Grant Application to the Zimin Institute for Engineering Solutions
Advancing Better Lives

Title:

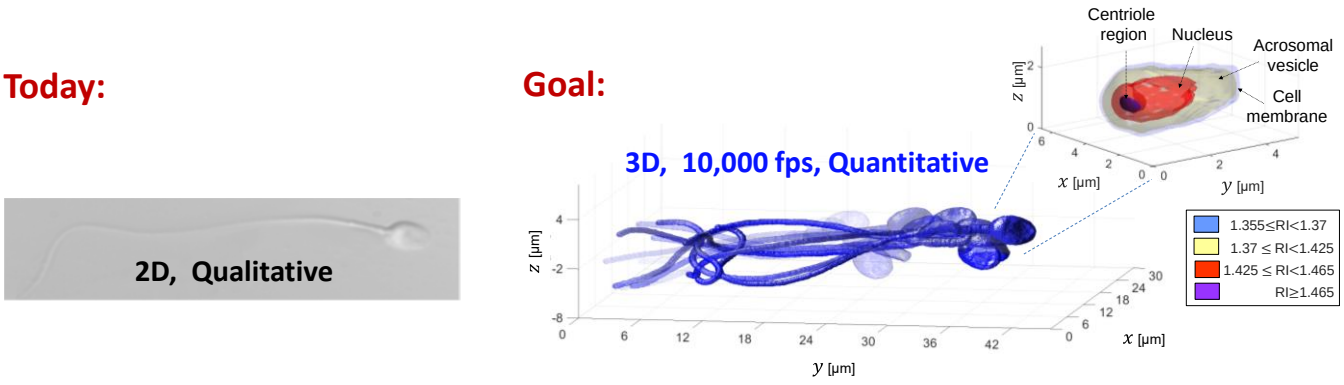
Human-sperm-cell full refractive-index mapping via ultra-rapid cell 3D tomography

PI:

Natan T. Shaked, *Department of Biomedical Engineering, Tel Aviv University*

Abstract:

Human sperm quality constantly declines, resulting in a worldwide fertility crisis. We will develop a new multidisciplinary microscopy approach for ultra-rapid 3D label-free fine-detailed imaging of live sperm cells during free swim without staining, with pioneering technological capabilities and revolutionary clinical implications. Our approach is based on optical interferometric tomography for ultra-rapid 3D refractive-index mapping with correlation with fluorescence nanoscopy. Success of this project is expected to end in a new understanding of how to choose the best sperm cells for in vitro fertilization (IVF) without the need for cell staining, as well as optimized ways to characterize male infertility problems and adapt personalized medicine treatments. There is no method today to image sperm cells in 3D during free swim in high resolution. The sperm cells not only move very fast, they are also mostly transparent under regular 2D light microscopy, and cell labeling is prohibited in human IVF. In the common procedure, the clinician chooses sperm cells without being able to characterize them well, while bypassing the mechanisms of natural selection of sperm cells in the woman's body, which results in low success rates. Our approach will enable dynamic-sperm 3D virtual staining, centriole imaging and DNA fragmentation analysis in live individual sperm cells without staining. This is expected to unmask the interplay between live sperm 3D dynamics and fine-detailed morphology and contents, potentially revealing new critical mechanisms, dramatically improving success rates in IVF.



