

Rapid and Miniaturized Robotic Crystallization Experiment using the Honeybee 963™ System

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Abstract:

An important consideration in protein crystallization screening is the limited availability of protein which makes the miniaturization of these experiments a necessity. Considerations such as dispense speed, protein concentration, protein/screening agent drop ratios, evaporation, etc. all need to be carefully analyzed when designing the crystallization experiment. The Honeybee 963™ enables rapid, precise and accurate dispensing of nanoliter (nl) volumes of screening and protein solutions. Strategies for experimental design will be discussed within the framework of the dispensing technologies that are utilized in the Honeybee 963. The ability to dispense up to three different proteins and/or protein concentrations coupled with the 96 tip dispensing head for screening solutions ensures evaporation is kept to a minimum and that protein usage is conserved.

Introduction:

The Honeybee 963 for automated sitting drop crystallization studies enables rapid, precise and accurate dispensing of nano-liter (nl) volumes of screening and protein solutions. Rapid dispensing to both reservoirs and shelves minimizes evaporation while precise and accurate dispensing allows for miniaturization and conserves precious protein.

The Honeybee 963 uses intuitive, menu-driven software which includes a plate library for simple switching between the many available plate types.

The system has a 96-tip dispense head that enables rapid transfer of all screening solutions from the source plate to the crystallization plate reservoirs and top shelves after a single aspiration step.

There are three independently controlled synQUAD™ channels for protein addition (protein tips). These tips enable rapid non-contact dispensing of up to three different protein solutions per run.

With these capabilities, the Honeybee 963 gives researchers the opportunity to consider a greater set of important parameters such as the drop size, sample concentrations, and drop ratios in their experimental design (Rupp, 2004).

System Capabilities:

System flexibility enables a greater range of parameters, besides chemical parameters, such as drop size, sample concentrations, and drop ratios, to be performed (Dunlop and Hazes, 2003; Carter, 1999). Experimental set up such as the following are possible:

- 1. Sample & Concentration Studies:** Three different proteins may be screened in a single experiment or alternatively, three different concentrations of the same protein may be screened to help find the optimal crystallization concentration.
- 2. Drop Size Studies:** Up to three different screening solution volumes may be dispensed onto the three top shelves of a crystallization plate. Alternatively, three different protein volumes may be dispensed onto the three top shelves in a single experiment (Figure 4) to ascertain correlations between drop size and probability of crystal formation.
- 3. Screening Solution & Protein Ratio Studies:** Different ratios of screening solutions and protein sample to help elucidate the optimal conditions for crystal formation of proteins that are difficult to crystallize. Up to three different volumes of protein may be dispensed by the three individually controlled protein tips.



Figure 1: The Honeybee 963 system includes a 96-tip dispense head, three independently controlled protein tips, and intuitive, menu-driven software.

synQUAD™ Technology

synQUAD dispense technology utilizes a micro-stepper motor controlled syringe coupled with a high speed solenoid valve to allow the accurate and precise non-contact dispensing of nanoliter volume drops. The syringe provides a constant pressure that exerts equal force on all drops ejected from the nozzle. Constant pressure combined with consistent

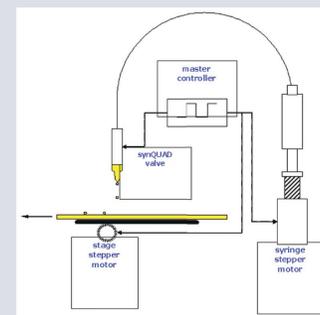
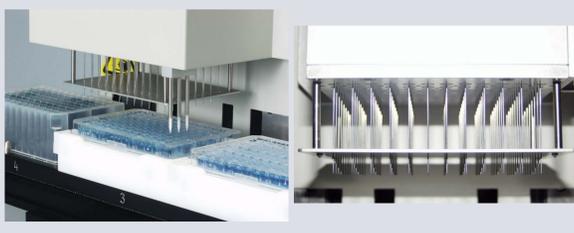


Figure 2: Schematic representation of synQUAD Technology-Protein Tips.

96-Tip Dispense Head Technology

Ninety-six stainless steel tips are attached directly to high precision syringes to provide simple, reliable, accurate and precise dispensing operations. Aspiration speed may be varied to accommodate different reagent viscosities, e.g. 30% 8K PEG. These syringes operate from 100 nl to 100 microliter (μl), allowing for the dispense of screening solutions into the main reservoirs and/or top shelves after a single aspiration step. If required, the washable, semi-flexible tips can be touched against the bottom of target wells to ensure even the smallest volumes are effectively dispensed. Spring loaded plate holders ensure the tips are never bent.

