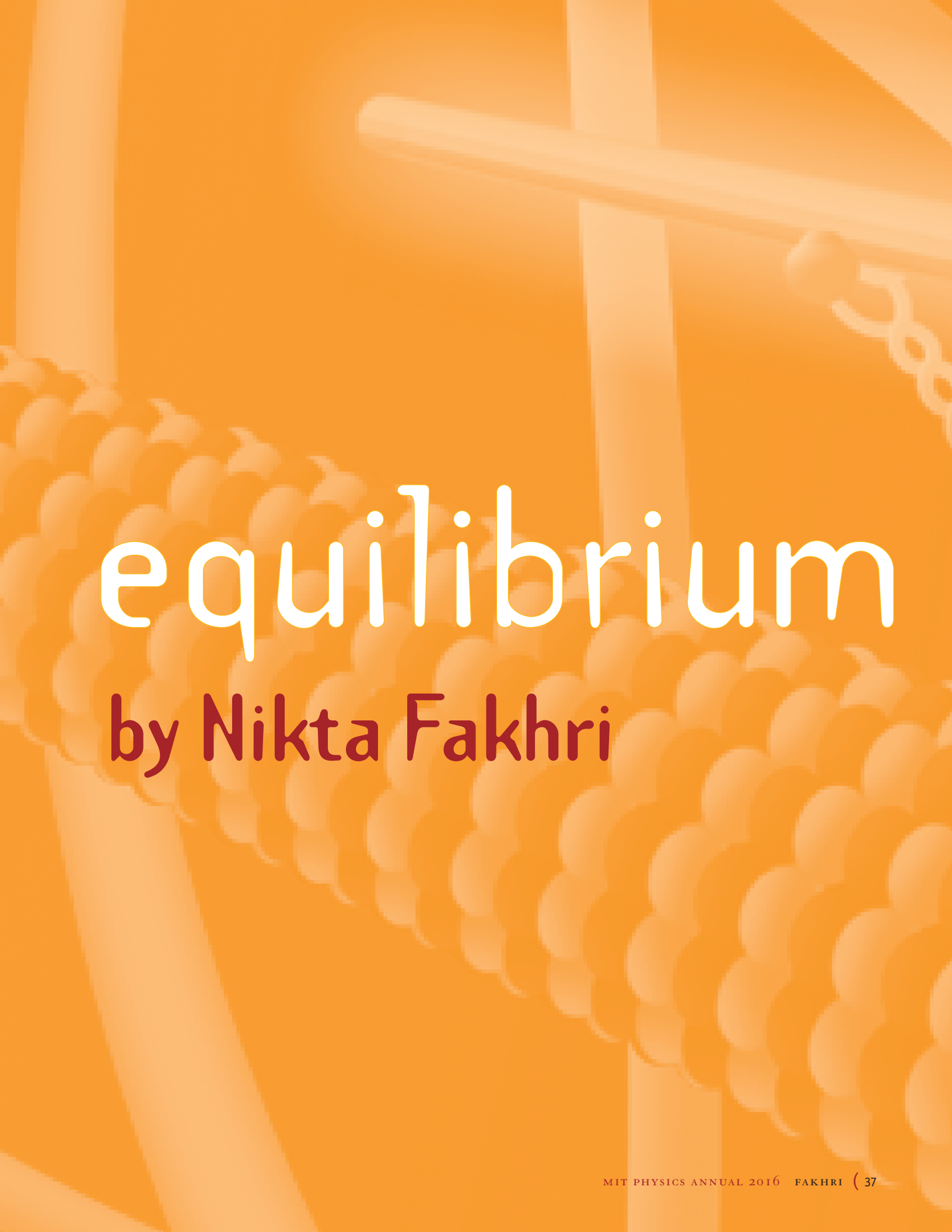
Life out of

Physical scientists have long been fascinated by the physical and chemical properties that distinguish living systems from inanimate matter. In his famous book, *What is Life?*, Erwin Schrödinger speculated on the notions of order, disorder and negative entropy and commented on the need for living systems to avoid thermodynamic equilibrium, which characterizes inanimate matter when it is left undisturbed. If an equilibrium state is one in which nothing seems to be happening, i.e., "equilibrium is death," then a key distinction of all living systems is that they operate out of thermodynamic equilibrium, which requires that they constantly burn energy, obtained from photosynthesis or metabolism, to stay alive.



