The 2nd Israeli Biophotonics Conference (IBPC-2)

December 1-2, 2015, Bar-Ilan University, Ramat-Gan, Israel

Abstracts

SESSION 1

Wide-field fast scatter spectral imaging of lumpectomy tissues during surgery

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Breast conserving surgery is routinely offered for localized breast malignancies, but there is significant uncertainty about if the entire cancer has been removed. Approximately 33% of all patients are recalled for a second re-excision because either residual cancer or DCIS was found on the specimen surface or within 1-2 mm of it, when analyzed in pathology in the days after the initial procedure. There is a need for an accurate surgeon assist device, which can determine the potential that the resected specimen is clear of cancer at the margins. This can be directly solved through a technological solution which is optimized for wide-field and volumetric scanning, coupled with computer-aided decision making.

Scatter imaging allows surface scanning through high-spatial frequency imaging of the tissue, which negates erroneous signals from blood, fluid or ink on the tissue surface, which is critically important for fast in situ imaging of large tissue fields. This is coupled with fast volumetric CT imaging in an integrated display software. The surface molecular-structural features are key to identifying potential cancer regions for the surgeon, as is the volumetric CT key to identifying the internal tracks of the cancer which were seen on mammography and can be used to help identify which faces of the specimen are closest to the internal lesion.

Validation studies are underway in a prospective trial using the system, to help determine the accuracy in tissue type identification. Taken together this will be one of the first comprehensive approaches to volumetric and surface scanning in a single package.

An Academic-Industry Partnership was created to realize this wide-field optical scatter spectroscopic imaging system, coupled to volumetric x-ray CT scanning. The package integrates a substantial pre-clinical experience by the PerkinElmer team, with substantial prototyping and clinical specimen imaging work of the Dartmouth team.

Coherent effects in multiple scattering of polarized light

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Bearing in mind the basic polarization formalism and coherent properties of light, we discuss the peculiarities of coherent polarized light propagation in multiply scattered tissue-like turbid media. Starting with a brief review of coherent effects of multiple scattering of light we discuss the directional awareness of circularly polarized light, propagating in the media exhibiting strong multiple scattering and investigate its use for characterization of anisotropy of scattering particles. Considering the propagation of circularly polarized light through a turbid medium where multiple scattering events occur, light backscattered an odd number of times will correspond to a reversal in helicity, and, thus, contribute the cross polarized portion of the detected signal. Whereas, light experienced an even number of backscattering events contributes to the co-polarized signal, i.e. even number of helicity changes have not changed the handedness of incident polarization of light. The developed phenomenological model is shown an excellent agreement with the results of phantom studies at the water solutions of polystyrene microspheres of a known size and concentration and with the results obtained by the polarization tracking Monte Carlo modelling. By analogy to the diffusing-wave spectroscopy we call this approach diffusing-wave polarimetry, and illustrate its utility in probing cancerous and non-cancerous tissues *in vitro*.

Rolling tomography: a complete optofluidics scrutiny of single cells and living micro-organism

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A novel optofluidics modality has been developed as simplified but effective full diagnostics tool for single cells while they flow along microfluidic sampling chanells. Phase contrast tomography can be easly retrivied by holography imaging approach. Results show how the approach can be adopted
for real blood screening.

SESSION 2

New photonic tools for cellular-scale interfacing with the retina

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Optical retinal prostheses for patients with outer-retinal degenerative diseases could interface directly with surviving retinal neurons in order to emulate a meaningful percept in the brain. Recently, we introduced an artificial photo-stimulation technique based on the projection of holographic patterns with high spatiotemporal resolution onto optogenetic probes, for selectively controlling large retinal neuronal populations in isolated mouse retinas.

Here, we describe recent developments aimed at further translation and testing of these solutions, which requires systems capable of imaging cellular-scale retinal structures in small optogenetic animal models *in vivo*. First, we present a system for targeting multiple optogenetic rodent retinal ganglion cells (RGCs) *in-vivo* with holographic patterns at a cellular resolution, while imaging the response in downstream circuits using calcium probes expressed in the visual cortex. Next, we use an intuitive optical model-based approach to analyze the requirements from an *in vivo* mouse retinal imaging system. Using these design criteria, we built a two-photon laser scanning system which yields exquisite images of optically sectioned, cellular-resolved fluorescent microstructures. The imaging is depth-scanned using an electronically tunable lens (ETL) integrated into the optical path is found to allow long-term repeated imaging, in a simple, widely accessible design.

This suite of new tools could enable artifact-free functional imaging of retinal and downstream neuronal activity in response to natural or artificial-optogenetic visual stimuli.

Impact of nanodiamonds on red blood cells microrheology at in vitro and in vivo applications

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Nanodiamonds (ND) are characterized by notable biocompatibility and possibilities of surface conjugation. This is why they recently attracted high attention in regard to their potential applications in biology and medicine, especially, for bioimaging and drug delivery. Remarkable fluorescence and Raman spectroscopic properties make ND an efficient biomarker for tracking the interactions of specific molecules with biological systems. Implementing ND presumes their administration into the organism, in particular, by injecting them intravenously into the blood circulation. In this paper, we present our results in studying the effects of ND on blood fluidity via interaction with different blood constituents.

Using different optical techniques (optical and fluorescence microscopy, Raman microspectroscopy, laser diffractometry, diffuse light scattering, etc.) we studied the interaction of ND of different sizes with blood plasma proteins and blood cells with special focus on red blood cells (RBC) in *in-vitro* and *in-vivo* conditions. We showed that ND do interact with RBC membrane in a concentration dependent manner. This interaction may, in certain conditions, influence the membrane structure and affect the RBC mechanical and microrheological properties, deformability and aggregation, which are the determinants of blood rheology and microcirculation. The obtained results imply that controlling the blood microrheologic properties is a necessary prerequisite for the optimization of ND application protocols.

This work was partially supported by the RFBR grants 12-02-92008-HHC, 12-02-01329, and the NSC grant 97-2923-M-259-001-MY3.

Numerical Modeling of tissue Ablation in laser angioplasty

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Laser angioplasty is one of the foremost common techniques in the treatment of peripheral atrial disease, and includes a nanosecond pulsed Xenon monocloride excimer laser source. During the procedure, the laser radiation (at 308 nm wavelength) is guided to the occluded site through optical fibers, and encounters fluid-phase media such as saline, blood, and iodine-based fluoroscopy contrast media. The interaction between the radiation and the latter two initiates pressure shockwaves, which may cause serious angiographic and clinical complications. Several methods are used to mitigate these effects (*e.g.*: dilution in saline, etc.). However, drawbacks such as renal complications due to accumulation of contrast media, and the inability to use the contrast media in real-time imaging during the ablation, reduce the technique's attractiveness. In order to find appropriate solutions, a comprehensive understanding of the given scenario (where the ablation site is surrounded by high optically-absorptive fluid-phase media) is required. A numerical model, which emphasizes thoughtfully the ablation trends, may meet this need. In our study, we numerically investigate the ablation effects in an aortic tissue surrounded alternately by blood, saline, and fluoroscopy contrast media, while the laser radiation is delivered through an optical fiber, in contact with the tissue. The simulations are based on Comsol Multi-physics© finite elements software, and considers both photo-mechanical and photo-thermal effects resulted due to the interaction with a nanosecond pulse in the UV. Our work sheds light on the ability to engineer the ablation parameters to minimize the effect of the pressure shockwaves.

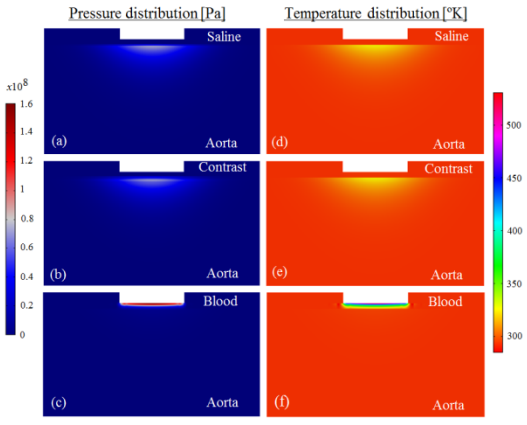

Fig. 1. Numerical simulations results of pressure and temperature distributions (the optical fiber at the center was omitted).

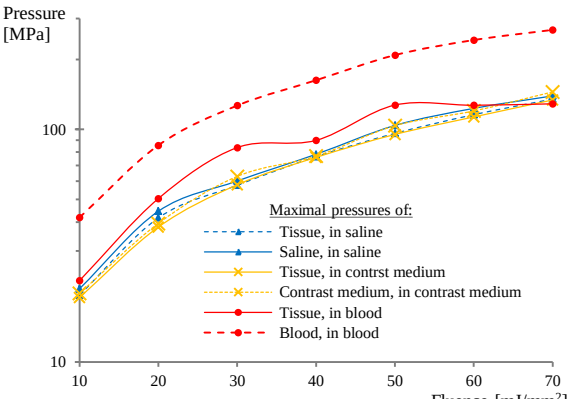

Fig. 2. Maximal pressures at the ablation site, for different fluence values, within 1 μs after the interaction

Mueller polarimetry as a tool for early cancer detection: measurements and simulations

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Multi-spectral backscattering Mueller matrix images of *ex-vivo* cervical and colon human tissues reveal the essential enhancement of contrast between healthy and cancerous zones, thus, demonstrating the potential of Mueller matrix imaging polarimetry to become a new efficient low-cost optical technique for early cancer diagnostics and proper cancer staging [1,2]. Obviously, the envisaged *in-vivo* implementation of this technique requires the deeper insights on the origin of polarimetric contrasts observed within the visible wavelength range.

