METHODS AND APPARATUS FOR PERFORMING ARRAY MICROCRYSTALLIZATIONS

BACKGROUND OF THE INVENTION



Field of the Invention

The present invention relates to methods and apparatuses for crystallizing molecules and, more particularly, to methods and apparatuses for automating the crystallization of molecules, particularly macromolecules such as proteins.

Description of Related Art

Fast progress in the area of genomics has provided explosively growing databases of information on genes of human and other organisms by mapping, sequencing and analyzing their genomes. Many genes that may be critical for identifying people predisposed to certain diseases such as cancer have been discovered and their biological functions have been assessed *in vitro* and/or *in vivo*. Recently, a new area of genomics, functional genomics, has been developed, which involves a genome wide analysis of gene function by using information and reagents from the genomic analysis and expressing the genes in various organisms such as yeast. Functional genomics has generated important information regarding the expression pattern of genes by using high throughput screening techniques such as DNA oligonucleotide chips for specific genes or high density microarrays. An understanding of the network of interactions between a protein expressed by a target gene and other macromolecules in the cell is also being expanded at an unprecedented rate by using efficient screening methods such as the yeast hybrid systems.

One of the ultimate goals of these genome projects is the development of efficacious therapeutics against proteins expressed by disease genes. Among various methods of drug discovery and development, structure-based drug development has become one of the most important approaches, thanks to



rapidly advancing computation techniques. It is well recognized that understanding of the detailed three-dimensional structure of a protein not only assists in rational drug design and development in the laboratory but also provides a well-defined target in high throughput drug screening by using computer-aided docking analysis.

Solving high resolution structures of protein in a high throughput fashion presents a major bottleneck in such a chain of genomics and drug development. High resolution structures of proteins are solved by X-ray crystallography, and more recently by using multi-dimensional NMR spectroscopy on high-field NMR machines for smaller proteins or peptides.

Various methods for X-ray crystallography have been developed, including the free interface diffusion method (Salemme, F. R. (1972) Arch. Biochem. Biophys. 151:533-539), vapor diffusion in the hanging or sitting drop method (McPherson, A. (1982) Preparation and Analysis of Protein Crystals, John Wiley and Son, New York, pp 82-127), and liquid dialysis (Bailey, K. (1940) Nature 145:934-935).

Presently, the hanging drop method is the most commonly used method for growing macromolecular crystals from solution, especially for protein crystals. Generally, a droplet containing a protein solution is spotted on a cover slip and suspended in a sealed chamber which contains a reservoir with a higher concentration of precipitating agent. Over time, the solution in the droplet equilibrates with the reservoir by diffusing water vapor from the droplet, thereby slowly increasing the concentration of the protein and precipitating agent within the droplet, which in turn results in precipitation or crystallization of the protein.

The process of growing crystals with high diffraction quality is time-consuming and involves trial-and-error experimentations on multiple solution variables such as pH, temperature, ionic strength, and specific concentrations of salts, organic additives, and detergents. In addition, the amount of highly purified protein is usually limited, multi-dimensional trials on these solution conditions is unrealistic, labor-intensive and costly.

A few automated crystallization systems have been developed based on the hanging drop methods, for example Cox, M.J. and Weber, P. C. (1987) J. Appl. Cryst. 20:366; and Ward, K. B. et al. (1988) J. Crystal Growth 90:325-339. A need exists for improved automated crystallization systems for proteins and other macromolecules.

SUMMARY OF THE INVENTION

The present invention relates to a method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule. The molecule may be a molecule for which an x-ray crystal structure is needed. Determining high-resolution structures of molecules by a high-throughput method such as the one of the present invention can be used to accelerate drug development. The molecule to be crystalized may also be a molecule for which a crystalline form of the molecule is needed. For example, it may be desirable to create a crystalline form of a molecule or to identify new crystalline forms of a molecule. In some instances, particular crystalline forms of a molecule may have more bioactive, dissolve faster, decompose less readily, and/or be easier to purify,

The molecule is preferably a macromolecule such as a protein but may also be other types of macromolecules. The molecule preferably has a molecular weight of at least 500 Daltons, more preferably at least 1000 Daltons, although smaller molecular weight molecules may also be crystallized.

In one embodiment, the method comprises: forming an array of microcrystallizations, each microcrystallization including a drop containing a molecule to be crystallized and a mother liquor solution whose composition varies within the array, the drop having a volume of less than 1 μL; storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and detecting molecule crystal formation in the drops.

In one variation, the method comprises: forming an array of microcrystallizations, each microcrystallization comprising a well including a mother liquor solution whose composition varies within the array, and drop region including a drop containing the molecule to be crystallized, the drop having a volume of less than 1 μL; storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and detecting molecule crystal formation in the drops.

In another variation, the method comprises: forming an array of microcrystallizations, each microcrystallization comprising a well including a mother liquor solution whose composition varies within the array, and a coverslip including a drop containing the molecule to be crystallized, the drop having a volume of less than 1 μL; storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and detecting molecule crystal formation in the drops.

In yet another variation, the method comprises: forming an array of microcrystallizations, each microcrystallization comprising a well including a mother liquor solution whose composition varies within the array, and sitting drop region including a drop containing the molecule to be crystallized, the drop having a volume of less than 1 μL; storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and detecting molecule crystal formation in the drops.

According to any of the above methods, the volume of the drop containing the molecule to be crystallized is less than about 1 μL, preferably less than about 750 nL, more preferably less than about 500 nL, and most preferably less than about 250 nL. In one variation, the drop volume is between 1 nL and 1000 nL, preferably between 1 nL - 750 nL, more preferably between 1 nL - 500 nL, more preferably between 1 nL - 250 nL, and most preferably between 10 nL - 250 nL.

The present invention also relates to plates for performing array microcrystallizations to determine suitable crystallization conditions for a molecule. According to one embodiment, the plate comprises an array of at

least 36 wells for holding a mother liquor solution, each well having a reservoir volume of less than about 500 μL, preferably less than about 400 μL, more preferably less than about 300 μL and optionally less than about 250 μL. Ranges of well volumes that may be used include, but are not limited to 25 μL - 500 μL and 25 μL - 300 μL. In one variation, the plate is designed to perform a hanging drop crystallization. In another variation, the plate is designed to perform a sitting drop crystallization and includes a mother liquor well as well as an adjacent sitting drop well.

The present invention also relates to various apparatuses for forming submicroliter drops used in an array microcrystallization to determine suitable crystallization conditions for a molecule.

In one embodiment, the apparatus comprises:

a platform on which a multiwell plate is positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to drop regions on the multiwell plate within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the drop regions within a volume range of less than about 25 nL.

In another embodiment the apparatus is designed for preparing submicroliter hanging drops on cover slips used in an array microcrystallization, the apparatus comprising:

a platform on which a multiwell plate is positionable;

a cover slip station on which a plurality of coverslips are positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to the plurality of coverslips within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the plurality of coverslips within a volume range of less than about 25 nL.

In yet another embodiment the apparatus is designed for preparing submicroliter sitting drops used in an array microcrystallization, the apparatus comprising:

a platform on which a multiwell plate is positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to drop regions on the multiwell plate within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the drop regions within a volume range of less than about 25 nL.

According to any of the above embodiments, the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL.

Also according to any of the above embodiments, a sensor may be included in the apparatus for preparing submicroliter drops which is detects whether mother liquor drops and/or molecule drops have been formed.

The mother liquor drop station and the molecule drop station are preferably each independently capable of delivering submicroliter volumes to at least four coverslips at a time, more preferably at least eight coverslips at a time.

The present invention also relates to methods for forming submicroliter drops for use in an array microcrystallization to determine suitable crystallization conditions for a molecule. According to one embodiment, the method includes: removing mother liquor from a plurality of wells of a multiwell plate; delivering submicroliter volumes of the mother liquor to drop regions of the multiwell plate within a volume range of less than about 25 nL; and delivering submicroliter volumes of a solution containing a molecule to be

crystallized to the drop regions of the multiwell plate within a volume range of less than about 25 nL; wherein a total volume of the submicroliter volumes delivered to each drop region is less than 1 μL.

According to another embodiment, the method is for a hanging drop crystallization and includes: taking a plurality of coverslips; removing mother liquor from a plurality of wells of a multiwell plate; delivering submicroliter volumes of the mother liquor to the plurality of coverslips within a volume range of less than about 25 nL; and delivering submicroliter volumes of a solution containing a molecule to be crystallized to the plurality of coverslips within a volume range of less than about 25 nL; wherein a total volume of the submicroliter volumes delivered to each coverslip is less than 1 μL.

According to another embodiment, the method is for a sitting drop crystallization and includes: removing mother liquor from a plurality of wells of a multiwell plate; delivering submicroliter volumes of the mother liquor to sitting drop regions of the multiwell plate within a volume range of less than about 25 nL; and delivering submicroliter volumes of a solution containing a molecule to be crystallized to the sitting drop regions within a volume range of less than about 25 nL; wherein a total volume of the submicroliter volumes delivered to each sitting drop region is less than 1 μL.

According to any of the above method embodiments, the total volume of the submicroliter volumes delivered is preferably less than about 750 nL, more preferably less than about 500 nL, and most preferably less than about 250 nL. It is noted that the drop volumes may be as small as 380 pL. The volumes delivered preferably range between 1 nL - 750 nL, more preferably between 1 nL - 500 nL, more preferably between 1 nL - 250 nL, and most preferably between 10 nL - 250 nL.

According to any of the above apparatus and method embodiments, the precision of the volumes delivered is preferably less than about 25 nL, more preferably less than 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL. The precision of the volumes delivered may also be between

380 pL and 25 nL, more preferably between 380pL and 20 nL, more preferably between 380pL and 15 nL, and most preferably between 380 pL and 10 nL.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a block diagram of a crystallization system according to the present invention.

Figure 2 illustrates a method for using the crystallization system of Figure 1 to perform a protein crystallization trial.

Figure 3A illustrates a top view of a multiwell plate which may be used to perform a hanging drop array microcrystallization.

Figure 3B is a sideview of the multiwell plate illustrated in Figure 3A.

