

# Single-Cell Westerns.

1,000 cells at a time.

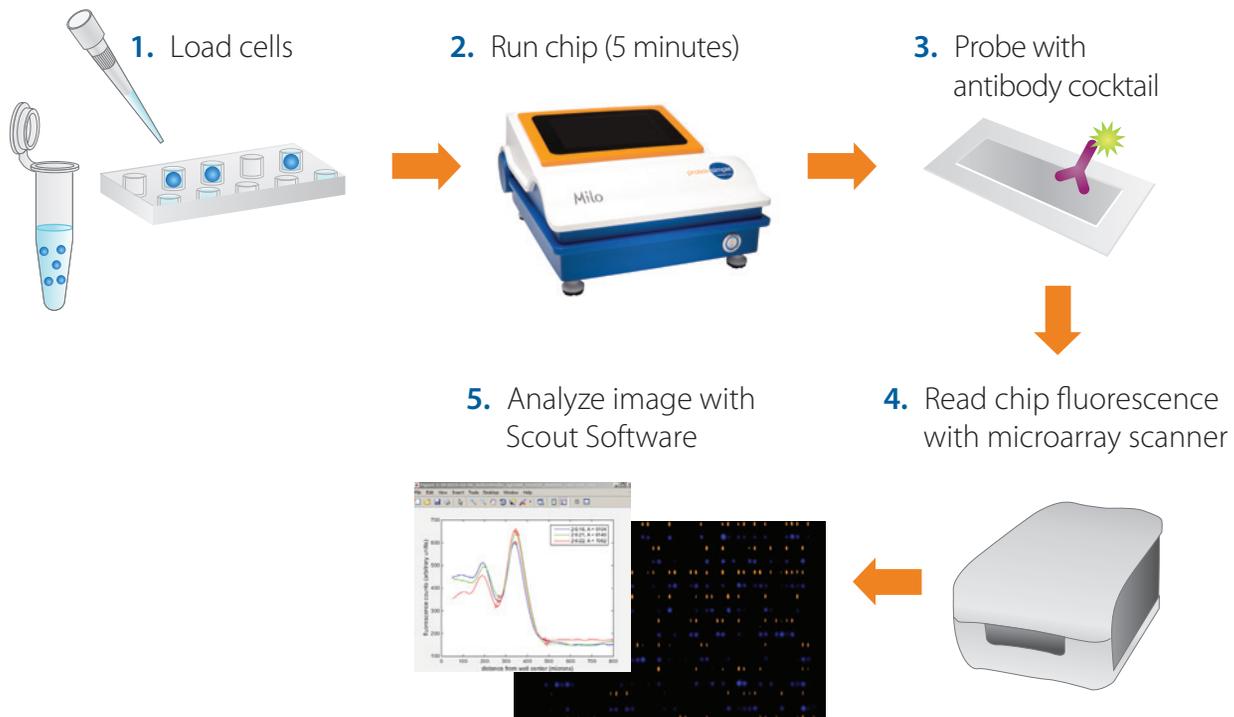
# Meet Milo.

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 makes measuring protein expression heterogeneity and identifying cellular subpopulations a breeze.



# One simple workflow.

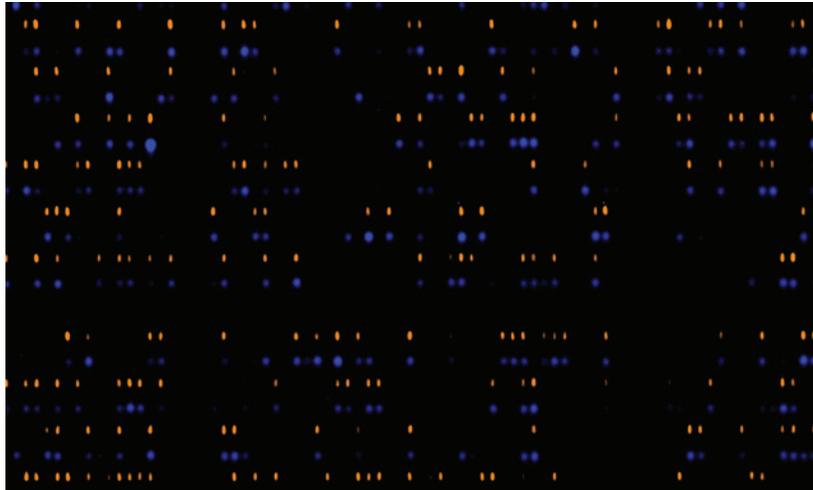
Single-Cell Westerns happen on scWest chips. Put your cell suspension on the chip and put it in Milo. He captures 1000+ cells, lyses them, runs an SDS-PAGE separation on every single cell, and immobilizes all your separated proteins in 5 minutes flat! Next, just probe your protein targets on-chip with standard primary and secondary antibodies. One simple process lets you measure diverse targets and detect surface and intracellular proteins at the same time!



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 rapid (~1 min) SDS-PAGE on each single-cell lysate and immobilizes the proteins in the gel. Probe with conventional antibodies in the probing chamber and image chip fluorescence. Scout Software analyzes images to extract data.

