

Cultured neuronal networks: Not so dull after all

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Cultured cells are one of the most powerful tools for biological investigations (1). Various cell types such as endothelial cells (cells that line the interior surface of blood vessels) and heart muscle cells, can be cultured in vitro and can be maintained for prolonged periods (weeks and months). While lacking the complexity of in-vivo system, they nonetheless exhibit rich and biologically relevant behaviors making them suited for examination of various cell activities such as metabolism, proliferation, cell programmed death and mechanical sensitivity, to name just a few.

What can be learnt from cultured neurons? Neurons, the main building blocks of the brain, are the core of our complex and mysterious mental facilities. Although individual neurons implement a variety of computational tasks (2), the collective activity of a network of neurons clearly surpasses the capacity of individual neurons to sustain and process information (3). Indeed, information processing in neuronal networks relies on the network's ability to generate temporal patterns of action potentials, the fundamental language of neuronal networks. These patterns have been intensively investigated at the individual neuron level, but the underlying principles of the collective network activity, such as the synchronization and coordination

between neurons, are largely unknown. Moreover, the interplay between the complex neuronal circuit's architecture (form) and its activity (function) is yet to be determined. The question is how much of the beauty and complexity of the brain can be retained in an artificially cultured neuronal system in which the neurons are uniformly distributed over a two-dimensional surface. Previous studies exploring the electrical activity of these cultured neuronal networks demonstrated fairly plain activity (4), amounting to network wide synchronous activity akin to well characterized synchronous brain activity during early development (5).

In our exploration we set to find whether cultured neural networks can exhibit more complex patterns of activity. Our underlying hypothesis was that function must follow form. We accordingly started by engineering our neurons into patterned networks with non-uniform architectures (Figure 1). We then set to explore the collective activity of these networks. While several other teams have used similar approaches in the past, we chose to base our patterning approach on innate neuronal migration processes by which developing brains self-organize. Therefore we were able to form extremely stable

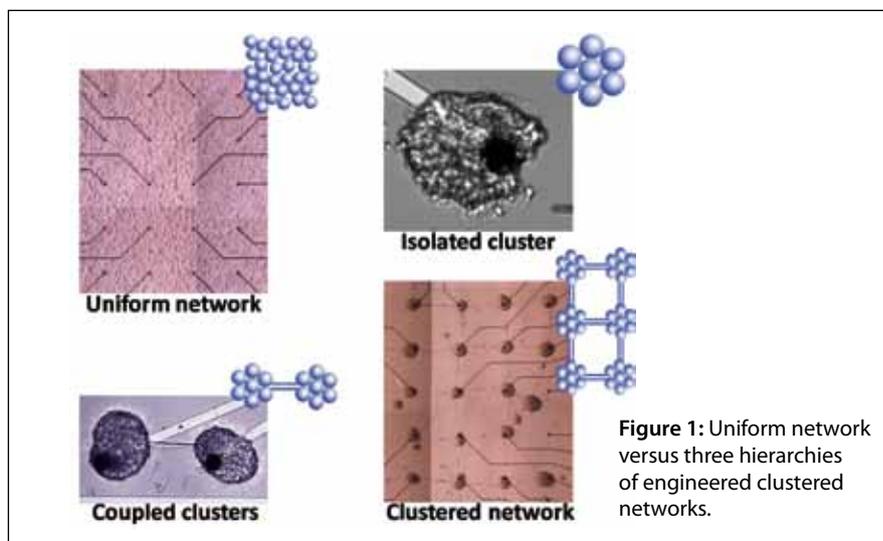


Figure 1: Uniform network versus three hierarchies of engineered clustered networks.

