TECHNICAL NOTE

# Biological and Technical Variation in IsoLight Single-Cell Proteomics Experiments

Technical and biological sources of variation and effects on single-cell cytokine secretion data on the IsoLight platform

**In this Technical Note we outline:**

- Replicate consistency: correlation of t-SNE cell subsets
- Sample-to-sample correlation: consistency of secretion intensity across all 32 measured cytokines

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## Introduction

The IsoLight automation platform is a hub for comprehensive functional profiling of each cell type across a large assay menu of single-cell chip and software products. The IsoLight's innovative and impactful end-to-end workflow covers a wide range of applications, from early optimization to applying key, correlative insights and visualizations.

Through single-cell technology, it has been discovered that subsets of cells that are phenotypically similar may actually be heterogenous. Therefore, biological and technical variation may be present, depending on the experimental design of a study. Several factors must be considered when designing an experiment. In this technical note, we will cover replicate consistency and sample-to-sample correlation on the IsoLight Platform.

## Replicate consistency: correlation of t-SNE cell subsets

CD8 T cell samples were stimulated with PMA/Ionomycin and prepared according to IsoCode Single-Cell Adaptive Immune: Human PBMC Protocol with PMA & Ionomycin. Replicate samples were pipetted onto four separate IsoCode chips and run simultaneously on the same IsoLight.

### Single-Cell t-SNE Similarity

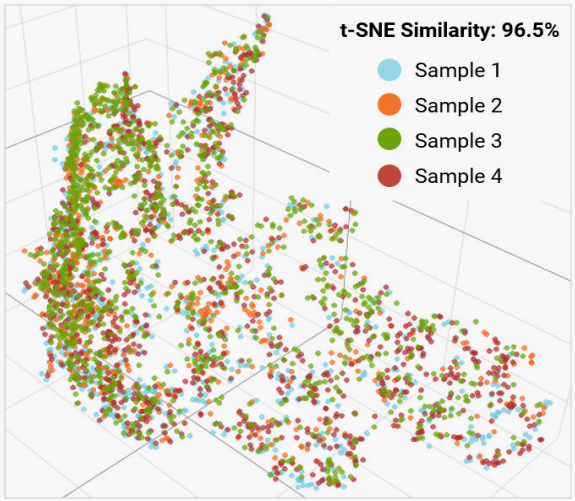


**Figure 1** | T-SNE plot of 4 replicate samples, showing consistency of distribution of single-cell secretions of each sample. The t-SNE similarity score indicates how closely matched the points of one replicate sample are to another replicate sample. On average, the sample-to-sample similarity across all sample pairs is 96.5%.

### t-SNE Similarity: Split View

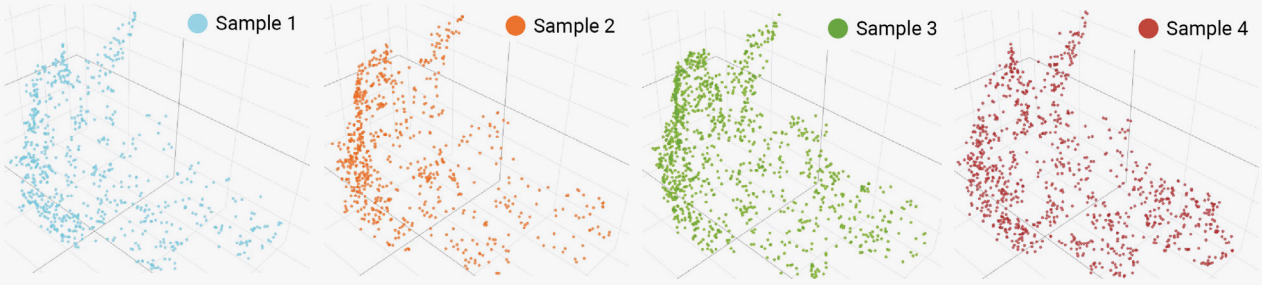


**Figure 2** | A split view of the t-SNE plot of 4 replicate samples (each sample shown individually), showing consistency of each sample's single-cell secretions of each sample. All four replicate samples showed a high degree of similarity with each other (shape of plot and density of points).

The presence of technical and biological variation was assessed using two methods: t-SNE† graph similarity and Pearson correlations between replilcate samples.

t-SNE plots were generated using all single-cell intensities of significantly secreted cytokines across the samples (Figure 1 and 2). The t-SNE similarity score indicates how closely matched the points of one replicate sample are to another replicate sample. All six sample to sample correlations ranged from R = 0.947 to R = 0.996. An aggregate similarity score was found across all pairs of the four replicate samples. On average, the sample-to-sample similarity across all sample pairs is 96.5%.

## Sample-to-sample correlation: consistency of secretion intensity

Sample-to-sample Pearson correlations were found using average single-cell signal intensity across 32 measured all cytokines (Figure 3 and 4). An aggregate average correlation was found across all pairs of the four replicate samples.

Each point in Figure 3 shows the average single-cell intensity from sample A and the average single-cell cytokine intensity from B. The average correlation across all pairs of samples was R = 0.974.

The four replicate samples were highly correlated and consistent, as evidenced by both of the applied metrics. Single-cell t-SNE plots of the four samples showed a high degree of consistency (Figures 1 and 2). The average single-cell intensities of each sample were highly correlated, as shown in Figures 3 and 4.

