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TECHNICAL NOTE

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Phase Contrast Microscopy Versus Differential Interference Contrast Microscopy as Applicable to the Observation of Spermatozoa

A review of available literature has shown something of a paucity of suggested and applied microscopic techniques on the identification of human spermatozoa in forensic casework. Phase contrast microscopy (PC) has been mentioned as the desirable technique by Kirk [*1*] and Kivela [*2*]. The advantages of PC as compared with bright-field are well known and would most certainly include simplifying the task of locating any spermatozoa present. Some other advantages are an apparent improvement in the morphological detail presented to the observer and some reduction in the fatigue associated with the eyestrain accompanying this frequently tedious task.

The purpose of this study was to evaluate the potential of incident light differential interference contrast (DIC) microscopy for the identification of human spermatozoa. Also, DIC was compared with PC in order to establish the pros and cons of each technique as applied to such examinations. The chosen criteria for these comparisons were (1) what microscopic details of the cell's structure were visible, (2) the quality of the observed image in terms of sharpness and freedom from interference by non-sperm cell particulate matter invariably present in forensic casework, and (3) the ability to work with substrates other than microscope slides.

Experimental Details

The two microscopes used in this study were available in the authors' laboratories. The microscopes used were a Leitz Orthoplan phase contrast microscope and an American Optical Co. Model D.I.C.V. microscope. Observations were made at ×200 and ×400 magnification for all the specimens.

Human semen reference specimens were obtained from laboratory personnel. Animal semen reference specimens were obtained through Dr. Martin Drost, Dr. Andrew Hendrichs, Dr. John Kendrick, Dr. Frank Ogasawara, Dr. Robert Parker, and Dr. Victor Shille of the University of California, Davis. The animal semen reference specimens used in this study were dog, monkey, and turkey.

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Dry mounts of each reference material were prepared on 25- by 75-mm microscope slides. Human seminal fluid was also applied to various opaque and semiopaque substrates. These were prepared in an effort to determine if spermatozoa could be observed directly on a substrate other than the customary microscope slides. The substrates chosen from those materials judged to be commonly encountered in routine casework were (1) a piece of cloth made of a 50:50 blend of nylon/acetate and believed to be similar to the materials used in the manufacture of women's underwear, (2) a piece of blue vinyl upholstery material similar to automotive seat coverings, (3) a piece of unfinished pine plywood, (4) a piece of green blotter paper, and (5) a piece of adhesive-backed, lead foil tape having a "Satin Finish."

Another substrate examined was a 25-mm diameter, 5-μm pore size, Uni-Pore® polycarbonate membrane filter. Human seminal fluid was not directly applied to this item as described above. Rather, the filter was used as an isolation device. A 1-cm^2 piece of seminal-stain-bearing cloth was excised from a three-year-old laboratory reference human semen stain; this was transferred to a 15-ml, conical centrifuge tube containing 1 ml of physiological (0.85% w/v) saline solution and allowed to soak for approximately 15 min. Following hydration of the stain-bearing cloth the centrifuge tube and its contents were subjected to ultrasonic cavitation [*3*] for 15 min in a Mettler Model ME 4.6 ultrasonic cleaner. The cloth was then removed and the extract transferred to the bottom of a 5-ml glass syringe which had been fitted with a 25-mm Uni-Pore membrane holder containing a 5-μm membrane filter. The syringe plunger was inserted into the syringe barrel and slowly depressed, forcing the liquid extract through the filter. The membrane was removed from the holder, placed on a microscope slide, and dried in an oven at 55°C.

Observations

The first observations were made with PC. The only substrate applicable to PC was the microscope slide. Observations could be performed on specimens that were dry mounted or covered with cover slips, or both, without loss of optical resolution. However, at ×400 magnification with PC it became difficult to resolve the various parts of the spermatozoon (head, neck piece, and tail). Staining did help to improve this condition somewhat; however, much was still desired in resolving the morphology of the spermatozoon. Another condition observed with PC was the bright halo effect of the Becke line [*4,5*] circumscribing particulate matter present in the dried stain extract. This caused a further loss of resolution when other particles were in close proximity to spermatozoa. When prepared slides were scanned at lower magnifications (×100) it was found that PC provided a more useful contrast between spermatozoa present and the background and thereby improved the chances of locating any spermatozoa present.

Using DIC the observer had a choice as to how much contrast would be introduced into the image formed. Moreover, there was a choice of either gray tones (black and white) or colors. Also, the presence of other particles near and about the located spermatozoon being studied did not interfere in any way with the resolution of morphological detail.

For the observation of spermatozoa on opaque substrates only, DIC was applicable because of the incident illumination. Human seminal fluid deposited on the nylon/acetate material was initially examined under lower magnification (×100). Encrustations of the dried seminal fluid were easily located on the threads of the fabric. These deposits were then examined at ×400 and spermatozoa were observed. This technique was also tried on cotton cloth with a limited amount of success because of the fuzzy nature of the cotton fabric used.

Human seminal fluid was applied to a swatch of blue vinyl upholstery material. Upon scanning the specimen it was necessary to continuously refocus because of the coarse

surface texture of the vinyl. Numerous spermatozoa were observed; however, greater difficulty was encountered in initially locating the spermatozoa. Once spermatozoa were located, identification was not difficult.

Human seminal fluid was applied to a piece of satin-finish lead foil tape. Upon scanning the surface substantial difficulties in locating spermatozoa were encountered. The metallic surface reflected considerable glare and the striations responsible for the satin finish produced alternating bright and dark bands. It was found that focusing very slightly above the surface during scanning facilitated locating spermatozoa. Although spermatozoa were located identification was most difficult.

Human seminal fluid was applied to a piece of pine plywood and to a piece of green blotter paper. Both of these substrates proved to have such irregular surfaces as to make locating the spermatozoa impossible.

The Uni-Pore membrane filter, prepared as described above, was examined and found to be a satisfactory substrate. The membrane filter was similar to glass slides in terms of locating and identifying spermatozoa. One very apparent advantage of the filter is the relative absence of the crystals that form upon drying stain extracts on microscope slides. This method appears to have some potential for application in forensic casework.

Summary

Phase contrast and incident light DIC each have advantages in certain situations. Phase at lower magnifications was better for screening prepared microscope slides, while DIC offered better morphologic rendition and the ability, in some cases, to work directly with the stain-bearing substrate. The latter is potentially beneficial in that there is no opportunity to lose or decapitate any spermatozoa present during extraction and mounting.

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References

[1] Kirk, P. L., *Crime Investigation: Physical Evidence and the Police Laboratory,* Interscience, New York, 1953, pp. 667-670.

[2] Kivela, E. W., "On Finding Spermatozoa in Suspected Seminal Stains," *Journal of Forensic Sciences,* Vol. 9, No. 1, Jan. 1964, pp. 138-139.

[3] Hueske, E. E., "Techniques for Extraction of Spermatozoa from Stained Clothing: A Critical Review," *Journal of Forensic Sciences,* Vol. 22, No. 3, July 1977, pp. 596-598.

[4] Gray, P., Ed., *Encyclopedia of Microscopy and Microtechnique,* Van Nostrand Reinhold Co., New York, 1973, p. 422.

[5] McCrone, W. C., Draftz, R. G., and Delly, J. G., *The Particle Atlas,* Ann Arbor Science Publishers, Ann Arbor, Mich., 1967, pp. 39-40.

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