Mueller matrix images of human colon tissue were simulated by Monte Carlo technique using multilayer optical model with different scattering and absorption coefficients of the layers. The results of simulations were compared with the experiments.

We demonstrated that measured values of depolarization for both circularly and linearly polarized incident light for healthy and anomalous tissues can be qualitatively reproduced only when small (compared to light wavelength) scatterers are incorporated into the optical model. The measured polarimetric response of cancerous zone was always less depolarizing compared to the healthy tissue for both linearly and circularly polarized light. We attribute it to the enhancement of absorption related to the tumor vascularization and to the decrease of scattering because of histologically confirmed break of tissue fine structure by cancer. The parametric studies of the model showed that both enhancement of absorption and decrease of scattering coefficients lead to better preservation of polarization of backscattered light. These results are consistent with the experimental trends.

[1] T. Novikova, A. Pierangelo, A. De Martino, A. Benali, P. Validire, "Polarimetric imaging for cancer diagnosis and staging", Optics and Photonics News, 26-32, (October 2012)
[2] A. Pierangelo, A. Nazac, A. Benali, P. Validire, H. Cohen, T. Novikova, B. Haj Ibrahim, S. Manhas, C. Fallet, M.-R. Antonelli, and A. De Martino "Polarimetric imaging of uterine cervix: a case study", Opt. Express 21(12), 14120–14130 (2013)

Monochomatic spectral imaging: principles and application for skin chromophore mapping

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Two most widespread techniques for obtaining spectral images currently are (i) spectral filtering of the target-reflected image under broadband (e.g. white) illumination [1] and (ii) capturing of the target image under spectrally narrowband illumination, e.g. by color LEDs [2]. Spectral bandwith related to such images typically is in the range 10...40 nm. If a set of spectral images is further processed to calculate parametric maps featuring specific target details (for instance - skin chromophore 2D-distribution), possibly narrow image-related spectral bands are preferred. Ideal option from this point would be monochromatic spectral imaging.

We have demonstrated the possibility to extract monochromatic spectral images from a single digital RGB image data set at simultaneous illumination of target by several discrete spectral lines [3,4]. This approach was successfully applied for fast single-snapshot mapping of three main skin chromophores under triple wavelength laser illumination [5,6]. Several aspects of technique for monochromatic spectral imaging will be discussed, including the specific illuminator designs and suitability of smartphones for quick mapping of skin chromophores [7].

This study was supported by Latvian National research program SOPHIS under the grant agreement # 10-4/VPP-4/11.

References

1. D. Yudovsky, A. Nouvong, K. Schomacker, L. Pilon, "Assessing diabetic foot ulcer development risk with hyperspectral tissue oximetry", *J. Biomed. Opt.* **16**, 026009 (2011).
2. D. Jakovels, U. Rubins, J. Spigulis, "RGB imaging system for mapping and monitoring of hemoglobin distribution in skin", *Proc. SPIE* **8158**, 81580R (2011).
3. WO 2013135311 A1 ”Method and device for imaging of spectral reflectance at several wavelength bands” (2012).
4. J.Spigulis, L.Elste,”Single snapshot RGB multispectral imaging at fixed wavelengths: proof of concept”, *Proc.SPIE* **8937**, 89370L (2014).
5. J.Spigulis, D.Jakovels, L.Elste**, “**Towards single snapshot multispectral skin assessment”, *Proc. SPIE* **8216**, 82160L (2012).
6. J.Spigulis, I.Oshina, “Snapshot RGB mapping of skin melanin and hemoglobin”, *J.Biomed.Opt*., 20(5), 050503 (2015).
7. I.Kuzmina, M.Lacis, J.Spigulis, A.Berzina, L.Valaine, “Study of smartphone suitability for mapping of skin chromophores”, *J.Biomed.Opt*., 20(9), 090503(2015).

Accurate Transcranial Dynamic Fluorescent Imaging Using Adaptive Time-Space Fourier Method

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In vivo imaging of cerebral vasculature is highly vital for medical researchers and clinicians. We recently introduced a simple and robust technique Transcranial Optical Vascular Imaging (TOVI) for dynamic fluorescent imaging of the brain vasculature that utilizes a standard fluorescent dyes, inexpensive micro-imaging and computation procedures.
The fluorescence imaging technique provides dynamic information from the intensity evolution. However, the structures of the region of interest show also rapid changes with time. Natural movements made during the image recording produce distortions that are unique in each frame.
These motions include rapid jerks or saccades, slower drifts, and high-frequency tremors perturbing the temporal analysis. Correction for these distortions is desirable to stabilize and achieve noise reduction in order to accurately study the dynamic of the structures as a function of time.
In the particular case of the dynamic fluorescence imaging, usual registration methods fail to stabilize the images due to the fast differing intensity of the structures. We show the reliability and the accuracy of the proposed novel application of time-space Fourier method to correct image motions and reduce the noise during intensity variations.

Acousto-optical interferometry for biological tissue imaging with increased SNR

Shira Zapesotsky, Darina Roitshtain and Natan Z. Shaked

We present a new method to quantify SNR in ultrasound-modulated optical tomography. The system is an interferometric system in combination with ultrasound modulation. It performs parallel speckle decorrelation, with optimum shot-noise sensitivity. The system separates the image from the shot-noise and can be used to preform imaging inside a scattering tissue.

SESSION 3

Optoacoustic imaging – opportunities and limitations

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Optoacoustic imaging is a promising non-ionizing hybrid imaging modality combining the high optical contrast and spectroscopic-based specificity of optical imaging with the high spatial resolution of ultrasound imaging. This allows simultaneously imaging anatomical and functional information of scattering tissue over a wide range of length scales from micrometers to centimeters with scalable spatial resolution from about 200 micrometers down to sub micrometer. Optoacoustics is based on the generation of broadband ultrasonic response from absorbing structures upon short-pulsed optical illumination. The ultrasonic waves propagate to the tissue surface where they are detected by an ultrasound receiver. When measuring the arrival time of the acoustic waves and knowing the speed of sound, an image of the absorbing structure can be reconstructed in much the same way a conventional pulse-echo ultrasound image is formed. To obtain, however, aberration free quantitative images knowledge about clutter and local variations in the fluence distribution as well as in the speed of sound is essential. Ideas for realizing a multimodal clinical diagnostic system combining conventional and Doppler ultrasound, optoacoustics, elastography and speed of sound measurements will be presented.

Label-free nonlinear photoacoustic nanoscopy and spectroscopy

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Super-resolution microscopy techniques have opened new opportunities to explore sub-cellular structures and dynamics not resolvable in conventional far-field microscopy. However, relying on staining with exogenous fluorescent markers, these techniques can sometimes introduce undesired artifacts to the image, mainly due to large tagging agent sizes and insufficient or variable labeling densities. By contrast, the use of endogenous pigments allows imaging of the intrinsic structures of biological samples with unaltered molecular constituents. Here, we persent label-free photoacoustic (PA) nanoscopy, which is exquisitely sensitive to optical absorption, with 88 nm resolution. At each scanning position, multiple PA signals are successively excited with increasing laser pulse energy. Owing to optical saturation or nonlinear thermal expansion, the PA amplitude depends on the incident optical fluence nonlinearly. The high-order dependence, quantified by polynomial fitting, provides super-resolution imaging with optical sectioning. In addition, we use a photoacoustic spectrometer to show how the nonlinear PA spectrum of different molecules (e.g., oxygenated and deoxygenated hemoglobin) has both wavelength and concentration dependence.

Measurement of blood oxygen saturation within a single capillary vessel

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Independent measurement of oxygen saturation of capillary blood is important for measuring the tissue oxygen consumption and could be utilized for diagnosing and monitoring patients with heart or lung diseases. In this work, we developed a simple, non-invasive technique for measuring the reflected spectra from individual capillary vessels within a human lip, allowing local measurement of the blood oxygen saturation. The optical setup includes a spatially incoherent broadband light that was focused onto a specific vessel below the lip surface. Backscattered light was imaged by a camera for identifying a target vessel and pointing the illumination beam directly at its cross section. Scattered light from the vessel was collected by a single-mode fiber and analyzed by a fast spectrometer. Spectra acquired from small capillary vessels within a volunteer lip showed the characteristic oxyhemoglobin absorption bands in real time and with a high signal-to-noise ratio. Measuring capillary oxygen saturation using this technique would potentially be more accurate compared to existing pulse oximetry techniques due to its insensitivity to the patient's skin color, pulse rate, motion, and medical condition. It could be used as a standalone technique for measuring tissue hypoxia or in conjunction with conventional pulse oximetry for a more accurate measurement of oxygen transport in the body.

Resonant femtosecond pulse irradiation of gold nanorods: effect of particles re-shaping on cell damage

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In recent years, irradiation of nanoparticle-targeted cells by resonant laser pulses has been proposed as an effective therapeutic tool for treating malignant tissues. Femtosecond pulses and gold nanorods are particularly attractive for this task, generating a variety of highly localized physical process using near infrared light, including acoustic shock waves, photoionization, and optical breakdown. Clearly, illumination of nanorod-targeted cells by several pulses is advantageous over a single pulse due to the cumulative damage of the resulting physical processes. Furthermore, the random angular distribution of the nanorods reduces the impact of a single, linearly polarized pulse and often requires additional pulses for optimal damage. Nevertheless, gradual loss of the resonance band due to particle reshaping after each pulse may prevent effective cumulative damage, resulting in expected loss of the therapeutic efficiency. In this work, we study the effect of particle re-shaping on the generation of cell damage by a short train of femtosecond pulses. Burkitt lymphoma B cells were coated with functionalized gold nanorods and irradiated by a sequence of 45 fs pulses at resonant central wavelength of 800 nm and repetition rate of 1 kHz. Cells were stained for necrosis using propidium iodide and their death was evaluated by time-lapse imaging followed by semi-automatic data analysis. Initial results show that cell death increases with the number of pulses at irradiance level above the ionization threshold (10^{12} W/cm^2), up to a certain plateau where sequences of more than five pulses show negligible incremental damage with each additional pulse. The talk will present our latest results, describe a quantitative model and discuss the implication of the results on a potential future therapeutic technology.

