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PUBLICATION SPOTLIGHT

SIMPLE WESTERN AND SINGLE-CELL WESTERNS ARE PROVEN, HIGH-IMPACT TECHNOLOGIES

INTRODUCTION

Western blotting is one of the most common techniques in molecular biology and proteomics. Since its development in 1979[1], the essential steps of the Western blot (gel electrophoresis, transfer and immunodetection) have remained constant with only minor modifications. While the goal of Western blotting is to reliably detect, and in some cases quantify, protein expression levels, the technique is prone to many pitfalls, including unusual or unexpected bands, no signal, faint bands or weak signal, high background on the blot, and patchy or uneven spots on the blot[2]. Therefore, the Western blot is considered only semi-quantitative at best.

BRINGING THE WESTERN BLOT INTO THE 21ST CENTURY

Since 2009, ProteinSimple has been the world leader in revolutionizing traditional western blotting through advances in automation, sensitivity, and single-cell resolution among others. In 2009, ProteinSimple launched the first capillary-based Western blotting instrument, NanoPro™. This was followed by a series of other instruments, including Wes™, Peggy Sue™, Sally Sue™, and Jess™, which launched in 2018. These platforms all enable capillary-based Western assays, so called Simple Western™ assays, which separate, immobilize and detect proteins directly in the capillary, eliminating the cumbersome gel separation and transfer step to blotting paper. This in turn eliminates many of the challenges encountered with traditional Western blot. Unlike traditional Western assays, Simple Western assays are fully automated, requiring no user intervention once samples are prepared, and they are fast, with data generated in as little as 3 hours. Importantly, the data that is generated is highly reproducible and fully quantitative.

More recently, another revolution in Western analysis occurred with the advent of Single-Cell Westerns, performed with Milo™ from ProteinSimple which launched in 2016. By scaling down Western analysis to a single-cell resolution across approximately 1,000 cells in a single run, Milo can reveal population heterogeneity in protein expression levels that are otherwise undetectable with traditional methods that analyze pooled populations of cells. For example, Milo can detect cell subpopulations that express none, low, medium, and high levels of a target protein, or subpopulations that express different isoforms of a protein or biomarkers. This is critical information that is undetectable by traditional Western analysis.

For researchers still relying on traditional Westerns, there is a need for robust imaging systems to ensure high quality data. ProteinSimple's Gel Documentation and Imaging Systems have been helping researchers get better and faster images of their gels for over 20 years. The high-performance FluorChem Imagers offer multi-mode functionality to image blots with high resolution, designed with a high dynamic range to image faint bands and bright bands without oversaturation. To date, these imagers and gel documentation systems are together featured in close to 30,000 publications worldwide, a number that is steadily increasing.

Here, we present evidence showing how these unique technology platforms have become well-established in the scientific literature, offering scientists access to cutting-edge technology with a proven track-record of high impact publications across a wide diversity of fields. Simple Westerns and automated imagers have seen quick and widespread adoption in all areas of scientific research, while the novel measurements enabled by Single-Cell Westerns have been featured in some of the world's leading scientific journals. Overall, this survey of the scientific literature demonstrates that Simple Westerns, Single-Cell Westerns, and Imaging systems are proven technologies at the cutting-edge of scientific research.

SIMPLE WESTERN IS A PROVEN TECHNOLOGY AND WELL-ESTABLISHED IN THE SCIENTIFIC LITERATURE

Since the invention of Simple Western technology, the number of publications using Simple Western has grown at nearly an exponential rate (**Figure 1**). This fact reflects the widespread demand the scientific community has for an automated, reproducible, and quantitative protein expression analysis platform. With these advantages over the traditional Western blot, the cumulative number of publications using Simple Western technology has reached more than 1000 to date (**Figure 1**). It should be noted that these numbers reflect only peer-reviewed primary research articles, and it is even larger when considering all publications, like patents, dissertations, and reviews. This strong publication track-record brings confidence that this technology provides consistent and quantitative results that are accepted in place of traditional western blotting data in all major academic journals.

