Microplate Surface Compounds that effect Assays and are removed by Plasma Cleaning

Paul Hensley and Scott Kunz, IonField Systems, Moorestown, NJ, USA

IonField SYSTEMS™

www.ionfieldsystems.com

Understanding Surfaces

IonField Systems in the course of developing the PlasmaKnife Microplate Cleaning System did a careful analysis of microplate surfaces and the chemicals on the surfaces of new microplates.

It doesn't seem unreasonable to expect that bulk commodity polymer materials, like polystyrene or COP, would all be the same. But as we began running SEM (scanning electron micrographs) and ToF-SIMS (time of flight secondary ion mass spectrometry) substantial differences become evident from the results. The differences include surface roughness – essentially the size of the roughness much like grades of sand paper; chemicals used to catalyze polymerization and prevent oxidation; and chemicals used in the molding process to control flow rate, flow evenness and improve release of the finished product from the mold. In all we found almost 30 chemicals in the three main brands we studies. Only a few were unique to any brand and differences between the brands were more the concentration of a core set of common chemical components.

Correlating with Results

The mix of chemicals are meaningless without assay data that provide comparative results using the brands that were tested.

To begin that analysis compounds were divided into two groups – those that the plasma treatment process reduced and those that did not change with treatment. The second group consists of compounds that are in the polymer matrix and as the surface is ablated by plasma, more of the same compound is exposed but varied in concentration by brand. In some instances there is more measured post treatment than when tested unused. The first group consists of compounds that are added at time of molding or segregate to the surface during the molding process. Chemicals with near identical concentrations in all brands are thought not to influence results.

Chemicals in the first group that were reduced include: NH4, C2H3O, C3H8N, C3H8N, C4H12N, C8H5O3, C20H40NO. Chemicals in the second group include basically all others measured except for Ti and Pb, both of which increased.

Patterning

Upon further testing of the differences in plasma effect when comparing one treatment with a course of 5 treatments, the results that some of what appeared stable between a new and single treatment were now being reduced further. And while the assays were slightly less noisy by the removal of the carbon compounds listed considerable noise remained. We then went back to the early SEM results and realized that the ion beam of the ToF-SIMS was not hitting a flat surface and the differences in the results for multiple spots on the same microplate were different because of surface area differences. And every spot and hence every well on a microplate has a unique surface area. The graph analysis in Figure 1 was run to compare all the brands.