Extremely sensitive dual imaging system in solid phantoms

Eran Barnoy, Dror Fixler, Rachela Popovtzer, Tsviya Nayhoz and Krishanu Ray

In our talk we will describe promising results from the combination of fluorescent lifetime imaging microscopy (FLIM) and diffusion reflection (DR) medical imaging techniques. Three different geometries of gold nanoparticles (GNPs) were prepared: spheres of 20nm diameter, rods (GNRs) of aspect ratio (AR) 2.5, and rods of AR 3.3. Each GNP geometry was then conjugated using PEG linkers estimated to be 10nm in length to each of 3 different fluorescent dyes: Fluorescein, Rhodamine B, and Sulforhodamine B. DR provided deep-volume measurements (up to 1cm) from within solid, tissue-imitating phantoms, indicating GNR presence corresponding to the light used by recording light scattered from the GNPs with increasing distance to a photodetector. FLIM imaged solutions as well as phantom surfaces, recording both the fluorescence lifetimes as well as the fluorescence intensities. Fluorescence quenching was observed for Fluorescein, while metal-enhanced fluorescence (MEF) was observed in Rhodamine B and Sulforhodamine B – the dyes with an absorption peak at a slightly longer wavelength than the GNP plasmon resonance peak. Our system is highly sensitive due to the increased intensity provided by MEF, and also because of the inherent sensitivity of both FLIM and DR. Together, these two modalities and MEF can provide a lot of meaningful information for molecular and functional imaging of biological samples.

Time multiplexing super resolution using Barker-based array

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We propose the use of a new encoding mask in order to improve the performance of the conventional time multiplexing super resolution method. The encoding is performed by a 2D Barker-based array, which is a 2D generalization of the standard 1D Barker code. The decoding process involves using a mismatched array. The 2D Barker-based array enables achieving two dimensional super resolution image using only one dimensional scan, by exploiting the unique cross correlation between the Barker-based array and the mismatched array. The cross correlation has a perfect peak to sidelobes ratio, making it ideal for the super resolution process. Also, instead of placing the 2D Barker-based array onto the object, we propose the projection of this array onto the object using a phase-only spatial light modulator. Projecting the array eliminates the need for printing it, mechanically shifting it, and having a direct contact with the object, which is not feasible in many imaging applications. 13 phase masks, which generate shifted Barker-based arrays, were designed using a revised Gerchberg-Saxton algorithm. A sequence of 13 low resolution images were captured using these phase masks, and were decoded using the mismatched arrays, resulting with a high resolution image. The proposed 2D Barker-based array, mismatched array, and the design process of the phase masks are presented, and the method is validated by a laboratory experiment.

Biomedical Optical Imaging and Biosensing Assisted by Liquid Crystal Devices

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A newly emerging field called LCs photonics is becoming more and more active in which the strong electrooptic properties of LCs are harnessed for photonic applications other than displays. Hence liquid crystal devices are under extensive study for photonic and optical non-display applications. One of the important areas where they can significantly improve applications is in optical imaging in which they can function as spatial light modulators for wavefront correction, tunable filtering for hyperspectral imaging, tunable focusing and polarization control for polarimetric imaging with their distinct advantage of being miniature and requiring low voltage and low power consumption. LCs can flow and fill small gaps; hence they can be integrated into nanophotonic structures in planar or cylindrical geometries such as in photonic crystal fibers and for optofluidics.

Recently we have been developing variety of specially designed LC devices integrated into imaging systems for specific applications such as (i) wideband tunable filters for hyperspectral imaging and frequency domain optical coherence tomography, (ii) discrete tunable filter for multispectral imaging, (iii) compact polarization rotator for polarimetric imaging, (iv) fast phase retarder for phase shift interferometry, (v) wideband achromatic waveplate, (vi) annular SLM for extended depth of focus, (vii) polarization independent LCFP tunable filter, and lately (vii) optically addressed SLMs. The main concepts of these devices and their functionality into imaging systems such as in skin cancer diagnosis, in optical biosensing using spectropolarimetric imaging and full field optical coherence tomography will be reviewed in this talk. [1-6]

Selected Publications:

1. Avner Safrani and I. Abdulhalim, Optics Letters, 34, 1801-3 (2009).
2. O. Aharon and I. Abdulhalim, Optics Express, 17, 11426-33 (2009).
3. A. Safrani, O. Aharon, S. Mor et.al., J. Biomed. Opt. 15, 026024-8p (2010).
4. O. Aharon, I. Abdulhalim, O. Arnon, L. Rosenberg, V. Dyomin, E. Silberstein, Differential optical spectropolarimetric imaging system assisted by liquid crystal devices for skin imaging, J. Biomedical Optics, 16(8), 086008-12p (2011).
5. A. Safrani and I. Abdulhalim, Optics Letter 37, 458 (2012).
6. I. Klapp, A. Solodar, I. Abdulhalim, Optics Letters, 39, 1414 (2014).
7. S. K. Srivastava and Ibrahim Abdulhalim, Opt. Lett. 40, 2425-28 (2015).
8. S. Issacs and I. Abdulhalim, Appl.Phys.Lett. 106, 193701-4 (2015).

SESSION 4

Fiber-optic Evanescent Wave Spectroscopy (FEWS) – A New Diagnostic Tool for the Early Detection of Melanoma

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There has been a steady growth in the number of malignant melanomas across all regions of the world. There is still a lack of adequate therapies for metastatic melanoma. Most patients survive if the melanoma is detected when its thickness is less than 0.3mm. On the other hand, most patients will not survive if the melanoma thickness at the time of diagnosis is more than 3mm. This is why early detection of melanoma is so critical. Diverse technologies to differentiate between benign and malignant pigmented lesions of the skin are under development. Yet, to this date, the only reliable tool for detecting melanoma is the eye of the experienced dermatologist. There is an urgent need to develop techniques that will enable less experience physicians to diagnose a suspicious lesion *in situ* and in real time. Fiber Evanescent Wave Spectroscopy (FEWS) is a very useful method for non-invasive and non-destructive biomedical diagnosis. We have developed a FEWS system that makes use of a Fourier Transform Infrared (FTIR) spectrometer and mid-IR transmitting $AgBr_xCl_{1-x}$ fibers. The evanescent wave penetration depth in the mid-IR is comparable with the thickness of the upper layer of the skin, and therefore the vibrational spectra of lipids, proteins and water can be easily analyzed. The FTIR-FEWS system is compact and easy to use, and it is ideal for the study of the spectroscopy of the skin in the mid-IR. We have started to use this system for a clinical study of the skin of patients, who had some suspicious skin lesions. Preliminary measurements were carried out both on the lesion and on neighboring healthy areas of the skin, showing some differences in the mid- IR absorption. Larger clinical trials are under way.

Comment: This research has been supported by the Ministry of Science.

Characterization and differentiation of viral infections in cell culture by Raman spectroscopy in tandem with advanced statistical methods

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Raman spectroscopy has been widely used for detection purposes in medicine and biology. It is a rapid, reagent-free, noninvasive, and non-destructive alternative for the analysis of cell biology systems.

Viruses are responsible for many of the human, animal and plant disorders. In humans and animals, viruses cause serious diseases that are considered life threatening. For successful therapy in most cases, early and reliable detection of these infections is critical. HSV-1 is the most common of the herpes viruses, causing mainly human cutaneous disorders. Detection of this virus infection at an early stage is important because the effective drug (acyclovir) that is used against this virus is more successful if employed at the early stages of the infection. Murine sarcoma virus (MuSV) is a retrovirus that can cause sarcoma tumors in rodents.

In our studies we evaluated the potential of using Raman spectroscopy for detection, identification and characterization of cell cultures infected with either the HSV-1 or the MuSV.

The Raman spectra of the different biological systems investigated in this study were very similar, with only slight variations that merged in the whole region. Therefore, advanced mathematical and statistical methods were applied for differentiation purposes. In this study, principal component analysis (PCA) and linear discriminant analysis (LDA) were used for the spectral analysis. After applying the PCA procedure, LDA was used as a classifier.

The classifier differentiated between the control and MuSV-infected NIH/3T3 cells with an 80-85% success rate and between the control and HSV-1 infected Vero cells with a 100 % sensitivity success rate.

In conclusion, this study proved the possibility of using Raman spectroscopy in tandem with multivariate analysis PCA and LDA to produce high success rates of detection and identification of viral infections in MuSV or HSV-1 infected cell cultures.

