Plasma Technology to Prevent Carryover of Nuclease Activity by a Pin Tool

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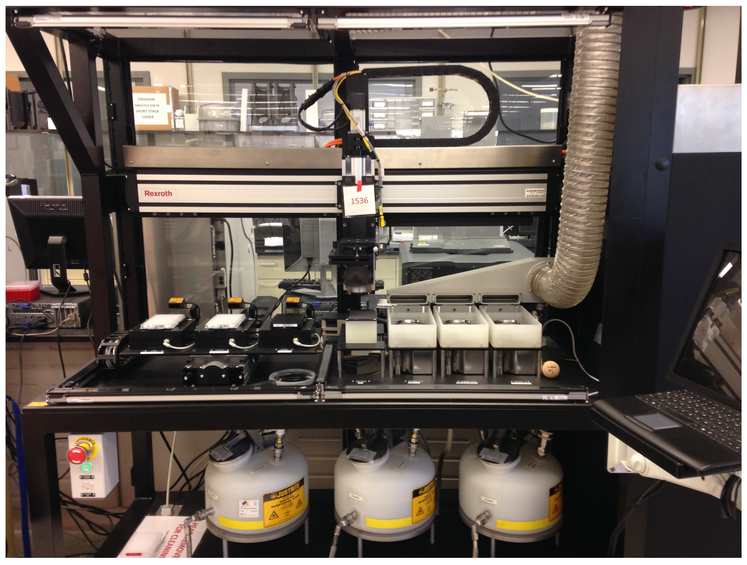
Abstract

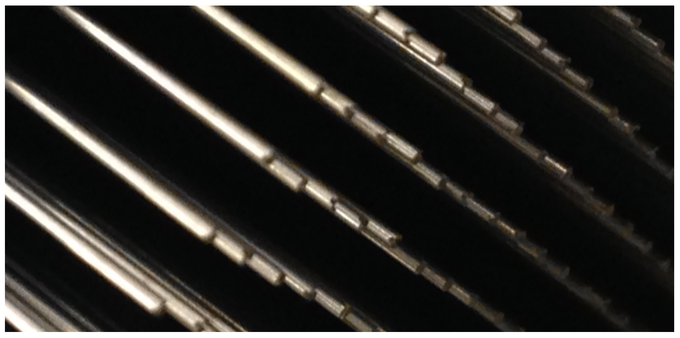
Pin tools are specialized liquid handling systems widely utilized to transfer thousands of experimental library compounds among microplates for high-throughput screening applications in drug discovery. The devices rely on an array of stainless steel pins with precisely shaped gaps to draw nanoliter volumes by a combination of capillary action and surface tension from a library source plate for delivery by diffusion into the wells of a recipient plate, in order to test the activities of individual compounds in biological assays. Advantages of the system include the high speed (< 60 seconds for an entire 1,536-well plate) and precision of the dispensers, as well as excellent reliability and low maintenance of the pin tools relative to other alternatives. The pins are cleaned by a sequence of the following wash steps: a) sonication for 50 seconds in de-ionized water (DI water); b) a 50/50 mixture of dimethyl sulfoxide/DI water with sonication for 70 seconds, and c) 100% methanol without sonication for 25 seconds, then drying in a vacuum station for 25 seconds. While this cleaning procedure is often sufficient, it failed to completely remove nucleases, which are enzymes that cleave nucleic acids. The carryover of molecules with biological activity is likely to introduce contaminants into both assay and compound library microplates and cause significant assay interference. Plasma technology has successfully been applied to clean and decontaminate a variety of materials utilized in biological research. The technology harnesses the reactivity of ozone, high energy oxygen, singlet oxygen and hydroxyl ions to rapidly ionize organic molecules and vaporize solvents. To determine if plasma could prevent the contamination of nuclease activity by a pin tool, we compared our current cleaning method against a 60 second cleaning with the TipCharger TC-1536, developed by IonField Systems, which utilizes plasma to clean 1,536 pin tools. Our results demonstrate that plasma technology is required to completely remove nucleases from the pins of a 1,536 pin tool. Additionally, repeated applications of the TipCharger improved the precision of Rhodamine B dispenses, lowering the coefficient of variation by 25% among 1,536 wells, from 16% to 12%. More broadly, the application of plasma technology to clean pin tools might prevent the contamination of microplates with other biologically and/or assay active molecules that are incompletely removed from pin tools by traditional cleaning solvents.

