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ОТСЛЕЖИВАНИЕ ПЛАВАНИЯ САМОДВИЖУЩИХСЯ СПЕРМАТОЗОИДОВ С ПОМОЩЬЮ КОМПЬЮТЕРНЫХ МЕТОДОВ АНАЛИЗА СПЕРМЫ ДЛЯ ОПРЕДЕЛЕНИЯ ВЛИЯНИЯ ВИБРАЦИИ НА СПЕРМАТОЗОИДЫ

Аннотация*: в статье отмечается, что подвижность сперматозоидов человека представляет большой интерес для исследователей и биологов, изучающих функции сперматозоидов, а также для врачей, занимающихся оценкой и лечением мужского бесплодия. Компьютерный спермоанализатор (CASA) используется для автоматического отслеживания путей плавания сперматозоидов в последовательностях изображений микроскопии с замедленной съемкой, соединяя точки между видеокадрами, что позволяет быстро автоматически определять параметры подвижности сперматозоидов для сотен клеток одновременно. Однако возможности обработки изображений и отслеживания сперматозоидов в современных приборах CASA могут быть расширены. Авторы выделили ограничения приборов CASA и предложили способы их смягчения. А также исследователи разработали алгортим – программный комплекс для определения линейной скорости на языке Java. Благодаря всем этим усилиям по дальнейшему совершенствованию вычислительных инструментов для CASA в обозримом будущем должны появиться гораздо более надежные и универсальные системы.*

Ключевые слова*: вероятностные модели, компьютерный спермоанализатор, модель сперматозоидов, режимы плавания сперматозоидов.*

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TRACKING THE SWIMMING OF SELF-PROPELLING SPERMATOZOA USING COMPUTER ASSISTED SEMEN ANALYSIS TECHNIQUES TO DETERMINE THE EFFECT OF VIBRATION ON SPERMATOZOIDS

Abstract*: human sperm motility is of great interest to researchers and biologists studying sperm function and to medical practitioners evaluating and treating male infertility. Computer-assisted semen analysis (CASA) instruments are used to trace the swimming paths of sperm automatically in time-lapse microscopy image sequences connect-the-dots between video frames, enabling rapid automatic quantification of sperm motility parameters for hundreds of cells at a time. The image processing and sperm tracking capabilities of today's CASA instruments can be enhanced However. In this paper we highlighted the limitations of (CASA) instruments and suggested ways to mitigate them from the literature. We have also our algorithm – program complex to determine linear velocity in Java. With all these efforts in further improving the computational tools for CASA, much more robust and versatile systems should be available in the foreseeable future.*

Keywords*: computer assisted semen analysis, probabilistic models, sperm model sperm swimming modes.*

1. Introduction:

Sperm cells are assigned a very difficult task of finding the egg, where only one in a million spermatozoa is capable of entering the Fallopian tubes in humans [1]. It is of great scientific interest to providing a deep understanding since this complex phenomenon helps mankind both in medical [2] and technological [3] advances. The journey of the spermatozoa toward the egg [4] have been of interest for researchers, including the biophysics of the sperm's swimming patterns, both from a kinematics [5] and a fluid dynamics [6] perspective.

Among these different directions of sperm research, one common need is to image and quantify the locomotion of sperm cells for scientific research, as well as for medical diagnostics and animal husbandry-related applications, since imaging of sperm is critical. Computer-Assisted Semen Analysis (CASA) systems and their algorithms continue to be of great interest to clinicians and andrology researchers1 [7]. Modern CASA systems «have been designed to objectively and quantitatively measure several aspects of sperm structure and function, aiming to provide high levels of intra- and

inter-laboratory consistency» [8]. To achieve this aim, methods of noise filtering, image segmentation, localization, multi-object tracking, and machine learning were employed [9].

This paper starts by giving a background on conventional computer assisted semen analysis and then we highlight the limitations of (CASA) instruments and suggested ways to mitigate them from the literature. We have also our algorithm – program complex to determine linear velocity in JavaScript. With all these efforts in further improving the computational tools for CASA, much more robust and versatile systems should be available in the foreseeable future.

2. Sperm tracking and computer-aided sperm analysis.

The widely used CASA system consists of an optical microscope (typically using a ×10 –×20 objective lens) with a digital camera attachment that records the microscopic images of the sperm samples[10], which are further processed with custom sperm analysis software (Figure 1a). The sperm cells are typically placed in a chamber that is 20 μm deep]11], confining their motion vertically, given that human sperm head is ∼4–5 μm wide and the flagellum is ∼40–50 μm long [12]. A stack of digital images are recorded, with the camera functioning at a frame rate that suffices to capture the locomotion of the sperm cells without temporal undersampling, e. g. > 60 fps for tracking human spermatozoa [13].

Sophisticated computational tools have been made available to process a stack of microscopic images to identify the sperm cells and connect their trajectories. Thresholding and image segmentation techniques [14] are widely used to find the sperm heads in digital frames, while advanced multi-object particle or cell tracking algorithms [15; 16] help register the positions of these sperm cells across the frame stack, building fully connected spatio-temporal trajectories of the spermatozoa (Figure 1b). Once these trajectories are determined, additional processing is applied to calculate several parameters used to assess sperm health and motility (Figure 1c), which include e. g. curvilinear velocity (VCL), average path velocity (VAP), the straightline velocity (VSL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF) [13], and the recently included fractal dimension

(D), among others [17]. These motility parameters form a much a better indicator of sperm functionality compared to conventional semen profile parameters, which only include the number of spermatozoa in a semen sample and the percentages of progressively motile and morphologically normal sperm.