Keywords: Raman spectroscopy, MuSV, HSV-1, PCA, LDA

Holographic phase texture changes in label-free live cell imaging as new biomarkers

Authors: Darina Roitshtain, Natan T. Shaked

We present a new analysis tool for studying texture changes in the quantitative phase maps, of live cells acquired by wide-field interferometry. The sensitivity of wide-field interferometry systems to small changes in refractive index enables visualizing cells and inner cell organelles without the using fluorescent dyes or other cell-invasive approaches, which may affect the measurement and require external labeling. Our label-free texture- analysis tool is based directly on the optical path delay profile of the sample and does not necessitate decoupling refractive index and thickness in the cell quantitative phase profile; thus, it can be calculated using a single-frame acquisition. Nowadays, it is not possible to detect small spaces inside living cells, such as vacuoles and lipid droplets, which has different refraction index using bright-field microscopy without labeling. Our experimental system includes low-coherence wide-field interferometer, combined with simultaneous florescence microscopy system for validation. We used this system and analysis tool for studying vacuole formation in sperm cells and lipid droplets formation in adipocytes. The latter demonstration is relevant for various cellular functions as lipid metabolism, protein storage and degradation to viral replication. These processes are functionally linked to several physiological and pathological conditions, including obesity and metabolic diseases. Quantification of these biological phenomena based on the texture changes in the cell phase map has a potential as a new cellular diagnosis tool.

Stimulated Emission (STED) pulsed microscope with supercontinuum laser source.

Eugene Brozgol, Liat Altman, Yuval Garini.

Many biological structures have important characteristics at the nanometer scale. Due to the limitations of electronic and optical microscopy, these structures are poorly understood.

In our present work we focus on building a Stimulated Emission Depletion (STED) microscope, based on a pulsed, super continuum laser source. Such a setup has unique characteristics, and will be utilized for studying the fine details of the telomere's structure in eukaryotic nuclei.

Here we present the basics of the pulsed, continuum laser STED setup.

Real-time movies of nerve signal propagation by sensing changes in birefringence with polarized light

Irving J. Bigio
Boston University

Action-potential-induced changes in the optical birefringence of nerve tissues may serve as a vehicle for minimally invasive neuroimaging methods with high spatiotemporal resolution, to aid in the study of neuronal activation patterns. With our improved instrumentation we have generated real-time “movies” of nerve impulse propagation in crustacean nerves, indicating promise for imaging neuronal activity in more complex systems. The temporal histories provide insights into the mechanisms of the crossed-polarized signal, which are not fully explained in prior literature.

SESSION 5

Advances in Optical Coherence Tomography

Prof. Martin Leahy

Optical Coherence Tomography (OCT) was originally applied to study and diagnose the eye where it allows 3D imaging of the structure in the retina and choroid. It typically used a broadband source centred around 850 nm approximately and a silicon photodetector which were inexpensive and accessible. The later availability of longer wavelength sources and detectors around 1060 and 1310 nm facilitated deeper imaging in scattering tissues such as the skin and arterial wall. This lecture will discuss functional extensions to OCT, including microcirculation and oxygen imaging as well as more recent developments aimed at extracting structural information on the nanoscale.

Enhanced Imaging and Sensing in Biophotonics: from UV to Terahertz

Prof. Valery V. Tuchin
Saratov State University, Russia

This paper presents fundamentals and advances of tissue optical clearing that provides enhanced imaging and sensing in tissue photonics in a wide wavelength range from UV to terahertz. The technology is based on controlling of tissue optical properties by using immersion technique via application of exogenous optical clearing agents (OCAs). Impact of an OCA and water transport in a tissue on temporal tissue optical properties will be analyzed. Tissue reversible dehydration and induced transverse and longitudinal shrinkage measured in vitro and in vivo will be discussed. The specific features of optical clearing of fibrous and cell-structured tissues are investigated using OCT, diffuse spectroscopy, photoacoustic imaging, and SHG. In terahertz range, a femtosecond laser spectrometer was used to prove dehydration mechanism of optical clearing. Enhancement of probing depth and image contrast in *in vitro*, *ex vivo*, and *in vivo* studies of a variety of human and animal tissues, including skin, fat, eye sclera, muscle, cerebral membrane, digestive tract tissue, cartilage, tendon, bone, blood vessels, and blood will be demonstrated. The technologies of effective OCA delivery, including hidden free diffusion, local heating, enforced tissue permeability (sonophoresis, laser or needle perforation), OCA encapsulation, and via blood and lymph vessel networking, will be also discussed. Impact of different OCAs on tissue structure, free/bound water balance and microcirculation will be analyzed. Experimental results on diffusivity of glucose, glycerol, PEG, Omnipaque^{TM} and other biocompatible clearing agents in normal and pathological tissues will be presented.

Optical wide field brain imaging

Ofer Levi
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Optical techniques are widely used in clinical settings and in biomedical research to interrogate bio-molecular interactions and to evaluate tissue dynamics. Miniature integrated optical systems for sensing and imaging can be portable, enabling long-term imaging studies in living tissues.

We present the development of a multi-modality optical neural imaging system, to image tissue blood flow velocity and oxygenation, using a fast CCD camera and miniature VCSEL illumination. We combined two techniques of laser speckle contrast imaging (LSCI) and intrinsic optical signal imaging (IOSI) simultaneously, using these compact laser sources, to monitor induced cortical ischemia in a full field format with high temporal acquisition rates. We have demonstrated tracking seizure activity, evaluating blood-brain barrier breaching, and integrating fast spatial light modulators for extended imaging depth and auto-focusing during brain imaging of flow dynamics. Our current studies include applying multi-modality optical imaging in seizure and stroke studies, and prototype designs, system optimization and evaluation for a low-cost portable imaging system as a minimally invasive method for long-term neurological studies in un-anesthetized animals. This system will provide a better understanding of the progression and treatment efficacy of various neurological disorders, in freely behaving animals.

Biography

Dr. Ofer Levi is an Associate Professor in the Institute of Biomaterials and Biomedical Engineering and the Edward S. Rogers Sr. Department of Electrical and Computer Engineering at the University of Toronto, currently on a Sabbatical leave at Stanford University. Dr. Levi received his Ph.D. in Physics from the Hebrew University of Jerusalem, Israel in 2000, and worked in 2000-2007 as a Postdoctoral Fellow and as a Research Associate at the Departments of Applied Physics and Electrical Engineering, Stanford University, CA. He serves as an Associate Editor in Biomedical Optics Express (OSA) and is a member of OSA, IEEE-Photonics, and SPIE. His recent research areas include biomedical imaging systems and optical bio-sensors based on semiconductor devices and nano-structures, and their application to bio-medical diagnostics, *in vivo* imaging, and study of bio-molecular interactions. More details can be found at http://biophotonics.utoronto.ca/

SESSION 6

Computational-Cannula Microscopy for brain imaging

Prof. Rajesh Menon

In our microscope, a surgical cannula transmits light from the sample (which can be inside the deep brain) to an output plane. Due to the internal reflections within the cannula, the rays from a point source are scrambled and generate a complex intensity distribution (point-spread function or PSF). This PSF is sensitive to the location of the point source, *i.e.*, it is space variant. Furthermore, since any complex self-luminous object (eg., any fluorescence labeled sample) is a linear superposition of numerous point sources, we can apply a variety of algorithms to reconstruct the object details with a knowledge of the space-variant PSFs.[1,2] We first calibrate the images formed by a single fluorescent microsphere at all possible locations in the sample plane, and then apply a nonlinear optimization algorithm to reconstruct the (unknown) distribution of point sources. In this presentation, I will discuss the application of this computational microscope to imaging inside the mouse brain at depths > 1mm.

High Detection Sensitivity of Skin Cancer at the THz by Parameters Optimization

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The detection of skin cancer at its early stages has drawmajor interest in the form of theoretical, numerical and experimental work during the few past decades. Various diagnostic methods aiming at various mechanisms and skin cancer artifacts detectable at different optical wavelengths have been suggested, some experimentally verified to some level. Among them two prominent approaches presenting high potential and applicability are sensing at the THz wavelengths region, exhibiting low scattering with long penetration lengths, and polarimetric techniques for pathological tissue differentiation presenting higher sensitivity and contrast than reflectometric techniques. As Monte-Carlo simulations have been widely demonstrated as a simplistic method for separately model various complex characteristics of light-tissue interactions and to accurately predict the behavior of the back-reflected electromagnetic field from the considered skin tissue models, a comprehensive MC simulation of radiative transfer in a complex skin tissue model have been developed to simultaneously consider various skin structural-optical properties such as its stratified structure, tissue optical dispersion modeling, surface roughness, embedded scatterers, and substructure organelles. Considering simultaneously a variety of tissue properties, we achieve a better light-tissue interaction modeling and more importantly a more realistic and accurate description of localized tissue and backscattered EM field variations related to the development of skin cancer. Furthermore, by combining THz with Mueller matrix imaging, the MC simulation is utilized for the determination of the optimal imaging configuration and parameters for highest detection sensitivity based on Mueller matrix differential analysis. These optimal imaging configurations and parameters can be a guide for the development of more sophisticated and sensitive polarimetric imaging diagnostic methods for early skin cancer detection. Sensitivity enhancement at the THz by the introduction of Parylene-C coated InN nano-particles into the skin tissue is also demonstrated. Current study is still ongoing and being further extended for improved modeling of the skin structure and light-tissue interactions.

High Resolution, Miniaturizeable, Optical Rotation Polarimeter for Continuous Glucose monitoring

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Optical rotation (OR) polarimetry is one method to track Glucose concentration *in vivo*. This can be achieved by implanting a miniaturized polarimeter in the human body, and tracking the Glucose level in the interstitial fluid. For the last few years we have been developing a novel OR polarimeter that is potentially useful for this purpose, as well as for other biomedical applications [1], [2]. This effort shall be described in the current paper.

