Solving the Challenges of Automated SPA YO_x Bead Dispensing

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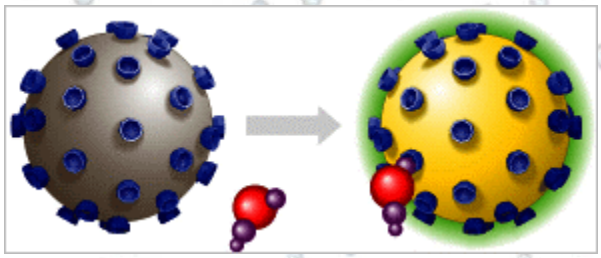
Background

Scintillation Proximity Assays (SPA) are characterized by the property that a signal is generated only when the radiolabeled molecules of interest are bound to the surface of the SPA bead. This signal is generated by the capture of the molecule onto the bead, thus bringing the radiolabel into close proximity with the scintillant contained within the bead.



(illustration courtesy of GE Healthcare)

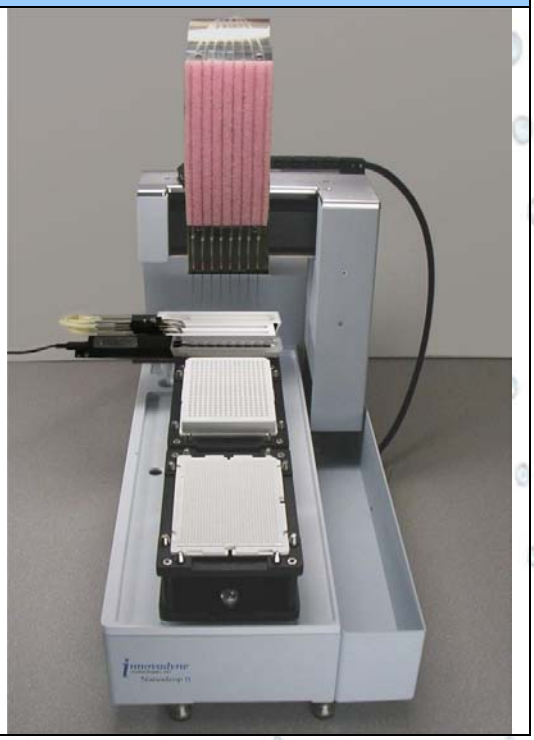
GE Healthcare's SPA Imaging Technology employs beads whose signal is a red-shifted fluorescence emission that minimizes interference from compound absorption, which typically occurs in the yellow and brown range of the spectrum. The LEADseeker Multimodality Imaging System from GE Healthcare is a high performance CCD imager that optimizes the performance of these beads by enhancing the sensitivity of the CCD chip toward the red portion of the spectrum.

SPA beads are available with both polystyrene and yttrium oxide (YO_x) cores. While the polystyrene beads allow for ease of handling and are readily adapted to automation, YO_x beads have superior scintillation counting efficiency and enhanced sensitivity. There are, however, some challenges involved in pipetting suspensions of YO_x beads and their adaptation for automated liquid handling for high throughput work. Many of the automation challenges result from the typically high density of YO_x beads (approximately 5 grams/mL), which causes them to settle out of suspension within a short period of time. Because of the many advantages found using YOx beads, many liquid handling companies have attempted the automated dispensing of these beads.

Liquid handlers with a flow-through design (valve in fluid path) can be expected to perform poorly or fail outright with YO_x beads because the passing of beads through valves and other moving parts creates a wide variety of maintenance issues, including valve malfunction and irreversible system failure. Non-contact aspirate-and-dispense systems are more appropriate devices for this kind of dispensing, but can still develop issues related to dispensing speed and cleanliness. Most often, liquid handlers have addressed these issues by dispensing only a few wells per aspiration and a small number of plates between replacements of the entire flow path of the instrument. This creates unacceptable throughput and cost for many institutions that require the use of these beads. Other technologies have been forced to dispense low bead concentrations (less than 30 mg/mL), which results in lower signal-to-noise values in high-density plate formats.