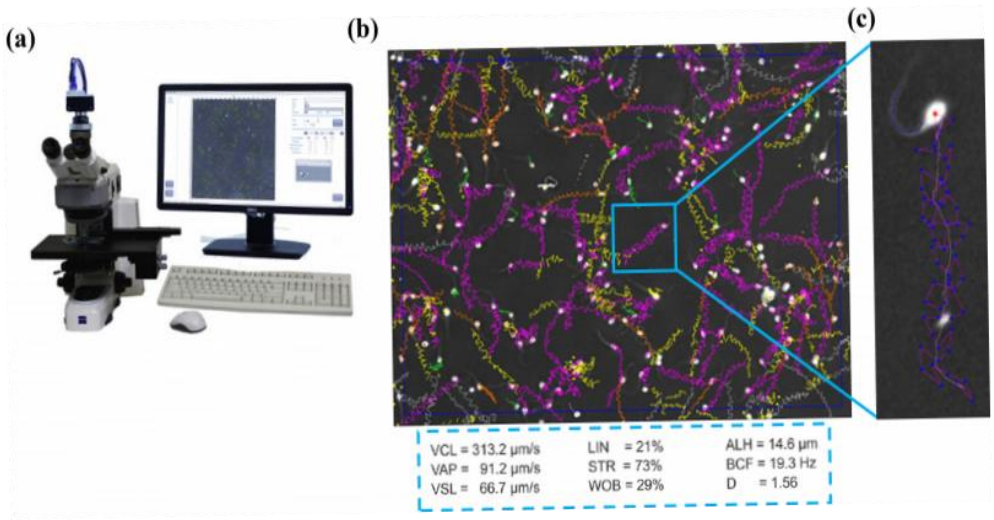

Fig. 1. (a) A standard CASA system [18]; (b) Reconstructed sperm trajectories over ~1 s, sampled at 60Hz. The purple tracks are identified to be hyperactive [17]; (c) A zoomed-in trajectory of a sperm with the corresponding motility parameters also listed [17]

The sperm cell has two main parts, the head and the flagellum. The flagellum is composed of midpiece, tail (also called principal piece), and end piece. The flagellum moves in a wave-like motion to propel the cell as shown in Figure 2.

