TECHNICAL NOTE

Performing Replicates and Understanding Lower Cellular Limits on the IsoLight System

Performing replicates with the fully automated IsoLight System

In this Technical Note we outline:

- Perfoming replicates on the fully automated IsoLight system
- Intra-Chip Variability on the IsoLight system
- Inter-Chip Variability on the IsoLight system
- Inter-IsoLight Variability across IsoLights

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Understanding lower cellular limits

The IsoLight's built-in incubator, advanced fluidics, and precision imaging, combined with the IsoSpeak software platform allows users to visualize, target, and utilize data from direct, functional cytokine profiling of single-cells in a fully automated workflow, reducing variability from user input.

Using the published and validated IsoCode Single-Cell Adaptive Immune: PBMC Protocol, sample replicates were prepared across 6 IsoCode Chips, and the chips were run on the fully automated IsoLight system. To understand the lower limits of the IsoLight system, a subset of 200 single-cells from each IsoCode chip were randomly selected, and the Intra-chip and Inter-chip Variability for both PSI and Polyfunctionality were calculated.

Intra-Chip Variability for 200 Cells

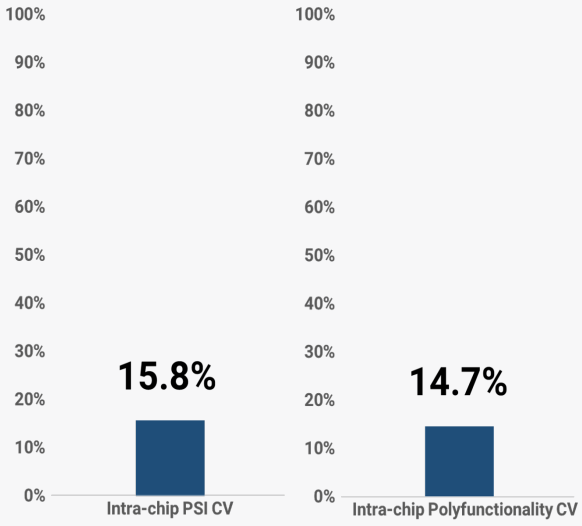

Figure 1 | **Intra-chip Variability**. The average intra-chip PSI CV was 15.8% across 6 chips.The average intra-chip polyfunctionality CV was 14.7% across 6 chips.

Inter-Chip Variability for 200 Cells

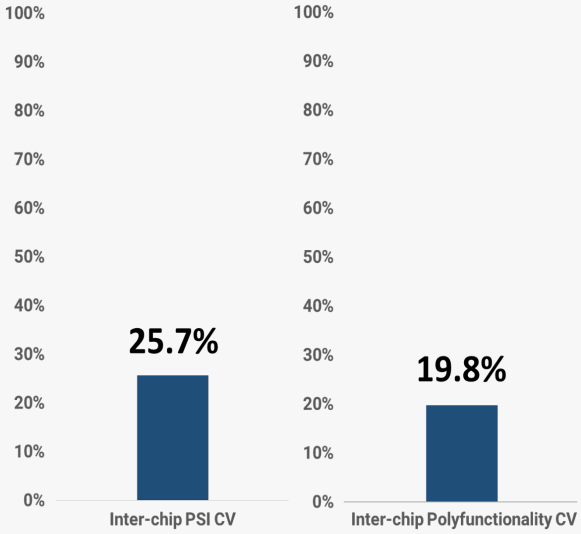

Figure 2 | **Inter-chip Variability**. The average inter-chip CV for 200 cells across 6 replicate chips for PSI was 25.7%.The average inter-chip CV for 200 cells across 6 replicate chips for Polyfunctionality was 19.8%.

To measure the intra-chip coefficient of variation (CV) of a sample, 4 random 200-single-cell subsamples were selected, and the PSI and polyfunctionality of each subsample was found (Figure 1). The average intra-chip PSI CV was 15.8% across 6 chips.The average intra-chip polyfunctionality CV was 14.7% across 6 chips.

