

**OASIS**

2007  
March 26-27  
מרכז הדיזיין, חיפה

The 11<sup>th</sup> Meeting on Optical Engineering and Science in Israel  
הכינוס האחד עשר לאופטיקה, אלקטרואופטיקה והנדסה אופטית

# Session 3

## Posters:

# OPTIMAL STRATEGIES FOR SPECTRAL UNMIXING WITH A HIGH RESOLUTION SPECTRAL MICROSCOP

Daniel Cohen<sup>1</sup>, Stanley R. Rotman<sup>1</sup> and Aryeh M. Weiss<sup>2</sup>

<sup>1</sup>*Ben-Gurion University in Ber-Sheva*

[dunicorn@gmail.com](mailto:dunicorn@gmail.com)

<sup>2</sup>*Bar Ilan University, Ramat Gan*

In biological microscopy, multiple fluorescent dyes are often used to simultaneously label various intracellular components. Moreover, it is often necessary to resolve small changes in the relative strength of two or more fluorescents .

However, the number of labels that can be resolved in one sample using standard fluorescence microscopy is limited because of the similarity of the spectral signatures of the fluorescent proteins and dyes which are presently available.

Spectral imaging and linear unmixing enable us to discriminate between fluorescent proteins or dyes with highly overlapping emission spectra i.e., spectral emissions which are not orthogonal to each other. Moreover, these methods can also be used to quantitate changes in relative emission from spectrally overlapping dyes, as is needed for methods such as FRET (Foerster Resonant Energy Transfer).

In this lecture, we will explain the spectral imaging method and present the latest methods of linear unmixing which are used to counter the problem of the non orthogonal spectral signatures. We will also demonstrate these ideas on spectra collected with a high resolution spectral microscope.

# **SENSITIVITY ENHANCEMENT OF GUIDED WAVE SURFACE PLASMON BASED BIOSENSORS**

**Amit Lahav and I. Abdulhalim\***

*Department of Electro-optic Engineering*

*Ben Gurion University, Beer Sheva 84105, Israel*

SPR (surface plasmon resonance) and GWSPR (guided wave SPR) are phenomena that are used in sensors as methods for investigating the contents of gases and biological or biochemical substances. They were widely investigated in the last two decades and are practically used in many applications today. We are investigating possibilities of improving their sensitivity and stability by modifying the multi-layered structure adjacent to the metal layer. Several configurations will be presented showing that in some cases it is possible to improve the sensitivity by one to two orders of magnitude. Surface electromagnetic waves appearing with one-dimensional photonic crystals are also evaluated for biosensing and compared to the SPR or the GWSPR sensor. Periodic structures containing both dielectric and metallic layers exhibit additional resonances that never investigated before.

## ACOUSTO-OPTICS TOMOGRAPHY ON LIVING MOUSE

A. Lev, M. Ohana\*, M. Rosilio\*, D. Issachar, S. Granovsky, Y. Eisen, B. Sfez  
*Soreq NRC, Electro-Optics Division, NRC Soreq, 81800 Yavne, Israel*  
[lev@soreq.gov.il](mailto:lev@soreq.gov.il), [bruno@soreq.gov.il](mailto:bruno@soreq.gov.il)

**Background:** The increasing success of functional medical imaging techniques such as PET drives the development of new techniques that can supply similar information in small clinics at lower price. It is well known that potentially optical methods(s) can give functional information such as blood concentration and saturation and can measure other pigments in the body. But in contrast with X-ray radiation, the optical waves strongly scatter inside the body, thus preventing imaging. We have developed an optical tomography technique that combines ultrasound and the scattered light to enable acquisition of 3D information inside the body.

Since the interpretation of optical functional images is not straightforward and can lead to misunderstanding of artifacts, we have decided to compare the optical images with images obtained using nuclear imaging.

**Methodology:** We have modified polystyrene microspheres dyed with a near-infrared dye (Duke Inc) by filling them with a radionuclide. The obtained microspheres were therefore contrast agents both for the optical and for the nuclear imaging methods. The microspheres were then injected in the mouse and imaged using a small gamma camera composed of CdZnTe (CZT) detectors. The mouse was then killed and a radionuclide analysis was performed. A new, similar mouse was then used under the same conditions and optically imaged using our method.