Innovadyne Technologies, Inc. has developed a method by which a high concentration of beads (up to 80 mg/mL) can rapidly be dispensed into 1536-well plates with no dispensing gradients (increasing or decreasing signal across the target path), little system maintenance and acceptable throughput.

Nanodrop II Liquid Handler with Harness Frame and Paddle Stirrer



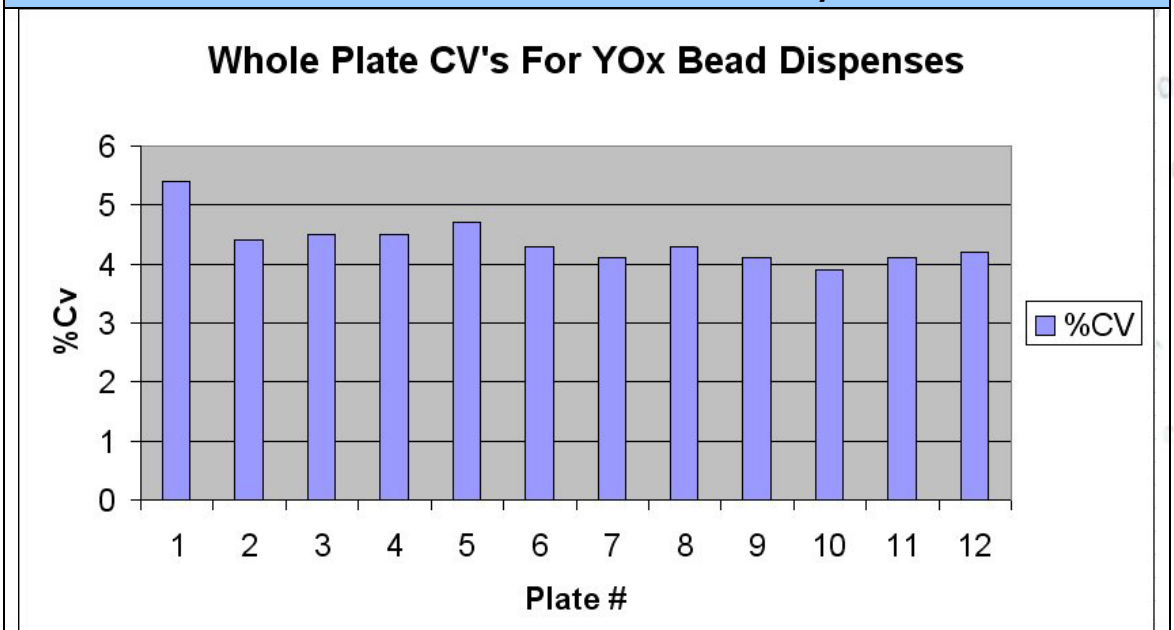
An Innovadyne Nanodrop™ liquid handler can be configured for bead dispensing by adding a paddle-wheel stirrer (manufactured by V&P Scientific, San Diego, CA) to keep beads in suspension in the reservoir, and a specialized fluidics harness frame to keep beads from settling in the tubing. It also utilizes slightly larger-bored dispensing tips. Because beads can scratch and stick to materials in the flow path, Innovadyne's design allows fouled tubing to be replaced with little effort or cost, making preventative maintenance both easy and effective. It is anticipated that the relatively inexpensive dispense tubing harness and tips will need to be periodically replaced as regular preventative maintenance. It should also be noted that the same instrument can be readily used for other dispensing tasks. Quick reconfiguration of the unit converts it from a bead dispenser to a conventional Nanodrop for other applications such as HTS, PCR, IC50 serial dilution, etc.

Multiple Plate Dispense Testing

At the GE Healthcare facility at Whitchurch, Cardiff (UK), twelve Greiner 1536-well plates were dispensed using the Innovadyne Nanodrop II platform. All plates were done consecutively and within a half day, dispensing to all wells of each plate and using all 8 tips at a time. A paddle-wheel stirring reservoir on the Nanodrop stage kept the beads in constant suspension. Into each well 1.25 µL of 80 mg/mL Streptavidin-coated YO_x beads (from GE Healthcare) were dispensed, resulting in a total number of 0.1 mg beads/well. These beads were then reacted with 3H-biotin. The resulting fluorescence was measured using a LEADseeker™ Multimodality Imaging System (GE Healthcare).

