Profile Heterogeneity with Single-Cell Westerns

ProteinSimple
www.proteinsimple.com
info@proteinsimple.com

Introduction

Milo™ is the world's first Single-Cell Western platform. He measures protein expression in ~1,000 single cells in a single run so you can profile heterogeneity in your samples. Milo can multiplex to measure multiple proteins per cell and uses conventional Western antibodies for detection – giving you access to the broadest set of antibody reagents for your assay.

Use Single-Cell Westerns to unlock the single-cell proteome and measure more of the proteome than is possible with any other single-cell technique.

How does Milo Work?

The heart of the Single-Cell Western system is the scWest chip (Figure 1), which can perform Westerns on ~1,000 single cells in parallel in a single run. Just load your cell suspension onto the chip and the scWest chip uses Poisson statistics to capture ~1,000 single-cells, with each single-cell contained in an individual microwell. Milo then lyses the captured cells and performs a fast (1 min) size-based SDS-PAGE separation on each single-cell lysate within the polyacrylamide gel. After separation, Milo immobilizes the protein bands into the polyacrylamide gel using UV light and a UV-sensitive chemistry included in the gel. You can then probe the scWest chip with your favorite conventional Western antibodies to measure up to 4 proteins per cell simultaneously. Typical workflows use unlabeled primary and fluorescent secondary antibodies. Finally, image the chip fluorescence in any open format microarray scanner capable of scanning standard glass microscope slide chip formats. A table of compatible scanners is available on the ProteinSimple website.

Finally, open the resulting chip images in Scout software which detects the separated peaks in each single-cell separation and quantifies peak area. Data is visualized using standard visualization tools. Probed scWest chips can be dried and archived. Re-probing of archived chips is possible months later.

Imaging scWest chips with the Innoscan 710 scanner

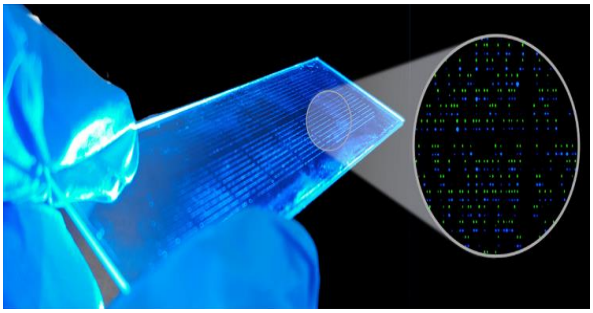

Figure 1: scWest chip contains 6,400 microwells patterned into a thin photoactive polyacrylamide gel

scWest chips can be imaged with any open format microarray scanner. If you don't have access to one already at your institute, ProteinSimple offers the InnoScan 710 microarray scanner which is capable of confocal 2 color scanning at 3 µm/pixel max scanning resolution, sequential or simultaneous scanning abilities, and a high sensitivity dual PMT system. The InnoScan 710 scanner includes a real-time autofocus system that makes the system adaptable to substrate distortion, thus making the entire read area completely uniform. The autofocus system combined with the confocal detection gives the scanner excellent