The Inter-chip CV from 6 replicate chips with a lower limit of 200 cells is shown in Figure 2. The average inter-chip CV for 200 cells across 6 replicate chips for PSI was 25.7%.The average inter-chip CV for 200 cells across 6 replicate chips for Polyfunctionality was 19.8%.

Replicates on the IsoLight System

To measure the inter-IsoLight CV, 28 replicates were tested across 4 IsoLights. The average inter-IsoLight PSI CV was 19% from 28 chips run across 4 IsoLights.The average inter-IsoLight polyfunctionality CV was 17% from 28 chips run across 4 IsoLights (Figure 3).

Inter-IsoLight Variability

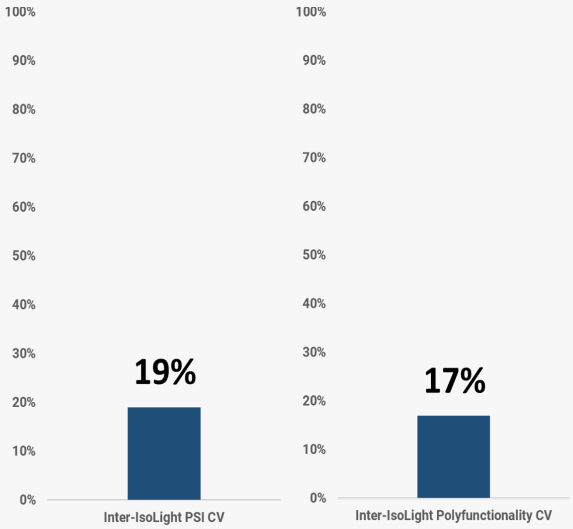

Figure 3 | **Inter-IsoLight Variability**. The average inter-IsoLight PSI CV was 19% from 28 chips run across 4 IsoLights.The average inter-IsoLight polyfunctionality CV was 17% from 28 chips run across 4 IsoLights.

Single-cell Cytokine Mapping t-SNE for 200 cells across 6 replicate chips shows consistent grouping of cell subsets across all 6 chips. (Figure 4).

This t-SNE mapping highights the consistent data generated by the IsoLight system with minimal levels of variability, even at lower limits (Figure 1,2).

Inter-chip Single-Cell Cytokine Mapping t-SNE across 6 replicates

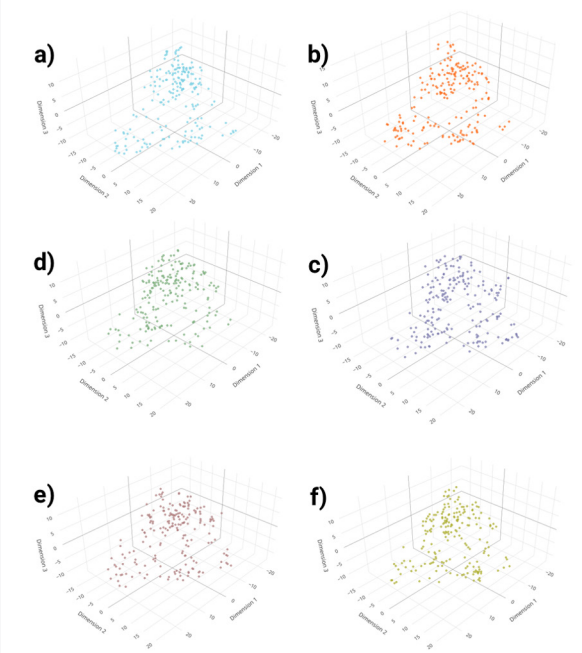

Figure 4 | **Single-cell Cytokine Mapping t-SNE.** The Single-cell Cytokine Mapping t-SNE for 200 cells across 6 replicate chips shows consistent grouping of cell subsets across all 6 chips.

The IsoLight system provides advanced, functional, single-cell characterization which can reveal correlative insights into true, functional, immune biology with an end-to-end automated workflow. At the lower cellular limits of 200 cells per chip, the IsoLight performs replicate chips with minimal variability.

Additionally, variability from hands-on user input is eliminated via the IsoLight system, revealing true functional immune biology to allow immune characterization, fitness metrics, and novel cellular discovery within the complex immune therapy discovery and development.