QUANTIFICATION OF HUMAN SPERM CONCENTRATION USING MACHINE LEARNING-BASED SPECTROPHOTOMETRY

[1]Haydarov Bekzod, [2]Kamalova Dilnoz, [3]Egamova Munira
[1,2]Assistant teachers of the Yangiyer branch of the Tashkent Institute of Chemical Technology
[3]Senior teacher of the Yangiyer branch of the Tashkent Institute of Chemical Technology
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Abstract. *Spectrophotometry is an indirect non-invasive and quantitative method for specifying materials with unknown contents based on absorption behavior. This paper presents the first application of artificial neural network in spectrophotometry for quantification of human sperm concentration. A well-trained full spectrum neural network (FSNN) model is developed by examining the absorption response of sperm samples from 41 human subjects to different light spectra (wavelength from 390 to 1100 nm). It is shown that this FSNN accurately estimates sperm concentration based on the full absorption spectrum with over 93% prediction accuracy, and provides 100% agreement with clinical assessments in differentiating the samples of healthy donor from patient samples. We suggest the machine learning-based spectrophotometry approach with the trained FSNN model as a rapid, low-cost, and powerful technique to quantify sperm concentration. The performance of this technique is superior to available spectrophotometry methods currently used for semen analysis and will provide novel research and clinical opportunities for tackling male infertility.*

Keywords: *semen analysis, sperm concentration, spectrophotometry, artificial neural network.*

INTRODUCTION

Infertility is on the rise worldwide [1, 2], affecting one in six couples with almost half of the cases caused by male infertility factors [3, 4]. Male infertility is commonly attributed to low sperm count (oligospermia), low sperm vitality (necrospermia), poor sperm motility (asthenospermia), and abnormal sperm morphology (teratospermia) [5]. Other quality metrics such as semen volume and DNA integrity are also shown to be correlated with male infertility, however, with less direct association [6]. Despite the rising trend of infertility, improvements in conventional diagnostic methods have been infrequent, and new technological development is required to allow for more accurate semen analysis outside of fertility clinics or for at- home testing [2, 7].

Semen analysis to quantify sperm concentration, vitality, motility, and morphology is crucial to infertility diagnosis [2, 8-10]. Current clinical semen analysis methods demonstrate about 89.6% sensitivity, with the ability to accurately diagnose 9 out of 10 infertile men [5]. Routinely, the first step in clinics is to quantify sperm concentration, motility and morphology as the three critical indicators [11]. In up to 90% of male infertility cases, low sperm concentration exhibits a strong positive correlation with other abnormal semen parameters [5, 12], and can be used as the first stand-alone step to diagnose male infertility. Moreover, sperm concentration for each individual changes on a daily basis [13], and for a reasonable judgement on the male fertility health, three cycles of semen analyses are to be conducted over the course of 2 to 3 weeks, each at least seven days apart [14]. Additionally, follow-up treatments need even more frequent analysis of semen to track subsequent changes in sperm concentration [14].

Conventional methods for sperm concentration analysis includes hemocytometry, computer-aided semen analysis (CASA), microfluidic methods, and spectrophotometry [16]. Hemocytometry is the most traditional and oldest method for quantifying cell concentration by counting the number of cells in a chamber of known volume. Besides being laborious and prone to the operator error, this method is the third most imprecise method for estimating sperm concentration [7]. CASA is the gold standard method for semen analysis in clinics. In CASA, captured image sequences from at least 100 sperm in the sample are processed in real-time using an advanced computer-based image processing unit to quantify sperm concentration and motility [8]. CASA system is expensive, while system-to- system variations between the image processing units can also influence calculated sperm concentrations [11]. Microfluidic methods [2], particularly in paper-based formats [3] and in combination with cell-phone based applications [10] have also provided new opportunities for low-cost and accessible semen analysis. While promising, lack of standardization and reproducibility have prevented adoption and/or commercialization of developed microfluidic technologies, mainly due to variation between different cell-phone-based imaging devices and challenges accessioned with handling delicate volumes of fluid. More recently, cell-phone based systems have been used as a capturing device to overcome issues associated with the lack of standardization procedures, providing a portable and more standard analyzing system.

