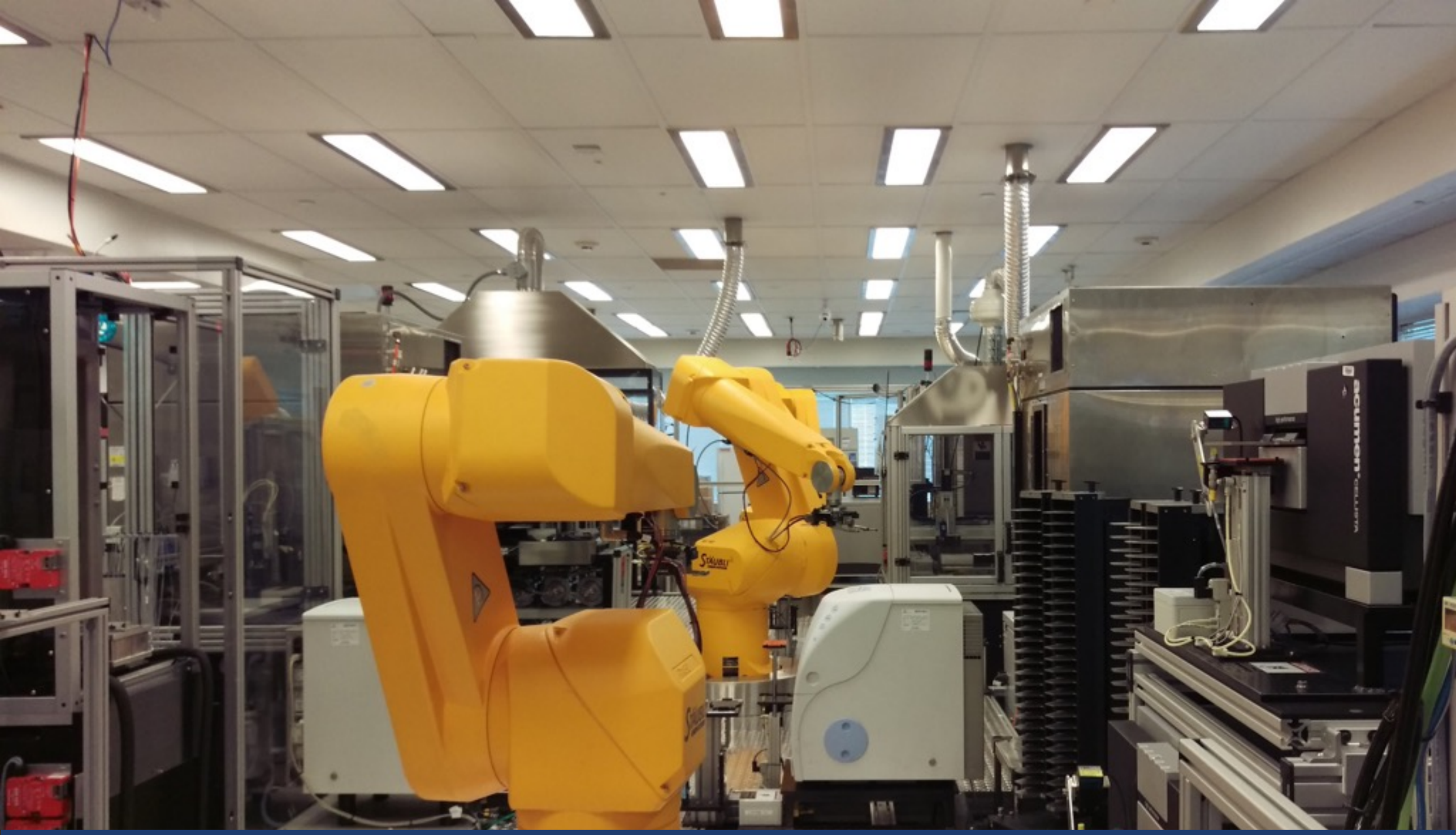


SLAS 2016 PRESENTATION

MICROPLATE SURFACES: HOW THEIR PROPERTIES AND CHEMICALS MAY AFFECT YOUR ASSAYS

BY PAUL HENSLEY, CEO, IONFIELD SYSTEMS

WHERE WE STARTED

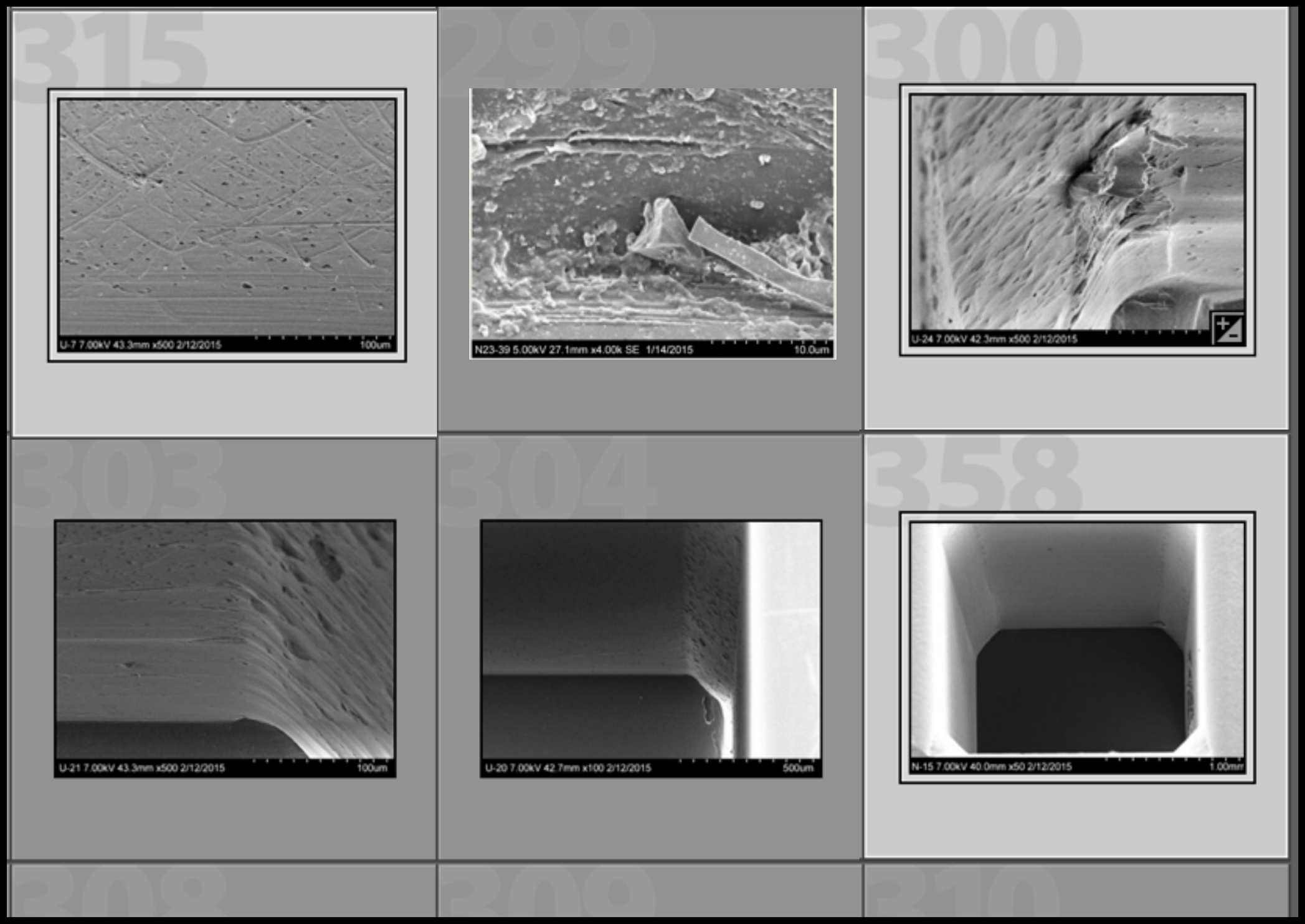
- A poster at SLAS 2015 by AstraZeneca and considerable anecdotal evidence from other labs that our plasma treatment process reduced CVs

