

APPLICATION NOTE: Four Corner Grid Screen Optimization with The ARI Scorpion

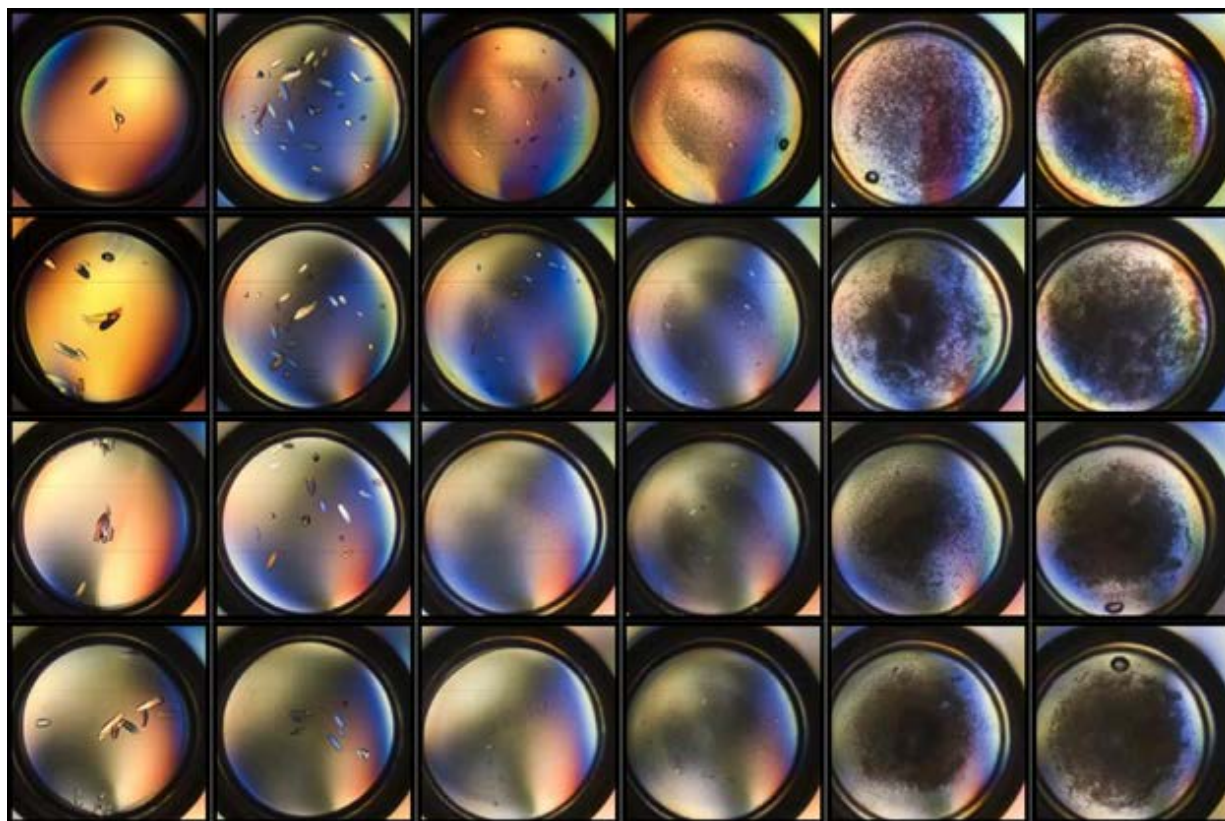
One continuous operation. Mixing of complex mixtures from stock reagents, dispensing four corners grid conditions, and full set up of experimental trays with protein crystallization grid experiment.

Background:

Great interest has recently been shown in the field of protein crystallization to develop new and readily available approaches for method design and screen building grid optimization experiments. The versatility of the new Scorpion robot from ARI can meet those demands, offering a superior choice to achieve the experimental goals of protein crystallization scientists. It is an efficient, cost-effective workstation purpose built to employ the positive attributes of an ultra-fast single channel pipetting machine.

For example “Automatic implementation of precise grid screens: the four corners method”, Hennessey et al., Acta Cryst. (2009) D65 1001- 1003, is an adaptation of standard grid building approaches, simple in principle, but made all the more robust by deploying a modern day fluid handling robotic system to a basic fluid handling experiment. The grid can be developed in the experimental tray or in larger volumes in remote tubes.

Here, The Scorpion performs all of the operations necessary to build optimization grids experiments. The instrument assembles complex mixtures from stock solutions in a large 15 ml tube format, then reads .csv files designed to mix a four corners matrix grid into the reservoir compartments of an INTELLI-PLATE® 24-4 way tray, and lastly can set up the experimental drop platform at routine small volume additions; thus completing all the necessary steps of a grid optimization experiment in a single session.

Corner 1Corner 2Corner 3Corner 4

1.5 ul Thermolysin at 25 mgs/ml + 1.5 ul reservoir condition incubated at room temperature. Four corners method builds 5 mls A1, A6, D1, and D6 as remote mixtures with sufficient volume to accommodate (2x) INTELLI-PLATE® 24-4 Way plates with a 400 ul reservoir for each experiment. Images captured at + 2 days with CrysCam scanner show the effects of increasing ammonium sulfate concentration from 1 to 2 molar along the X axis, and a pH shift with Tris buffer from pH 8.0 to pH 9.0 down the Y axis. The system proves to have a narrow crystallization outcome under these conditions.