Spectrophotometry is a precise method for quantifying cell concentration and is routinely used for estimating sperm concentration in wild and captive animals such as bull, horse and aquatic species. This method is based on measuring the transmission of light through a known volume of sample, in which light absorption values at specific wavelengths are used to quantify sample concentration [12]. According to Beer-Lambert law, the absorption magnitude, A, is equal to the log ratio of transmitted light intensity, I, to the initial light intensity, I_o, as $A=\log(I_0/I)$ [26]. Using this method, sperm concentration in unknown samples is estimated based on a previously established linear correlation between absorption intensity (recorded at a set wavelength) and sperm concentration. This method has been applied to quantify bull sperm concentration, demonstrating good agreement with that of the hemocytometry method. While spectrophotometry is rapid, accessible and well-suited to process a large number of samples, the method is very time sensitive as the absorption reading varies significantly with time. Moreover, the optimal wavelength for the most accurate prediction of sperm concentration varies from species to species and/or setups. For examples, this method has been applied to quantify sperm concentration by recording the absorption intensity at 400 nm for ray-finned fish and bull, at 480 nm for salmonid fish, and at 550 nm for roosters. This variability reduces the prediction accuracy of the methods where the most appropriate wavelength is not applied [12], preventing its widespread application, particularly for human semen analysis. Moreover, due to inherent nature of spectrophotometry, the sensitivity of the method is influenced by the concentration of somatic cells and debris in the raw semen sample that additionally absorb light and result in overestimating sperm concentration compared to conventional CASA systems.

Here, using a UV-Visible spectrophotometer, we introduce a well-trained ANN approach to evaluate human sperm concentration, by establishing a correlation between the full absorption spectrum and sperm concentration. This method is the first application of artificial neural network in spectrophotometry for sperm concentration analysis, and by using the full absorption spectrum rather than only one optimal wavelength, significantly improves over the traditional spectrophotometry-based methods to predict sperm concentration. Due to the inherent nature of spectrophotometry, our method is rapid and low-cost with over 93% accuracy in predicting sperm concentration, providing identical clinical outcomes for tested patient and donor samples.

MATERIAL AND METHODS

sample preparation

The semen samples were collected from 41 male subjects between 30 and 45 years old who were referred to Avicenna Infertility Clinic for treatment, with ~15% of male partners experiencing infertility issues and the samples covering a wide range of patient and donor samples. All subjects signed an informed consent form and the study was approved by the ethical Committee of Avicenna Research Institute (Tehran, Iran). The study excluded subjects with a history of round cells ($\geq 1 \times 10^6$ per ml), radiation, smoking, alcohol consumption, drug abuse, systemic disease, leukocytospermia, and varicocele. Semen samples were collected after 2–5 days of sexual abstinence, liquefied and prepared by standard swim-up technique. In brief, 1 ml of the semen specimen was gently mixed with 1 ml of Ham's F10 medium (Sigma, USA) supplemented with 3% human serum albumin (HSA) (Sigma, USA) and centrifuged at 330 g for 10 min. The supernatant was removed and 2 ml of Ham's F-10 medium supplemented with 3% HSA was added to the pellet. Subsequently, a MedeaLab CASA system (MTG, Germany, Ver. 4.1) with a MD-30 CCD camera and a sperm motility analyzer (VT Sperm 3.1) was used for clinical measurement of sperm concentration and progressive motility, according to World Health Organization (WHO) guidelines [40].

UV-VISIBLE SPECTROPHOTOMETRY

Absorption magnitudes versus wavelength were recorded using UV-Visible Spectrophotometer (Perkin Elmer, Lambda 25), as shown in Figure 1a. To calibrate the device, sample and base cuvettes were both filled with Ham's F-10 medium supplemented with 3% HSA, and the machine was zeroed. The recorded absorption spectrum was used as a reference to be subtracted from sperm sample spectra. The sample cuvette was then loaded with 0.5 mL of washed semen sample. To prevent inaccurate readings due to probable and gradual settlement of semen samples, the sample was remixed by gently shaking the closed-cap sample cuvette prior to each measurement. The wavelength was swept from 390 nm to 1100 nm (1 nm interval), and the measurement was repeated twice for each sample (sample volume was divided into two equal parts and tested separately). Each test took about 5 minutes, and all the experiments were performed at 11 am to minimize the sensitivity of the method to the time-frame. Furthermore, linear regressions between sperm absorbance and concentration were generated at 5 absorption peaks.