The configuration of our polarimeter is mainly based upon a self-reference optical mechanisms, which facilitates high sensitivity for the OR angular signal. The reference signal compensates for background noises, which in many cases can be more than an order of magnitude larger than the rotation signal itself.

The current limit of detection of our lab prototype is 20 micro-degrees. For 10mm optical path length, this translates to ~0.5 mg/dL Glucose concentration.

The optical configuration is relatively simple (see figure 1), and consist of only few components. This potentially facilitates miniaturization, and eventually an implantable polarimeter.

In this paper, we shall present the principles, modelling and experimental results.

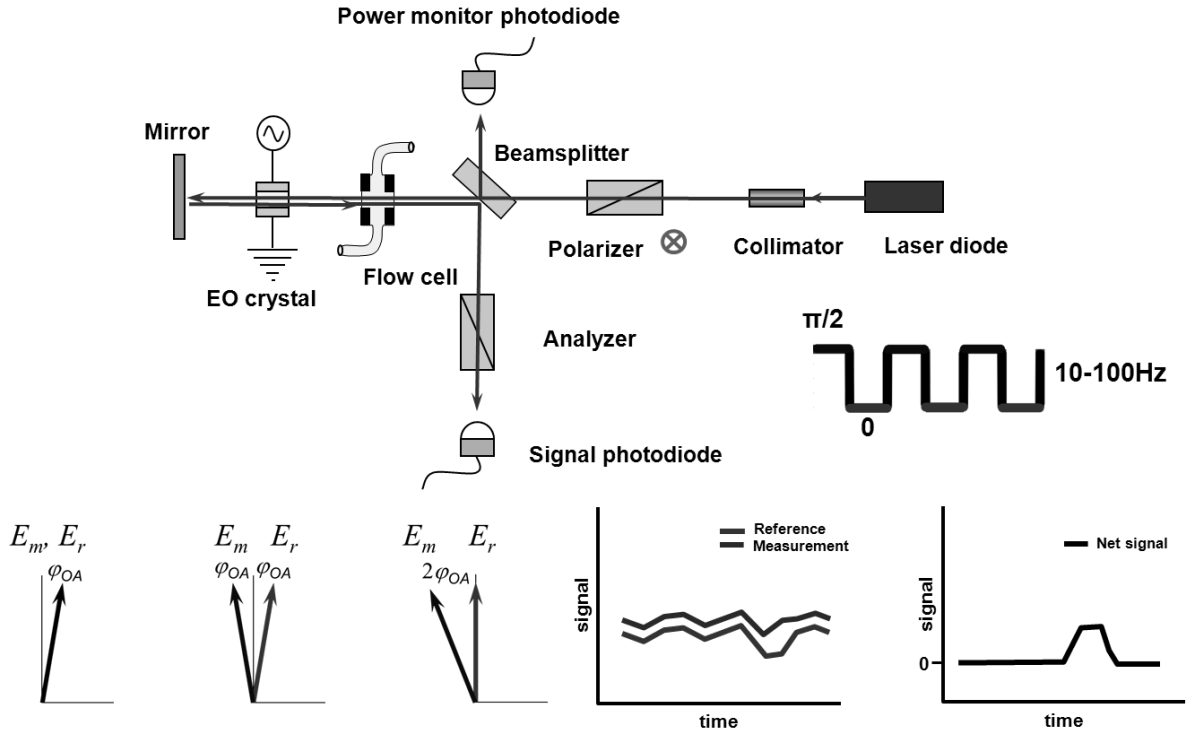

Fig1. Configuration of the Optical Rotation Polarimeter, and the method of extracting the referenced rotation signal and its subtraction from the OR measurement signal.

1. D. Goldberg and Z. Weissman, *Appl. Opt.* **53**, 577 (2014).
2. US patent **8,576,405**

Three-Photon Adaptive optics Fluorescence Microscopy

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Multiphoton fluorescence microscopy is a well-established technique for deep-tissue imaging with subcellular resolution. Three-photon fluorescence microscopy (3PM) when combined with long wavelength excitation was shown to allow deeper imaging than two-photon fluorescence microscopy (2PM) in biological tissues, such as mouse brain, because out-of-focus background light can be further reduced due to the higher order nonlinear excitation. As was demonstrated in 2PM systems, imaging depth and resolution can be improved by aberration correction using adaptive optics techniques which are based on shaping the scanning beam using a spatial light modulator (SLM). In this way, it is possible to compensate for tissue low order aberration and to some extent, to compensate for tissue scattering. Recently, it was theoretically shown that the compensation for signal degradation due to aberrations or scattering will be much more significant in higher order nonlinear imaging such as 3PM than in 2PM, which should improve both imaging depth and resolution in tissues.

Here, we present what is to our knowledge the first 3PM adaptive optics microscopy system. Soliton self-frequency shift was used to create a femtosecond source at ~ 1660 nm and a microelectromechanical (MEMS) SLM serves as the wavefront shaping device. We perturb the 1020 segment SLM using an orthogonal Walsh- sequence basis set with a modified version of three-point phase shifting interferometry. The nonlinearity of the fluorescence signal used for feedback ensures that the signal will increase when the spot size decreases, allowing compensation of phase errors in an iterative optimization process without direct phase measurement. We demonstrated *700 improvement in fluorescence beads signal, and *25 improvement for dye pool signal after aberrations correction.

We anticipate that the signal improvement shown here, will serve as a significant enhancement to current 3PM, allowing imaging deeper and with better resolution in biological tissues.

Passively Q-switched Tm-doped laser for bio-medical applications

Arik Korenfeld ,Daniel Sebbag and Salman Noach

Department of applied physics, Electro Optics Engineering Faculty, Jerusalem College of Technology, Jerusalem, 91160, Israel

Tm-based lasers at 1936nm and 1885nm in CW and passive Q-switch pulsed operation were developed.
High pulse energies, peak powers and good agreement with the model were achieved.
Laser interaction with tissue tested and showed successful ablation.

Lasers operating in the 2 μm region, especially pulsed lasers, have been proposed for applications in a wide variety of fields. The significant absorption in water and therefore in human tissue causes these lasers to be attractive for medical applications, such as surgery laser lithotripsy, laser angioplasty and ophthalmic procedures. Lasers at 2 μm work as a pump source for non-linear crystals or for Cr:ZnSe lasers and optical parametric oscillators. 2 μm laser sources are also attractive for military applications: this region is both eye-safe and shows low atmospheric absorption, making it an excellent wavelength for remote sensing, laser radar, infra-red countermeasures, and optical communications [1, 2].

In this paper Tm-based lasers will be presented at wavelengths of 1936 and 1885nm in CW and pulsed operation. For the laser experiments, a linear resonator was used, as seen in Figure 1. Maximum output power of ~3W in CW operation was achieved for pump power of 12W with slope efficiency of 30%. The pulses were obtained using a passive Q-switch with saturable absorber crystals Cr:ZnSe and Cr:ZnS. The experiments included Cr:ZnSe with initial transfers of T_0 = 93% and 85% and Cr:ZnS crystal with an initial transfer of T_0 = 89%.

High pulse energies of up to 4.31 mJ for Tm:YLF[3] and 1.85 mJ for Tm:YAP[4], correspond peak powers of 200 kW and 52 kW, respectively. Maximum average power reached 2.2 W. Concerning the three saturable absorbers, their measured pulse energies, pulse durations at FWHM and pulse repetition frequencies as a function of absorbed pump power are presented in Figure 2, together with the calculated peak powers.

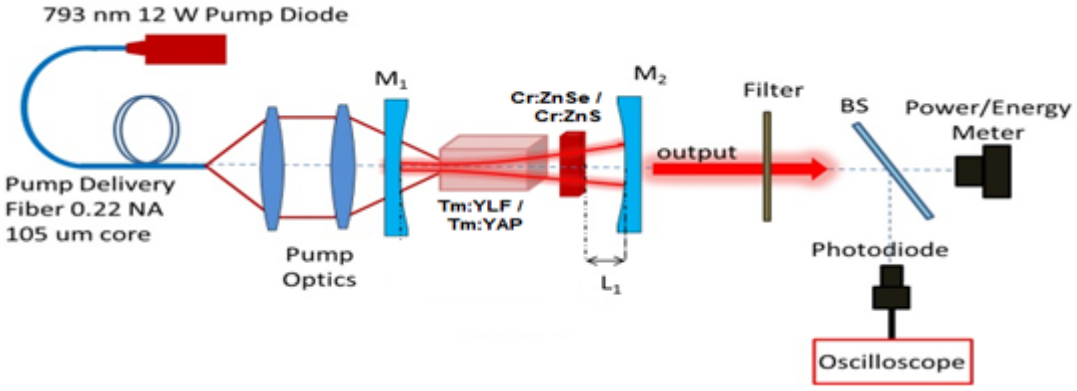

Figure 1. Schematic of the laser experimental setup

Experimental results showed good agreement with mathematical model that was developed. The model based on rate equations of the system and was extended to take into account additional effects.

