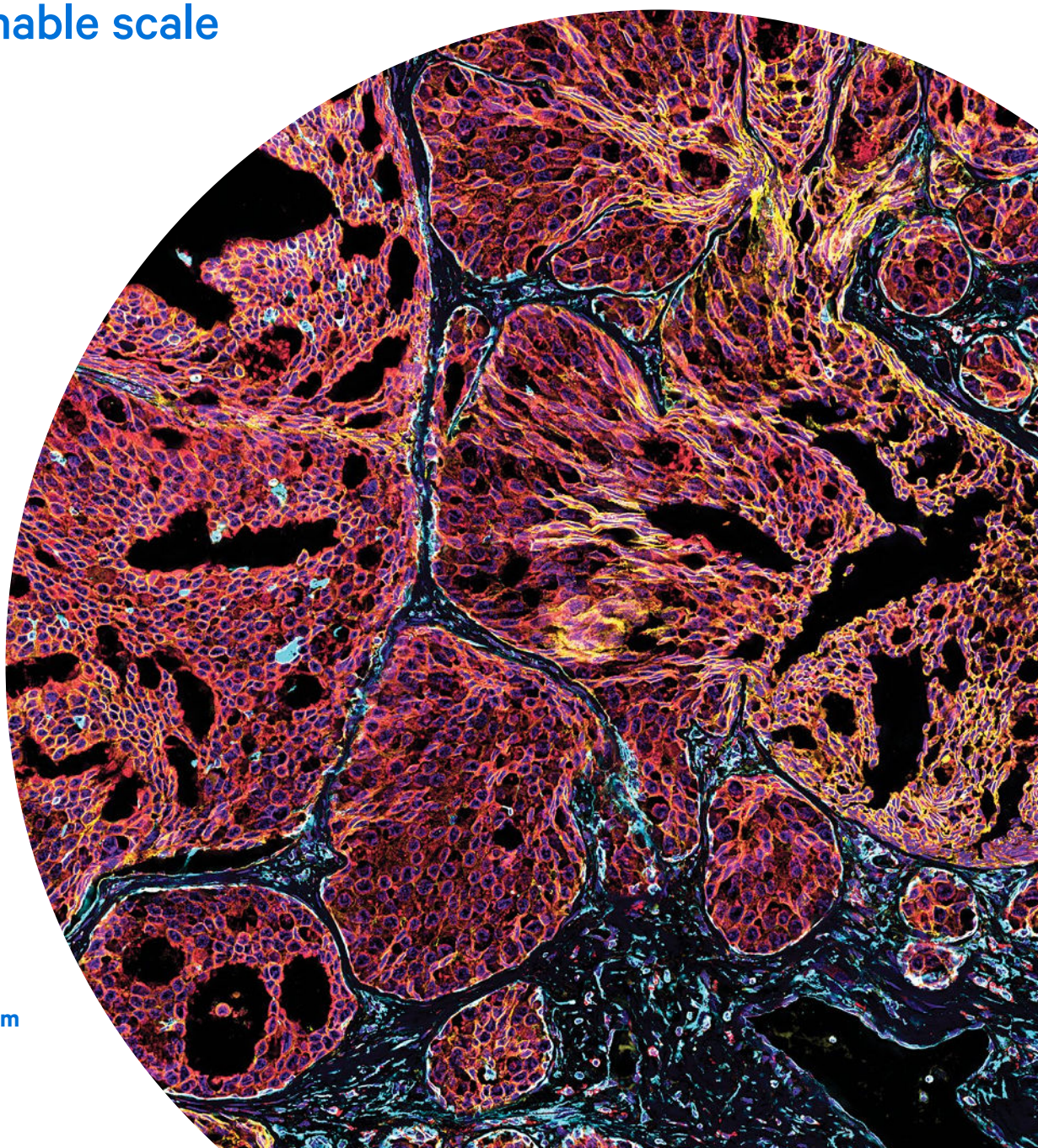
10x GENOMICS

Atera Grant Application Resource

Spatial designed for unimaginable scale



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Atera In Situ

Summary statement

The Atera platform (Atera) is an end-to-end solution (hereafter referred to as "Atera") from 10x Genomics that enables high-throughput subcellular mapping of the whole transcriptome (up to 19,000 genes). Compatible with fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) tissues, the platform includes the Atera instrument, a versatile and easy-to-use instrument; sensitive and specific chemistry; multimodal cell segmentation; a menu of customizable panels; and software. Data can be analyzed and visualized with the intuitive Sentira cloud analysis platform or community-developed analysis tools. As with other 10x Genomics products, Atera has a robust roadmap to enhance the core platform with more capabilities and analytes.

No instrument purchase is required to run Atera projects. Catalyst Research Services let researchers submit samples directly to 10x Genomics and receive whole transcriptome spatial (Atera WTA panels) or midplex spatial (Atera Select panels) analysis data. Catalyst Research Services scales to the size of the project, from pilot studies to large sample cohorts.

Overview

The advancements of single cell spatial imaging analysis provided by Atera give scientists the capability to view their samples with subcellular resolution and at a sensitivity and plex like never before, allowing simultaneous profiling of up to 19,000 genes, each in the context of their spatial localization. This type of analysis enables applications such as locating and typing cells within their biological context, addressing questions about cell–cell communication, profiling cellular microenvironments, and identifying rare cell infiltration. Therefore, Atera provides a new level of spatial discovery power and precision that expands upon insights from high-performance single cell RNA-sequencing and spatial transcriptomics technology, without compromising on plex or resolution.

