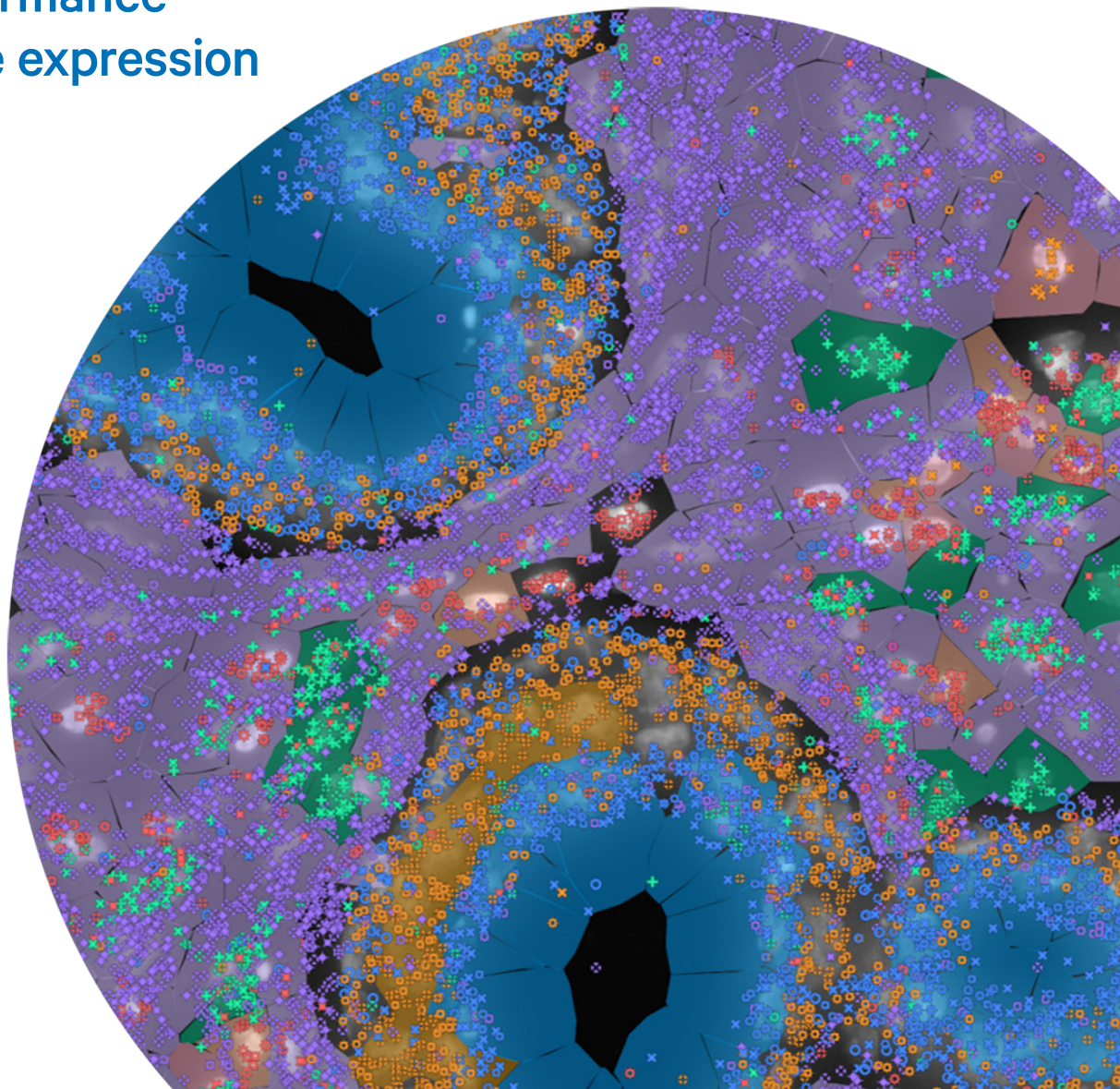![10x Genomics logo]

Xenium In Situ Grant Application Resource

High-performance in situ gene expression mapping

10xgenomics.com

Xenium In Situ

Summary statement

Xenium is an end-to-end platform from 10x Genomics that enables high-throughput subcellular mapping of 100s to 1,000s of RNA targets alongside multiplexed protein in the same tissue section. Compatible with fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) tissues, the platform includes the Xenium Analyzer, a versatile and easy-to-use instrument; sensitive and specific chemistry; a diverse menu of customizable panels; and software. Data can be visualized with the intuitive Xenium Explorer or community-developed analysis tools. As with other 10x Genomics products, the Xenium platform has a robust roadmap to enhance the core platform with more capabilities and analytes.

Overview

The advancements of targeted high-plex in situ analysis provided by Xenium gives scientists the capability to view their samples with subcellular resolution and at a depth like never before, by profiling up to 1,000s of targets, each in the context of their spatial localization patterns. This type of analysis enables studies to not only locate and type cells within their biological context, but also address questions about cell–cell communication, profile cellular microenvironments, and identify rare cell infiltration. Therefore, Xenium In Situ, provides another powerful tool that can be used alongside insights gained from Chromium Single Cell and Visium Spatial.

The Xenium platform is a complete end-to-end solution that enables scientists to visualize, quantify, and analyze gene expression and protein in FF and FFPE-preserved tissue sections immobilized onto a Xenium slide. A diverse menu of validated, customizable fit-for-purpose gene panels, as well as fully custom and multi-tissue panels, are available for highly sensitive profiling of genes of interest.

