Interferometric phase microscopy for label-free morphological evaluation of sperm cells

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Objective: To compare label-free interferometric phase microscopy (IPM) to label-free and label-based bright-field microscopy (BFM) in evaluating sperm cell morphology. This comparison helps in evaluating the potential of IPM for clinical sperm analysis without staining.
Design: Comparison of imaging modalities.
Setting: University laboratory.
Patient(s): Sperm samples were obtained from healthy sperm donors.
Intervention(s): We evaluated 350 sperm cells, using portable IPM and BFM, according to World Health Organization (WHO) criteria. The parameters evaluated were length and width of the sperm head and midpiece; size and width of the acrosome; head, midpiece, and tail configuration; and general normality of the cell.
Main Outcome Measure(s): Continuous variables were compared using the Student's *t* test. Categorical variables were compared with the χ^2 test of independence. Sensitivity and specificity of IPM and label-free BFM were calculated and compared with label-based BFM.
Result(s): No statistical differences were found between IPM and label-based BFM in the WHO criteria. In contrast, IPM measurements of head and midpiece width and acrosome area were different from those of label-free BFM. Sensitivity and specificity of IPM were higher than those of label-free BFM for the WHO criteria.
Conclusion(s): Label-free IPM can identify sperm cell abnormalities, with an excellent correlation with label-based BFM, and with higher accuracy compared with label-free BFM. Further prospective clinical trials are required to enable IPM as part of clinical sperm selection procedures. (Fertil Steril® 2015;104: 43–7. ©2015 by American Society for Reproductive Medicine.)
Key Words: Interferometry, phase microscopy, male fertility, sperm analysis

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After the introduction of in vitro fertilization (IVF) extensive research was conducted to identify the morphologies of the oocyte and fetus as a prognostic tool (1). Fewer studies were conducted on the ability of sperm cell morphology to predict the success rates of natural fertilization, intrauterine insemination, IVF, and IVF with intracytoplasmic sperm injection (ICSI) (2–4). Typically, sperm cells are imaged optically using bright-field microscopy (BFM) and chosen according to World Health Organization (WHO) guidelines (5). Recently, new methods were developed for identifying finer properties of sperm cells that are not shown with BFM (e.g., surface charge selection [6]). Most of these methods involve biochemical preparations that might change the viability of the cells and thus preclude their use in IVF.

Without staining, sperm cells are nearly transparent under BFM, because their optical properties differ only slightly from those of their surroundings, resulting in a weak image contrast. An internal contrast mechanism that can be used when imaging sperm cells is their refractive index. The light beam that passes through the sperm cells is delayed, because the cells have a slightly higher refractive index compared with their surroundings. Regular, intensity-based detectors are not fast enough to record this delay directly.

Phase imaging methods, on the other hand, use the optical interference phenomenon to record the delays in the

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passage of light through the sample, and they are able to create label-free contrast in the image. However, conventional phase-contrast imaging methods for sperm cells, such as Zernike's phase contrast (7), and Nomarski's differential interference contrast (DIC), which is the basis for the motile sperm organelle morphology examination (MSOME) technique (8), are not fully quantitative, because they do not create meaningful contrast on all points of the measured sperm. In addition, these techniques present significant imaging artifacts, especially near the cell edges, which may yield incorrect morphological assays.

Interferometric phase microscopy (IPM) (9) is a holographic imaging method, which allows for a fully quantitative measurement of the cell optical thickness (i.e., the product of the refractive index and the physical thickness) on all the sperm spatial points. This method requires a lower illumination power and presents high throughput because capturing is done in a single exposure and without scanning. Holographic imaging has been identified previously as a tool for sperm measurements (10, 11). However, until recently, most IPM setups were bulky, expensive, and hard to operate. Recently, we have developed a portable and easy-to-operate IPM module, which can be attached to existing clinical microscopes and provide label-free, quantitative contrast for cell samples (12, 13).

Sperm cell morphology is known to be an indicator of its fertilization potential (2–4). Therefore, improved noninvasive morphological assays for sperm cells are needed. These assays are expected to be especially important for cases in which cell labeling is not recommended. One specific example is IVF with ICSI for sperm cells of infertile males. In these cases, quantitative morphological imaging might be the best predictor for choosing the most suitable sperm cell for injection into the oocyte.

To date, however, no research has compared label-free IPM with BFM, using the WHO criteria. The aim of the present study is to compare IPM with BFM, in sperm cell evaluation, to examine the potential validity of this method as a clinical tool for sperm analysis.

MATERIALS AND METHODS

Sample Preparation and Imaging

After institutional ethics committee approval, normal and pathologic sperm samples were received from the male fertility clinic at Chaim Sheba Medical Center. After the samples were collected, using methods in accordance with the WHO manual (5), a drop of 5–10 μl of fresh semen was smeared onto a clean microscope slide (24 × 50 mm) and left to dry for 5 minutes. Before the sample was imaged, it was fixed in 98% ethanol for 10 minutes.

Morphological evaluation was performed on the same sperm cells, using IPM (see next section) and BFM with an inverted microscope (Axio Observer D1, Zeiss). To find the same sperm in different imaging modalities, each slide was painted with a 2 × 2 point grid. After the samples were imaged without labeling (in label-free BFM and IPM), they were stained with Quick Stain (Biological Industries), left to dry for 15 minutes, and imaged again in label-based BFM. All measurements were performed by one of the investigators, who is a trained urologist, accompanied by the biomedical engineer investigator, who built the optical systems, to allow optimal imaging results.

Interferometric Phase Microscopy System

The proposed IPM optical system is depicted in Supplemental Figure 1 (available online). Briefly, the system is comprised of our previously developed portable interferometric module (τ interferometric) (13), connected at the exit of a regular inverted microscope.

In this system, a partially monochromatic light source (6.2-nm–wavelength bandwidth) illuminates an existing inverted microscope for sperm analysis. The τ portable interferometric module is a small box, connected at the port of the microscope output (where the camera is usually positioned), and projects an interference pattern on the digital camera, allowing quantitative phase acquisition. This interference is created by projecting two beams onto the camera with a small angle between them: a sample beam, which is the regular magnified image of the sperm sample; and a reference beam, which does not contain the sample information. The interference pattern is acquired by a regular digital camera in a single exposure, and can be digitally processed, by a conventional computer in real time, to the optical thickness map of the sperm, allowing a fully quantitative contrast image of the sperm sample, without any labeling.

Unlike previous IPM setups that require custom-built microscopes, expensive equipment, and difficult alignment, our setup is robust, portable to existing clinical microscopes, and easy to align. A detailed description of the setup and the following digital image analysis is given in Supplemental Appendix 1 (available online).

Statistical Analysis

The following variables were collected using IPM, label-free BFM, and labeled-based BFM: length and width of sperm head, number and relative size of head vacuoles, width and relative size of the acrosome, and length and width of the midpiece. Furthermore, qualitative assessment of the form of the midpiece, length and form of the tail, and general form of the sperm cell were also gathered.

Continuous variables were presented as mean (±SD) and evaluated by the paired Student's t test and the Wilcoxon signed rank test, as applicable. Categorical variables were presented as percentages and evaluated with the χ^2 test of independence and McNemar's test, as needed. In addition, sensitivity and specificity of IPM and label-free BFM were calculated, and compared with label-based BFM. The number of sperm cells needed for evaluation was chosen so as to be able to identify a 10% difference in any of the continuous variables at $P<.05$ and at a power of 80%. Statistical analysis was performed with SPSS, version 21 (IBM).

RESULTS

Figure 1A and B show a typical interferogram obtained using the IPM system. The bending of the interference fringes over