MULTIMODAL MICROSCOPY FOR THE DETECTION OF SINGLE-CELL IMMUNE RESPONSE

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Recent advances in biology have shown the importance of advancing the ability to perform measurements at single-cell level, as numerous cellular behaviors are highly heterogeneous and cannot be accurately characterized through bulk measurements that only represent an average response over a large population. Measurements at single-cell level however raise the issues of sensitivity and throughput, as it can be technically challenging and/or costly to retrieve information from single cells. Numerous efficient approaches have been recently developed, ranging from the study of cell surface receptors with fluorescence tags or single-cell sequencing; these methods however are invasive, by involving either the use of multiple tags, and/or the destruction of the original specimen.

We developed a label-free multimodal system that combines Raman spectroscopy and quantitative phase microscopy (QPM) that allows the simultaneous acquisition of images as well as vibrational spectra at single-cell level noninvasively, and devised a method to retrieve spectra representative of the whole cellular content at high-throughput by averaging signals emitted from a region in which the excitation beam is rapidly scanned [1]. Based on these measurements, it has been possible to identify different cell types with high statistical significance.
We employed these measurements, both QPM and Raman, to extract features representative of the cellular morphology and molecular content, respectively. We then applied these label-free features with machine learning algorithms to study the immune response of macrophage cells, and detect the activation state at single-cell level within populations of genetically identical cells [2]. This method proves to be sensitive enough to retrieve a dose-response behavior with morphological indicators, and to allow the detection of selective activation pathways through the partial inhibition of the signaling cascade.

We also present more recent results, where we demonstrated the validity of our approach also in the case of primary cells [3], where we are able to distinguish both the activation state but also the origin of macrophages extracted from the peritoneal cavity, where cellular sub-types from different developmental origins are present.

REFERENCES

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