The platform includes the Xenium Analyzer, a robust, versatile instrument for high-throughput analysis, that comes with onboard analysis capabilities to process image data, localize RNA and protein signals, and perform secondary analysis. You can also easily transfer data off the instrument to perform visualization and further analysis with 10x Genomics–provided software or third-party tools of your choice. Additionally, researchers have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting.

Xenium In Situ platform

Sample preparation

The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to preserve and access the RNA with circularizable DNA probes. The DNA probes are flanked by two regions that independently hybridize to the target RNA and also contain a gene-specific barcode sequence. Ligation of the probe ends to each other then generates a circular DNA probe which is enzymatically amplified. In the event that one part of the probe experiences off-target binding, ligation will not occur, thus suppressing off-target signals and ensuring high specificity.

Multiplexed protein detection, a proven capability that will be enabled on the platform in the future, will leverage DNA-barcoded antibodies for labeling of protein targets in each tissue section.

The Xenium workflow is also non-destructive. With the sample morphology intact at the end of the workflow, both hematoxylin & eosin (H&E) and immunofluorescence (IF) staining are possible on the same tissue section, enabling the direct comparison of Xenium transcriptional data to morphological data.
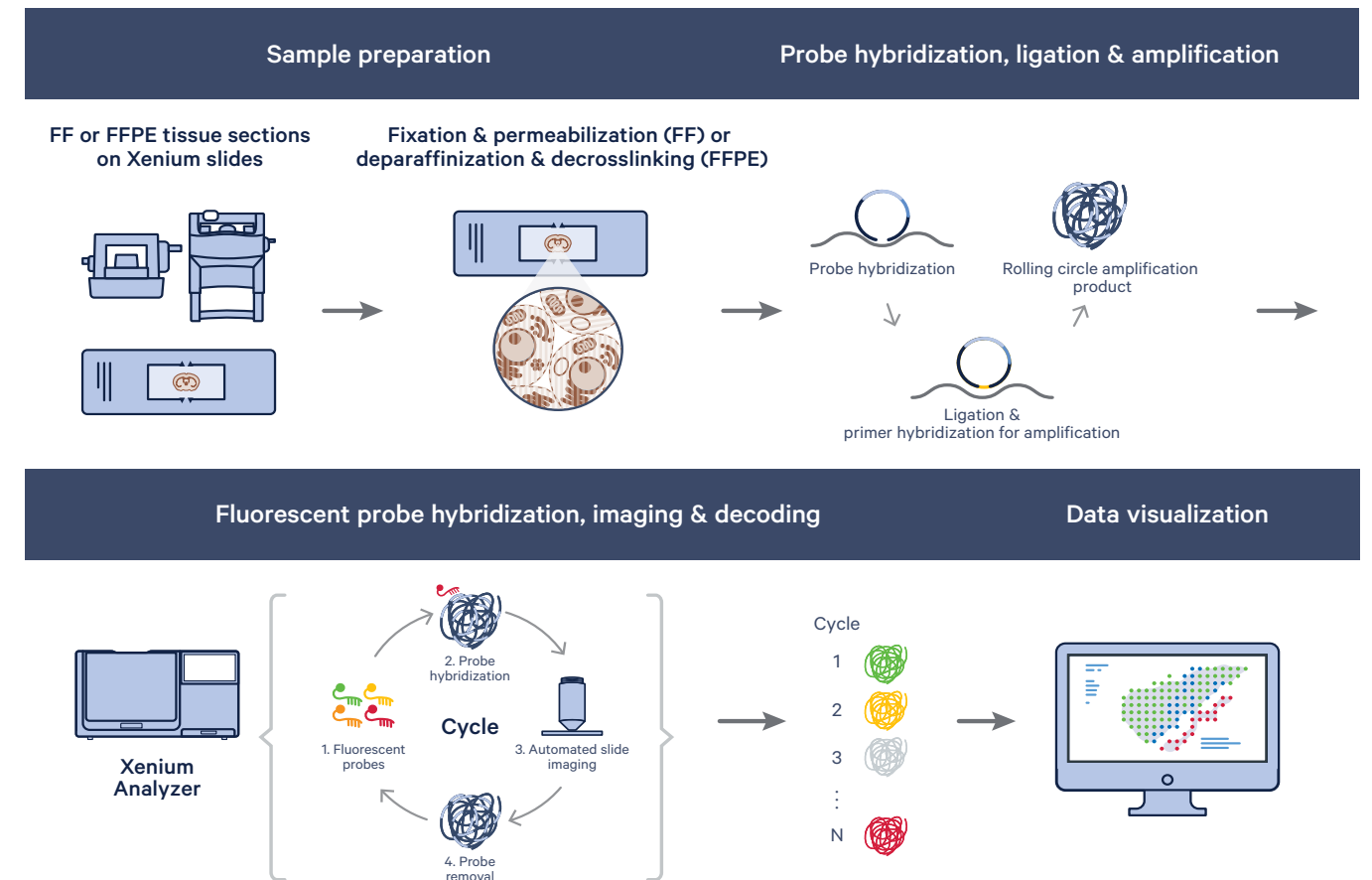

Figure 1. Workflow overview for Xenium In Situ Gene Expression assays.

