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Whole-organ profiling of cancer metastasis by tissue clearing and light-sheet fluorescent microscopy

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It has been recognized that interactions between cancer cells and stroma are important for survival of cancer cells and metastasis at distant organs. In addition, the complexity within tumor microenvironment contributes to resistance of conventional therapy. Therefore, despite decades of cancer researches, it is still difficult to elucidate the complexity of tumor microenvironment at whole organ or whole body level. To understand tumor microenvironment and overcome cancer, it is necessary to detect and quantify sparsely distributed metastatic cells throughout the body or organ at single-cell resolution.

Here, we demonstrate that CUBIC (clear, unobstructed brain/body imaging cocktails and computational analysis)-based cancer (CUBIC-Cancer) analysis with refractive-indices (RI) optimized protocol enables comprehensive cancer cell profiling in whole body and organs. We applied CUBIC-Cancer analysis to a dozen mouse models using several cancer cells and spatio-temporal quantification of metastatic cancer progression at single-cell resolution. As a result, metastatic foci can be observed and quantified through whole organ or whole body at single-cell resolution. In addition, three-dimensional (3D) monitoring revealed that the patterns of metastasis were dependent upon cancer cell or metastatic organs. Comparing two cancer cell lines, we quantified the difference of metastatic processes between angiogenesis and vessel cooption. Whole-organ profiling with single-cell resolution also enables to quantify the early steps of lung metastasis formation and rejection.CUBIC-Cancer analysis suggests that the epithelial-mesenchymal transition promotes not only extravasation but also cell survival at metastatic sites. CUBIC-Cancer analysis is applicable to pharmacotherapeutic profiling of anti-tumor drugs. CUBIC-Cancer analysis is compatible with in vivo bioluminescence imaging and 2D histology.We suggest that a scalable analytical pipeline with these three modalities may contribute to addressing currently incurable metastatic diseases.