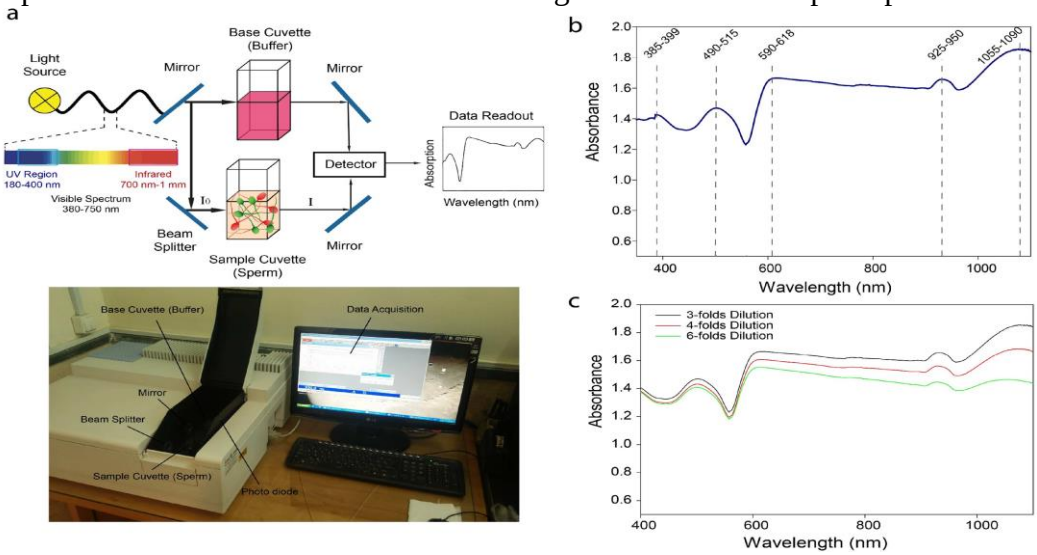

Figure 1. (a) UV-Visible spectrophotometry experimental setup. (b) A representative absorption spectrum for a tested semen specimen, demonstrating 5 absorption peaks at 390, 502, 615, 929, and 1075 nm. (c) The effect of sample dilution on absorption intensity for a semen specimen with the initial raw concentration of 70 Million/ml.

Absorption pattern is identical and only lower absorption and complex but related datasets. Two neural networks, each with a two-layer backpropagation network, were developed using Matlab R2019b software for predicting sperm concentration from absorption spectrum (Figure 2), one based on absorption peaks (5 peaks at 390, 502, 615, 929, and 1075 nm) and the other one based on the full absorption spectrum. The choice of backpropagation was because it is the most popular training algorithm for adjusting network weights and biases. Among the 41 tested human semen specimens, 26 samples were used in the model development phase (70% for training, 15% for testing and 15% for validation), and an additional 15 samples (previously unseen by the model) were used for external validation, ensuring the generality of the trained network for prediction and avoid overfitting of the data. The optimum number of neurons in each hidden layer was also optimized through assessing 400 ANN structures (for 5 times per structure for a total of 2000 cases) to achieve the lowest RMSE for best prediction accuracy (Table S1 and Table S2 in the Supplementary Material). It is noteworthy that the prediction accuracy was defined as the percentage ratio between predicted concentrations from the model and the clinical values (100 × model value/clinical value), as a clear metric to demonstrate how closely the estimated concentration from the model compares with the clinical measurements. To validate the performance of the model, 13-fold cross validation was also performed on the dataset, with the results demonstrating an average classification accuracy of 92% for the dataset A multi-input selected peak neural network (SPNN) model was trained by using peak absorptions for each of the 26 training samples as the input dataset and their corresponding sperm concentration as the output dataset (Figure 2a). The inputs were the product of peak absorption wavelengths (W_i=390, 502, 615, 929 and 1075 nm) and the corresponding normalized absorption values (Abs_i). The maximum absorption value among all tested samples was used for normalization. Absorption measurement at the peak wavelengths introduces the smallest error due to their relatively high signal-to-noise ratio, improving measurement accuracy especially for relatively small absorbance values. A full spectrum neural network (FSNN) model was also trained by using the full absorption spectrum for each training sample as the input (711 data points per sample) and the corresponding sperm concentration as the output (Figure 2b).

