High-purity sorting of bacterially-infected cells on Highway1

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Introduction

The isolation of infected cells is essential for studying infectious diseases such as tuberculosis, malaria, and HIV. Some work can be carried out on fixed samples, which reduces the biosafety risk of sorting, but for live samples, droplet-based sorting is less practical due to the generation of aerosols in the sorting process.

The Highway1 cell sorter is a microfluidic cartridge-based sorter that utilises patented VACS™ (Vortex Actuation Cell Sorting) technology to gently sort target cells in a sterile manner (Figure 1). The fluidics are entirely contained within a disposable cartridge, meaning no aerosols are generated, and the sample never comes into direct contact with the instrument. This removes the requirement for cumbersome cleaning protocols. Once used, the cartridge can simply be disposed of.

Here, we present a simple workflow (Workflow 1. Figure 2) for the selection human monocyte-derived macrophages (hMDMs) infected with *M. tuberculosis* H37Ra using the Highway1 cartridge-based cell sorter. In this workflow, we validate the output purity of target cells using the BD FACSDiscover™ S8 and identify sub-populations of bacterially-infected hMDMs using imaging cytometry. Workflow 2. shows how sorting with the Highway1 could be applied to live bacterially-infected hMDMs.

Designed with biosafety, sterility and ease of use in mind, the Highway1 is the ideal sorter for isolating cells at higher containment levels.

Figure 1: *The Highway 1 cell sorter and sterile cartridges.*

In collaboration with the Research Center Borstel, we present a workflow to identify and sort macrophages infected with *M. tuberculosis* H37Ra on Highway1 in a fast, sterile manner, which can easily be applied to higher-containment level sorting. Here, an aliquot of purified output was analysed using imaging cytometry to assess purity, as well as pathogen localisation within the cell.

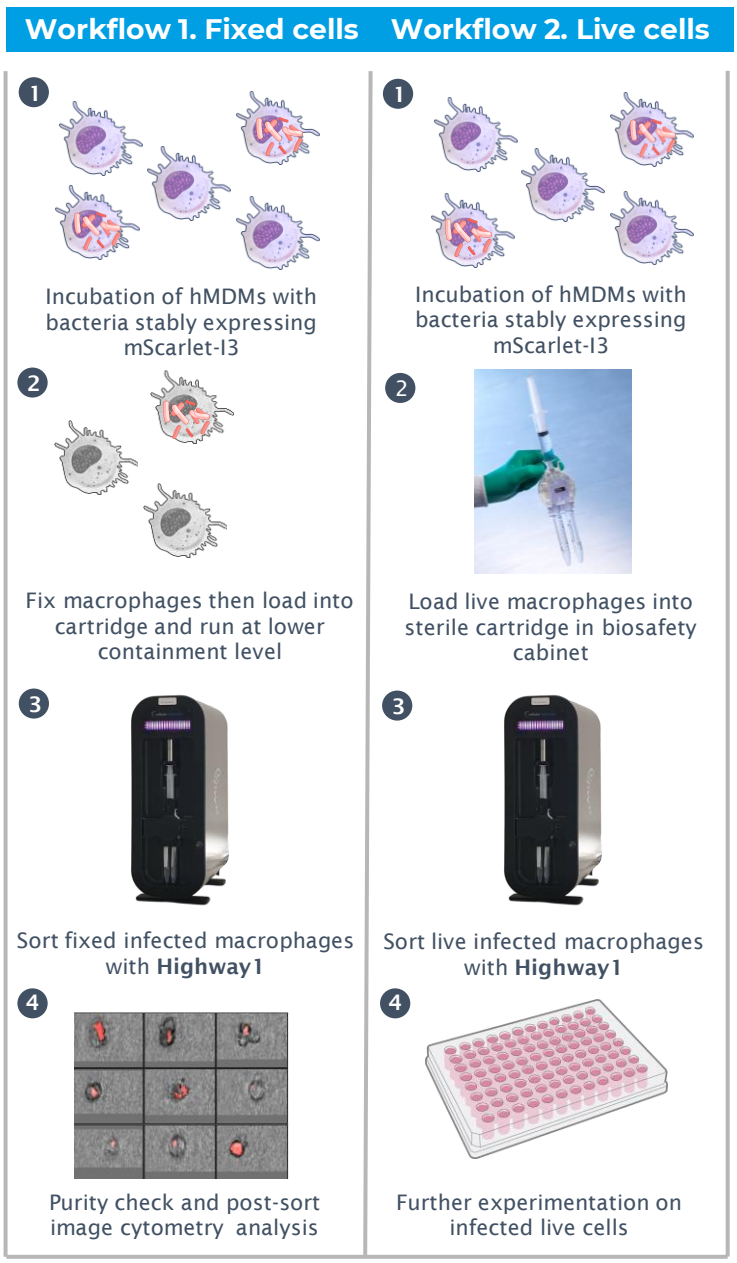

Figure 2: *Workflow (1) was used to sort human monocyte-derived macrophages (hMDMs) infected with M. tuberculosis* H37Ra. *Workflow (2) highlights how Highway1 could be used to sort live infected cells at higher containment levels.*

Illustrations from NIAID NIH BIOART Source.

