Discovering Cancer Heterogeneity by Image Analysis-linked Genomics using Phenotype-based High-throughput Laser-aided Isolation and Sequencing (PHLI-seq)

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Populations of cancer cells display serious heterogeneity in their phenotypic traits, which is important in both scientific and clinical aspects as it is deeply related to carcinogenesis and clinical outcomes. However, profiling genetic information in tumor cells *en masse* averages out variability between each tumor cells. Therefore, heterogeneous genetic information in tumor cells should be accessed by isolating single-cell or a minimal number of neighboring cells in tumor tissue into different reactors to separate their genetic information from that of surrounding populations.

To enable this, we have developed Phenotype-based High-throughput Laser-aided Isolation and Sequencing (PHLI-seq) technology to isolate each cancer cell using a single laser pulse (~ 1 isolate/second). The isolated cells then underwent whole genome amplification and sequencing. The PHLI-seq system is equipped with an infrared nanosecond pulse laser and a discharging layer for cancer cell isolation. We have also developed software for automation, which can be used by hospital pathologists or laboratory researchers to analyze cells remotely.

As a first application, we present a spatially resolved sequencing in three-dimensional cancer tissue to create a high-definition genomic map in space. Spatial information of genetic information is important, because cancer cells evolve through various geographic conditions and microenvironment of tissue, and can act differently depending on their location in the tumor. We applied PHLI-seq to breast cancer tissues to analyze genome-wide copy number alterations (CNA) and single nucleotide variants (SNV), and map each isolated cell's genomic data to the tissue's original location. Finally, we constructed a cancer genome map in 3D space of a breast cancer and visualized it using 3D visualization software. This study would provide new insights into cancer cell heterogeneity in relation to the spatial location of cancer cells.

By merging PHLI-seq and microfluidics, we also developed Laser-induced Isolation of Microstructures on Optomechanically-transferrable chip and sequencing (LIMO-seq). Using LIMO-seq we separated single circulating tumor cells and analyzed each of their whole genomes to explore their heterogeneous CNA. We applied LIMO-seq to the whole blood of a breast cancer patient and visualized the genomes of the separated rare cells. LIMO-seq, if applied to transcriptomics and other microfluidic platforms, will be a powerful tool to uncover many biological questions.