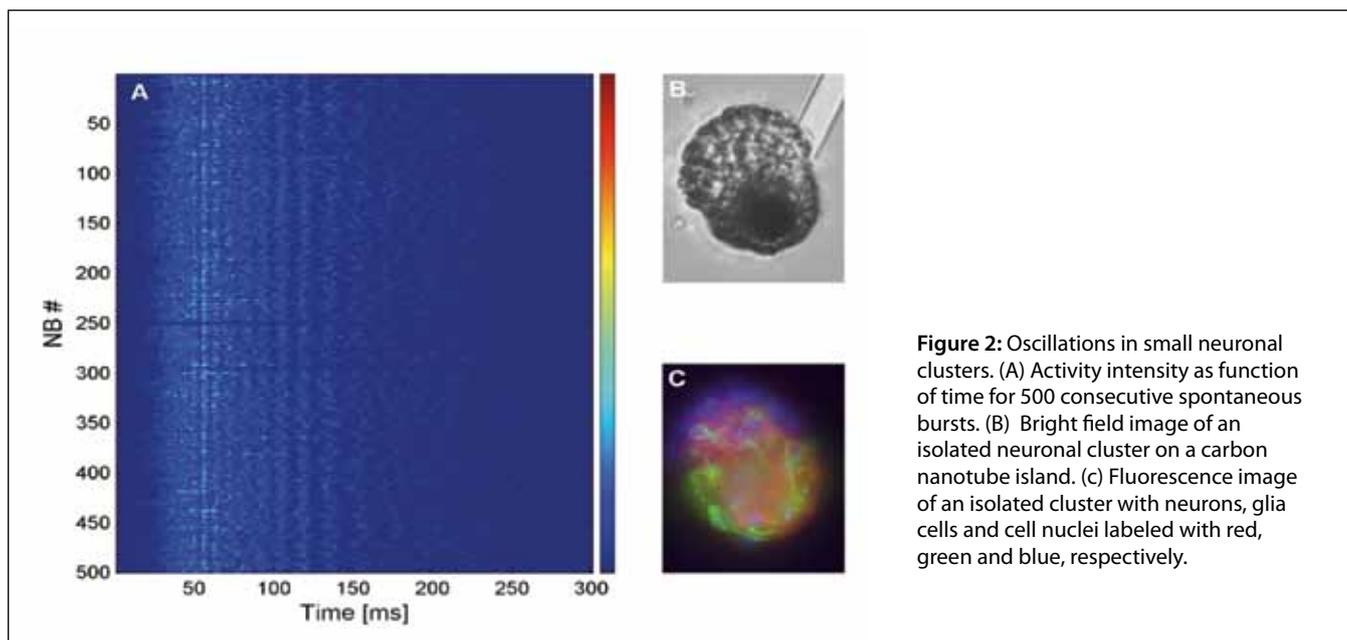


Figure 2: Oscillations in small neuronal clusters. (A) Activity intensity as function of time for 500 consecutive spontaneous bursts. (B) Bright field image of an isolated neuronal cluster on a carbon nanotube island. (c) Fluorescence image of an isolated cluster with neurons, glia cells and cell nuclei labeled with red, green and blue, respectively.

cultures which exhibit clear and robust electrical activity. One of the main contributing factors to our successful patterning method is the use of carbon nanotubes (as well as other materials) as a platform for constructing small neuronal clusters which we can then investigate with great fidelity (6). We also used these special carbon nanotubes as electrodes to record the electrical activity of the neuronal clusters.

From the outset it became clearly apparent that patterned networks exhibit markedly different activity characteristics than those of uniform networks. To focus the investigation we begun by exploring the activity of very small populations of cells (7); These are isolated neuronal clusters made of only few tens to few hundreds of cells. Our investigation focused on simple, yet fundamental questions: What is the minimal number of cells needed to exhibit collective dynamics? What are the internal temporal characteristics of such dynamics and how do the temporal features of network activity alternate upon crossover from minimal networks to large networks?

By analyzing the data from clusters of different sizes we were able to assert that small clusters made of as few as 40

cells already exhibit spontaneous collective events. The duration and rate of the network events scale with cluster size but converge to that of large uniform networks. More interestingly, these collective events are characterized by innate synchronous network oscillations in the range of 25 to 100 Hz (Figure 2). The consistent emergence of similar activity across networks of different size and morphology, suggests that neuronal clusters self-regulate their activity to sustain network bursts with internal oscillatory features. We therefore suggest that clusters of few tens of cells can serve as a minimal but sufficient functional network, capable of sustaining oscillatory activity with frequencies similar to those observed in-vivo. The underlying principles of this activity is currently under investigation.

