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An automated image-collection system for crystallization experiments using SBS standard microplates

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As part of a structural genomics platform in a university laboratory, a low-cost in-house-developed automated imaging system for SBS microplate experiments has been designed and constructed. The imaging system can scan a microplate in 2–6 min for a 96-well plate depending on the plate layout and scanning options. A web-based crystallization database system has been developed, enabling users to follow their crystallization experiments from a web browser. As the system has been designed and built by students and crystallographers using commercially available parts, this report is aimed to serve as a do-it-yourself example for laboratory robotics.

1. Introduction

A number of structural genomics consortia have developed high-throughput (HTP) pipelines containing devices for automation, miniaturization and parallelization (Adams *et al.*, 2003; Lesley *et al.*, 2002). These well funded activities have pushed the limits and explored new structural biology frontiers.

Various universities with structural biology laboratories have also adapted their methods to obtain a higher throughput, although with less funding and automation (Segelke *et al.*, 2004). The need for automation and method development in a university environment is of a different character to that in a fully designed HTP pipeline, as many experiments may still be carried out manually. In this partly automated environment, individual students may have many parallel experiments running and although the automated steps may reduce the workload, the amount of experimental data will be greatly increased. Thus, the function of automatically harvesting, storing and analyzing these data must be taken into consideration.

We have designed and built a low-cost automated imaging system in-house as a part of our laboratory's structural genomics platform, as described in detail by Su *et al.* (2006). The aim has been to increase throughput and collect crystallization data into an online database. The system is logically and mechanically simple, but leaves room for further additions and developments. The basic idea for designing such a system is 'simplicity = robustness = low cost'. The system accepts SBS footprint microplates (ANSI/SBS 1-2004; American National Standards Institute/Society for Biomolecular Sciences, Danbury, USA), making it compatible with many other automated systems. The software is developed in-house and consists of robot-controlling software and an image-management system.

Developing the system and software allowed customization to our individual requirements and the opportunity to try different kinds of crystallization monitoring systems. Apart from the automatic image collection of hanging/sitting-drop crystallization experiments, functions for collecting images for crystal-growth movies from parallel experiments on a single microplate have been explored. Another considerable benefit has been the full freedom for system integration into our laboratory information-management system (hereafter referred to as LIMS), which was also developed in-house (Su *et al.*, 2006).

