affymetrix
Biology for a better world

excellence

Enabling single cell analysis
from exploration to validation

Real insight starts with single cells.

Don't settle for average.

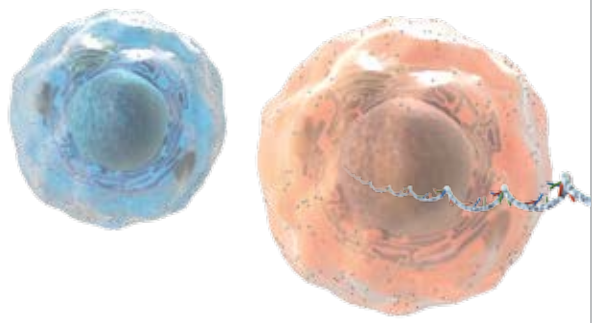
eBioscience GeneChip USB

Refuse to be average

Cellular heterogeneity is present in all biological samples, including gene expression differences between cells, low- to high-protein levels in cell populations, or unique morphological attributes of individual cells within tissues. Paradoxically, most of our understanding of gene expression is based upon bulk population averages. This has advanced our understanding of general biological function and the identification of informative signatures. However, bulk analysis often leads to conclusions that assume averages reflect the dominant biological mechanism operating within an entire population. Using such measurements and assumptions can mask the presence of rare cellular attributes or average an important natural bimodal distribution of distinct cellular behaviors. Furthermore, it ignores essential cell-to-cell and spatial differences. To fully understand how gene expression heterogeneity contributes to biological function, a single-cell analysis approach must be applied.

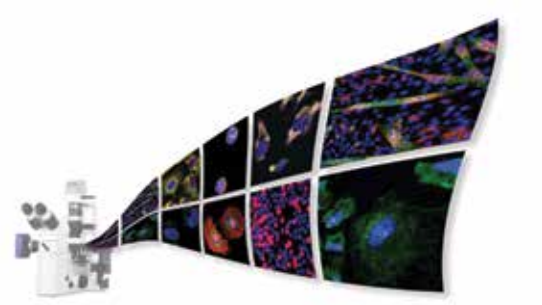
Reveal gene expression heterogeniety using single-cell platforms.

Combined RNA and protein profiling



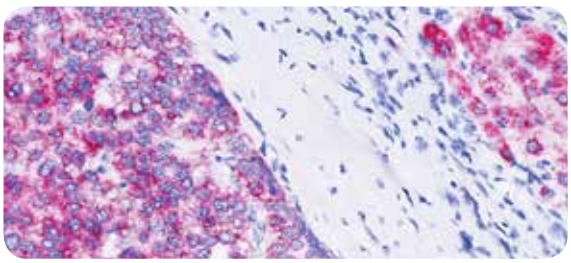
PrimeFlow® RNA Assay

Subcellular localization



ViewRNA® ISH Cell Assay

Morphological context



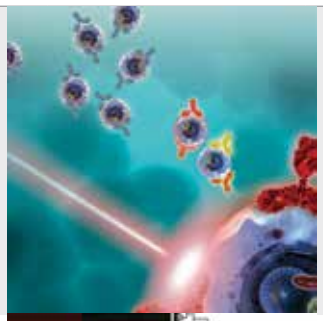
ViewRNA® ISH Tissue Assay

Whole-transcriptome profiling

GeneChip® WT Pico Kit

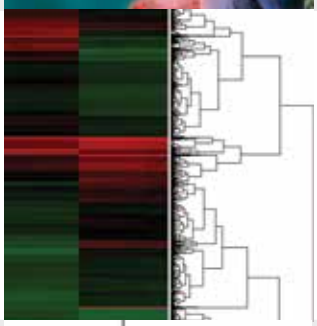
Supporting the single-cell workflow from exploration to validation

Isolation



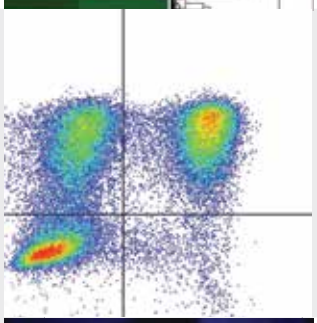
Flow cytometry antibodies
The broadest portfolio of fluorochrome-conjugated antibodies, supported by exceptional technical and panel design support