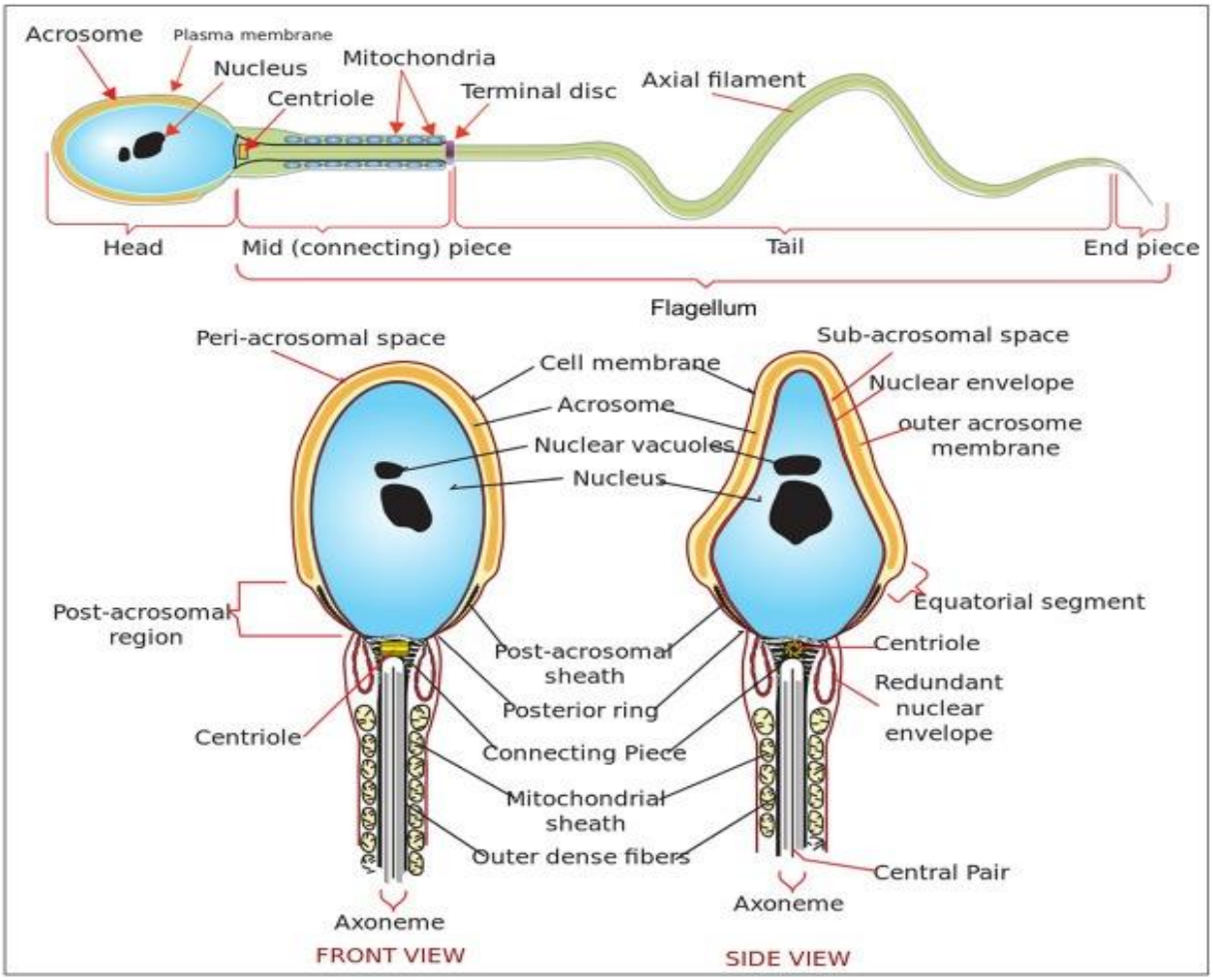

Fig. 2. Diagram of a human sperm cell36. (Top) Main components of a sperm cell. (Bottom) Front and side view of sperm head and midpiece

Limitations of CASA Systems

1. A major challenge in developing and validating CASA systems is the accurate assessment and comparison of their semen analysis methods to the ground truth. In order to validate a CASA system, this process needs to take place across a representative sample of the expected semen images. For real-life samples, the ground truth is often unknown, *motivating* the use of high-quality image simulations with modifiable parameters for validation of CASA systems and algorithms.

2. One of the problems that current CASA systems face is when two or more spermatozoa collide spatially (or come very close to each other) during the imaging process [18; 19; 56; 57], confusing the trajectory calculation.

It is widely recognized that the image processing and sperm tracking capabilities of today's CASA instruments can be enhanced [20; 1]. In particular, most CASA instruments cannot reconstruct reliably the paths of two or more sperm swimming in close proximity or whose paths intersect. This shortcoming requires substantial user invention and tuning. Often, the trajectories of a significant number of sperm involved in cell-to-cell collisions and near-misses are excluded from analysis by CASA algorithms. Since higher velocity sperm are more likely to be involved in apparent collisions, their exclusion tends to bias motility measurements toward slower sperm. To reduce the probability of cell collisions, samples are often diluted (typically to less than 20×106 sperm/mL) or analysis is limited to short video clips (typically less than 1–2 sec).

Finally, CASA motility analysis can vary significantly when different system settings are used [21; 11], making it difficult to compare results obtained with the same instrument in different studies. These difficulties, combined with the prohibitively high cost of acquiring and maintaining a CASA instrument, have often outweighed the benefits of automation.

3 – There are other limitations of conventional CASA systems that are directly related to optical imaging hardware. A conventional optical microscope with objective lenses is used to image the sperm cells in CASA systems, where there is an inherent trade-off between spatial resolution and field of view (FOV or sample volume). Furthermore, such microscopes are relatively bulky and expensive, limiting the CASA setups to laboratory settings.

3. Discussion

In this section we will introduce interventions to help mitigate the above-mentioned limitation of CASA systems.

3.1. Sperm Collisions

A technology from air traffic control systems, i. e. the joint probabilistic data association filter, has been recently employed [22] to better handle such cell collisions.

Probabilistic models have also been used to trace the low-visibility sperm flagellum effectively [23] along with maximum intensity region and optical flow algorithms [24].

We have developed a fully automated, robust, multi-sperm tracking algorithm. It has the demonstrated capability to detect and track simultaneously hundreds of sperm cells in recorded videos while accurately measuring motility parameters over time and with minimal operator intervention. Sperm measurement-to-track association conflicts occurring during real and apparent cell-to-cell collisions were reconciled by adapting and applying the joint probabilistic data association filter (JPDAF) [25; 15], representing a mature technology employed in air traffic control systems. This approach uses independent Kalman filters to estimate the position and velocity of each sperm tracked, and enables accurate tracking of sperm in undiluted specimens over periods significantly longer than 1 sec (typically 15–45 sec at 200× magnification).

Unlike most CASA instruments which measure motility parameters only once per sperm, our algorithm continually measured all parameters along the entirety of each reconstructed sperm trajectory, revealing interesting temporal changes in individual sperm motility not evident in population statistics.

A block diagram of the algorithm is shown in Figure 1(a) (the implementation currently uses MATLAB). The input to the algorithm is a sequence of time-lapse images currently encoded either as an MP4 or AVI video file. The output of the algorithm is a database file containing the set of reconstructed swimming paths for each sperm tracked and corresponding values for a host of parameters of interest. These data are visualized as parameter scatter plots, trajectory path histories, and annotated movies.