Research Project Description

1. Introduction

Almost one in six couples faces infertility, defined as being unsuccessful in efforts to conceive over the course of one full year. Male infertility factors contribute to approximately 50% of all cases [1]. In vitro fertilization (IVF) allows fertilization of a female egg by a male sperm outside the female body. The selection of sperm cells possessing normal morphology and motility is crucial especially for intracytoplasmic sperm injection (ICSI), a very common type of IVF and the leading one in developed countries, in which the clinician chooses a single sperm cell using a micropipette and injects it into the female egg in a dish. The physiological mechanisms of the woman's body for natural selection of sperm cells are bypassed in ICSI, and it is impossible to predict which individual sperm cell is most likely to fertilize the egg naturally and result in a healthy child. The risk of fertilization by a defected sperm is even higher in IVF and ICSI than in natural fertilization, since the incidence of sperm abnormalities, including DNA fragmentation, is typically higher in men who require these procedures [2]. The fertilized egg is then incubated for up to six days, during which it can be inspected, before returning it to the woman's womb. The problem with this current practice is that about 10 eggs are obtained in each IVF cycle in a surgery, after the woman has been treated with hormones, and it is not uncommon that all the fertilized eggs do not develop into good quality embryos, or, even if the embryos are thought to have developed properly in the dish, the pregnancy spontaneously aborts. Unfortunately, much less effort is invested in analyzing sperm cells than eggs. The World Health Organization (WHO) provides criteria for classification of sperm cells after staining [3]. However, since **stains cannot be used in human IVF**, as these may damage sperm viability, clinicians examine and select sperm cells using imaging methods that only provide gross 2D morphology and possess distinctive imaging aberrations that occlude important morphological details. Therefore, sperm mobility and progressivity are largely used to select the most potent sperm cells in ICSI, but due to the 2D imaging methods used, one cannot well characterize the 3D movement of the sperm. All of this leads to a lack of consistency in sperm selections performed by different clinicians, as well as a **large margin of human error**. Sperm cells with **fragmented DNA** [2] or with **non-functional centrioles** [4,5] may fertilize the egg, which will not result in normal development. **Fluorescence nanoscopy** can detect the centrioles, and other important structures inside the sperm, as well as indicate DNA fragmentation. However, it is not fast enough to be performed on live sperm cells during 3D movement, and it is not permitted in human IVF due to using staining. **The biological mechanism that connects sperm movement, morphology and contents to fertilization potential and normal pregnancy is not completely understood** [4-7]. Recent models analyzing sperm spatial

movement use rough assumptions taken based on 2D dynamic optical imaging [8], or on cryo-electron microscopy of the sperm obtained for fixed cells [9]. **There is no method today that can provide high-resolution 3D imaging of individual sperm cells during free swim, even when using cell labeling, preventing full quantification of sperm biological functions and its evaluation.**

State of the art in my lab: My group's recent discoveries show that clinic-ready interferometric imaging systems [10-16] can image sperm cells with excellent contrast without staining by reconstructing the cell 2D topographic phase map [17-22], possess potential for detecting DNA fragmentation in sperm cells without staining [21,22], as well as can virtually stain them in 2D [23] (see Fig. 1, published recently in *PNAS*). We have shown that these techniques enable automatic classification of sperm cells [19,20,22], and provide various new quantitative parameters of sperm cells, such as their individual organelle dry masses and volumes, which have not been previously available to clinicians [17,18].

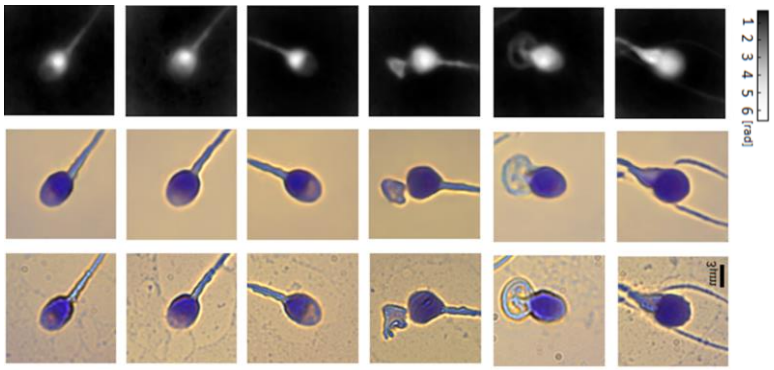


Figure 1. Preliminary results [23]: 2D virtual staining (without chemical labeling) of individual sperm cells using single-projection interferometry. We used the label-free quantitative phase profiles of the cells as inputs to a neural network that performed the mapping, after being trained with the coinciding chemical staining images. The first row shows the quantitative phase images extracted from the single holograms. The second row shows the coinciding virtual stained images, generated by the network. The third row shows the coinciding bright-field chemically stained images of the same sperm cells that the network did not see; yet it could present the cells as if they were stained.