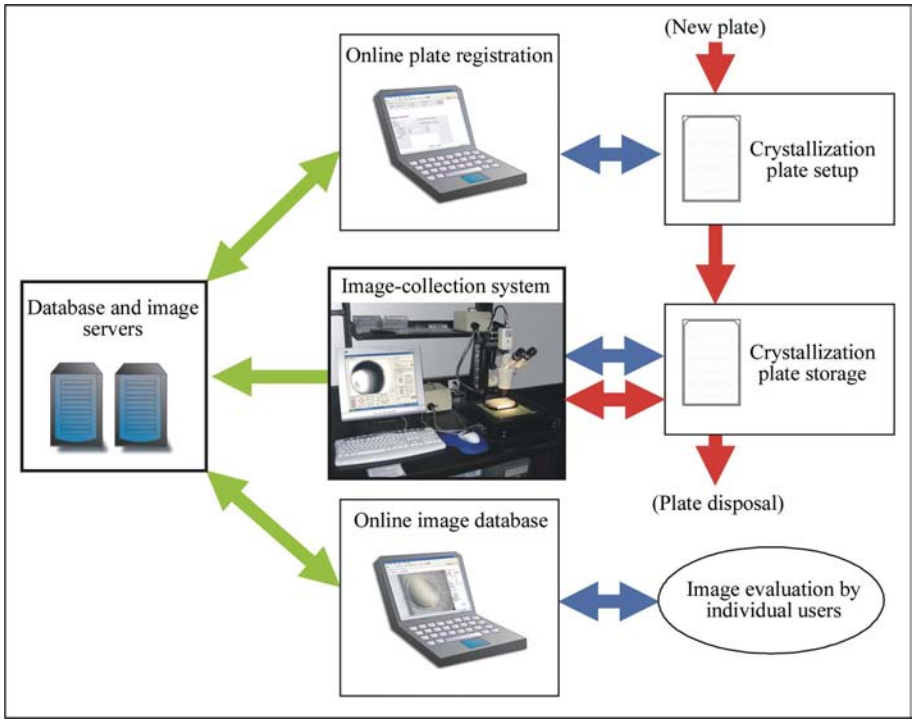


Figure 1
System overview. The red arrows show the physical movement of the microplates throughout the crystallization experiments. The blue arrows show the user interacting with the imaging system either *via* a web interface or by using the imaging robot, represented by the picture in the middle. The green arrows show the flow of image and database information between the imaging robot, user computers and servers.

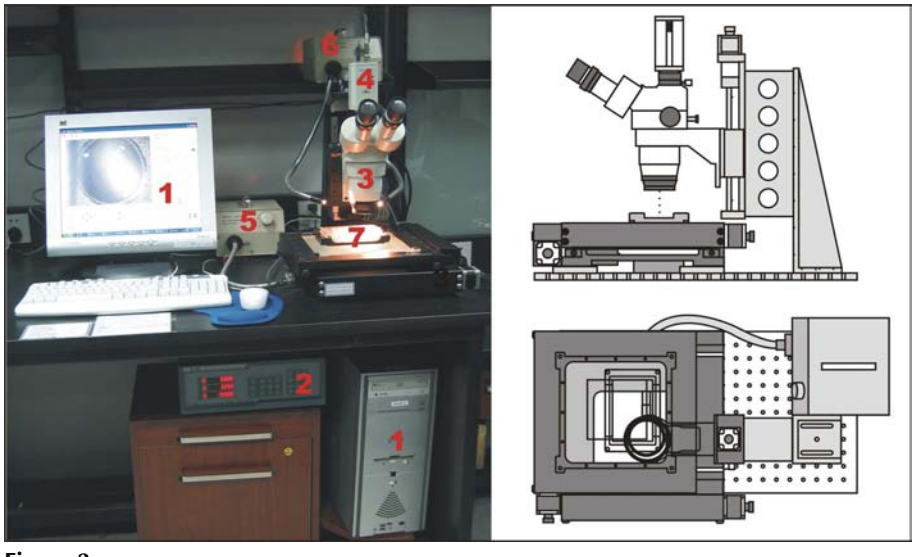


Figure 2
Imaging-system components. 1, Computer and monitor; 2, stepper motor controller; 3, microscope; 4, CCD camera; 5, bottom light source; 6, top light source; 7, SBS plate holder and microplate. The robot schematics on the right show a more detailed view of the system. For clarity, the side view is displayed without a light source and the top view without a microscope and camera.

2. Materials and methods

2.1. Automatic imaging system overview

As shown in the system overview (Fig. 1), the imaging system is mainly composed of two subsystems: the image-collection system and the image-management system.

Using the imaging robot and its controlling software, the image-collection system automatically collects images from crystallization experiments set up on SBS footprint microplates.

The image-management system gives the user access to the collected images through a web interface and also handles the experimental information provided by the user. Physically, the system consists of two servers connected to the laboratory local network and to the internet.

This logical division of the system makes it possible to have multiple image-collection systems connected to one image-management system.