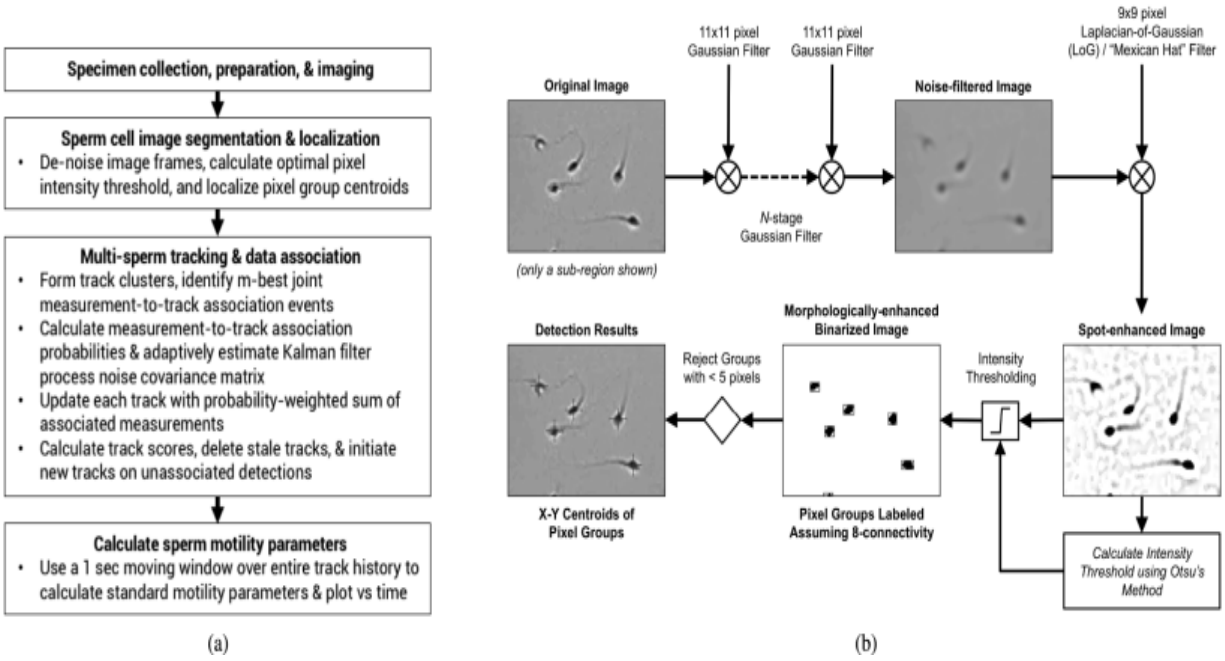

Fig. 3. Sperm tracking algorithm

3.2. Simulation

Simulations have the potential to help in developing automated semen analysis systems, and in comparing different candidate algorithms to each other.

To generate a simulation of semen sample video for assessment and validation, it is important to have following: (1) a model for the image of a sperm cell (to generate sperm cells in the simulated semen image) and (2) a model for the sperm cell movement that defines how the semen image changes over time.

(Ji won Chi, et al.) provided models for the image of a sperm cell and for four (4) different swimming modes of sperm cells (circular, linear mean, hyperactive, and im-motile). And generated images in a format similar to the ones used by CASA systems as shown in Figure 4.

Sperm image Model

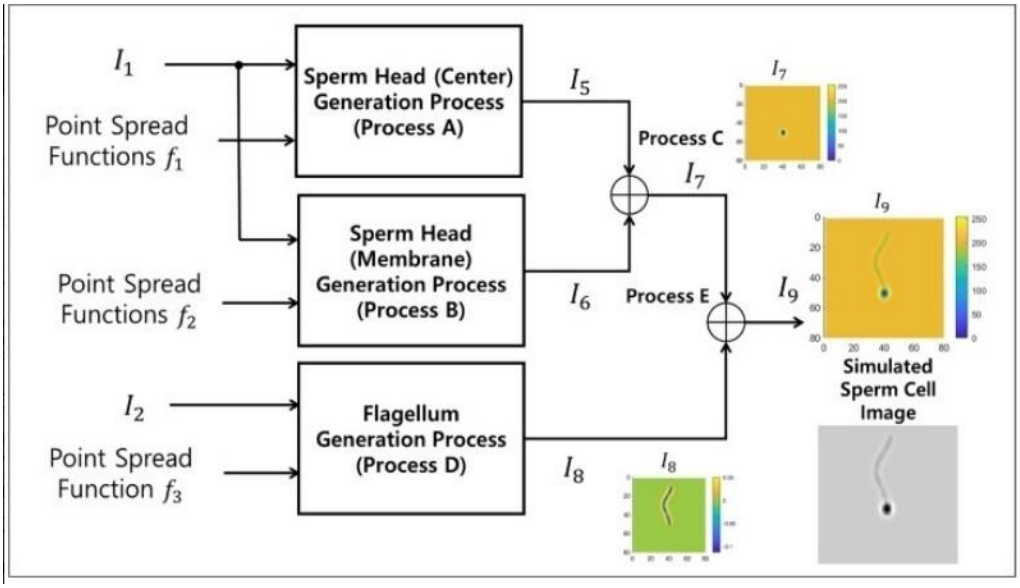

Fig. 4. Flowchart of sperm image generation process

(Ci et Al.) presented a model of 2-D (top-down) view of a sperm cell and of four (4) different swimming modes generated by observing the swimming paths of real human sperm cells. Shown in Figure 5 and 6.

