cancer. We use Forster Resonance Energy Transfer (FRET) with intensity-based measurements to study the structural transformations of integrins. FRET with fluorescence lifetime-dependent cytometry can be used to observe these structural transformations and illustrate the heterogeneity of integrin conformational states across the cell population. We expect bent conformations that are engaged in FRET to have shorter lifetimes compared to the extended state, where longer lifetimes are expected.

Methods: To test our hypothesis, a human cell line (U937), stably transfected with formyl peptide receptor has been cultured using standard tissue culture techniques. First, cells were treated with a fluorescein analog bound to a Leu-Asp-Val-peptide derivative (LDV-FITC). This compound specifically binds to the headgroup of the VLA-4 integrin and serves as fluorescence donor. Next, a red fluorescent lipid dye, PKH-26, was added to the cell suspension. PKH-26 serves as FRET acceptor, which quenches the LDV-FITC in the resting bent conformation due to close proximity between the headgroup and membrane-incorporated PKH-26. The cells are subsequently treated with n-formyl-Met-Leu-Phe-Phe (fMLPP) to initiate activation of the integrins into the extended conformation. This results in the immediate de-quenching of the FITC donor fluorescence. At each stage of cell treatment, flow cytometry measurements were made using standard cytometry and lifetime-dependent cytometry.

Results: With ten repeated measurements, the average fluorescence lifetime of LDV-FITC in a non-quenched state was 4.00 +/- 0.03 ns. When engaged in FRET, the average fluorescence lifetime of LDV-FITC is 3.1 +/- 0.35. After activation (de-quenching of the LDV-FITC), the fluorescence lifetimes are 3.4 +/- 0.55 ns. In any given cell, a distribution of integrin conformations exist and is reflected in standard cytometry data.

Conclusion: The results suggest that FRET between two molecules can be detected using lifetime-based flow cytometry. Current data suggests the occurrence of significant heterogeneity in integrin conformational states in the cell population. Incomplete restoration of the average lifetime after activation may be associated with this heterogeneity. The ability to measure fluorescence lifetimes is promising for future cell signaling studies, because it can lead to the development of quantitative approaches to analyze the population statistics of activated molecules. More work is necessary to estimate the fraction of integrins in various states for each individual cell. We are refining flow cytometers to enable this through a technique called 'multi-frequency flow cytometry.'

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The Stem Cell Orchestra – A Non-narrative Approach to Communicate Experimental Processes

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Translation of basic stem-cell research into therapeutics demands reproducibility and identification of subsequent experimental processes variations, in order to assure cellular quality and efficacy. However, many methodologies of stem cell research remain highly idiosyncratic; and the traditional narrative (textual, verbal or video) approaches still lack the ability to identify or assess process variations. As a result, both the reproducibility and standardisation of the processes remain an elusive goal. Addressing this reality, here, we introduce a non-narrative audio-visual approach to assess process variations that utilise provenance information. Provenance embodies the history of an object or data with chronological description of the 5Ws - who, where, when, what and why. Provenance has been extensively used in the manufacturing industry to determine the quality of a product, by providing better interpretation, assessment, attribution and authentication via these 5Ws. For this project, provenance information was acquired from our open source software ProtocolNavigator, which is a virtual laboratory environment where researchers can emulate their laboratory activities, and the software automatically annotates the provenance information of the process and depicts a time-integrated interactive experimental map, that includes action patterns, manipulations and data acquisition icons. Interlinking and traversing through this map, a provenance trail for samples becomes apparent and the underlying process emerges visualised across a timeline. Sonification, the use of non-speech audio to convey or contextualize complex spatiotemporal information, has been used as a kinaesthetic learning approach to identify the sources of process loss, determination of critical steps, and most importantly assessment method for process improvement – fundamental perquisite for reproducibility. Using a parameter mapping sonification technique we have transformed multi-dimensional provenance information (eg. activity, location) into auditory dimensions (eg. pitch and rhythm) to facilitate the auditory perception for metadata interpretation. The goal has been to generate a language independent approach so that researchers from any discipline can abstract knowledge and discover relationships between process variation and sample quality. Parallel to this qualitative approach, we are also developing a quantitative approach based around provenance data model (PROV-DM) and associated algorithms. Together this comprehensive approach will enable us to scope the depth and breadth of process variability in stem cell research and lead to the establishment of "best practice" in qualitative as well as quantifiable terms. This will not only facilitate safe therapeutics development, but provide a 'trust calculator' for data derived from cellular studies.

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Barcoding and Tracking Stem Cells

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Background and Aims: Understanding how mesenchymal progenitor cells (MPCs) behave as part of a micro-community is fundamental to our knowledge of endogenous tissue repair processes. We have derived populations of MPCs from the dental pulp (DPPCs). Clonally isolated and heterogeneous cell populations have been characterised by expression of MPC markers and their ability to proliferate and differentiate in 2D. Qtracker705® (Qdot) are far-red fluorescent nanoparticles that can label and track single cells according to the innate proliferative capacity. As cells divide, Qdots re-segregate between resulting daughter cells such that the total fluorescent signal of both daughter cells is equal to the originating cell. This approach provides a method to select and separate different cell populations based on the simple biophotonic readout. The overall aim of this study is to develop a barcoding method that will enable the identification (by flow and image cytometry) of specific cell populations within both heterogeneous 2D and 3D environments.

Methods: DPPCs were cultured in α-MEM (10% Foetal bovine serum, 100μM L-ascorbate) and labelled with 4nM Qdots. Two defined windows were sorted based on width and amplitude of fluorescence signal to obtain a narrow sort window in order to mathematically de-convolve the subsequent signal attenuation. Cells were then seeded for onward culture and Qdot signal re-distribution was measured again at 48, 72 and 144 hours. The resultant Qdot profiles were further interrogated with a second sort at 72 hours to reveal specific populations based on unique regions across the fluorescence spectrum. RNA was extracted