system that includes storage and imaging of crystallization plates, and liquid-formulation robots.

Honey Bee and Phoenix robots contain a single (or several) non-contact channel that dispenses protein solution and a 96-channel dispenser head that dispenses the crystallization solutions (50 nl to 100 µl). The 96-channel head transfers crystallization solutions from a 96-well deep-well plate into the reservoir and crystallization drop in a 96-well crystallization plate (Fig. 4.4.3.1*e*). A single channel transfers protein solution into each of the 96-well drops one by one without touching the precipitate drops. The plate is then sealed with a clear film by the user. The Mosquito liquid-handling robot can set up drops with a hanging-drop geometry, and is more popular with membrane protein crystallization (detergent solutions have a tendency to adhere to the side of the drop well in sitting-drop geometry). The Mosquito uses only disposable tips capable of dispensing 20 nl–1.2 µl volume; thus the user can control the location of drop deposition more precisely because the disposable tips can touch the drop. The use of disposable tips also prevents cross-contamination between samples, and washing steps between samples are eliminated.

The Fluidigm TOPAZ system utilizes a new technology, the crystallization screen chip, in which protein sample and reagent solutions are automatically loaded into diffusion chambers within the protein screen chip and the two solutions mixed by free interface diffusion, as opposed to vapour diffusion or microbatch techniques (Thorsen *et al.*, 2002). A very small amount of protein is required for a crystallization screen, *i.e.* as little as 1.0 µl protein solution for 96 trials. Crystals obtained from the protein chip are generally too small for X-ray data collection, and thus need to be scaled up to obtain diffraction-quality crystals.

Progress of crystallization trials from large numbers of 96-well plates can be monitored using an imaging robot to take pictures of individual crystallization drops. The resulting images can then be analysed either manually or using automatic crystal recognition systems at specified time intervals (Markley *et al.*, 2009). Remote viewing of recorded crystal pictures is also available over the web. Each recorded image is linked to crystallization conditions for evaluation and scoring of the crystallization conditions (Fig. 4.4.3.1*f*). Minstrel (Rigaku), CrystalFarm (Bruker) or HomeBase (The Automation Partnership) systems offer integrated systems for plate storage and imaging.

Crystallization conditions that initially produced crystals should be optimized to improve crystal growth and quality. A liquid-handling robot can be used to make screens in 96-well deep-well plates. A liquid-formulation robot has been developed for protein crystallization to make grid screens of 96-well deep-well conditions (*e.g.* Alchemist from Rigaku). The crystallization conditions stored in an imaging robot are linked to the liquid-formulation software, and can be used to formulate 96-well screen conditions for optimization experiments. Other optimization methods such as crystallization in gels, control of nucleation using oil mixtures or microporous materials, and seeding experiments can also be employed in an HT fashion (Chayen, 2003; Georgiev *et al.*, 2006; Sugahara *et al.*, 2008).

### 4.4.6. Synchrotron data collection

High-brilliance beamlines at modern synchrotrons have significantly reduced the time required for X-ray data collection, and complete data sets can often be collected within minutes. Thus, the time required for crystal mounting and centring is no longer negligible. Automatic crystal mounting and centring allow users to remotely mount crystals for crystal evaluation and data collection without entering the experimental hutch (Manjasetty *et al.*, 2008; Sharff & Jhoti, 2003; Sugahara *et al.*, 2008).

Automated crystal mounting allows the screening of many crystals for diffraction quality and then goes back to the best diffraction-quality crystals for full data collection. The mounting robot picks up frozen crystals in a pin from a Dewar, puts them on a goniometer and retrieves the pin after the diffraction test. The automated crystal-mounting robots have primarily been developed for use at synchrotron sources, although a commercial version of an automatic sample-mounting robot is now available that can be used with a home-source X-ray generator (ACTOR from Rigaku and cryogenic sample changer from Marresearch). Fully automated crystal alignment is not yet available, but semi-automated crystal centring by clicking a mouse to indicate the intended centre of the crystal is used at most beamlines.

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