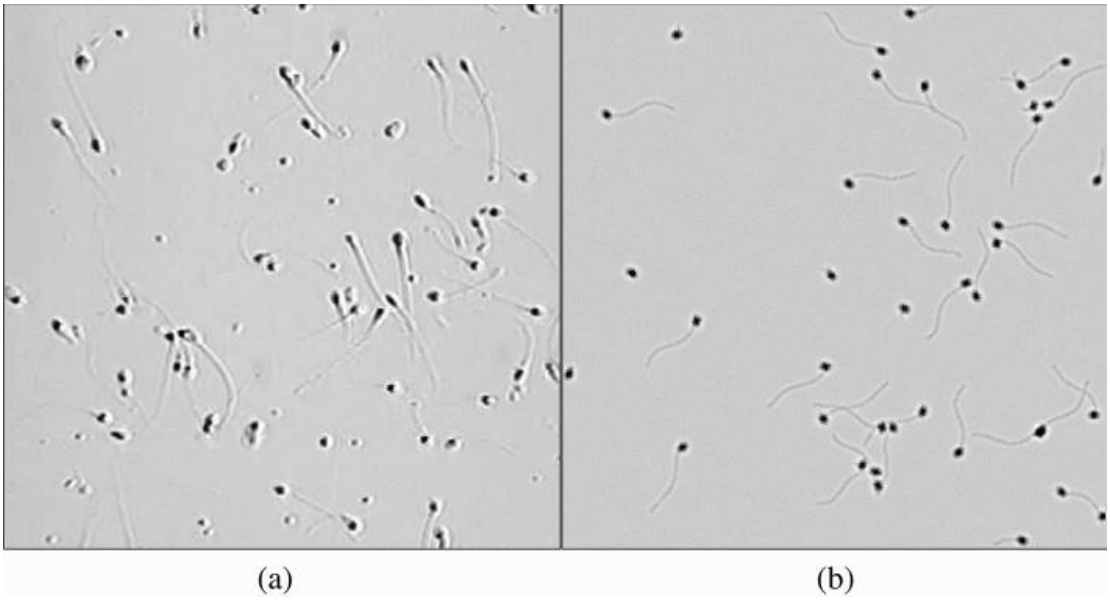

Fig. 5. (a) Image of real human semen sample. (b) Image of simulated semen sample

Swimming modes

The swimming models resented in describe how the position of the head and the flagellum change over time and they categorize the sperm movement into the following four swimming modes:

1) circular swim;

2) linear mean swim;

3) hyperactivated;

4) immotile, or dead.

Figure 5 shows simulated tracks for the four swimming modes (4 seconds long). The tracks indicate the location of the head of a cell and the arrow indicates the direction of movement.

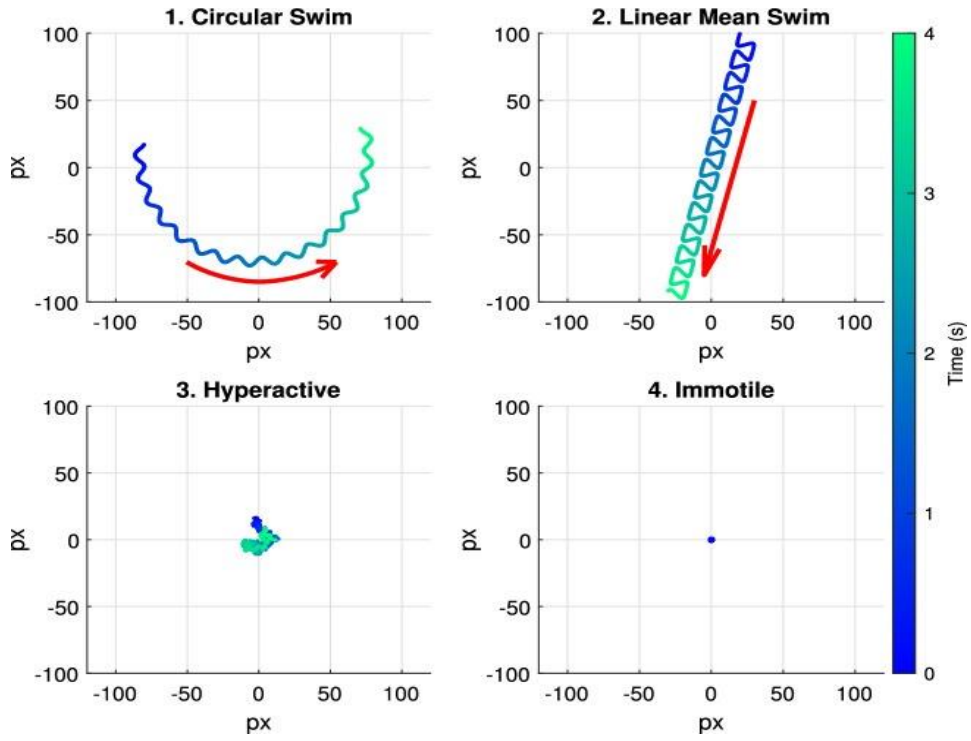

Fig. 6. Simulated swimming path of the four swimming modes: circular swim, linear mean swim, hyperactive, and immotile. The color bar indicates the color of a track with respect to time. The duration of movement is 4 s

3.3. Holographic imaging of sperm locomotion

As a cost-effective and portable alternative to lens-based optical microscopy tools, computational on-chip holography [26; 60] offers a unique opportunity to image and

track sperm cells without using any lenses or other bulky optical components [27]. This alternative computational imaging platform only consists of an inexpensive complementary metal-oxide-semiconductor (CMOS) imaging sensor, which is widely used in consumer electronics products including mobile phones, and a simple partially coherent light source such as a light-emitting diode (LED) (Figure 7a). The semen sample is placed very close to the CMOS image sensor (<1 mm vertical distance between the sample and the sensor plane) and the light source is placed further above (e. g. ~4–5 cm) with the use of a large diameter (100 μm) aperture/pinhole (Figure 7b) that improves the spatial coherence of the light illuminating the sample [27].