Panels

The Xenium platform uses targeted panels to detect gene and protein expression at the subcellular level, and has been built for the flexibility needed to address different research questions in a variety of samples. Multiple panel options are available so you can find the one to address your specific research needs.

RNA panels

10x Genomics is developing a menu of off-the-shelf panels featuring validated, biologically relevant targets. Some predesigned panels focus on addressing a specific research need, while multi-tissue panels are able to perform cell typing across multiple tissue types. Each Xenium panel currently includes a few hundred genes with the option to customize with up to 100 additional genes specific to each project. The maximum combined plexy of off-the-shelf panels paired with a custom panel is ~400 genes.

10x Genomics also offers fully custom panels for human or mouse samples. These panels offer researchers the complete flexibility of selecting up to 300 genes. These are designed using the same algorithm as the off-the-shelf panels.

Future product extensions will expand the Xenium panels menu for additional species, tissues, and research applications. The platform has the capability for detection of over 1,000 genes and the built-in capability to detect isoforms, which will be leveraged in future panels.

Protein panels

Future product extensions will include protein panels as a part of the Xenium product line.

Decoding on Xenium Analyzer

Figure 2. Xenium Analyzer instrument.

The Xenium Analyzer (Figure 2) fully automates and integrates sample handling, liquid handling, and wide-field epifluorescence imaging. Data processing is performed in parallel to a run, allowing data outputs to be prepared quickly for interpretation. The imager uses a high numerical aperture and a fast area scan camera with a low read noise sensor to achieve ~200 nm-per-pixel resolution. The imager's field of view is 600 x 720 µm, and the Z stacks are acquired with a 0.75-µm step size across the whole tissue thickness. The standard tissue thickness is typically 5 µm for FFPE sections and 10 µm for FF sections.

The acquired images are processed through the Xenium Analyzer's onboard analysis software to enable single-molecule localization with a lateral (XY-axis) precision within < 30 nm and about < 100 nm z-axis precision. Up to two slides, each with an imageable area of about 12 x 24 mm, can be loaded onto the Xenium Analyzer per run. This large imageable area allows the flexibility to place multiple sections and samples on each slide.

Analyte detection and image acquisition are performed on the Xenium Analyzer in cycles. The reagents, including fluorescently labeled probes for the detection of RNA and protein molecules, are automatically cycled in, incubated, imaged, and removed by the instrument. An optical signature specific to each RNA transcript within the sample is generated, enabling identification of the target gene. At the end of the instrument run, the data is integrated to build a spatial map of the transcripts across the tissue section.

Software—data management and analysis

Xenium analysis software allows for immediate exploration of the assay's subcellular readout. This functionality is delivered in two parts: (1) comprehensive onboard analysis and (2) off-instrument analysis that leverages the exploration-ready output generated by the Xenium Analyzer (Figure 3).
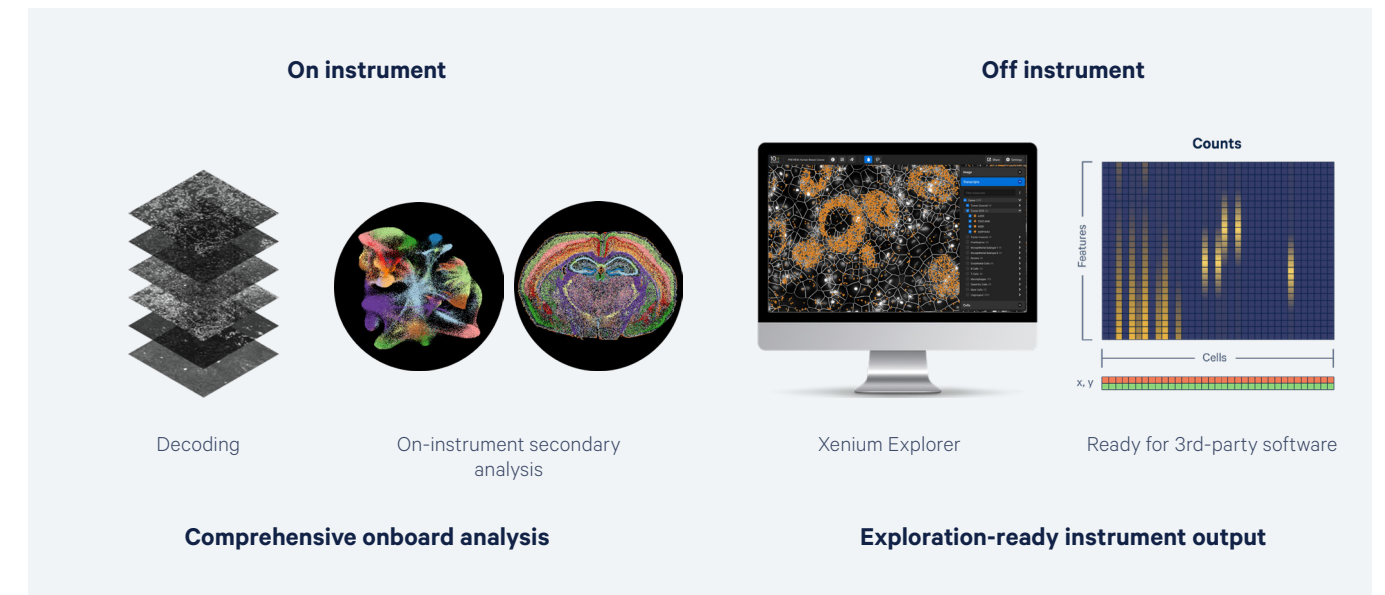

Figure 3. Xenium's streamlined onboard and off-instrument data analysis.