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

The platform includes the Atera instrument, a robust, versatile instrument enabling high-throughput analysis, that comes with onboard analysis capabilities to process image data, localize RNA signals, and perform secondary analysis. You can also easily transfer data off the instrument to Sentira or third-party storage to perform visualization and further analysis. Additionally, researchers have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting. This extends to in-depth guidance on data analysis and interpretation.

Researchers who want to run an Atera project without purchasing an instrument can send samples to Catalyst Research Services to be run in 10x Genomics' service lab, with data delivery via Sentira and available analysis support.

Atera In Situ platform

Sample preparation

The Atera workflow starts with sectioning tissues onto a standard glass slide (Figure 1). The sections are then treated to preserve and access the RNA with circularizable DNA probes. The DNA probes contain two terminal 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. The tissue is then stained with interior, nuclear, and membrane stains for multimodal cell segmentation.

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

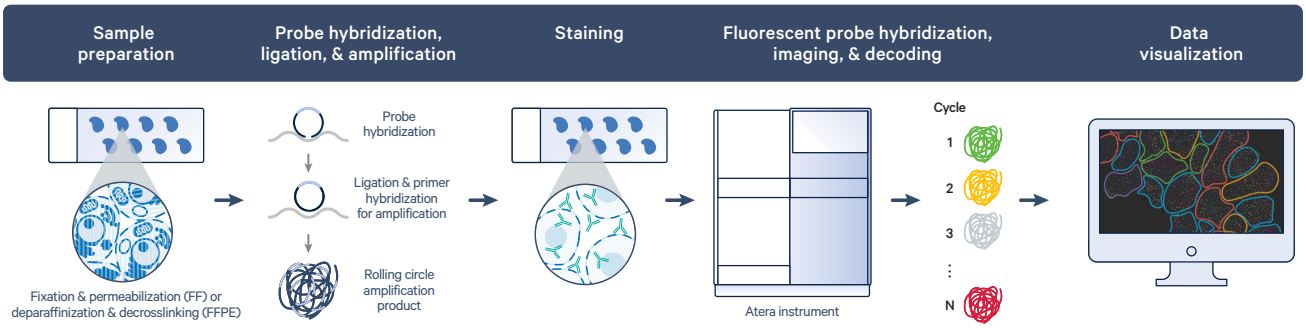


Figure 1. One universal workflow for unmatched, whole transcriptome spatial insights from your fresh frozen and FFPE tissue. After sectioning tissue onto a standard glass slide, probes from your chosen panel are bound, ligated, and amplified on-slide using a universal workflow. Following multimodal tissue staining, slides are then loaded onto the Atera instrument where fluorescence imaging, decoding, and analysis is performed automatically on-instrument.

