Using Fluorescence Multiplexing and Spectral Unmixing to Characterise the Haematopoietic Stem Cell Microenvironment

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The role of the stem cell microenvironments which consists of supporting cells residing in close proximity with the stem cells in the regulation of stem cell behaviour are well established. However, there has been less success in the characterisation of these microenvironments and the interactions between these cells and the stem and progenitor cells. The limitations in studying stem cell niches *in situ* are largely due to the complexity in identifying stem cells and niche cells on a single section, requiring multiple markers; a minimum of 5 to identify stem cell populations excluding markers for the microenvironments. Whilst multi-labelling is simple with fluorescence activated cell sorting (FACS), it does not characterise the microenvironment. Interactions can be visualised with immunofluorescence, but the degree of complexity increases exponentially with every added marker. To address the limitations in the field, we use fluorescence multiplexing with tyramide signal amplification and spectral unmixing to characterise the stem cell microenvironment.

Formalin fixed paraffin embedded tissue sections of mouse bone marrow are labelled with antibody against the marker of interest, then tyramide-conjugated fluorophore covalently binds to the tissue localised at the marker of interest. The antibody complex but not the covalently bound fluorophore is then removed with heat treatment, allowing the labelling of a new marker with a pair of different antibody and fluorophore. The process is repeated until all markers have been labelled, and primary antibodies from the same species can be used together on the same sample (Figure 1). Imaging is performed on a confocal microscope (Nikon A1R or Vectra Imaging System) using a spectral detector and spectral unmixing is used to identify the unique spectral fingerprint of each fluorophore within the tissue. The combination of these two techniques allows for the labelling of at least six cell markers plus DAPI. Images are then analysed using ImageJ or InForm Software.

This technology allows the visualisation and quantification of the interactions between stem and progenitor cells with their microenvironments in 2D and potentially 3D. We have achieved the labelling of paraffin embedded mouse bone marrow tissue with 5 markers for haematopoietic stem cells (HSCs) and multipotent progenitor (MPP) cells and a microenvironment marker. Using image analysis, we were able to identify different stem and progenitor cell populations based on the expression of the markers used in the sample (Figure 2). The flexibility of this technique allows it to be readily adapted to identify other cells/markers of interest (including reporter mice). It can be used for frozen or paraffin sections and is a valuable tool for researchers studying a wide range of stem cells in different tissues, including human biopsies.

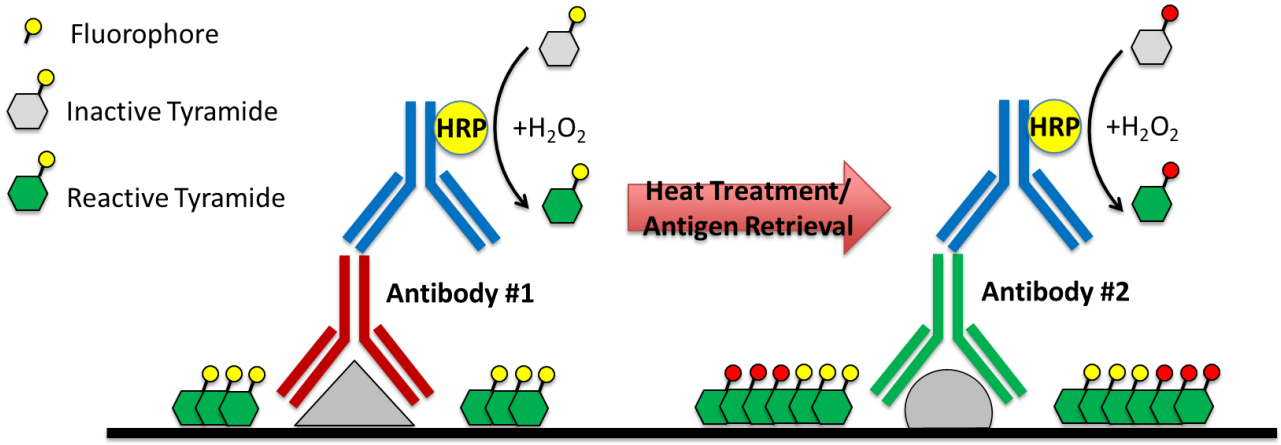

Figure 1. Fluorescence multiplexing using tyramide signal amplification.

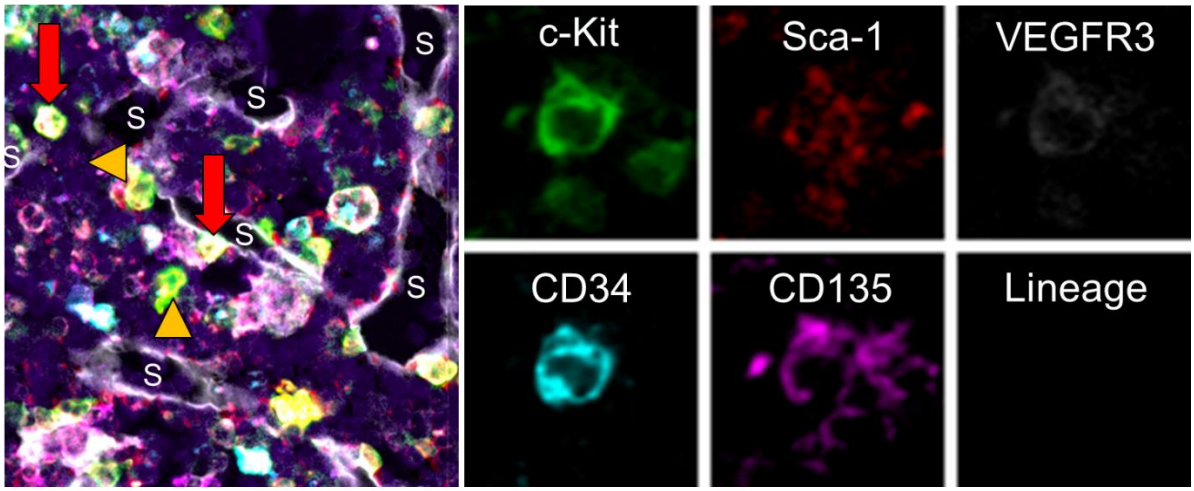

Figure 2. Opal multiplexing permits the identification of a minimum of 6 different antigens. Shown is a mouse tibia bone marrow section stained with antibodies against bone marrow sinusoids (S) and antibodies to identify HSCs (orange arrowheads) and MPPs (red arrows). Lineage positive cells are purple. Individual antibodies are shown on an MPP in the panels on the right.

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