The first step of Xenium onboard analysis is the decoding of optical signatures into transcripts. As described in the "Decoding on Xenium Analyzer" section, chemistry and imaging cycles consist of fluorescent oligos hybridizing to amplified products. In successive cycles, fluorescent oligos bind to rolling circle amplification products and images are acquired. It is the unique optical signature of a transcript across cycles—as it lights up in different channels—that allows the onboard image processing to decode and determine transcript localization across the tissue. The next step of Xenium onboard analysis is cell segmentation, where it applies a deep learning approach to nuclear segmentation, followed by expansion to infer full cell boundaries (Figure 4). Xenium onboard analysis will also apply the deep learning approach to full cell morphology stains as a future capability of the platform. The final step of the on-instrument analysis workflow is assignment of transcripts to cells and computation of additional secondary analyses, including clustering, dimensionality reduction, and differential expression.
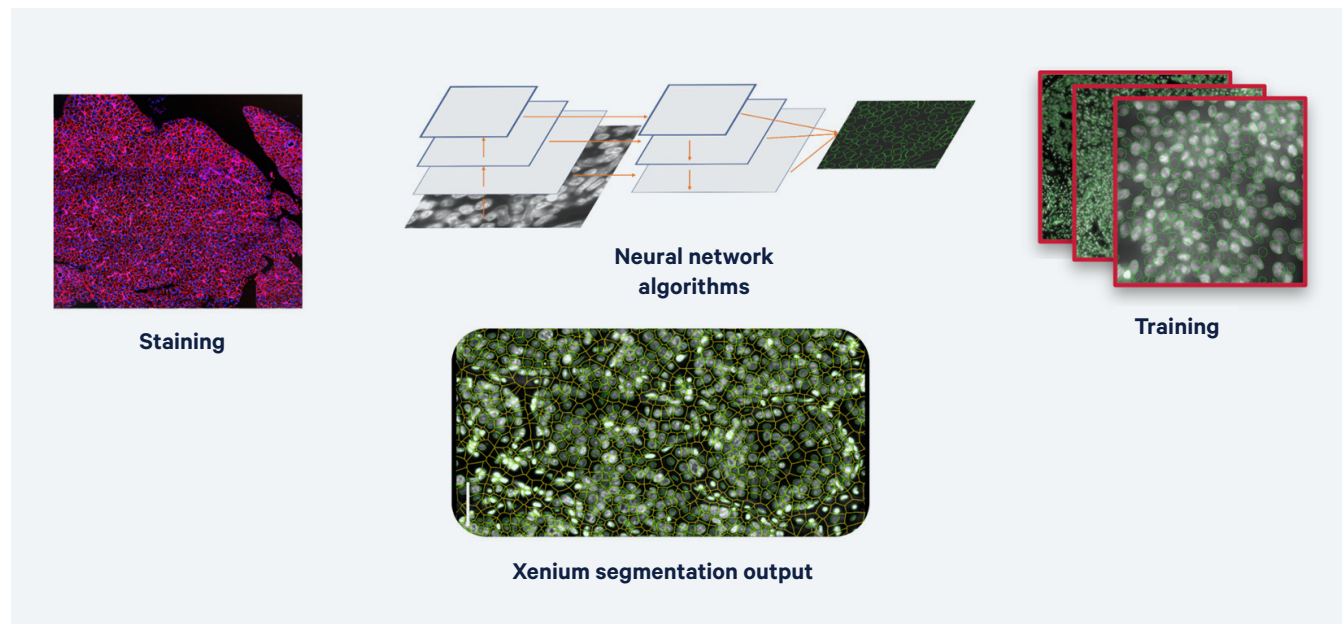

Figure 4. Xenium's integrated, deep learning approach to cell segmentation.

Directly after an experiment run, the Xenium Analyzer output can be easily transferred off-instrument for interactive visualization using the Xenium Explorer desktop application or community-developed tools without the burden of heavy data processing off-instrument (Figure 3). Xenium Explorer is a data visualization application for exploring, QCing, and analyzing Xenium In Situ data. It is designed to allow map-like panning and zooming across high-resolution morphology images and transcript data, and comparison of multiple views side-by-side in separate instances. It offers easy navigation of gene lists to view expression at subcellular scale in addition to facilitating tissue-scale comparisons.

In the future, the Xenium platform will integrate with 10x Genomics Cloud Analysis with the option to automatically transfer the onboard analysis output to local storage or to the 10x Genomics Cloud Analysis, where Xenium data will be stored, visualized, and further analyzed collaboratively.

Training and support

With purchase and acquisition of the Xenium Analyzer, 10x Genomics provides on-site installation and calibration of the instrument by a qualified Field Service Engineer (FSE). Comprehensive training for users is performed by a trained Field Application Scientist (FAS). Training topics include sample preparation, instrument operation, data interpretation, and data analysis. After completion of training, the customer will receive comprehensive support from our Technical Support, FAS, FSE, and Applied Bioinformatics teams, covering all aspects of the workflow, the consumables, the instrument, and the software. Local support is available in each geographic region.