Panels

Atera uses whole transcriptome and targeted panels to detect gene expression at the subcellular level. It has been built with the flexibility needed to span both discovery and targeted analysis applications, enabling researchers to answer different 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 offers a menu of pre-designed panels featuring validated, biologically relevant targets. Some pre-designed panels focus on addressing a specific research need, while multi-tissue panels are able to perform cell typing across multiple tissue types. Currently available pre-designed Atera panels range from 1,000-gene Atera Select panels to 18,000-gene Atera WTA (whole transcriptome) panels.

10x Genomics also offers fully custom panels for Atera. These panels offer researchers the complete flexibility of selecting up to 1,000 genes. These custom gene targets are validated using the same algorithm as the pre-designed panels. Custom panels are modular and stackable on top of existing pre-designed panels. Currently, Atera Select and Atera WTA panels can be customized by adding one additional 1,000-gene panel. Two custom 1,000-gene panels can also be stacked for a fully custom 2,000-gene panel, with the capability for stacking three 1,000-gene panels coming soon.

Imaging on Atera



Figure 2. Atera instrument.

The Atera instrument (Figure 2) fully automates and integrates sample handling, liquid handling, and wide-field epifluorescence imaging. 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 1 x 1 mm, 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.

Atera performs onboard analysis after a run completes, and onboard analysis can be parallelized with a subsequent run. Data outputs are egressed from the instrument, delivering insights directly from Atera. The acquired images are processed through the Atera instrument's onboard analysis software to enable single-molecule localization with a lateral (XY-axis) precision of 20 nm and <150 nm Z-axis precision. Up to four slides, each with an imageable area of 625 mm^2, can be loaded onto the Atera instrument 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 Atera instrument in cycles. The reagents, including fluorescently labeled probes for the detection of the amplified probe binding to RNA, 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.

Data management and analysis

Atera's analysis platform enables exploration of the assay's subcellular readout. This functionality is delivered in two parts: (1) comprehensive onboard analysis after completion of the imaging and decoding steps and (2) off-instrument analysis that leverages the exploration-ready output generated by the Atera instrument software.

The first step of Atera onboard analysis is the decoding of optical signatures into transcripts. 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 Atera onboard analysis is cell segmentation. The tissue is stained with a single mix containing multiple stains targeting nuclei, cell boundary, cell interior RNA, and cell interior protein targets. Then, Atera onboard analysis software leverages the morphological information provided by these stains to perform precise, multimodal cell segmentation. The final step of the on-instrument analysis workflow is assignment of transcripts to cells and computation of additional analyses, including clustering, dimensionality reduction, and differential expression.

Immediately following an experiment, the Atera instrument output can be easily egressed for interactive visualization using Sentira analysis and visualization or community-developed tools. The cloud-based Sentira software includes a data visualization application for exploring, QCing, and analyzing Atera spatial data. It is designed to allow map-like panning and zooming across high-resolution morphology images, cells, and transcript and protein data, offering easy navigation of gene lists and cell groups to explore spatial- and cell-type-specific patterns of gene expression in addition to facilitating tissue-scale comparisons.

Finally, our analysis pipelines offer you increased flexibility in analyzing your data by enabling you to relabel transcripts, resegment cells, and import third-party cell segmentation, H&E images, or IF images to reassign transcripts and visualize results in Sentira.

Atera projects through Catalyst Research Services

10x Genomics offers the Catalyst Research Services program. 10x is committed to enabling broad access to Atera, with a robust Atera service provider network and enabling customers to submit samples to our Catalyst Research Services. Customers can submit samples to 10x, choose any pre-designed Atera RNA panel or design their own custom panel, and receive high-quality Atera data for their unique studies.

Training and support

After purchase and acquisition of Atera, 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, consumables, instrument, software, and analysis. Local support is available in each geographic region.