# Full-on quantitative data.

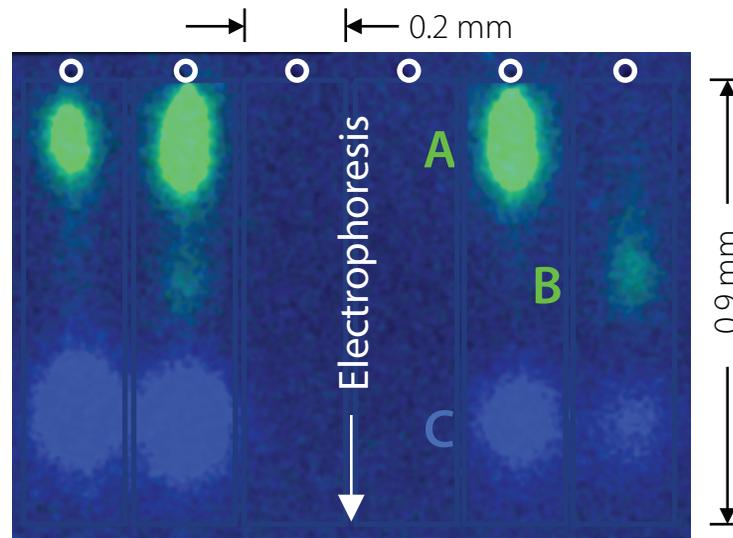
You can image probed scWest chips with any open-platform fluorescence microarray scanner. Then just hand the images over to Scout Software to identify each target protein per cell and quantitate abundance of each one. And the best part? Archive your scWest chip and re-probe for new targets up to nine months later!



An array of Single-Cell Westerns on an scWest chip probed for two protein targets — one in orange and one in blue.

# See four proteins per cell.

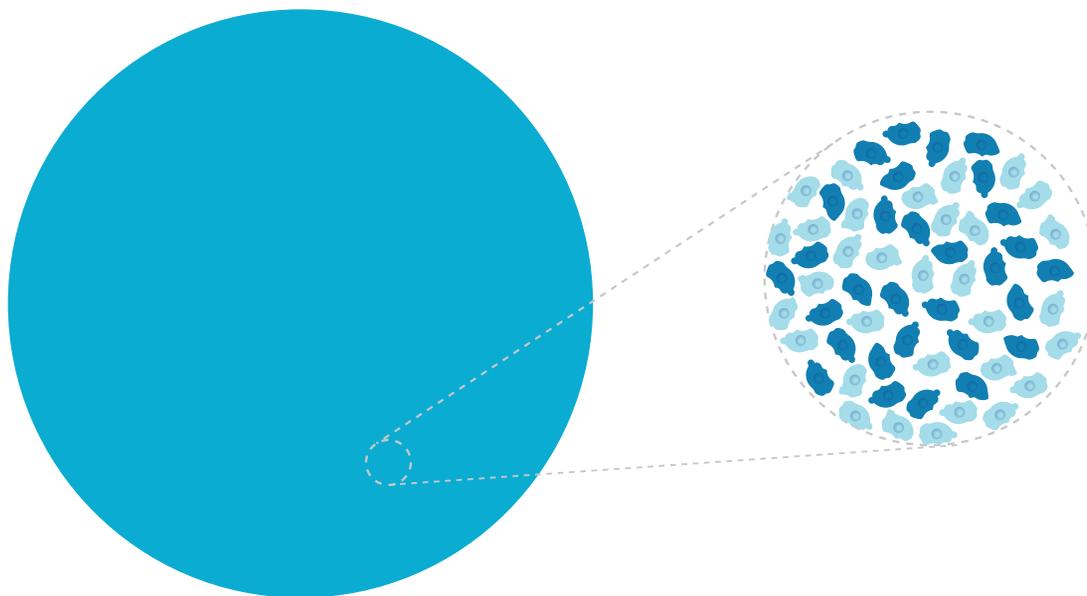
Multiplexing? Just probe the scWest chip with your favorite cocktail of primary antibodies and measure up to four proteins in every single cell. You can use molecular weight differences or distinct spectral channels to differentiate your targets. No matter what color you image in — Scout Software makes 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. One cell was captured in each microwell at the top of each electrophoresis lane on an scWest chip. Each single-cell lysate was then separated, immobilized, probed with a cocktail of antibodies and then the scWest chip was imaged.

# Lots of applications.

Milo lets you study the target expression heterogeneity of tumors, identify differentiated stem cell subtypes and measure intracellular signaling pathway activation — including phosphorylated targets or transcription factors that are hard to do with flow. You can also validate your single-cell RNA results with the protein expression information he gives you. If you need to find out how efficient your genetically engineered CRISPR, transduction, or transfection cell methods are, he'll let you do that too!



Bulk measurements can't interrogate individual cells within a population. Single-Cell Westerns show you when subpopulations are present or when your cells are responding differently to a stimulus.

# Requirements & compatibility.

- **Sample type:** Suspension containing >10,000 cells
- **Cell diameter:** 7–25  $\mu\text{m}$
- **Cell type:** Mammalian cells; globular in suspension and unfixed
- **Antibody requirement:** Standard, commercial unlabeled primaries and fluorescent secondaries
- **Other equipment needed:** Open-format fluorescence microarray scanner capable of 10  $\mu\text{m}$  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
- **Workflow time:** 4–6 hours

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