Additional activity features were revealed following the coupling of two clusters into a network (Figure 1 – coupled clusters). Whereas both uniform networks and isolated clusters showed either sporadic or synchronized activity, coupled clusters exhibited also partially synchronized activity. Activity either propagated between clusters yielding mutual network events or was confined to only one of the clusters showing individual behavior. The manifestation of

such gated activation was amplified in networks of many connected clusters (Figure 1 – clustered network). In these networks, the propagation of collective events was also observed. However, these events varied in size and intensity, from confined activation in single clusters to massive recruitment of the whole network producing complex activation and propagation patterns.

Clearly, the complexity of neurons in culture is a far cry from that of even the simplest in-vivo neuronal circuit. Yet, by controlling their organization, new and exciting features can be revealed and may shed light at how these networks develop and function. Clearly, cultured neurons are not so plain after all.

References

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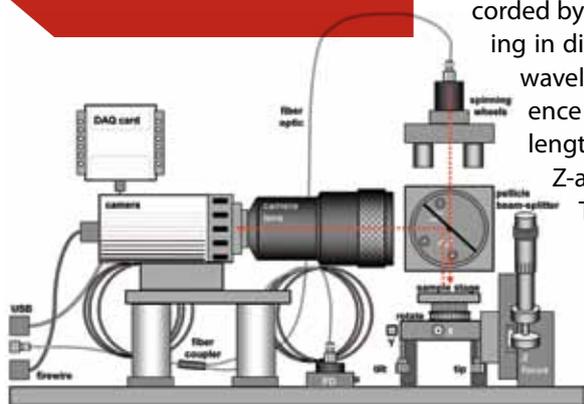
The Nanotechnology Center 7th Annual Workshop



On February 1st the Nano center researchers and students went north to Ha'Goshrim resort hotel for the Fred Chaul workshop of the Center for Nanoscience and Nanotechnology. This is the 7th annual workshop of the center, which by now has become a tradition. While enjoying wonderful food and deserts we heard 19 talks, looked at many posters and most importantly mingled both socially and scientifically. The talks spanned the wide spectrum of disciplines associated with the center: from solid state NMR for complex systems, through uses of nano-particles for probing immune response or for catalyzing fuel cell reactions, to quantum phase transitions in superconductors. Two fascinating plenary talks were given by Dror Seliktar (Technion) and

Moty Heiblum (Weizmann). This year we have increased the number of student-presented talks to 5 full-length oral presentations, chosen among the submitted abstracts by the scientific committee. The poster flash session was very lively as always, with 21 short spotlights by poster-presenting students. Three "best-poster" prizes were awarded. You can now enjoy video recordings of all the presentations at the Tel Aviv University TAUVID channel on YouTube. Finally, we should mention the pleasant and collaborative environment during the meeting which reflects the spirit of the nano center as a place for sharing not only equipment and facilities but also ideas and knowledge.

New equipment in the Center



SRIB is a new label-free sensing technique based on optical phase differences that result due to accumulation of biological material on solid substrates. The working-principle of the technique is the quantification of interferometric signals to accurately measure the optical thickness of a transparent film. The phase difference, causing the interference, results from the layered structure of the substrate - a silicon wafer with a top layer (ca. 10-20 μm) of thermally grown silicon dioxide (SiO_2) and the buried reference-surface at the SiO_2 - Si interface. A laser beam illuminating the wafer is reflected from the two different layers (the top surface of the chip and the reference interface) and recorded by the camera. These two reflections interfere with each other, resulting in differences in the magnitude of the total reflected light at a specific wavelength. These differences depend on the optical path-length difference (OPD) between the reflecting surfaces and the interrogating wavelength. Hence, variation in light intensity is translated into differences in Z-axis thickness of the transparent film, at sub-nanometer resolution. The transparent film can consist of the SiO_2 layer plus any additional material bound to its surface. Applying a spot of antigen to the top surface creates a thin protein layer that causes a local increase of the OPD (oxide thickness plus bound protein). The increased OPD in turn causes measurable phase retardation. Further retardation occurs upon binding of antibody to the antigen, where each binding event is detected as a measurable change in local thickness.