- The inability to determine the source of the “noise” our process removes and if systemic or random

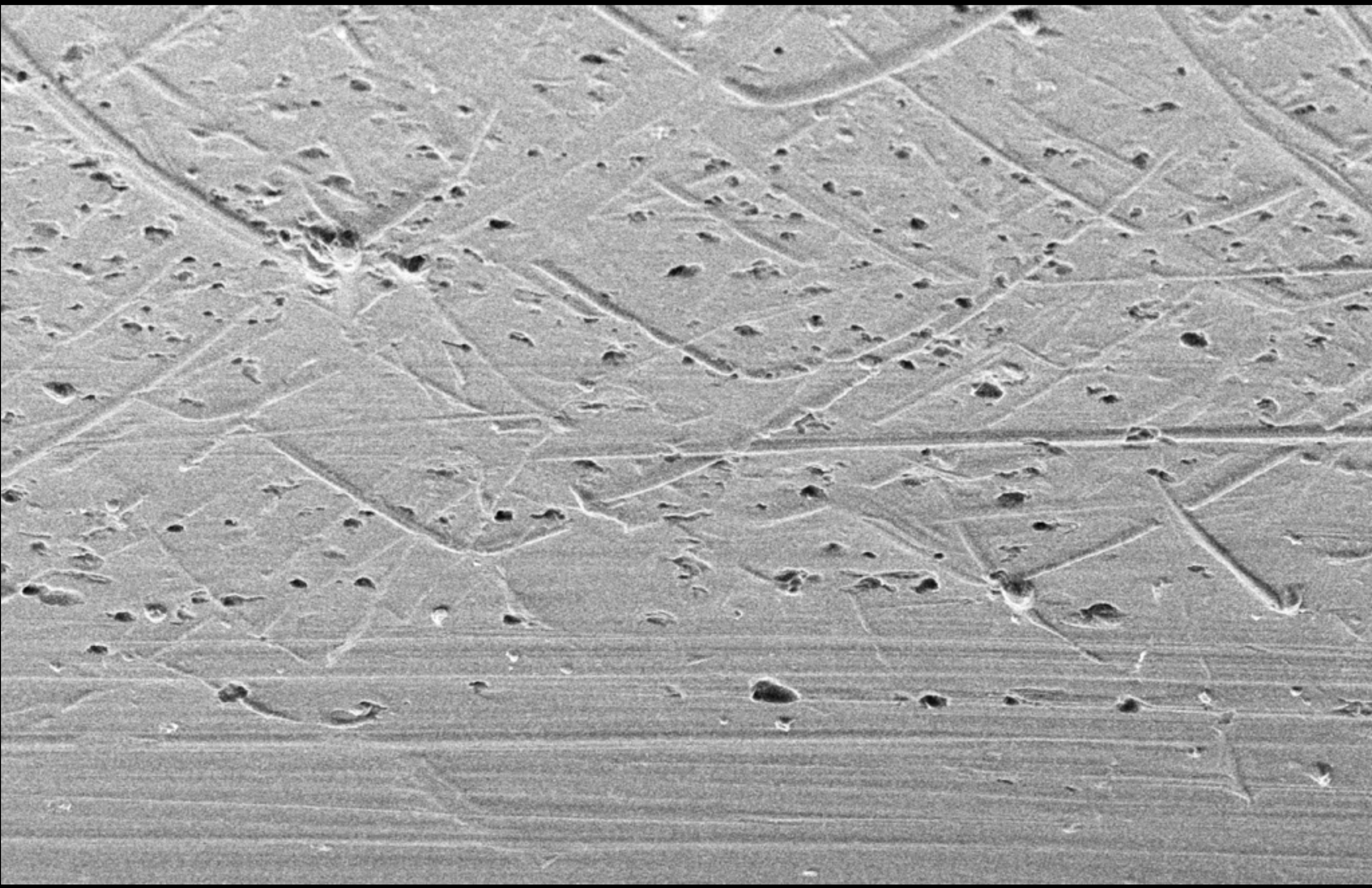
- Planned to run SEM, then look at surface chemicals with XPS, ToF-SIMS or other technique

SEM identified four distinct problems with new, unused plates: Surface Debris, Inclusions, Roughness, and Exclusions

