

**W0441**

**Rapid Preparation of Custom Grid Screens for Crystal Growth Optimization Using Standardized Pipetting Maps.** Anne B. Senger, Timothy C. Mueser, Dept. of Chemistry, The University of Toledo, Toledo, OH 43606.

High throughput crystallization involves the use of sparse matrix crystal screens, high density format crystallization trays, and crystallization robots. Screening methods define initial starting conditions but these conditions typically need to be optimized before diffraction experiments can be conducted. The optimization of crystallization hits requires a series of grid searches, commonly referred to as expansion trays, which investigate the importance of each chemical component. A standard optimization strategy utilizes coarse concentration gradients to vary a single chemical parameter around the initial crystallization conditions. The best conditions are then setup with a shallow concentration gradient to prepare the diffraction quality crystals. Expansion trays are typically prepared by pipetting stock solutions into individual wells, requiring numerous volume calculations and multiple pipetting steps. This process is complicated by pipetting errors due to differences in viscosity, small volumes with large dilutions, evaporation and poor mixing.

Here we present a method for the rapid preparation of custom grid screen crystallization trays using standardized pipetting maps. To simplify the preparation of expansion and production trays, we prepare only the initial and final solutions that define the endpoints of the gradient. We then pour the tray using a standardized pipetting map. Step gradients can be prepared rapidly by adding decreasing amounts of the initial (A) solution to consecutive wells followed by adding the final (B) solution in increasing volumes. The same volumes are used regardless of the components and we can prepare both shallow and coarse A/B gradients rapidly and accurately. The pipetting errors are confined within the parameters defined by the A and B solutions. Programmable electronic pipettes can be used to prepare the well solutions and can also be adapted to robotic liquid handlers. This method alleviates many of the problems encountered when pipetting the wells individually.