ADVANCING PLASMA CLEANING: METHODS FOR OPTIMIZING DESIGN OF PLASMAKNIFE™ FOR MICROPLATE CLEANING

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Abstract

IonField Systems' TipCharger uses room temperature plasmas to decontaminate and clean tips and pins used for liquid handling of DNA, protein and small organic molecules. The treatment is complete in seconds, and does not impact pipetting accuracy or assay fidelity. We now seek to utilize room temperature plasmas to accomplish the same results with multiwell assay plates by designing the PlasmaKnife™ workstation. Here we describe methods developed to test the effectiveness of instrument designs and ongoing improvements to~~to~~ optimize configuration of the workstation. We show the sensitivity limits encountered using standard tannic acid assays, and will present a novel luciferase assay protocol for extremely low levels of carryover detection developed for current and future optimization. PlasmaKnife™ will decrease hazardous waste generation and screening costs in the pharmaceutical industry and decrease contamination problems in molecular biology and forensics laboratories, improving outcomes and assay robustness throughout the life science research community.

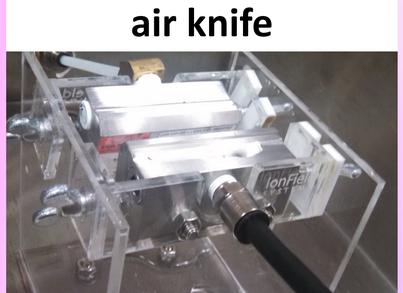
Ion Plasma Technology

- Plasma, the forth state of matter, is energetically charged gas.
- Plasma can be generated by dielectric barrier discharge in air, using a high frequency, high voltage, low amperage electrical field.
- The interaction of plasma with air generates oxidizing radicals that break down all organic materials.
- Studies using IonField Systems TipCharger shows that 30 second plasma treatment breaks down organic DNA and other organics, releasing nontoxic gas byproducts.

PlasmaKnife cleaning modules

A. Plate wash module

Nozzle assembly & air knife



Assembled unit

B. Plasma cleaning module

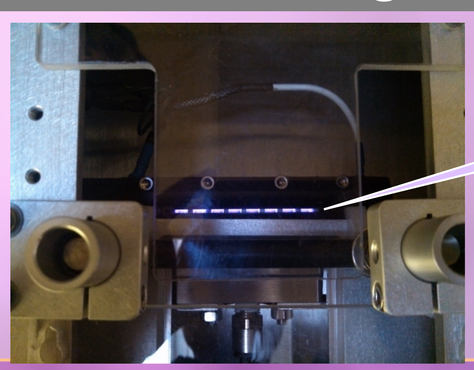
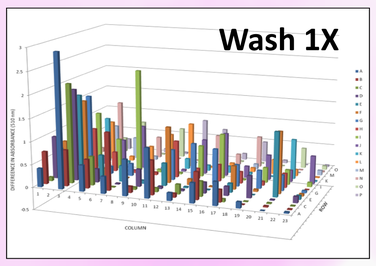
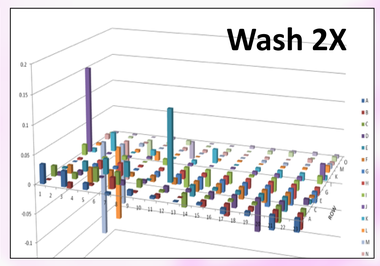


FIGURE 1: Optimizing wash number in carriage transport prototype (stream angle 75°)

Procedure

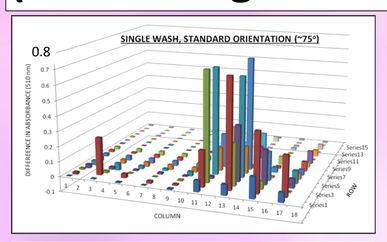
- ➢200 nmol Tannic Acid (in methanol) deposited on selected wells in 384-well Corning 3702 polystyrene plates – dried
- ➢ Plates washed, assayed using colorimetric assay

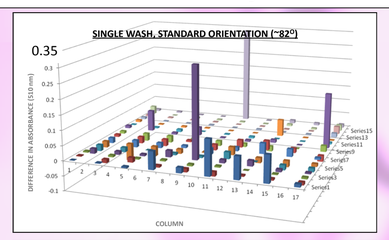


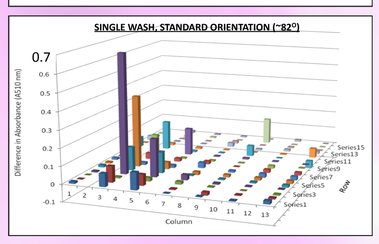


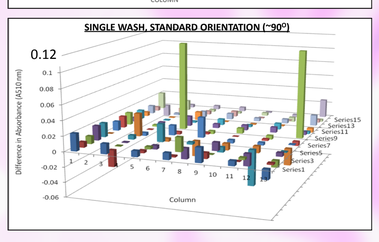
CONCLUSIONS: Single wash leaves sufficient residue for optimization studies.