Foundational technology

The Xenium platform is based upon foundational technologies acquired from ReadCoor and Cartana, which stem from developments in the laboratories of George Church and Mats Nilsson, respectively, combined with proprietary developments from 10x Genomics. The resulting proprietary 10x Genomics in situ technology features sensitivity, specificity, and throughput that are improved many-fold over the foundational technologies.

Representative data

The following datasets were generated using our Xenium Analyzer in conjunction with a 313-plex human breast tissue panel on an FFPE-preserved human breast invasive carcinoma tissue section and compared with a hematoxylin and eosin (H&E)-stained section from the same block, which had been previously annotated by a pathologist and run through the Visium Spatial Gene Expression for FFPE workflow and analysis pipelines.

Xenium data

The analysis of an FFPE-preserved human breast invasive carcinoma resulted in a spatial map of the transcripts with x, y, and z coordinates. Nuclei boundaries were identified through DAPI staining, and the cell boundaries were estimated by expanding the nuclei edges. RNA transcripts were assigned to specific cells and a gene-by-cell matrix was generated. Based on each cell's RNA content and the markers making up the panel, cell types were then assigned (Figure 5). For example, FASN was used to identify invasive tumor cells, and POSTN was used to identify stromal cells. Cells were colored by their assigned cell types. This resulted in a spatial map of cells, identified by type, with the assigned transcripts.
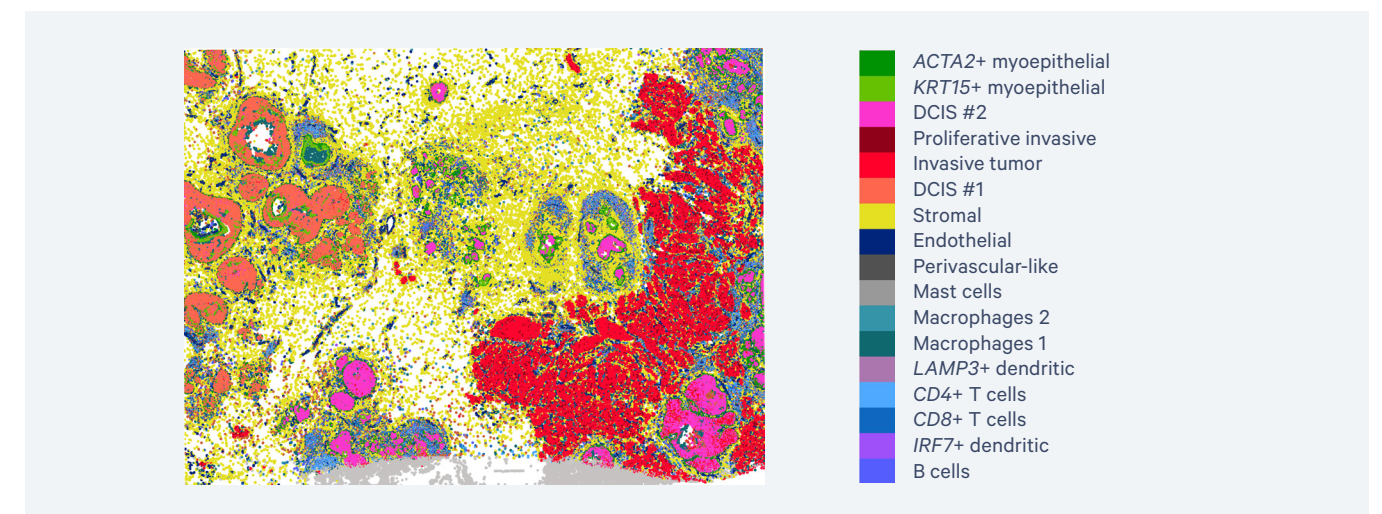

Figure 5. Xenium data provide extremely high-resolution single cell information with spatial localization from a targeted panel of genes.

