Single-Cell Westerns

Unlock the single-cell proteome.

proteinsimple
a bio-techne brand

Meet Milo.

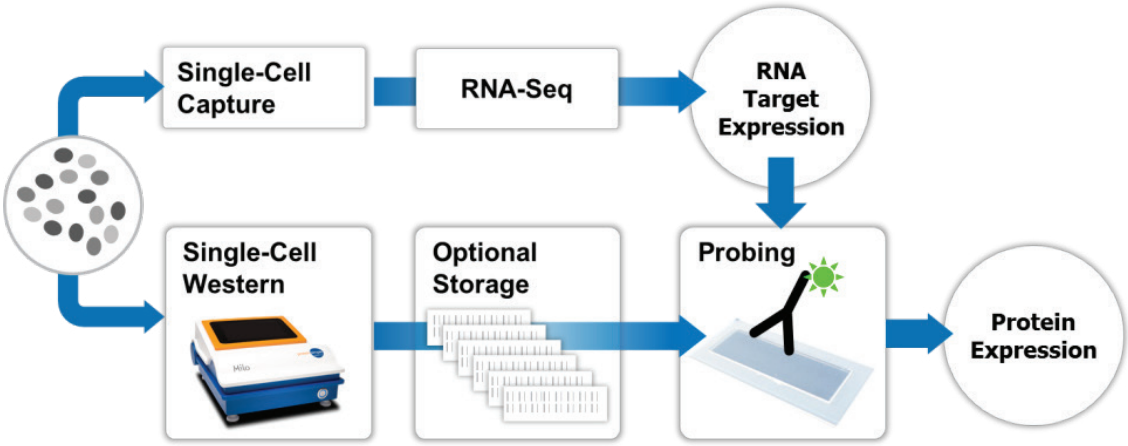
He does Single-Cell Westerns that'll let you measure protein expression in thousands of single cells in a single run. You'll get it all done in 4–6 hours with no overnight transfer step. And you can use off-the-shelf primary Western antibodies too — try that with flow cytometry! Milo measures protein expression heterogeneity and identifies cell subpopulations so you can make measurements you can't make any other way and uncover novel insights about your samples!

How can Milo help you?

- Validate single-cell RNA data at the protein level
- Measure proteins that don't have good flow antibodies
- Simplify challenging flow assays (e.g., transcription factors)
- Measure protein isoform heterogeneity
- Analyze samples with low cell numbers (e.g., FACS-sorted cells or rare stem cells)
- Quantify transfection efficiency
- Reveal heterogeneity obscured by bulk Westerns

Validate single-cell RNA data at the protein level.

Milo provides protein heterogeneity information to validate your single-cell RNA data. Since mRNA levels do not always correlate with functional protein levels, pairing single-cell RNA data with single-cell protein expression data is critical to making accurate and complete conclusions about cellular function. Because Milo uses the large Western catalog of antibodies & can easily measure proteins irrespective of their location in or on a cell, Milo is the only platform with the versatility to detect diverse targets that are discovered in your sequencing runs. Plus, scWest chips can be archived for up to 9 months after you run them on Milo so you have plenty of time to get your sequencing results back before you have to probe for your targets of interest.



Simplify and augment your flow and mass cytometry assays.

MORE ANTIBODIES

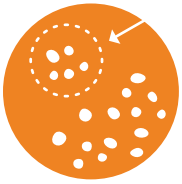
Struggling to find a good flow or mass cytometry antibody for your target of interest?
Milo is an open platform so you can use the large commercial catalog of Western antibodies which is 10-100x larger than the flow cytometry antibody catalog.

SIMPLIFY WORKFLOWS

Need to speed up your assay development time?
Eliminate the fixation and permeabilization steps of flow cytometry! Milo chemically lyses the cells captured on the scWest chip before analysis to gain access to intracellular and intranuclear compartments more easily than with flow cytometry. By lysing the cells, Milo can detect challenging proteins like transcription factors and even methylated histones!

RESOLVE ISOFORMS

Want to study protein isoform heterogeneity?
Milo's molecular weight sizing step can resolve protein isoforms that differ in molecular weight, allowing you to measure how many cells in your sample express one isoform, the other isoform, or both isoforms. Try that with flow!