A 1536 Pin Tool Station

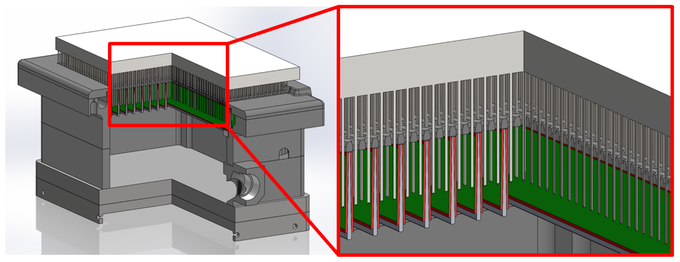


The 1536 Pin Tool Station used for this study. Assay plates are inserted into the three nests on the left. Library compound plates are placed in first nest (far left), control compound plates are placed in the second nest (middle) and assay plates are placed in the third nest (right). The pin washing station is located on the right, with de-ionized water in the first reservoir (left), a 50/50 mixture of dimethyl sulfoxide/de-ionized water in the second reservoir (middle), and 100% methanol in the third reservoir (far right). The vacuum station is located in the center of the station directly under the pin tool head. The de-ionized water and solvents are continuously circulated from the three large storage containers underneath the Pin Tool Station platform.

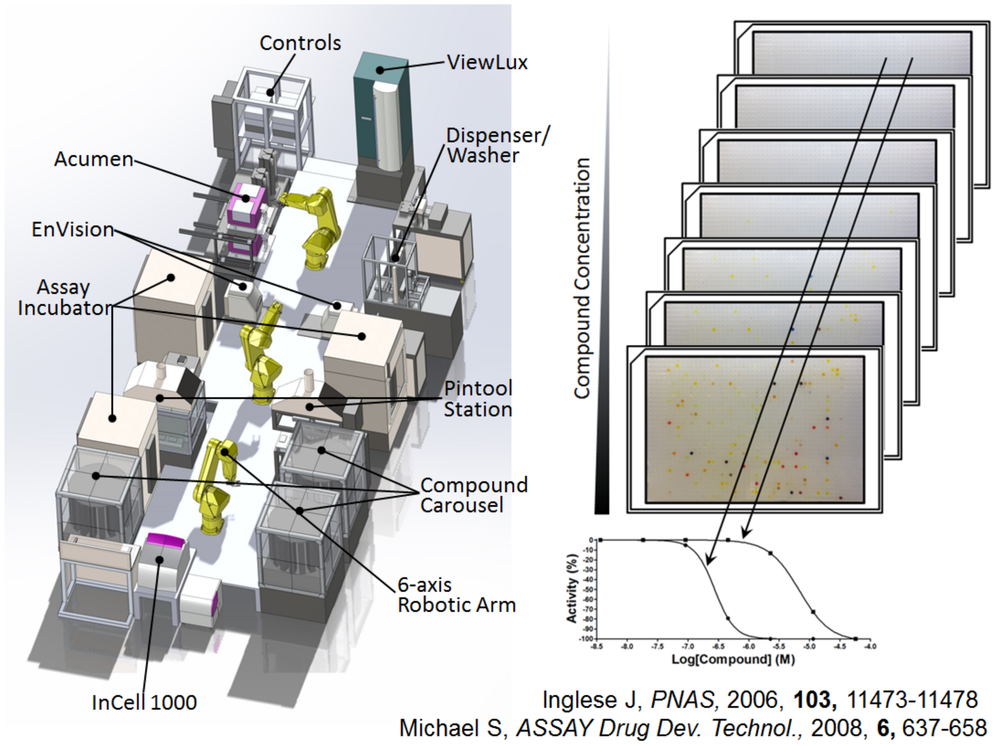
Cleaning Pin Tools with Plasma Technology



A magnified view of the stainless steel pins from a 1536 pin tool. The precisely shaped gaps draw and dispense 23 nL solution volumes.