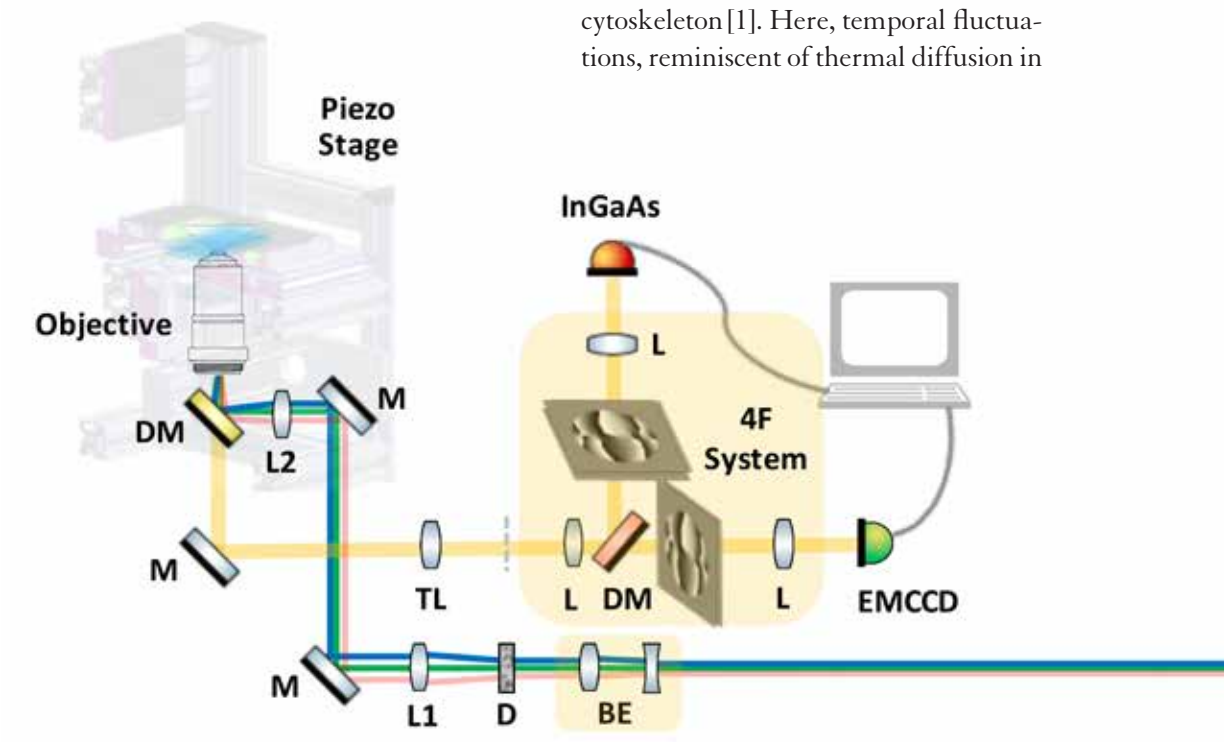
equilibrium

by Nikta Fakhri

Cells are the elementary building block of living systems. The cytoplasm of animal cells consists of a highly dynamic biopolymer composite known as the CYTOSKELETON. Its mechanical properties are dominated by filamentous protein polymers: microtubules, F-actin, and intermediate filaments. Metabolism maintains a chemical non-equilibrium that energizes this mechanical framework of cells. Molecules self-organize into complex machineries and patterns on all length and time scales to drive functions as various as intracellular transport, cell locomotion, and muscle contraction.

Generally, dynamics in cells are scale-dependent. At short times (microseconds to milliseconds), motion does not directly imply non-equilibrium because the thermal bombardment at the temperatures organisms live at is enough to make molecules and even parts of cells move and fluctuate quite vigorously. This is known as the BROWNIAN MOTION. This term derives from the botanist Robert Brown who observed random motions of pollen grains in a fluid in 1828. This kind of motion is general to sufficiently small particles suspended in a fluid. By observing different kinds of particles, he concluded that the motion was not dependent on the type of particles. We now know that this motion is due to the ever-present thermal fluctuations in the fluid itself. In a series of papers beginning in 1905, Einstein gave a theoretical basis for Brownian motion.

