Thermo Scientific Matrix Hydra Platforms for Automated Nanoscale Applications

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ABSTRACT

Performing biological applications at sub micro liter volumes facilitates processing large numbers of samples in routine biological laboratories in an economical fashion. A prerequisite for the miniaturized automated experimentation is the availability of a reliable bench top instrument with a user-friendly interface. The Thermo Scientific Matrix Hydra group of instruments offers a great flexibility to perform a variety of scientific applications. Coupled with the Hydras is also an optional single channel eDrop module. The Hydra offers 96/384 liquid dispenses ranging from sub-micro liter to hundreds of micro liters of volumes in a contact dispensing mode. The eDrop module, in conjunction with Hydra enables non contact dispensing of liquids via a single channel micro dispenser. The ability to use the 96/384 contact and single channel non contact dispensers in a single operational process makes the Hydra platforms ideal for several miniaturized biological applications. The 96/384 channel dispenses use positive displacement mechanism and the single channel non contact dispense occurs via a friction free electro magnetic bellow mechanism. Aside from these robust mechanisms, several other instrument capabilities coupled with versatile software programming features enable users to easily modulate programs according to their specific requirements. The instrument has been validated to dispense various types of biological reagents and finds applications in several scientific experiments. The successful use of Hydras in automating polymerase chain reactions, protein crystallization trials and protein assays demonstrates the potential of Hydra to target a gamut of other applications. Here, we discuss the salient features of Thermo Scientific Matrix Hydra bench top platforms and present their use in performing automated biological applications.

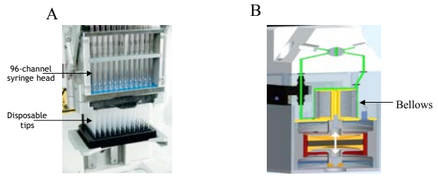
INTRODUCTION

Starting with the user-interface, the dispense mechanisms and instrument features of Hydra automation platforms is presented. Details of the instrument parameters is discussed in the below individual sections. Results of different scientific applications performed on the platforms is shown in the respective figure legends. The platforms and applications offer tailored guidelines to users wanting to automate their specific processes.

I. User Interface: The ControlMate Software

ControlMate software: The main application screen of the ControlMate programming interface consists of a menu which is similar to the Windows interface. The sequence editor of ControlMate consists of drag and drop icons that are used for easy programming. Each command is further controlled by a dialog box via which the parameters such as heights and speeds can be controlled. The commands can also be grouped into a series of repetitive steps to perform a process. Global values can be assigned or external procedures can be called wherever required.

II. Dispense mechanisms of Hydras



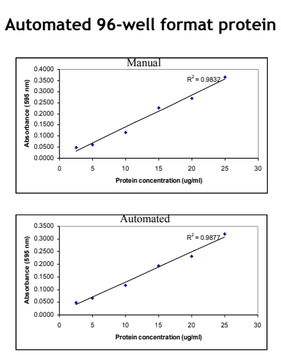
Dispense mechanisms of Hydra eDrop: *Panel A* shows the 96-well dispense mechanism of Hydra. The mechanism of aspiration and dispenses is brought about by 96 syringes. The 96-channel head is available with non disposable nozzles or disposable tips. The nozzles in non disposable units are rigid and available as stainless steel and duraflex models. The disposable tips (DARTs: Disposable Automation Research Tips) are arranged in a magazine of 96/384 with various volume ranges. *Panel B* shows a cross section of the electromagnetic bellows which aid in non contact dispensing by the single channel nozzle in the eDrop module. The upward and downward movement of the bellows bring about the non contact dispenses. The movement of the bellows is controlled by the ControlMate software.

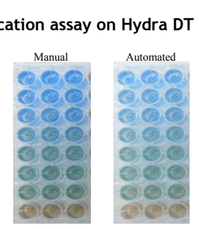
III. The Hydra instruments

The Thermo Scientific Matrix Hydra Automation Platforms: The Thermo Scientific Matrix Hydra platforms are compact benchtop instruments designed to perform sub-microliter volume liquid handling operations. They are equipped with 96 or 384-channel contact dispenser and manual/software controlled interfaces. The syringe-based 96-channel microdispenser functions by positive displacement mechanism. The Hydra II versions have syringe based nozzles for liquid dispension and the Hydra DT versions accommodate disposable tips namely disposable automation research tips (DARTs). The Hydra platform can also be equipped with a single channel non contact dispenser, the eDrop. In contrast to 96-channel dispenses, the single-channel noncontact liquid dispense is facilitated by an electro magnetic bellow (EMB) based system. Combined use of these contact and noncontact microdispensers enables users to perform both single and multiwell dispenses with an integrated protocol. Fine control of the performance of the Hydra platforms is achieved by optimization of parameters of aspirate and dispense via software. The platforms can have variable stage positions. Lateral movements of the stage and vertical movements of the single-channel microdispenser are programmed into the ControlMate software by select and drop features that allow easy protocol creation and editing. With all these variable capabilities, these instruments have been used to setup different biological experiments.