Foundational technology

The Atera platform builds upon foundational capabilities that the Xenium In Situ platform brought to the spatial biology market. Atera technology features sensitivity, specificity, and throughput advancements that are improved many-fold over the technologies used in the Xenium Analyzer instrument. The original Xenium In Situ platform was based on proprietary developments from 10x Genomics and technologies acquired by 10x Genomics.

Representative data

The following datasets were generated using Atera in conjunction with an Atera WTA Human panel on an FFPE-preserved human stage III-B invasive squamous cell carcinoma of the cervix tissue section, and then on serial sections from FFPE-preserved human testis.

Atera data

The analysis of an FFPE-preserved human squamous cell carcinoma of the cervix sample resulted in a spatial map of the transcripts with X, Y, and Z coordinates. Nuclei boundaries and cell boundaries were identified through multimodal cell segmentation, which entails DAPI staining plus interior and membrane staining. RNA transcripts were assigned to specific cells, and a gene-by-cell matrix was generated. Based on the differential gene expression analysis from graph-based clustering, cell types were annotated (Figure 3). Well-differentiated tumor cells were identified via their resemblance to the stratified epithelium of the ectocervix. Hypoxic tumor cells were identified via known markers, such as *CA9*, *HIF1A*, and *NDRG1*. *ODAM* was a novel biomarker found to be highly enriched in hypoxic cells, whereas *FGFR3* was highly enriched in the proliferative (*MKI67*$^{+}$) parabasal cells.

