

Whole Mouse Cryo-imaging of Single Stem Cells in Cardiovascular Cell Therapy

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We developed and applied a cryo-imaging system which provides single-cell detection of fluorescently labeled stem cells throughout a mouse. Alternating between sectioning and imaging, the robotic system collects tiled anatomical color brightfield and molecular fluorescence block face images, creating >60 GB data volumes at 10 μ m-scale. Specialized software provides seamless multi-scale visualization, detection of fluorescent cell clusters, and the number of cells per cluster. We have now applied the system to various experiments on homing/engraftment/differentiation using fluorescent reporters and quantum dot labeling.

Here we illustrate capabilities in cardiovascular therapeutics. 100,000 MSCs labeled with quantum dots were injected via tail vein in mice with or without myocardial infarction (MI). Using interactive visualization software (Fig 1), we examined cell distribution at scales from whole mouse to organ to tissue to single cell. Dividing numbers of cells in heart to lung gave a delivery ratio (R_D). Stem cell homing was evident (Fig 2). At Day1-Day4, R_D (with, without MI) was (0.17 \pm 0.02,0.02), (0.15 \pm 0.02,0.04), (0.21 \pm 0.03, 0.03), and (0.28 \pm 0.03,0.05), respectively. Over time, cells near the infarct remained whereas other cells disappeared. From Day1-Day5, the percent of cells in the heart, in and near the infarct were 1%, 17%, 18%, 30%, and 44%, respectively. At Day1, histograms of cells per cluster (heart, lung) were remarkably consistent: (73%,75%), (15%,16%), (6%,6%) for 1's, 2's, and 3's, respectively, providing ample hypotheses for additional investigation. There were some few (4%) large clusters (≥ 5 cells) in the lung not in the heart, probably indicating filtered cell "clumps." Over time, clusters had more cells indicative of cell division. With only ≈ 800 cells in the heart with a mean minimum distance 130 μ m, there is significant cardiac recovery, arguing for a very strong paracrine effect from each cell. Cryo-imaging promises to be an important tool for the study of stem cell biology, delivery, homing factors, differentiation, etc.

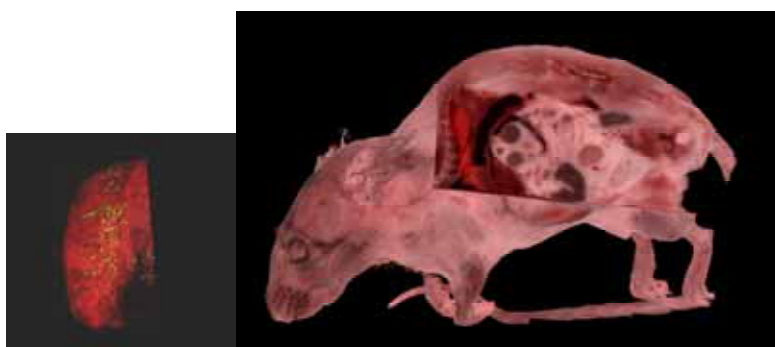


Fig 1. Whole mouse cryo-image with insert showing lung and MSCs labeled in green. Fully automated volume rendering allows one to image at multiple scales from whole mouse to a single cell.

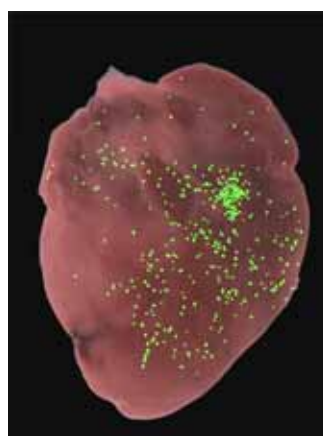


Fig 2. MSCs in heart at Day1 following MI.