2.2. The imaging robot

The imaging robot was designed and assembled in-house. It contains the following components (Fig. 2): a Zolix SC300-3A stepper motor controller (factory upgraded to SCA300-3B), a Zolix TSAW100x100-XY-A translation table (Zolix, Beijing, People's Republic of China) with a custom frosted glass plate with mounted Beckman SBS plate holder (Beckman Coulter, Inc., Fullerton, USA), a Zolix TSA100-B Translation stage, a Motic SMZ-168TL microscope with an added 2× additional objective and 2× photo adapter (Motic China Group Co. Ltd, Xiamen, People's Republic of China), an Ikegata Ik-CP450 1/3′ Color Digital CCD PAL camera (Ikegata Corporation, Japanese Electronic Components, Japan), custom spacers, two multi-position fibre-optic illuminator systems (Motic China

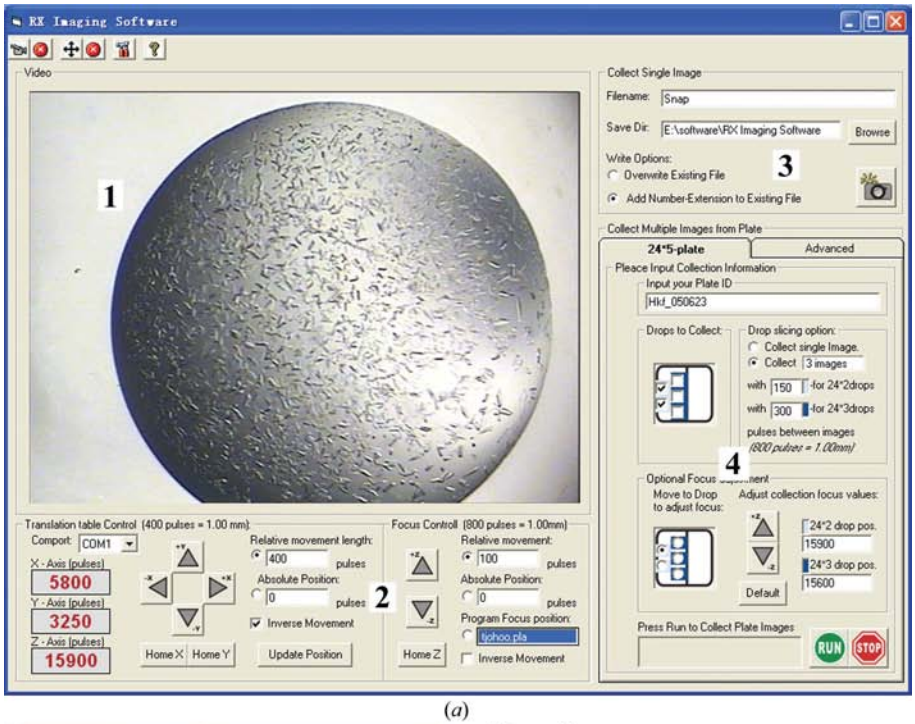
Group Co. Ltd, Xiamen, People's Republic of China) and a Windows PC with a Imavision CG300 video input card (China Daheng Group Inc, Beijing, People's Republic of China).

The stepper motor controller is connected to the computer through the RS-232 serial port and the CCD camera through a 75 Ω BNC coaxial cable connected to the video input card. The TSAW100x100-XY-A translation table has a 100 × 100 mm travel range (custom upgradeable to 110 × 110 mm to enable sample collection from any standard SBS microplate), a smallest step size of 1.25 µm and a repeatability accuracy of 3 µm. The TSA100-B translation stage has a 100 mm travel range, a smallest step size of 1.25 µm and a repeatability accuracy of less then 3 µm.