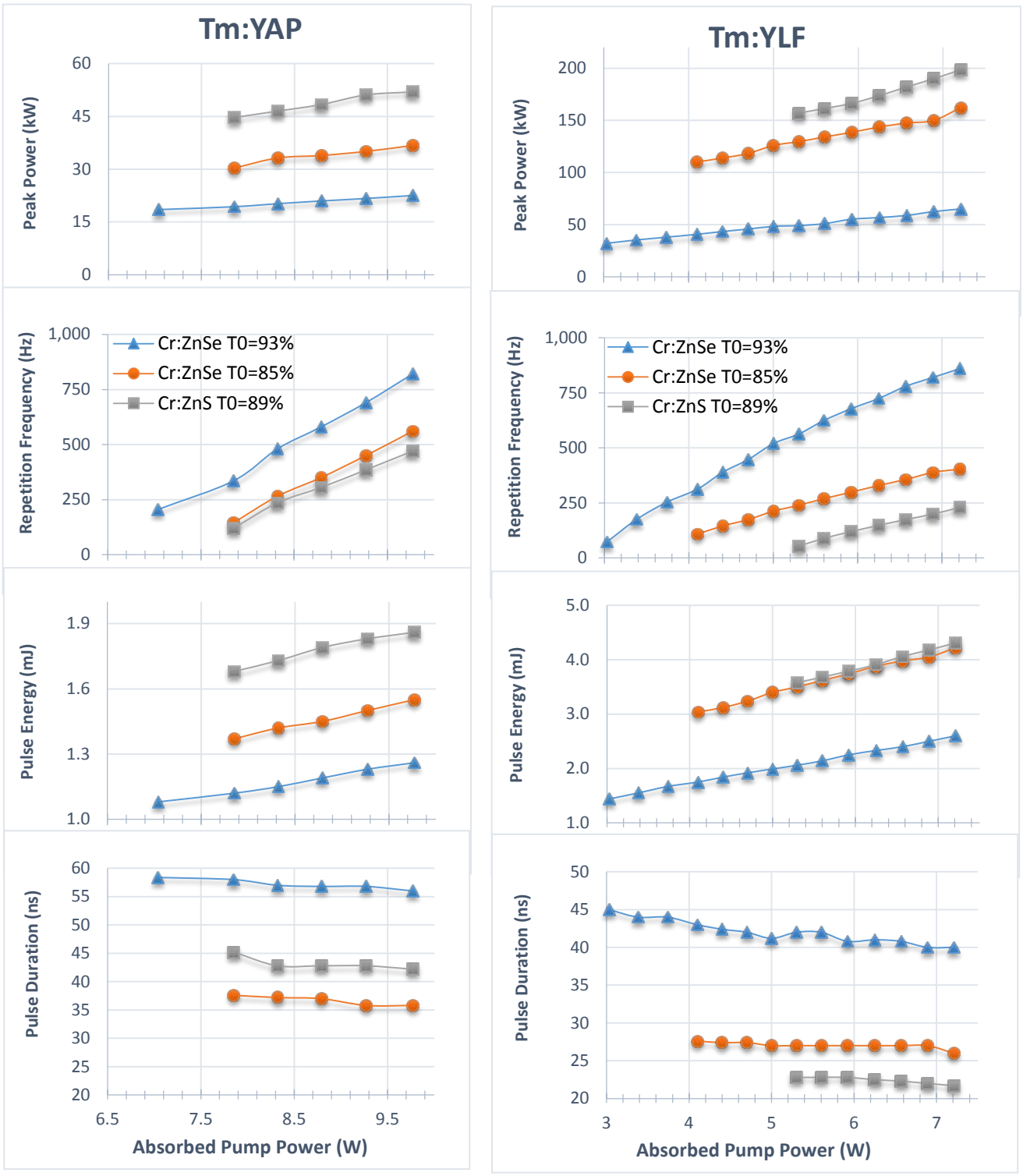

Figure 2. Pulse parameters for the passive Q switched Tm:YAP and Tm:YLF laser.

The next step was to examine the laser radiation interaction with biological tissues. In order to focus the beam emitted from the resonator a system including lenses and mirrors was built (see Figure 3). For the purpose of experiments pig intestinal tissue, chicken skin and cow's shoulder tissues were examined. The experiments were conducted both in CW and pulsed operation. Tissue samples were sent to histological examination to diagnose the tissue penetration depth and thermal environmental damage caused. The histology results of some of the tested samples showed successful ablation. Examples for histology results can be seen in Figures 5-6.

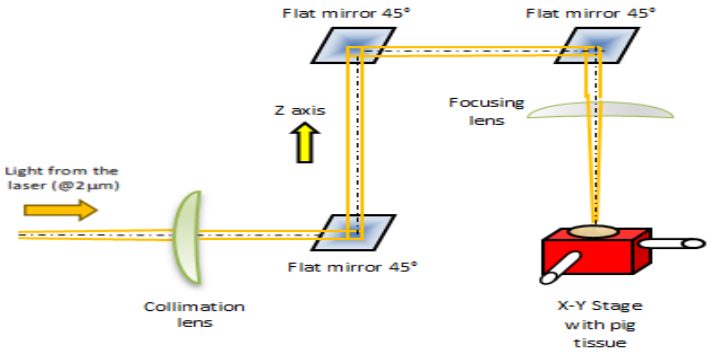

Figure 3. Schematic of the clinical experiments setup

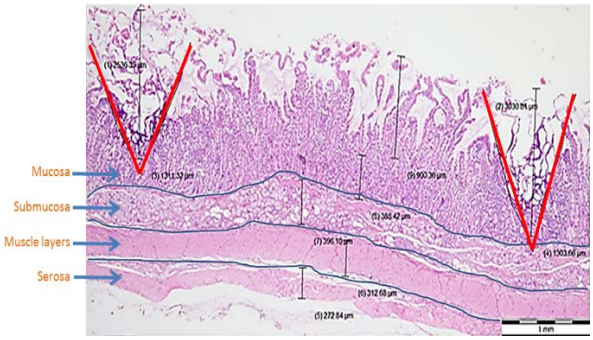

Figure 4. Typical histology image for CW operation. Zones hit by the laser and position of the pig tissue layers marked.

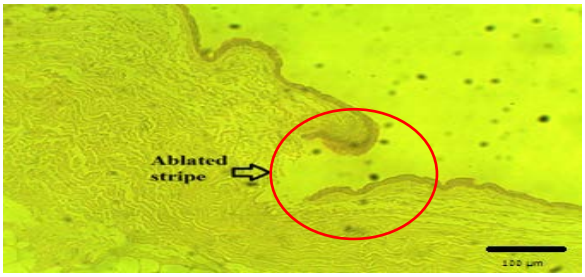

Figure 5. Chicken skin tissue cross-section of an ablated stripe. No thermal damage was indicated.

References

[1] Carrig T J 2004 Novel pulsed solid state sources for laser remote sensing *Opt. Eng.* **5620** 187–98

[2] Godard A 2007 Infrared (2–12 *μ*m) solid-state laser sources: a review *C. R. Phys.* **8** 1100–28

[3]. Arik Korenfeld , Daniel Sebbag , Udi Ben-Ami, Eran Shalom, Gilad Marcus and Salman Noach. " High pulse energy passive Q-switching of diode-pumped Tm:YLF laser by Cr:ZnSe". *Laser Phys. Lett.* **4,12** 2015.

[4]Daniel Sebbag, Arik Korenfeld, Udi Ben-Ami, Eran Shalom and Salman Noach "Diode End-Pumped Passively Q-switched Tm:YAP Laser with 1.85 mJ Pulse Energy". Optics Letters **40**(7) 1250-1253

Photonic biomedical mapping via manipulated magnetic nano particles

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Our main goal is to develop a new speckle based imaging modality for biological applications. The method is based on detection and manipulation of targeted conjugated magnetic nanoparticles (MNPs), which can specifically target cells or other live tissue, and form a concentrated assembly yielding speckle imaging capabilities. The MNPs detection technique employs temporal tracking of secondary speckle patterns while applying a magnetic excitation field that oscillates the particles. We experimentally demonstrate that placing MNPs in an alternating current (AC) electromagnetic field gives rise to modulation of incident light scattered from the material. It was found, via *in vitro* and two *ex vivo* experiments, that the resultant modulation spectrum of the speckle patterns is directly associated with the chemical properties of the MNPs and demonstrates the potential of the proposed speckle imaging method for detecting the presence of arterial plaque.

Real time tomographic phase microscopy of live cells using CUDA programming

Gili Dardikman and Natan T. Shaked

We present highly parallel and efficient algorithms for real-time reconstruction of the quantitative three-dimensional (3-D) refractive-index maps of biological cells without labeling, as obtained from the interferometric projections acquired by tomographic phase microscopy (TPM). The new algorithms are implemented on the graphic processing unit (GPU) of the computer using CUDA programming environment. The reconstruction process includes two main parts. First, we used parallel complex wave-front reconstruction of the TPM-based interferometric projections acquired at various angles. The complex wave front reconstructions are done on the GPU in parallel, while minimizing the calculation time of the Fourier transforms and phase unwrapping needed. Next, we implemented on the GPU in parallel the 3-D refractive index map retrieval using the TPM filtered-back projection algorithm. The incorporation of algorithms that are inherently parallel with a programming environment such as Nvidia's CUDA makes it possible to obtain real-time processing rate, and enables high-throughput platform for label-free, 3-D cell visualization and diagnosis.

Reflectance confocal microscopy of red blood cells: simulation and experiment

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The properties of red blood cells are remarkable indicators of the body's physiological condition; their density could indicate anemia or polycythemia, their absorption spectrum correlates with blood oxygenation, and their morphology is highly sensitive to various pathologic states including iron deficiency, ovalocytosis, and sickle cell disease. Therefore, measuring the morphology of red blood cells is important for clinical diagnosis, providing valuable indications on a patient's health. In this work, we simulated the appearance of normal red blood cells under a reflectance confocal microscope and discovered unique relations between the cells' morphological parameters and the resulting characteristic interference patterns. The simulation results showed good agreement with *in vitro* reflectance confocal images of red blood cells, acquired using spectrally encoded flow cytometry (SEFC) that imaged the cells during linear flow and without artificial staining. By matching the simulated patterns to the SEFC images of the cells, the cells' three-dimensional shapes were evaluated and their volumes were calculated. Potential applications include measurement of the mean corpuscular volume, cell morphological abnormalities, cell stiffness under mechanical stimuli, and the detection of various hematological diseases.