However, interferometric imaging provides just a 2D topographic map. It does not have **intracellular sectioning capability** and it cannot provide the *x–y–z* 3D image. To be able to obtain the full 3D image, **interferometric tomography** is used, where many angular interferometric projections are collected and processed to generate the 3D RI cellular map. There are two approaches for obtaining these projections: rotating the entire sample or scanning the illumination. Nevertheless, none of these previous interferometric tomographic methods can cope with the problem of obtaining high-resolution 3D label-free imaging of ultra-rapid dynamic cells, like sperm cells swimming freely.

Recent advances in my lab: In Ref. [24], which was recently published in *Science Advances*, we proposed a novel preliminary concept for **ultra-rapid dynamic acquisition of live and unstained sperm cells in 3D**. This is the first time that live sperm head internal structure and flagellum 3D fine-detailed dynamics are imaged simultaneously during sperm free swim. This is a very challenging task due to the

fine-structures / fast-dynamics duality characterizing individual sperm cells. Even when using confocal fluorescence microscopy, which is not allowed in human IVF, nobody, to the best of my knowledge, could provide such fine 3D details when imaging live sperm cells during free swim, due to the need to scan and collect the 3D image, as the scanning is much slower than the sperm dynamics. Our new technique is based on optical computed tomography with ultra-rapid interferometric imaging of sperm cells, on the natural head rotation of the sperm cell during free swim, and a novel set of reconstruction algorithms. This technique is able to reconstruct sperm cell 3D movement and dynamics, including the full fine-detailed 3D rotation of the sperm tail and head. The method utilizes an internal contrast mechanism of live cells in watery medium, the refractive index (RI) of the cell, which varies for the different cellular organelles, providing the ability to image the 3D high-resolution RI profile of individual sperm cells during free swim (see Fig. 2). Surprisingly, we detected **very high RI values in the centriole area**, **an organelle with great clinical value,** which can only be detected with fluorescence nanoscopy. We have not verified yet that this is indeed the centriole.