Figure 3C illustrates a top view of a multiwell plate which may be used to perform a sitting drop array microcrystallization.

Figure 3D is a sideview of the multiwell plate illustrated in Figure 3C.

Figure 3E is a cross section of a multiwell plate with a plate cover.

Figures 4A-4J illustrate the various stations which can be included in a mother liquor delivery system.

Figure 4A is a sideview of a plate loading station looking across a plate track positioned adjacent to the plate loading station.

Figure 4B is a sideview of a plate loading station looking along the longitudinal axis of the plate track.

Figure 4C is a sideview of a bar code reading station for reading a bar code included on a multiwell plate.

Figure 4D is a sideview of a sealing medium station for applying a sealing medium to an upper edge of wells defined in a multiwell plate.

Figure 4E is a sideview of a plate cover removal station for removing a plate cover from a multiwell plate.

Figure 4F is a topview of a mother liquor delivery station.

Figure 4G is a topview of a delivery block for delivery of a mother liquor into multiwell plates.

Figure 4H is a cross section of a delivery block for delivery of a mother liquor into a multiwell plates.

Figure 4I is a sideview of a mother liquor source storage bank.

Figure 4J is a sideview of a syringe pump for delivering a mother liquor from a mother liquor source to a fluid injector.

Figures 5A-5E illustrate the various stations which can be included in a drop formation system.

Figure 5A is a top view of a drop formation station.

Figure 5B is a sideview of the drop formation station.

Figure 5C is a sideview of a pipette holder.

Figure 5D is a sideview of a well cover holder.

Figure 5E is a sideview of a well cover magazine for storing well covers to be positioned over the wells in a multiwell plate.

Figures 6A-6I illustrate operation of the drop formation station.

Figure 6A illustrates a drop formation station in the rest position.

Figure 6B illustrate the drop formation station with a multiwell plate has been moved into position for drop formation and a pipette holder is moved into position over the wash basin.

Figure 6C illustrates the pipette holder moved into position over a column of wells in the multiwell plate.

Figure 6D illustrates the pipette holder moved into position over the well cover holder.

Figure 6E illustrates the pipette holder returned to its rest position and a protein delivery pipette moved into position over a well cover.

Figure 6F illustrates the protein delivery pipette moved into its rest position and the cover holder inverted and moved into position over the column of wells on the multiwell plate.

Figure 6G illustrates hanging drops suspended from well covers over the wells of a plate.

Figure 6H illustrates the cover holder moved into position over a well cover storage component.

Figure 6I illustrates the cover holder returned to its rest position.

Figures 7A-7G illustrate operation of the drop formation station to form sitting drops.

Figure 7A illustrates a drop formation station in the rest position.

Figure 7B illustrate the drop formation station with a multiwell plate adapted to perform a sitting drop array microcrystallization in position for drop formation and a pipette holder moved into position over the wash basin.

Figure 7C illustrates the pipette holder moved into position over a column of wells in the multiwell plate.

Figure 7D illustrates pipettes in the pipette holder aligned with the well regions of wells in a column of the plate.

Figure 7E illustrates pipettes in the pipette holder aligned with the sitting drop regions of wells in a column of the plate.

Figure 7F illustrates the protein delivery pipette moved into position over the sitting drop region of a well in the column of wells.

Figure 7G illustrates a sitting drop formed in the sitting drop region of a well.

Figure 8A is a sideview of a plate track with a pin extending above the plate track from a pin carriage positioned beneath the plate track.

Figure 8B is a sideview of a plate track with a pin of Figure 8A withdrawn beneath the plate track.

Figure 8C is a sideview of a transport assembly having a plurality of pin carriages.

Figure 9 illustrates the composition of 480 mother liquor solutions for a preferred coarse screen.

Figures 10A-10D illustrate formation of crystals in different drops sized from 40 nL to 1000 nL.

Figure 11 lists the mother liquor compositions for 24 mother liquors used in the fine screen stage of a crystallization trial.

DETAILED DESCRIPTION

The present invention relates to a method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule. The molecule is preferably a macromolecule, such as a protein. Other types of molecules and macromolecules may also be crystallized according to the present invention. The molecule preferably has a molecular weight of at least 500 Daltons, more preferably at least 1000 Daltons, although it is noted that the invention can be applied to molecules with lower molecular weights.

The method involves forming an array of microcrystallizations where each microcrystallization includes a drop containing a molecule to be crystallized and a mother liquor solution whose composition varies within the array, the drop having a volume of less than 1 μL. The array of microcrystallizations are stored under conditions suitable for molecule crystals to form in the drops in the array. Molecule crystal formation is then detected in the drops. As will be described herein, this method can be employed in any crystallization method involving drops, including, but not limited to hanging drop crystallization methods and sitting drop crystallization methods. Example sitting drop crystallization methods are provided in U.S. Pat. No. 5,096, 676 (McPherson et al.) and U. S. Pat No. 5,419,278 (Carter).

An important feature of the present invention is the utilization of small drop volumes. For example, the volume of the drop containing the molecule to be crystallized is less than about 1 μL, preferably less than about 750 nL, more preferably less than about 500 nL, and most preferably less than about 250 nL. In one variation, the drop volume is between 1 nL and 1000 nL, preferably between 1 nL - 750 nL, more preferably between 1 nL - 500 nL, more preferably between 1 nL - 250 nL, and most preferably between 10 nL - 250 nL.

Applicants believe that the rate of crystallization is dependent on the drop volume where crystals form faster when smaller drop volumes are used. As a result, crystals can be formed more rapidly by using the smaller drop

volumes used in the present invention. This significantly increases the throughput rate of the method for determining crystallization conditions.

Without being bound by theory, it is believed that smaller drops will equilibrate faster than larger drops and that this causes crystals to form more rapidly. The rate of equilibration is believed to be related to a relationship between the rate of drop evaporation and drop volume. Meanwhile, the rate of drop evaporation is dependent on drop surface area. The surface area of a drop does not decrease linearly with the drop's volume. As a result, a larger drop having twenty times the volume of a smaller drop (e.g., 1 μL vs. 50 nL) will have significantly less than twenty times the surface area of the smaller drop. By reducing drop volume, one is able to improve the relationship between the rate of drop evaporation (surface area dependent) and drop volume, thereby accelerating equilibration and crystal formation.

A further advantage of the present invention is that smaller drop volumes allow less molecule to be used to perform each crystallization trial. As a result, a greater number of crystallization trials can be performed using the same amount of molecule. This is of great significance when it is difficult to obtain the molecule to be crystallized and when a large number of crystallization trials are needed in order to successfully crystallize the molecule.

It is frequently difficult to produce and purify the molecule being crystallized. In the case of protein crystallization, it can require one to two weeks of lab work to produce and purify enough protein to perform 48 crystallization trials using drops greater than 1 μL in size. By reducing the drop volume and hence the amount of molecule used per crystallization trial, it becomes feasible to significantly increase the number of crystallization trials that can be performed. As a result, it becomes feasible to take a more combinatorial, shotgun approach to molecule crystallization trials since the pressure to conserve molecule usage is reduced. By contrast, prior to the present invention's utilization of sub microliter drop volumes, a need existed to minimize the number of trials that were performed at one time due to a shortage of available molecule.

By reducing the drop volume, the number of microcrystallizations that can be performed in the array is increased. The number of microcrystallizations in the array is typically greater than 48, preferably greater than 96, more preferably greater than 144, most preferably greater than 192. It is noted that the number of microcrystallizations in the array can also exceed 288 or 384. For example, an apparatus for preparing arrays which include 480 microcrystallizations is described herein.

Increasing the number of microcrystallizations that can be performed in the array also allows a greater number of different stock solutions to be used to form the mother liquor solutions used in the array. For example, forming the array of microcrystallizations can include using greater than 48 stock solutions to form the mother liquor solutions used in the array. Optionally, greater than 96, more preferably greater than 144, most preferably greater than 192 different stock solutions may be used. It is noted that the number of stock solutions can also exceed 288 or 384. For example, an apparatus described herein uses 480 different stock solutions.

Smaller volumes of mother liquor may also be used in the wells. The volume of mother liquor used in the wells is preferably less than about 500 μL, preferably less than about 400 μL, more preferably less than about 300 μL and optionally less than about 250 μL. Ranges of mother liquor volumes that may be used include, but are not limited to 25 μL - 500 μL and 25 μL - 300 μL. In this regard, forming the array of microcrystallizations may include forming the microcrystallizations in a plate including a plurality of wells each having a volume less than about 500 μL, preferably less than about 400 μL, more preferably less than about 300 μL.

The use of small volumes of mother liquor allows the wells in multiwell plates to be made smaller, thereby allowing more wells to be positioned on a multiwell plate per unit area. For example, the 48 well plates having a well volume less than about 500 μL has approximately the same footprint as 24 well plates typically used to perform protein crystallization. Further reduction of the mother liquor volumes may be employed in order to further reduce plate sizes.

By utilizing small drop volumes, a significantly greater number of crystallization trials can be performed using the same amount of molecule. As a result, it is feasible to perform a greater number of crystallization trials, which in turn allows the mother liquor solution to be more widely varied in its composition. This allows the mother liquor solution to be formed of 1, 2, 3, 4, 5, 6 or more components which are varied within the array.

Also according to this method, the array of microcrystallizations is formed of one or more multiwell plates. Each plate preferably has at least 24 wells, more preferably at least 36 wells, and most preferably at least 48 wells. By utilizing less mother liquor, smaller wells can be used which allows the same size plate to contain more wells.

Also according to this method, detecting crystal formation can include characterizing the crystal formed (needle, cube, etc.), the size of the crystal, and the quality of the crystal's structure. Characterization of the crystal can be performed manually, or by taking images of the drops and analyzing those images for the structure of crystals contained within those drops.

The method can also include identifying the compositions of those mother liquor solutions in which crystals were detected and performing additional crystallization trials where the formulation of the mother liquor solutions in which crystals were detected is further varied.

Figure 1 illustrates a crystallization system 10 for performing a crystallization trial. The crystallization system 10 can be divided into various stations 12 described below. During operation of the crystallization system 10, multiwell plates are positioned on a plate track 14. A transport assembly (not shown) moves the multiwell plates along the plate track 14 to the various stations 12.