The power of Simple Western has been harnessed in all areas of biological and biomedical research, including oncology, drug discovery, and fundamental cell biology, from academic, government and commercial laboratories. **Figure 2** provides an overview of the research areas that Simple Western assays have contributed to. To learn more about how Simple Western is used in the scientific literature, see our Publication Spotlight. To see how Simple Western is used in select research areas, see our Publication Spotlights on the following topics:

- Cancer
- Targeted protein degradation
- Neuroscience

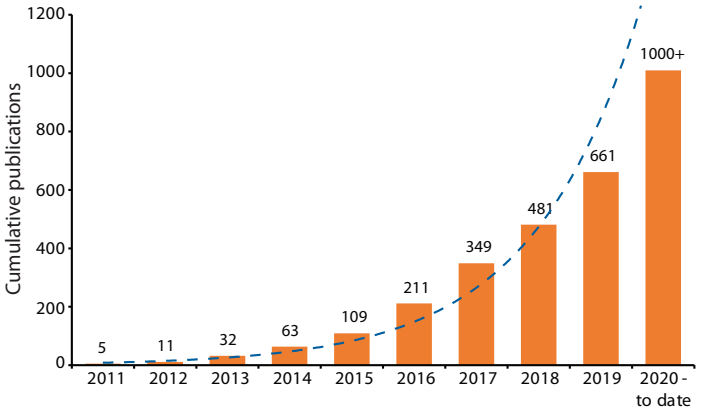

FIGURE 1. Cumulative yearly peer reviewed Simple Western publications. The dashed line represents an exponential line of fit.

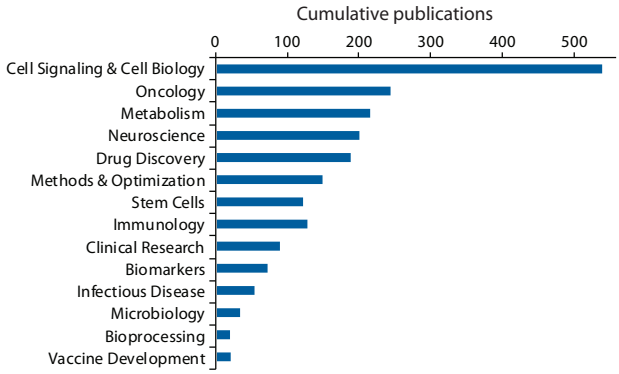

FIGURE 2. Simple Western publications by research area.

SIMPLE WESTERN CHARGE SHEDS NEW LIGHT ON PROTEIN BIOCHEMISTRY

The traditional Western blot is typically thought of as a size-based separation technique, requiring protein denaturation to reach an unfolded confirmation prior to separation based on molecular weight. Therefore, it is hardly surprising that **Simple Western Size** assays make up approximately **75%** of all Simple Western publications.

However, Simple Western assays are not limited to size-based separation. The resolving power of capillary isoelectric focusing (cIEF) to separate proteins in their native confirmation based on their isoelectric points can also be used in Simple Western assays, called **Simple Western Charge**. While cIEF is a common technique that detects proteins by native absorbance or fluorescence, it is severely limited in sensitivity and specificity, frequently requiring sample pretreatment and concentration. By contrast, Simple Western Charge on Peggy Sue and NanoPro 1000 combines cIEF with immunodetection seamlessly back-to-back in the capillary, thereby greatly enhancing sensitivity and specificity, while reducing sample size requirements. Another major advantage of Simple Western Charge is that it can resolve isoforms resulting from post-translational modifications like phosphorylation[3,4] or glycosylation[5,6] that are difficult or impossible to resolve by Simple Western Size (**Figure 3**). Thus, Simple Western Charge represents an underexploited technique to gain new insights into protein biochemistry. See our Publication Spotlight to learn more about how Simple Western Charge drives research forward.