Between milliseconds and seconds, thermal motion might still be relevant, but there is mounting evidence that the motion of larger objects couples to stress fluctuations in the cytoskeleton [1]. Here, temporal fluctuations, reminiscent of thermal diffusion in



liquids, can arise from non-equilibrium dynamics in the viscoelastic cytoskeleton. (Viscoelasticity is the property of materials that exhibit both viscous and elastic characteristics when undergoing deformation.) This is important because, in contrast to thermal fluctuations, non-thermal fluctuations can generate spontaneous motion even in the absence of an external driving force.

On longer time scales, from minutes to hours, directed transport and larger-scale collective motions are the dominant modes of transport in cells. Quite generally, the spectrum of fluctuations can serve as a key signature of non-equilibrium behavior and a powerful tool to analyze the underlying mechanism.

To claim that the inside of a living cell is dynamic is a truism. But what may be easy to say is not necessarily easy to study. Understanding motions within cells and their underlying mechanisms requires techniques that can interrogate dynamics over a wide range of temporal and spatial scales.

In this article, I describe my lab's interdisciplinary approach at the junction of biophysics, statistical physics and nanotechnology for the development of such methods, and the discoveries we have made along the way. Particularly, I will focus on introducing a novel technique based on the unique near-infrared (near-IR) fluorescence properties of single-walled carbon nanotubes that will serve as multiscale "stealth" reporters of individual and collective motions and organization in living systems *(Figure 1)*.

Seeing is believing

Imagine aliens sent to the earth on a mission to study the behavior of human beings. From the spaceship, they monitor the human distributions on Earth. They report an average concentration map where spots correspond to the metropolitan areas. Such average information is stationary and statistically precise. However, it intrinsically hides fluctuations such as the mobility between cities. It also completely neglects the

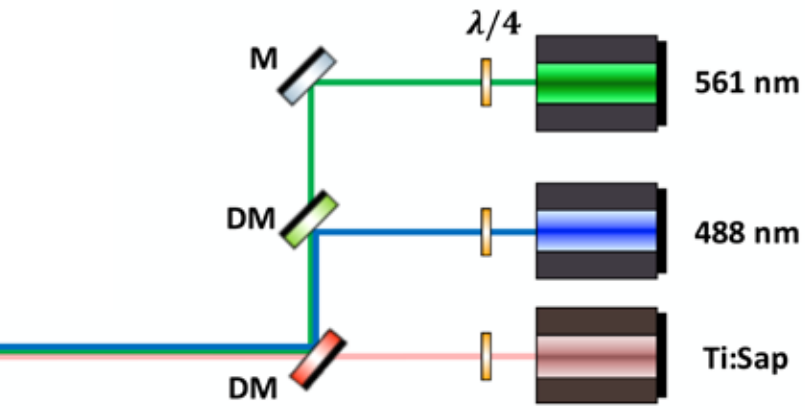


FIGURE 1

Schematic of the custom-built *near-IR fluorescence microscopy setup designed in Fakhri Lab. Three lasers are combined into a single collinear beam path using long-pass dichroic mirrors. The position of the ground glass diffuser is conjugated to the back focal plane of the objective lens. The resulting random-phase wavefront enables speckle illumination for optical sectioning. Fluorescent light collected by the objective lens enters the two-channel 4F system which contains two Double-Helix point spread function phase masks for 3D imaging. [Credit: Yoon Jung]*

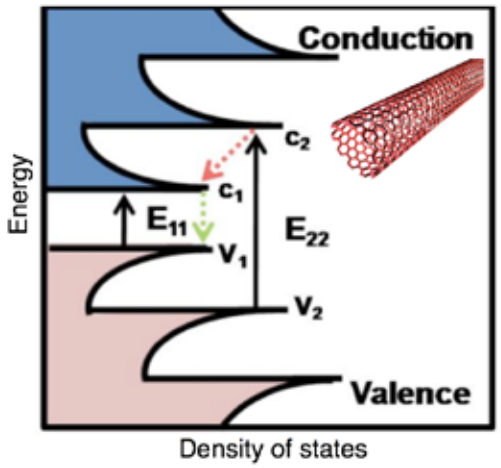


FIGURE 2

Schematic of the electronic density *of states for the π-electrons in a semiconducting SWNT.*

individuals moving inside the city, as this activity does not affect the total population. The natural conclusion from this measurement would be that a human being is a static form of life! So what would aliens have to do to better understand human beings? The best course of action would be to send a few disguised agents or to kidnap some human beings, tag them and put them back on the Earth. By tracking the motion of these individuals for a long lapse of time they can deduce dynamic features of human life otherwise invisible!