DATA ANALYSIS

Absorption spectra from a total of 41 semen specimens were recorded, out of which 26 samples were used for establishing the calibration curves and training the artificial neural networks and the other 15 samples were used as unknown samples for testing the external validity. To validate the performance of the ANN models, 13-fold cross validation was also performed on the dataset, with the results demonstrating an average classification accuracy of 92%. For cross validation, the dataset was randomly partitioned into 13 parts, where 12 parts formed the training set and the remaining part formed the test set. Each 13-fold cross validation procedure was repeated three times to ensure

RESULTS AND DISCUSSION

absorption-sperm concentration correlation

Figure 1b shows a representative absorption spectrum for a human sample with 70 million per millilitre (M/ml) sperm concentration, demonstrating 5 absorption peaks at 390, 502, 615, 929, and 1075 nm. To characterize the effect of sperm concentration on absorption spectrum, the semen specimen (with 70 M/ml sperm concentration) was also diluted 3-, 4-, and 6- folds and absorption spectra were recorded (Figure 1c). Recorded absorption spectra for diluted samples followed the same pattern as the original sample but shifted down by diluting the sample. Specifically, the absorption peak positions were almost independent of the applied dilution (i.e. change in sperm concentration), however, the peak absorptions were considerably influenced and shifted down for

lower sperm concentrations. As shown in Figure 1c, the change in the peak absorption values was more pronounced at higher wavelengths, particularly for peaks at wavelength higher than 600 nm. Specifically, by diluting the sample 4- and 6-folds, the absorption magnitude at wavelength higher than 600 nm decreased on average by 6% and 13%, respectively, but only by 2.5% and 4% at lower wavelengths (<600 nm). Compared to the sample with 3-folds dilution, the peaks at 1084 nm decreased considerably by 10% and 28% by diluting the sample 4- and 6-folds, respectively. As a result of this nonlinear change, the maximum absorption was observed for the 612 nm peak by diluting the sample 6-folds, instead of the maximum peak at 1075 nm for samples of higher sperm concentrations. The results demonstrate that the absorbed light intensity is correlated with sperm concentration, shifting down for samples with lower concentrations.

To establish a correlation between sperm concentration and absorption intensity, 26 human samples with sperm concentrations ranging from 4 M/ml to 70 M/ml and sperm motility ranging from 10% to 65% were tested (Figure 3). For samples of different concentrations, 5 absorption peaks were observed, shifting by less than 5% to be at 385-399 nm, 490-515 nm, 590-618 nm, 925-950 nm, and 1055-1090 nm.

All of the tested samples demonstrated the visible range peak at 590-618 nm, but with lower peak absorption intensities for lower sperm concentrations. For samples with sperm concentration lower than 20 M/ml (Figure 3a), the 490-515 nm peak mostly disappeared, and the first dominant absorption peak was observed at 590-618 nm. Moreover, the two absorption peaks at 925-950 nm and 1055-1090 nm were also only observed for samples with concentrations above 40 M/ml, but not for samples of lower concentrations. The absorption behaviour was also compared for samples of different concentrations but the same motility of 50% (obtained from clinical measurements), as shown in Figure 3b. Samples with fixed sperm motility of 50% demonstrated the same behavior, and absorption spectra shifted down at lower concentrations. It is noteworthy that, while absorption spectra were influenced by sperm concentration, no clear and comprehensive correlation between any of the individual absorption peaks and sperm concentration was observed.