The scattered light from each sperm cell interferes with the background light, creating holographic interference patterns (Figure 7c) that are captured by the CMOS image sensor. These interference patterns (or holograms) encode both the amplitude (Figure 7e) and the phase information (Figure 7f) of the object (Figure 7d), which could be digitally reconstructed through various back-propagation techniques [26; 27] effectively replacing the objective lens in a conventional optical microscope with rapid computation.

Such a simple imaging platform not only offers a very robust, field-portable, and cost-effective alternative to CASA platforms, but it also allows highthroughput imaging and tracking of spermatozoa over a large FOV (e. g. ~24–30 mm2) (Figure 2b). Unlike its lens-based counterparts, for a lensfree on-chip microscope the imaging FOV is decoupled from spatial resolution, and is only limited by the active area of the image sensor chip, which in our mobile phone cameras typically range between 20 and 30 mm 2, more than 20-fold larger than the FOV of a typical ×10-×20 objective lens.

Using this on-chip holographic imaging framework, an automated semen analysis platform that only weighs 46 g (Figure 7a) was created to measure the concentrations of both motile and immotile human sperm cells, along with the VSL distributions of the cells and the flagellum morphology of the immotile cells [27]. With a USB-based interface used to connect to a laptop computer, this portable and cost-effective computational microscope is a powerful alternative to CASA systems in resource-limited or

field settings, relevant for animal husbandry applications, where it would be quite challenging to operate a conventional optical microscope.

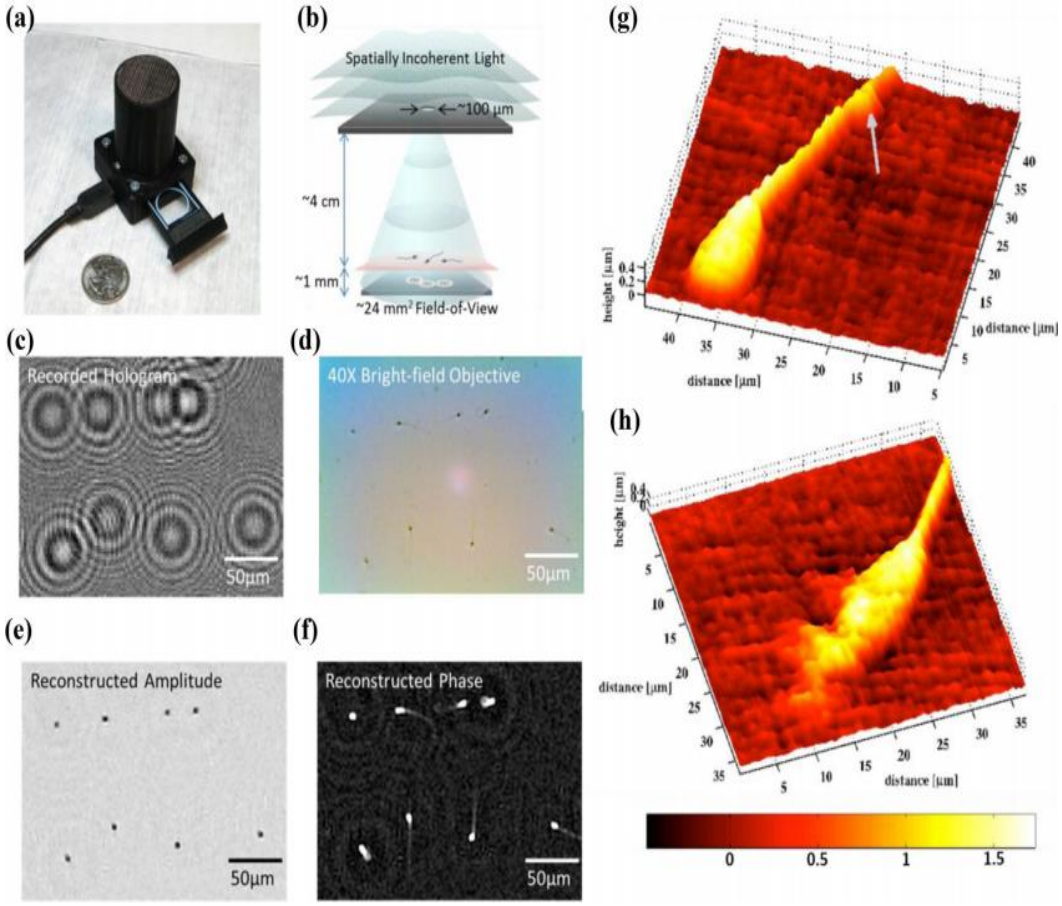

Fig. 7. Lightweight holographic imaging platform

3.4. Our Algorthm

For the purpose of studies effect of acoustic and mechanical vibration on motility of sperm cells we developed this fit – to – purpose algorithm and coded it JavaScript.to guage sperm linear velocity before and past exposure to mechanical stimulation.

Here are the outlines.

The experiment

First of all, by the properties of the lenses and video we need to specify the dimensions of the frame in metric system. For this example, let’s assume that the frame of the video shows you rectangle with 800 micrometers (horizontal) and 600 micrometers (vertical) of examining surface.

Knowing this, capture a video of the movement of a single spermatozoid (Let’s name Y).

Calculations.

Now we have a video of sperm Y f let's assume that it moved four different steps different direction three seconds and then stops.as shown in Figure 8 below.