Exploration and biomarker identification

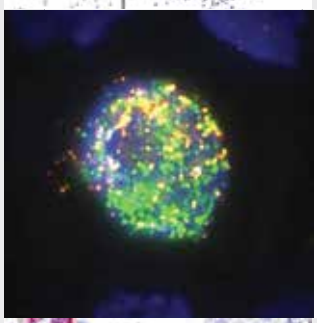


GeneChip® Transcriptome-view Pico Solution
The simplest, most flexible, rapid, and cost-effective method to deliver the broadest measurement of transcriptome-wide expression changes using **as few as 10 cells**

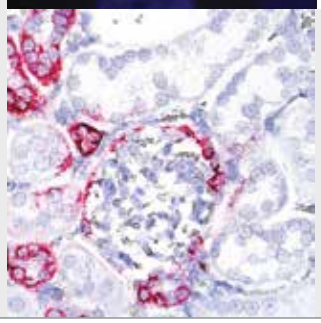
Validation without transcript amplification



PrimeFlow™ RNA Assay
Simultaneous detection of RNA and protein expression in millions of single cells by flow cytometry



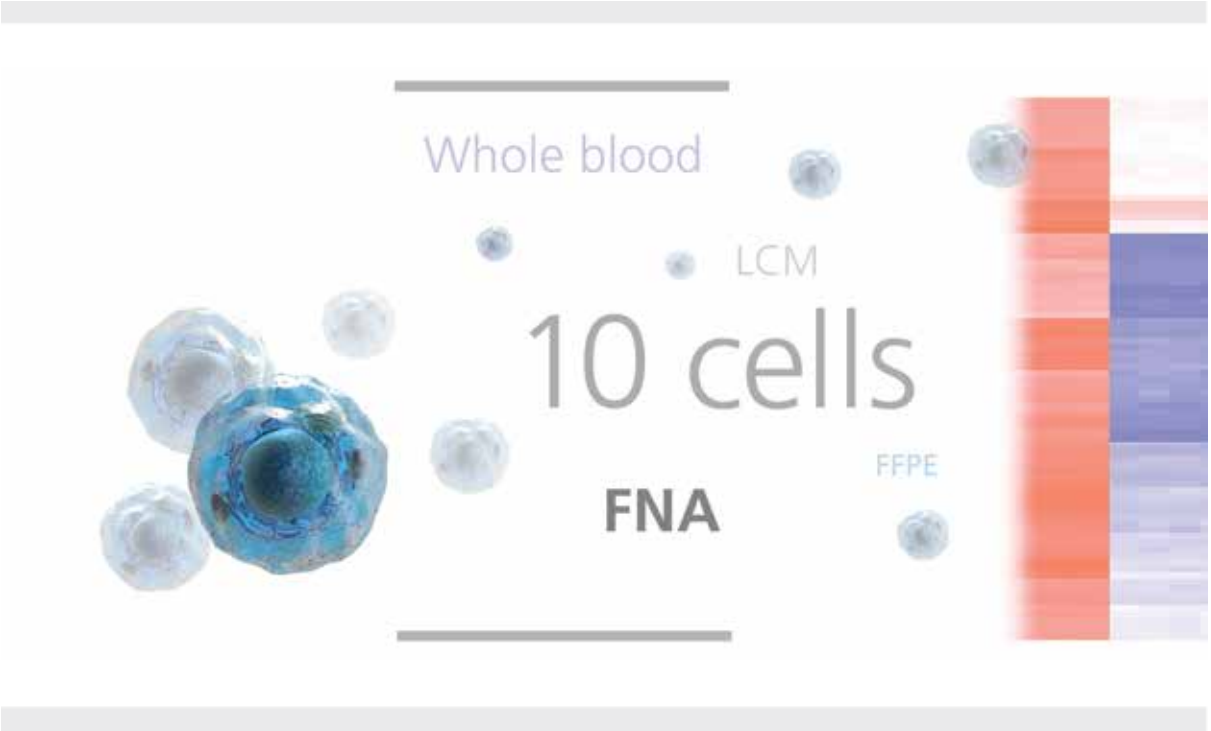
ViewRNA® ISH Cell Assay
RNA visualization with single-copy sensitivity and single-cell resolution



