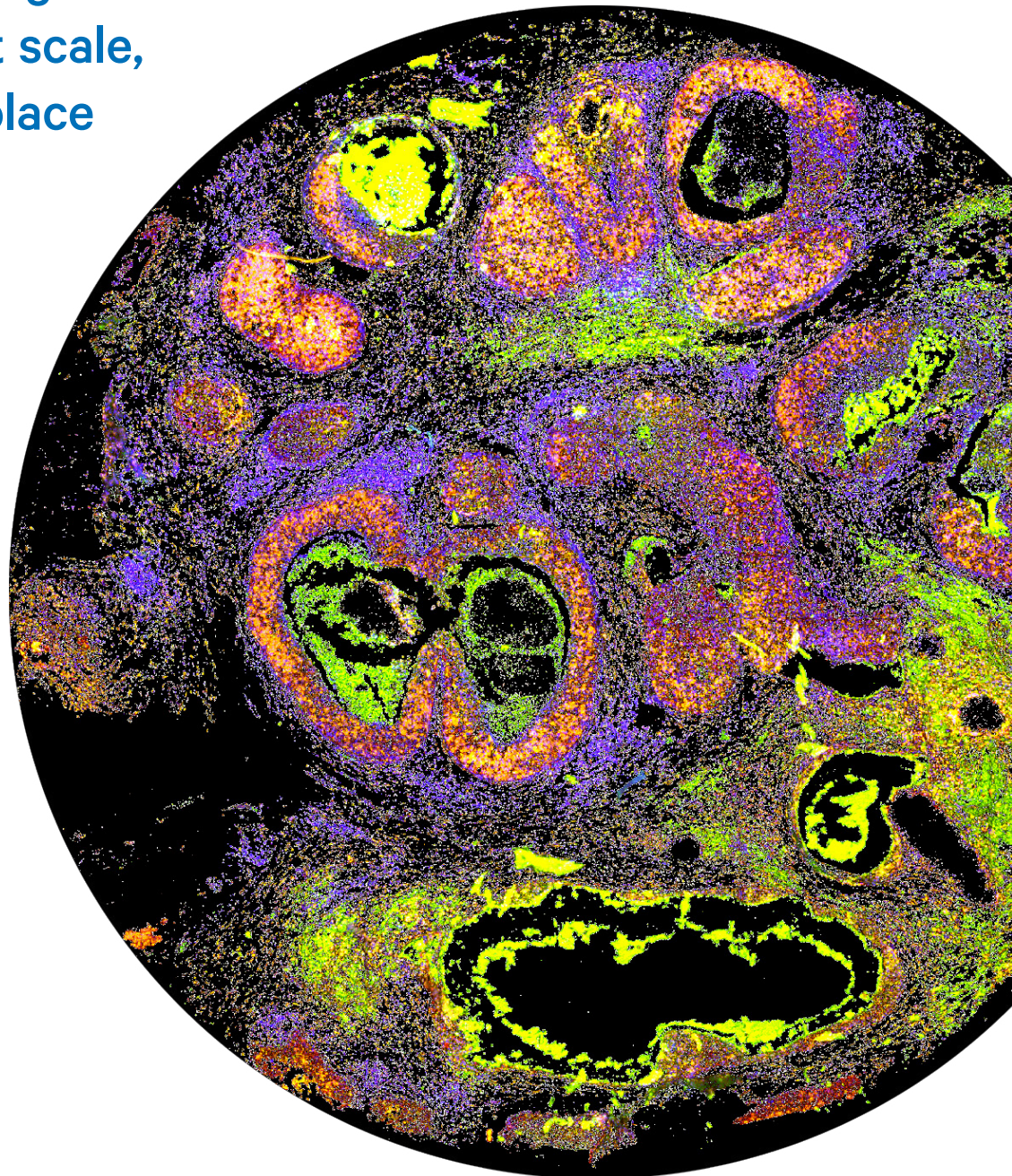


# Xenium Analyzer

In situ profiling—  
just the right scale,  
in the right place



# Xenium Analyzer

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## Summary statement

Xenium is an end-to-end platform from 10x Genomics that provides highly sensitive, targeted gene and protein expression information at subcellular resolution. The platform includes the Xenium Analyzer, a versatile instrument for fully automated high-throughput in situ analysis; consumables; panels; and software. Additionally, the platform is backed by the full support of 10x Genomics expertise, from tissue preparation to data analysis. It is based on substantial proprietary developments made to foundational technologies from ReadCoor and Cartana, which were acquired by 10x Genomics. The Xenium Analyzer combines the power of single molecule RNA and protein detection with powerful optics, data acquisition, and decoding technology to rapidly detect analytes at high plexy and at subcellular resolution across entire tissue sections. The platform is designed to be compatible with both fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) tissues. As with other 10x Genomics products, the Xenium platform will have a robust roadmap to enhance the core platform with more capabilities and analytes, such as simultaneous RNA and protein detection.

## Platform overview

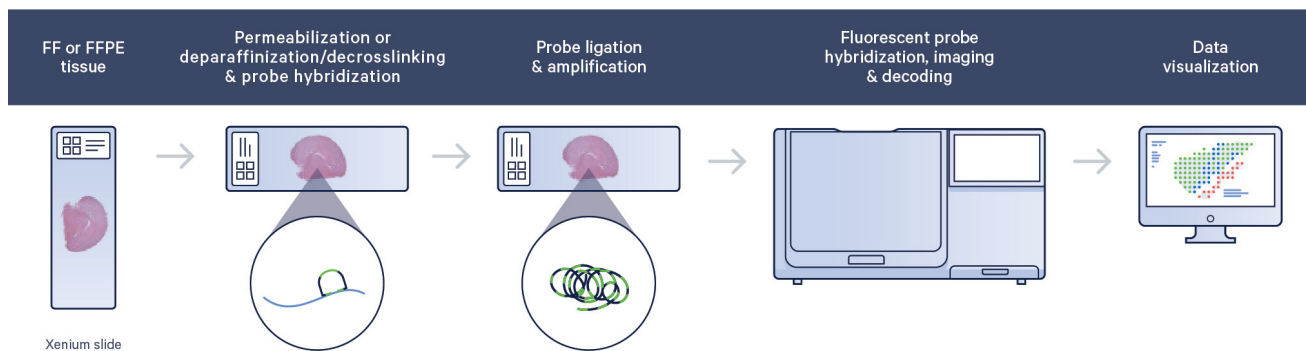
Assays such as Chromium and Visium give researchers the ability to profile the whole transcriptome of single cells or entire tissue sections, respectively. Targeted in situ analysis can be used in follow-up studies to not only locate and type cells in their biological context, but also address a variety of specific questions based on previous knowledge of the sample acquired from Chromium and Visium data.

The Xenium platform enables scientists to visualize, quantify, and analyze gene expression and protein abundance in FF- and FFPE-preserved tissue sections immobilized onto a Xenium slide. A menu of tissue- and research-specific gene panels, along with the ability to design custom gene sets, are available for highly sensitive profiling of analytes of interest. The platform leverages the 10x Genomics suite of analysis tools and pipelines to process and visualize Xenium data. Additionally, researchers have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting.

The platform is a complete end-to-end solution, including a robust, fully automated instrument for high-throughput analysis. The Xenium Analyzer 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.



## Workflow overview



**Figure 1. Xenium workflow.** Start with sectioning FF or FFPE tissues onto a Xenium slide (each slide has an imageable area of 12 mm by 24 mm). Sections are then treated to access the RNA for labeling with circularizable DNA probes. Probe ligation generates a circular DNA probe which is enzymatically amplified. Place the slide in the Xenium Analyzer where the sample then undergoes successive rounds of fluorescent probe hybridization, imaging, and removal; creating bright, easy to image signal with a high signal-to-noise ratio. An optical signature specific to each gene is generated, enabling target gene identification. Finally, a spatial map of the transcripts is built across the entire tissue section.