Correlation data

To validate that the human breast panel for Xenium accurately captured the biological heterogeneity of the sample, the gene expression data from Xenium was compared to the expected cell types. The expected cell types in the sample were identified using Chromium Gene Expression Flex data generated from the same breast cancer block. When the Chromium Gene Expression Flex data was filtered to the 313 genes used in the Xenium human breast panel, the same cell-type populations were identified, confirming that the Xenium human breast panel captured the biological heterogeneity in the breast cancer sample (Figure 6).
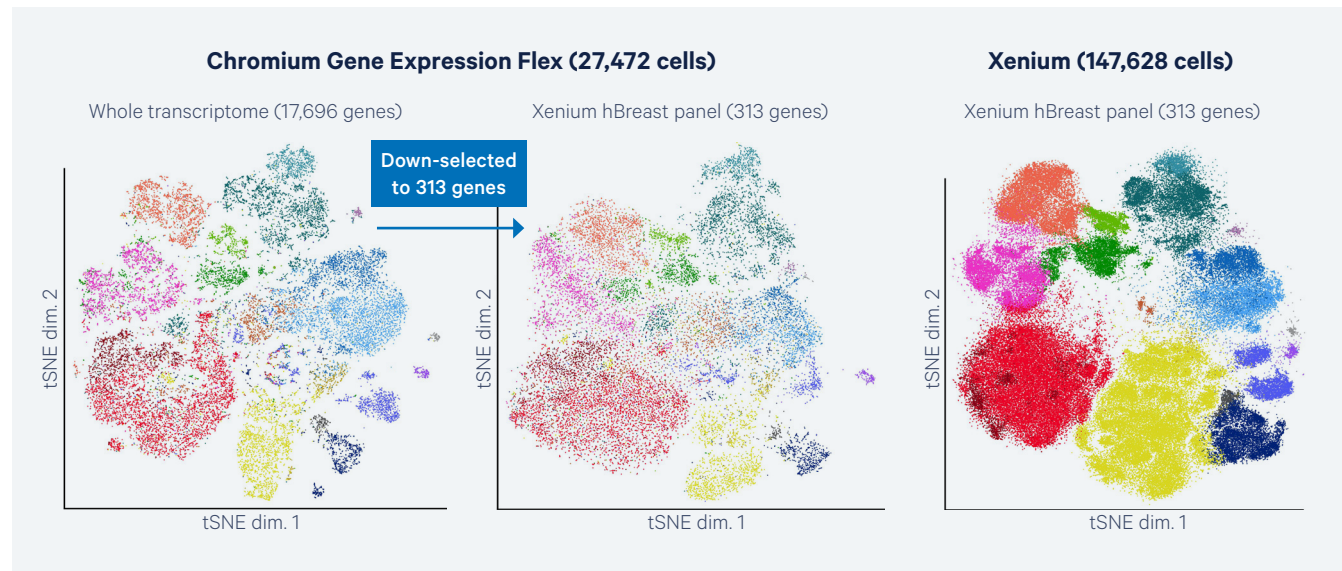
Figure 6. Xenium captures the full biological heterogeneity observed in Chromium Gene Expression Flex data.

Xenium also accurately captures spatial localization of transcripts and correlates well with the corresponding morphology as seen in the H&E image (Figure 7). To assess accurate capture of the spatial components of Xenium data, we were able to compare it with an image of the same tissue section H&E-stained post Xenium workflow. These images showed the same morphology. Xenium data also correlates well with the whole transcriptome data generated by Visium. Comparing the expression of three hormone receptor genes between Visium (detected through NGS) and Xenium (detected through microscopy at subcellular resolution) data showed a strong correlation of spatial localization of these transcripts. Further, because of the subcellular resolution of Xenium, only after these three genes were located via Xenium were we able to identify the same patterns in Visium.

Taken together, these data demonstrate that the Xenium platform can reproduce the cellular heterogeneity captured by our Chromium platform, the spatial morphology shown during H&E staining, as well as the spatial distribution of gene expression observed with our Visium platform—all within the same platform.
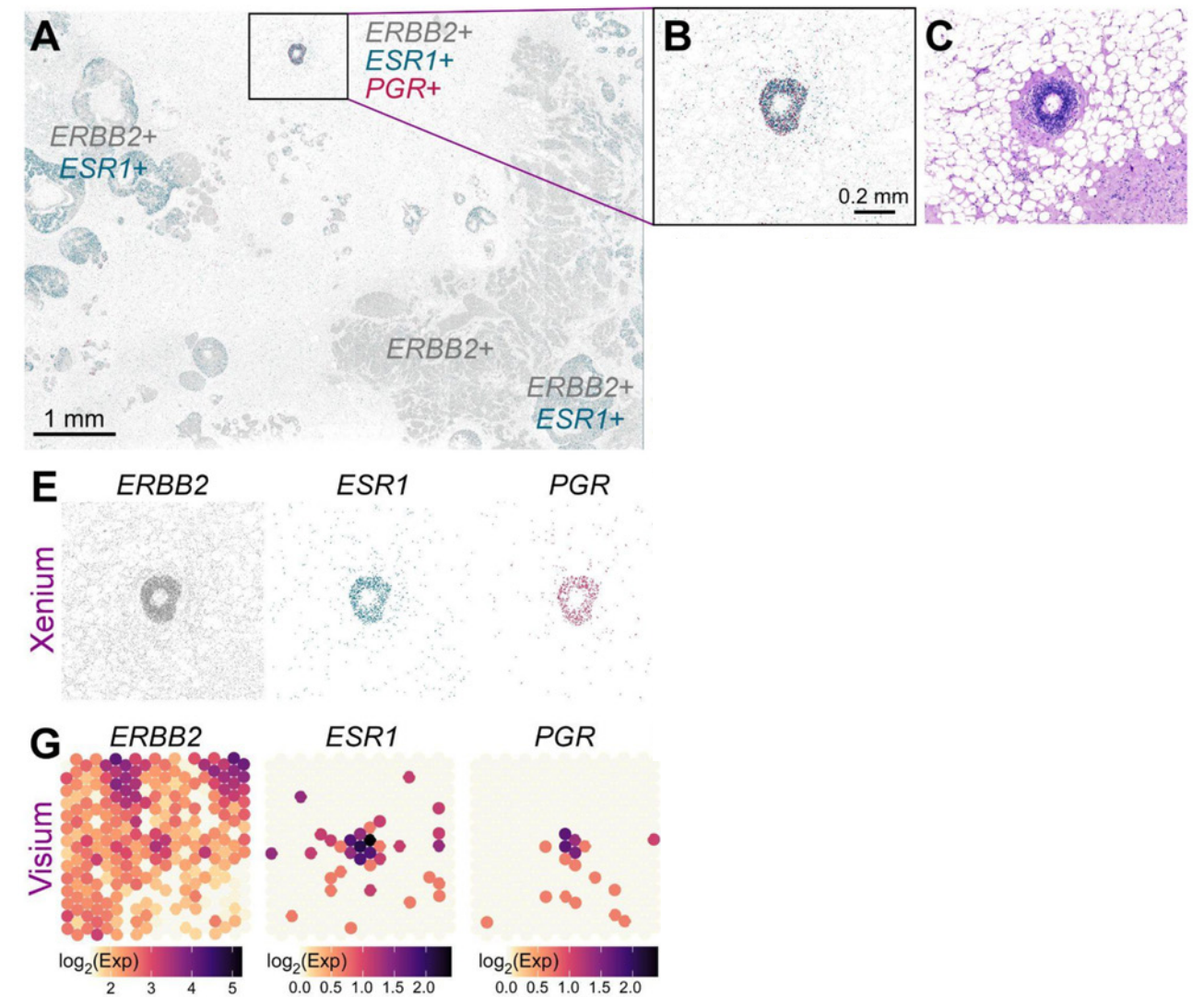
Figure 7. Xenium reproduces the morphology seen in H&E images and spatial gene expression data obtained with Visium. A. Xenium spatial plot for *ERBB2* (HER2- gray), *ESR1* (estrogen receptor - green), and *PGR* (progesterone receptor - magenta) decoded transcripts. **B.** Closer view of triple-positive ROI. **C.** Corresponding H&E image. **E.** Individual Xenium spatial plots from (B). **G.** Triple-positive region is identified in Visium (given a priori knowledge from Xenium.