Bifurcation Topology Amplification

Ron Lifshitz

Ron Lifshitz from the School of Physics, in collaboration with the groups of Michael Roukes and Michael Cross at Caltech, have recently described a novel and generic amplification scheme based on inducing dynamical changes to the topology of a simple bifurcation diagram. Previous amplification schemes have utilized static bifurcations in nonlinear dynamical systems for amplification; biasing the operating point at locations where the response of such systems is highly sensitive to input. In a new publication [1], which was highlighted in Physics [2], the authors proposed and demonstrated a conceptually new approach, in which tiny variations in an input signal change the overall topology of the bifurcation diagram. This, in turn, leads to a dramatic change in the overall response of the system. They have demonstrated this new principle with a dynamical system consisting of a pair of coupled, parametrically-driven high frequency nanoelectromechanical systems (NEMS) resonators. Without coupling, owing to the fact that parametric response occurs at half the driving frequency, the two resonators are free to oscillate either in-phase or out-of-phase, giving rise to a so-called pitchfork bifurcation. However, the slightest coupling between the resonators breaks this symmetry, and

Innate Synchronous Oscillations in Freely-Organized Small Neuronal Circuits

M. Shein Idelson, E. Ben-Jacob E, Y. Hanein

Information processing in neuronal networks relies on the network's ability to generate temporal patterns of action potentials. These patterns have been intensively investigated at the individual neuron level, but the underlying principles of the collective network activity, such as the synchronization and coordination between neurons, are largely unknown. In a recent project we utilized micro-patterning techniques to engineer neuronal clusters of different sizes in order to systematically study the neuronal activity of small neuronal populations. We focus on simple, yet fundamental questions: What is the minimal number of cells needed to exhibit collective dynamics? What are the internal temporal characteristics of such dynamics and how do the temporal features of network activity alternate upon crossover from minimal networks to large networks?

We found that small clusters made of as few as 40 cells already exhibit spontaneous collective events characterized by innate synchronous network oscillations in the range of 25 to 100 Hz. The duration and rate of the network events scale with cluster size but converge to that of large uniform networks. The consistent emergence of similar activity across networks of different size and morphologies, suggests that neuronal clusters self-regulate their activity to sustain network bursts with internal oscillatory features. We therefore suggest that clusters of few tens of cells can serve as a minimal but sufficient functional network, capable of sustaining oscillatory activity with frequencies similar to those observed in-vivo.

More details: Shein Idelson M, Ben-Jacob E, Hanein Y, PLoS ONE 5(12), e14443 (2010)

alters the topology of the pitchfork bifurcation in one of two ways, depending – with exceptional sensitivity – on whether this coupling is attractive or repulsive. By controlling this coupling through the application of a small input signal, one obtains a novel amplifier, which the authors have termed a Bifurcation-Topology Amplifier (BTA).

In the reported work Lifshitz and his co-workers first introduce the BTA conceptually in a broad theoretical context, and then provide a detailed account of a first experimental implementation of the BTA using a pair of coupled NEMS resonators. The resonators are parametrically driven using piezoelectric actuation, their coupling is tuned by a small external input voltage, and their response is subsequently measured using optical interferometry – all in a room-temperature, table-top setup. Their BTA is capable of measuring a signal that

corresponds to a fraction of an electron charge applied to each resonator's conducting layer. This makes the first BTA already one of the most sensitive room-temperature mechanical charge detectors demonstrated to date.

The principles that underlie bifurcation topology amplification are simple and generic. Accordingly, the authors believe that their scheme may be applicable to a wide variety of physical systems – ranging from mechanical resonators to such systems as laser cavities, superconducting resonators, coupled Josephson junctions, and possibly even oscillating chemical and biological systems.

[1] R.B. Karabalin, Ron Lifshitz, M.C. Cross, M.H. Matheny, S.C. Masmanidis, and M.L. Roukes. Phys. Rev. Lett. 106 (2011) 094102.

[2] M.J. Dykman, Physics 4 (2011) 16.