ViewRNA® Tissue Assays
Transcript visualization and quantification within the tissue microenvironment using manual or automated assays

Whole transcriptome profiling

GeneChip® WT Pico Kit



Traditional whole-transcriptome expression analysis depends on samples derived from a relatively large number of cells. However, gene expression measurements from these large cell populations represent an average that can often mask key differences between subpopulations. To better understand the biological significance of these differences in gene expression, researchers are increasingly focusing on smaller, characterized subsets of cells.

Modern technologies, including fluorescence-activated cell sorting (FACS) using fluorochrome-conjugated antibodies and laser-capture microdissection (LCM), enable characterization and subsequent isolation of small cell populations. Due to their small size, however, these samples usually produce insufficient RNA to obtain robust expression data from current microarray or RNA-Seq assays.

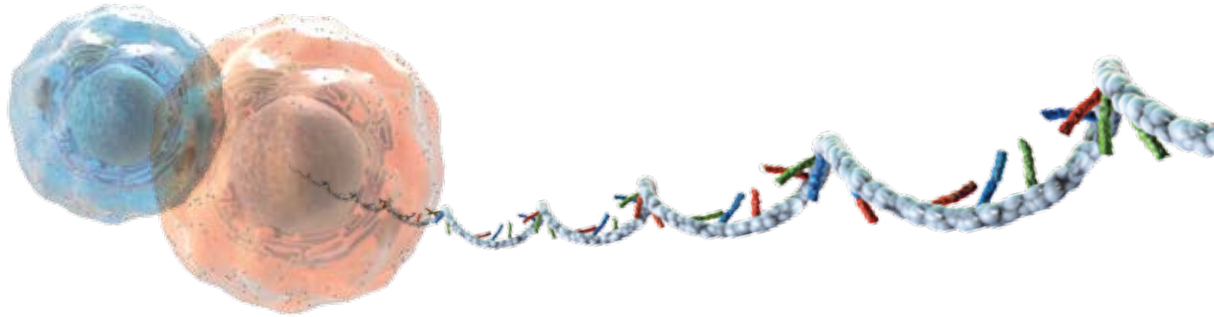
Overcoming these sample-input challenges, the GeneChip® Transcriptome-view Pico assays provide the most powerful and flexible solutions for measuring a broad range of expression changes from as little as 10 cells.

Obtain ultimate flexibility for small quantities of RNA.

- Reveal the biology in small subpopulation of cells using as little as 100 pg of total RNA (approximately 10 cells).
- Benefit from total flexibility with a single solution for various key sample types, including cultured cells, fresh/fresh frozen or formalin-fixed paraffin-embedded (FFPE) tissues, and whole blood, without the need for globin or ribosomal RNA removal.
- Use GeneChip® Transcriptome-view Pico assays with whole-transcriptome arrays from Affymetrix, including GeneChip® Human Transcriptome Array 2.0 and Mouse Transcriptome Array 1.0, as well as mid-plex Luminex based QuantiGene® Plex Assays.
- Perform multi-layered analysis to accurately measure gene- or transcript-level expression of coding and long non-coding RNA.
- Interpret data to insights in minutes using free, highly visual, intuitive data analysis software.
- Obtain the highest accuracy with an assay that ensures strand specificity is preserved.