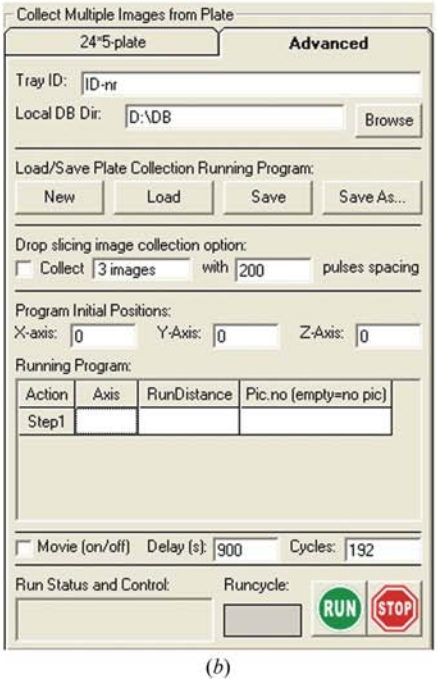
2.3. Robot software

The imaging robot-controlling software has a windows-styled graphical interface (Fig. 3) and was developed in Visual Basic (Microsoft Corporation, Redmond, USA). A user can input collection strategies, running programs for automatic image collections within the permitted range of movement, enabling the system to theoretically work with any SBS sitting-drop microplate (Fig. 3*b*). The software can control all settings of the stepping motor controller directly from the program interface. The controlling software is independent of the crystallization database software, but by providing the microplate ID before the automatic image collection, the database will locate the image file.

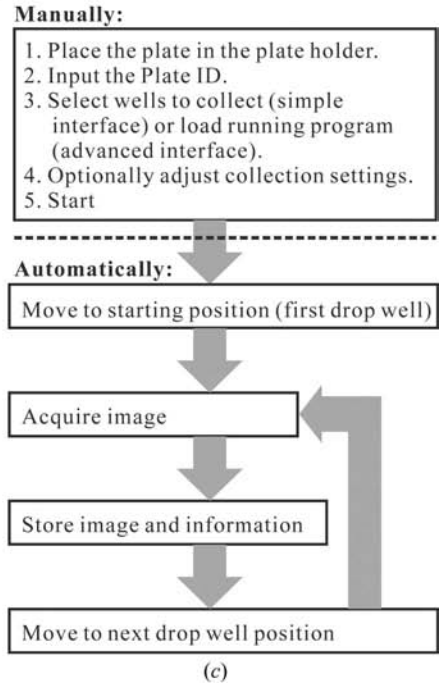
The software video window can display a live video stream from the microscope CCD camera. The software incorporates a snapshot function, which enables the user to take pictures



(*a*)



(*b*)



(*c*)

Figure 3
The main window of the robot controller software (*a*) consists of five parts: 1, the video window; 2, the user manual control interface; 3, the snapshot window; 4, the automatic collection program interface (simplified interface selected; the advanced interface is shown in Fig. 3*b*); 5, connection, settings and help buttons. The advanced collection interface (*b*) can be used both to start a plate-collection program and to input a new one. The running program variables are start position (x, y, z), relative positions for the movement program, image-collection option linked to the drop well name, slicing option with slicing distance setting and collection run cycling with a delay (for movie functions). Variables such as initial translation velocity, constant translation velocity and translation acceleration are set for each individual axis (x, y, z) in the settings menu and are not specific to the running program. A simple collection scheme (*c*) illustrates the actions of the user and the robot during a collection run. The user has to place the plate in the correct orientation in the plate holder, manually input the plate ID and mark which wells to collect (or load the appropriate image-collection program in the advanced collection window) and press the 'Run' button (labelled 4 in Fig. 3*a*). The robot will follow the selected image-collection program and move to the starting position. It will capture an image at the first well position and save it, with plate ID, drop well name, date and time information in a shared *SAMBA* directory. It will then move to the next image-capture position listed in the collection program and repeat the procedure until the running program has finished.

manually. It also incorporates a movie function for parallel monitoring of crystal-growth experiments on a single microplate.

2.3.1. The image-slicing function. The option of capturing several images from the same drop at different focusing positions, referred to as slicing, is included in the software. During an automated image collection, the focus position is based on the software's focus settings for the microplate type used in the experiment, possibly with an optional user adjustment for the specific collection. The slicing function is to ensure that focused images will be collected even if the software settings for a microplate differ slightly from the physical positions of an individual microplate or if any pitch in the translation system affects the focusing position. 'Slicing' instead of 'auto-focusing' is one of the features that makes this system simple and fast yet robust.