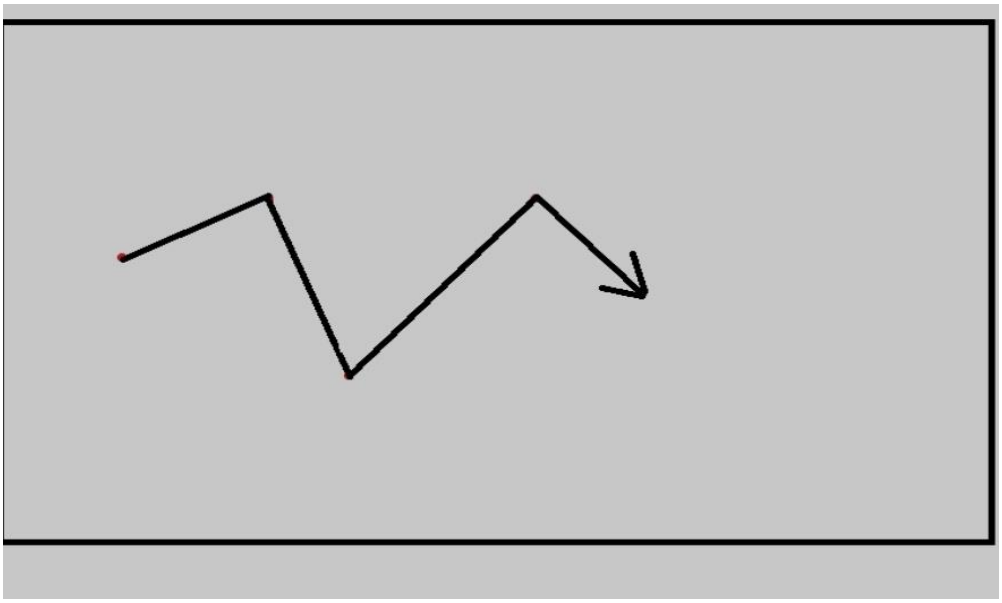

Fig. 8. Sperm Y moving 4 steps

As assumed above the video shows a rectangle of 800 Mm X 600 Mm. Now we can draw a mesh over it with 800 squares in horizontal and 600 squares in vertical. Which means that each square in the mesh will represent 1 square micrometer of the surface. And that, my friend, is a coordinate axis now.

Having that done, we need to find the spots where the spermatozoid starts movement, changes directions and stops. Figure (9).

Let’s say those spots are.

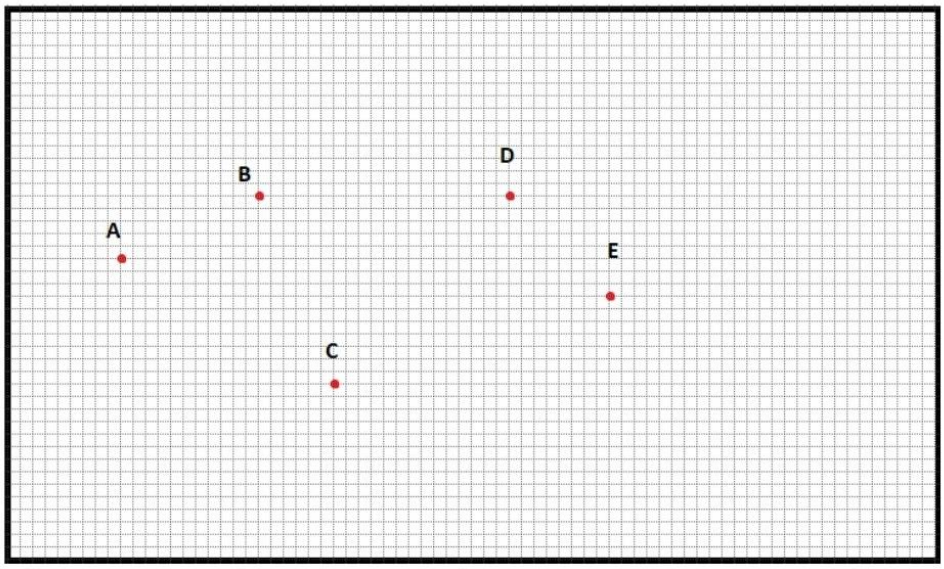

Fig. 9. Tracking Sperm Movement

From here you can calculate the coordinates of each spot. For this example, I will use fake numbers.

A(10, 30)

B(20, 35)

C(30,15)

D(45,35)

E(55,20)

Now we need to calculate the distance between all spots *AB*, *BC*, *CD* and *DE*. using this formula:

$$AB = \sqrt{(x_b - x_a)^2 + (y_b - y_a)^2}$$

Now we have values:

AB = 11.18 Mm;

BC = 22.36 Mm;

CD = 15 Mm;

DE = 18.02 Mm.

Those are the distances that sperm Y passed.

Speed Calculation

We will need to find out the framerate of the video. Usually, it's 24 frames per second. But we need to check it out from your microscope video capturing properties.

We also need some video editing application that can show video frame by frame.

We put this video and the mesh into an application and count how many frames it took for each distance to be passed by Sperm Y.

Tactics on AB distance.

Let's assume that it took *5* frames for Sperm Y to pass *AB* distance (11.18 Mm). By dividing distance by number of frames it took we will get a distance that Sperm Y had covered in a single frame.

11.18 / 5 = 2.24.

That means it covered 2.24 Micrometers in every single frame.