The Xenium workflow starts with sectioning tissues onto a Xenium slide (Figure 1). The sections are then treated to access the RNA and protein for labeling with circularizable DNA probes and DNA-barcoded antibodies, respectively. The DNA probes are flanked by two regions that independently hybridize to the target RNA. They also contain a gene-specific barcode sequence. Ligation of the probe ends on the targeted RNA region generates a circular DNA probe which is enzymatically amplified. This ligation ensures a unique level of probe specificity to the target region. Protein detection, a future capability of the platform, will leverage fluorescent probe hybridization to detect the DNA-barcoded antibodies for each labeled protein in the tissue section.

Up to two slides are then placed into the Xenium Analyzer. The enzymatically amplified DNA probes attached to RNA are bound with fluorescent oligos, creating a bright, easy-to-image signal with a high signal-to-noise ratio. The sample undergoes successive rounds of fluorescent probe hybridization, imaging, and removal. An optical signature specific to each gene is generated, enabling identification of the target gene. Proteins are further detected through hybridization of fluorescent probes to the DNA barcode of the antibody. A spatial map of the transcripts and proteins is built across the entire tissue section. Cell boundaries are inferred through nuclear segmentation or identified through cell morphology staining. Finally, cells are mapped onto the image and transcripts and proteins are assigned to cells.

## Xenium platform

### Sample preparation

The Xenium workflow, which supports both FF and FFPE tissues, starts by sectioning the desired tissues onto a Xenium slide. These sections undergo treatments, such as deparaffinization and permeabilization, to make the RNA and protein analytes accessible. The RNA is labeled with circularizable DNA probes containing two regions that hybridize to the target RNA and a third region that encodes a gene-specific barcode. The two ends of the probes bind the target RNA and are ligated to generate a circular DNA probe. In the event that one part of the probe experiences an off-target binding event, ligation will not occur, suppressing off-target signals and ensuring high specificity. Following ligation, the circularized probe is enzymatically amplified, generating multiple copies of the gene-specific barcode for each RNA binding event. This results in a stronger signal that is easy to image and has a high signal-to-noise ratio, which is particularly important when working with samples, such as FFPE tissues, that have high autofluorescence.

## Decoding on Xenium Analyzer



**Figure 2. Xenium Analyzer.**

The Xenium Analyzer (Figure 2) is fully automated and includes parallel data processing to quickly prepare data outputs 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 field of view is 600 x 900  $\mu\text{m}$  and the Z stacks are acquired with a 0.75  $\mu\text{m}$  step size across the whole tissue thickness. The standard tissue thickness is typically 5  $\mu\text{m}$  for FFPE and 10  $\mu\text{m}$  for FF. The acquired images are processed through the Xenium Analysis software to enable single molecule detection with sub-50 nm localization resolution. Additionally, the instrument integrates sample handling, liquid handling, and wide-field epifluorescence imaging. Up to two slides, each with an imageable area of about 12 x 24 mm, can be loaded onto the Xenium Analyzer. This large imageable area allows scientists 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 detecting RNA and protein molecules, are automatically cycled in, incubated, imaged, and removed by the instrument.

Following the binding of fluorescent oligos to the amplified barcode sequence, the sample undergoes successive rounds of fluorescent probe hybridization, imaging, and probe removal to generate an optical signature specific to each gene. At the end of the instrument run, the data is integrated to build a spatial map of the transcripts across the tissue section. The imager uses a high numerical aperture and a fast area scan camera with a low read noise sensor to achieve high sensitivity and resolution.

## Panels

The Xenium platform uses targeted panels to detect gene and protein expression at the subcellular level. The diversity of gene and protein expression in different sample types is best addressed by tissue- and application-specific panels with additional customization options. The off-the-shelf panels are being built using a data-driven approach that combines extensive cell atlasing studies with manual curation and invaluable input from research area experts.