The crystallization system 10 also includes a processing unit 16 in electrical communication with the various stations 12. Suitable processing units 16 for use with the crystallization system 10 include, but are not limited to, PCs and computer workstations. The processing unit 16 includes process control logic for controlling the operation of each station and the transport assembly.

An operator can use one or more user interfaces to interact with, disengage and/or to alter the process control logic. Suitable user interfaces include, but are not limited to computer monitors, keyboards, mouses, and trackballs.

During operation of the crystallization system 10, the transport assembly moves a multiwell plate past the stations 12 which each perform a particular function. For instance, the crystallization system 10 includes a plate loading station 18 where multiwell plates are sequentially loaded onto the plate track 14. The crystallization system 10 also includes a bar code reading station 20 where a bar code on the multiwell plates can be read. The crystallization system 10 further includes a sealing medium station 22. The sealing medium station 22 can be used to apply a sealing medium to the multiwell plates. Specifically, the sealing medium can be applied to the upper edge of liquid receiving wells defined in each multiwell plate. The sealing medium serves to form a seal between the upper edges of each well and a well cover, commonly referred to as a coverslip, which is positioned over each well at a later station of the crystallization system 10. The crystallization system 10 also includes a plate cover removal station 24 where plate covers 44 are delivered to or removed from the multiwell plates.

The crystallization system 10 also includes a mother liquor delivery station 26 where mother liquors are delivered into the wells defined in the multiwell plates. Different mother liquors can be delivered into different wells or the same mother liquor can be delivered into more than one well. Further, mother liquor can be delivered into a portion of the wells on a single multiwell plate so the remaining wells are empty.

The crystallization system 10 also includes a drop formation station 28 where mother liquors from the various wells are used to form one or more drops on a plurality of coverslips that will be placed over the wells. The drop formation station 28 also adds a solution containing the molecule to be crystallized to the coverslips. Once drops containing mother liquor and the molecule to be crystallized are formed on the coverslips, the coverslips are

positioned over each well such that the one or more drops hang from the coverslip into the well. These drops are called hanging drops.

It is noted that the drop formation station can be readily adapted to form sitting drops in a sitting drop regions of a multiwell plate by delivering mother liquors from the various wells and the solution containing the molecule to be crystallized to the sitting drop regions.

The crystallization system 10 also includes a plate cover delivery station 29 where plate covers 44 can be positioned on each multiwell plate. The multiwell plate can then by transported to a plate unloading station 30 where the multiwell plates can be removed from the plate track 14 and stored.

Although the crystallization system 10 illustrated in Figure 1 has the various stations 12 positioned around a single plate track 14, it is noted that the various stations 12 can be divided into one or more sub-systems, each optionally having its own track. It is further noted that many of the stations 12 may optionally be included or excluded from the crystallization system 10. Further, the stations 12 can be positioned in a sequence other than the sequence illustrated in Figure 1. For instance, the plate cover removal station 24 can be positioned before the bar code reading station 20. Additionally, several of the described functions can be carried out at a single station. For instance, a plate cover delivery station 29 can be formed integrally with the drop formation station 28 or the plate unloading station 30.

The above stations 12 can be included in a single system or can each be included in different independent sub-systems. For instance, the tray loading station, bar code reading station 20, sealing medium station 22 and mother liquor delivery station 26 can be included in a single mother liquor delivery system 31 while the drop formation station 28 and the plate unloading station 30 can be included in an independent drop formation system 32. Additionally, the functions associated with a particular station need not be carried out during operation of the crystallization system 10. For instance, the mother liquors can be delivered into the wells of a multiwell plate by an external apparatus before the multiwell plate enters the crystallization system 10. In such an instance,

when a multiwell plate already containing mother liquor reaches the mother liquor delivery station 26, the mother liquor delivery station 26 can be operated to not deliver mother liquors into the wells.

After a microcrystallization array has been prepared by processing a multiwell plate through a crystallization system 10 such as the one illustrated in Figure 1, drops in the microcrystallization array can be observed for the formation of crystals. When crystals are formed in a drop of a particular well, the quality of crystals within the drop can be graded for various characteristics such as shape, size or time for crystal formation. When the mother liquors used in each well are different, the crystal grades can be compared to determine which mother liquor was associated with the most desirable crystals. Accordingly, each well serves as a different crystallization experiment which produces results which can be compared with the results of other crystallization experiments.

A crystallization trial includes comparing the results of several crystallization experiments in order to optimize the composition of the mother liquor used for crystallization of a particular molecule. Figure 2 illustrates a technique for performing a crystallization trial. A coarse screen is performed at an initial stage of the trial. In Figure 2, the crystallization experiments associated with the coarse screen are illustrated as a plurality of boxes set out in three different arrays which are labeled CS_1, CS_2 and CS_3. In each array two variables of the mother liquor composition are incrementally varied as shown along the x and y axis associated with each array. For instance, in CS_1 several crystallization experiments are performed where the pH is varied from 2-8 in intervals of 2 and the % $(NH_4)_2SO_4$ is varied from 20-80 in intervals of 20.

The crystallization experiments in the coarse screen are analyzed to select one or more crystallization experiments which yield the best crystals or, if no crystals form, the best crystal-like precipitate. A coarse screen experiment selected as producing a promising crystal or crystal-like precipitate is illustrated as a black box in Figure 2. Fine screens are then performed for the crystallization experiments selected through the course screen.

A fine screen crystallization experiment is performed by designing a crystallization array based on the mother liquor composition used in a crystallization experiment selected through the course screen, indicated in Figure 2 as the array labeled FS_1. The compositions of the mother liquors used in the fine screen crystallization array are selected by making small variations in the composition of the mother liquor used in the selected experiment from the course screen. For example, if the mother liquors used in the course screen had a pH between 2-8 and the mother liquor in the selected crystallization had a pH of 4.0, the mother liquors used in the fine screen experiments might have a pH between 3.4 to 4.6. Further, by focusing the array around mother liquors having a pH of about 4, one can reduce the incremental change in the value in the fine screen FS_1. For instance, the incremental change in the pH during the coarse screen CS_2 shown in Figure 2 is 2.0 while the incremental change of the pH during the fine screen also shown in Figure 2 is 0.4.

Crystals formed in each crystallization experiment in the fine screen are analyzed in order to select the one or more crystallization experiments yielding the best crystals or crystal-like precipitate. A crystallization experiment selected during the fine screen experiment is illustrated in Figure 2 as a box having an X. If the crystals formed during the fine screen are of a sufficiently high quality, one might isolate the crystals formed in the experiment and perform x-ray diffraction on the isolated crystals to resolve the molecule's crystal structure. Alternatively, one might use the mother liquor used in the selected fine screen experiment in order to grow additional crystals. However, if the crystals formed during the fine screen are not of a sufficiently high quality, the mother liquor can be further optimized by taking the mother liquor used in the selected fine screen experiment as the starting point for an additional fine screen. Figure 2 illustrates a second array of fine screen crystallization experiments labeled FS_2. It is noted that this iterative process of selecting a fine screen experiment and performing a finer screen array based on a selected experiment can be repeated until a suitable mother liquor is identified for use in preparing crystals.

The microcrystallization methods and apparatuses of the present invention may be used to perform the course screen array experiments described in regard to Figure 2 in order to analyze a larger set of mother liquors than had previously been feasible with drop sizes larger than 1 microliter. It is noted that the fine screen array experiments may also be performed using the microcrystallization methods and apparatuses of the present invention or may be performed where drop sizes are larger than 1 microliter.

Figure 3A illustrates a top view of a multiwell plate 34 which may be used with the methods and apparatuses of the present invention to perform a hanging drop array microcrystallization. As illustrated, the multiwell plate 34 includes a support structure 36 defining wells 38 arranged in 6 columns and 8 rows. Although Figure 3A illustrates a multiwell plate 34 with a total of 48 wells 38, the multiwell plate 34 can include a different number of wells 38.

Figure 3B provides a sideview of the multiwell plate 34 illustrated in Figure 3A. Each well 38 includes an upper edge 40 extending above the support structure 36. The upper edge 40 is preferably wide enough that a layer of a sealing medium, such as grease, can be applied to the upper edge 40. The support structure 36 preferably has a geometry which allows multiwell plates 34 to be stacked on top of one another without one multiwell plate 34 interfering with the well contents of an adjacent multiwell plate 34.

Figure 3C illustrates a top view of a multiwell plate 34 which may be used with the methods and apparatuses of the present invention to perform a sitting drop array microcrystallization. As illustrated, the multiwell plate 34 includes a support structure 36 defining 48 wells 38 arranged in 6 columns and 8 rows. Although a multiwell plate 34 with 48 wells 38 is illustrated the multiwell plate 34 can include a different number of wells 38. A well region 41 is adjacent to a sitting drop region 42. Although the sitting drop region 42 is illustrated as being centrally positioned within the well 38, the sitting drop region 42 can be positioned to one side of the well 38.

Figure 3D provides a sideview of the multiwell plate 34 illustrated in Figure 3C. The sitting drop region 42 extends upward from the bottom of the

well 38. As illustrated by the cut-away, the sitting drop region 42 can include a recess where a sitting drop can be formed. Each well 38 includes an upper edge 40 extending above the support structure 36. The upper edge 40 is preferably wide enough that a layer of a sealing medium, such as grease, can be applied to the upper edge 40. The support structure 36 preferably has a geometry which allows multiwell plates 34 to be stacked on top of one another without one multiwell plate 34 interfering with the well 38 contents of an adjacent multiwell plate 34.

A plate cover 44 can be positioned over each multiwell plate 34 as illustrated in Figure 3E. The plate cover 44 can be designed so the cover rests on the upper edges 40 of the wells 38. As illustrated in Figure 3E an insert 46 can be positioned between the plate cover 44 and the multiwell plate 34 so the insert 46 rests on the upper edges 40 of the wells 38. The insert 46 can be removable from the plate cover 44 or can be permanently attached to the plate cover 44. The insert 46 can be formed from a flexible material so the insert 46 provides a seal between the insert 46 and the upper edges 40 of the wells 38 in order to reduce evaporation from the wells 38. Suitable materials for the insert 46 include, but are not limited to, soft rubbers and other gasket material.