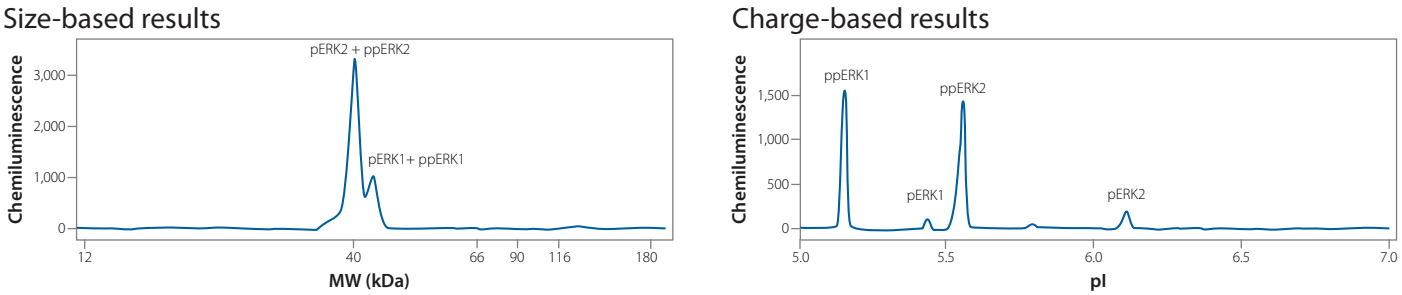

FIGURE 3. Simple Western Size data (left) and Simple Western Charge data (right). The same HeLa lysate sample, stimulated with epidermal growth factor, was analyzed and probed with an anti-phospho specific ERK antibody in both size-based and charge-based assays. The size-based electropherogram shows detection of total phosphorylated ERK2 (pERK2 + ppERK2), a quantifiable large peak; and total phosphorylated ERK1 (pERK1 + ppERK1), a smaller peak, at their correct molecular weights. The charge-based electropherogram provides more in-depth characterization of the single and dual phosphorylated isoforms of ERK1 and ERK2 at their expected pIs, demonstrating that the dual phosphorylated isoforms are the dominant species present in the lysate.

SINGLE-CELL WESTERNS ARE OVERWHELMINGLY PUBLISHED IN THE WORLD'S MOST PREMIER JOURNALS

Single-Cell Westerns on the Milo platform are a newer technology than Simple Western, yet Milo has proven its ability to provide new and profound scientific insight that traditional Westerns cannot, particularly insight on population heterogeneity. This fact is reflected in the rank of the journals that feature articles with Single-Cell Western data with the vast majority (74%) of articles featuring Single-Cell Western data in top journals with impact factors of 7 or greater including *Science*, *Cell*, *Nature Methods*, *Nature Communications* and *Cell Reports* (**Figure 4**). In fact, 32% of Single-Cell Western publications are in journals considered here to be in the premier category with an impact factor greater than 21. These data showcase the power that Single-Cell Westerns have in enabling entirely new measurements and insights into cellular biology and disease pathogenesis, driving forward cutting-edge scientific research.

Like Simple Western, Single-Cell Westerns have been published in a variety of different research areas including developmental biology, oncology, stem cells, immunology and infectious disease (**Figure 5**). Because of Milo's ability to provide insight into population heterogeneity, Single-Cell Westerns are a complementary technique to Simple Western, and the two assays work together to provide a high-resolution understanding of protein expression in complex samples. For example, when profiling the tumor microenvironment, Simple Western and Single-Cell Westerns synergize to provide critical answers to 1) what type of immune cell populations are present in a sample and then 2) what percentage of cells in that sample make up a specific immune cell subtype. This workflow gives you a population-level and single-cell-level perspective on each sample analyzed, a much faster time to result than the traditional approach you may currently be using, while requiring only small sample sizes. For more information, see our Application Note on this topic.

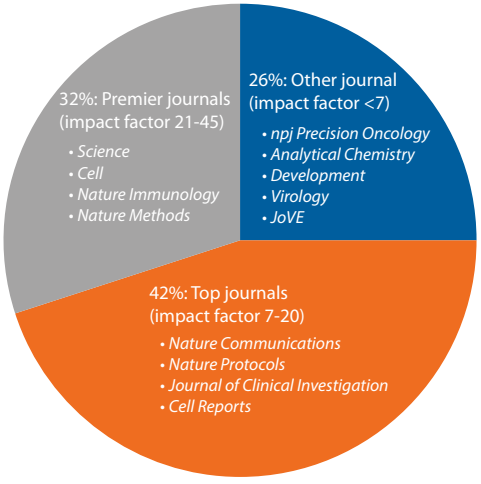

FIGURE 4. Cumulative Single-Cell Western publications by impact factors. Here, top journals are considered as those with impact factors between 7 and 20, and premier journals are considered those with impact factors of 21 or greater. Representative journals from each category are listed.