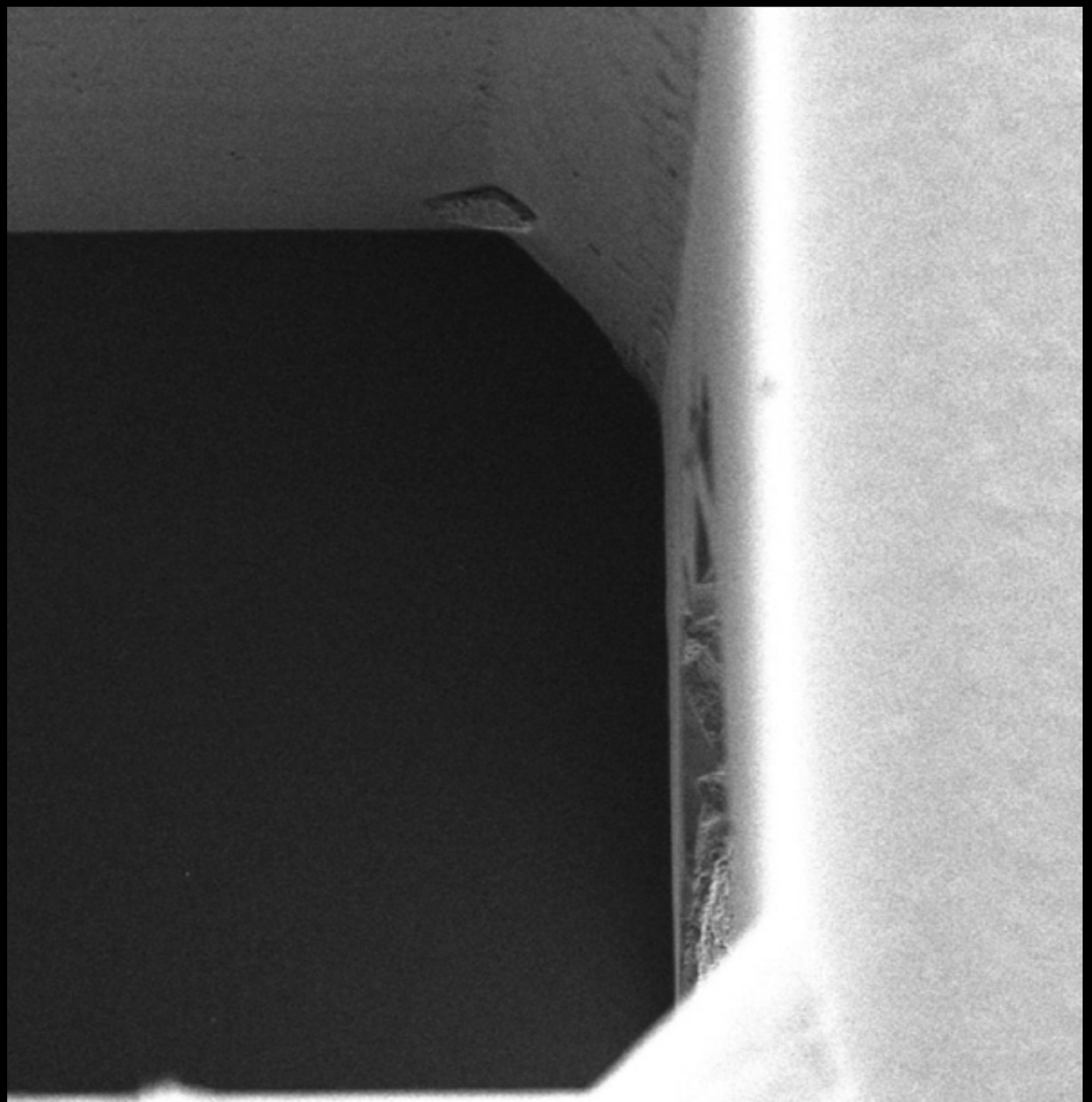
“Microplate RESIDUE” makes each well unique



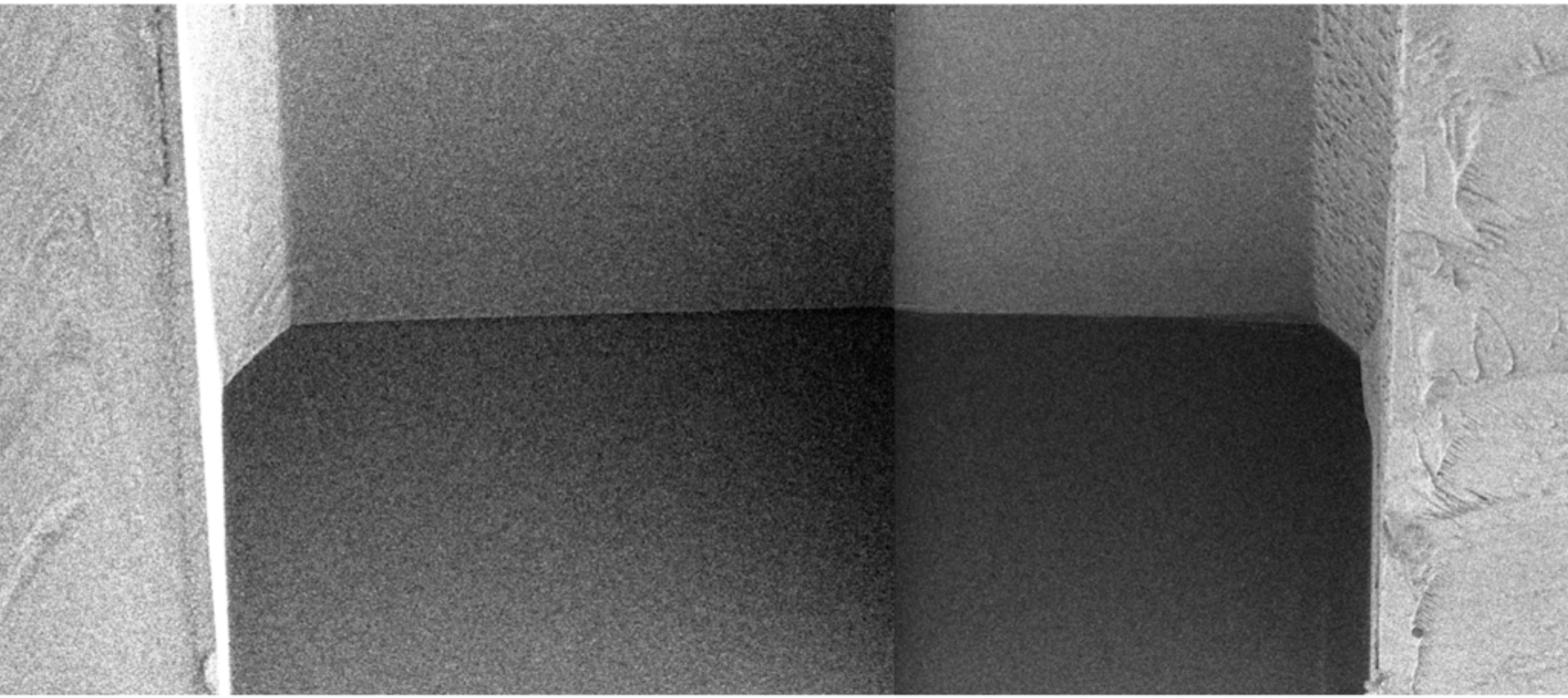
Upper Left



Lower Right



SEM shows that plasma treatment
smooths surface flakes.



WHAT WE KNEW BEFORE CHEMICAL ANALYSIS

- Methods development scientists believe different brand microplates running the same assay may give different results

