Germany,

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which at that time was among the world-leading factories for the production of microscopes and other optical instruments. Years later, remembering the first contact with the firm, Zernike wrote: “With the phase contrast method still in the first somewhat primitive stage, I went in 1932 to the Zeiss works in Jena to demonstrate it. It was not received with as much enthusiasm as I had expected.” [7]. This behavior Zernike attributed to the prevailing view within Zeiss management that “everything worth knowing or trying in microscopy had been already achieved” [7] by the physicist Ernst Abbe (1840–1905), whose theoretical and technical innovations in optical theory, carried out in the period 1870–1890, had led to major advancements in microscopy [7]. However, in spite of this somewhat discouraging beginning, Zernike did not abandon his project, so that: “Zeiss in Jena slowly continued with the design of instruments. After several of my visits, after some years of developmental work, and after further delay by the war, they brought out phase contrast objectives and accessories in 1942.” [7].

Thus, in the following years PCM started being tested, validated, and developed by many leading optical laboratories all over the world. This led to the production of PC components by several optical factories besides Zeiss, and to the application of PCM in various fields including industry, biology, and medicine, as was well described in a monograph published in 1951 [9].

As the result of all this, in 1953, Zernike was awarded the Nobel Prize in Physics.

3. PCM for the examination of U-sed in the period 1950–1982

3.1. The 1950s

Brief mentions of the *potential*, but still unproved, utility of PCM for U-sed examination, especially for the identification of casts, date back to 1948 [10,11]. However, still in 1952, the advantages of the new technique had not been fully proved and the PC equipment was considered “expensive and cumbersome to use” by Richard W. Lippman (1916–1959), a pupil of Thomas Addis (1881–1949) in his successful and influential Atlas on urine microscopy [12].

In 1957, however, an atlas on U-sed was published in Spain, which demonstrated, for the first time and in a sound way, the advantages PCM could offer over BFM [13]. Written by Luis Daufí Moreso (1927–2013), associate professor of General Patology at Barcelona University, and Francesco Preto Albajés, head of the clinical laboratory of the same institution, the atlas contained 137 microscopic photographs, 48 of which were obtained with PCM. In the authors' view, PCM was better than BFM for hyaline casts, mucus and “many other particles”, which enabled “better quantitative and qualitative results”, as many photographs of various particles, obtained with the two techniques, demonstrated. However, the authors also recognized that PCM supplied very poor images of crystals, which appeared as illuminated masses with undefined contours, for which reason the book included photographs of crystals obtained only with BFM. In spite of this limitation, the authors did believe in the major advantages of PCM, and this was one of the main reasons which induced them to write their book, as they stated in the “Introduction”. However, the atlas was in Spanish, was not translated into other languages, and had only a local circulation and influence, if any. This may well explain why this interesting and innovative, for its time, monograph is totally forgotten today.

3.2. The 1960s

The first contribution of this period was contained in the volume “Actualités Néphrologiques de l'Hôpital Necker 1967” [14]. This collected the texts of the presentations made during an update course on nephrology, organized annually since 1960 by the team of Necker hospital, Paris. The contribution, in French, was written by the Belgian nephrologist Paul Michielsen, who in the period 1956–1961 had worked as “foreign resident” at Necker hospital [15]. Once back in his home town of Leuven, where he had become the head of the renal Division, Michielsen bought a Reichert PCM for the examination of U-sed of his renal patients, a task which he entrusted to a young collaborator, Didier Hauglustaine [15]. The text “The examination of the urinary sediment” described in detail both methodological aspects and particles, which were shown by 23 black and white figures, all obtained by PCM. The author considered this technique as the best tool for the examination of U-sed, since it offered an “excellent resolution” and was free from the technical problems associated with stain techniques, largely used at that time. Today, we can hypothesize that Michielsen's presentation and paper were the first occasions for the many nephrologists who were attracted by the Necker course from many areas of the word to hear and read about PCM and U-sed.

A landmark of the period was the paper “Identification of elements of urinary sediment with phase-contrast microscopy”, which was published in 1968 in the Journal of the American Medical Association [16]. It was authored by a biochemical technician and two medical doctors from the Department of Medicine of Chicago University Hospital, Illinois. The corresponding author was Robert M. Kark (1910–2002), who is remembered today by the nephrological community for having introduced and developed renal biopsy in the USA in the early 1950s [17]. The paper in JAMA was certainly due to Kark's long-lasting interest in urinalysis, as the booklet “A Primer of Urinalysis” he had published with his collaborators in 1963 demonstrates [18]. The article contained an explanation on how and why the authors began using PCM, which we believe worth reporting here in its integrity: “During the past few years, we have been engaged in systematic studies of the urinary sediment, and we have been seeking methods for recognizing and separating cells which stem from the kidney from those arising in the urinary conduit. We have great difficulty in trying to reproduce accurately, by photographs, the elements in the sediments we wished to demonstrate to our students. Ultimately we were persuaded to take photographs with phase microscopy. We were astonished at the clarity of prints and amazed at the definition of objects we viewed. Since then, we have taught staff, fellows, residents, students and technicians to examine the urinary sediment with phase microscopy.” The paper went on to describe in detail the limitations of BFM, which were also demonstrated by the means of five figures in which hyaline casts, lipid-containing casts, renal tubular epithelial cells, erythrocytes, and bacteria were shown by both BFM and PCM (Fig. 1). The paper ended with the following sentence: “It is our belief that phase contrast microscopy should be used for routine study of urinary sediments, both in the physician's office and in the hospital laboratory”. This already 50 years ago.

In the same year, German urologists tested the utility of PCM in the identification of neoplastic urothelial cells in patients with bladder carcinoma [19]. The results obtained with PCM were similar to those obtained with stains. This led to the conclusion that PCM could be used as a reliable screening tool for the detection of urothelial atypical cells. This concept was confirmed not only by other investigators of the period (see below) but also in a recent article published in this journal [20].

3.3. The 1970s

In 1971, a new monograph emphasizing the added value of PCM in U-sed examination was published in Denmark [21]. This was written by two Danish authors, Edwin S. Spencer, a clinician, and Ib Pedersen, a representative of an optical company in Copenhagen. The monograph, whose text was in English and was meant for international distribution, was comprised of 78 pages and 42 black and white photographs of 25 different particles, most of which were shown by both BF and PC microscopy, to demonstrate once again the superiority of PCM. Some fatty particles and crystals also came with polarized light images, while for some crystals only BF images were supplied, maybe for the same reason