**Results:** The imaging results with the CZT gamma camera show that the microspheres were concentrated essentially in the liver and in the kidneys, as shown with the nuclear imaging. The optical imaging clearly revealed these two organs, however we could not establish that we imaged the microspheres, but rather directly the high level of blood concentration.

**Conclusion:** We have demonstrated for the first time high resolution optical imaging in living mice (resolution: 2 mm, imaging depth: 2 cm) in vivo. We have compared these images with nuclear imaging and showed an excellent agreement.

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\*Also with Jerusalem College of Technology, Jerusalem, Israel

# **OPTICAL WAVEGUIDE BIOCHEMICAL SENSORS BASED ON TRANSITION OF MODES AT ABRUPT DISCONTINUITY**

**R.Levy, S.Ruschin**

*Department of Physical Electronics, School of Electrical Engineering*

*Faculty of Engineering, Tel-Aviv University, Tel-Aviv 69978 Israel,*

*ronenle1@tau.ac.il*

Integrated optical devices have been increasingly used for chemical and biochemical sensing applications due to their features such as high sensitivity, miniaturization, mechanical stability, and freedom from electromagnetic interference. Some of the widely adopted configurations used for these sensors are phase sensing in a Mach Zehnder Interferometer and attenuation sensing in an SPR configuration.

In this presentation we shall describe a new scheme of enhanced sensitivity for Integrated Optics sensors based on transition of modes at abrupt discontinuity. This method allows combined sensing of modal changes including amplitude and phase in SPR sensors. This method is also applicable to phase sensors, allowing a single channel phase sensing without the need of a reference leg. Calculated values show sensitivities of an order of magnitude higher compared to previously reported SPR sensors.

The scheme is extremely simple, consisting of a single waveguide structure supporting two guided modes acting as sensor section. The two modes are made to interfere by an abrupt discontinuity of the sensing waveguide into a thinner single-mode output waveguide. The geometrical parameters of the entire scheme are chosen as to produce a delicate balance very sensitive to small changes in the waveguide's environment.

Additional sensitivity and extended working range can be gained by allowing external control of the differential phase between the propagating modes.

# **AC COUPLED MULTI-WAVELENGTH DETECTION FOR SPECTRAL PHOTOPLETHYSMOGRAPHY AND PHOTO- ACOUSTIC EXPERIMENTS**

**German Tsvilikhovski, Boris Epstein and I. Abdulhalim\***

*Department of Electro-optics Engineering*

*Ben Gurion University of the Negev*

*Beer Sheva 84105*

Multi – wavelength photo-acoustics (PA) and photoplethysmography (PPG) techniques are believed to give comprehensive clinical information by measuring small changes in blood content in arteries and capillaries. The signal measured in the PA and PPG has however large dc content which is usually removed by filtering of low frequency components. When simultaneous multi-wavelength or spectral detection is required the detector is not a single pixel, rather a large array of small detectors such as a CCD or PIN diode array. There are several issues involved in the design of multi–wavelength PA or PPG such as the light source, filtering and amplification, in order to obtain clean informative signal. Possibilities of using existing spectrometers as well as new designs for such AC coupled spectrometers are being considered. We shall present the design and some preliminary results of measurements using transmitted signals through the human finger.

[\\*abdulhlm@bgu.ac.il](mailto:*abdulhlm@bgu.ac.il)

# **A FIBER-OPTIC SENSOR FOR MEASURING RESIRATORY CHANGES IN CHEST-CIRCUMFERENCE**

**M. Pinchas, A. Avraham, I. Faib, A. Babchenko, S. Mizrahi and M. Nitzan**

*Department of Applied Physics/ Medical Engineering, Jerusalem College of Technology,  
Jerusalem, Israel. E-mail: Nitzan@jct.ac.il*

The significance of the respiratory-induced fluctuations in the heart-rate and in the arterial blood pressure is their relationship to sympathetic and parasympathetic activity. In order to study the temporal relationship between respiration and peripheral hemodynamics, a novel simple sensor for the measurement of the respiratory-induced changes in chest-circumference has been developed, and was used simultaneously with the photoplethysmographic (PPG) signal, which reflects the cardiac-induced increase in the tissue blood volume during systole. The sensor principle is based on the dependence of light transmission through bent optic-fiber on its radius of curvature. Part of the light, which is totally reflected in and transmitted through straight optic-fiber, escapes through its cladding when the fiber is bent, because for some light rays the angle to the normal of a light ray is lower than the critical angle.