As illustrated in Figure 3A, the multiwell plate 34 can include a bar code 48 formed on the support structure 36. The multiwell plate 34 can also include a surface 50 sized to receive a bar code sticker. Alternatively, a bar code can be formed on a plate cover 44 or the plate cover 44 can include a surface sized to receive a bar code sticker. When the multiwell plate 34 or plate cover 44 includes a surface for receiving a bar code sticker, the bar code sticker is preferably removable from the multiwell plate 34 so different bar codes can be fixed to a single multiwell plate 34. As will be discussed later, these bar codes can be used to identify the multiwell plate 34 and/or the contents of the multiwell plate 34 to the system control logic.

Figures 4A-4J illustrate embodiments of the various stations 12 of the mother liquor delivery system 31 illustrated in Figure 1. Figures 4A and 4B illustrate a plate loading station 18 for sequentially loading multiwell plates 34

onto a plate track 14. Figure 4A is a sideview of the plate loading station 18 looking across the plate track 14 and Figure 4B is a sideview of the plate loading station 18 looking down the longitudinal axis of the plate track 14. The plate loading station 18 includes a tower 52 positioned over the plate track 14 so the plate track 14 extends outward from the base of the tower 52. The tower 52 includes a chute 54 sized to receive a stack of multiwell plates 34 arranged one on top of another.

The plate loading station 18 also includes plate lowering mechanics (not shown) which can be engaged to lower a multiwell plate 34 at the bottom of the stack onto the plate track 14. The action of gravity moves a new multiwell plate 34 into the position of the multiwell plate 34 lowered onto the plate track 14. The clearance between the plate tower 52 and the plate track 14 is enough for the plate transport assembly to transport the multiwell plate 34 lowered onto the plate track 14 out from under the tower 52 as illustrated in Figure 4A. Once the multiwell plate 34 has been transported from beneath the tower 52, plate lowering mechanics can be re-engaged so a new multiwell plate 34 at the bottom of the chute 54 is also loaded onto the plate track 14. Because the plate loading station 18 can hold several multiwell plates 34 and sequentially position each multiwell plate 34 on the plate track 14, the mother liquor delivery system 31 can process many multiwell plates 34 without an operator manually positioning each multiwell plate 34 on the plate track 14.

The plate loading station 18 can be easily adapted into a plate 34 unloading station by operating the plate lowering mechanics in reverse. This reverse operation causes a multiwell plate 34 located beneath the tower 52 to be raised from the plate track 14 and added to the stack of multiwell plates 34 stored within the chute 54.

Figure 4C illustrates a bar code reading station 20. A bar code reader 56 is positioned alongside the plate track 14. The bar code reader 56 is directed toward the plate track 14 at an angle which permits the bar code reader 56 to read a bar code 48 on a multiwell plate 34 on the plate track 14. As described above, these bar codes can formed on the multiwell plate 34 or can be included

on a bar code sticker to be placed on the multiwell plates 34. The bar code reader 56 is monitored by the system control logic which associates each bar code with a particular multiwell plate 34 and/or with particular characteristics of a multiwell plate 34. Suitable characteristics include, but are not limited to, the number of wells 38 in the multiwell plate 34, the volume of the wells 38 in the multiwell plate 34, whether the multiwell plate 34 includes a plate cover 44, etc.

The characteristics of a multiwell plate 34 can be administratively entered in order to indicate information about the multiwell plate 34 to the system control logic. For instance, a user can enter characteristics such as identifying particular mother liquids to be delivered into particular wells 38 on a multiwell plate 34. Further, if an operator uses an external method to deliver mother liquids into the wells 38 of the multiwell plate 34, the user can indicate this to the system control logic. Because various mother liquids are already present in the wells 38 of the multiwell plate 34, the system control logic can override mother liquid delivery station in order to avoid delivering additional mother liquids to the wells 38 of the multiwell plate 34.

As a multiwell plate 34 moves through the mother liquor delivery system 31, the drop formation system 32 and/or through the crystallization system 10, the characteristics associated with the multiwell plate 34 can optionally be modified by the system control logic in order to reflect the changing status of the multiwell plate 34. For example, the system control logic can note when mother liquor has been added, or when drops have been formed.

Figure 4D illustrates a sealing medium station 22. The sealing medium station 22 includes a sealing member 60 suspended over the plate track 14 at a height which permits multiwell plates 34 to be moved under the sealing member 60. The sealing member 60 includes a sealing surface 62 with a plurality of sealing medium injectors 64 arranged so each sealing medium injector can be concurrently aligned with a well 38 in each multiwell plate 34. The sealing medium injectors 64 are in hydraulic communication with a sealing medium source 66. Accordingly, a sealing medium can be delivered from the sealing

medium source 66 to the portion of the sealing surface 62 adjacent to the sealing medium injectors 64.

The sealing member 60 can be coupled with actuators for moving the sealing member 60 relative to the wells 38 on a multiwell plate 34. The sealing member 60 can be moved vertically over a multiwell plate 34 as illustrated by the arrows labeled A. The sealing member 60 can also be translated laterally relative to a multiwell plate 34 as illustrated by the arrows labeled B. Suitable actuators include, but are not limited to, pneumatic pistons, hydraulic pistons and electrically driven motors.

In operation of the sealing medium station 22, the plate transport assembly transports a multiwell plate 34 into a position where the wells 38 in the multiwell plate 34 are positioned beneath the sealing medium injectors 64. The sealing member 60 is lowered until the sealing surface 62 is in contact with the upper edge 40 of the wells 38 on the multiwell plate 34. Because the wells 38 of the multiwell plate 34 are aligned with the sealing medium injectors 64 before the downward motion of the sealing member 60, the upper edge 40 of each well 38 encircles a sealing medium injector. Once, the sealing surface 62 is in contact with the upper edges 40 of the wells 38, the sealing member 60 is laterally translated. The lateral translation causes the sealing member 60 to follow a circular path along an edge of the well 38, although other paths may also be used depending on the geometry of the wells 38.. This lateral translation transfers the sealing medium delivered to the sealing surface 62 to the upper edge 40 of each well 38.

The amount of sealing medium transferred to the upper edge 40 of each well 38 depends on the amount of sealing medium present on the sealing surface 62 adjacent to the sealing medium injectors 64. The amount of sealing medium delivered to the upper edges 40 of the wells 38 should be sufficient to create a substantially airtight seal between a coverslip and the upper edge 40 of the well 38. Suitable sealing mediums include, but are not limited to, grease and vasaline.

It is noted in regard to the sealing medium station 22 that the station may be readily adapted for use with hanging drop array crystallizations as well as with sitting drop array crystallizations. In regard to each type of drop array crystallization, an airtight seal should be formed between the edges of a well 38 and a coverslip or other form of covering member which is placed over the well 38.

Figure 4E is a sideview of a plate cover 44 removal station 24 positioned adjacent a plate track 14. The plate cover 44 removal station 24 includes a carriage 68 configured to move vertically as illustrated by the arrow labeled A and laterally as illustrated by the arrow labeled B. A plurality of vacuum fittings 70 are coupled with the carriage 68 and are in pneumatic communication with a releasable vacuum source. Suitable vacuum fitting include, but are not limited to, rubber fittings having a cup shape and including a vacuum port in pneumatic communication with a vacuum source.

During operation of the plate cover removal station 24, the plate transport assembly moves a multiwell plate 34 into position next to the plate cover removal station 24. If the multiwell plate 34 has a plate cover 44, the carriage 68 is moved laterally until each of the vacuum fittings 70 are positioned over the multiwell plate 34. The carriage 68 is lowered until at least a portion of the vacuum fittings 70 are in contact with the plate cover 44. The vacuum source is activated in order to immobilize the plate cover 44 relative to the carriage 68. The carriage 68 is then raised to its original height. The vertical motion of the carriage 68 lifts the plate cover 44 from the multiwell plate 34. The carriage 68 is then moved laterally until the carriage 68 is positioned over a plate cover storage component 72. The carriage 68 is lowered into the plate cover storage component 72 and the vacuum source disengaged in order to drop the plate cover 44 into the plate cover storage component 72. Finally, the carriage 68 is then returned to its original position.

The plate cover removal station 24 can be adapted to a plate cover delivery station 29 by operating the plate cover removal station 24 in reverse. The reverse operation causes a plate cover 44 to be removed from the plate

cover storage component 72 and then placed on a multiwell plate 34. When a crystallization system 10 uses both a plate cover removal station 24 and a plate cover delivery station 29, the plate covers 44 used with the plate cover removal station 24 can be the same as or different from the plate covers 44 used with the plate cover delivery station 29.

Figure 4F is a top view of a mother liquor delivery station 26 where a mother liquor is delivered into the wells 38 of a multiwell plate 34. The mother liquor delivery station 26 includes a plurality of delivery shuttles 74. Each shuttle includes a delivery block 76 configured to slide along block supports 78. The delivery blocks 76 are coupled with block actuators 80 to slide the delivery blocks 76 in a lateral direction relative to the plate track 14 as illustrated by the arrow labeled A. Suitable block actuators 80 include, but are not limited to, pneumatic pistons, hydraulic pistons and electric motors.

Figure 4G provides a top view of a delivery block 76. A plurality of lumens 82 extend through the delivery block 76. The lumens 82 are divided into a first delivery group 84 and a second delivery group 86. A fluid injector 88, such as a syringe, can be removably positioned in each of the lumens 82 as illustrated in Figure 4H. The lumens 82 in each delivery group 84, 86 are arranged on the delivery block 76 so each fluid injector 88 can be concurrently aligned with a different well 38 of a multiwell plate 34. Accordingly, the number of lumens 82 in each delivery group 84, 86 is preferably equal to the number of wells 38 in the multiwell plate 34. For instance, when the multiwell plates 34 include 48 wells 38, each delivery group 84, 86 preferably includes 48 lumens 82.