2.4. The image-management system

To maintain the collected images and make them accessible to users, a web-based server has been developed. Users can reach images and related information *via* an internet connection or the laboratory local network. The server is mainly composed of two parts: experimental data management and user management. The experimental data-management part not only records images, plate information and evaluation of images from the user, but also organizes information and generates statistics automatically (Fig. 4). To avoid users accidentally changing other users' data, user management was enforced on the server. This also enhances the safety of the system. Except for the administrator, who has all rights to access any part of the system, only the user that registered a specific microplate experiment can update or delete its information from the database. We have, however, made it possible for all users to browse all available images. The server system can work as a stand-alone system, but we have chosen to incorporate the user-authentication function under our web-based LIMS (Su *et al.*, 2006) to obtain the benefit of using our laboratory's existing user identities.

Running on a Linux Fedora Core 1 computer, the image-management system uses Apache HTTP Server v.2.0 (The Apache Software Foundation; http://httpd.apache.org/) for http support, a MySQL 5.0 database (MySQL AB, Uppsala, Sweden; http://www.mysql.com/) to provide secure database access and a PHP5 script (The PHP Group; http://www.php.net/) to build the web interface to communicate with the database. Considering the large number of data generated by the image-collection system, the storage was set up stand-alone on another Linux Fedora Core 4 server. *SAMBA* (The