Combined RNA and protein profiling

PrimeFlow™ RNA Assay

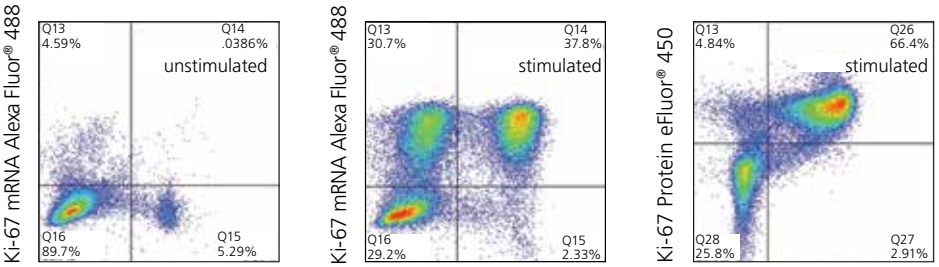


Flow cytometry, with its ability to acquire and analyze millions of individual single cells simultaneously, to use multiplexing capabilities and to detect both cell surface and intracellular proteins in a straightforward workflow, has long been the standard for characterizing heterogeneous cell populations. Nevertheless, flow cytometry is constrained by the availability and adequacy of antibodies to measure all analytes of interest. Non-coding RNA, messenger RNA, viral transcripts, unique model organisms, and targets for which antibody development is troublesome have not been able to utilize the power of flow cytometry, and have historically required that researchers conduct numerous disconnected experiments to analyze these cellular systems.

With the launch of the novel PrimeFlow™ RNA Assay, scientists can now reveal the dynamics of RNA and protein expression simultaneously within millions of single cells. This assay employs a proprietary fluorescent *in situ* hybridization (FISH) and branched DNA (bDNA) amplification technique for simultaneous detection of up to three RNA transcripts in a single cell using a standard flow cytometer. RNA detection may be combined with intracellular and cell surface antibody staining to elevate the understanding of single-cell dynamics to a new dimension.

Detect RNA and protein at the single-cell level by flow cytometry.

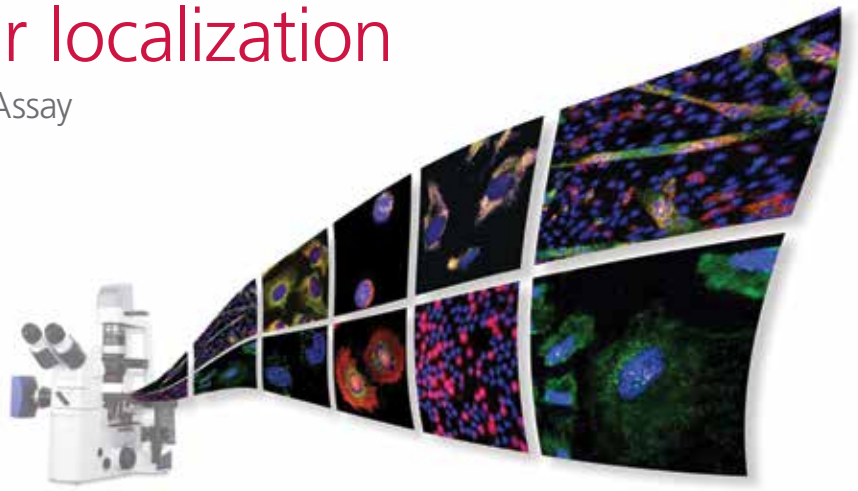
- Unmask gene expression heterogeneity at the single-cell level.
- Correlate RNA and protein level in the same cell.
- Detect non-coding RNA in cellular subsets.
- Evaluate viral RNA in infected cells.
- Analyze mRNA expression when antibody selection is limited.



PrimeFlow™ RNA Assay in action.
C57Bl/6 splenocytes were unstimulated (left) or stimulated for 2 days with Anti-Mouse CD3 and CD28 Functional Grade Purified Antibodies (cat. no. 16-0031 and cat. no. 16-0281 [middle and right]) and in the presence of Protein Transport Inhibitor Cocktail (cat. no. 00-4980) for the last 3 hours of culture, followed by analysis using the PrimeFlow™ RNA Assay (cat. no. 88-18001). Cells were fixed and permeabilized using the PrimeFlow™ RNA Assay buffers and protocol, then intracellularly stained with Anti-Mouse CD8a PE-eFluor® 610 (cat. no. 61-0081), and Anti-Mouse Ki-67 eFluor® 450 (cat. no. 48-5698). Cells were then hybridized with Type 4 Mouse Ki-67 Alexa Fluor® 488 (cat. no. VB4-16518).