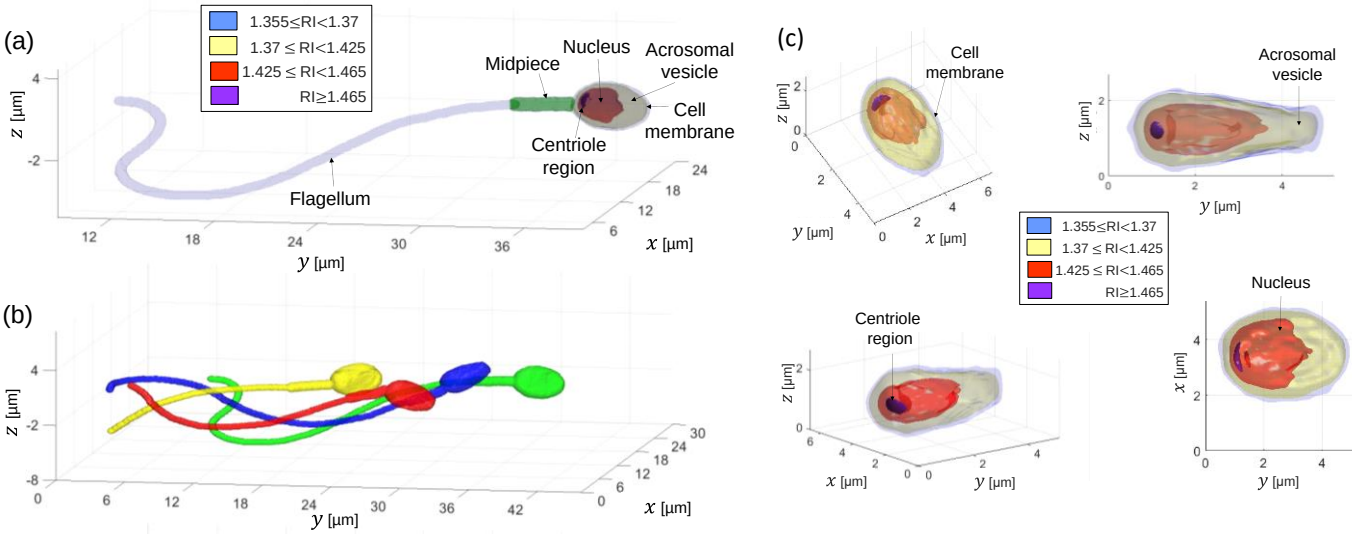


Figure 2. High-resolution label-free dynamic 3D imaging of a sperm cell swimming freely. (a) A single frame from the 3D motion in Movie S3 in Ref. [24], revealing the internal structure of the sperm cell. Light purple indicates the cell membrane (1.355≤RI<1.37), green indicates the midpiece (RI = 1.383), yellow indicates the acrosomal vesicle (1.37≤RI<1.425), red indicated the nucleus (1.425≤RI<1.465), and dark purple indicates RI≥1.465 (centriole region). (b) Overlay of four frames from the 3D motion in Movie S3 in Ref. [24]. (c) The sperm cell head 3D RI reconstruction from various perspectives. RI, Refractive index.

2. Project goal

Despite our recent results providing preliminary indications of detecting high-impact components in sperm cells with distinctive RI values, the current system lacks 3D fluorescence and thus we **cannot perform the required mapping of the resulting 3D RI profile** in order to verify our high-impact

findings. To solve this problem, the main objective of the proposed project is to perform 3D RI mapping of human sperm cells. This will be done by **correlating the 3D profiles of human sperm cells resulted from our dynamic interferometric approach with fluorescence nanoscopy, in the first time for human sperm cells**. The next section elaborates the different stages in reaching the project goal.

3. Methodology

System development – high-resolution interferometric tomography with a cell micromanipulator: A partially coherent light beam will illuminate the sample in an inverted microscope. The magnified sample beam at the exit of the microscope will enter an external interferometric module, producing off-axis wide-field interference, allowing sensing the complex wavefront of the sample. All perspective complex-wavefront projections will be used to reconstruct the 3D RI distribution through the optical diffraction tomography theory [24]. For the first time, the system will be integrated with a single-cell micro-manipulator, allowing catch single cells and transfer them to another imaging system.

Interferometric tomography of rapidly swimming sperm cells: Human sperm samples will be obtained from 50 human donors. I have the university ethical approvals for bringing sperm donors to my lab and performing the proposed imaging experiments. Sperm cells provide access to their perspective projection during their natural swimming. Supported by preliminary results [24], by modeling the sperm cell head as an ellipsoid, we will accurately retrieve all three projection angles from each frame (pitch, roll and yaw), based on the minor and major radii of the cell head. All off-axis interferometric projections, experimentally acquired during the free swim, will then be processed into the 3D RI cell distribution. In parallel, the sperm tail will be reconstructed from each complex wavefront by digital refocusing.

3D super-resolved imaging for 3D RI mapping: Following interferometric tomography, 3D fluorescence microscopy of sperm cells will be performed inside and outside the interferometric imaging system, and the 3D sperm images from multiple modalities will be co-registered for 3D RI super-resolved mapping with organelles specificity. We will label the plasma membrane, the mitochondria, the nucleus, and the microtubulin [25]. The integrated confocal spinning-disk fluorescence imaging system, providing ~250 nm *x-y* resolution, will enable imaging with molecular specificity of the thin structures in immobilized or heavily slowed down stained sperm cells. In this case, both stain-free interferometric tomography and 3D super-resolved fluorescent imaging will be done on the same system, allowing measuring a large number of cells. Next, we will use the cell micromanipulator to catch sperm cells and image them using an external fluorescence STED-based nanoscopy system (~40 nm resolution) located in the Imaging Center of the School of Medicine. This validation, performed following our live-cell dynamic imaging, will **reveal small details below the optical diffraction limit**, for **quantifying our dynamic-**

