Latrunculin B;(iv) lipoplex mobility increased. Indeed, within each motion category (i.e. directed or Brownian), the diffusion coefficients were, in general, higher than the corresponding values obtained in NT cells. However, a very precise trend could not be found probably due to the low accuracy of experimental data;(v) within experimental error, the mean velocities were in the same range of those obtained in NT cells;(vi) the calculated asphericities were lower than that calculated in NT cells and were found to be close to the theoretical random walk value.

1926-Pos Board B696
Mitochondria - A Potential Roadblock for Axonal Transport
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Axonal transport of materials in neurons is an important process that directly affects the survival of neurons. Moreover, defects in this process have been linked to various neurodegenerative diseases. This work examines the interaction between mitochondria and cargoes transported along axon of dorsal root ganglion neurons using two-color imaging on a pseudo-TIRF setup. The result shows that cargoes transported along axon are more likely to slow down and pause in the vicinity of mitochondria. This propensity increases when drug is used to induce mitochondrial swelling. This can be explained as the large size of mitochondria (relative to the thin shaft of axon) can potentially inhibit cargo transport in axon through steric hindrance. Moreover, this finding also indicates that swollen mitochondria observed in some neurogenerative diseases may be one of the causes for axonal transport failure.

1927-Pos Board B697
Correlative Real-Time and Super Resolution Imaging of Mitochondrial Dynamics
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Mitochondria are energy producing organelles that play essential functions in all aspects of cell biology. In most mammalian cells mitochondria exist in the form of a highly dynamic interconnected network. Live cell imaging experiments revealed that this network undergoes dramatic shape re-organization as motor proteins actively pull the mitochondrial membrane. In addition, mitochondria that exist as individual organelles can be actively transported from one location to another. Mitochondrial shape changes and their active transport involve microtubules and play key roles in mitochondrial function However, several questions remain unanswered regarding the dynamic behavior of mitochondria: how often do mitochondria change tracks as they are transported along microtubules, how do the underlying microtubule tracks influence the observed shape changes and can mitochondrial membrane be pulled along multiple microtubule tracks.
In order to relate the dynamic events of the mitochondrial network to the underlying cytoskeleton, we developed a sequential live-cell and super-resolution imaging approach. Live cell imaging allowed us to track the movement of mitochondria and characterize their dynamic behavior. We could stop the dynamics at a specific time point by *in situ* fixation on the microscope stage. Subsequent immuno-staining followed by super-resolution imaging using Stochastic Optical Reconstruction Microscopy (STORM) allowed us to obtain a high resolution (~20 nm) image of the underlying microtubule network. The microtubule network was stabilized through treatment with low concentrations of drugs (nocodazole and taxol) and did not change appreciably during live cell imaging. As a result, we could overlay the dynamic behavior of mitochondria with the high resolution images of microtubule tracks by using fiduciary markers for alignment. This approach allowed us to correlate mitochondrial dynamics and the microtubule tracks at high resolution in order to answer the questions that we pose above.

1928-Pos Board B698
Intraflagellar Transport Powers Flagellar Surface Motility in Chlamydomonas
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Chlamydomonas reinhardtii is a unicellular biflagellate alga that exhibits whole-cell gliding motility and directed transport of flagellar membrane glycoproteins (FMG) on the flagellar membrane. There are indications that gliding motility and FMG movement are manifestations of intraflagellar transport (IFT), in which kinesin-2 and dynein-1b move large arrays of proteinaceous particles to and from the distal end of the flagellum. It is of much interest to determine whether IFT plays a role in dynamic flagellar turnover and whole cell motility. We have studied all three types of motility in live Chlamydomonas cells by a combination of advanced single-molecule fluorescence and force microscopy techniques. We have observed that FMGs rapidly associate and dissociate from IFT cargos and are transported by IFT machinery back and forth along the flagella. Individual retrograde IFT trains transiently pause, presumably due to adhesion of FMGs to a glass surface. Forces generated by dynein-1b motors attached to the paused IFT trains pull the whole cell in the opposite direction relative to the substrate. A single IFT train is transported by at least 4–5 motors on average in each direction, and opposite polarity motors do not interfere with each other along the length of the flagellum. The results have suggested that IFT is a highly regulated bidirectional transport, which also generates force for flagellar surface motility in Chlamydomonas.

1929-Pos Board B699
Axonal Traffic Control in Live Neurons by Tailor-Designed Magnetic Forces
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The axon acts as a conduit for organized transport of material, between the cell body and the synapse, which is essential for the function and survival of neurons. Axonal traffic jams caused by local accumulation of cargo have been implicated in many neurodegenerative diseases. In order to study the neuronal response to axonal traffic jams we need new noninvasive assays capable of A) slowing/stalling axonal cargo by external forces to induce controlled traffic jams B) monitoring the perturbed transport and the ensuing neuronal response in real time. Here, we present an integrated methodology based on microfluidic neuron culture, high-gradient magnetic trapping and multi-color TIRF imaging that permits external control of axonal traffic in live neurons via magnetic forces. We fabricated a novel microfluidic device for neuron culture by patterned electrodeposition of soft micromagnets (permalloy) on glass coverslips. In the presence of an external magnetizing field, the soft micromagnetic pattern gives rise to local zones of high magnetic gradients. By culturing neurons in this device, with axons aligned along these high gradient zones, we can exert pN forces on axonal endosomes carrying magnetic nanoparticles (MNPs, 50 nm). The magnetic forces counter the molecular motor forces to physically stall the endosomes, which leads to axonal traffic jams. The axonal growth and the delivery of MNP-loaded axonal endosomes along the high gradient zones are achieved by microfluidic compartmentalization of neuron culture. We have successfully A) compartmentalized DRG neurons in prototype magnetic devices B) characterized lectin-mediated axonal transport of 50 nm MNPs by pseudo-TIRF imaging, with/without external magnetic forces C) demonstrated the magnetic induction of controlled axonal traffic jams. These advances can potentially unravel the cooperative mechanics of multi-motor axonal transport and also elucidate the generic links between traffic jams, axonal swellings and neurodegeneration.

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Study of BDNF Transcytosis in Hippocampal Neurons
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Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays important roles in neuron survival, development and synaptic efficacy. It is believed that exogenous BDNF binding to TrkB activates three major signaling pathways: MAPK, PI3K and PLC-gamma. One important issue under these regulation mechanisms is whether and how BDNF is transported into neurons and its intracellular translocation. Furthermore, whether such exogenous BDNF is released and taken up by another neuron and involved in neuron to neuron communication has not been studied. In this work, BDNF conjugated to quantum dot is traced after it is taken by hippocampal neurons. A compartmentalized microfluidic device has been designed to separate axons and dendrites from each other. By applying Qdot-BDNF only to the axon chamber and observing some Qdot-BDNF leaving from the dendrite chamber, it is clearly proved that BDNF up-taken from the axon terminal can be translocated all the way to dendrites. Qdot-BDNF entering and leaving the soma has also been directly observed. Whether BDNF is released from the axon or dendrite terminal is under investigation. It is hoped that at the end of this work, an overall picture of the whole regulation cycle of exogenous BDNF and whether its role as a chemical communicator through the neuronal network can be clearly shown.