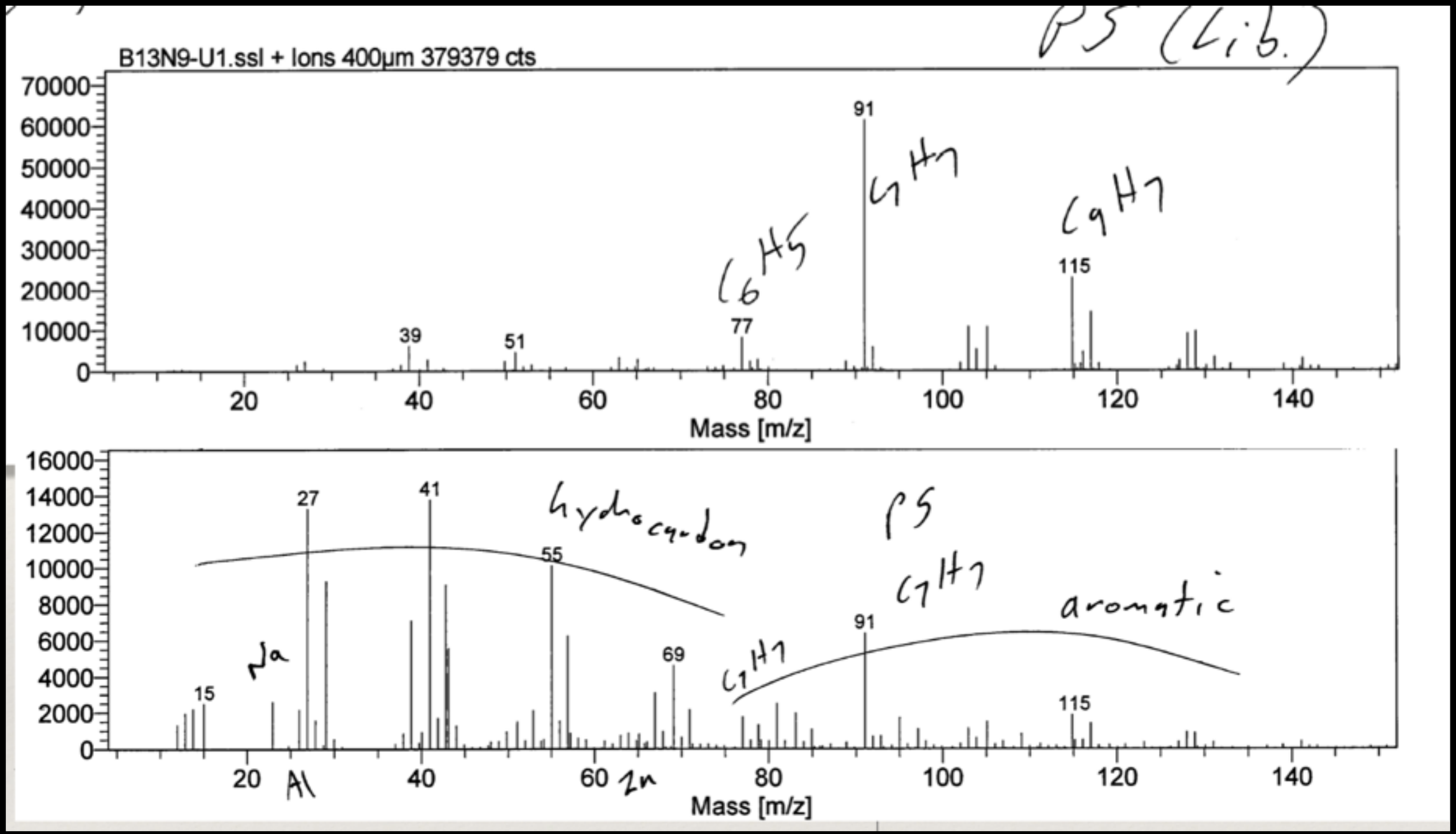
- HTS groups report lot changes can impact assay performance

- The SLAS 2015 Interest Group focused on surface and leachables as the possible explanation

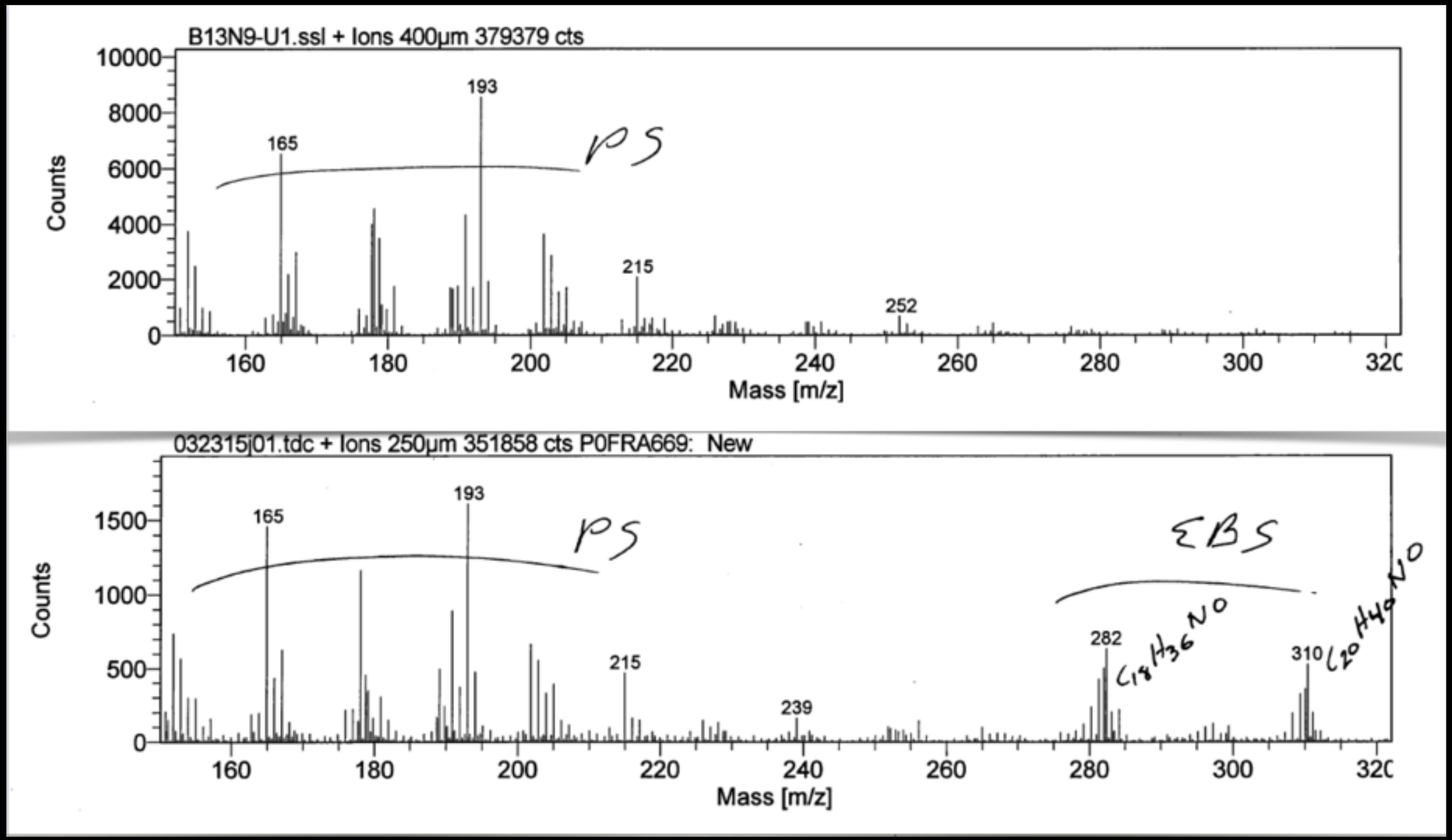
PROCESS & METHODS

- ToF-SIMS was selected for maximum resolution
- What's on the surface might affect your assay
- Samples were hit with positive ions

