Supporting Information

Multiplexed Ion Beam Imaging Readout of Single-Cell Immunoblotting

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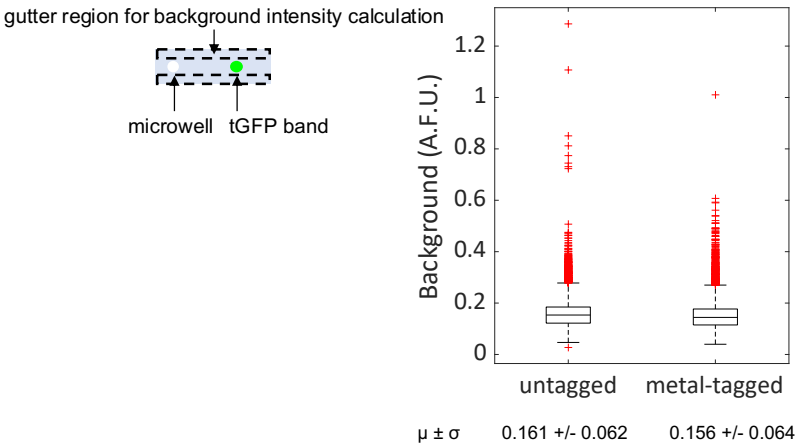
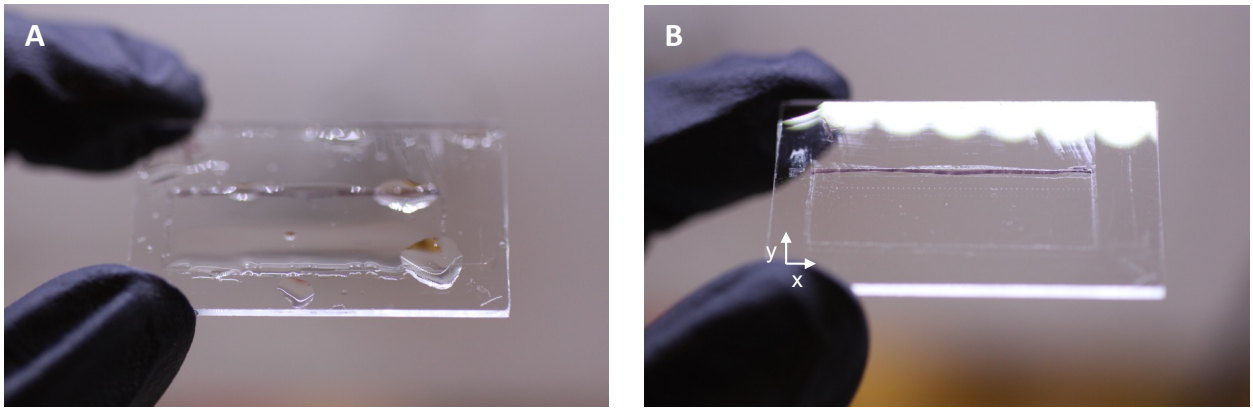


Supplemental Figure S1. Background signal for metal-tagged antibodies. Boxplot of background signal from the same results depicted in main text Figure 2D. Background intensity was calculated from a gutter region of each analyzed ROI chosen as 3-4 standard deviations away from the peak center as depicted in the schematic. Horizontal line in the boxplot is the median (higher for gels immunoprobed with untagged 1° Ab, Mann−Whitney U-test p-value <0.0005) and box edges are at 25th and 75th percentile. Mean and standard deviation of data is displayed below plot. Difference in background signal in scWB for metal-tagged antibody versus untagged antibody configuration is statistically significant, but small. $n_{Untagged}$ = 849 cells, $n_{Metal\text{-}tagged}$ = 728 cells.

