pyTASBE – A Python Toolbox to Revolutionize 2D Cell-Cell Communication Analysis through FACs and Microscopy Data Integration

Katherine Kiwimagi Chen

In the study of complex multi-cellular systems, including synthetic cell-cell communication networks, precise measurements of individual cells and their spatial relationships over time are crucial. Flow cytometry and fluorescent microscopy are two widely used techniques for collecting single-cell data in these systems, each with its own strengths and limitations. Flow cytometry offers high precision but lacks spatial and temporal information, while fluorescent microscopy excels in providing spatial and temporal measurements but falls short in precision. This discrepancy poses a significant challenge when comparing outputs from these two methods, as they are not directly comparable without proper normalization. Even after normalization, inherent non-linearities between measurement modalities often result in comparisons that are qualitative at best. To address this issue, we introduce a software script based on Cytoflow to map both flow cytometry and microscopy data to a standardized unit: Molecules of Equivalent Fluorescein
(MEFL). This approach allows for a more meaningful comparison between the two techniques while also highlighting the limitations of such comparisons. By standardizing measurements to MEFL, we demonstrate how this conversion to physical units can account for non-linearities in current measurement techniques. This standardization is crucial, as we show that without it, certain statistical tests may not be valid when applied to data from these different methodologies. This work contributes to the field by providing a tool for more accurate and quantitative comparisons between flow cytometry and fluorescent microscopy data, potentially enhancing our understanding of multi-cellular systems and improving the reliability of analyses in this domain.