Test case I Thermolysin screen build:

We have successfully performed the assembly of grids deploying the four corners screen method as an example of the machines capability.

We used Thermolysin because it is readily crystallized according to the protocol at Hampton Research (http://hamptonresearch.com/documents/product/hr000655_7-098_user_guide.coa_-_website_version.pdf). The condition involved incremental shift in percentage of ammonium sulfate from 1 to 2 molar along the X axis and discrete pH shifts from pH 8.0 to pH 9.0 along the Y axis. A four corners grid mixing approach set up the experimental ARI INTELLI-PLATE® 24-4 way plate reservoirs.

The Scorpion sets up the four corners screen slightly differently than would be done by hand. We chose to reduce the amount of steps and tips that are being used during the construction of the grid. The four corners screen is implemented in an Excel spreadsheet that has the .csv exported and is then capable of being read in by the Scorpion's software.

Thermolysin crystallization standard condition:

1.5 M Ammonium sulfate (AS), 12% Glycerol, 0.1 M Tris pH 8.5

The following reagents were mixed at large volume in 4 remote 15 ml reagent tubes and then the grid is built in the reservoirs of the experimental plate:

Corner 1: 1 M AS, 12% Glycerol, 0.1 M Tris pH 8.0

Corner 2: 2 M AS, 12% Glycerol, 0.1 M Tris pH 8.0

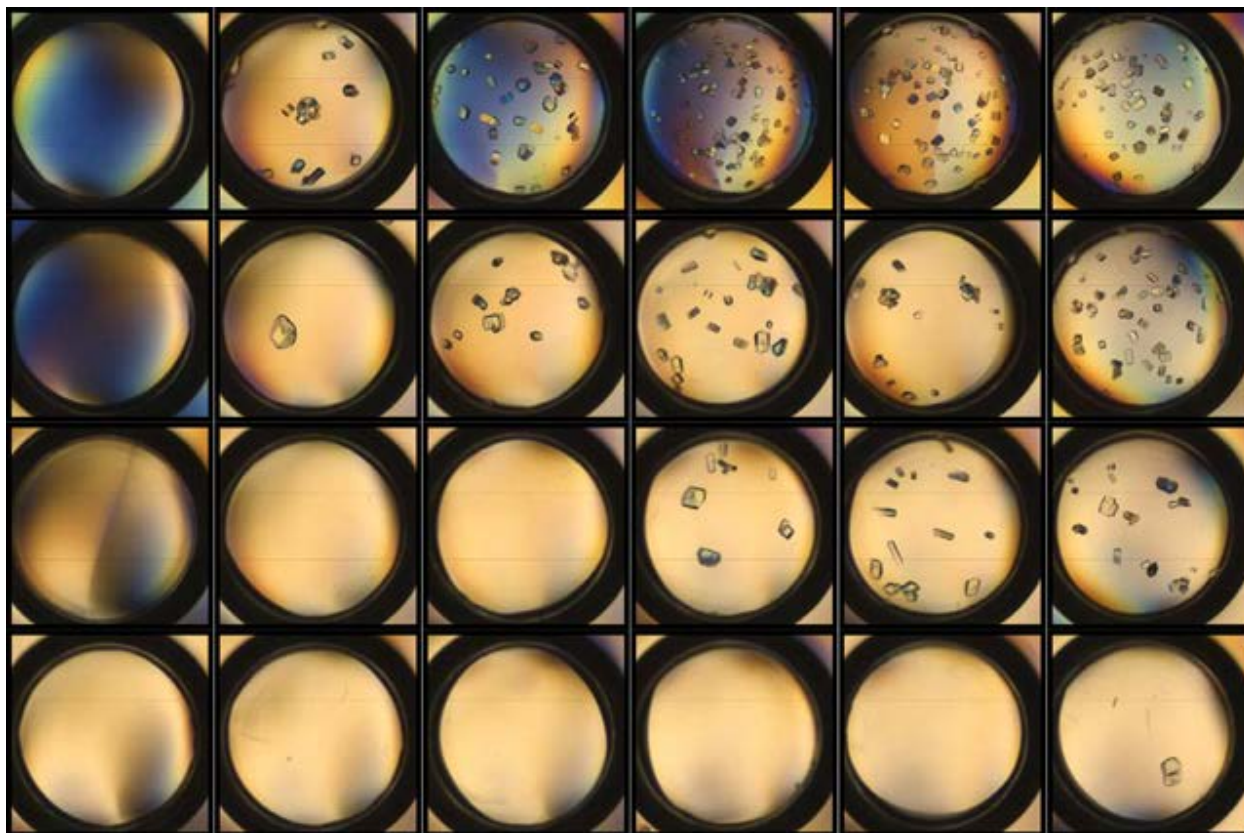
Corner 3: 1 M AS, 12% Glycerol, 0.1 M Tris pH 9.0

Corner 4: 2 M AS, 12% Glycerol, 0.1M Tris pH 9.0

Test case II Lysozyme Four Corners Grid Screen build:

We also used Lysozyme because it is readily crystallized according to the protocol at Hampton Research

(http://hamptonresearch.com/documents/product/hr002676_new_user_guide_6.12.12.pdf). This condition is one of the many listed standard conditions for Lysozyme to crystallize in. A four corners grid mixing approach again set up the experiment in the ARI INTELLI-PLATE® 24-4 way plate.

Corner 1**Corner 2****Corner 3****Corner 4**