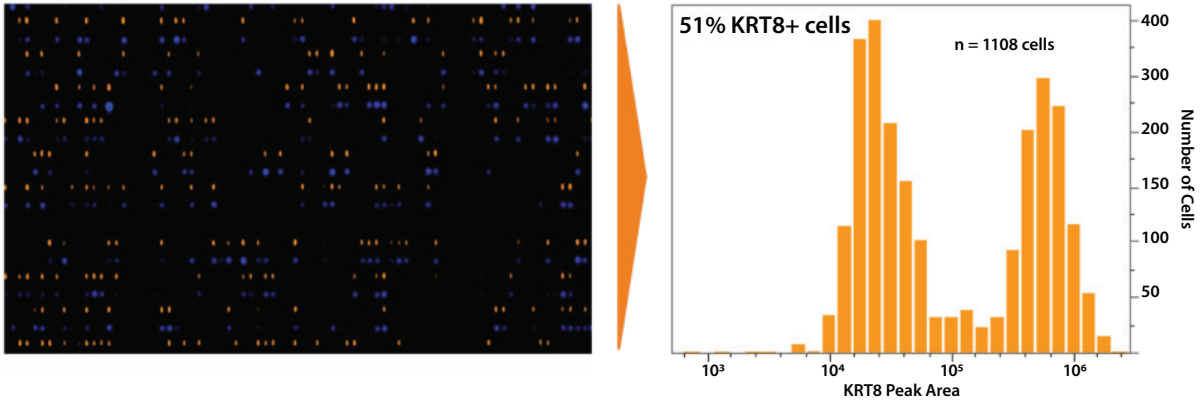


LOW CELL NUMBERS

Does your sample contain low cell numbers?
Milo can analyze samples which contain as low as 10,000 single-cells so you no longer have to have collect millions of cells to analyze protein expression. Characterize your highly enriched FACS-sorted cells or rare stem cells. The number of cells analyzed per run scales with the number of cells loaded so even lower abundance samples are possible.

Profile heterogeneity with quantitative data.

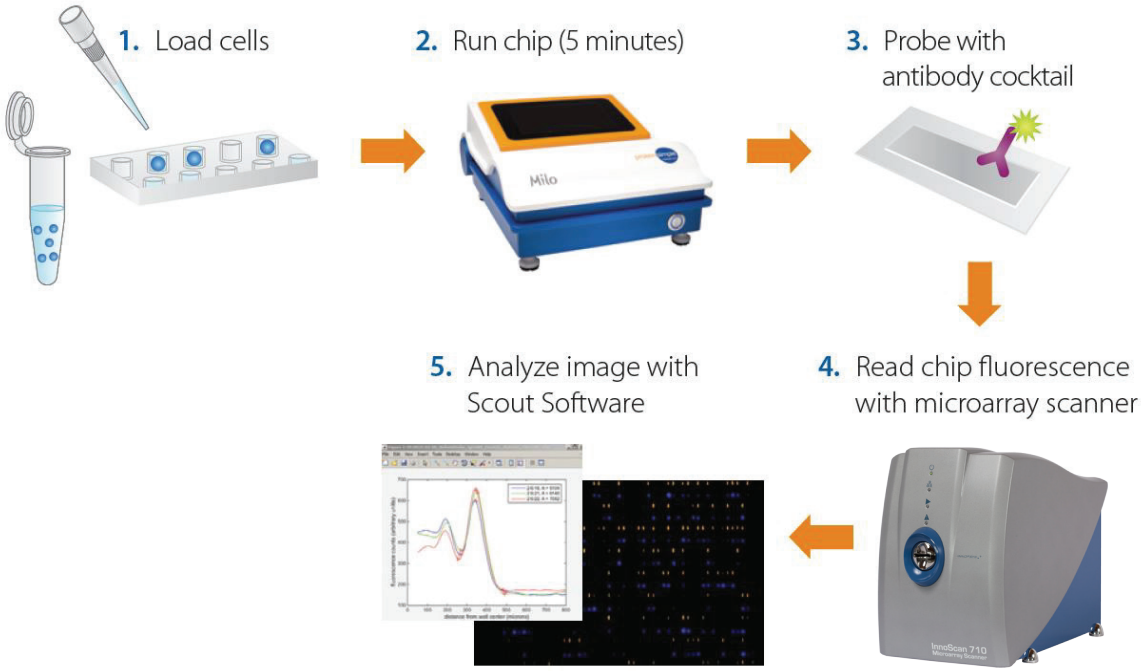
You can image probed scWest chips with any open-format fluorescence microarray scanner including the InnoScan 710 two-color or InnoScan 1100 three-color scanners. Then just upload the images in Scout Software to identify the peak(s) for your target(s) of interest in each lane and quantitate abundance of each target in each single-cell. Create histograms to see how expression of your protein target(s) varies across your sample or to study protein expression in specific subpopulations of cells. Milo can even be used to quantify transfection efficiency in low efficiency systems.