## Average Sample-to-Sample Signal Correlation

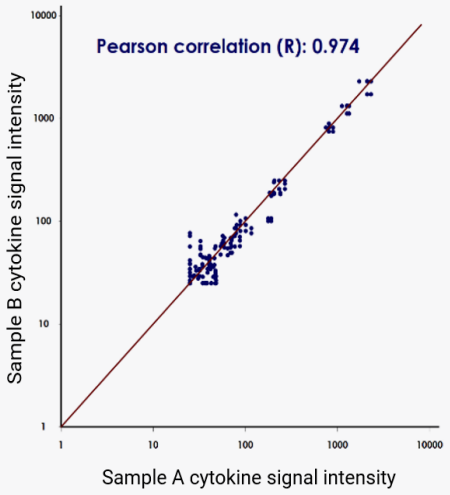


**Figure 3** | Sample-to-sample correlation plot of 4 replicate samples (all pairs of samples), showing consistency of secretion intensities of each cytokine in sample A versus sample B. Sample-to-sample Pearson correlations were found using average single-cell signal intensity across all 32 Single-Cell Adaptive Immune cytokines. Each point shows the average single-cell intensity from sample A and the average single-cell cytokine intensity from B. The average correlation across all pairs of samples was R = 0.974.

## Sample-to-Sample Correlations: Split View

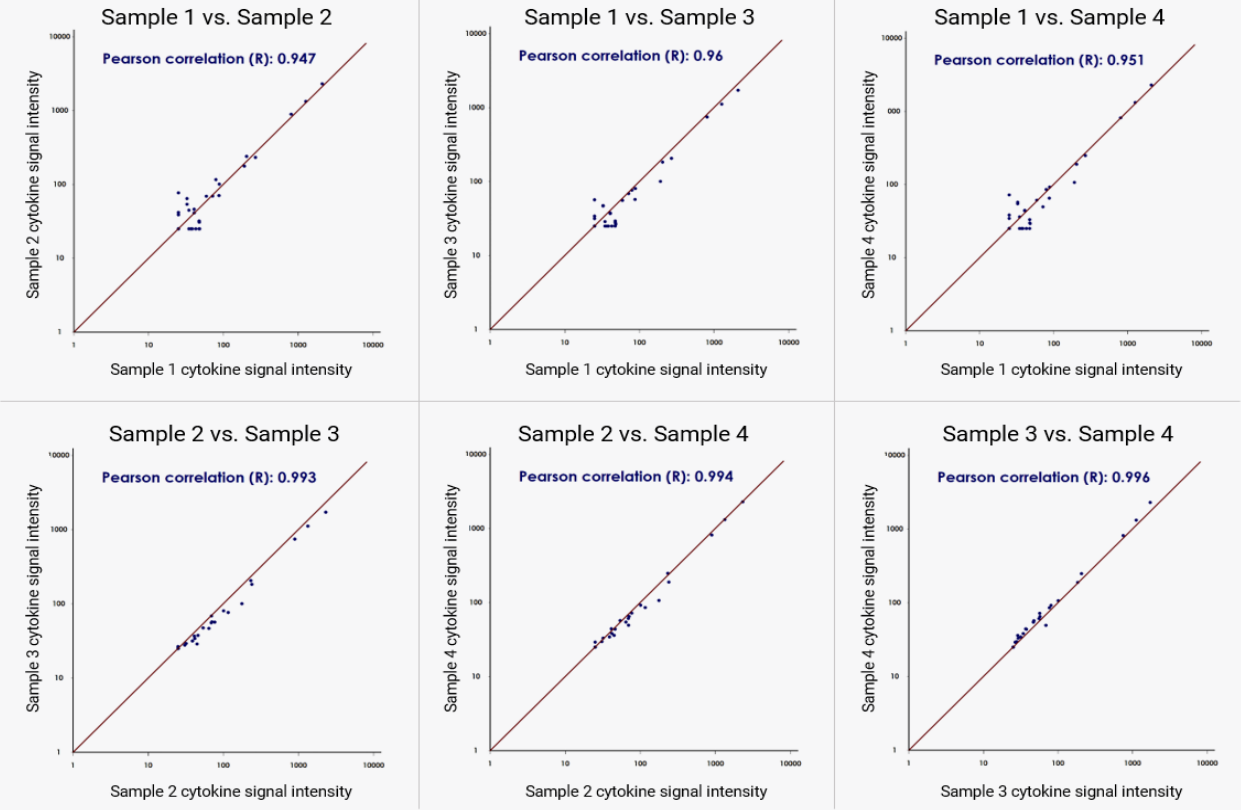


**Figure 4 |** Sample-to-sample correlation plots of each pair of replicate samples from the set of four tested replicates, illustrating the consistency of secretion intensities of each cytokine in sample A versus sample B. Sample-to-sample Pearson correlations were found using average single-cell signal intensity across all 32 Single-Cell Adaptive Immune cytokines. All 6 sample to sample correlations ranged from R = 0.947 to R = 0.996. (average of 0.974).

## Conclusion

In this technical note, technical and biological sources of variation were evaluated for their effects on single-cell cytokine secretion data from the IsoLight platform. The nearly identical resulting single-cell t-SNE plots and single-cell intensity levels across all four replicates indicate very low levels of variability.