Closed Orbits and Light Trapping in Anti-Symmetrically Mirrored Waveguide Layouts

Shlomo Ruschin, Ohad Dahan and Amos Hardy

Negative refractive index materials (NIM) have been continuously promoting investigation over past years due to a host of remarkable effects. We investigated configurations of waveguides composed by both, regular dielectrics and negative-index materials disposed in an anti-symmetric way with respect to the optical axis [1]. We demonstrated the existence of closed orbits within both a geometrical and electromagnetic modal pictures, as can be seen in the figures below. Furthermore, when cores are allowed different widths, continuous control of the group velocity of the propagating light is possible.

More details: S. Ruschin, Opt. Express 18, 20681 (2010)

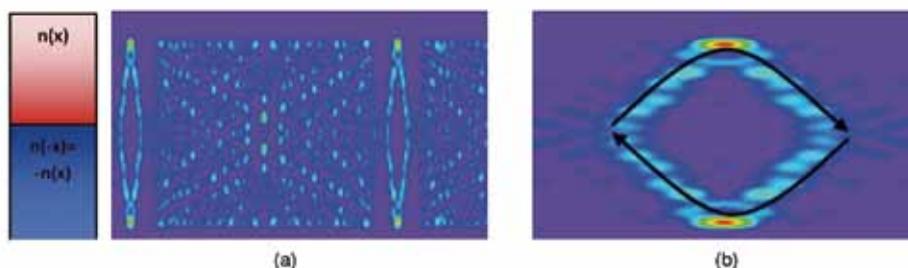


Figure 1: Multimodal patterns in the interface between anti-symmetrically mirrored materials showing closed orbits. (b) Is an enlarged portion of an orbit in (a).

Thermoplastic Nano-Imprinting Lithography for the Formation of Patterned Thin Layers of P(VDF-TrFE-CFE) and Poly(dimethylsiloxane) (PDMS)

Leeya Engel, Jenny Shklovsky, David Schreiber, Yelena Sverdlov, Slava Krylov, Yosi Shacham-Diamand

Thermoplastic Nano-Imprinting Lithography (T-NIL) has been used to form patterned, ultra thin layers from two kinds of polymer, P(VDF-TrFE-CFE) and poly(dimethylsiloxane) (PDMS). The periodic features formed on the micron scale layers are $2 \mu\text{m}$ wide and $1.5 \mu\text{m}$ high. The critical advantage of the T-NIL process for polymers is that the formation of the thin layers is simultaneous with the patterning as opposed to using separate processes for each step. The deposition of a hydrophobic silane monolayer on a Si stamp makes such imprinting possible by reducing the adhesion between the polymer and stamp. Silanizing both substrate and stamp using this technique has enabled the application of this method

to the fabrication of micron-scale flat membranes of PDMS that are free-standing.

PDMS is an optically transparent, fully biocompatible, dielectric polymer and is the most popular elastomer used in MEMS. Poly(vinylidene fluoride), commonly known as PVDF, and its copolymers are the most widely exploited polymers that exhibit ferroelectric behavior. Recently, a novel PVDF terpolymer, P(VDF-TrFE-CFE) [poly(vinylidene fluoride - trifluoroethylene - chlorofluoroethylene)], has been shown to exhibit large electrostrictive strains. This along with its high modulus ($>0.4\text{GPa}$) make the terpolymer attractive for Microelectromechanical Systems (MEMS) actuation.

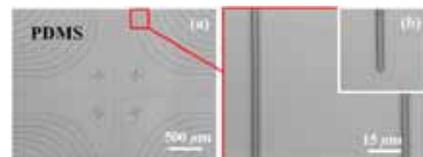


Figure 1: Optical photographs of imprinted PDMS stripes on a Si substrate: (a) a part of a chip, (b) a close up of one of the stripes