Each fluid injector 88 is in fluid communication with a mother liquor source. More than one fluid injector 88 can be in fluid communication with a single mother liquor source. However, each fluid injector 88 is preferably in fluid communication with a different mother liquor source. Figure 4H illustrates a mother liquor delivery station 26 having five first delivery groups 84 and five second delivery groups 86 which each include 48 fluid injectors 88.

Accordingly, 480 mother liquor sources are required when each fluid injector 88 is in fluid communication with a different mother liquor source.

Figure 4I is a sideview of a mother liquor source storage bank 90 for holding different mother liquor sources 92. The bank 90 includes source holders 94 arranged in five columns and four rows. Each source holder 94 can hold a plurality of mother liquor sources 92 and can be slid in and out of the bank 90 to provide easy access to the mother liquor sources 92 being held by a single source holder 94. When each multiwell plate 34 has 48 wells 38, each source holder 94 preferably holds 12 different mother liquor sources 92. Accordingly, each column contains 48 mother liquor sources 92 which can each be in fluid communication with a different fluid injector 88 included in the same delivery group 84, 86. As a result, each column of mother liquor sources 92 can be associated with a single delivery group 84, 86. A mother liquor source bank 90 can be included on each side of the plate track 14. The mother liquor sources 92 positioned on one side of the plate track 14 can be in fluid communication with the delivery groups 84, 86 nearest that side of the track while the mother liquor sources 92 positioned on the opposing side of the plate track 14 can be in fluid communication with the delivery groups 84, 86 on the opposing side of the plate track 14.

During operation of the mother liquor delivery station 26, the plate transport assembly moves a multiwell plate 34 beneath a particular one of the delivery blocks 76. The block actuators 80 can then move the delivery blocks 76 so the injectors in a particular delivery group 84, 86 are aligned with the wells 38 in the multiwell plate 34. The particular delivery block 76 and the particular delivery group 84, 86 are associated with the mother liquors which the operator desires to be delivered into the wells 38 of the multiwell plate 34. The mother liquors are then delivered from the mother liquor sources 92 through the fluid injectors 88 and into the wells 38 which are aligned with the mother liquors. The delivery of mother liquid into each of the wells 38 can occur concurrently and the same volume of mother liquor is preferably delivered into each of the wells 38.

Figure 4J illustrates a syringe pump for delivering mother liquor from mother liquor sources 92 into a well 38 of a multiwell plate 34 through a fluid injector 88. Mother liquor flows from a mother liquor source 92 to the fluid injector 88 through a fluid conduit 96. The fluid conduit 96 is in fluid communication with a syringe 98 positioned between the mother liquor source 92 and the fluid injector 88. The volume within the syringe 100 can be mechanically compressed and expanded as illustrated by the arrow labeled A. A first pinch bar 102 is positioned on an input side of the syringe 104 and a second pinch bar 106 is positioned on an output side of the syringe 108. The first pinch bar 102 and the second pinch bar 106 are coupled with a rocker bar 110. In Figure 4J, the rocker bar 110 occupies a first position where the first pinch bar 102 has pinched the fluid conduit 96 shut on the input side of side of the syringe while the output side of the syringe 108 remains unobstructed. The rocker bar 110 can occupy a second position where the second pinch bar 106 has pinched the fluid conduit 96 shut on the output side of the syringe 108 while the output side of the syringe 108 remains unobstructed. The rocker bar 110 can be automatically moved between the first and second positions as illustrated by the arrow labeled B.

During operation of the syringe pump, the rocker bar 110 occupies the first position and the volume within the syringe 100 is expanded by the amount of mother liquor to be delivered into a well 38 from the fluid injector 88. Because the fluid conduit 96 on the output side of the syringe 108 is pinched closed, the expansion of the volume within the syringe 100 by a particular amount causes that particular amount to be withdrawn from the mother liquor source 92. The rocker bar 110 is then moved to the second position and the volume within the syringe 100 compressed by the amount of mother liquor to be delivered into the well 38 through the fluid injector 88. Because the fluid conduit 96 on the input side of the syringe 104 is closed, the compression of the volume within the syringe 100 by the particular amount causes that particular amount to flow through the fluid injector 88 and into the associated well 38.

The mother liquor delivery section discussed above is for illustrative purposes only and many variations are possible. For instance, a mother liquor delivery station 26 can include more than five delivery shuttles 74 or as few as one. Further, each delivery shuttle 74 can include more than two delivery groups or a few as one. When a delivery shuttle 74 includes a single delivery group, the block actuators 80 can be eliminated and the delivery shuttles 74 can be stationary relative to the plate track 14. Additionally, the combination of the plate track 14 movement and the delivery block 76 movement can be used to position a particular fluid injector 88 over a particular well 38 and the mother liquors can be sequentially delivered into the wells 38. Accordingly, a particular mother liquor can be delivered into a particular well 38.

Figures 5A-5E illustrate various stations 12 that may be included in a drop formation system 32. It is noted that the drop formation system 32 illustrated in regard to Figures 5A-5E is adapted for a hanging drop array crystallization. The drop formation system 32 can be readily modified for a sitting drop array crystallization by causing the mother liquor drops and molecule solution drops to be deposited on a sitting drop region 42 of a multiwell plate 34, such as the one illustrated in Figure 3C, as opposed to on a coverslip.

Figure 5A is a top view of a drop formation station 28 and Figure 5B is a sideview of the drop formation station 28. The drop formation station 28 includes a wash basin 112 through which a cleansing solution can be flowed. Suitable cleansing solutions include, but are not limited to, water. The drop formation station 28 also includes a molecule solution storage component 114 having one or more molecule solution wells 116 for storing solutions containing the molecule to the crystallized. The molecule solution wells 116 can be capped for storing the molecule solutions when the drop formation system 32 is not in operation. The molecule solution storage component 114 can be refrigerated in order to provide cooling to the molecule solution within the molecule solution wells 116. For example, when the molecule solution is a molecule solution, the solution is preferably kept at 3-4 °C whether the drop formation station 28 is or

is not in operation. The drop formation station 28 also includes syringe pumps 118 and a coverslip storage component 120 for storing coverslips.

The drop formation station 28 also includes a pipette holder 122 configured to move vertically as indicated by the arrow labeled A and laterally as indicated by the arrows labeled B. The pipette holder's 122 lateral range of motion allows the pipette holder 122 to move to a variety of positions including a position over the wash basin 112 and a position over the coverslip holder 124. The drop formation station 28 also includes a coverslip holder 124 configured to be inverted as indicated by the arrow labeled C. The coverslip holder 124 can move vertically as indicated by the arrow labeled D and laterally as indicated by the arrows labeled E. The pipette holder's 122 lateral range of motion allows the pipette holder 122 to move to a variety of positions including a position over the coverslip storage component 120 and several positions over the plate track 14. The drop formation station 28 also includes a molecule delivery pipette 126 which is configured to move vertically as indicated by the arrow labeled F, laterally as indicated by the arrow labeled G and longitudinally as indicated by the arrow labeled H. The longitudinal and lateral ranges of motion allow the molecule delivery pipette 126 to be moved to a variety of positions including a position over each molecule solution well and a plurality of positions over the coverslip holder 124.

The above movements can be achieved by coupling the pipette holder 122, coverslip holder 124 and the molecule delivery pipette 126 to a variety of different actuators. Suitable actuators include, but are not limited to, pneumatic pistons, hydraulic pistons and a variety of motors.

Figure 5C is a sideview of a pipette holder 122. The pipette holder 122 includes a pipette support frame 128. The pipette support frame 128 holds a number of pipettes 130 equal to the number of wells 38 in a column of a multiwell plate 34. The pipettes 130 are held at a spacing which approximates the spacing between the wells 38 in the column of the multiwell plate 34. This spacing permits each pipette 130 to be concurrently aligned with a different well 38 in the column.

Each pipette 130 includes a valve 132 and a conduit 134 extending from the valve 132 to a syringe pump 118. The syringe pump 118 can be used to draw fluid into the pipettes 130 and to drive fluid out of the pipettes 130. The valve 132 is configured to deliver drops of a particular size from the pipette 130. These drops are delivered from the pipette 130 until a desired total volume is delivered from the pipette 130. Suitable valves 132 include, but are not limited to, piezoelectric valves and solenoid valves which can be configured to deliver drops as small as 380 pL. This allows production of mother liquor drops as small as 380 pL. Further reduction in the drop size delivered by these pipettes 130 may also be possible, would be desired, and is intended to fall within the scope of the present invention.

The pipette arrangement used for the molecule delivery pipette 126 is similar to the pipette arrangement used for the pipettes 130 within the pipette holder 122. Accordingly, the molecule delivery pipette 126 also includes a valve 132 and a conduit 134 extending from the valve 132 to a syringe pump 118. The molecule delivery pipette 126 is able to produce molecule solution drops as small as 380 pL. Further reduction in the drop size delivered may also be possible, would be desired, and is intended to fall within the scope of the present invention.

Figure 5D is a sideview of a coverslip holder 124. The coverslip holder 124 includes a frame 136 which supports a plurality of support cups 138 shaped to removably hold coverslips at a spacing which approximates the spacing between the wells 38 in a column of the multiwell plate 34. This spacing permits each coverslip to be concurrently aligned with a different well 38 in a column of the multiwell plate 34.

The support cups 138 can include an attachment mechanism 140 for immobilizing the coverslips in place relative to the support cups 138. The attachment mechanism 140 serves to keep the coverslips in place when the coverslip holder 124 is inverted. However, the attachment mechanisms 140 can release the coverslips at a desired moment. Suitable coverslip holder 124 attachment mechanisms 140 include, but are not limited to, a vacuum source in

pneumatic communication with vacuum ports positioned in the support cups 138. Pulling a vacuum through the vacuum ports serves to keep the coverslips in place on the coverslip holder 124. However, when the coverslip holder 124 is inverted, the vacuum can be released by disengaging the vacuum source or reversing the vacuum. The release of the vacuum releases the coverslips from the coverslip holder 124.