### RNA panels

10x Genomics is developing a menu of off-the-shelf panels specific to certain tissues and applications. These modular panels can be combined to fit the needs of individual projects. Each Xenium panel is expected to have a few hundred genes with the option to order up to 100 custom add-on genes specific to each project. The maximum combined plexy with one to two off-the-shelf panels paired with a custom panel is 400 genes. The platform itself is being developed for a future plexy capability of ~1,000 genes. Future product extensions will expand Xenium panels for additional species, tissues, and research applications. They are also expected to allow for custom capabilities per the needs of researchers.

### Protein panels

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

## Software—data management and analysis

The Xenium platform includes onboard analysis capabilities that process image data, localize analyte signals, and perform secondary analysis to assign these markers to cells—producing a feature-cell matrix. In secondary analysis, nuclei and cell morphology information is used to identify the cell boundaries in the tissue section. Onboard analysis happens in parallel to instrument runs. A panel design portal is also included to enable experimental design and customization of experiments.

The Xenium Analyzer also includes pipelines and visualization tools for downstream analysis that enable scientists to explore Xenium data. This gives scientists access to data that is ready for interpretation quickly. Resulting data from the Xenium Analyzer can be easily transferred off instrument and combined with underlying tissue morphology, cell boundaries, and beyond for visualization and further analysis with 10x Genomics–provided software. Additionally, the data is output in open standard file formats, allowing researchers to use third-party software and community tools to perform data analysis.

In the future, the Xenium platform will integrate with the 10x Genomics Cloud. Scientists will have the option to automatically transfer instrument output to local storage or to the 10x Genomics Cloud. Scientists can leverage the 10x Genomics Cloud infrastructure to store, visualize, and analyze the image-based data.

## Training and support

With purchase and acquisition of the Xenium Analyzer, 10x Genomics will provide on-site installation and calibration of the instrument by a qualified Field Service Engineer (FSE). Comprehensive training for users will be offered 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

These datasets were generated using our Xenium Analyzer in conjunction with a 200-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 for FFPE workflow and analysis pipelines.

### Xenium data

A human breast invasive carcinoma FFPE tissue was analyzed using a gene panel containing 200 genes. This 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 (Figure 3). 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. For example, *ERBB2* was used to identify tumor cells and *DCN*, which codes for decorin, was used to identify stromal cells. Lymphocytes and macrophages were identified by CD markers. Cells were colored by their assigned cell types. This resulted in a spatial map of cells, identified by type, with the transcripts that have been assigned.