sperm label-free RI imaging capabilities. We will then design a **correlative software platform** that can **co-register 3D sperm images** from multiple modalities by 3D correlations [26]. This will also allow us performing label-based **DNA fragmentation** assays, for evaluating our label-free imaging capabilities.

4. Significance and suitability for the call for proposals

Infertility is a very common condition; one in six couples faces it. Some populations, including Japan and Spain, keep disappearing. Many people are seeking medical interventions in order to fulfil their natural, organic urge to have children. Sperm quality constantly declines [27]. Sperm cells under conventional light microscopy are mostly transparent, and cell staining is not allowed. This project suggests developing a new dynamic 3D label-free fine-detailed imaging of live sperm cells during free swim without staining. Success of this research is expected to end in a **new understanding of how to choose the best sperm cells for ICSI without the need for cell staining**, as well as **optimized ways to characterize male infertility problems** and adapt personalized medicine treatments. **There is no full biological understanding of how sperm 3D movement, normal morphology and contents are all connected and correlated with successful fertilization**. ICSI is an expensive procedure and only 20-25% of the ICSI treatments end in live births [6]. By **mapping the cell RI in 3D, in the first time for human sperm cells**, my project is expected to provide new abilities to **analyze the nanoscopic centrioles and DNA fragmentation** in highly dynamic sperm cells without cell staining. Without centrioles originating from the sperm cell, the fertilized egg will stop its development after several days. Centrioles can be measured by electron microscopy [28] and by optical fluorescence nanoscopy [25], but these techniques are inaccessible in most clinics, and cannot be performed for live cells during IVF. Even in normal semen samples, approximately 20% of the sperm cells have fragmented DNA [29,30]. Sperm DNA fragmentation has been associated with reduced fertilization rates, reduced embryo quality, reduced pregnancy rates and increased miscarriage rates [7]. Thus, sperm cells with fragmented DNA should not be selected for IVF. Detecting DNA fragmentation requires molecular staining, which cannot be carried out during human IVF. This research will also affect various **other research fields, beyond the scope of the current proposal**. For example, our ability to image individual sperm cells dynamically in 3D can be used for **building improved analytic models for the sperm tail and head mechanics** [31,32] bringing to efficient **biomimetic micro-robotic swimmers** and **drug carrier devices** in vivo.

This is **interdisciplinary bio-convergence project** involving engineering, physics, life science and medicine. I have well-equipped optical and biological labs with the state-of-the-art optical microscopy equipment. In additions to engineers, my group contains experienced PhD biologists, Dr. Itay Barnea and Dr. Mattan Levi, who is also a clinical embryologist, as well as a senior urologist (Dr. Miki Haifler, MD).

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Requested Budget
Scholarships/salaries

Senior postdoc (PhD Biology) – 40%, salary	$14,300
PhD student – 50%, scholarship	$10,900
MSc student 1 – 100%, scholarship	$14,500
MSc student 2 – 100%, scholarship	$14,500

The senior postdoc (PhD Biology) will process the sperm samples. The optical system will be adjusted by the PhD student and MSc student 1. The data will be acquired by MSc student 1. The 3D processing will be implemented by MSc student 2.

Equipment, software, and disposables

Cell micro-manipulator	$12,800
Optical imaging system (optics and mechanics)	$17,100
Computers and software	$9,100
Biological samples and dishes	$2,000
Internal services (STED Imaging)	$2800
Publication costs	$2,000

We will need to purchase a single-cell micro-manipulator to enable transferring sperm cells after label-free interferometric tomography to the fluorescence STED imaging system located in the Medicine Faculty's imaging facility. Additional small optical components (lenses, mirrors, micro-mechanics, etc.) will be needed for adjusting an existing optical system for the proposed experiments. Biological samples, dishes, reagents, and microscopy disposables will be routinely required. Internal services for STED imaging on hourly basis and publication costs for 1-2 journal papers are also requested.