Figure 5E is a sideview of a coverslip storage component 120 which includes a plurality of magazines 142 sized to hold coverslips 144 stacked on top of one another. The stack of coverslips 144 within the magazine 142 can be biased upward until the coverslip 144 on the top of the stack is near the top of the magazine 142. The spacing between the magazines 142 approximates the spacing between the support cups 138 of the coverslip holder 124. This spacing permits each magazine 142 to be concurrently aligned with a different support cup 138 of the coverslip holder 124. Accordingly, a coverslip 144 from each magazine 142 can also be aligned with a different support cup 138.

Figures 6A-6I illustrate a method for operating the drop formation station 28 to form hanging drops in each of the wells 38 of a multiwell plate 34. The figures are described with respect to crystallization of a protein, however, the same method can be used for crystallization of other types of molecules. Figure 6A illustrates a drop formation station 28 in the rest position which can be occupied when the drop formation station 28 is not in use or between multiwell plates 34 being transported into the drop formation station 28. In the rest position, coverslips 144 are attached to the coverslip holder 124 which is positioned to one side of the plate track 14 and the pipette holder 122 is positioned to the opposing side of the plate track 14.

Figure 6B illustrates a multiwell plate 34 moved into position for drop formation and the pipette holder 122 moved into position over the wash basin 112 for priming of the pipettes 130. The pipette holder 122 is lowered until the pipette tips are within a cleansing solutions within the wash basin 112. Cleansing fluid is aspirated from the wash basin 112 and the pipette holder 122 is raised to remove the pipette tips from the cleansing solution. The cleansing

fluid is then expelled from the pipettes 130. The process of aspiration and expulsion can be repeated as often as is necessary to achieve a properly primed pipettes 130.

Figure 6C illustrates the pipette holder 122 moved into position over a column of wells 38 in the plate 34. The pipette holder 122 is positioned so each pipette tip is aligned with a different well 38 in the column. Accordingly, each pipette 130 is associated with a particular well 38. The pipette holder 122 is lowered until each pipette tip is positioned within the mother liquor in the associated well 38. A portion of the mother liquor is aspirated from each well 38 associated with a pipette tip. The actuators then lift the pipettes 130 upward to remove the pipette tips from the wells 38. A portion of the aspirated mother liquors are then expelled from each pipette 130. The expelled mother liquors fall back into the associated well 38.

Figure 6D illustrates the pipette holder 122 moved over the coverslip holder 124 and is positioned so each pipette tip is aligned with a different support cup 138. The support cups 138 are each holding a coverslip 144 upside down and the attachment mechanism 140 is engaged to immobilize the coverslips 144 relative to the support cups 138. One or more drops of mother liquor is expelled from each pipette 130 onto the associated coverslips 144. As a result, one more drops of the mother liquor from a particular well 38 is delivered onto a particular coverslip 144.

The drops of mother liquor are expelled onto the coverslips 144 until a desired volume of mother liquor has been delivered onto each coverslip 144. The total volume of the drops delivered onto the coverslips 144 is strictly controlled. As discussed previously, a feature of the present invention is the ability to deliver small volumes precisely which enables small drop volumes to be used. With devices which can deliver volumes as low as 380 pL, volumes can be delivered with great precision. The precision of the volumes delivered is preferably less than about 25 nL, more preferably less than 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL. The precision of the volumes delivered may also be between 380pL and 25 nL, more

preferably between 380pL and 20 nL, more preferably between 380pL and 15 nL, and most preferably between 380pL and 10 nL.

Figure 6E illustrates the pipette holder 122 returned to the rest position which was illustrated in Figure 6A. The molecule delivery pipette 126 is moved into position over a coverslip 144. Before being moved into position over the coverslip 144, the molecule delivery pipette 126 was lowered into a particular molecule solution well and a volume of the molecule solution aspirated. Once the molecule delivery pipette 126 is in position over the coverslip 144, drops of the molecule solution are delivered onto the mother liquor which was previously delivered onto the coverslip 144. The drops of molecule solution are delivered until a desired volume of molecule solution is achieved on the coverslip 144. The precision of the volumes delivered is preferably less than about 25 nL, more preferably less than 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL. The precision of the volumes delivered may also be between 2 and 25 nL, more preferably between 2 and 20 nL, more preferably between 2 and 15 nL, and most preferably between 2 and 10 nL.

The mother liquor drops and the protein drops may be delivered in any order. Once both drops are delivered, the drops combine to form a hanging drop to be studied for crystal formation.

After forming a hanging drop on the coverslip 144, the molecule delivery pipette 126 proceeds to the next coverslip 144 until a hanging drop is formed on each coverslip 144. The molecule delivery pipette 126 then returns to the position over the molecule solution well which was the source for the molecule solution used to create the hanging drops. The molecule solution remaining in the molecule delivery pipette 126 is expelled into the molecule solution well.

Figure 6F illustrates the molecule delivery pipette 126 returned to its rest position as illustrated in Figure 6A. Figure 6F also illustrates the coverslip holder 124 inverted and moved into position over the column of wells 38 on the multiwell plate 34. The coverslip holder 124 is positioned so each coverslip 144 is aligned with a different well 38 in the column. Specifically, a given

coverslip 144 is aligned with the well 38 which was the source of the mother liquor used to create the hanging drop on the given well 38.

The coverslip holder 124 is lowered until the coverslips 144 contact the upper edges 40 of the associated wells 38. The sealing medium which was previously applied to the upper edge 40 of the wells 38 causes a seal to be formed between the coverslips 144 and the upper edges 40 of the associated wells 38. The attachment mechanism 140 is released and the coverslip holder 124 is raised to leave each coverslip 144 in place over an associated well 38. The hanging drop hangs from the coverslips 144 into the wells 38 as illustrated in Figure 6G.

Figure 6H illustrates the coverslip holder 124 moved into position over the coverslip storage component 120. The coverslip holder 124 is positioned so each support cup 138 is aligned with a magazine 142 in the coverslip storage component 120. Accordingly, each support cup 138 is associated with the top coverslip 144 in each magazine 142. The coverslip holder 124 is lowered until each support cup 138 contacts a coverslip 144 within the associated magazine 142. The attachment mechanism 140 is engaged to immobilize the contacted coverslips 144 relative to the associated support cups 138.

Figure 6I illustrates the coverslip holder 124 returned to its rest position. The top coverslip 144 from each magazine 142 discussed with respect to Figure 6G is attached to the associated support cup 138.

The steps described with respect to Figures 6A-6I result in a hanging drop being formed in each well 38 of a single column of wells 38. These steps are repeated until a hanging drop is formed in the wells 38 of each column of the multiwell plate 34. Once a hanging drop is formed in each of the wells 38, the multiwell plate 34 can be moved to the next station.

The crystallization system 10 described above can be adapted to form sitting drops. This adaptation can be made with changes to the mother liquor delivery station 26 and the drop formation station 28. For instance, the mother liquor delivery station 26 is adapted to deliver mother liquor into the well regions 41 of a multiwell plate 34 adapted to perform a sitting drop array

microcrystallization such as the multiwell plate 34 illustrated in Figure 3C. Specifically, the fluid injectors 88 of the mother liquor delivery station 26 must be aligned with the well regions 41 before the mother liquor is delivered into the wells 38 of the multiwell plate 34. This alignment permits delivery of the mother liquors into the well region 41 of each well 38 without delivering the mother liquors onto the sitting drop region 42 of each well 38.

Adapting the crystallization system 10 to form sitting drops also includes adapting the drop formation station 28 to form sitting drops. The drop formation station 28 can include each of the components illustrated in Figure 5A-5E arranged with the same spatial relationships illustrated in Figures 5A-5E. However, the method of operating these components varies from the method illustrated in Figures 6A-6I. Figures 7A-7G illustrate a method for operating the drop formation station 28 to form sitting drops in each well 38 of a multiwell plate 34 adapted to perform a sitting drop array microcrystallization.

The figures are described with respect to crystallization of a protein, however, the same method can be used for crystallization of other types of molecules. Figure 7A illustrates the drop formation station 28 in the same rest position illustrated in Figure 6A. Figure 7B illustrates a multiwell plate 34 adapted to perform a sitting drop array microcrystallization moved into position for sitting drop formation. Accordingly, each well 38 in the multiwell plate 34 includes a well region 41 adjacent to a sitting drop region 42. Figure 7B also illustrates the pipette holder 122 moved into position over the wash basin 112 for priming of the pipettes. The pipettes are primed as described with respect to Figure 6B.

Figure 7C illustrates the pipette holder 122 moved into position over a column of wells 38 in the multiwell plate 34. The pipette holder 122 is positioned so each pipette tip is aligned with the well region 41 in a different well 38 in the column as illustrated in Figure 7D. Accordingly, each pipette is associated with a particular well 38. The pipette holder 122 is lowered until the tip of each pipette is positioned in the mother liquor which was previously delivered into the well region 41 of the associated well 38. A portion of the

mother liquor is aspirated from each well region 41 associated with a pipette tip. The actuators then lift the pipette upward to remove the pipette tips from the wells 38. A portion of the aspirated mother liquors are then expelled from each pipette. The expelled mother liquors fall back into the associated well regions 41.

The pipette holder 122 is then moved so each pipette tip is aligned with the sitting drop region 42 in a different well 38 in the column as illustrated in Figure 7E. One or more drops of mother liquor is expelled from each pipette onto the associated sitting drop region 42. As a result, one more drops of the mother liquor from a particular well region 41 is delivered onto the sitting drop region 42 of the same well 38. The drops of mother liquor are expelled onto the sitting drop region 42 until a desired volume of mother liquor has been delivered onto each sitting drop region 42. The total volume of the drops delivered onto the coverslips 144 is strictly controlled. As discussed previously, a feature of the present invention is the ability to deliver small volumes precisely which enables small drop volumes to be used. With devices which can deliver volumes as low as 380 pL, volumes can be delivered with great precision. The precision of the volumes delivered is preferably less than about 25 nL, more preferably less than 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL. The precision of the volumes delivered may also be between 380pL and 25 nL, more preferably between 380pL and 20 nL, more preferably between 380pL and 15 nL, and most preferably between 380pL and 10 nL.