SESSION 7

Plasmonic femtosecond photoionization for nanometer scale radiation therapy

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Targeting individual cells within a heterogeneous tissue is a key challenge in cancer therapy, motivating numerous new approaches for cancer treatment that complement the shortcomings of conventional therapies. The small dimensions of isolated cell clusters require highly localized interactions that could be driven by focused laser beams; however, light-tissue interactions often involve macroscopic processes that may harm healthy nearby tissue. Here, we present a new technique for specific targeting of living cells using nanometric plasmonic photoionization. Using only a single, intense femtosecond laser pulse and specifically designed functional gold nanorods, we experimentally demonstrate rapid and effective cell death that is nonlinearly dependent on pulse duration and irradiance. The experimental results are supported by a detailed physical model for the pulse-particle-medium interactions. A good correlation is found between the simulated number and energy of the photo-generated free electrons and the observed cell death rates, suggesting that photoionization plays the dominant role in cell death.

Laser Bonding of Tissues

Abraham Katzir, School of Physics and Astronomy, Tel Aviv University, Israel

Laser welding of an incision in tissue involves the approximation of the edges and heating them, point by point, with a laser beam. In laser soldering, some biological solder (e.g. albumin) is applied on the approximated edges before the laser heating. Although there has been great interest in both of these methods for bonding of incisions and bonding of tissues, it has not gained wide acceptance by surgeons. We argue that the main obstacle has been the lack of temperature control, which led to weak bonding. We developed over the years a laser bonding system based on two optical fibers. One fiber was used to deliver the laser beam to heat a spot on the incision, be it a near-IR or mid-IR laser. A second fiber was an infrared transmitting AgBrCl fiber that delivered the mid-IR emitted from the heated spot onto a mid-IR detector. The signal generated by this detector was proportional to the temperature T of the heated spot and made it possible to monitor and control T. We found that if T was too low, the bonding was weak, and if T was too high, there was significant thermal damage. Optimally, each spot should be heated to T=60-65C for 6-10 seconds. The system was successfully used for laser soldering of incisions in the skin, dura, urinary bladder, cornea and kidney in live animals. It was also used clinically for soldering of incisions in the skin. We inserted the two fibers mentioned above into the body of a large pig via an endoscope and carried out endoscopic soldering. This would make it possible to carry out endoscopic bonding of incisions or tissues, during surgical procedures that make use of various endoscopic systems or even robotic systems. Laser soldering offers several advantages: it is more reproducible, it results in a watertight seal, and it leaves less scarring than suturing.

Study of chromatin organization in live cells by using advanced fluorescence imaging techniques.

I. Bronshtein, E. Kepten, S. Berezin, I. Kanter, M. Lindner, S. Gonzalo, R. Foisner, Y. Shav-Tal and Y. Garini

The organization of the genome in the nucleus is believed to be crucial for cellular functions. It's known that chromosomes fold into distinct territories, but little is known about mechanisms of chromosome territory maintenance. We describe the critical impact of lamin A and lamin A-associated polypeptide LAP2α on chromatin dynamics, demonstrating their prominent role in maintaining a genomic organization.

We used Single Particle Tracking to characterize the diffusion properties of different genomic regions in live cells. Chromatin diffusion in normal cells is found to be slow and anomalous; in vast contrast, depletion of lamin A protein results in faster dynamics and the chromatin diffusion transforms from anomalous to normal diffusion. Depletion of LAP2α plays a reciprocal role, as it slows the chromatin dynamics and does not change the diffusion type.

Continuous photobleaching measurements revealed that LAP2α down-regulates binding of lamin A to the chromatin.

We suggest that constrained chromatin mobility decreases probability for genomic aberrations such as translocations and insertions. We applied Spectral Karyotype technique to $Lmna^{+/+}$ and $Lmna^{-/-}$ cell lines and we found that $Lmna^{-/-}$ cells have 30% higher frequency of genomic aberrations compared to cells that express lamin A protein.

These observations strongly suggest that the dynamics of chromatin in the nucleus is mediated by lamin A and LAP2α proteins. We propose that is restrained by lamin A protein through chromatin-chromatin bindings and controlled by LAP2α. Our model naturally provides rigidity to the nucleus and explains the mechanism that maintains the chromosomal territories.

Cellular superresolved imaging using temporally flickering nanoparticles

Tali Ilovitsh, Yossi Danan, Rinat Meir, Amihai Meiri and Zeev Zalevsky; BIU

This work presents a novel method that enables the simultaneous superresolved imaging of multiple types of gold nanoparticles (GNPs) that label targets of interest in biological samples. The method utilizes a lock-in technique at which the imaging of the sample is done using a number of time-modulated modulated laser beams that match the number of the types of GNPs that label a given sample, and resulting in the excitation of the temporal flickering of the scattered light at known temporal frequencies. The final image where the GNPs are spatially separated is obtained using post processing where the proper spectral components corresponding to the different modulation frequencies are extracted. The proposed method enables the detection of overlapping types of GNPs, at significantly sub-diffraction distances, making it attractive for super resolving localization microscopy techniques. In addition, by targeting each type of GNPs to different areas within the sample, the cite-specified areas can be simultaneously imaged. Furthermore, the spatial separation of the GNPs can be done even at poor signal to noise (SNR) conditions, where the inspected signal is indistinguishable in the given noisy environment.

Super resolved optical system for objects with finite sizes using circular gratings

Asaf Ilovitsh,[1,*] Vicente Mico,[2] and Zeev Zalevsky[1]

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We present a real time all optical super resolution (SR) method for exceeding the diffraction limit of an imaging system which has a circular aperture. In Field of view (FOV) SR the resolution improvement is achieved by exploiting unused parts of the FOV. Diffractive gratings are used in order to optically encode (and later on decode) the high resolution spatial data that is diffraction limited by the aperture. The high resolution information is also encoded into other areas in the FOV, thus there is a trade-off between the possible resolution improvement and the size of the inspected object. In our method, the SR is obtained using two fixed circular symmetric gratings which are placed in predetermined positions. The proposed method has several advantages over previous ones, where 2D Dammann Cartesian gratings were used for the SR process. Mathematically, the proposed circular gratings are 1D gratings as they are only radius dependent. As such, their design is simplified. In addition, since the gratings are rotating angle independent, the optical setup requires less calibration and alignment. Furthermore, the circular gratings generate synthetic circular duplications of the aperture. Thus, they seem to be the best choice for an optical system which has a circular aperture. The method is applicable for both spatially coherent and incoherent illuminations, as well as for white light illumination. The proposed method is presented analytically, demonstrated via numerical simulations, and validated by laboratory experiments.

SESSION 8

Using ultrasound modulated light for non invasive physiological monitoring

Dr. Michal Balberg
Tel Aviv University, Israel

Modulating coherent light with ultrasound waves, via the acousto-optic effect, enables localization of optical measurements in deep tissue. This localization is important for measurement of regional blood flow and oxygen saturation in tissue, particularly in the brain, where conventional near infrared spectroscopy is contaminated by signals from superficial, extracerebral tissue.We present experimental data from tissue mimicking phantom models and validation with Monte-Carlo simulations that demonstrate the ability to non-invasively monitor variations in blood flow in deep layers. In addition, clinical data demonstrating the value of for monitoring localized variations in cerebral blood flow will be presented.

Quantitative measurements in blood tissue by light scattering

Alberto Bilenca; BGU

Light scattering in biomedical research is used for characterizing tissue and for studying

their dynamical and mechanical properties. In the talk, we will present camera-phone-based laser speckle imaging to measure physiological parameters such as heart rate and blood perfusion noninvasively with applications in burn diagnosis.

Best practices of European instruments for technology R&D in Bio Photonics

Aviv Zeevi Balasiano
Israel Europe R&D Directorate

Around 52 M euro is available for funding in the Bio photonics field in the next two years through the horizon2020 and the photonics sensing program.
This lecture will demonstrate best practices of technology transfer through several European funding tools in the Bio photonics field.
The h2020 new funding instruments present a unique method based on international consortium for R&D.
The lecture also presents future opportunities for israeli entities and technology transfer in bio photonics the next two years .
In the last two years around 2M euro was delivered to relevant Israeli companies in the field: optiQGain Ltd., Opticul Diagnostics Ltd., BrightWay Vision ,Spring BioMed ,ELfi-Tech Ltd.

The lecture also present the European strategic research map in Bio photonics as developed by the photonics21 platform; new cost-effective methods for improved diagnosis and therapy and to control water and food quality, thereby reducing diseases caused by contamination

A novel method for sensing metastatic cells in the CSF of pediatric population with medulloblastoma by frequency domain FLIM system

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Brain tumors are the second leading cause of cancer-related deaths in children, after leukemia. Patients with cancer in the central nervous system (CNS) have a very low recovery rate. Today known imaging and cytology techniques are not always sensitive enough for an early detection of both tumor and its metastatic spread, moreover the detection takes a relatively long time. Medulloblastoma (MB) is the most common malignant brain tumor in children. The aim of our talk is to present the frequency domain fluorescence lifetime imaging microscopy (FD-FLIM) system as a possible method for an early detection of MB and its metastatic spread in the cerebrospinal fluids (CSF) within the pediatric population.