FLUORESCENCE MICROSCOPY is an essential technique in biological research to study the function of proteins and organelles. Particularly with the development of genetically encoded fluorescent probes and high-speed high-photon-efficiency cameras, we now have subcellular spatial resolution and single molecule sensitivity *in vitro*. In living cells, however, molecular imaging has been limited to high resolution static maps or short trajectories. Moreover, signal-to-noise ratios tend to be marginal because of cellular background fluorescence. Hence, live fluorescence imaging has been limited in providing dynamic information for living processes. A multiscale probing of the dynamics in cells to obtain a complete and accurate understanding of the biological processes requires (*i*) inert, non-perturbing and highly stable fluorescent probes; and (*ii*) high signal-to-noise ratio in imaging.

The wand

SINGLE-WALLED CARBON NANOTUBES (SWNTs) are prototypical quasi one-dimensional materials composed of carbon, with walls only one atom thick and tens of atoms in circumference [2]. Every carbon atom lies on the surface of the nanotube and forms part of a strong covalently bonded structure. SWNTs are typically about 1 nm in diameter but have lengths from hundreds of nanometers to several microns, with long-range crystalline periodicity retained along the tube axis. Perhaps the most exciting characteristics of carbon nanotubes are their unusual mechanical and electronic properties [2].The electronic structures of nanotubes also lead to extremely useful optical characteristics. *Figure 2* shows a schematic of the electronic density of states expected for the π-electrons in a semiconducting SWNT. The spikes in the density of states, called van Hove singularities, arise from the quasi one-dimensional character of electronic motions in systems with very high aspect ratios. Each van Hove singularity is labeled with the index of the sub-band to which it belongs. Semiconducting SWNTs have direct bandgaps, and optical selection rules allow light that is polarized parallel to the tube axis to excite transitions between corresponding sub-bands in the valence and conduction bands. An important optical characteristic is the near-IR fluorescence (fluorescence is the property of absorbing light of short wavelength and emitting light of longer wavelength) of individually dispersed SWNTs in aqueous media [3,4]. When the

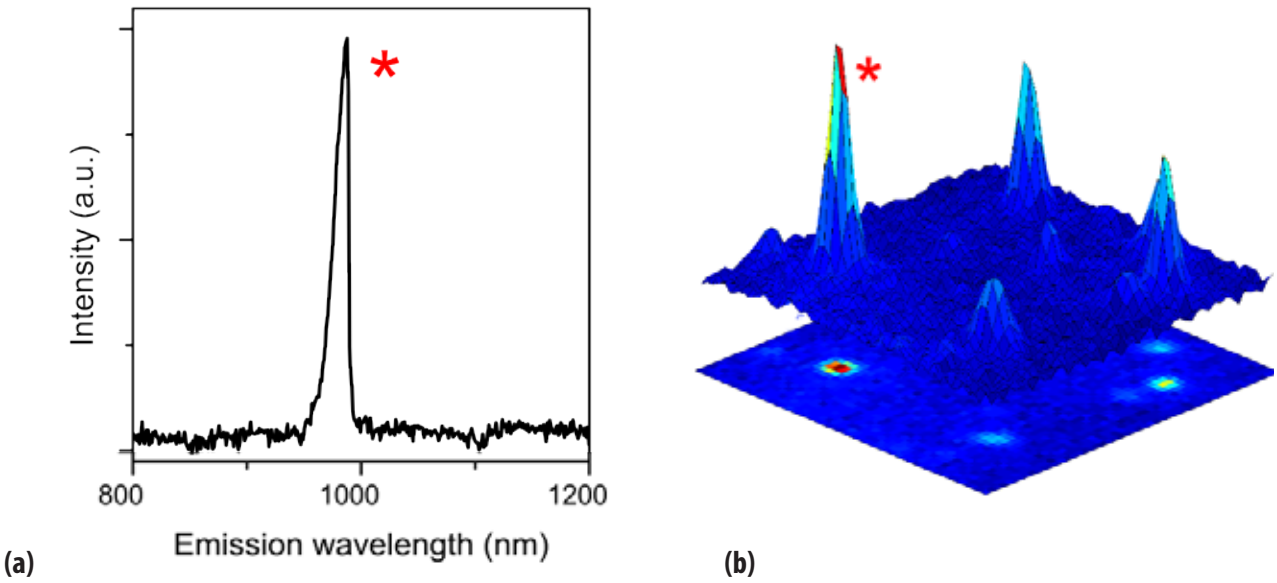


FIGURE 3
(a) Individual SWNT emission spectrum *with peak at 985 nm, implying a specific chiral angle with a diameter of 0.76 nm.*
(b) A single frame of individual SWNT-labeled *kinesins showing high image contrast. Heat map color code indicates relative intensity [1].*