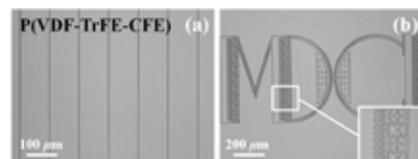
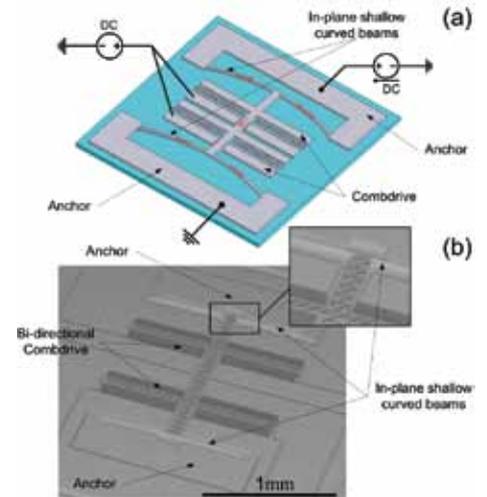


Figure 2: Optical photographs of thermally imprinted P(VDF-TrFE-CFE) features on Si substrate: (a) stripes (b) logo of TAU MEMS Design and Characterization Laboratory

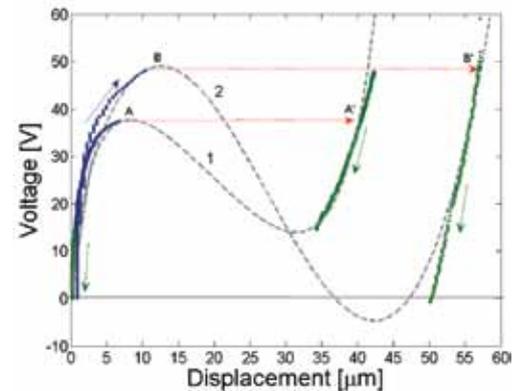
Electrothermal bistability tuning in micro actuators

Yuval Gerson, Slava Krylov, B. Rob Ilic

Bistability, namely, the existence of different stable configurations at the same loading, is a beneficial feature in many MEMS based applications. One of the most common examples is an initially curved beam loaded by a transverse force. Sufficiently curved beams may remain latched in the buckled configuration even at zero force/voltage – the feature which is beneficial in applications. In this case however a force in the opposite direction should be applied in order to release the device and an additional electrode should be provided, which complicates the design. Since bistable devices are extremely sensitive to the structure's geometry, low relative tolerances of micromachining and uncertainty in ambient conditions poses a challenge when designing these devices. The ability to adjust stability properties is therefore an important feature. In this work which is in collaboration with Y. Gerson and B. Ilic from Cornell university we report on an approach allowing simple yet efficient tuning of the bistability properties in large displacement electrostatic micro actuators. The devices fabricated from silicon on insulator (SOI) wafers using Deep Reactive Ion Etching (DRIE) based process incorporate elastic suspension realized as a pair of curved beams and are operated electrostatically by a combdrive transducer. The curvature of the beams and therefore the stability characteristics of the suspension are controlled by passing a current through the suspension and resistive heating the beams material. Experimental results, which are in good agreement with the model predictions, demonstrate the feasibility of the suggested approach and show that the application of a small tuning current increases the device deflection from 40 μm to 55 μm , allows adjustment of the critical voltages and deflections and makes it possible the control of latching without an additional electrode. The approach can be efficiently implemented in electrical and optical switches, threshold inertial and mass sensors and nonvolatile memory devices where the use of long displacement actuators with adjustable bistability range is beneficial.



(a) Schematics of the device and the electrical connection. Red arrows represent the direction of the current (b) Scanning Electron Microscope micrographs of the fabricated device.



Experimental limit point buckling curves (markers) and finite element model results (dashed lines) for different values of the tuning current: 1-zero current; 2 -5.5mA. Blue/green arrows illustrate the direction of loading/unloading.

Emission of azimuthally polarized beams from radial Bragg nanolasers

Ori Weiss and Jacob Scheuer

Cylindrical vector beams (CVB) are unique beams which exhibit spatially dependent polarization state. Such beams exhibit linear polarization at each point across the beam profile but with varying orientation according to the beam type. Such beams have unique properties and diverse applications such as plasmons excitation, Plasmonic imaging, focusing beyond the diffraction limit and optical trapping.