Fluorescence lifetime (FLT) is considered more advanced sensing method than the classical fluorescence intensity (FI) as it is not exposed to many of its artifacts. Furthermore FLT provides a means of probing changes in the local fluorophore's environment such as viscosity and pH. In frequency domain fluorescence lifetime imaging microscopy (FD-FLIM) the FLT is extracted from the amplitude attenuation and the phase shift between the FI emission and the exciting light source. This leads FLT measurements to provide a means of sensing changes in the local fluorophore physical, chemical and biological environment. Cancerous cells are known for changing several environmental factors include pH and the viscosity. As a result they can theoretically be sensed by the FLIM system.

In our talk we will present for the first time the variations of the FLT of DAPI in medulloblastoma (MB) patients. The cells were extracted from tumor and cerebrospinal fluids (CSF) of children diagnosed with MB following nuclear staining of DAPI via FD-FLIM technology. The FLT of cells from the original tumors and the metastatic cells was greatly extended (median 5.73ns, 6.47ns respectively; p-value=$8.231*10^{-9}$) relate to the normal/medium FLT measured in inflammatory samples from non-oncology pediatric patients who served as controls (median 2.6ns; p-value<$2.2*10^{-16}$). In addition short FLT value was measured in samples from children post treatment – either chemotherapy or craniospinal radiation (median 1.6ns; p-value<$2.2*10^{-16}$). These findings may pave the way for better detection of the tumors and metastatic cells, and may guide personally tailored treatment, improve outcome and increase survival as well as for others biomedical applications.

Full scattering profile for detecting physiological tissue properties

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Light reflectance and transmission from soft tissue has been utilized in noninvasive clinical measurement devices such as the photoplethysmograph (PPG) and reflectance pulse oximeter. Most methods of near infrared (NIR) spectroscopy focus on the volume reflectance from a semi-infinite sample, while very few measure transmission.
We have previously shown that examining the full scattering profile (FSP), which is the angular distribution of exiting photons, provides more comprehensive information when measuring from a cylindrical tissue, such as earlobe, fingertip and pinched tissue. Our hypothesis is that during respiration the change in blood vessel diameter is more significant than the change in optical properties.
In our first work [1] we have shown by Monte Carlo simulation that the FSP is less affected by larger blood vessel because of the "shielding effect", which decreases the effective scattering coefficient.
Furthermore, an isobaric point was found, which is not dependent on changes in the reduced scattering coefficient. The angle corresponding to this isobaric point linearly depends on the tissue diameter [2].
Lately we have investigated the role of multiple scattering and absorption on the FSP [3]. First we defined the range in which multiple scattering occurs for different tissue diameters. Next we demonstrated that the absorption linearly influences the intensity at each angle of the FSP and, more importantly, the absorption does not change the position of the isobaric point. Furthermore, this linear dependency also exists in multiple scattering, although the slope of this linear relation varies according to the scattering coefficient.
The findings of this work demonstrate a realistic model for optical tissue measurements such as NIR spectroscopy, PPG and pulse oximetery.

[1] H. Duadi, D. Fixler, and R. Popovtzer, “Dependence of light scattering profile in tissue on blood vessel diameter and distribution: a computer simulation study,” J. Biomed. Opt. 18(11), 111408 (2013).
[2] H. Duadi, I. Feder, and D. Fixler, “Linear dependency of full scattering profile isobaric point on tissue diameter,” J. Biomed. Opt. 19(2), 026007 (2014).
[3] H. Duadi and D. Fixler, “Influence of multiple scattering and absorption on the full scattering profile and the isobaric point in tissue,” J. Biomed. Opt. 20(5), 056010 (2015).

Three dimensional imaging using phase retrieval with two focus planes

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This work presents a technique for a full 3D imaging of gold-nanoparticles (GNPs) tagged cells using only two images, rather than many images per volume as is currently needed for 3D optical sectioning microscopy. The proposed approach is based on the Gerchberg-Saxton (GS) phase retrieval algorithm. The reconstructed field is free space propagated to all other focus planes using post processing, and the 2D z-stack is merged to create a 3D image of the sample with high fidelity. Since the method requires the capturing of two images only, it can be suitable for 3D live cell imaging. In addition, the method can yield a specific volume mapping within the cell by targeting the GNPs into this specific volume. The GNPs are excited by a laser illumination at a wavelength corresponding to their spectral absorption peak, and their scattering is being imaged at two different focus planes. The method is generic and applicable to all wavelengths, given GNPs with absorption peak that matches the laser's wavelength. The proposed concept also has the main advantage of retrieving the phase information while being a non-interferometric configuration.

Experimental evaluation of cMUT and PZT transducers in receive only mode for Photoacoustic imaging.

Omri Warshavski

Capacitive Micro machined Ultrasound Transducers (cMUT) are an alternative and promising developing technology that complement conventional ultrasound transducer technologies. The unique characteristics of cMUT, such as broadband response, design flexibility and natural packaging capabilities with Integrated circuit technology make them highly suitable for Optoacoustic signals detection and Photoacoustic imaging.

Commercial piezoelectric transducers that are commonly used in Photoacoustic or Photoacoustic systems are by design dedicated to perform both for transmit and receive operations.

cMUT transducers which offer higher degree of control and design flexibility over the transducer performances can specifically be optimized for “reception only” mode and have the potential to enhance the global performances of Photoacoustic\Optoacoustic architecture.

This work presents an experimental comparative analysis of the reception characteristics of individual elements from various cMUT and PZT 1D transducer arrays for superficial imaging. The benchmarking is focused on receive sensitivity, frequency response, angular response and noise equivalent pressure (SNR). We used a dedicate Analog Front End which enables high degree of adjustability and optimization of the reception chain parameters for different transducer types. The elementary RF signal analysis is followed by a K-Wave based simulation that links influence of tested transducer receive performances to the final image quality.

The goal of the characterization is to establish reliable requirements to support the development of two-dimensional CMUT array for real time, high frame rate three-dimensional Photoacoustic Imaging systems.

Experimental evaluation of cMUT and PZT transducers in receive only mode for Photoacoustic imaging. 89 words abstract

Capacitive Micro machined Ultrasound transducers (cMUT) is an alternative, rapidly developing technology which compliments conventional ultrasound transducer technologies. The unique characterizations of cMUT, such as broadband response, design flexibility and inherent\natural integer-ability with semiconductor technology makes them highly suitable for utilization for detection of Photoacoustic\Optoacoustic signals. This work presents an experimental based comparative analysis of the (reception) performances of cMUT and PZT transducers in terms of sensitivity, frequency response and angular\directivity response, followed by simulation based analysis of the influence of transducer the receive parameters on the image quality.

Experimental system of the full scattering profile of circular phantoms

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Human tissue is one of the most complex optical media since it is turbid and nonhomogeneous. Optical methods of sensing physiological tissue state base on light-tissue interaction are non-invasive, inexpensive and simplistic and therefore are very useful. Most of the optical methods are focused on the reflection light from the tissue, which is describes as a semi-infinite medium, while very few use the transmitted light.

We suggest a new optical method for sensing physiological tissue state, based on the collection of the ejected light at all exit angles, to receive the full scattering profile. We simulate the light propagation in homogenous and heterogeneous cylindrical tissues and obtain the full scattering profile. In addition we built a unique set-up for noninvasive encircled measurement. We use a laser, a photodetector and tissues-like phantoms presenting different diameters and different reduced scattering coefficients. Our method reveals an isobaric point, which is independent of the optical properties and linearly depends on the exact tissue geometry.

In addition, the blood vessels in human tissues are the main cause of light absorbing and also scattering. Therefore, the effect of blood vessels on light-tissue interactions is essential for biomedical applications based on optically sensing, such as oxygen saturation, blood perfusion and blood pressure. We present experimental measurements of the full scattering profile of heterogenic cylindrical phantoms which include blood vessels. We show the vessel diameter influence on the full scattering profile, and found higher reflection intensity for larger vessel diameters, despite the blood volume kept constant, accordance to the shielding effect. These findings can be useful for biomedical applications such as non-invasive and simple diagnostic of the fingertip joint, ear lobe and pinched tissues.

K-factor image deshadowing for three-dimensional microscopy

Tali Ilovitsh, Aryeh Weiss, Amihai Meiri, Carl. G. Ebeling, Aliza Amiel, Hila Katz, Batya Menessa Green and Zeev Zalevsky; BIU

This work presents a novel use of the nonlinear image decomposition technique called K-factor that reshapes the three dimensional (3D) point spread function (PSF) of an XYZ image stack into a narrow Gaussian profile. The experimentally obtained PSF of a Z-stack raw data that is acquired by a widefield microscope has a more elaborate shape that is given by the Gibson and Lanni model. This shape increases the computational complexity associated with the localization routine, when used in localization microscopy techniques. Furthermore, due to its nature, this PSF spreads over a larger volume, making the problem of overlapping emitters detection more pronounced. The ability to use Gaussian fitting with high accuracy on 3D data can facilitate the computational complexity, hence reduce the processing time required for the generation of the 3D superresolved image. In addition it allows the detection of overlapping PSFs and reduces the effects of the penetration of out of focus PSFs into in focused PSFs, therefore enables the increase in the activated fluorophore density by ~50%. The algorithm was tested both on simulated data and experimentally, where it yielded an increase in the localization accuracy by ~60% with compare to regular Gaussian fitting, and improved the minimal resolvable distance between overlapping PSFs by ~50% .