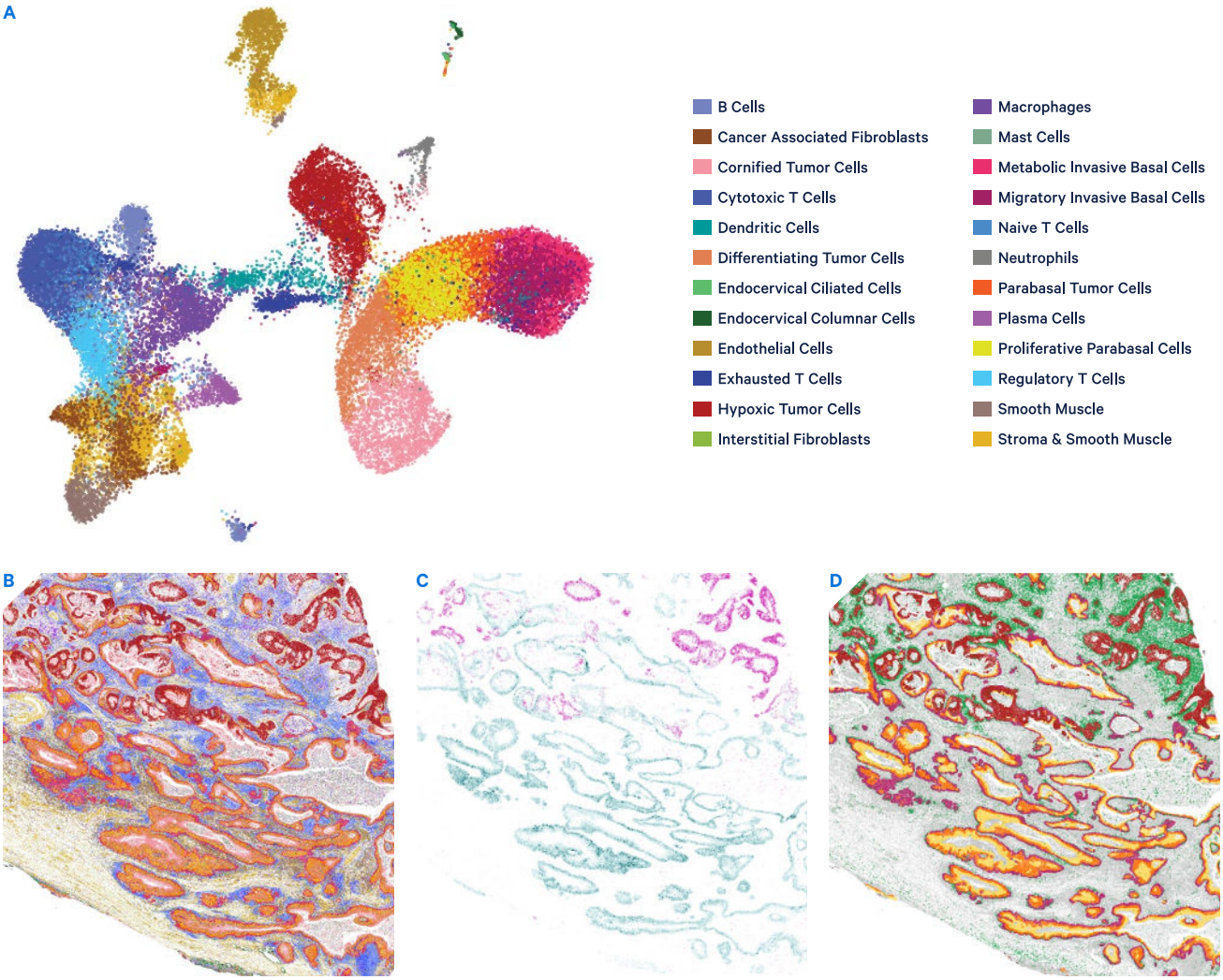


Figure 3. Atera data provides extremely high-resolution single cell information with spatial localization from a whole transcriptome panel. **A.** Representative UMAP (717,576 cells detected and 1.04B transcripts over ≥Q20) from FFPE stage III-B invasive squamous cell carcinoma of the human cervix using the Atera WTA Human panel. **B.** Representative spatial distribution of cells from UMAP (A) in the cervical cancer section. **C.** Precise localization of unique tumor states in the cervix tumor microenvironment leveraging biomarkers, *ODAM* (pink), indicative of hypoxic tumor, and *FGFR3* (teal), indicative of proliferative parabasal tumor cells. **D.** Niche analysis for cell types detected in the cervical tumor microenvironment with Atera WTA (hypoxic tumor [red], well-differentiated tumor [orange, yellow, pink]) reveals *INHBA* and *COMP* (green) as novel hypoxia-associated gene expression markers from a heterogeneous immune cell niche.

Performance data

To validate the robustness and sensitivity of the Atera WTA Human panel, gene expression data from an Atera run on FFPE human testis was compared to gene expression data from the same sample block run with the Chromium Flex Apex assay (Figure 4). The data demonstrated a strong correlation of transcripts per cell, with the Atera WTA assay showing ~1.3x median sensitivity compared to Flex Apex. A strong correlation was also seen for multiple replicates of FFPE human testis across several Atera runs and between replicate sections on the same slide.

Taken together, this data verifies the high performance of the Atera platform, with equivalent, if not higher, sensitivity in FFPE tissues compared to the Chromium Flex Apex assay, but with the added capability of mapping spatial morphology and whole transcriptome gene expression in the same tissue section.