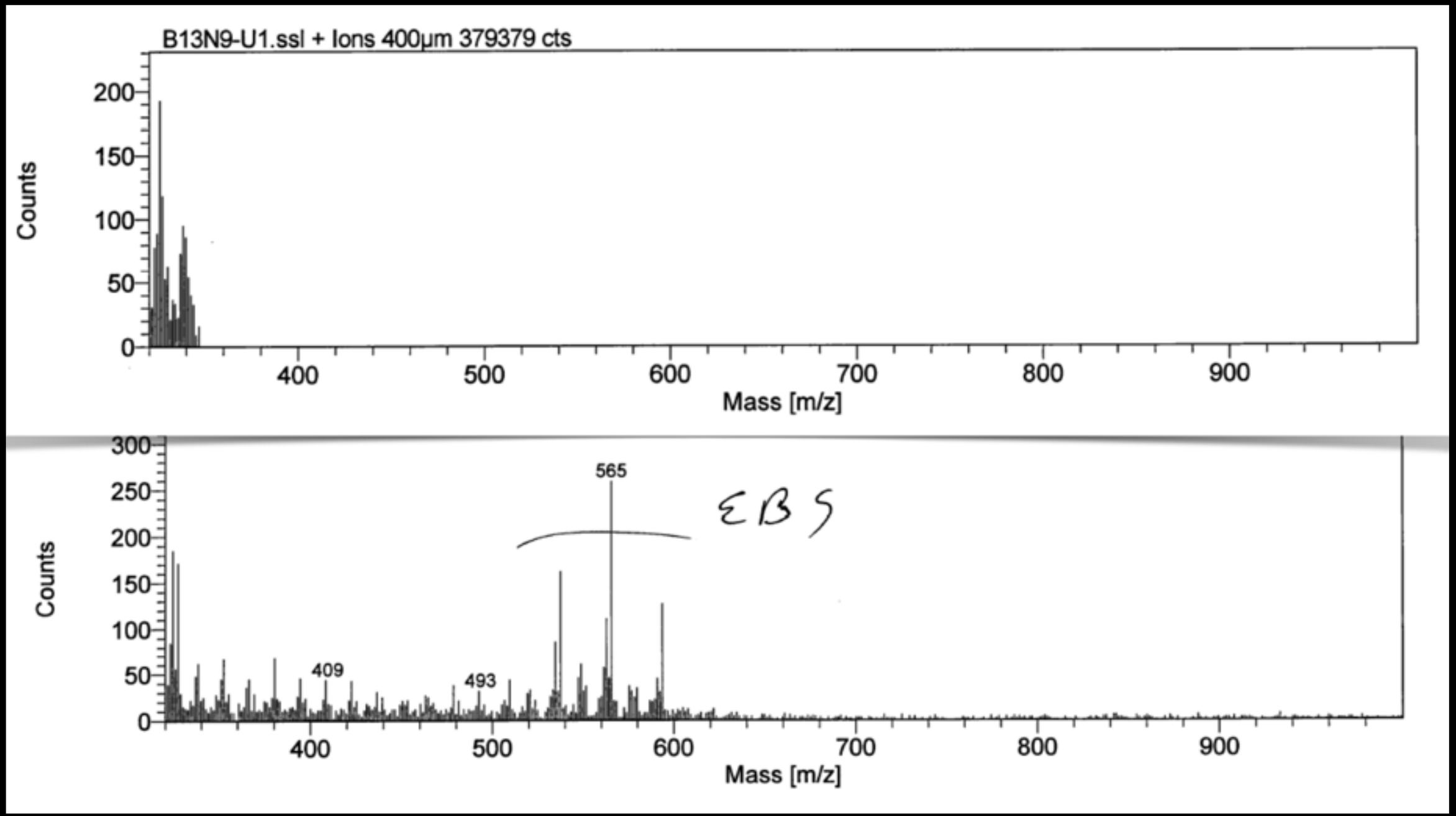
ToF-SIMS - Top PS standard, Bottom new 1536 Plate



ToF-SIMS, 150 to 320



ToF-SIMS, 350 to 1000



Identified + Ions in 9 Different Plates

ammonium						Pro	TMAH
18	23	27	39	40	64	70	74
NH4	Na	Al	K	Ca	Zn	C4H8N	C4H12N
0.08	13.14	6.71	0.04	1.75	3.42	2.89	0.09
0.18	29.07	17.35	0.03	3.57	5.23	1.99	0.15
8.42	1.34	0.26	0.09	0.01	0.01	2.55	7.49
4.83	84.56	19.11	1.01	0.10	0.19	2.87	1.59
4.81	99.50	11.80	0.86	0.06	0.17	2.10	2.18
7.11	5.03	0.24	1.40	16.13	0.36	2.32	14.62
5.26	4.63	1.28	0.66	28.61	0.72	1.68	7.71
0.08	27.25	9.79	0.03	2.58	4.65	2.37	0.08
0.04	22.40	6.10	2.90	0.15	3.66	0.52	0.26
7.21	0.20	0.01	0.08	0.01	0.02	2.08	9.43
3.42	0.35	0.01	1.06	0.50	0.01	1.68	4.20
0.11	47.30	27.76	9.59	0.25	8.56	1.56	0.18
8.42	99.50	27.76	9.59	28.61	8.56	2.87	14.62
0.08	0.20	0.01	0.03	0.01	0.01	0.52	0.08
105	498	2776	320	2861	856	6	183

Identified + Ions in 9 Different Plates (cont)

	Phe	amine	PDMS	phthalate		EBS	Irgafos
107	120	130	147	149	208	310	648
Ag	C8H10N	C8H20N	Si2OC5H15	C8H5O3	Pb	C20H40NO	C42H64PO3
0.02	0.21	0.09	0.35	0.40	0.03	2.40	0.03
0.02	0.15	0.07	0.29	0.19	0.02	1.96	0.02
0.06	1.07	4.18	2.47	2.43	0.06	0.01	0.00
0.02	0.54	5.36	1.53	4.19	0.11	0.02	0.01
0.03	0.59	2.22	1.65	1.21	0.14	0.00	0.00
0.03	0.58	4.82	1.68	2.81	0.16	0.01	0.00
0.04	0.52	3.83	1.27	1.85	0.19	0.01	0.01
0.03	0.24	0.09	0.21	0.21	0.02	2.42	0.01
0.03	0.06	0.20	0.92	0.34	0.04	0.00	0.00
0.05	0.85	5.96	1.64	1.59	0.07	0.02	0.00
0.04	1.20	5.01	1.91	1.80	0.09	0.02	0.01
0.05	0.07	0.07	0.89	0.25	0.07	0.00	0.02
0.06	1.20	5.96	2.47	4.19	0.19	2.42	0.03
0.02	0.15	0.07	0.21	0.19	0.02	0.01	0.00
3	8	85	12	22	10	242	30

A CELLULAR ASSAY: FIRST USE — THE CHALLENGE

- 500 Be2C cells in 6 uL are added to all columns except #1
- 2 mM bortezomib (Velcade®)[1] is added to columns 2, 3
- Other cytotoxic compounds are titrated in stripes
- Plates are incubated for 72 hours, treated with CellTiter-Glo®[2] then read in a ViewLux®[3]