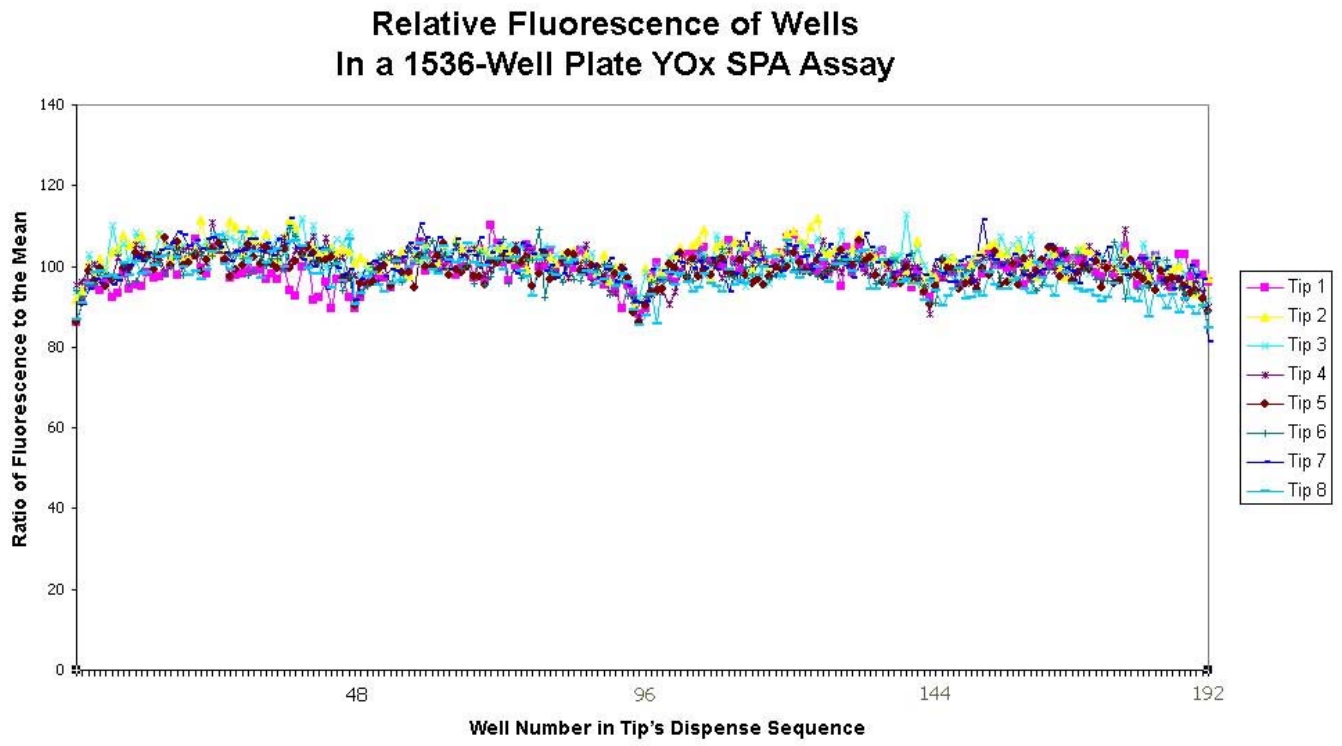
The dispensing precision results are shown in Figure 2. As can be seen in the graph, whole plate Cv's ran between 3.9% and 5.4% with no overall trends seen in the dispensing. Signal-to-noise values in excess of 10:1 were observed. Also, no tips or solenoids showed signs of clogging during this period, and no degradation of system performance was observed in the course of this study. It should be noted that previous publications with other technologies have not demonstrated the ability to successfully automate the dispense of YO_x beads at high concentrations to all wells of a 1536-well plate.

Fig. 2 -- Dispensing Precision for Each 1536-Well Plate Run in a 12-Consecutive Plate Study



The following graph (Figure 3) shows the fluorescence data from a representative single plate in the study (Plate 12). The slight dip in measured fluorescence as each tip dispensed in the first and last wells in a row (near positions 48, 96, 144 and 192 in each sequence) is a correctable edge effect typical of 1536-well fluorescence measurement, and is not a result of dispense volume variance.