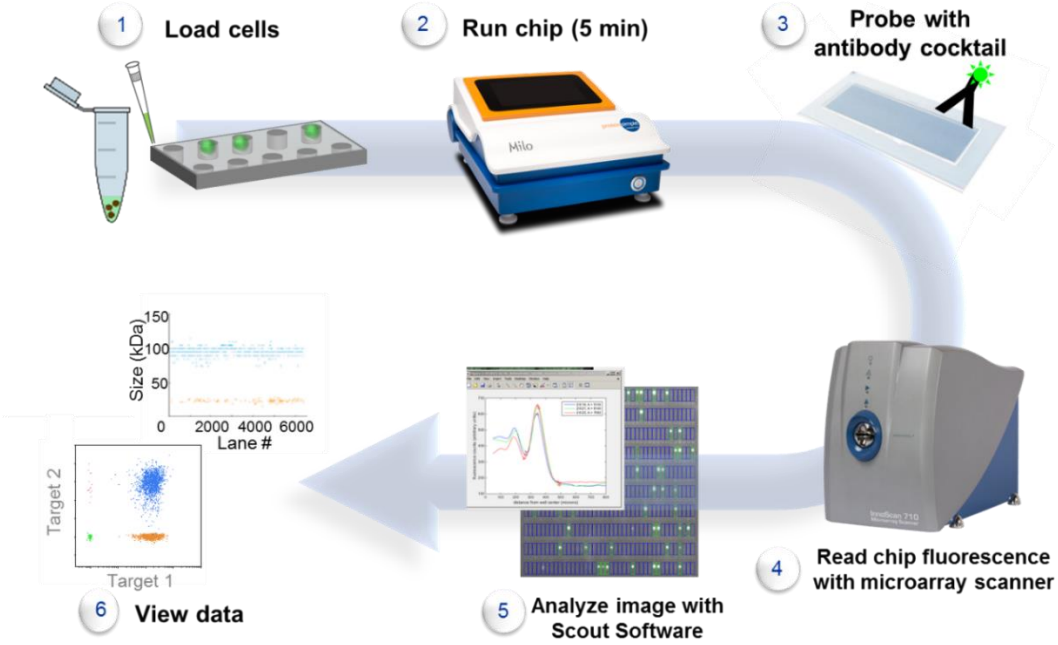

Figure 2: Single-Cell Western workflow

photometric properties including sensitivity and signal-to-noise ratios. With a rapid, 3.5 min full chip scanning time, this scanner has the best combination of sensitivity,scan time and resolution needed to scan scWest chips. When purchased with a Milo system, ProteinSimple also offers worldwide support.

Table 1: Innoscan 710 scanner specifications

Detection type	**2 PMT / Confocal with real-time autofocus**
Resolution	3 µm/pixel
Dynamic range	> 4 orders of magnitude > 6 orders of magnitude in XDR mode
Scanning time (2-color at 10 µm/pixel	3.5 min.
Excitation wavelength	635 nm and 532 nm
Loader	Single slide or 24-slide autoloader
Software	Mapix (image acquisition and image processing software)

Why Single-Cell Westerns?

Milo is a flexible protein analysis tool and unlike conventional Westerns, it will give you data at the single cell level -- providing you with deeper insight into cell-to-cell variation in your sample.

Below we highlight just a few of the ways that Milo can be used to advance your research.

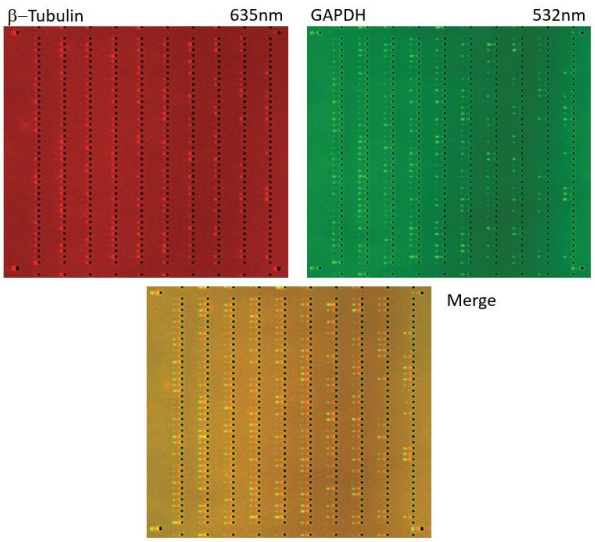

Figure 3: Two color and merged fluorescence images of 400 microwell block on an scWest chip from Innoscan 710 scanner

Profile Heterogeneity in Complex Samples

Many cell populations, such as tumor cells or differentiating stem cells are heterogeneous. Milo can quantify how protein expression levels vary across the cells in your sample and the percentage of cells in your sample that are target positive. In Figure 4, you can see a distribution of KRT8 expression in a dissociated breast tissue sample. One subpopulation of cells has low KRT8 expression and one subpopulation has high KRT8 expression. Approximately 51% of cells analyzed expressed KRT8.

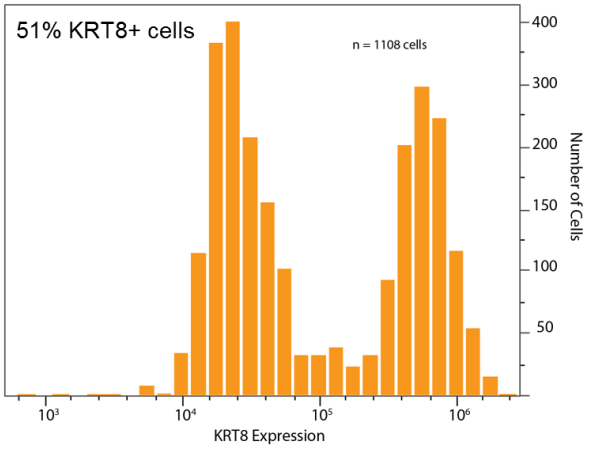

Figure 4: Heterogeneity in KRT8 expression

Validate Single-Cell RNA targets

Everyone knows that RNA and protein levels do not always correlate. Milo provides protein validation of single-cell RNA-seq data so you can have confidence that your single-cell gene expression results are functionally expressed at the protein level. By using the large catalog of conventional western antibodies for detection, Milo has the versatility to detect any of the RNA targets discovered in your sequencing run. Plus, scWest chips can be archived for up to 9 months after you run them so you have plenty of time to get your sequencing results back before you have to probe for your targets of interest.