Internal view of 1536 format pins inside the TipCharger Cleaning Station. Electrodes have a 5,000V differential and are covered with an Alumina dielectric barrier. The ceramic prevents arcing and allows electron streamer formation that provide the energy to generate the atmospheric pressure plasma that fills the space between them.



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The National Center for Advancing Translational Sciences applies the most recent technologies to screening large compound libraries in order to identify small molecule modulators of a variety of biological targets that are relevant to human diseases. These small molecule modulators can then be developed for use as research tools or as starting points for therapeutic development. The robotic screening platform, represented in the schematic on the left, is set up for ultra-high throughput with 1536-well plates. Screening assays are optimized to utilize the lowest possible volumes and the platform is equiped with reagent dispensers capable of delivering nanoliter volumes. Also within the platform are a variety of plate reading instruments capable of different types of detection, such as absorbance, fluorescence and luminescence as well as image-based cell phenotypes. Primary screens are performed in a dose-response format as shown schematically on the right, with each compound tested as a dilution series. This approach delivers concentration response data directly from the primary screen, which includes information such as the shape of the dose response curve, potency and efficacy. Having these data derived from the primary screen allows better-informed selections of hits to progress to the next stages and significantly lowers the rate of false positives.

Plate 1: Direct Pin Transfer of Nucleases or Empty Well Control

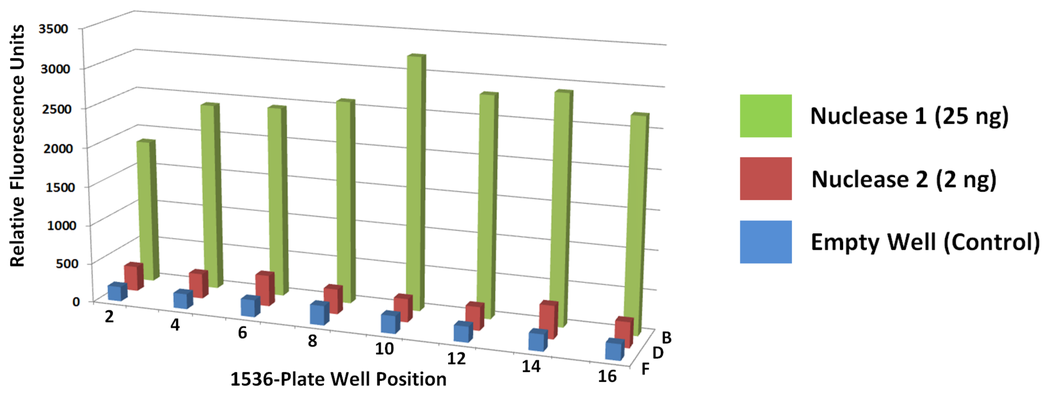

Plate A: Contact with Pins Cleaned by the 1536 TipCharger

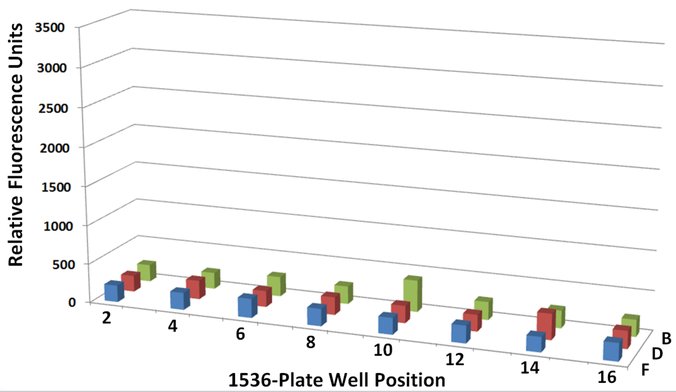

Plate B: Contact with Pins Cleaned by DMSO/Sonication and MeOH