applied to liposomes that have been under investigation for decades as nanocarriers for drugs. First we studied their aggregation in an artificial blood circulation system. By functionalizing the liposomes with sufficient amounts of polyethyleneglycol, aggregation could be substantially reduced. In vivo experiments demonstrated that large aggregates are the first ones to be removed from the blood circulation, while small particles have a longer circulation time. Finally, we have demonstrated that the fSPT sizing technique can be used for identifying and sizing natural cell-derived microparticles in human plasma. In conclusion, fSPT sizing has the potential to become an important tool in pharmacy and biomedical diagnostics, as well as any other field where the characterisation of nanomatter in complex fluids is of critical importance.
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1712-Pos Board B622
High-Throughput Screening of Response Kinetics of Genetically-Encoded Metal Sensors with Microfluidics Technology
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Metal sensors, chromophores which generate responses upon metal-ion binding, are indispensible tools to study the metal homeostasis of the cell. Compared to the traditional dye-based metal sensors, the fluorescent protein (FP)-based FRET sensors offer many distinctive advantages. They are fully genetically encoded and targeted to specific cell location, capable of controlled expression, and provide intensity-independent ratiometric measurement. Yet at present the performances of the FP-based sensors, such as the dynamic range, metal selectivity, and metal affinity, need to be markedly improved to meet the requirements and challenges of their cellular application. By combining a sensor library design strategy, the aim of our work is to screen and sort the FP-based sensors for improved photophysical and photochemical properties using a high-throughput microfluidics method. We have developed a microfluidic platform that combines high-throughput screening with versatile optical FRET detection, fast mixing, and laser trapping techniques on individual mammalian cells. We demonstrated that the FRET responses of the sensors can readily be measured and differentiated on our cytometer with a rate of >20 cells/second. At this throughput a typical small targeted sensor library (~10^5 clones) can be sorted within a time frame of several hours. Using this technique we also investigated the response kinetics of various Ca^{2+} and Zn^{2+} sensors expressed at different cell locations upon exposure to metal ions in the microfluidic environment. Dissection of the relationship between sensor kinetics, construction, and location will shed light on the mechanism and dynamics of sensor function and ion transportation in the cell. The high-throughput characteristics of the microfluidics approach, integrated with fast optical detection with ms time-resolution over a broad time window (spanning orders of magnitudes from ms to seconds), can be readily adapted to other high-fidelity kinetics-based screening or sorting applications.

1713-Pos Board B623
Confocal Linear and Nonlinear Photothermal Microscopy of Intrinsic and Exogenous Probes in Live Cells
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We report first biological application of advanced confocal photothermal (PT) microscopy using pump-probe thermal-lens schematic in linear and nonlinear modes. In linear mode the optimal spatial matching of pump-probe beam waists and a pinhole (typical for thermal lens) provided confocal detection schematic. The 3-D sample images were obtained by X-Y rapid scanning at different axial positions. In addition, laser-induced nanobubbles around overheated absorbing zones provided nonlinear PT signal amplification in beam focus area only. This nonlinear effect can be accompanied by laser burning of absorption background. These phenomena enhanced 3-D capability of PT imaging and provided a tool for spectral selective erasing of not desired absorption background. The use of a tunable nanosecond optical parametric oscillator as a pump beam gives an opportunity to acquire multispectral 3-D PT images in the spectral range of 410 - 2200 nm. The PT microscope has demonstrated capabilities for label-free imaging and spectral identification of cytochrome *c* and melanin as intrinsic probes in breast cancer and melanoma cells, respectively. Moreover, high absorption sensitivity and spectral specificity allowed detection and identification of conventional fluorophores (e.g., MitoTracker or GFP). PT microscopy was used for visualization of cell-nanoparticle interaction with a focus on folate conjugated quantum dots and anti-Melanoma (MCSP) antibody conjugated 10-nm magnetic beads. Cytochrome *c* demonstrated capacity to serve as intrinsic PT marker of oxidative stress in live *C. elegans* at a single cell level. The features of described PT microscope compared to existing conventional PT and fluorescent techniques are further highlighted.

1714-Pos Board B624
Use of Parallel Flow for Angiogenesis and Cancer Cell Invasion in Microfluidic Devices Using Real-Time Microscopy
Carolyn G. Conant, Michael Schwartz, Cristian Ionescu-Zanetti.
Understanding the molecular and cellular mechanisms of cancer progression from primary malignancy to metastatic disease is critical to development of successful treatments. Cell invasion from the primary tumor and tumor-induced angiogenesis are just two of many phenomena contributing to the pathology of metastatic disease. Both biological processes involve migration and transmigration of cells in response to chemoattractants. *In vitro* analysis of these phenomena typically involves deposition of a basement membrane derived matrix (Matrigel) in a transwell plate insert. This allows quantitation of either invasion or angiogenesis, but the inserts can be cumbersome to process. Here, we demonstrate a microfluidic based method to follow angiogenesis or cell invasion in real time. The method enables acquisition of high content data by microscopy using microfluidic flow cells which are optimized for imaging. The channels can be filled with matrix and the device's fluid exchange capabilities facilitate introduction of cells and compounds. Using this technique, we tested endothelial cell response to bFGF and fumagilin. It was shown that bFGF impregnated in the matrix promoted angiogenesis while fumagilin abrogated the effect. We also investigated invasion of serum starved HT1080 and MCF-7 cells into FBS containing matrix and found that as predicted only HT1080 cells successfully invaded the matrix. Using these experimental conditions, the method is amenable to screening many cell-types or many simultaneous conditions or compounds by microscopy in the solid or liquid matrix of a microfluidic channel.

1715-Pos Board B625
Novel Photo-Switchable Green Fluorescent Proteins for Biological Imaging
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Fluorescent proteins that can be reversibly photoswitched between a fluorescent and a nonfluorescent state have found numerous applications in microscopy biotechnology and cell biology as probes for protein tracking and sub-diffraction resolution localization. Here we introduced a series of photo-reversible green fluorescent proteins with different switch-rates and photo-chemical characters, which could be used for different applications such as pulse-chase imaging and photo-activated localization microscopy (PALM). Also the proteins can be used to study the photo-switch mechanism because all the mutants differ from each other by only a single amino acid residue at the same position.

1716-Pos Board B626
The Study of Human Dermal Fibroblasts at the Cellular Level by Imaging Infrared Microspectroscopy
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Human Dermal Fibroblasts have been grown under standard conditions, harvested, and applied to infrared transparent windows to allow spatially resolved imaging of individual cells using resolved Fourier transform infrared (FTIR) imaging microspectroscopy. Spatially resolved infrared spectroscopic images of these cells are used to detect the spatial distribution of chemical species within the cell such as protein, lipid, and collagen. This is done without the use of probe molecules that might perturb the native state of the component molecules. These microspectroscopic images are compared to visual images of the same cells providing points of reference and specific information as to the location for molecules of interest within the cell. Additionally, these techniques are applied to fibroblasts grown in the presence of proprietary peptides designed to promote collagen production in fibroblasts. A collagen marker band at 1340 cm^{-1} in the infrared spectrum is used to determine the collagen presence and define it's spatial distribution within both peptide treated and control populations of fibroblast cells. The comparison between peptide treated and control cells, by this spectroscopic imaging technique are presented and discussed. Progress and pitfalls for this work as determined thus far are presented. These results also find support from ultrastructural observations on fibroblasts treated with peptides by Transmission Electron Microscopy (TEM), and will be presented.