The respiration sensor is composed of an elastic chest belt, on which a bent multimode optic-fiber, of variable radius of curvature was connected. Light from an infrared LED is transmitted through the optic-fiber and measured by a PIN diode photodetector. The radius of curvature increases during inspiration, resulting in higher light transmission. The respiration sensor was found to be sensitive enough for the measurement of respiration-induced chest circumference changes, and was even capable of detecting the small changes in chest circumference induced by aorta movement during cardiac systole.

Two PPG devices, composed of infrared LED and photodetector were attached to a finger and to the cheek. The PPG photodetector showed oscillations in the heart rate – the PPG signal. Simultaneous measurements by the PPG sensors and the depth-of-breath sensor demonstrated increase of the PPG baseline during inspiration, for both finger and cheek. Since PPG baseline is inversely related to tissue blood volume, our examinations demonstrated decrease of tissue blood volume during inspiration, probably due to higher sympathetic activity. Generally, no difference was found between finger and cheek respiratory-induced fluctuations, though the finger is innervated only by the sympathetic nervous system, while the cheek is innervated by both the sympathetic and the parasympathetic nervous systems.

## **SCHEMATIC EYE MODEL**

**Alexander Sudakov and I. Abdulhalim\***

*Department of Electro-optic Engineering  
Ben Gurion University, Beer Sheva 84105, Israel*

The importance of having most accurate human eye model is a main problem in wide areas like instrumental optics, spatial vision, optometry, eye surgery, etc. Implementation of eye model in optical design tool helps to perform analysis of different eye components, study more about complex human eye system, predict estimated results, and save practical experiments. We present detailed analysis of several schematic human eye models and compare their performance in terms of the MTF, aberrations and dispersions using ray trace optical design software. The models considered in this work are Emsley, Gullstrand (No. 1 and No. 2), Le Grand, Koojimaan, and a newly reported one by Siedlecki-Kasprzak that takes into account the radial variation of the lens refractive index. We propose a new model that provides better description of the image and the aberrations. One of our purposes is to determine if eye models can simulate the visual performance of normal human eyes under conditions of varying low myopic blur, pupil size, and contrast.

[\\*abdulhlm@bgu.ac.il](mailto:*abdulhlm@bgu.ac.il)

# OPTICAL COHERENCE TOMOGRAPHY WITH ANNULAR APERTURE

**Lior Liraz, Ron Friedman and I. Abdulhalim\***

*Department of Electro-optics Engineering*

*Ben Gurion University of the Negev*

*Beer Sheva 84105*

[abdulhlm@bgu.ac.il](mailto:abdulhlm@bgu.ac.il)

In Optical Coherence Tomography (OCT), both high axial resolution and high lateral resolution are desired. While axial (Z) resolution is achieved by a broadband source, lateral (XY) resolution is achieved by high NA objective in the sample arm. Unfortunately, there is a trade-off between the two resolutions: increasing the objective NA, decreases the spatial coherence length, hence decreasing the depth of field i.e. increasing axial resolution. However, it is known [1], that annular aperture increases the depth of field. We propose here to incorporate annular lens within an OCT system in order to achieve high lateral resolution with minimal affection on the axial resolution. A MATLAB<sup>TM</sup> model was developed, simulating various annular apertures and showing the predicted enhancement when incorporating such apertures. Experimental setup was built to verify the simulations.

[1] S. Ledesma, J. C. Escalera, J. Campos, J. Mazzaferri, M. J. Yzuel, "High depth of focus by combining annular lenses," Opt Commun Vol 266 (2006) 6-12

\*abdulhlm@bgu.ac.il