Subcellular localization

ViewRNA® ISH Cell Assay

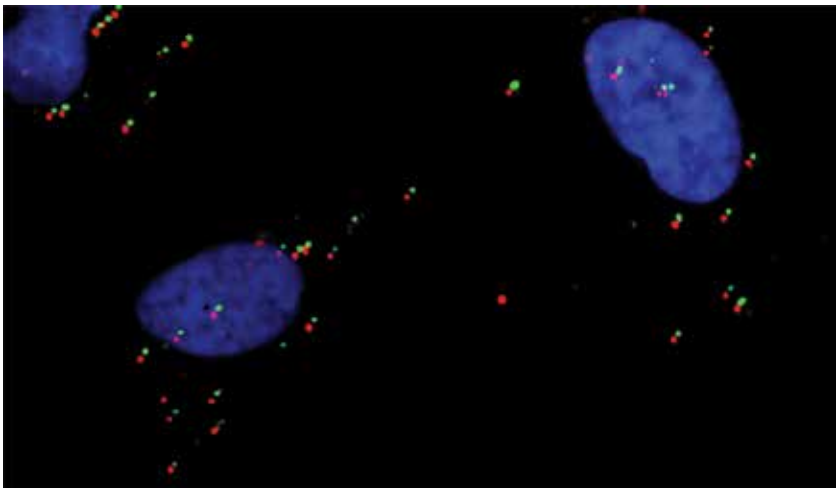


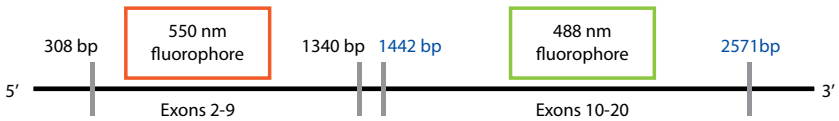
Fluorescent *in situ* hybridization (FISH) is a powerful technique that allows specific visualization of RNA targets in fixed cells at single-cell resolution. Traditional FISH techniques that rely on oligonucleotides directly labeled with a few fluorophores are generally limited by high background and low detection sensitivity due to non-specific binding and insufficient signal-to-noise ratios.

The ViewRNA® ISH Cell Assays incorporates a proprietary probe design and branched DNA (bDNA) signal amplification technology that results in excellent specificity, low background, and high signal-to-noise ratios, and makes ViewRNA ISH Cell Assay the most sensitive and specific RNA ISH method on the market. The assay enables simultaneous visualization of up to four RNA transcripts down to a single RNA molecule in single cells. Under equivalent imaging conditions, the ViewRNA ISH Cell Assay is 100 times brighter than traditional FISH, creating at minimum a 2–3 times higher signal-to-noise ratio.[1]

Visualize RNA in cells with single-molecule sensitivity and single-cell resolution.

- Analyze transcriptional heterogeneity.
- Study single cells for non-coding RNAs, including micro RNA (miRNA) and long non-coding RNA (lncRNA).
- Track, visualize, and detect genomic viral RNA.
- Provide high-throughput, single-cell quantitation and imaging of four RNA targets.