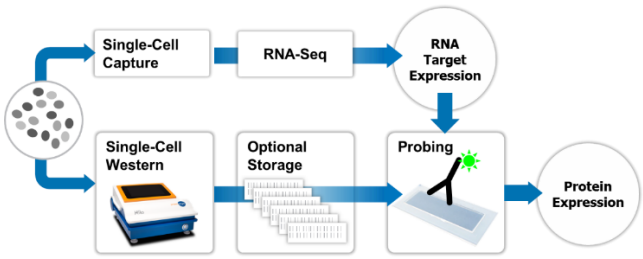

Figure 5: Milo can be run in parallel with single-cell RNA-seq workflows to get protein validation of your single-cell RNA-seq data on the same sample.

Measure protein isoform heterogeneity

Milo's molecular weight sizing step can resolve protein isoforms that differ in molecular weight, allowing researchers to measure how many cells in each sample express one isoform, the other isoform, or both isoforms.

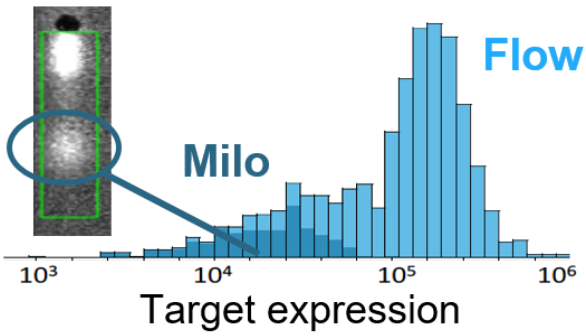

Figure 6: Two protein isoforms detected by the same antibody can be resolved by molecular weight on Milo, allowing users to integrate the signal from only their protein isoform band of interest. Since flow cytometry lacks this molecular weight sizing capability, it would integrate signal from both protein isoform bands.

Simplify phospho-flow

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. This enables detection of challenging proteins like transcription factors and even methylated histones (Figure 7)! You

can also use Milo's sizing step to separate out non-specific binding, something that is commonly seen with phospho-specific antibodies and which flow cytometry can't resolve. Stop hunting for flow-validated antibodies and let Milo simplify your assay development workflows by using the large catalog of Western antibodies for detection.

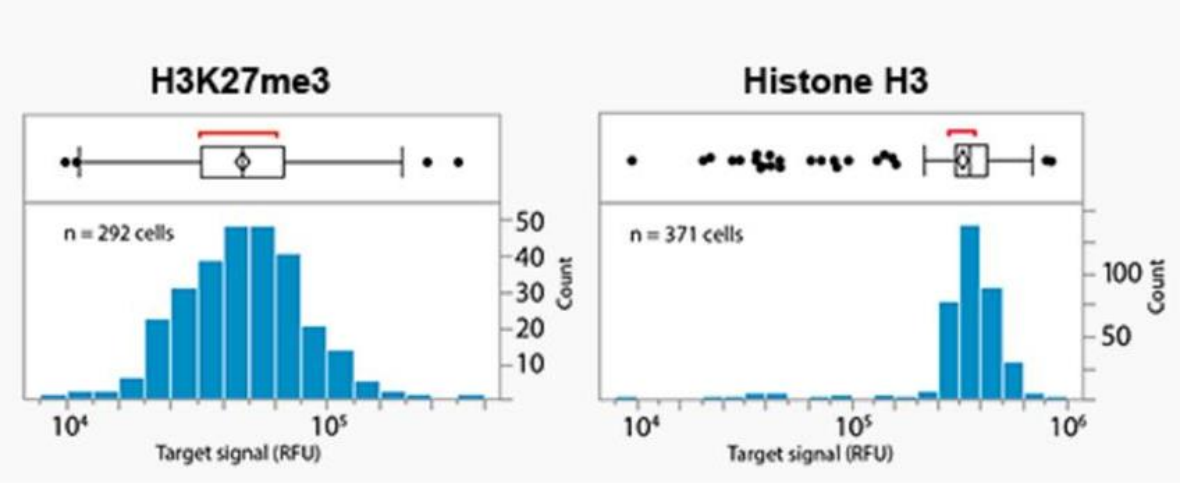

Figure 7: Single-Cell Westerns reveal variation in expression of methylated and total Histone H3

INNOPSYS
Carbonne - FRANCE
+33 561 971 974
contact@innopsys.fr
Chicago, IL - USA
+1 312 235 3587
contact@innopsys.com
www.innopsys.com