Trademarks 1 - Takeda, 2 - Promega, 3 PerkinElmer

Cellular Assay Results

	Control	1st Use	Treated	1st Use	Treated
	6653	**1652**	**1652**	**1654**	**1654**
Plate AVE	12210	7298	12138	7293	11532
SD	746	537	699	590	700
CV	6.1%	7.4%	5.8%	8.1%	6.1%

	1st Use	Treated	1st Use	Treated
	6558	**6558**	**6560**	**6560**
Plate AVE	5870	10004	6036	10132
SD	530	594	522	530
CV	9.0%	5.9%	8.6%	5.2%

	1st Use	Treated	1st Use	Treated
	0542	**0542**	**0544**	**0544**
Plate AVE	5280	7710	5012	7667
SD	468	770	440	781
CV	8.9%	10.0%	8.8%	10.2%

Plate 1652 — First Use

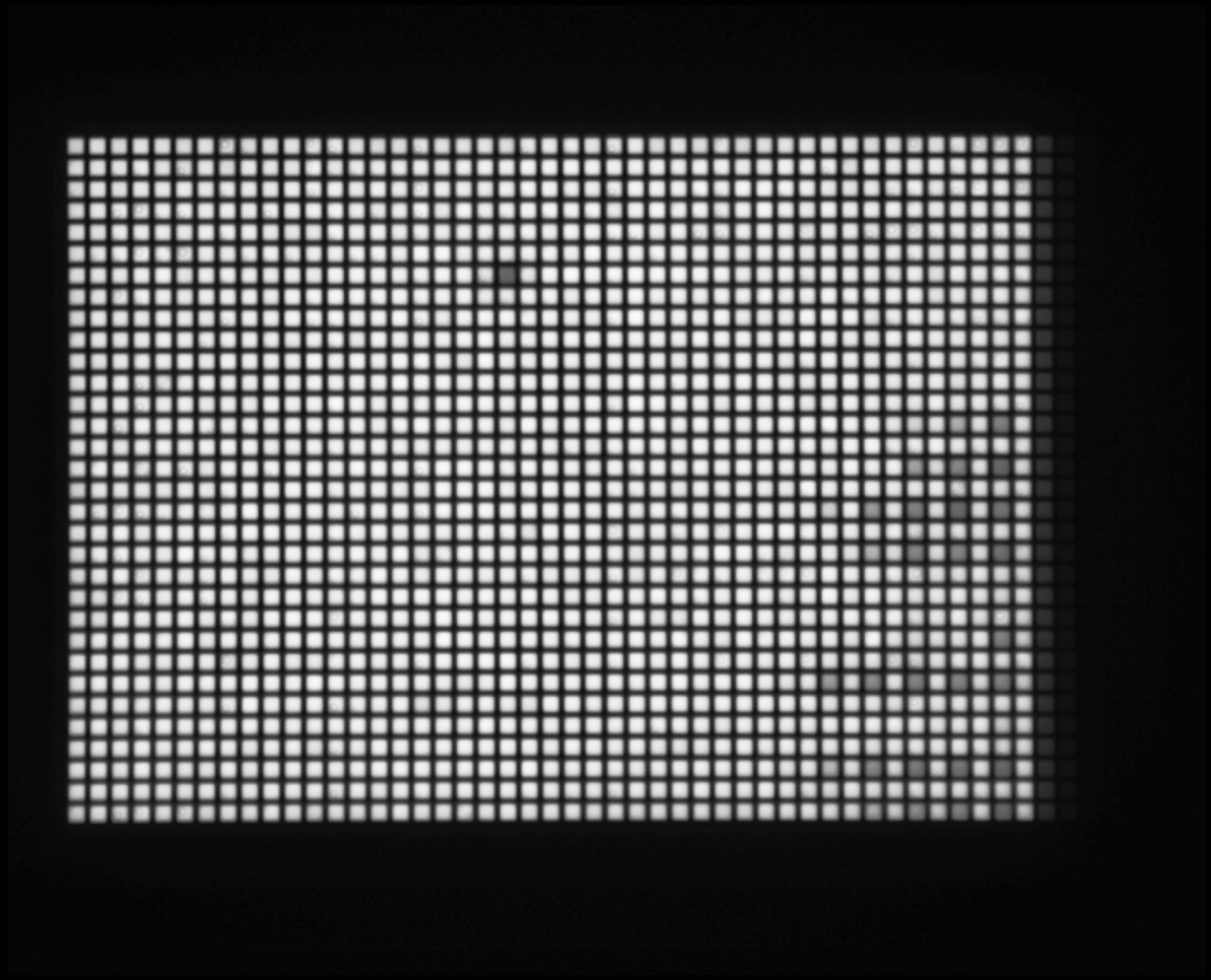
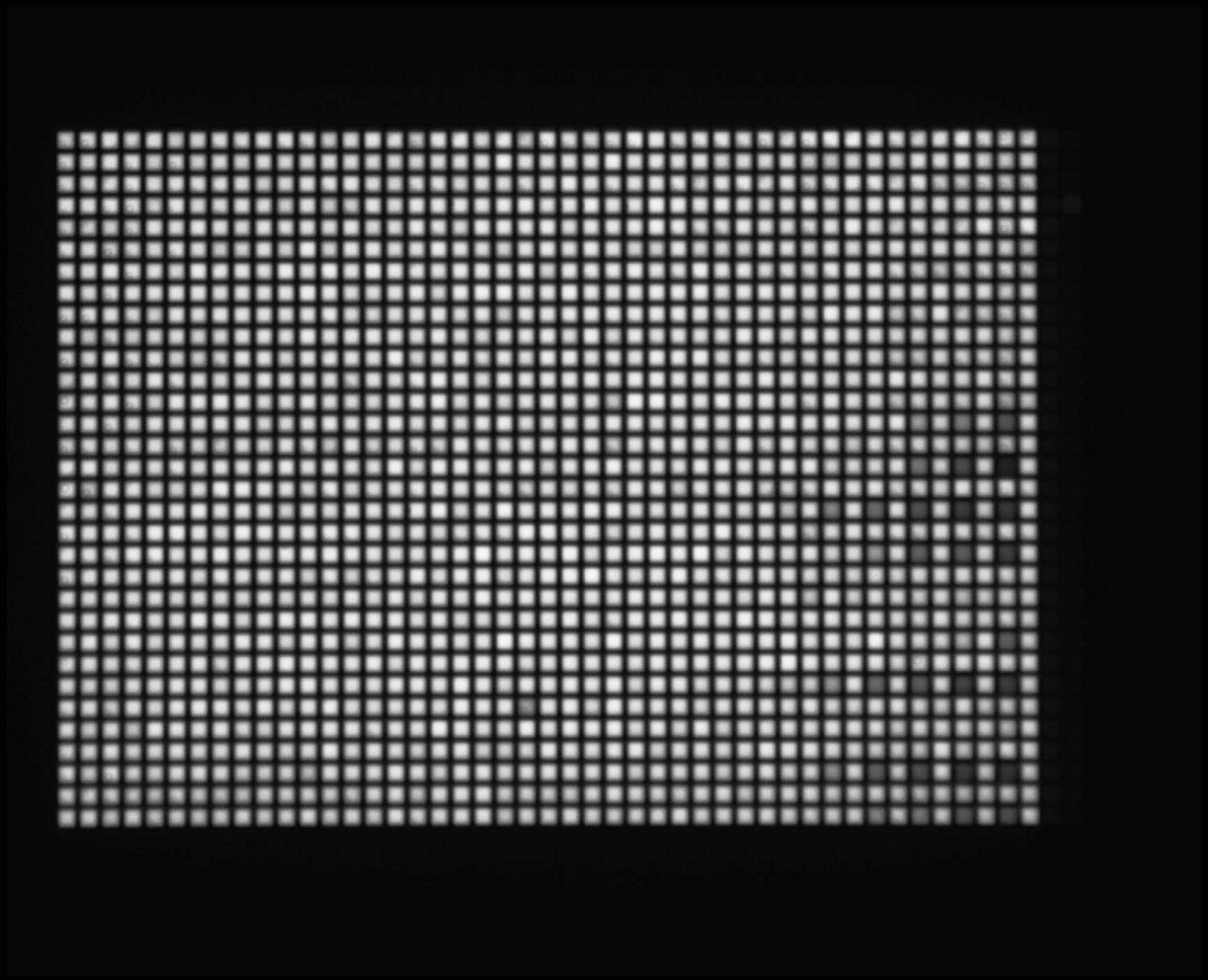