Figure 3: Side and front views of the 96-tip dispense head. The three protein tips are also visible in the side view.



Results:

Photo of sample drops on a Crystallization plate dispensed with the Honeybee 963 System.



Figure 4: Enlarged image of a single well on a Greiner 963 Crystallization plate with three drops of 10% Tartrazine dye solution. Drop volumes are 300 nl, 200 nl and 100 nl respectively.

The entire crystallization plate is filled with screening solutions and protein samples in less than two minutes thus minimizing evaporation while maintaining a total CV of less than 10%.



Figure 5: Actual photo of a Greiner 963 Crystallization plate with each main reservoir containing 80 μl of blue dye solution and three different drop volumes of 10% Tartrazine dye solution on the three top shelves. The drop volume in each top shelf is 300 nl, 200 nl and 100 nl respectively. Drops were dispensed with the Honeybee 963 System.

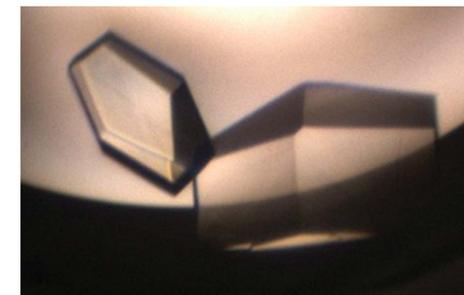


Figure 6: Image of crystals from experiment setup with a Honeybee system.

References:

- Carter, C.W.J.** (1999) Crystallization of Nucleic Acids and Proteins, Oxford University Press, Inc, New York, NY.
- Dunlop, K.V. and Hazes, B.** (2003) Acta Crystallogr, D59, 1797-1800.
- Hazes, B. and Price, L.** (2005) Acta Crystallogr, D61, 1165-1171.
- Rupp, B.** (2004) Crystallization Strategies for Structural Genomics, International University Line.

Summary:

The Honeybee 963 gives researchers the necessary tool to dispense drops rapidly, in order to prevent evaporation, and to dispense nanoliter volume drops, in order to conserve protein. The system is dedicated for protein crystallization and utilizes fast, accurate and proven liquid handling technology without the requirement to purchase disposable tips. This compact system has been designed to fit on a standard bench top. The Software has a graphical user interface that is both intuitive and menu driven enabling straight forward programming of any sitting drop crystallization studies. Consequently, the Honeybee 963 is very simple to use and requires minimal maintenance.

Honeybee 963 Software

The Honeybee 963 Software was designed with the laboratory user in mind. All software options are intuitive and menu driven thus do not require users to have any prior knowledge of computer programming.

The main Graphical User Interface (GUI) shows the types of lab-ware chosen (Source plate, Crystallization plates, and Wash station), operation button for each station, (Aspirate, Dispense, and Wash operations) and the current protocol (Figure 7a).

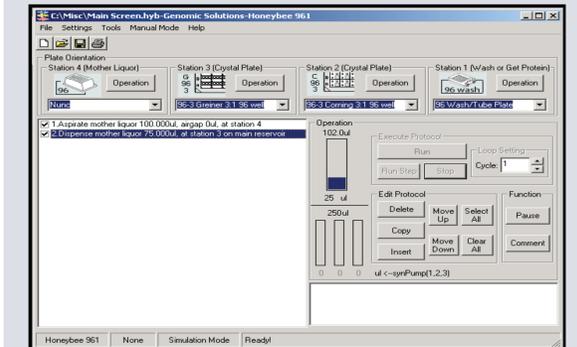
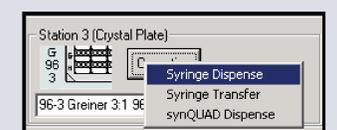


Figure 7a. Main Graphical User Interface Window.

When the operation button for the Crystal Plate stations is selected the menu options available for these stations is displayed. Both crystal plate positions may be used to set up two plates in a single protocol. The options are: 1.) dispensing of the screening solutions aspirated from the source plate; 2.) transferring of screening solutions from the main reservoir in the crystal plate to the top shelves of the same crystal plate; 3.) dispensing of protein samples onto the top shelves of the crystallization plates using the Protein tips (Figure 7b). A protocol is written as a user selects and customizes operation options in the desired sequence.

Figure 7b. Operations selection menu.



The Protein tips dispense window allows the custom selection of dispense drop volumes, top shelf positions to be dispensed onto, and dispense speed during the operation (Figure 7c). The protein dispense operation may be for the entire plate or for any specific row(s) on a plate. Furthermore, the protein drops may be dispensed directly on top of the screening solution drops or dispensed partially off center of the screening solution drops to delay the mixing of protein and screening droplets.

Figure 7c. Protein Tips Dispense Window.