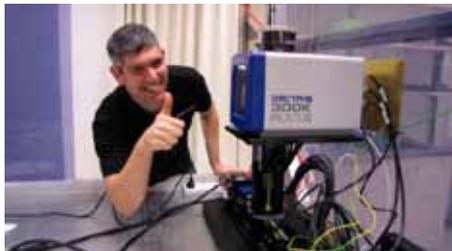
CVBs are highly interesting and useful but are rather difficult to realize. The most common approach for the generation of such beams is using birefringent masks converting linear polarization beams into CVBs.

Ori Weiss and Koby Scheuer from the School of EE have shown that CVB are naturally emitted from radial Bragg lasers (RBLs) – a unique class of nanolasers which employs radial distributed

feedback for lateral confinement and exhibit ultra-small modal volume, high Q-factor and single mode lasing. This discovery provides an attractive route for efficient and simple generation of (multiple) CVBs without the need for complex optical setup and alignment

More details: O. Weiss and J. Scheuer, Appl. Phys. Lett. 97, 251108 (2010).

New faces in the Center



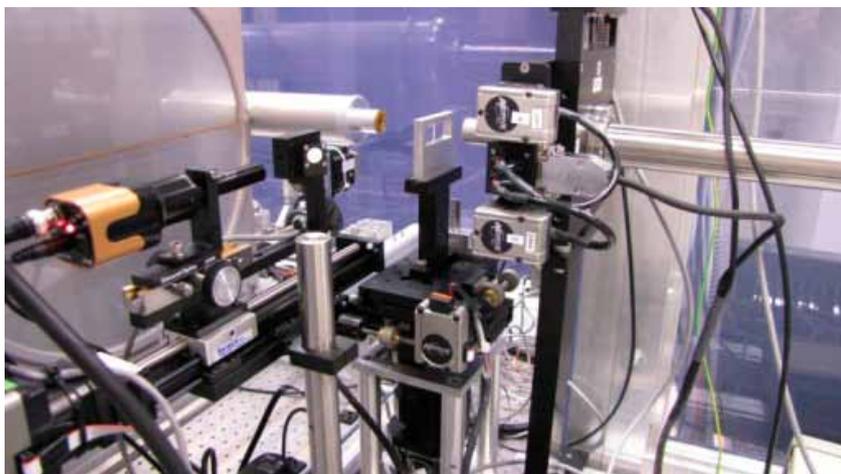
The new lab for nano-bio-physics and small angle scattering is open!

The recent recruitment of Dr. Roy Beck-Barkai to the school of physics and Astronomy as a core member of the center for nanoscience & nanotechnology

has been distinguished by the opening of a set of new laboratories for bio-physics in the center floor. The laboratory is interested in the intermolecular forces and interactions responsible for supramolecular nanometric complex assembly as they are extremely important for the proper function of many biological systems.