Finally, to benchmark the sensitivity of the Xenium assay, it was compared against the median gene sensitivity of Chromium Gene Expression Flex. Comparing median gene expression from both technologies ensured no bias from high or low expressors. The number of transcripts per cell for Xenium was compared to the number of UMIs per cell for Chromium Gene Expression Flex. Overall, Xenium was 1.4x more sensitive than 10x Genomics' most sensitive single cell assay, Chromium Gene Expression Flex (Figure 8).
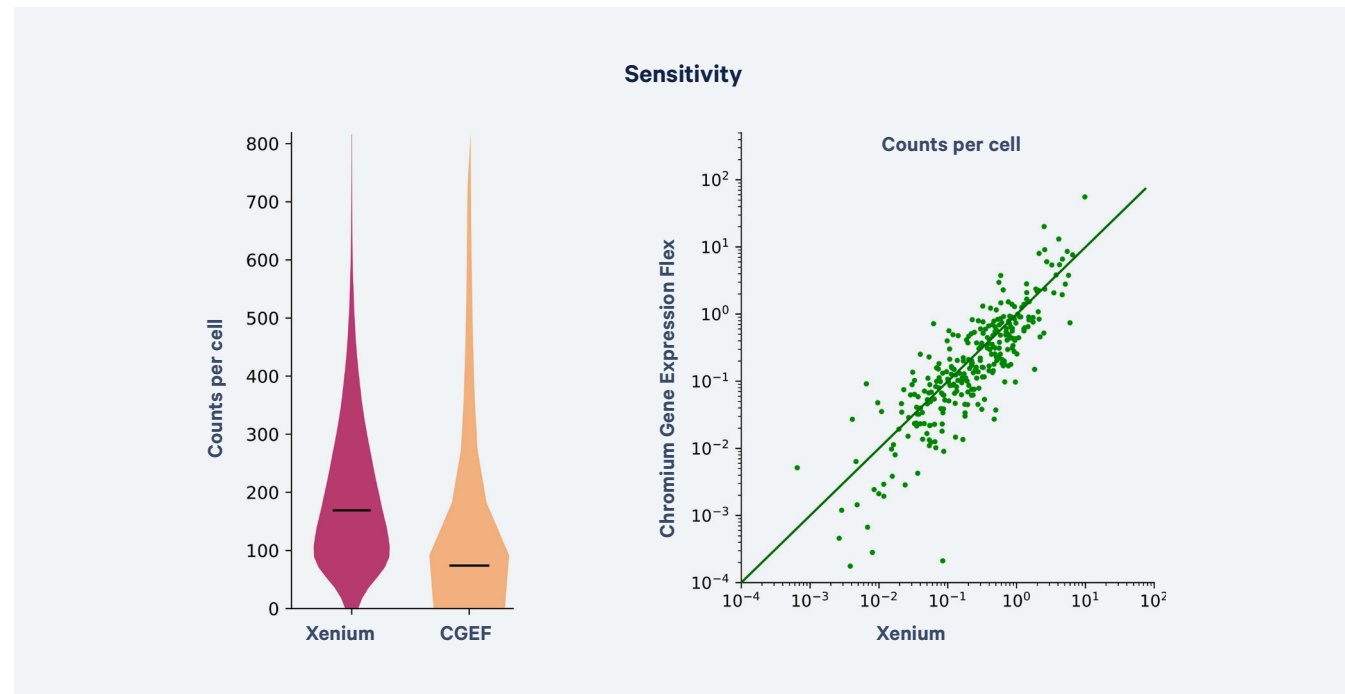
Figure 8. Benchmarking Xenium specificity, sensitivity, and resolution against Chromium scFFPE-seq. Chromium Gene Expression Flex data down-selected for only the 313 genes that appear on the Xenium gene panel. Violin and scatter plots showing the total number of transcript counts per cell detected in the Xenium data vs. UMIs per cell in the scFFPE-seq data.