Plate 0544 — First Use



THE SAME CELLULAR ASSAY: AFTER PLASMA TREATMENT—TREATED PLATES

- Again, 500 Be2C cells in 6 uL are added to all columns, except #1, of plate (Note: 1 month has elapsed and plates are not pinned)
- No bortezomib is added; no other compounds are added
- The plates are incubated for 72 hours and processed identically
- Data are compared to determine if there is carryover of cytotoxic compounds
- A new Control Plate was run with the treated plates

Control Plate 6653

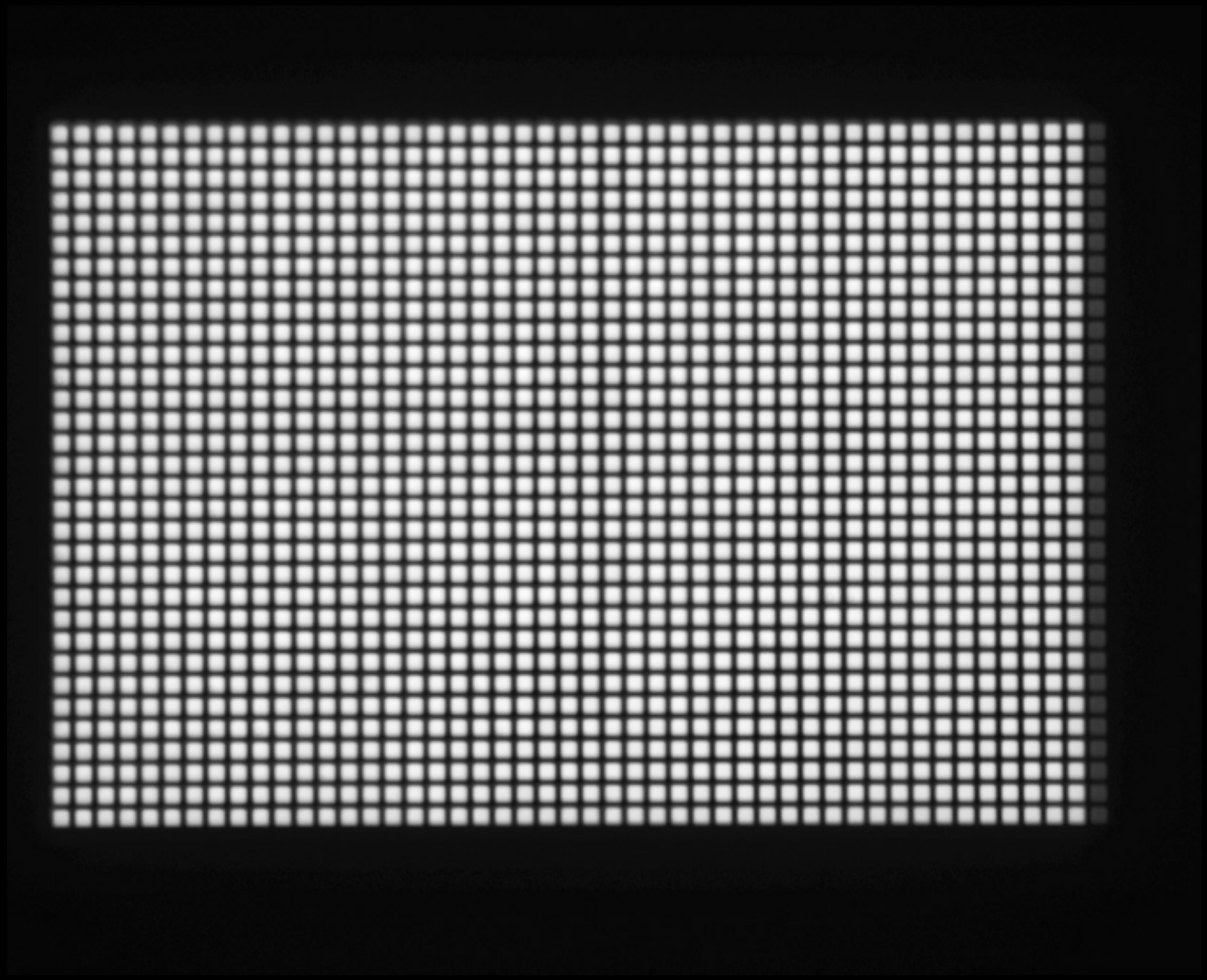


Plate 1652 — Second Use After Treatment

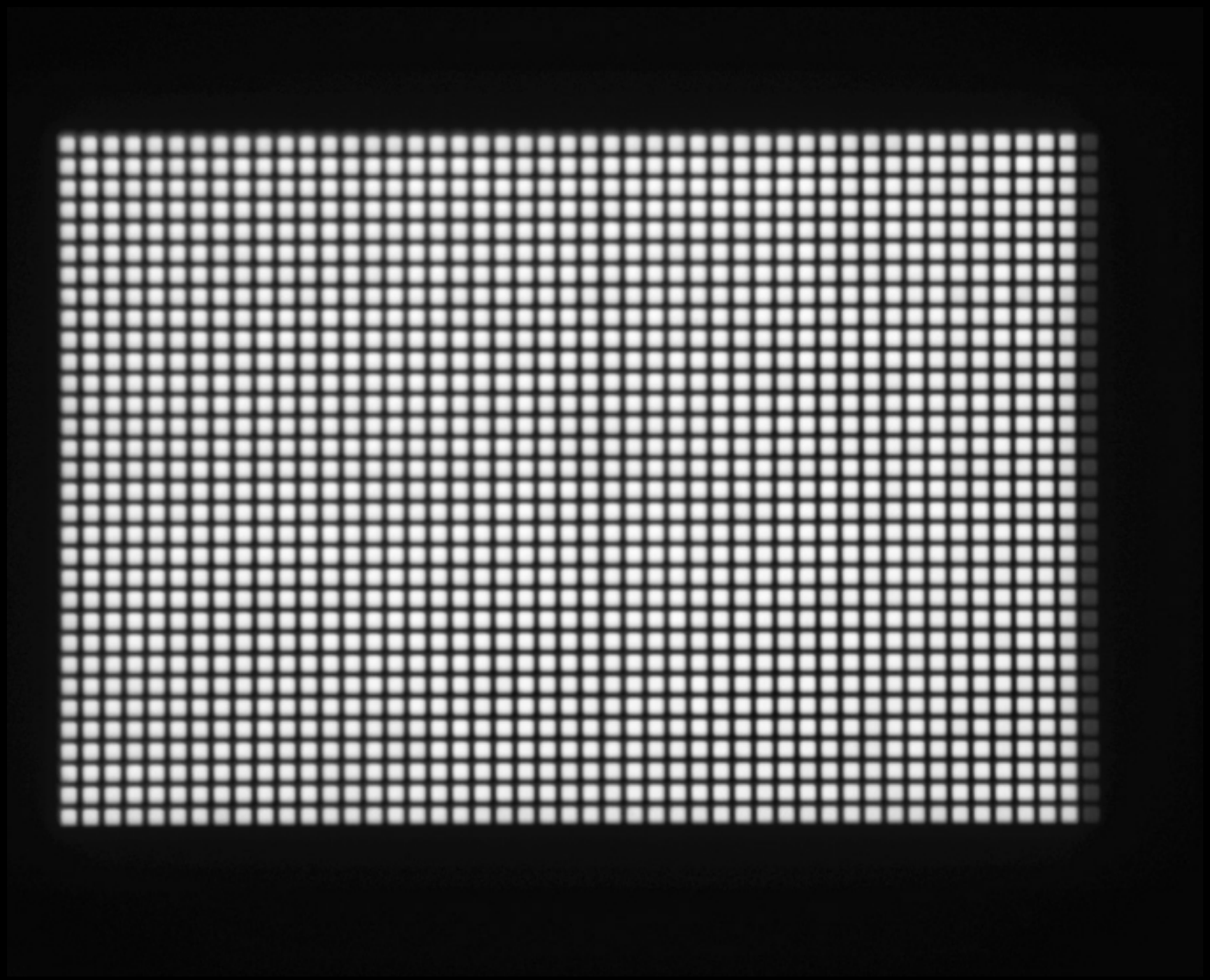
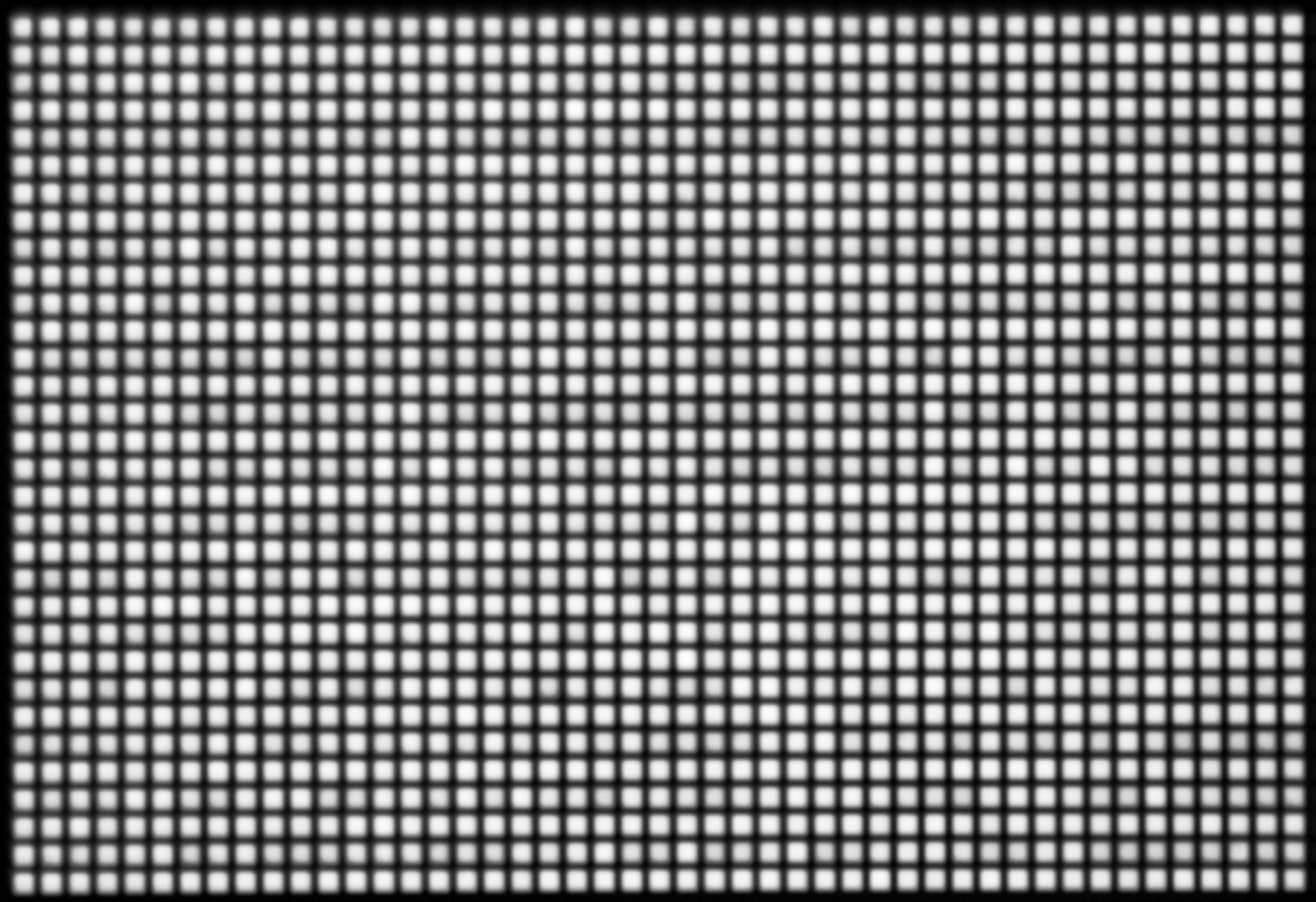


Plate 0544 — Second Use After Treatment



WHAT CHEMICALS ARE ON 0544?

- In higher concentration than other microplates
  - Zn
  - EBS (a lubricant mixture)
  - IRGAFOS (an antioxidant)

CONCLUSIONS

- Chemicals appear to play a role in the significantly lower luciferase reaction results as shown by 0544 and 0542 in new and plasma treated microplates

- Microplate RESIDUE — both physical and chemical — may affect many assays; generally reducing assay signal

- Plasma treatment is effective preventing carryover; none of the plasma treated plates indicate the presence of any cytotoxic compounds from the first use

- Plasma treated microplates performed as well as, and in some cases slightly better than, the new microplates

ACKNOWLEDGEMENTS

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