

Workshop VI: Quantitative Imaging Cytometry of the Bone Marrow Microenvironment

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Sustained production of all mature blood cell types relies on the continuous proliferation and differentiation of a rare population of self-renewing, multipotent hematopoietic stem cells (HSCs) inside the bone marrow (BM). Upon entering the differentiation pathway, HSCs progress from primitive, multi-lineage potential progenitors through more restricted progenitors, finally giving rise to fully functional, mature blood cells. As a result, the BM constitutes an extremely complex and diverse environment, harboring a vast array of hematopoietic cells and distinct populations of stromal cells of mesenchymal origin, as well as cells involved in bone metabolism and an intricate vascular network. How the diverse cell-fate decisions, as well as differentiation and maturation processes resulting in the strictly regulated production of different hematopoietic cell lineages, are controlled in such an apparently unorganized tissue architecture remains poorly defined to date.

Quantitative imaging allows a detailed understanding, unachievable with conventional imaging techniques, of the spatial organization of BM tissue underlying the sophisticated regulation of hematopoiesis. We have employed Laser Scanning Cytometry (LSC) to analyze the localization of distinct hematopoietic populations inside whole longitudinal sections of murine femoral BM cavities. In this workshop, we will review the technical details associated with optimal sample preparation, immunofluorescent staining, protocol design and quantification of cell distribution and vascular structures in bone marrow sections via LSC. Data on HSPC as well as B cell progenitor localization and niche components will be presented.