Applications

The Xenium platform allows researchers to deepen the understanding of both healthy and diseased tissue through high-plex RNA and multiplexed protein detection at subcellular resolution. Previous versions of the technology have been used for:

• Cell-type mapping for Alzheimer's disease (1)

• Spatial characterization of tissue based on multiple marker genes for multiple cell types (2, 3)

• Spatiotemporal characterization of a complex developmental process and underlying transcriptional cell states (4)

• Validation of single cell sequencing and in situ sequencing data to deepen understanding of the molecular foundations of tissue organization (5)

• Characterization of the cellular composition and differentially expressed genes of distinct tumor microenvironments of interest in a ductal carcinoma in situ sample (6)

The data in the publications listed in references 1–5 above are representative of the applications of in situ technology in general and are not a representation of the sensitivity and resolution of the 10x Genomics Xenium platform. Since acquiring Cartana and ReadCoor, 10x Genomics has invested in the continuous development of the in situ technologies described in the examples above. The Xenium Analyzer combines the foundational technologies with proprietary improvements to deliver a platform that has increased resolution and sensitivity when compared to the data above. It also features significant improvements to the platform's ease of use and data-generation time.

Justification for using the Xenium platform for your research

The Xenium platform offers many advantages, making it the ideal product for analyzing high-plexy spatial gene expression and multiplexed protein information at subcellular resolution. At the core of the platform is the high-throughput Xenium Analyzer instrument, which performs fully automated labeling, imaging, and onboard data analysis.

• **Robust yet flexible core platform**—Xenium is a robust and flexible core platform that can be used for a variety of tissues, sample types, and applications. Additionally, the platform is compatible with many other capabilities that can be leveraged in the future for the simultaneous detection of RNA and protein on the same section, increased plexy to detect up to 1,000 analytes simultaneously, alternative forms of cell segmentation, additional analysis features, and more.

• **Sample input flexibility**—The Xenium Analyzer is compatible with fresh frozen and formalin-fixed, paraffin-embedded tissues samples.

• **High plexy and subcellular resolution**—The Xenium platform currently enables the detection of up to 400 RNA transcripts at subcellular resolution and is designed to allow increased plexy detection of 1,000s of analytes simultaneously in the future.

• **Curated gene panels with custom capabilities**—The Xenium platform utilizes off-the-shelf panels for biologically relevant targets, which are built using a data-driven approach that combines extensive cell atlasing studies with manual curation and invaluable input from research area experts. Xenium panels can be combined and customized with tailored gene sets delivered ready to use in the Xenium assay. Additionally, 10x Genomics is committed to expanding the Xenium off-the-shelf panel menu to include more tissue types over time.

• **Highly specific probe chemistry**—The Xenium platform's unique probe chemistry, which requires dual hybridization and ligation stringency, enables highly specific binding and the potential to target expressed SNPs and isoforms.

• **Ease of use**—The Xenium platform is a complete and intuitive solution that includes reagents required to prepare samples for analysis as well as instrumentation for image acquisition and software for onboard processing on the Xenium Analyzer. The desktop Xenium Explorer software allows for visualization and interpretation of the data.

• **Best-in-class throughput**—The Xenium Analyzer performs high-plexy imaging at the whole-section scale. Each Xenium slide has a large 12 x 24 mm imageable area, and two slides can be loaded and analyzed simultaneously. The individual throughput is determined by the sections contained within the imageable area. For example, if the tissue sections are 10 x 10 mm, each slide can accommodate two sections, allowing for four sections to be run simultaneously. With as little as two days of instrument run time, the weekly throughput is 12 sections per week.

- **Software to accelerate data analysis and insight**—10x Genomics provides comprehensive onboard analysis via the Xenium Analyzer as well as state-of-the-art data visualization with the Xenium Explorer desktop software. Onboard analysis output includes primary and secondary analysis—including cell segmentation, transcript assignment to cells, and clustering results. This allows immediate visualization and exploration of gene expression at subcellular and tissue scale in Xenium Explorer without requiring further off-instrument processing. Data is also provided in open standard file formats, allowing scientists the freedom to use other tools of their choice for custom analyses.

- **Broad support resources**—10x Genomics provides comprehensive support resources, ranging from our Technical Support Scientists, Field Application Scientists, Field Service Engineers, and Applied Bioinformatics Scientists, who are trained in Xenium workflow, instrumentation, and analysis, to freely available videos and documents that guide new users through the Xenium workflow.

- **Certified product quality**—10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.

Note: The information presented in this document includes 10x Genomics, Inc.'s estimates regarding the performance and specifications of the Xenium platform. An internet connection is required for the installation and use of Xenium instruments. 10x Genomics collects certain system logs generated by Xenium instruments, which may be used by 10x Genomics for the purposes of monitoring and improving product performance. Such logs do not include any biological data regarding experimental samples. In addition, when you contact 10x Genomics for troubleshooting or other technical support for your Xenium instrument, 10x Genomics personnel may remotely access the instrument for the purposes of providing such support. Remote access is currently required for most forms of 10x Genomics technical support. For further information, please consult with your 10x Genomics sales representative.

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Resources

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