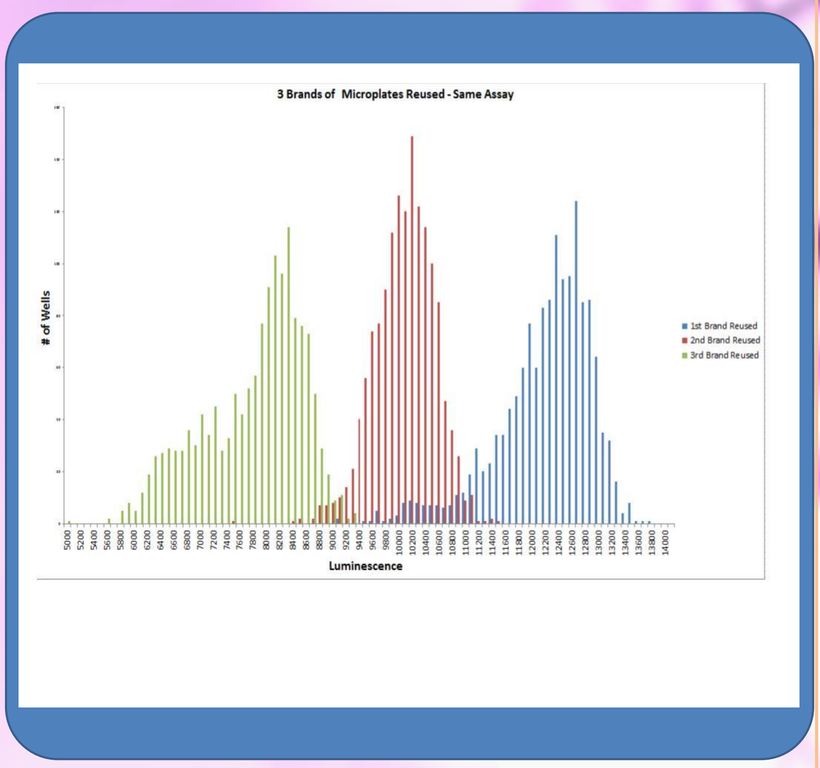

Figure 1. All brands, results of cell assay, well count by result

Key Experiment

An experiment to test the effectiveness of plasma as a cleaning agent failed spectacularly. There was no carryover, so all wells in the three brands we had performed SEM and ToF-SIMS on now had a full plate identical assay run. In theory, the result from every well should be the same. The only variable is the microplate. As this is a luminescence assay, there will be differences between plate brands due to the measurement process; and as a standard curve was not run, that difference can not be determined for this experiment. But the differences in assay readout are striking both between plate brands and within individual plates.

Chemical Patterns

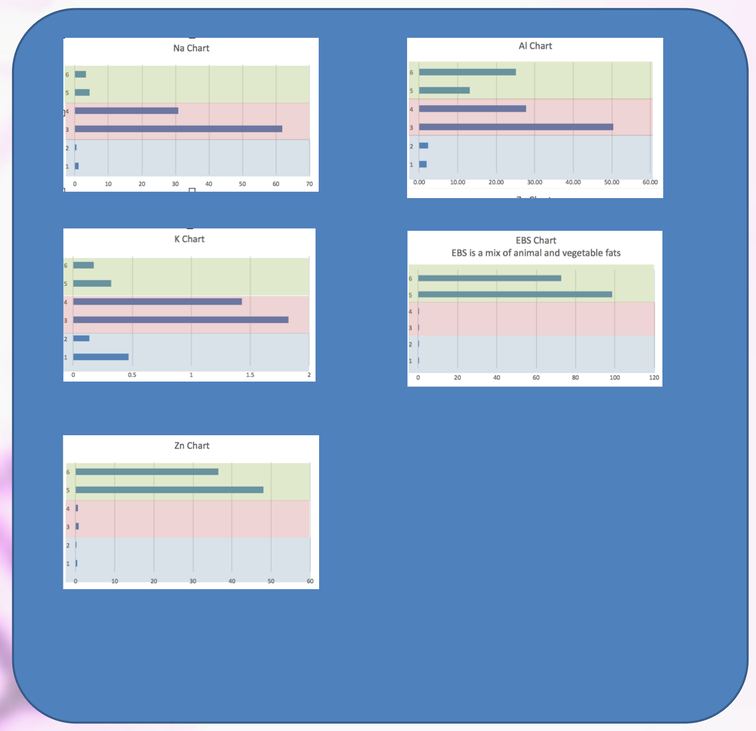

Figure 2. Relative concentrations by Brand Na, K, Zn, Al. EBS

Very obviously, Brand 1 (blue bars in Fig.1 and blue area in Fig.2) has the lowest levels of 4 of these ions and about the same K as Brand 3. This assay was performed post plasma treatment so the compounds in Group 1 are reduced. The CV for Brand 1 was 6%, quite low for a cell assay. The CV for Brand 2 was almost identical; Brand 3's CV was almost 10% - it was the only brand with EBS and while EBS concentration was lowered, as shown in the graph, much remained even after multiple treatments. The pairs of ToF-SIMS results show good consistency of these key ions post plasma.

Conclusions

- Surface chemicals on microplates effect assay results.
- Plasma treatment reduces hydrocarbon compounds improving assay precision.
- Comparison of plate pairs show more consistency of non-removed ions, further reducing assay noise and increasing precision.
- Careful selection of a microplate to use for an assay can have a substantial impact on the dynamic range and noise.