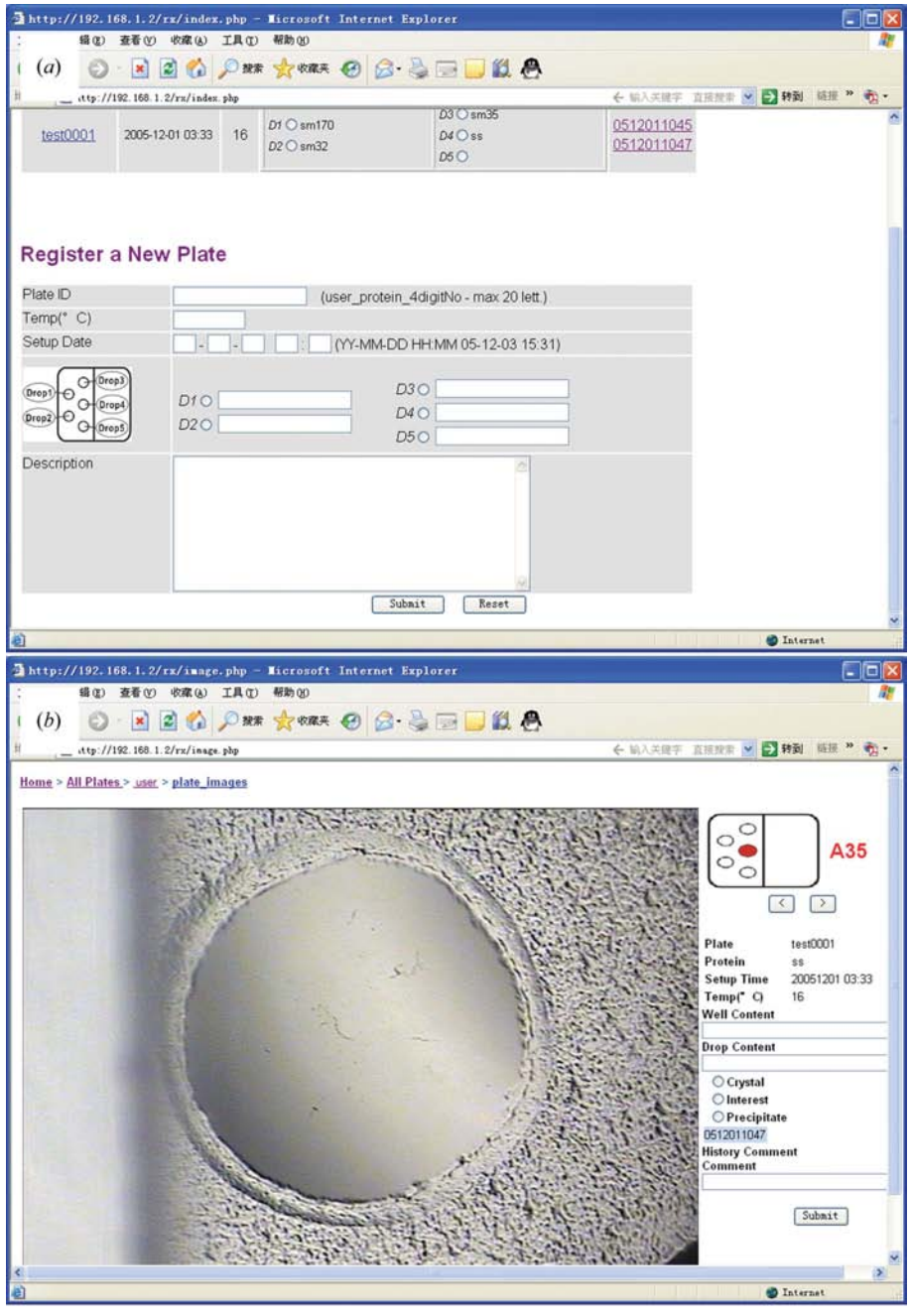


Figure 4
The image-management web interface allows the user to register new microplates for crystallization experiments and handles the information provided by the user. The plate-registration window (*a*) is designed to be quick to use and requires very little information input. The plate-images window (*b*) enables the user to view collected images from all collected microplates and to add information to the plates registered with the user's own user identity.

Samba Team; http://www.samba.org/) was used to share files between the storage computer and the robot's Windows computer. All the software used under Linux is completely free. It is also possible to transplant the whole system into a Windows environment without making any major changes to the source code.

3. Results and discussion

3.1. Image-collection system

The function of the image-collection system is to help users collect images from SBS microplate crystallization experiments in a simple way and to transfer them into the image-management system for subsequent evaluation. The image quality must be good enough to evaluate the crystallization experiments, as shown in Fig. 5.

3.1.1. The imaging robot. The movement range, smallest step size and accuracy of the *xyz* translation have proven to be good enough to capture images from the various SBS microplates used for crystallization experiments. The visual area, which is between 10.4 × 7.8 mm (minimum zoom) and 1.65 × 1.24 mm (maximum zoom), has proven to be sufficient for the collection of crystallization experiments in which total drop volumes ranging from 1 to 4 µl have been used. Since the zoom must be adjusted manually, a standard zoom setting is used for the automatic collection programs. The optics of the system consist of a stereo microscope with a CCD camera using a mono channel so that the sample is viewed from a small angle. When the focus distance is changed, this angle will result in a slight sideways movement of the focus position. This has not proved to be a problem so far, since the collected images have a 4:3 ratio (768 × 576 pixels, width × height) and a little sideways movement has little impact on clearly imaging the drops. However, a mono-optical system will be used in the system for subsequent versions of the robot.

The collection time for a 96-well plate is about 2 min if one image is taken for each well position.

3.1.2. Robot-controlling software. The robot-controlling software has been designed to be simple to learn and use once the user has gained a general understanding of the system as a whole. The live video stream from the CCD camera makes controlled manual movement in the *x*/*y*/*z* directions straightforward and the snapshot function enables manual image collection with 'what you see is what you get' quality.

The simplified interface for an automated collection lets the user start an automatic collection for the most commonly used microplate type in the laboratory in a few simple steps (the interface can be seen in Fig. 3). Trial runs have proven that the whole system is quite simple to learn and use. The advanced collection interface lets the user load an existing collection program or input a new custom collection program for new plate types or for custom collections.

Since the software uses fixed positions for image acquisition, it enables the user to visually inspect and adjust the starting position and initial focus before starting an automatic image collection. Apart from when a microplate is damaged or skewed, we have found that this approach performs well.