Total: 100,000 $

25% matching will be given in kind via providing 1/2 of the student and postdoc salaries.

Curriculum Vitae

- **PERSONAL INFORMATION**

Shaked, Natan Tzvi
Department of Biomedical Engineering, Tel Aviv University Ramat Aviv, Israel
Date of Birth: 10/10/1976. Status: Married +3
www.eng.tau.ac.il/~omni

- **EDUCATION**

2004 – 2008	**PhD, Electrical and Computer Engineering** Ben Gurion University of the Negev, Beer Sheva, Israel
2002 – 2004	**MSc (Summa Cum Laude), Electrical and Computer Engineering** Ben Gurion University of the Negev, Beer Sheva, Israel
1998 – 2002	**BSc, Electrical and Computer Engineering** Ben Gurion University of the Negev, Beer Sheva, Israel

- **POSITIONS**

July 2019 – Present	**Professor** Department of Biomedical Engineering Tel Aviv University, Tel Aviv, Israel
Apr. 2011–July 2015	**Senior Lecturer (2011-2015), Associate Professor (2015-2019)** Department of Biomedical Engineering Tel Aviv University, Tel Aviv, Israel
Oct. 2010 – Apr. 2011	**Visiting Assistant Professor** Department of Biomedical Engineering Duke University, Durham, North Carolina, USA
Oct. 2008 – Sept. 2010	**Israel Science Foundation (ISF) Bikura Postdoctoral Scholar** Department of Biomedical Engineering Duke University, Durham, North Carolina, USA
2002 – 2008	**Instructor / Teaching Assistant** Head Instructor of Electro-Optics and Advanced Computers Teaching Labs Department of Electrical and Computer Engineering Ben Gurion University of the Negev, Beer Sheva, Israel

- **PRIZES AND AWARDS**

2021	**SPIE Fellow; OPTICA (OSA) Fellow** *"For significant contributions in biomedical holography, developing clinical portable holographic modules, and for novel holographic multiplexing and machine-learning approaches."*
2020	Israel Science Foundation (ISF) Grant
2015	ERC Starting Grant (+ 2 ERC Proof of Concept Grants)
2011,2015	German-Israeli Foundation (GIF) Grants
2014	U.S.-Israel Binational Science Foundation (BSF) – Young Scientist Track
2014	Excellent Lecturer Award, Department of Biomedical Engineering, Tel Aviv University
2013	Outstanding Reviewer Award, Optical Society of America (OSA)
2012	Marie Curie Career Integration Grants (CIG)

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS**

2011 – Present	Supervised 12 postdocs, and 32 graduate students Department of Biomedical Engineering, Tel Aviv University, Tel Aviv, Israel

- **TEACHING ACTIVITIES**

Course Lecturer, Department of Biomedical Engineering, Tel Aviv University, Israel

2014,2016,2018 Advanced Interferometric Methods in Biomed. (New grad. course I developed)
2012 – 2021 Electromagnetic Fields and Waves for Biomed. (New undergrad. course I developed)
2012,2013 Lasers and Optics in Medicine (Undergrad. course)
2016 – 2021 Intro. to Cellular Optical Coherent Imaging (New undergrad. course I developed)
2012,2013,2015,2019 Advanced Optical Microscopy in Biomed. (New grad. course I developed)