Knowing that this video has a 24fps framerate, we multiply distance of a single frame by count of frames in a second 2.24 x 24 = 53.76.

And there we have it. Sperm Y speed on an *AB* distance was *53.76 Mm/s*.

Now we need to do the same trick in all other distances *BC, CD, DE*.

Roughly speaking Let's assume they are:

AB = 53.76 Mm/s;

BC = 52.30 Mm/s;

CD = 54.24 Mm/s;

DE = 50.11 Mm/s.

By adding all speeds together and dividing result by count of distances you we will get the average speed.

(53.76+52.30+54.24+50.11)/4 = 52.6.

SO! 52.6Mm/s (micrometers per second) is an average speed of spermatozoid Y movement.

4. Conclusion

With all these efforts in further improving the computational tools for CASA, much more robust and versatile systems should be available in the near future.

1. The simulation models and the software presented in (Chi et al.) work serve as a powerful new tool for developing and enhancing CASA systems and algorithms.

Using this new tool, stronger and more robust CASA systems can be developed. The use of such systems is an attractive alternative to manual semen collection and assessment. Specifically, clinicians can generate and demonstrate to students and technicians a variety of images that represent different types of observed semen samples; this task can be accomplished without having to collect, process, and record human semen images. The simulated images can also be used to train technicians, by comparing the results of their analyses of the images to the simulations ground truth.

2. Using JPDAFthis algorithm, hundreds of human sperm can automatically be tracked and we can measure their dynamic swimming parameters over time, with minimal user intervention, and without sample dilution. Unlike existing computer assisted semen analysis (CASA) instruments, the algorithm has the capability to track sperm swimming in close proximity and during apparent cell-to-cell collisions.

Appendix A

Java Code

Source code of the project contains several Java classes. The whole algorithm is described in a logic below:

Seed: Describes each individual sperm with it’s own positions on the screen and contains the calculated speeds of it on each distance.

```
public class Seed
{
List<SeedPosition> seedPositions = new ArrayList<>();
String id;
List<SeedPositionsSpeed> positionsSpeeds;
public Seed()
{
this.id = UUID.randomUUID().toString();
}
public List<SeedPosition> getSeedPositions()
{
return seedPositions;
```

```
}
public void setSeedPositions(List<SeedPosition> seedPositions)
{
this.seedPositions = seedPositions;
}
public List<SeedPositionsSpeed> getPositionsSpeeds()
{
return positionsSpeeds;
}
public void setPositionsSpeeds(List<SeedPositionsSpeed> positionsSpeeds)
{ this.positionsSpeeds = positionsSpeeds;
}
}
```

SeedPosition: Contains the position of each sperm at certain timestamp:

```
public class SeedPosition
{
long timeMills;
double x;
double y;
public SeedPosition(long timeMills, double x, double y)
{
this.timeMills = timeMills;
this.x = x;
this.y = y;
}
public long getTimeMills()
{
return timeMills;
}
public void setTimeMills(long timeMills)
```

```
{
this.timeMills = timeMills;
}
public double getX()
{
return x;
}
public void setX(double x)
{
this.x = x;
}
public double getY()
{
return y;
}
public void setY(double y)
{
this.y = y;
}
}
```

SeedPositionSpeed: Contains distance and time for each movement of each sperm.

```
public class SeedPositionsSpeed
{
double distance;
Long time;
public SeedPositionsSpeed(double distance, Long time)
{
this.distance = distance;
this.time = time;
```

```
}
public double getDistance()
{
return distance;
}
public void setDistance(Long distance)
{
this.distance = distance;
}
public Long getTime()
{
return time;
}
public void setTime(Long time)
{
this.time = time;
}
}
```

SpeedCalculator: Utility class that helps to calculate speed of a certain sperm from all of it’s positions and times

```
public class SpeedCalculator
{
public static void calculate(Seed seed)
{
List<SeedPositionsSpeed> speeds = new ArrayList<>();
for (int i = 0; i < seed.getSeedPositions().size(); i++)
{
if (i!= seed.getSeedPositions().size() – 1)
{
SeedPosition firstPosition = seed.getSeedPositions().get(i);
```

```
SeedPosition secondPosition = seed.getSeedPositions().get(i + 1);
double distance = calculateDistance(firstPosition.x, secondPosition.x, firstPosition.y,
secondPosition.y);
Long time = calculateTime(firstPosition.timeMills, secondPosition.timeMills);
SeedPositionsSpeed speed = new SeedPositionsSpeed(distance, time);
speeds.add(speed);
}
}
seed.setPositionsSpeeds(speeds);
}
public static double calculateDistance(double x1, double x2, double y1, double y2)
{
double powOfX = Math.pow(x2 – x1, 2);
double powOfY = Math.pow(y2 – y1,2);
double distance = Math.sqrt(powOfY + powOfX);
return distance;
}
public static long calculateTime(long time1, long time2)
{
return time2 – time1;
}
}
```

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7. (Otsu) Binarization of image using Otsu's thresholding followed by morphological enhancements (closing, dilation, and erosion) 48.

8. (Adaptive) Binarization of image using the adaptive thresholding method of Bradley49 with the sensitivity defined to be 0.8, followed by morphological enhancements (closing, dilation, and erosion).

9. (Spot-enhancement) Binarization of spot-enhanced image using Otsu's thresholding, followed by morphological enhancements (closing, dilation, and erosion) (method proposed in Urbano et al. 3).

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