Figure 7F illustrates the pipette holder 122 returned to the rest position which was illustrated in Figure 7A. The molecule delivery pipette 126 is moved into position over a sitting drop region 42 in a well 38 of the column. Before being moved into position over the well 38, the molecule delivery pipette 126 was lowered into a particular molecule solution well and a volume of the molecule solution aspirated. Once the molecule delivery pipette 126 is in position over the sitting drop region 42, drops of the molecule solution are delivered onto the mother liquor which was previously delivered onto the

coverslip 144. The drops of molecule solution are delivered until a desired volume of molecule solution is achieved on the sitting drop region 42. The precision of the volumes delivered is preferably less than about 25 nL, more preferably less than 20 nL, more preferably less than 15 nL, and most preferably less than 10 nL. The precision of the volumes delivered may also be between 380 pL and 25 nL, more preferably between 380 pL and 20 nL, more preferably between 380 pL and 15 nL, and most preferably between 380 pL and 10 nL.

The mother liquor drops and the protein drops may be delivered in any order. Once both drops are delivered, the drops combine to form a sitting drop to be studied for crystal formation. Figure 7G illustrates a sitting drops formed on the sitting drop region 42 of a well 38. After forming the sitting drop on the sitting drop region 42, the molecule delivery pipette 126 proceeds to the sitting drop region 42 in the next well 38 until a sitting drop is formed in each well 38 of the column. The molecule delivery pipette 126 then returns to the position over the molecule solution well which was the source for the molecule solution used to create the sitting drops. The molecule solution remaining in the molecule delivery pipette 126 is expelled into the molecule solution well.

After formation of the sitting drop, the coverslips 144 are positioned over the wells 38 having sitting drops, new cover slips are loaded onto the coverslip holder 124 and the drop formation station 28 is returned to the rest position as described above with respect to Figure 6F-6I.

The steps described with respect to Figures 7A-7G result in a sitting drop being formed in each well 38 of a single column of wells 38. These steps are repeated until a hanging drop is formed in the wells 38 of each column of the multiwell plate 34. Once a hanging drop is formed in each of the wells 38, the multiwell plate 34 can be moved to the next station.

Although Figures 6A-7G illustrate a method for operating the drop formation station to form sitting drops and hanging drops, the hanging drop station be easily adapted to other crystallization techniques, other well geometries and/or other multiwell plate geometries.

It is noted that the apparatuses described in regard to Figures 6A-7G may optionally include one or more sensors which can detect whether mother liquor drops and/or molecule drops have been formed. An example of a suitable sensor is a LED sensor.

While many plate tracks 14 and transport assemblies can be used with the above stations 12, Figures 8A-8C illustrate a preferred embodiment of a plate track 14 for transporting multiwell plates 34 between the above stations 12. Figures 8A and 8B are sideviews of a plate track 14 looking down the longitudinal axis of the plate track 14. The plate track 14 includes two spaced apart plate supports 158. A pin 160 extends upward from a pin carriage 162 positioned beneath the plate track 14. The carriage includes mechanics which can be actuated to extend the pin 160 above the plate track 14 as illustrated in Figure 8A or to withdraw the pin 160 below the plate track 14 as illustrated in Figure 8B.

Figure 8C is a lateral sideview of a plate track 14 and transport assembly with a plurality of multiwell plates 34 present on the plate track 14. The transport assembly includes a first pin carriage 162A, a second pin carriage 162B and a third pin carriage 162C. Each of the pin carriages 162A, 162B, 162C is configured to move along the longitudinal axis of the plate track 14 as illustrated by the arrows labeled A, B and C. The brackets at the ends of the arrows indicate the range of motion of each pin carriage 162A, 162B, 162C.

The first pin carriage 162A and the third pin carriage 162C include a plurality of pins 160. The pins are located along the pin carriage 162A, 162C with an approximately constant displacement between adjacent pins 160. The pin carriage 162A, 162C serves to maintain the displacement between the pins 160 during movement of the pin carriage 162A, 162C.

Each pin 160 is illustrated in the extended position, however, the pins in one pin carriage 162 can be withdrawn while the pins 160 in another pin carriage 162 are extended. In another embodiment, a portion of the pins 160 in a single pin carriage 162 can be extended while another portion of the pins 160 within the same pin carriage 162 are withdrawn.

An air gap 166 is formed between the pin carriages 162A, 162B, 162C and each of the multiwell plates 34 positioned on the plate track 14 so the pin carriages 162A, 162B, 162C do not contact the bottom surface of the multiwell plates 34. As a result, when the pins 160 are withdrawn, the pin carriages 162A, 162B, 162C can be moved along the longitudinal axis of the plate track 14 without moving multiwell plate 34 on the plate track 14. When the pin 160 is extended and the pin carriage 162 is moved along the longitudinal axis of the plate track 14, the pin 160 pushes any multiwell plate 34 obstructing the pin's travel along the longitudinal axis of the plate track 14.

As described above, the plate transport assembly is used to transport the multiwell plates 34 from station to station along the plate track 14. Various positions along the plate track 14 can be associated with a particular station of the crystallization system 10. For instance, when a multiwell plate 34 is located at position P_1, a bar code on the multiwell plate 34 can be read by a bar code station and when a multiwell plate 34 is located at position P_2, a sealing medium station 22 can be used to apply a sealing medium to the upper edge 40 of the wells 38 of the multiwell plate 34. Further, when a multiwell plate 34 is positioned at P_3, the multiwell plate 34 can be positioned beneath one of the delivery shuttles 74 of a mother liquor delivery station 26.

The following description describes a method for using the above transport assembly for advancing the multiwell plate 34 labeled T_1 from the position labeled P_1 to the position labeled P_2 to the position labeled P_3. Once the multiwell plate T_1 is located at position P_1, the pins 160 in the first pin carriage 162A are withdrawn below the plate track 14. The first pin carriage 162A is then moved to the left and the pins 160 extended above the plate track 14 as illustrated in Figure 8C. The multiwell plate T_1 can then be moved from position P_1 to position P_2 by moving the first pin carriage 162A to the right until the multiwell plate T_1 is positioned at position P_2. The pins 160 are then withdrawn below the plate track 14 and the first pin carriage 162A is moved back to is original position and the pins 160 are again extended above the plate track 14. The pins 160 in the second pin carriage 162B are withdrawn below

the plate track 14 and the second pin carriage 162B is moved to the left of the multiwell plate T_1. The pins 160 in the second pin carriage 162B are then extended above the plate track 14 and the second pin carriage 162B moved to the right until the multiwell plate T_1 is located at position P_3.

The plurality of pin carriages 162 illustrated in Figure 8C allows a multiwell plate 34 at one station to be processed through the crystallization system 10 independently of another multiwell plate 34 being processed through the crystallization system 10. For instance, a first multiwell plate 34 can be advanced from P_1 to P_2 while a second multiwell plate 34 remains in place at P_3. As a result, when P_1, P_2 and P_3 are each associated with different stations 12, multiwell plates 34 can be processed through different stations 12 at different rates. Further, different pin carriages 162 which make up a transport assembly can be included with independent systems which are assembled together to form the system. For instance, the first pin carriage 162A and the second pin carriage 162B can be included in a mother liquor deliver system and the third pin carriage 162C can be included in a drop formation system 32.

Crystal formation can be detected by examining each drop for the formation of crystals. In a preferred embodiment, crystals are detected and graded in the various wells for crystal quality. This may be done manually or by an automated device. Diversified Scientific, Inc. of Birmingham, Alabama manufactures CRYSTALSCORE™ which may be used to automate the scoring of crystal formation.

As described above, the system can be used to performed crystallization trials where various mother liquor are screened for their ability to crystallize a protein of interest. The crystallization trials frequently include a coarse screen followed by one or more fine screens. While the mother liquors used for the fine screens are often dependent on the results of the coarse screen, the mother liquors used for the coarse screen can be standard for each crystallization trial.

When the mother liquors are used to crystallize proteins, a preferred coarse screen preferably consists of the 15 sub-screens listed in Table 1. The number of mother liquors included in each sub-screen is also listed in Table 1.

The composition of the mother liquors included each of these sub-screens is listed in Figure 9. Mother liquors having the listed compositions can be obtained from Hampton Research of Laguna Niguel, California.

As illustrated in Table 1, a total of 480 mother liquors are associated with the sub-screens of the preferred coarse screen. Since 480 mother liquors are included in the coarse screen and since each plate preferably includes 48 wells, the coarse screen can be performed by processing only 10 plates through the system. Further, the sub-screens generally include 24 or 48 mother liquors. Accordingly, each plate can include from one to two sub-screens.

TABLE 1

Screen	Number of mother liquors
Crystal screen I	48
Crystal screen II	48
Grid ammonium sulfate	24
Grid MPD	24
Grid sodium chloride	24
Grid PEG6000	24
Grid PEG/lithium chloride	24
Sodium/potassium phosphate	24
PEG/ion screen	48
Membrane protein screen	48
Detergent screen I	24
Detergent screen II	24
Detergent screen III	24
Cryo screen	48
Low ionic strength screen	24

Each of the mother liquors used for the coarse screen can be stored in one or more of the mother liquor storage banks. However, the number of

mother liquors which may be needed for different fine screens is large enough that storage of these mother liquors impractical. Accordingly, the system can also include a station which forms the fine screen mother liquors from stock solutions and then delivers them into the wells of a plate. Alternatively, one or more external systems can be used to create the fine screen mother liquors from stock solutions and to deliver these mother liquors into the wells of one or more plates. These plates can then be processed through the system.

When an external system is used to form and deliver fine screen mother liquors, the system control logic needs to override the mother liquor delivery station in order to avoid doubling up on the delivery of mother liquor into the wells of a plate. As a result, the system control logic must be informed when a plate which already has mother liquor is in the system. An operator can use a user interface to inform the system control logic which one of the plates already has mother liquors delivered into the wells. Alternatively, an operator use a plate having a bar code which indicates that mother liquors are already present in the wells of the plate.

Example 1

The system described above was used in a plurality of lysozyme crystallization experiments where lysozyme was crystallized in a mother liquor composition including 100 mM sodium acetate and 10% sodium chloride at a pH of 4.6. The volume of the hanging drop formed by the drop formation station was different for each experiment. Figures 10A-10D respectively illustrate crystal formed in hanging drops of 40 nL, 100 nL, 200 nL and 1000 nL. The crystals were formed regardless of the reduction in drop size. As a result, the system can be used with submicroliter hanging drop volumes.