RESULTS

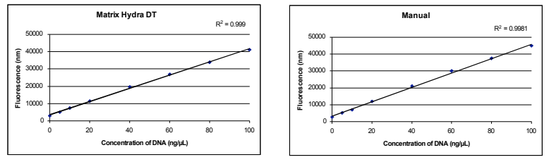
I. Automated 96-well format protein quantification assay on Hydra DT





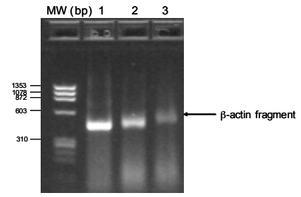
Automation of the protein assays: Automation of the Coomassie protein assay on the Hydra DT automation platform is shown. The experiment was performed in a 96-well format with 150 µl volumes. The graphs showing the standard curves and plate pictures generated by manual and automated methods are indicated. A significant correlation is observed between manual and automated methods.

II. Automated 96-well DNA quantification assay on Hydra DT



Automation of the DNA assays: Automation of a DNA standard curve using the Invitrogen Pico green kit by manual method and on the Hydra DT automation platform is shown. Standard curves are generated with 200 µl BR reagent and 10 µl of DNA stock solutions from the kit (Pico green QuantiT dsDNA kit, Invitrogen). A significant correlation is observed between manual and automated methods.

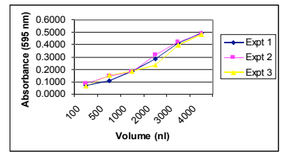
III. Automated PCR setup using the Hydra eDrop



A generic automated process for PCR has been setup with the Hydra eDrop using twenty microliter volumes. The setup is validated by subjecting the reaction to amplify β-actin gene using human genomic DNA as a template. The process has been found reliable and significantly reduced manual intervention.

DNA agarose gel showing amplification of partial β-actin gene from human genomic DNA. Samples 1, 2 and 3 indicate 246, 2.46 and 0.246 µg/ml of the template genomic DNA used in the reaction respectively. The PCR setup was automated using the Hydra eDrop and the PCR was performed using a thermocycler.

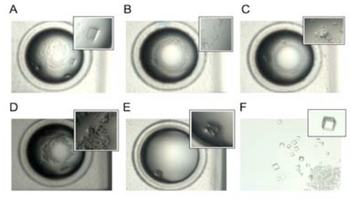
IV. Hydra eDrop for automated protein assays



An automated protein dye binding assay was performed on the Hydra eDrop. The experiment demonstrates the use of Hydra eDrop to perform 96-well and single channel dispenses to automate the protein assay. Miniaturizing the assay to sub-microliter volumes will be economical in higher throughput experimentation.

A series of 0.1 to 4 µl volumes of protein sample (1 mg/ml) was aspirated and dispensed using the single channel micro dispenser of Hydra eDrop. 200 µl of Bradford reagent was added to all the wells from a reservoir in two steps of 100 µl each time with the help of Hydra 96-channel micro dispenser. The plate was subjected to 2 min. shaking followed by 8 min. incubation at room temperature and the absorbance was recorded at 595 nm. An average of eight absorbance readings from three independent experiments is plotted.

V. Automated protein crystallization trials using the Hydra eDrop



Obtaining suitable crystals for X-ray crystallography is one of the rate-limiting steps in macromolecular crystallography. The Hydra eDrop has successfully been used for setting up automated crystallization trials with 400 nl volumes. This work was performed at Yale University in the laboratories of Dr. Titus Boggon. (Ref: Tal Murthy, Yongcheng Wang, Colin Reynolds and Titus Boggon. JALA, Aug 12, vol 4; - 213-218).

Crystallization of a rhomboid family intramembrane protease, GlpG, using the Thermo Scientific Matrix Hydra eDrop: Initial crystallization trials discovered five crystal hits using the Hampton Research SaltRX HT kit, (Hampton Research cat. number HR2-136). Panels A through E show the crystal hits. The crystallization screen conditions for each panel were, **A,** 1.5 M Ammonium Chloride, 0.1 M Bis-Tris Propane, pH 7.0, **B,** 3.5 M Ammonium Chloride, 0.1 M Bis-Tris Propane, pH 7.0, **C,** 3.5 M Ammonium Chloride, 0.1 M Tris, pH 8.5, **D,** 3.2 M Sodium Chloride, 0.1 M Bis-Tris Propane, pH 7.0, **E,** 4.0 M Sodium Nitrate, 0.1 M Bis-Tris Propane pH 7.0. Panel **F** shows crystals resulting from optimization of initial crystal conditions shown in Panel D. The optimized precipitant conditions, 3.0 M NaCl, 100 mM Bis-Tris propane, pH 7.0, resulted in diffraction-quality three-dimensional crystals with dimensions of approximately, 100 x 100 x 100 µm.

CONCLUSION

The Hydra platforms are designed with accuracy and precision to serve as bench top automation platforms. A user-friendly software interface offers drag and drop features for easy programming. The dispense mechanisms of Hydra and some generic biological applications are presented here. The automation can be readily tailored to perform several other user defined applications.

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