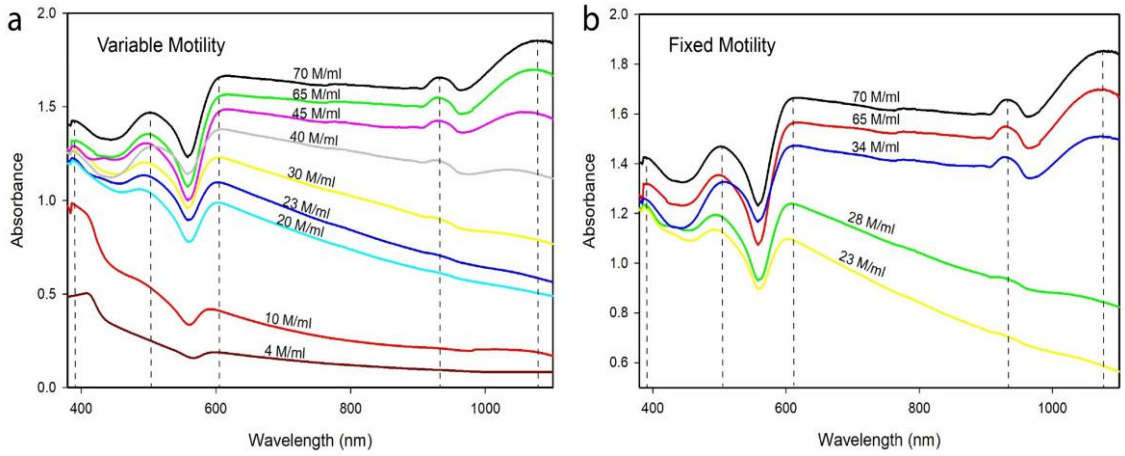

Figure 3. Absorption spectra for human semen specimens of different concentrations. (a) Absorption spectra for 9 semen specimens ranging in sperm concentration from 4 M/ml to 70 M/ml and in motility from 10% to 65%.

Full spectrum neural network (fsnn) model

To develop a comprehensive prediction tool, a full spectrum neural network (FSNN) model was constructed that estimates sperm concentration based on the full absorption spectrum (711 data points per sample, Figure 2b). The minimum RMSE of 0.086 was achieved for the FSNN model with Levenberge-Marquardt (LM) as the training method and with Tansig and Logsig as the transfer functions for the first and second hidden layers respectively. The best FSNN model

maps 711 input variables (absorption values and corresponding wavelengths) onto a single output (sperm concentration) with 12 neurons in the first hidden layer and 20 neurons in the second hidden layer (denoted as 711:12:20:1), listed as structure number 240 in Table S1 in Supplementary Material. Figure 5b compares sperm concentrations from clinical measurements with values predicted by the FSNN model, for the same 15 human samples tested with the SPNN model. Sperm concentration from the FSNN model strongly correlated ($R2=0.98$, $P\leq0.0001$) with clinical measurements, with predicted values within 7% of the clinical measurements (93% prediction accuracy). The FSNN model demonstrated over 7% and 15% improvement in prediction accuracy as compared with the SPNN and simple regression models, respectively. The FSNN model accurately estimates sperm concentration by fully accounting for the non-linear correlation between sperm concentration and absorption spectrum, including the change in the dominant absorption peak and the shift in the peak position for samples of different concentrations. It is noteworthy that, both SPNN and FSNN models were also trained and evaluated to also estimate sperm motility from recorded absorption spectra for clinically tested samples with sperm concentrations ranging from 4 M/ml to 70 M/ml and sperm motility ranging from 10% to 65% (i.e. ANN models with sperm concentration and motility as output parameters). However, the models were incapable of predicting sperm motility and estimated concentrations were independent of sperm motility.

To evaluate the performance of the developed models to estimate sperm concentration, Figure 6 compares sperm concentrations from the linear regression, SPNN and FSNN models against clinically measured values, for patient and donor samples ranging in concentration from 5 to 65 M/ml.

CONCLUSIONS

In summary, we demonstrated a machine learning-based spectrophotometry approach for quantifying human sperm concentration. The full spectrum neural network (FSNN) model accurately estimates sperm concentration based on the full absorption spectrum by establishing a weighted correlation graph to fully account for the non-linear correlation between absorption spectrum and sperm concentration. Sperm concentration from the FSNN model strongly correlated ($R2=0.98$) with clinical measurements and were within 7% of the clinical values, demonstrating a prediction accuracy of 93% with over 15% improvement over the linear regression model. The FSNN model provided 100% agreement with clinical measurements in terms of clinical outcome for patients to accurately distinguish between donor and patient samples. However, increasing sample representativeness in terms of sperm concentration by increasing the sample size, could possibly increase the prediction accuracy of our method and better validate the model for clinical adoption. Collectively, the FSNN model provides a rapid and powerful tool for quantifying sperm concentration, improving on current spectrophotometry methods for semen analysis and providing novel opportunities for male infertility diagnosis. While increasing the sample size, can better inform the model for estimating sperm concentration and further increase the prediction accuracy, the FSSN model still demonstrates its performance for male infertility diagnosis. Utilizing the novel machine learning-based spectrophotometric approach is also suggested for samples with DNA fragmentation in search for an effective diagnostic tool.

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