Fig. 3 Relative Fluorescence of 1536-Well Plate YO_x SPA Assay

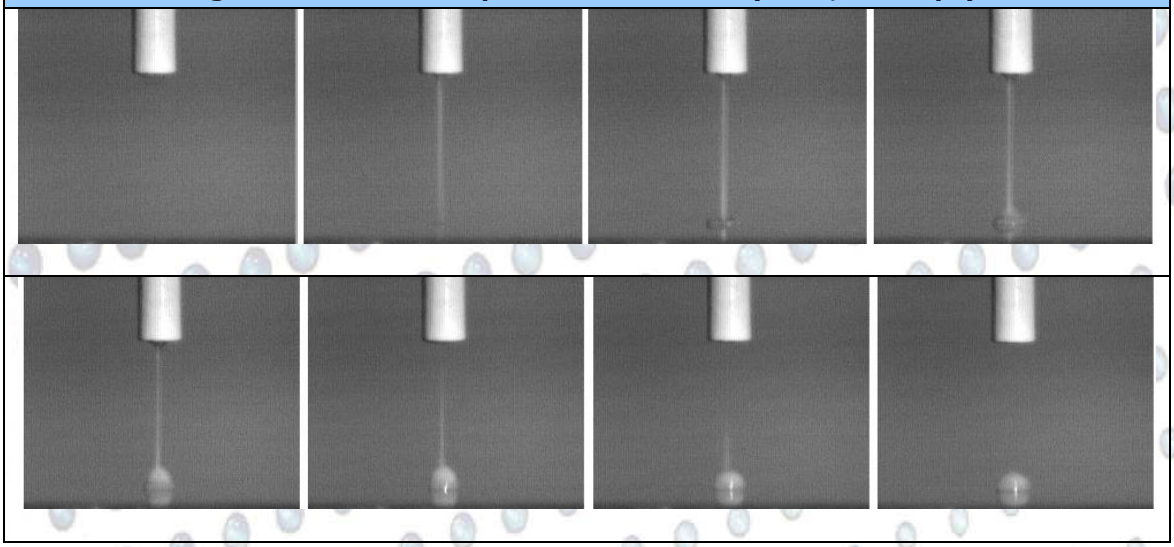


Representative 1536-well plate relative fluorescence (Plate 12 from the 12-plate study) from dispensing 1.25 uL of 80 mg/mL YO_x SPA beads (GE Healthcare).

High-Speed Photography

A high-speed video camera was set up to generate stop-action photographs of YO_x SPA bead dispenses made with the Nanodrop II configured for bead dispensing. The dispenses consisted of a 250 nL drop at a concentration of 80 mg/mL, dispensed to a glass slide.

Fig. 4 Non-contact dispense of YO_x beads (200X, 1000 fps).



The series of frames shows the stream as it emerges from the tip and the drop as it forms on the glass slide. Note that the stream is almost perfectly vertical, does not spread out near the bottom, and does not splash.

Conclusions

Innovadyne Technologies, Inc. and GE Healthcare have succeeded in using the Nanodrop II system to overcome the challenges of automated YO_x bead dispensing. To prevent bead settling in the reagent reservoir and dispense tubing, a paddlewheel stirrer was utilized as well as a protocol that maximized aspiration and dispensing speeds. Clogging issues were eliminated by utilizing wider bore tips and a proprietary fast bead-clearing procedure between runs. Minor adjustments to experimental protocol resulted in multiple 1536-well plates with no dispense gradient. Maintenance issues were minimized by the inherent design of the Nanodrop instrument, which includes no moving parts in the flow path as well as easily changeable tip and dispense tubing harnesses.

The unprecedented data obtained proves that excellent whole-plate Cv's (3.9% to 5.4%) can be obtained in a 1536-well format using highly concentrated YO_x bead suspensions. The reliability, ease of dispensing, and high signal-to-noise values (greater than 10:1) of this assay protocol would directly transfer to higher quality assay data.