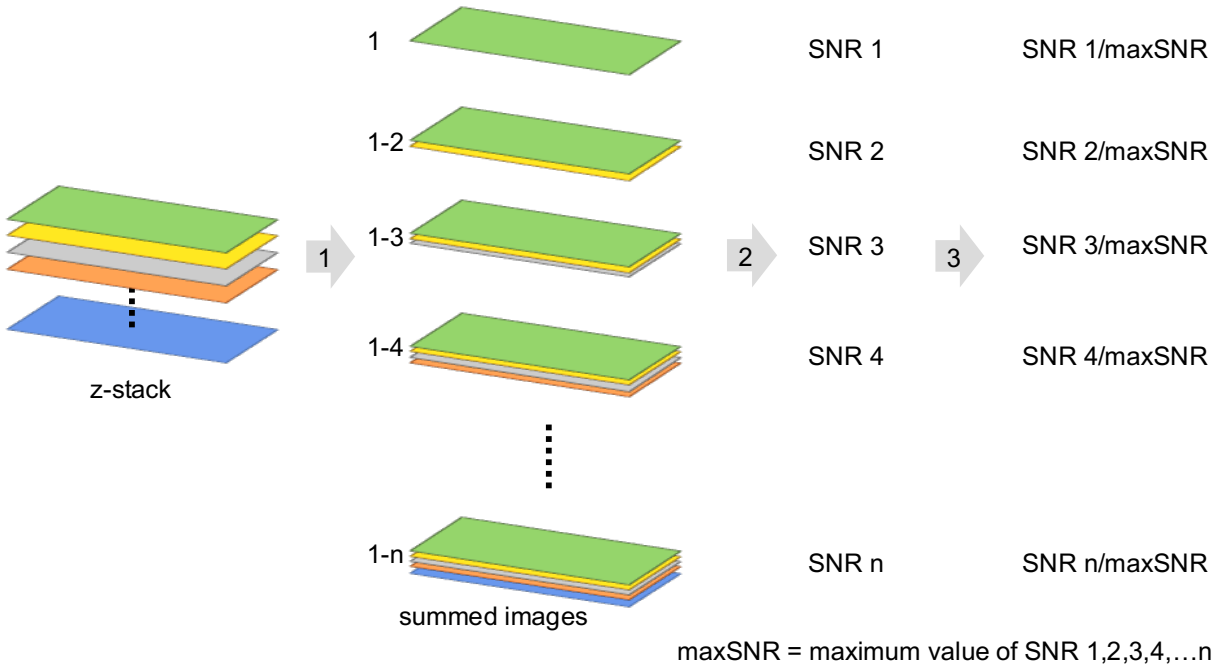


Supplemental Figure S2. PA gel on glass slide is dehydrated before MIBI-TOF. (A) Hydrated scIEF chip on half of a standard microscope slide. (B) Dehydrated scIEF chip. Single row of microwells is visible.

Note S1. Calculation of normalized SNR

We calculated a normalized SNR in Figure 3C of increasingly summed confocal slices to investigate the relationship between percent of gel depth imaged and SNR, since percent of gel depth imaged is a tunable parameter in MIBI-TOF. With an in-house Matlab script, we performed the following:

1. From each confocal z-stack of a single scIB lane with n slices, we summed slices 1, 1-2, 1-3, 1-4…1-n with slice 1 being the top layer of the gel and slice n being the bottom layer of the gel (gel-microscope slide interface). The result was n images with the first image being just the top layer of the gel and the nth image being the sum of the entirety of the gel over its depth.
2. For each of the n images, we performed background subtraction, Gaussian fitting, and calculated SNR as previously described[1]. Images with protein bands with SNR < 3 were disregarded.
3. Since cell-to-cell variation resulted in large differences in absolute SNR values, we normalized each SNR value by the maximum SNR within the n images for each cell, which allowed improved side-by-side comparison of the biological replicates.



Supplemental Table S1. Composition of IEF lid gel. Components of the 3-part lid gel used for lysis and electrophoresis in the scIEF assay.

Lid gel components	pH 4 anolyte boundary condition	Focusing region	pH 10 catholyte boundary condition
Polyacrylamide gel	• 15 %T • 3.3 %C • 0.2% VA-086	• 15 %T • 3.3 %C • 0.2% VA-086	• 15 %T • 3.3 %C • 0.2% VA-086
IEF reagents and detergents		• 1% final ZOOM™ Carrier Ampholytes pH 4-7 • 1% (v/v) TritonX-100 • 3.6% (w/v) CHAPS • 0.0125% (w/v) digitonin • 7 M urea • 2 M thiourea	
Boundary conditions	• 13.6 mM pKa 3.6 immobiline • 6.4 mM pKa 9.3 immobiline		• 5.6 mM pKa 3.6 immobiline • 14.4 mM pKa 9.3 immobiline

Supplemental Table S2. Imaging conditions and depth rasterized data.

Fig	Current (nA)	FOV size (μm)	dwell time (ms)	# planes	pixels	Ion dose/plane (nA×hr/mm^2)	Ion dose total (nA×hr/mm^2)	Average depth rasterized (μm)
4B, 5	21.9	200	4	1	256	39.87	39.87	1.48
4B	21.9	200	4	2	256	39.87	79.74	1.73
4B	9.5	400	1	10	256	1.08	10.81	0.13
4B	47	400	4	1	256	21.39	21.39	0.44

Reference

1. Kang, C.-C. *et al.* Single cell–resolution western blotting. *Nat. Protoc.* **11**, (2016).