scattering samples in both excitation modes. These experiments showed again the better performances of TPE compared to single photon excitation.

(1) Cella Zanacchi et al. Proc. of SPIE, vol.7903 (2011)
(2) Planchon et al. Nature Methods 8, 417-423, (2011)
(3) Truong et al. Nature Methods 8,757-760, (2011)

**982-Pos Board B768**

**Angular Morhpomechanics in the Establishment of Multicellular Architecture**

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We address a fundamental question: what are the physical laws that govern the assembly of acinar structures? We report a novel type of human cell motility where single cells undergo multiple rotations. This motion is maintained as these cells cohesively divide to assemble into polarized multicellular spherical structures (acini) when placed in a 3D basement membrane surrogate gel. We link the functional relevance of coherent angular motion (CAMo) to spherical architecture, and determine the importance of molecules involved in cell-cell adhesion and tissue polarity to the outcome. CAMo is observed in both primary human cells and established breast cell lines where the final realized geometry is spherical. Breast cancer cells do not display CAMo but are randomly motile. Upon 'phenotypic reversion' of malignant cells, both CAMo motility and correct architecture are restored

**983-Pos Board B769**

**Separation of NADPH and NADH Fluorescence Emission in Live Cells using Flim**

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Observing changes in the intensity of the endogenous fluorescence of the enzymatic cofactors NADH and NADPH has been used to monitor the metabolic state of cells and tissues for upwards of half a century. The fluorescence arising from NADH and NADPH is spectrally identical, leading to the observed combined fluorescence being labelled NAD(P)H.

In this work, we investigate the application of fluorescence lifetime imaging microscopy (FLIM) to separate the fluorescence arising from NADPH from that of NADH in live cells.

The fluorescence lifetime of NADH in solution (~0.4ns) increases more than 5-fold when NADH is enzyme bound, with a precise lifetime governed by the particular enzyme to which the cofactor is bound.

We have used FLIM to measure the fluorescence lifetime of NAD(P)H in HEK293 cells overexpressing NAD-kinase, in which NADPH levels are elevated ~15-fold compared to NAD-kinase knock down cells. The fluorescence lifetime of free NAD(P)H remains unchanged at ~0.4ns, but the fluorescence lifetime arising from enzyme-bound NADPH was significantly increased to $3.9(\pm 0.4)$ns compared to its value in the knock down cells, $3.0(\pm 0.2)$ns.

FLIM studies of mammalian cochlear explant cultures also revealed a layer of supporting cells which showed a similar long lifetime of $3.6(\pm 0.1)$ns, suggesting that these cells exhibit elevated NADPH. This lifetime was significantly reduced by biochemical interventions to decrease NADPH (pentose phosphate pathway inhibition), supporting this conclusion.

These results represent a first step towards a functional imaging protocol for separating NADH and NADPH levels in live tissue.

**984-Pos Board B770**

**Comparing Photodamage Induced by Confocal Microscopy and Light Sheet Fluorescence Imaging of Zebrafish Skeletal Development**

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Understanding the formation of multicellular structures during embryogenesis requires high quality three dimensional microscopy over long periods of time, a task for which confocal fluorescence microscopy has become the standard technique. Extended viewing of developing structures in vivo, however, exposes the organism to prolonged illumination that runs the risk of being detrimental to the processes being studied. Furthermore, the effects of phototoxicity may not be apparent over the course of an experiment, as they may become significant well before any photobleaching of the sample is observed. Selective plane illumination microscopy (SPIM), or ''light sheet'' microscopy, has been developed as an imaging platform that can address the issues of phototoxicity, acquisition rate, and photon efficiency present in confocal methods of imaging. While it is known that SPIM requires orders of magnitude less light intensity than confocal techniques for similar imaging conditions, it has not been previously demonstrated that this difference is relevant to maintaining normal biological function. Using the development of the opercle, a cranio-facial structure in larval zebrafish (Danio rerio), we have tested the effects of long term imaging using a commercial spinning disk confocal microscope as compared to our own self-built SPIM instrument. Examining a range of exposure times and scan durations of several hours, we find that confocal imaging can generate significantly abnormal growth of bone forming cells (sp7:eGFP transgenic), especially if high signal to noise is desired. In contrast, we find normal opercle development with SPIM throughout the entire examined range of conditions.

**985-Pos Board B771**

**Fluorescent Live Cell Imaging Under Pressure**

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Perturbing equilibria of molecular interactions within a cellular environment provides a versatile method for dissecting the cellular functions of proteins. To date this has been achieved by modulating the temperature of the sample under investigation. When the sample contains a temperature sensitive mutant protein it allows this method to be used to investigate specific protein functions. However increasing temperature often has associated disadvantages of having non-reversible and destructive effects upon the cell/molecule under investigation.

Hydrostatic pressure offers a number of advantages over temperature as a variable for probing protein-protein interactions. High pressure does not usually disrupt native protein structure at pressures below ~ 2GPa. The effects are fully reversible; pressure can be applied and released from a sample extremely rapidly in the sub-millisecond; and the sample reaches uniform equilibrium pressure quickly, which is in contrast to conventional temperature techniques, which can take several minutes. The ability to rapidly increase and decrease pressure also allows the opportunity to determine whether the proteins/cells under investigation are irreversibly effected/damaged during the experiment.

We have recently developed a pressure chamber which allows rapid modulation of the hydrostatic pressure acting upon a biological sample and is used in combination with a conventional magnification fluorescence live-cell imaging system with up to 60x objective magnification. This system can be used to establish the effect pressure has upon the organisation and function of a plethora of cellular processes and protein-protein interactions.

Having established conditions which have no observable effect upon yeast cell viability, we are now using this live cell pressure perturbation imaging system to alter the dynamic behaviour of the actin and microtubule cytoskeleton, and thereby probe their regulation and function.

**986-Pos Board B772**

**Visualizing Hyroynamic Transgene Delivery and Expression in Live Mammalian Kidneys**

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While gene transfer has been difficult to accomplish in the kidney, reports have indicated that tissue cavitation may facilitate such genetic modifications. Accordingly, we investigated this theory, and designed and characterized a method that utilizes renal vein-guided, retrograde pressurized injections to illicit prompt transgene expression in mammalian kidneys. In devising this approach we hypothesized that the hydrodynamic forces generated from pressured injections were sufficient to facilitate the widespread delivery of exogenous matter throughout the kidney. As a consequence, this infusion process would assist the passage of transgenes across epithelial and endothelial tissue layers, and ultimately support their cellular uptake. We tested this hypothesis by introducing naked plasmid DNA and baculovirus vectors, which express fluorescent proteins, into 60 rodent kidneys. Gene expression was then examined in live animals using intravital multiphoton fluorescence microscopy, and in vitro with confocal laser scanning microscopy and electron microscopy. We recorded transgene expression in various glomerular, tubular and vascular segments throughout a period of 3 weeks. Moreover, we were able to generate transient, yet stable targeted and non-specific genetic modifications. Overall, these results outline a potentially attractive approach for renal gene transfer in live animals.