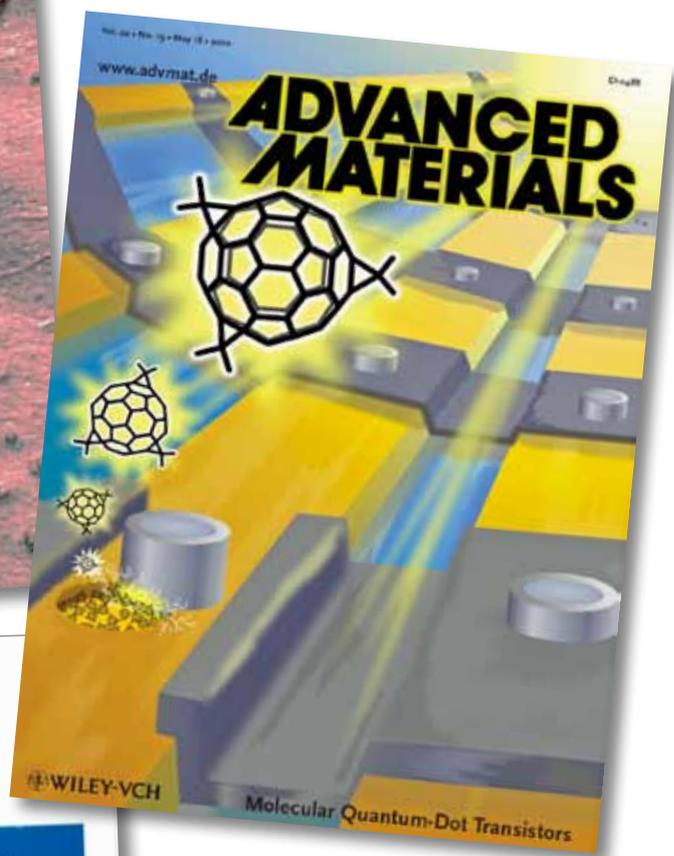
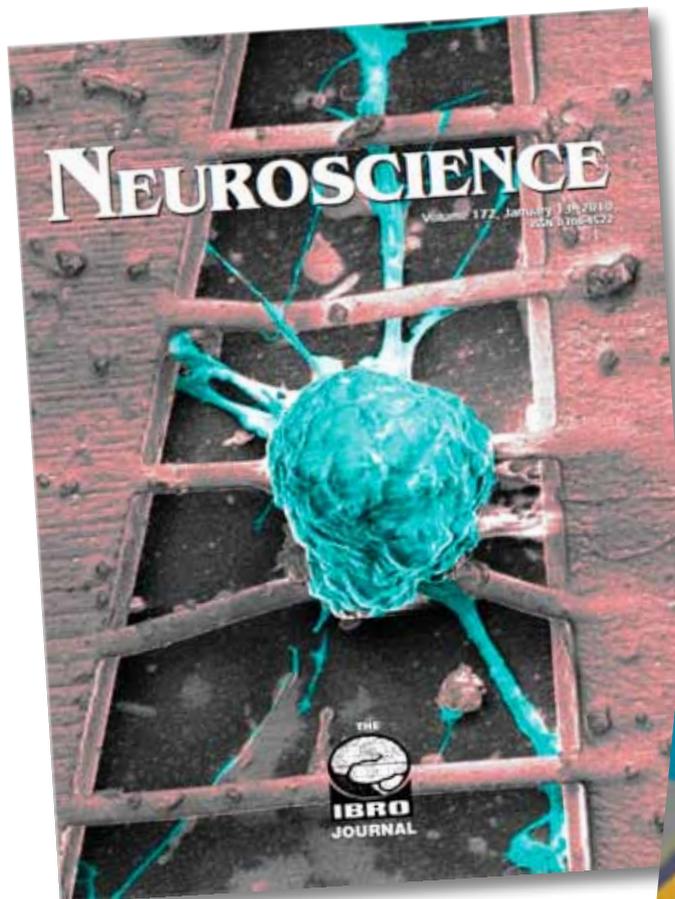
In addition to a biochemistry & molecular wet laboratory which include protein purification HPLC setup, and a couple of optical microscopy units, Dr. Beck-Barkai's laboratories consist of a new, top-of-the-line nanoscience characterization tool named Small Angle X-ray Scattering (SAXS). This characterization tool is unique as it has capabilities to measure repeating nanometric structures in non-destructive manner, without staining or other perturbation, in a various conditions such as aqua mediums, gel state or embedded on surfaces.

The SAXS system, with a cost of over \$700,000 is composed of a Genix (Xenocs) Cu x-ray micro-source, 3D x-ray optics, motorized and temperature controlled sample stage, 5 meter long x-ray enclosure and two x-ray detectors: solid state high speed Pilatus 300K (Dectris) and image-plate Mar345 (Mar-research). The combined detectors setup allows simultaneous structural measurements over extensive length span (0.1-100 nm). The setup has been installed and tested during Dec. 2010 and is now fully functional and operational.



Prizes

- Mark Shein, a PhD student in the faculty of engineering, received the best poster award in the Frontiers in Neuroengineering conference 2010.
- Giora Beit-Ya'akov, an MSc student in the school of EE, has been awarded the TAU Marian Gertner Institute fellowship for Medical Nanosystems.
- Inbal Friedler, a postdoctoral researcher in the school of EE has been awarded the Dan-David foundation scholarship for an outstanding post-doc.
- Assaf Yaakovovitz, a PhD student in the faculty of engineering, has been awarded the Wolf foundation scholarship for an outstanding graduate student.



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