photon energy of the excitation source matches the second van Hove transition energy of one of the SWNT species in the sample, the resulting optical absorption generates a hole in its second valence sub-band and an electron in its second conduction sub-band. The electrons and holes relax through phonon emission to the first sub-bands. Then a small fraction of the excited nanotubes emits near-IR fluorescence through radiative electron-hole recombination across the semiconducting band gap.

The near-IR fluorescence of SWNTs is extremely favorable for biological applications for two reasons. First, unlike conventional organic fluorophores, carbon nanotubes are resistant to photobleaching by intense irradiation. (Photobleaching is the photochemical alteration of a dye or a fluorophore molecule such that it permanently is unable to fluoresce.) Nanotube emission therefore may be collected steadily for extended periods under strong optical excitation. Second, the fluorescence of SWNTs occurs in the near-IR at wavelengths at which there is normally little background emission from biological samples. This allows the SWNT fluorescence to be detected with high selectivity and optical contrast. Moreover, due to their extreme aspect ratio they can easily "reptate" into crowded and confined environments without perturbing the surroundings. (Reptation is the thermal motion of very long linear, entangled macromolecules in polymer melts or concentrated polymer solutions [5].)

We use two-dimensional InGaAs arrays as detectors in SWNT near-IR fluorescence microscopy to simultaneously capture emission from many thousands of image pixels. This makes it possible to record image sequences with millisecond time resolution to track the translational, rotational, and bending motions of single SWNTs in fluid media [6]. Because their spectroscopic transitions are strongly polarized, nanotube orientations are also easily determined with optical methods. The spatial resolution of far field optical microscopy in this spectral range is limited to about less than a 1 μm, which is much coarser than that attained with scanning probe or electron microscopies. However, the versatility provided by time-resolved selective imaging in complex media makes this method a valuable probe of some important biological processes *(Figure 3)*.