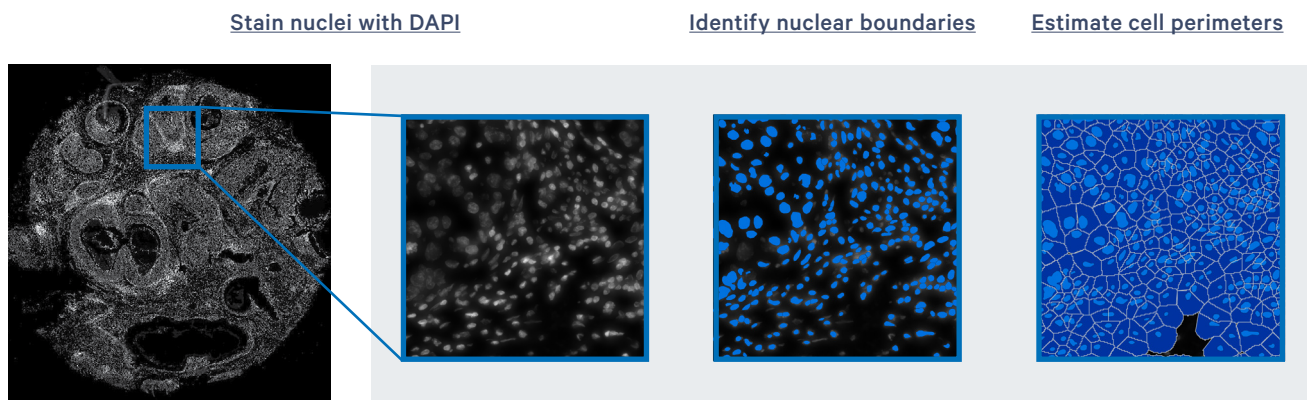


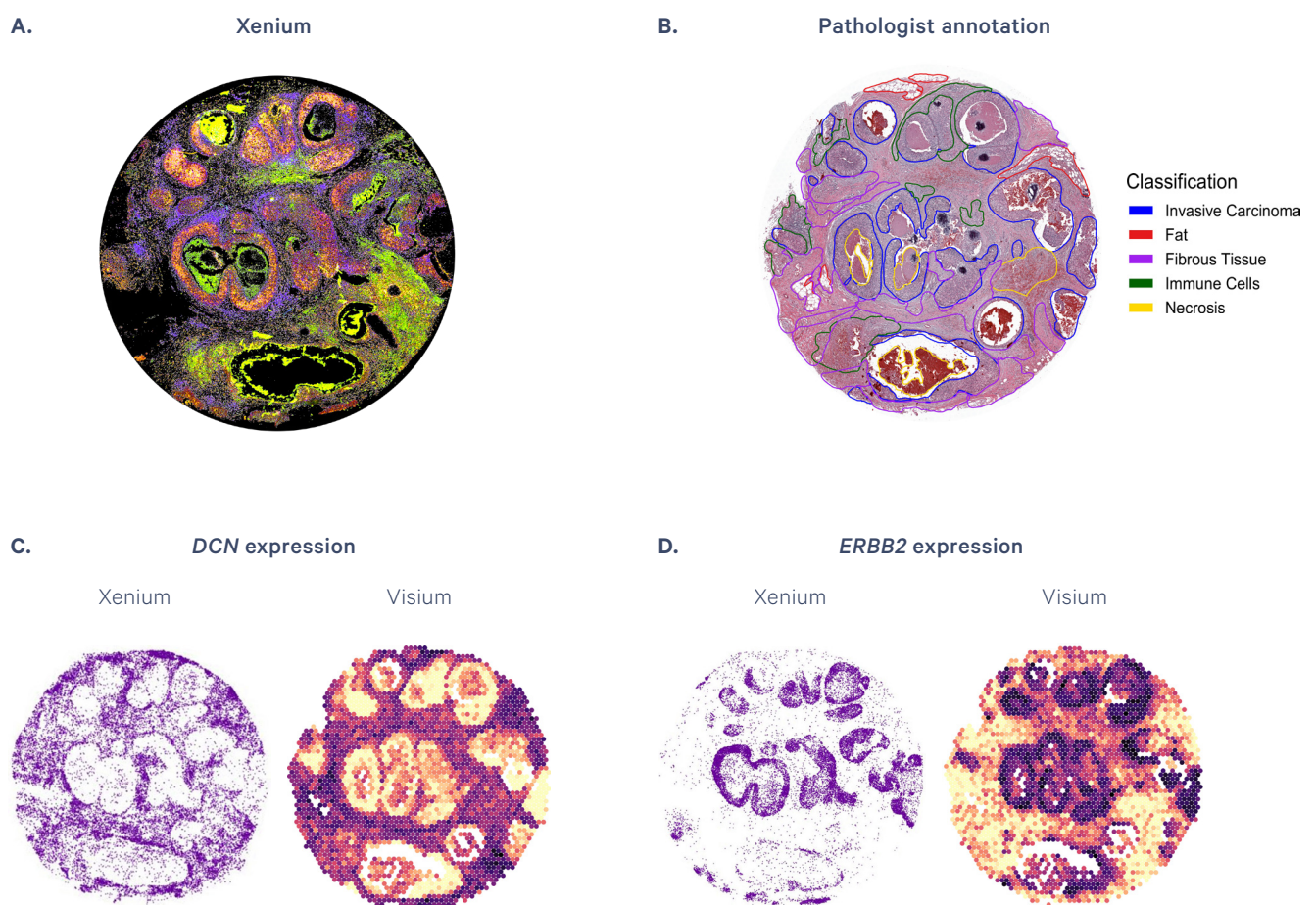
Figure 3. Defining cell boundaries using DAPI nuclei staining.