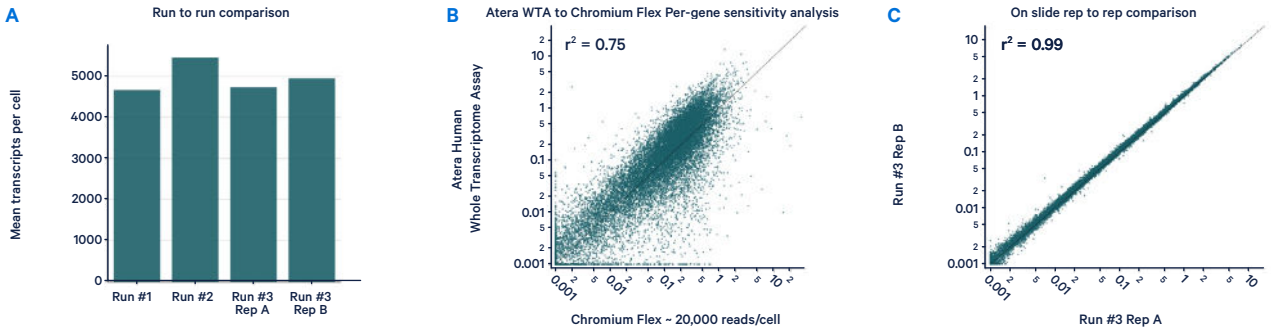


Figure 4. Reproducibility and sensitivity of Atera in FFPE human testis. A. Sensitivity (transcript count per cell) of multiple replicates across several runs. **B.** Representative plot of transcripts per cell comparing Atera WTA to 10x Flex Apex. **C.** Representative plot of a correlation of transcript counts between two technical replicates using Atera WTA on the same slide.

Justification for using Atera for your research

Atera offers many advantages, making it the ideal product for analyzing high-plex spatial gene expression with sub-100 nm resolution. At the core of the platform is the high-throughput Atera instrument, which performs fully automated labeling, imaging, and onboard data analysis.

- **Robust yet flexible core platform**—Atera is a robust and flexible core platform that can be used for a variety of tissues, sample types, and applications. Additionally, the platform is built to enable third-party analysis features and future multiomic capabilities.

- **Unified sample input compatibility**—The Atera sample prep workflow is compatible with both fresh frozen and FFPE tissue, requires no optimization (even across diverse tissue types), and is non-destructive, which allows the same section to be H&E- or IF-stained post-workflow.

- **High plex and subcellular resolution**—Atera currently enables detection of up to 18,000+ transcripts with 20-nm XY localization and high spatial fidelity.

- **Curated gene panels with custom capabilities**—Atera utilizes pre-designed 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. Atera panels can be combined with custom panels targeting tailored gene sets, including isoforms, exogenous sequences like gRNAs and barcodes, CAR T-cell transcripts, viruses, and more, delivered ready to use in Atera assays.

- **Highly specific probe chemistry**—Atera's unique probe chemistry, which leverages a dual hybridization and ligation stringency, enables highly specific binding and the potential to target expressed SNPs and isoforms.

- **Ease of use**—The Atera platform is a complete and intuitive solution that includes reagents required to prepare samples for analysis, instrumentation for image acquisition, and software for onboard processing on the Atera instrument. Sentira Analysis Software allows for visualization and interpretation of the data.

- **Best-in-class throughput**—The Atera instrument performs high-plex imaging at the whole-section scale. Each off-the-shelf slide has a large 625 mm^2 imageable area, and up to four slides can be loaded and analyzed simultaneously. Throughput for a specific experiment is determined by the panel plex and the total tissue area you're analyzing in a single run. For a 1,000-plex Atera Select panel on 16 cm^2 of tissue (4 slides with 4 cm^2 tissue per slide), using multimodal cell segmentation, the Atera instrument runtime takes approximately 29 hours. For our 18,000-plex Atera WTA panel, using the same tissue area and segmentation, the instrument runtime takes approximately 7 days, and can scale throughput to over 800 1 cm^2 samples per year.

- **Software to accelerate data analysis and insight**—10x Genomics provides comprehensive onboard secondary analysis via the Atera instrument as well as state-of-the-art data visualization with Sentira Spatial cloud analysis software. Onboard analysis output includes cell segmentation, transcript assignment to cells, and clustering results. This allows visualization and exploration of gene expression at subcellular and tissue scale on 10x Cloud 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 access**—A growing core lab footprint and Catalyst Research Services enables Atera WTA and Atera Midplex projects without an instrument purchase.

- **Comprehensive support resources**—10x Genomics provides resources ranging from our Technical Support Scientists, Field Application Scientists, Field Service Engineers, and Applied Bioinformatics Scientists, who are trained in the Atera workflow, instrumentation, and analysis. Additionally, freely available videos and documents can easily guide you through the Atera workflow.

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

Note: An internet connection is required for the installation and use of Atera instruments. 10x Genomics collects certain system logs generated by Atera 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 Atera 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.

Resources

Technology overview page
10xgenomics.com/platforms/atera

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