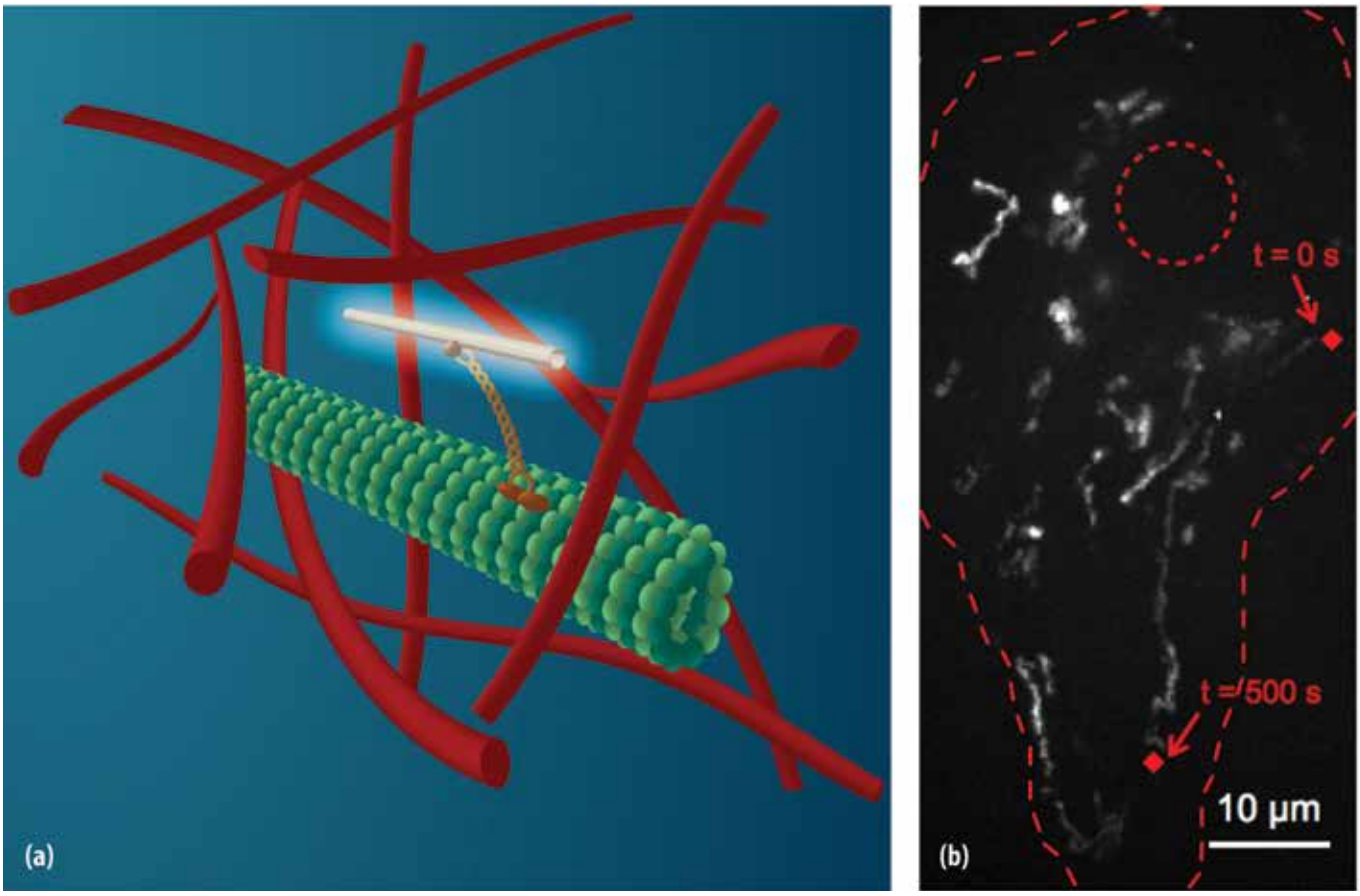


FIGURE 4

(a) Schematic of an SWNT bound to a kinesin motor. *SWNT-labeled kinesin motor moving along a microtubule embedded in an actin-myosin network. [Courtesy of Dutch Data Design.]*

(b) Tracks of SWNT-labeled kinesin motor proteins *in a cell shown as 2D maximum-intensity projection. Nucleus and cell periphery are outlined with red dashed and dotted lines, respectively. Red diamonds mark beginning and end of the 8.3-min trajectory of a particular SWNT-kinesin [1].*

The nanotube's guide to the cell

Cells have developed a differentiated delivery system to sustain their specific functions and morphology (the form and structure of an organism). This intracellular transport mechanism is spatially and temporally controlled by microtubule-dependent MOTOR PROTEINS [7]. Motor proteins are good reporters of dynamics from the molecular scale upward because they drive many cellular motions. The kinesin superfamily of motor proteins transports various cargo such as membrane organelles, protein complexes, and mRNAs along the microtubule highways. It plays significant roles for various mechanisms fundamental for life, such as brain wiring; higher brain functions such as memory and learning; activity-dependent neuronal survival during brain development; and for the determination of important developmental processes such as left-right asymmetry formation and suppression of tumorigenesis (formation of a tumor). To track the dynamics of the cytoskeleton without introducing invasive probes, we specifically targeted short SWNTs (~100 to 300 nm) to the endogenous kinesin-1 motor in living cells [1]. The key to using these probes to monitor intracellular dynamics is to find a way to specifically label a particular protein within the cell. To do this, we first maintain the SWNTs in a dispersed state in the intracellular milieu in such a way that the cell is unharmed