An array of Single-Cell Westerns on an scWest chip probed for two protein targets — one in orange and one in blue. Scout extracts peak area for each protein band so you can quantify how protein expression varies across each single-cell analyzed!

One simple workflow.

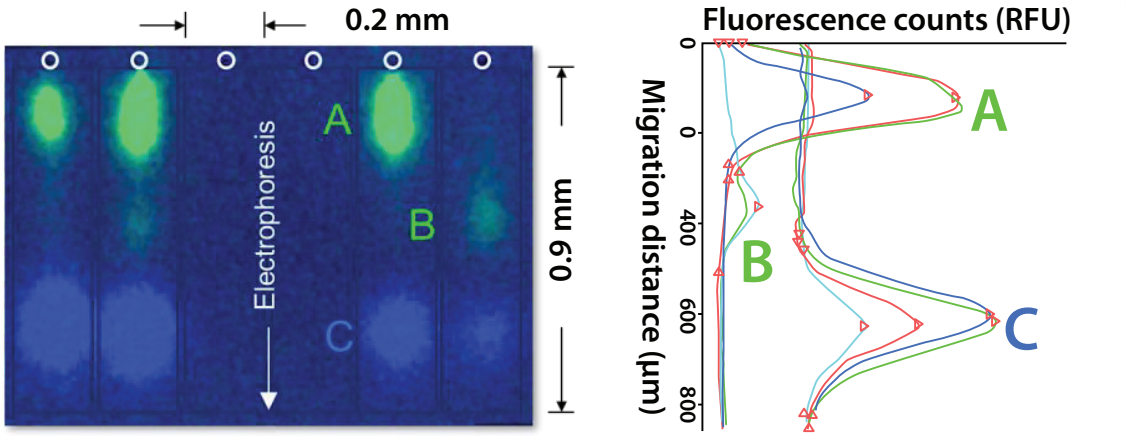
Single-Cell Westerns happen on scWest chips. Put your cell suspension on the chip and put the chip in Milo. He captures 1000+ cells, lyses them, runs an SDS-PAGE separation on every single-cell lysate, and immobilizes all your separated proteins in 5 minutes flat! Next, just probe your protein targets on-chip with standard primary and fluorescent secondary antibodies and image chip fluorescence. Scout will then automate your data analysis for you! One simple process lets you measure diverse targets and profile heterogeneity in your complex samples!



Add 1 mL of a single-cell suspension onto an scWest chip. Individual cells settle into microwells patterned into the pre-cast polyacrylamide gel. Milo lyses the cells, does a rapid (~1 min) SDS-PAGE separation on each single-cell lysate and immobilizes the proteins in the gel. Probe with conventional Western primary and fluorescent secondary antibodies in the probing chamber and image chip fluorescence. Scout Software analyzes images to extract peak area for each target detected in each single-cell lysate.

Multiplex 12+ proteins per cell.

Multiplexing? Just probe the scWest chip with your favorite cocktail of primary antibodies and measure 12 or more proteins in every single cell. You can use molecular weight differences or distinct spectral channels to differentiate your targets in each probing round. scWest chips can also be stripped and reprobed up to 9 times so you can design highly multiplexed studies that match or exceed the most powerful flow cytometers. No matter what color or order you image your targets in, Scout Software makes sure all your proteins are detected in each cell.



Single-Cell Western of SCO cells where three proteins of differing molecular weights are detected in two spectral channels. A single-cell is captured in each microwell at the top of each electrophoresis lane. Here, 4 of 6 lanes contained a cell. Each single-cell lysate was then separated, immobilized, probed with a cocktail of antibodies and then the scWest chip was imaged in two colors. Scout creates fluorescence intensity plots to identify target peaks and calculates area under the curve to quantify abundance of each protein of interest.

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Performance & specifications.

REQUIREMENTS & COMBATIBILITY

- **Sample type:** Suspension containing >10,000 cells
- **Cell diameter:** 7–25 µm in suspension
- **Cell type:** Mammalian cells; globular in suspension and unfixed
- **Antibody requirement:** Standard unlabeled primaries and fluorescent secondaries
- **Other equipment needed:** Open-format fluorescence microarray scanner capable of 5 µm resolution

PERFORMANCE & SPECIFICATIONS

- **Typical cell dilutions** yield capture and analysis of 1,000–2,000 cells per scWest chip
- **Molecular weight (MW) range:** 15–175 kDa
- **MW resolution:** 10% differences in distinct spectral channels, as low as 30% differences in same spectral channel
- **Typical target multiplexing:** Up to four proteins per cell by spectral and size-based multiplexing. Twelve-plus proteins per cell using stripping & reprobing.
- **Workflow time:** 4–6 hours

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