XYZeq: A better map of cell diversity

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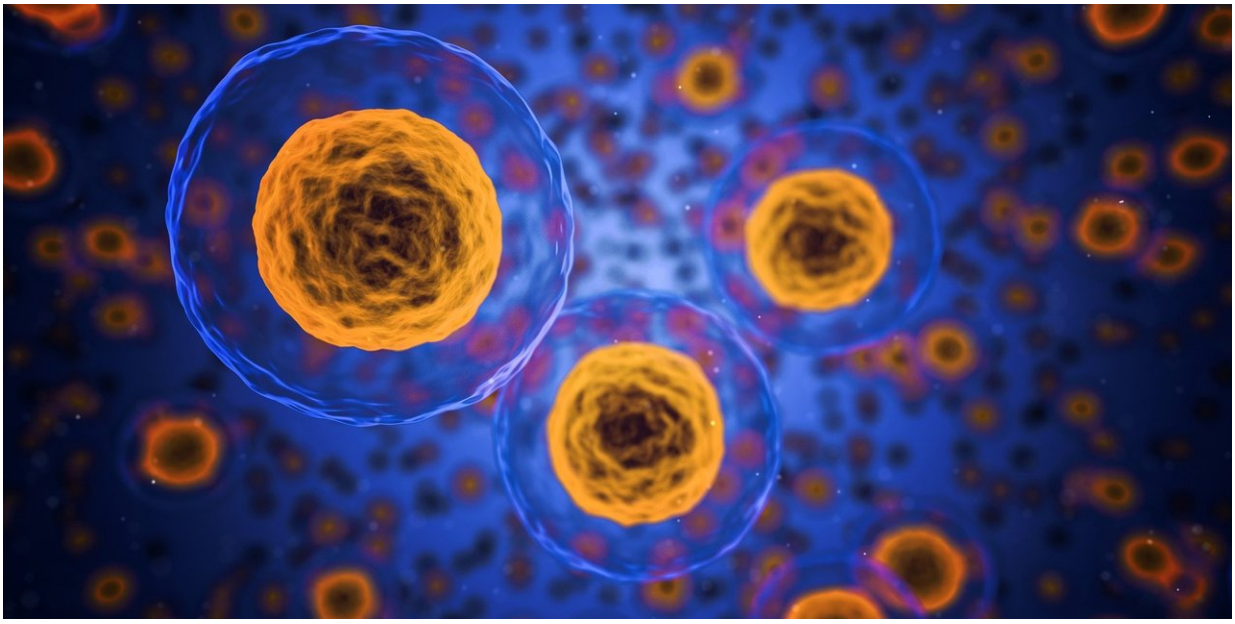

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Not all cancer cells within a tumor are created equal; nor do all immune cells (or all liver or brain cells) in your body have the same job. Much of their function depends on their location. Now, researchers at Gladstone Institutes, UC San Francisco (UCSF), and UC Berkeley have developed a more efficient method than ever before to simultaneously map the specialized diversity and spatial location of individual cells within a tissue or a tumor.

The technique, called XYZeq, was described online this week in the

journal *Science Advances.* It involves segmenting a tissue into a microscopic grid before analyzing RNA from intact cells in each square of the grid, in order to gain a clear understanding of how each particular cell is functioning within its spatial location. This offers new insight into the organization of tissues and the interplay between different cell types during disease, including in cancers.

"What we have built is essentially a way to combine microscopy and single-cell analysis by sequencing," says Chun Jimmie Ye, Ph.D., associate professor of medicine at UCSF and lead author of the paper. "This technology gets us closer to being able to create a modern-day atlas of the human body. It lets us see not only what cells are included in a specific tissue, but where they are located within that tissue, what their relationships are, and how that changes with disease."

"I think we're actually taking a step toward this being the way tissues are analyzed to diagnose, characterize, or study disease; this is the pathology of the future," says Alex Marson, MD, Ph.D., one of the study's senior authors who is the director of the Gladstone-UCSF Institute of Genomic Immunology, and associate professor of medicine at UCSF.

Over the last decade, the advent of single-cell sequencing—which lets researchers analyze (or sequence) all the DNA or RNA contained in one cell at the same time—has shed new light on the diversity of cells within tissues. However, this technique, as well as experimentally integrating data about the cells' location, remains challenging using currently available methods. So, while researchers could see that a tissue contained a great diversity of cells, they didn't know how that diverse mixture of cells was arranged.

"What most people had been doing until now was taking a whole tissue, grinding it up, and then getting single-cell data from that mixture," says Youjin Lee, a postdoctoral scholar at Gladstone and co-first author of

the new study. "Once the tissue is ground up, all information about the cells' spatial relationships is lost. With this new approach, we retain information about where each cell came from."

In XYZeq, a slice of tissue is placed on a slide that divides the tissue into hundreds of "microwells," each about the size of a grain of salt. Each cell in the tissue gets tagged with a molecular barcode—a short stretch of DNA—that's unique to the microwell it's contained in, like a neighborhood zip code. The cells are then mixed up and assigned a second barcode to ensure that each cell within a given square has its own unique identifier, like a street address within the neighborhood. Finally, the RNA from each cell is sequenced, and the results retain both barcodes to tell the researchers exactly where in the tissue it came from.

To test the utility and effectiveness of XYZeq, the team sampled tissue from mice with liver and spleen tumors. Their new approach let them visualize how cancer cells and healthy cells were arranged next to each other in the tissue samples.

However, that basic division—which could have been seen using other methods—wasn't all they could see. The team also found that some cell types located in the vicinity of the liver tumor weren't evenly spaced out; immune cells and specific types of stem cells were clustered in certain regions of the tumor. Moreover, certain stem cells had different levels of some RNA molecules depending on how far they resided from the tumor.

"This is a pattern we never would have been able to see without the spatial information that XYZeq conveys," says Derek Bogdanoff, graduate student at UCSF and co-first author of the study.

The researchers aren't sure yet what this pattern means, but they hint at the possibility that molecular signals generated by or near the tumor

affect what nearby cells do.

This is the kind of spatial information that XYZeq was created to show. And the approach not only has promise in helping researchers untangle the roles of cells in the complex environment around a cancerous tumor, but in revealing cellular patterns in other organs and diseases. The brain, for instance, contains diverse cells whose physical arrangement influences how they communicate and store information.

To develop the XYZeq system, the researchers had to build each individual component, which required a large investment in time and machinery that is not available to all research groups. So, the team is now working on ways to scale up the technology to make it more accessible to other scientists.

"This technology generates spatially localized single-cell data that can be applied to tissues from different diseases," says Eric Chow, Ph.D., an assistant professor of biochemistry at UCSF and a senior author of the paper. "Eventually, that will help us move toward being able to use it in clinical settings as well."

More information: Youjin Lee et al, XYZeq: Spatially resolved single-cell RNA sequencing reveals expression heterogeneity in the tumor microenvironment, *Science Advances* (2021). DOI: 10.1126/sciadv.abg4755

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