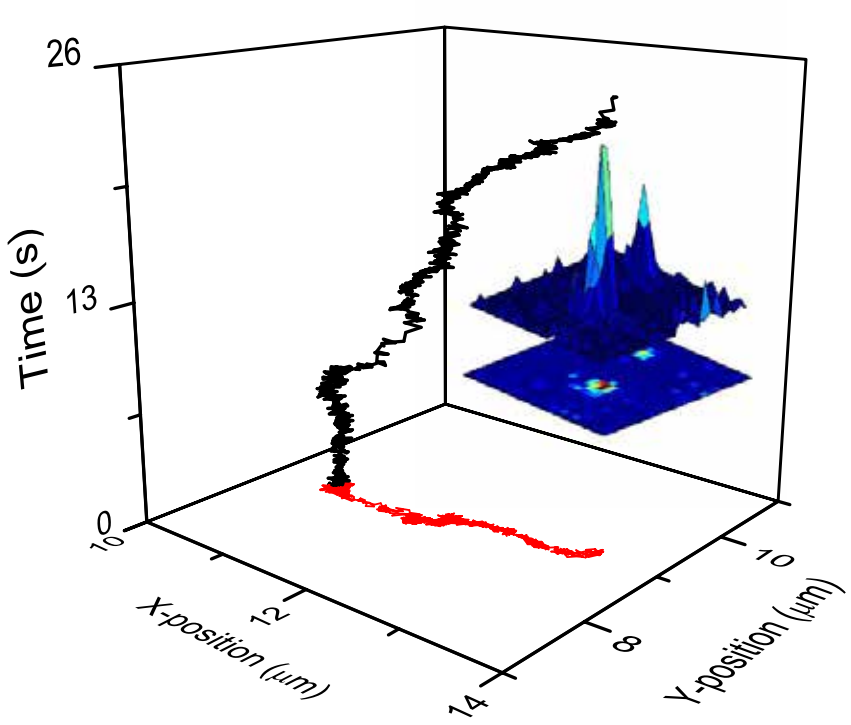


FIGURE 5
***Track of a SWNT-labeled kinesin in a cell**; 5 ms frame time. Inset: a single frame of individual SWNT-labeled kinesins showing high image contrast at 5 ms acquisition time. Heat map color code indicates relative intensity [1].*

and nanotube fluorescence remains stable. Then we introduce them into the cells via electroporation (the process of introducing a substance into cells using a pulse of electricity to briefly open the pores in the cell membranes) and covalently couple the SWNTs to the molecular motor kinesin. The first thing we observed is that a substantial fraction of the nanotubes displayed directed motion—SWNT-labeled kinesin walking along the microtubules—with the rest moving randomly but in a locally constrained fashion *(Figure 4)*.

The exceptional properties of SWNTs means that they can be tracked within cells for as long as 2-3 hours, with a precision of tens of nanometers, and over timescales spanning five orders of magnitude. As a consequence, not only could we monitor single kinesin molecules moving along cellular microtubules but we could probe other non-equilibrium intracellular movements as well.

The power of theoretical physics

One surprising discovery was that some of the seemingly random movements of the nanotubes contained interesting information. At intermediate timescales between fast thermal motion and (relatively) slow-directed kinesin tracking along microtubules, we observed movements of the probes transverse to the microtubule tracks that we were able to attribute, on the basis of a theoretical model, to non-equilibrium movements of the microtubules themselves. Furthermore, we identified the underlying cause of these movements to be non-equilibrium stochastic stress fluctuations in the cytoskeleton [1].

With this elegant use of nanotubes, a picture thus begins to emerge of a very mobile cytoplasm in which motors and their cargo move along the cytoskeleton and in which active, motor-driven non-directed movements constantly buffet intracellular structures, as well *(Figure 5)*.

FIGURE 6
Fakhri Lab at MIT *(front row): visitor, graduate student Yoon Jung, Prof. Nikta Fakhri, UROP student Aina Martinez i Zurita; (back row): alumnus Jacob Gold, graduate students James Pelletier, Junang Li, Alexandru Bacanu, and Tzer Han Tan.*

The future is bright

Non-equilibrium driving can boost not only intracellular transport, but also the fidelity of transcription, chemotaxis, and the accuracy of sensory perception. To understand cell function, it is thus important to map the spectrum of fluctuations and to determine whether particular cellular processes result from non-equilibrium activity [1,8]. With nanotubes as the beacons of light, the Fakhri Lab *(Figure 6)* hopes to gain fundamental insights into the inner workings of the cell.

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PROFESSOR NIKTA FAKHRI *has pioneered the use and development of fluorescent single-walled carbon nanotubes as probes in soft matter and biophysics. Her research group focuses on combining concepts from physics, biology and engineering to decode non-equilibrium mechanisms in active living matter; to exploit these mechanisms for engineering functional active materials; and to identify universal behavior in this broad class of internally driven systems.*

Fakhri joined the MIT Physics Department as an Assistant Professor in January 2015. She completed her undergraduate degree at Sharif University of Technology, Tehran, Iran, and her PhD at Rice University. Before coming to MIT, she was a Human Frontier Science Program postdoctoral fellow at Georg-August-Universität in Göttingen, Germany. Her recent awards include a 2016 Human Frontier Science Program Career Development Award and a Scialog Fellowship from The Research Corporation for Scientific Advancement/The Gordon and Betty Moore Foundation.