- **ORGANISATION OF SCIENTIFIC MEETINGS (SELECTED)**

2019-2023 *Main Conference Chair* and *Conference Founder* (annual conference)
SPIE Label-Free Biomedical Imaging and Sensing, SPIE Photonics West, San Francisco, 4 days
2019 *Conference Chair*. Biomedical Engineering, Annual Meeting of the Israel Society for Medical and Biological Engineering, Haifa, Israel. 250 submissions. 2 days
2014 *Conference co-Chair* and *Conference Organizer*
Biomedical Optical Imaging and Laser Manipulations, Tel Aviv, Israel

- **OTHER PROFESSIONAL ACTIVITIES**

- *Chair of the IEEE Chapter on Engineering in Medicine and Biology in Israel*
- *OSA (Fellow), SPIE (Fellow), IEEE (Senior)*
- *Founder and Faculty Advisor of the SPIE Student Chapter of Tel Aviv University*

- **JOURNAL PUBLICATIONS (SELECTED)**

Refereed journal papers: 101 Times cited: 4494 h-index (Scholar): 39
Conference presentations: 183 (40 keynote/invited) Patents: 15

Bolded author names indicate me and my students/postdocs * Most relevant for this grant

1. ***G. Dardikman-Yoffe**, **S. K. Mirsky**, **I. Barnea**, and **N. T. Shaked**, "Four-dimensional acquisition of freely swimming human sperm cells without staining," *Science Advances* **[IF 14.980]** 4, 1600205, 2020.
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3. **M. Nasser**, **M. Levi**, **S. K. Mirski**, **G. Dardkiman**, and **N. T. Shaked**, "Prediction of sperm progression in three dimensions using rapid optical imaging and dynamic mechanical modeling," *Cells* **[IF 7.666]** 11, 1319, 2022.
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10. **P. Girshovitz** and **N. T. Shaked**, "Doubling the field of view in off-axis low-coherence interferometric imaging," *Nature – Light: Science and Applications* **[IF 20.257]** 3, e151, 1-9, 2014.

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אוניברסיטת תל אביב

December 31, 2022

To: Zimin Institute for Engineering Solutions
Advancing Better Lives
Tel Aviv University

Dear respectful committee members,

Re: **Endorsement by the Dean – Natan T. Shaked's Grant Application**

I am writing this letter to fully support the grant application submitted by Prof. Natan T. Shaked from the Biomedical Engineering Department at Tel Aviv University for the Zimin Institute for Engineering Solutions Advancing Better Lives.

Prof. Shaked's proposal deals with developing novel tools for sperm 3D imaging without chemical cell staining, presenting the **fastest 3D sperm imaging existing today**. This unique imaging technique allows acquiring the internal structure of sperm cells during extremely rapid movement, allowing their deep biophysical and medical analysis as never possible before. I strongly believe that this research is expected to **open new horizons in the field of in vitro feralization (IVF)**. Preliminary results from this research were lately published by Prof. Shaked in very high impact journals such as **PNAS** and **Science Advances**. The proposed project is truly **multi-disciplinary**, involving optics, engineering, image processing, biology, and medicine.

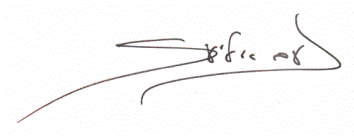
Prof. Shaked is an internationally renowned expert in the field of quantitative biomedical imaging techniques and directs a **large research group and well-equipped experimental labs**. In addition, he currently serves as the **Chair** of the Department of Biomedical Engineering. Prof. Shaked is the coauthor of **more than 100 journal papers and 180 conference papers**. In the past, he won the prestigious ERC Starting Grant, as well as other grants. He has lately received a great international

recognition, becoming a **Fellow Member** (the highest membership type reserved for renowned scientists) in both leading international societies in his field, the SPIE and OPTICA (previously OSA, the Optical Society of America).

To conclude, I find Prof. Shaked's project **highly innovative**, and I expect this research to bring to important discoveries with **high impact on lives of many patients** suffering from fertility problems.

I am fully committed to provide Prof. Shaked the resources and time to perform proposed project. Thank you for considering Prof. Shaked's application.

Sincerely,



Professor Noam Eliaz

Dean