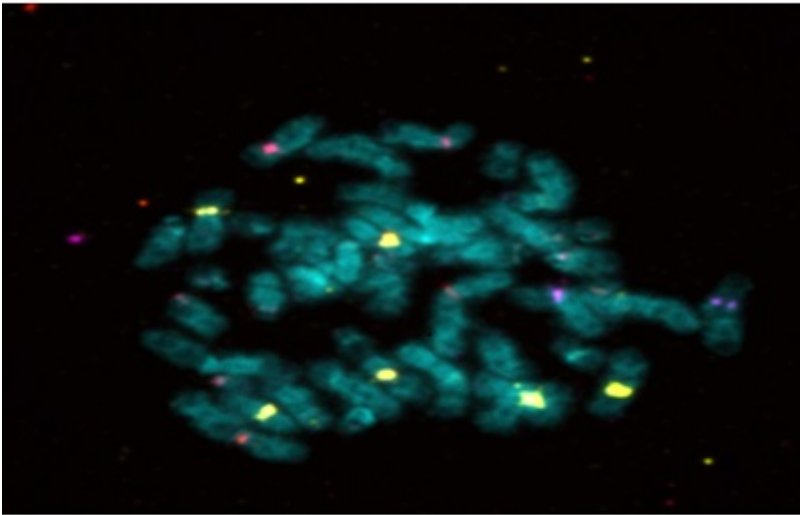


## Spatial Multiplexing with Fluorescent “Rings”

The innovation is a highly multiplexed spatial imaging approach that only requires a single round of imaging. The cellular biomarkers are labeled using a distinct fluorescent signal pattern, a fluorescent “ring”, that is deposited in situ at the site of each targeted biomolecule.



### What is the Problem?

Fluorescence microscopy can be used to characterize the spatial organization of cellular biomolecules such as chromosomal DNA, messenger RNAs, and proteins, which are essential for all cellular processes. To image more cellular biomarkers than the number of imaging channels supported by fluorescent microscopes, many methods have been developed that rely on iterative rounds of signal generation, imaging, and signal removal. These methods are complex and costly to implement because the multiple rounds of imaging require dedicated hardware and analysis solutions. As a result, these highly multiplexed imaging methods are typically not used in routine diagnostic settings and are incompatible with high value patient tissue samples.

### What is the Solution?

The solution is a highly multiplexed spatial imaging approach that only requires a single round of imaging. The cellular biomarkers are labeled using a distinct fluorescent signal pattern, a

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fluorescent “ring”, that is deposited in situ at the site of each targeted biomolecule. This approach uses in situ hybridization probes to target RNA/DNA molecules and oligonucleotide (oligo) conjugated antibodies to target protein molecules. These probes recruit an oligo conjugated to a peroxidase enzyme for programmed rounds of signal deposition at the target, resulting in “rings” of additional signal around the initial deposited signal that serve as a barcode.

### **What is the Competitive Advantage?**

The competitive advantage of this technology lies in its ability to image cellular biomarkers in a high-throughput manner with only a single round of imaging. With the fluorescent “ring” approach, the barcoding and imaging are decoupled. This approach greatly increases throughput, decreases costs, and makes highly multiplexed biomarker imaging more readily accessible to a broad range of commercially and clinically relevant samples. As the global spatial omics market is valued at \$320.8 million in 2023 with an expected CAGR of 8.2%, there is a significant opportunity for this technology to advance the field of spatial transcriptomics and proteomics.