Example 2

The system described above was used in a crystallization trial where the mother liquor for crystallizing lysozyme was optimized. During the coarse screen, 480 crystallization experiments were performed using each of the 480 mother liquors disclosed in Figure 9. The results from each of the 480 experiments were compared to one another to identify one or more crystallization experiments yielding crystals with the most desirable characteristics. One of the identified coarse screen experiments was associated with a mother liquor composed of 30% MPD (+/- 2-methyl-2,4-pentanediol), 100 mM sodium acetate, 20 mM calcium chloride, at pH 4.6.

A fine screen consisting of 24 crystallization experiments was then performed. The composition of the mother liquors associated with each of the 24 crystallization experiments was selected relative to the composition of the mother liquor associated with the identified coarse screen experiment. The compositions of the 24 mother liquors selected for the crystallization experiments of the fine screen are listed in Figure 11. The concentrations of certain components in each of the 24 mother liquors matched the concentration of these components in the identified coarse screen experiment. For instance, the mother liquor associated with the identified coarse screen experiment and the mother liquors for each of the fine screen crystallization experiments were all about 30% MPD and 100 mM sodium acetate. The concentrations of other components in the 24 mother liquors were varied over a range which encompassed the concentration of these same components in the identified coarse screen experiment. For instance, the concentration of calcium chloride was 20 mM in the identified coarse screen experiment but was varied from 12.5-27.5 mM in the 24 mother liquors. Similarly, the pH was 4.6 in the identified coarse screen crystallization experiment but was varied from 4.1 to 5.1 in the 24 mother liquors.

Each of the 24 fine screen crystallization experiments were compared to one another to identify the one or more crystallization experiments yielding the most desirable characteristics.

The foregoing examples and description of preferred embodiments of the present invention are provided for the purposes of illustration and description. The examples and preferred embodiments, however, are not intended to be exhaustive or to limit the invention to the precise forms disclosed. Obviously, many modifications and variations will be apparent to practitioners skilled in this art. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application, thereby enabling others skilled in the art to understand the invention for various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the following claims and their equivalents.

What is claimed is:

1. A method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule, the method comprising:

forming an array of microcrystallizations, each microcrystallization comprising

a drop comprising a mother liquor solution whose composition varies within the array and a molecule to be crystallized, the drop having a volume of less than 1 μL;

storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and

detecting molecule crystal formation in the drops.

2. The method according to claim 1 wherein the molecule is a macromolecule.

3. The method according to claim 1 wherein the molecule is a protein.

4. The method according to claim 1 wherein the macromolecule has a molecular weight of at least 500 daltons.

5. The method according to claim 1 wherein the drop has a volume of less than about 750 nL.

6. The method according to claim 1 wherein the drop has a volume of less than about 500 nL.

7. The method according to claim 1 wherein the drop has a volume of less than about 250 nL.

8. The method according to claim 1 wherein the drop has a volume of between about 1 nL - 750 nL.

9. The method according to claim 1 wherein the drop has a volume of between about 1 nL - 500 nL.

10. The method according to claim 1 wherein the drop has a volume of between about 1 nL - 250 nL.

11. The method according to claim 1 wherein the wells include less than about 500 μL of mother liquor solution.

12. The method according to claim 1 wherein the wells include less than about 250 μL of mother liquor solution.

13. The method according to claim 1 wherein the wells include mother liquor solutions having at least 4 components which are varied within the array.

14. The method according to claim 1 wherein the wells include mother liquor solutions having at least 5 components which are varied within the array.

15. The method according to claim 1 wherein the array includes greater than 96 microcrystallizations.

16. The method according to claim 1 wherein the array includes greater than 192 microcrystallizations.

17. The method according to claim 1 wherein forming the array of microcrystallizations includes using greater than 48 stock solutions to form the mother liquor solutions used in the array.

18. The method according to claim 1 wherein forming the array of microcrystallizations includes using greater than 96 stock solutions to form the mother liquor solutions used in the array.

19. The method according to claim 1 wherein forming the array of microcrystallizations includes using greater than 192 stock solutions to form the mother liquor solutions used in the array.

20. The method according to claim 1 wherein forming the array of microcrystallizations includes forming the drops within a volume range of less than about 25 nL.

21. The method according to claim 1 wherein forming the array of microcrystallizations includes forming the drops within a volume range of less than about 20 nL.

22. The method according to claim 1 wherein forming the array of microcrystallizations includes forming the drops within a volume range of less than about 15 nL.

23. A method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule, the method comprising:

forming an array of microcrystallizations, each microcrystallization comprising a hanging drop comprising a mother liquor solution whose composition varies within the array and a molecule to be crystallized, the drop having a volume of less than 1 μL;

storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and

detecting molecule crystal formation in the drops.

24. A method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule, the method comprising:

forming an array of microcrystallizations, each microcrystallization comprising a sitting drop comprising a mother liquor solution whose composition varies within the array and a molecule to be crystallized, the drop having a volume of less than 1 μL;

storing the array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and

detecting molecule crystal formation in the drops.

25. A method for performing array microcrystallizations to determine suitable crystallization conditions for a molecule, the method comprising:

forming a first array of microcrystallizations, each microcrystallization comprising a drop comprising a mother liquor solution whose composition varies within the array and a molecule to be crystallized, the drop having a volume of less than 1 μL;

storing the first array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array;

detecting molecule crystal formation in the drops;

forming a second array of microcrystallizations, each microcrystallization comprising a drop comprising a molecule to be crystallized and a mother liquor solution whose composition varies within the array and is based on one or more mother liquor solutions used in the first array in which molecule crystals were detected;

storing the second array of microcrystallizations under conditions suitable for molecule crystals to form in the drops in the array; and

detecting molecule crystal formation in the drops.

26. The method according to claim 25 wherein drop is a hanging drop.

27. The method according to claim 25 wherein drop is a sitting drop.

28. The method according to claim 25 wherein drops formed in the second array have a volume of less than 1 μL.

29. An apparatus for forming submicroliter drops in an array microcrystallization to determine suitable crystallization conditions for a molecule, the apparatus comprising:

a platform on which a multiwell plate is positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to drop regions on the multiwell plate within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the drop regions within a volume range of less than about 25 nL.

30. The apparatus according to claim 29 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 20 nL.

31. The apparatus according to claim 29 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 15 nL.

32. The apparatus according to claim 29 wherein the mother liquor drop station and the molecule drop station each include a piezoelectric valve or a solenoid valve.

33. An apparatus for forming submicroliter hanging drops on cover slips used in an array microcrystallization to determine suitable crystallization conditions for a molecule, the apparatus comprising:

a platform on which a multiwell plate is positionable;

a cover slip station on which a plurality of coverslips are positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to the plurality of coverslips within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the plurality of coverslips within a volume range of less than about 25 nL.

34. The apparatus according to claim 33 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 20 nL.

35. The apparatus according to claim 33 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 15 nL.

36. The apparatus according to claim 33 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes to at least four coverslips at a time.

37. The apparatus according to claim 33 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes to at least eight coverslips at a time.

38. An apparatus for forming submicroliter drops in an array microcrystallization to determine suitable crystallization conditions for a molecule, the apparatus comprising:

a platform on which a multiwell plate is positionable;

a mother liquor drop station capable of removing mother liquor from a plurality of wells of the multiwell plate and delivering submicroliter volumes of mother liquor to sitting drop regions on the multiwell plate within a volume range of less than about 25 nL; and

a molecule drop station capable of delivering submicroliter volumes of a solution containing a molecule to be crystallized to the sitting drop regions within a volume range of less than about 25 nL.

39. The apparatus according to claim 38 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 20 nL.

40. The apparatus according to claim 38 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 15 nL.

41. The apparatus according to claim 38 wherein the mother liquor drop station and the molecule drop station are each capable of delivering submicroliter volumes within a volume range of less than about 10 nL.

42. A method for forming submicroliter drops for use in an array microcrystallization to determine suitable crystallization conditions for a molecule, the method comprising:

delivering to a plurality of drop regions within a volume range of less than about 25 nL submicroliter volumes of mother liquor solutions whose compositions vary within the array; and

delivering to the plurality of drop regions submicroliter volumes of a solution containing a molecule to be crystallized within a volume range of less than about 25 nL;

wherein a total volume of the submicroliter volumes delivered to each drop region is less than 1 μL.

43. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is less than about 750 nL.

44. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is less than about 500 nL.

45. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is less than about 250 nL.

46. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is between about 1 nL - 750 nL.

47. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is between about 1 nL - 500 nL.

48. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region between about 1 nL - 250 nL.

49. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is within a volume range of less than about 20 nL.

50. The method according to claim 42 wherein the submicroliter volumes delivered to each drop region is within a volume range of less than about 15 nL.

51. A method for forming submicroliter hanging drops on cover slips

for use in an array microcrystallization to determine suitable crystallization conditions for a molecule, the method comprising:

taking a plurality of coverslips;

removing mother liquor from a plurality of wells of a multiwell plate;

delivering submicroliter volumes of the mother liquor to the plurality of coverslips within a volume range of less than about 25 nL; and

delivering submicroliter volumes of a solution containing a molecule to be crystallized to the plurality of coverslips within a volume range of less than about 25 nL;

wherein a total volume of the submicroliter volumes delivered to each coverslip is less than 1 μL.

52. A method for forming submicroliter sitting drops for use in an array microcrystallization to determine suitable crystallization conditions for a molecule, the method comprising:

taking a multiwell plate comprising a plurality of regions, each region including a sitting drop region and a well comprising a mother liquor solution whose composition varies within the regions of the multiwell plate;

delivering submicroliter volumes of the mother liquor solutions from the wells to the sitting drop regions within a volume range of less than about 25 nL; and

delivering submicroliter volumes of a solution containing a molecule to be crystallized to the sitting drop regions within a volume range of less than about 25 nL;

wherein a total volume of the submicroliter volumes delivered to each sitting drop region is less than 1 μL.