Bacterially-infected cell sorting on Highway1

Human monocyte-derived macroplages (hMDMs) from two donors, D1 and D2, were incubated with *M. tuberculosis* H37Ra stably expressing mScarlet-I3, allowing bacterially-infected hMDMs to be identified by the presence of the fluorescent reporter. Cells were then fixed and resuspended in the recommended sorting buffer. For sorting, cells were gated upon on FSC-A vs SSC-A, then doublets were excluded using FSC-W vs FSC-H. Although mScarlet-I3 is normally excited using a 561nm laser, the 488nm laser on Highway1 was still suitable with only 12% excitation maximum. mScarlet-I3 was detected using FL2 A - 585/29 – mScarlet-I3 against FL1 A – 525/40 - autofluorescence (Figure 3). The cells from the donors were sorted in Purity Mode on Highway1.

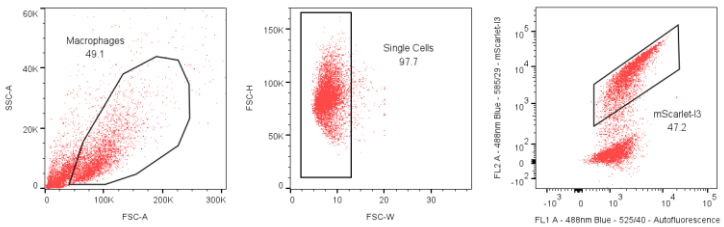

Figure 3: *Gating strategy for bacterially-infected hMDM. mScarlet-I3 was excited by the 488nm laser and emission was detected in FL2 with 585/29 bandpass filter.*

Post-sort analysis using imaging cytometry

Once sorted, the purity of each sample was analysed on Highway1 (Figure 4.) and the BD FACSDiscover™ S8, which both confirmed a purity of >97%. Images of sorted cells were also acquired on the BD FACSDiscover™ S8 and compared to a control (Figure 5). Using this imaging capability, a range of parameters, including localisation, can be used for analysis or sorting of fixed samples. Furthermore, utilising Highway1's high-speed enrichment mode (up to 37,000/s) prior to the image-based sorting, reduces sort time whilst enabling in depth-cell analysis.

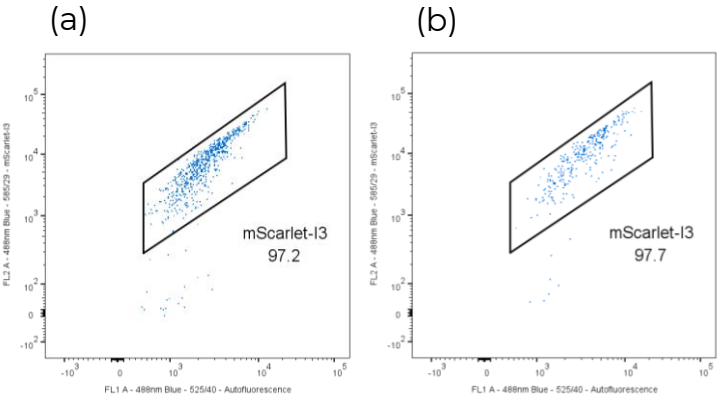

Figure 4: *Ouput purity of bacterially-infected hMDM was >97%. Post-sort purity of both samples D1 (a) and D2 (b) was analysed on the Highway1 (shown here) and the BD S8 FACS Discover.*

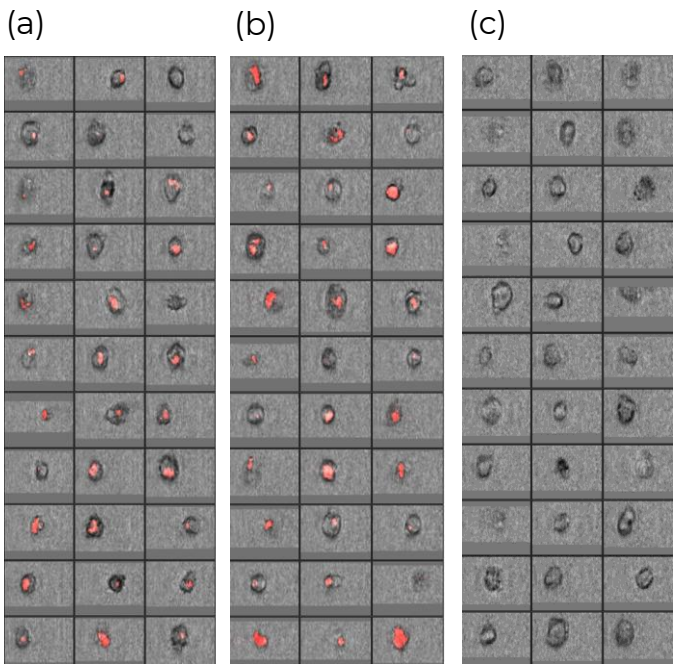

Figure 5: *Image-based analysis of bacterially-infected hMDM was performed on the BD FACSDiscover™ S8. The sorted fractions of D1 (a) and D2 (b) show the presence of bacteria within the hMDMs, as indicated by the reporter mScarlet-I3. Using image-based cytometry allows for a range of parameters to be analysed, such as localisation and diffusivity. The control (not infected) hMDMs are shown in (c).*

Summary

- Here we show mScarlet-I3 being excited by the 488nm Blue on Highway1. This may be applicable to other fluorescent proteins normally excited by the 561nm Yellow/Green laser (e.g. dsRed, mCherry, dTomato).
- The Highway1 sorted fixed hMDMs infected with *M. tuberculosis* H37Ra to over 97% purity.
- The sorted output was run on the BD FACSDiscover™ S8 to validate purity and to visualise localisation of bacteria within the cells.
- This workflow demonstrates the Highway1 is well-suited for higher containment level sorting as it is cartridge-based and generates no aerosols, reducing the risk to users.

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