## Correlation Data

H&E staining of a tissue section from the same FFPE breast cancer block showed distinct tumor features. Pathologist annotation of this FFPE section identified invasive carcinoma surrounded by fibrous tissue, adipose tissue, and tumor necrosis. The Xenium data correlates well with the features of the invasive carcinoma annotated by the pathologist (Figure 4).

This same section was next processed using the Visium Spatial Gene Expression for FFPE assay to compare the spatial distribution seen with Visium data to the profiles obtained with Xenium. A strong correlation between the spatial distribution of genes observed in Visium (detected through NGS) and Xenium (detected by microscopy at subcellular resolution) data was observed (Figure 4).

Taken together, these data demonstrate that the Xenium platform can reproduce the cell typing and annotation of a pathologist-annotated H&E stained image as well as the spatial distribution of gene expression observed with our Visium platform.



**Figure 4. Correlation of Xenium data with pathological annotation and Visium gene expression data.** **A.** Human breast cancer FFPE section was analyzed with Xenium using a gene panel containing 200 genes. **B.** H&E stained section showing distinct tumor features as annotated by a pathologist. **C.** Side by side comparison of *DCN* expression observed by Xenium (left) and Visium (right). **D.** Side by side comparison of *ERBB2* expression observed by Xenium (left) and Visium (right).



## Applications

The Xenium platform will allow researchers to deepen the understanding of both healthy and diseased tissue through high-plex RNA and 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)

The data in the publications listed 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 ReadCoo, 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-plex spatial gene expression and protein information at subcellular resolution. At the core of the platform is the fully automated, high-throughput Xenium Analyzer instrument, which performs 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 designed in a way that enables the addition of novel capabilities in the future, including additional tissue- and disease-specific panels, the simultaneous detection of RNA and protein on the same section, and an increase in plexy to detect up to 1,000 analytes simultaneously.
- **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 is expected to enable the detection of up to 400 RNA transcripts at subcellular resolution and is designed to enable an increased plexy detection of up to 1,000 analytes simultaneously in the future.
- **Curated gene panels with custom capabilities**—The Xenium platform utilizes off-the-shelf tissue and research panels 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.



- **Ease of use**—The Xenium platform is an easy-to-use complete solution that includes reagents required to prepare samples for analysis, image acquisition, and processing on the Xenium Analyzer, as well as 10x Genomics software to analyze and interpret the data.
- **Best-in-class throughput**—The Xenium Analyzer performs high-plexity 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 mm x 10 mm, each slide can accommodate two sections and four sections can be run simultaneously. With approximately two days of instrument run time, the weekly throughput is then 12 sections/week.
- **Comprehensive data analysis solution**—10x Genomics provides data analysis pipelines for analysis and reanalysis, as well as state-of-the-art software for data visualization. Data from the Xenium Analyzer can be easily transferred off instrument, and combined with underlying tissue morphology, cell boundaries, and more for visualization. Further analysis is supported by 10x Genomics–provided software. Data is provided in industry standard file formats, allowing scientists the freedom to use other tools of their choice.
- **Cell-type assignment**—10x Genomics software assigns each detected transcript or protein to a cell and enables scientists to annotate the cell-type based on their chosen gene panel.
- **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 to-be-launched 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

Technology overview page

[10xgenomics.com/in-situ-technology](https://10xgenomics.com/in-situ-technology)

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