FIGURE 2: Optimizing wash head angle in carriage transport prototype (stream angle 75° to 82° to 90°)









CONCLUSIONS: Single wash at 90° improves washing efficiency.

FIGURE 3: Evaluating Roller Transport Prototype using Tannic Acid Assay

Procedure

- ➢200 nmol Tannic Acid (in methanol) deposited on selected wells in 384-well Corning 3702 polystyrene plates – dried
- ➢ Plates washed (right side up plate & upside down orientation) , assayed using colorimetric assay

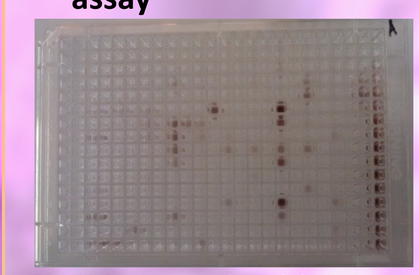


PLATE A: 30 second wash in 20% ethanol (use fresh wash buffer), right side up plate orientation

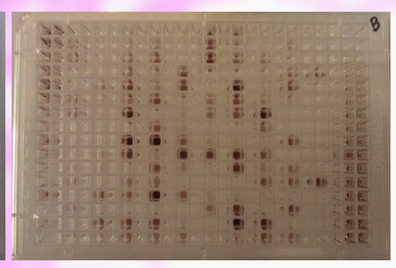


PLATE B: 30 second wash in 20% ethanol (use fresh wash buffer), upside down plate orientation

CONCLUSIONS: Washing from top (right side up plate orientation) appears superior.

Sensitivity of tannic acid assay

- Plates coated with 200 nmol tannic acid
- Residual tannic acid (A_{510} =0.1) equals 0.6 nmol
- Cleaned 99.7% of tannic acid, but retains 3.6 x 10^{14} molecules of contaminant – seek better assay sensitivity before evaluating plasma

LUMINESCENCE OFFER BETTER DYNAMIC RANGE

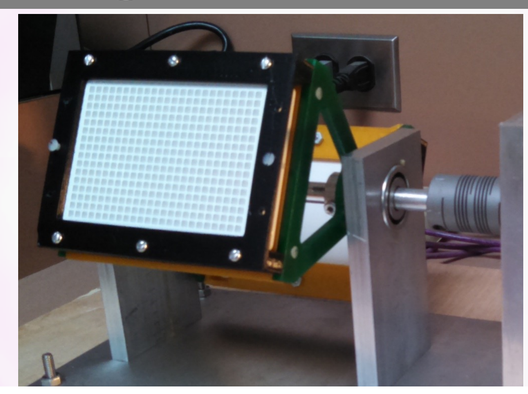
Issues with carriage transport & roller transport cleaning modules

- Roller transport offers simplified engineering solutions

BUT

- Rollers provide increased surface area for contamination from wash buffers

C. Centrifugal Plate wash module



- Improved removal of residual liquid

FIGURE 4: Evaluating plate wash using luciferase assay

Procedure

- ➢Plates coated with 10 ul luciferin (10 nmol) in the presence of 1% yeast extract
- ➢ Plates dried under warm air (2 hours)
- ➢ Wash 30 seconds with 20% ethanol using improved prototype (wash plates right side up)
- ➢ Dry by centrifugation, 10 seconds, 1100 rpm
- ➢ Assay for residual luciferin
  - 2.45 pmol of 10 nmol - > 99.975% clean

1% DIFCO Bacto™ yeast extract

CONCLUSION:

- Washing perpendicular to plate improves single pass wash efficacy (square well, flat bottom plates)
- Colorimetric tannic acid assay insufficient to continue improving cleaning efficacy
- Luminescence shows promise for supporting continuing instrument improvements