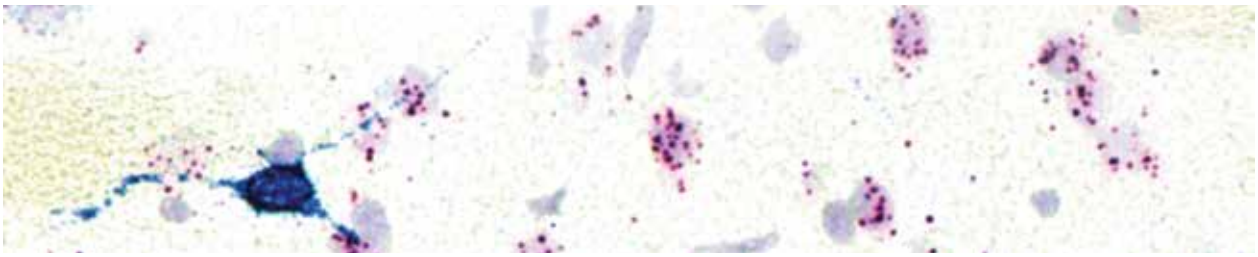


Validation of single-molecule detection.
To demonstrate single-molecule sensitivity, wherein one dot is equivalent to one target molecule, two Probe Sets were designed to target different regions of the ERBB2 (Her2) mRNA. One Probe Set targeted the region from exons 2-9 (Alexa Fluor® 546, Red Dot) and the other Probe Set targeted the region from exons 10-20 (Alexa Fluor®488, Green Dot). Although resolving these two signals is not possible on a single target, images were captured slightly offset to enable visualization of both signals. If one dot is equivalent to detection of one target, one would expect to see pairs of red and green dots, as is evident in the image. Nuclei (blue) were stained with DAPI.

[1] Battich N, *et al.* Image-based transcriptomics in thousands of single human cells at single molecule resolution. *Nature Methods* **10**(11):1127–1133 (2013).

Morphological context

ViewRNA® ISH Tissue Assay



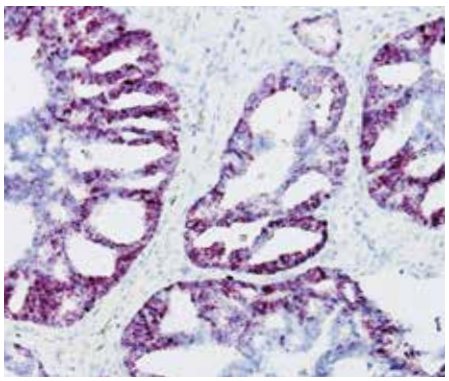
Microscopy is a widely used imaging method to analyze biological systems. It can reveal the abundance, distribution, and localization of biomarkers within a tissue, providing insight into cellular structure, mechanisms, and microenvironments. Detailed morphological analysis is vital to understanding and validating biomarkers of interest in both research and clinical settings. Historically, quantitation of RNA expression *in situ* had limited utility due to low sensitivity of non-radioactive formats, complicated workflows, and the inability to analyze multiple biomarkers simultaneously. With the availability of the ViewRNA® proprietary probe set design and branched DNA (bDNA) signal amplification, the reliance on radioactive probes is eliminated, and it further enables analysis of multiple analytes simultaneously within the same slide.

ViewRNA Assays bring a new level of power and simplicity to RNA ISH, enabling the visualization of targets not easily localized using other technologies.

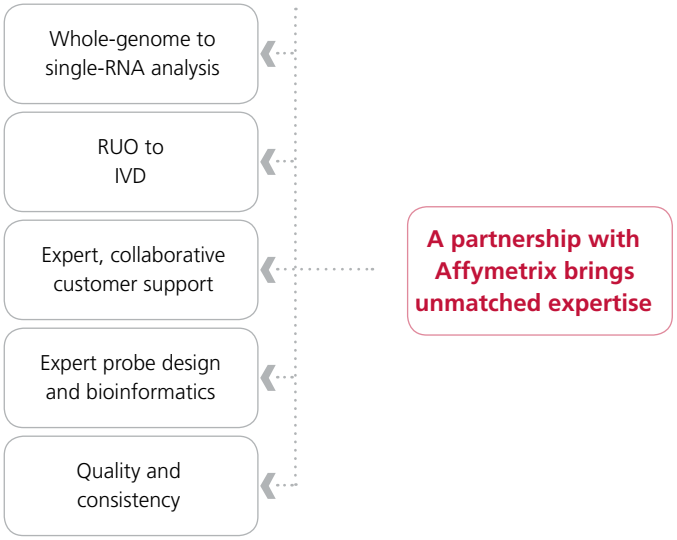
- Secreted proteins
- Targets for which antibodies are not readily available
- Long non-coding RNAs
- Complimentary validation of immunohistochemistry (IHC) data
- Works with many tissues for manual and automated processing

Affymetrix is your partner from bench to bedside.

Affymetrix understands what is needed to develop an assay from early research to validation. We are committed to accelerating biomarker discovery across the single-cell analysis spectrum supporting translational research, pharmaceutical discovery and development, and clinical assay development.



Alpha-methylacyl coenzyme A racemase (AMACR; red) in human prostatic adenocarcinoma.



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