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Engineering a metabolomics workflow to capture single-cell diversity in 3D models

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Stellingen (Propositions)

Behorende bij het proefschrift

Engineering a metabolomics workflow to capture single-cell diversity in 3D models

1. Biological translation of single-cell metabolomics depends as much on mass spectrometry data processing innovations such as filtering standards and clustering algorithms as on analytical chemistry advancements in biochemical assays [Chapter II].

2. Improved analytical selectivity of metabolites without sacrificing throughput enables biological discoveries of pathways that cannot be recovered through computational analysis of data alone [Chapter III].

3. Precision medicine based on metabolomics requires mass spectrometry measurements with single-cell resolution in physiologically relevant models [Chapter IV].

4. The implementation of organoids in clinical and research settings requires the standardization of parameters in biological ones such as organoid size and microenvironment factors [Chapter V].

5. "High-throughput is imperative for single-cell analysis because a large number of single cells must be analyzed to obtain statistically significant results" *(Shi, X. et al., Chemical Science, 2026)*. While current methods of sampling and measuring indeed have relatively low throughput, translation into biological conclusions require a higher level of confidence that can only be achieved with improved quantitative analysis as well as higher throughput.

6. Metabolomics is useful because the metabolome is sensitive to minute changes in the microenvironment, and there is great emphasis on the sampling method to limit disruptions *(Alseekh, S. et al., Nature Methods, 2021)*. However, the sampling methods are not the only influence on the microenvironment. To take advantage of the full potential of metabolomics, cells should be measured as close to their complex native state as possible, which primarily includes multiple cell types, extra cellular matrix, and fluid flow.

7. “Metabolome localization in brain organoids using MSI is a promising area of research that has the potential to shed new light on the complex processes that govern brain development by identifying specific lipid signatures, metabolic routes, and metabolic cell fate trajectories” *(Cappuccio, G. et al., Frontiers in Molecular Biosciences, 2023)*. As it is clear that organoids are complex structures with inherent heterogeneity, MSI is not the only tool that should be developed. One of the next steps is to shift the focus from mass spectrometry advancements to sampling advancements such as the development of a device to isolate and rotate organoids to sample from different sections of the organoid.

8. “Drift tube-based ion mobility spectrometry and high-field asymmetric ion mobility (FAIMS) can increase selectivity and separate or filter singly charged species from the multiply charged peptides that are generally used for identification and quantification in bottom-up proteomics” *(Kelly, R., Molecular & Cellular Proteomics, 2020)*. FAIMS is optimized for proteomics because of the ability to filter singly charged ions, however, it has potential in single-cell metabolomics if an acquisition method can incorporate both direct infusion and FAIMS in one measurement.

9. “Things are only impossible until they're not.” Captain Jean Luc Picard

10. “We're all battling fear, we're all battling doubt.” Slipknot

11. “Success is often achieved by those who don't know that failure is inevitable.” Coco Chanel

Laura Ann Castaneda
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