antibodies without prior fixation. Hepatocytes take up asialoorosomucoid (ASOR) through receptor mediated endocytosis using the asialoglycoprotein receptor (ASGPR). Fluorescent early endosomes that contain both ASOR and ASGPR were able to move along MTs in our chamber and undergo fission resulting in sorting. Immunofluorescence revealed that kinesins and Rab4 were associated with these vesicles. Motility and fission were eliminated by 1 mM AMP-PNP (kinesin inhibitor) [illegible] sin and Rab4 antibodies. [illegible] esicle motility and fission. [illegible] wn that endosomal sorting [illegible] proteins can be assigned [illegible] in the sorting process.

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530.2

The Fate of Parafusin Related Protein (PRP1) in *T. gondii* During Ca^{2+}-regulated Exocytosis and Invasion

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The apicomplexan protozoan *Toxoplasma gondii* is an obligate intracellular parasite. *T. gondii* contains three types of secretory organelles. Exocytosis of its two apical secretory organelles is essential for invasion. Ca^{2+}-dependent release of the apical vesicles, micronemes, takes place immediately after binding of parasite to host cell. Earlier studies have demonstrated that PRP1 localizes principally to micronemes. This has now been confirmed by immuno-EM. Fluorescence light microscopy showed that PRP1 localization changes upon *in vitro* ethanol-induced exocytosis as well as upon *in vivo T. gondii* invasion of host cells. 2 min after inoculation of parasites to host cells, fluorescence intensity of both PRP1 and micronemes content (Mic3) labeling decreased 10-fold and localization changed. The intensity of microneme content labeling returned to pre-invasion level after 15 min. After 1 h the intensity of PRP1 labeling increased above the level prior to invasion. The fluorescence increased considerably for both PRP1 and microneme content until the parasites began to lyse the host cells after 40 h. This suggests that PRP1 and parafusin have a conserved function in Ca^{2+}-regulated exocytic processes.

530.3

3D visualization of tumor spheroids for correlative microscopy of video hologram images

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Full frame optical coherence imaging (FFOCI) can produce real time holographic images at resolutions of up to 10 im. Rat osteogenic sarcoma tumor spheroids were used as a model tissue in order to develop this technology for imaging biological tissues and to interpret the images produced by FFOCI. As the tumor spheroid grows, cells within the spheroid undergo apoptosis and necrosis, which results in the production of intercellular voids. The number and distribution of these voids as determined by 3D reconstruction of serial sectioned spheroids were compared with images produced by FFOCI. The 3D reconstructions demonstrated patchy necrotic spaces with a similar pattern and spatial distribution to structures observed in the holographic images. The images produced by FFOCI likely reflect these regions of apoptosis/necrosis and may indicate regions of changing optical density.

530.4

Calcein-AM: A Useful Probe to measure redox potential in living cells

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We have incidentally observed that changes in fluorescence from cells loaded with calcein acetoxy-methylester (AM), a viability probe used in confocal microscopy (CLSM), parallel fluctuations in intracellular redox potential. We demonstrate here by fluorimetry in CLSM that well known oxidants, such as methylmercury, ethanol, and phorbol esters, increase calcein signal in different experimental models, such as, C6 glioma cells, CAPAN-1 pancreatic duct cells, and human fibroblasts respectively. The removal of the cell-permeant form calcein-AM from the culture medium renders cell signal insensitive to oxidative stimuli, as a result of the back diffusion of the dye into the medium. Moreover, in a cell-free system, high signal of free calcein does not change with oxidant treatment, while the low emission of calcein-AM shows a linear dose-dependent increase, indicating that the AM form is the actual sensor. Consistently, fluorescence increase upon oxidant treatment is proportional to the extracellular concentration of calcein-AM, a result compatible with saturation of intracellular esterases. These data point to calcein-AM as a redox sensor that can be applied in microscopy of living cells and fluorimetry.

530.5

Novel Applications of Confocal Microscopy in Lung Toxicology

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Confocal microscopy (CM) allows optical sectioning of a sample without physical damage. We examined the deposition of inhaled asbestos and quantified lung injury after intratracheal amiodarone (AD) treatment with CM. Exposed lungs were: 1) airway fixed, stained with lucifer yellow (LY; 0.1 mg/ml), and embedded in plastic; or 2) inflated with ethidium homodimer (EH; 0.4 mM), cryopreserved, and sectioned. Images of asbestos deposited in human lung parenchyma were reconstructed in 3-D. Asbestos fiber orientation in situ was obtained while preserving anatomic relationships and fiber geometry. Chronic lung injury in rats was quantified by measuring the connective tissue area stained by LY as an indicator of alveolar wall thickening. AD induced a fibrotic response at 28 days post-treatment. For acute cytotoxicity, AD compromised the integrity of cell membranes as evidenced by a marked increase in the number of cells labeled with EH. Damage was more prevalent in the airways at 15 min, but by 3 days, the injury was localized to the parenchyma. CM can quantify toxic responses and identify locations of damage without physical disruption of tissue and may be useful in the analysis of human biopsy samples.

530.6

Non-specific bindings of riboprobes at medial longitudinal fasciculus in rat hindbrain by in situ hybridization

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We detected non-specific bindings for different riboprobes at the medial longitudinal fasciculus (mlf) in Sprague-Dawley rat hindbrain by in situ hybridization (ISH). Antisense and sense probes for both glucose transporter 2 (GLUT2) and glucokinase (GK), and sense probes for dopamine transporter were used for ISH at the area of mlf. All five probes were labeled with digoxigenin and detected by either fluorescence or non-fluorescence method. Strong signals showed at mlf (Bregma –14.30mm to –13.50mm) in the hindbrain for all the five probes. We confirmed that there were no antisense transcripts of GLUT2 and GK in the brain by RT-PCR. Thus, the signals at mlf were from non-specific binding. Oligonucleotides complementary to sequences of vectors included in the probes did not block these non-specific bindings. However, using commercially available blocking reagent, instead of normal sheep serum, in the blocking step greatly decreased the non-specific bindings. Thus, the non-specific bindings at mlf may be due to anti-digoxigenin antibodies. Conclusion: Hindbrain mlf area is susceptible to non-specific binding during in situ hybridization.

530.7

Immunocytochemical localization of cathepsin B in injured gastrocenemius muscle cells

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Rat gastrocnemius showed increased protein degradation (+75- 115%) at 48 h after traumatic injury. Injured muscle showed increased cathepsin B activity (+327%) and the mRNA encoding cathepsin B (+670%), Cathepsin L (+298%), Cathepsin H (+159%) and Cathepsin C (+268%). In the insitu hybridization, cathepsin BmRNA localized to the mononuclear cell infiltrate in injured muscle and only background levels of hybridization were observed either over muscle cells in injured tissue or in uninjured muscle. Immuno-gold and electron microscopy showed specific staining for Cathepsin B only in the lysosome-like structures in the cell of

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