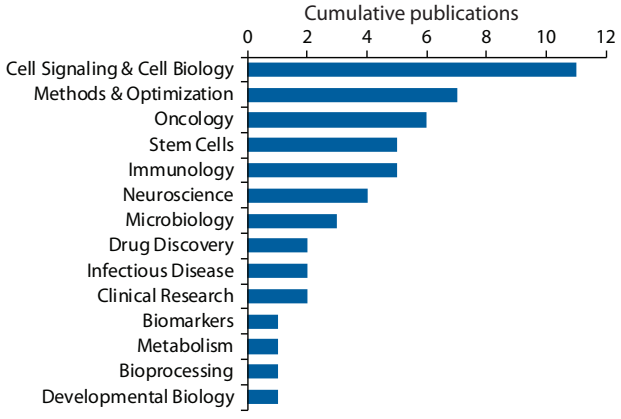

FIGURE 5. Single-Cell Western publications by research area.

HIGH-PERFORMANCE IMAGING: 30,000 PUBLICATIONS CAN'T BE WRONG

For traditional Western blot users, imaging the blot is the last critical step to obtain protein expression data, and it can be a sticking point that may require troubleshooting. For example, detecting faint and strong signals on the same blot can be tricky, requiring multiple exposures and experimentation to find just the right exposure time without oversaturation. To address this, the FluorChem Imagers offer a high-dynamic range so that faint and strong signals can be detected simultaneously in a single exposure without oversaturation. These high-performance imagers are also multi-modal, with options for fluorescence and chemiluminescence detection, among others. The high performance of these imagers & gel documentation systems is together reflected in the nearly 30,000 publications that feature their data, a figure that is steadily growing (**Figure 6**). Because of their versatility, these imagers have been harnessed for applications beyond the Western blot, including DNA gels, colony counting, and imaging Proteome Profile Antibody Arrays from R&D Systems.

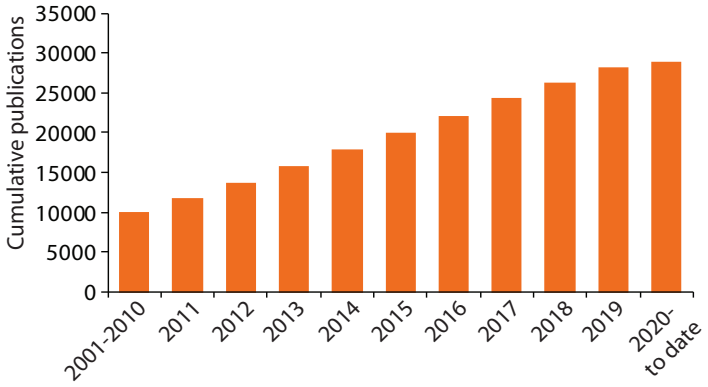

FIGURE 6. Cumulative publications featuring data from FluorChem and Alphalmager systems.

THE FUTURE OF WESTERN BLOTTING

In just over a decade, ProteinSimple has revolutionized the traditional Western blot. Simple Western has become ubiquitous in all fields of biological and biomedical research, with approximately 70 new publications each year on average. It's clear that the automation, quantitation, reproducibility, and small sample size requirements Simple Western provides were sorely needed to replace the cumbersome and labor-intensive Western blot. While traditional Western blot has had a 30-year head start, Simple Western has enjoyed nearly exponential growth in number of publications per year, and if this trend continues, it is not unreasonable to speculate that Simple Western will eventually rival the traditional Western blot in utilization across laboratories worldwide. Likewise, Single-Cell Western assays promise to provide a new layer of insight into protein expression heterogeneity across populations. The novel measurements it enables is recognized by its heavy representation in the premier journals of the scientific community. Finally, traditional Western users can take advantage of the optimization in imaging capability offered by the FluorChem Imagers. Taken together, these well-established technologies have a proven track-record of high impact publications that demonstrate how these next-generation Western blotting technologies are enabling cutting-edge biological and biomedical research.

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