3.1.3. Image quality. The PAL-quality CCD camera offers a resolution of 768 × 576 pixels. The camera can stream live video to the software. The captured images are saved directly from the video stream buffer as Windows bitmap images (Microsoft Corporation, Redmond, USA) with a file size of about 1.3 Mb. If batch compressed to JPEG format, the size will be reduced to 50–150 kb, depending on image characteristics and the compression ratio. The contrast and white balance is adjusted automatically by the CCD camera.

The resolution of the images is lower than that of many modern digital cameras, but for most experiments we have found it can provide enough information for the user to be able to evaluate the experimental data. We have found that the contrast, light sources and focus of the images are of greater importance (Fig. 5).

3.1.4. Slicing option. The slicing option enables the user to obtain a few focused images of each drop, even if the microplate height is uneven. With the standard slicing distance settings, it is unusual with our system that the user does not obtain at least two useful images out from the three collected from each drop.

The greatest advantage of the slicing method, however, has proven to be the

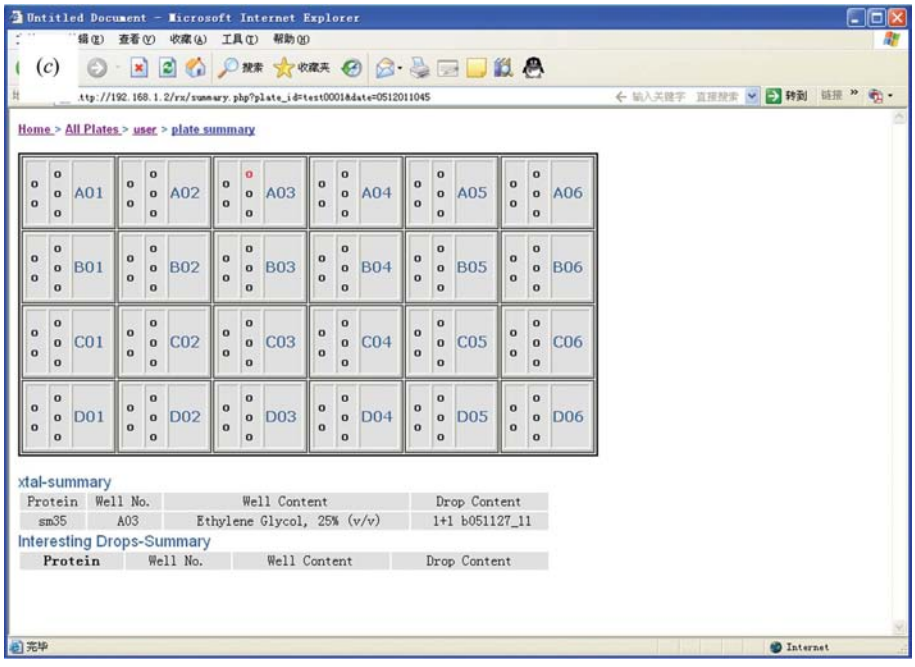


Figure 4 (continued)
(*c*) Plate information can be visualized in a summary window.

ability to collect more information from a drop than a single 'auto-focused' image would when the depth of the drop is greater than the focusing depth (Fig. 6).

3.2. Image-management system

By incorporating the image-management system into the in-house-developed LIMS system, users have been able to reach and use the image-management system through the laboratory web page with existing user identities. This, together with the simple web interface, has helped laboratory members to quickly learn and adapt to the system.

The web pages for evaluating the collected images perform very well when accessed from a computer connected to the laboratory's local network. Image browsing has very little lag time, making it convenient to quickly go through crystallization results. This is a consequnce of the laboratory local network using 100 Mbit switches and 1000 Mbit connections to servers.

The amount of information the user needs to input is low, making the experiments very easy and quick to register and follow. Most users have simply chosen to register an information summary for the whole crystallization experiment at microplate registration (such as protein information and screen type) and thereafter only add additional information to drops that show interesting results.

Figure 5
Sitting drop (2 µl) image collected by the imaging system at PAL resolution (768 × 576 pixels).

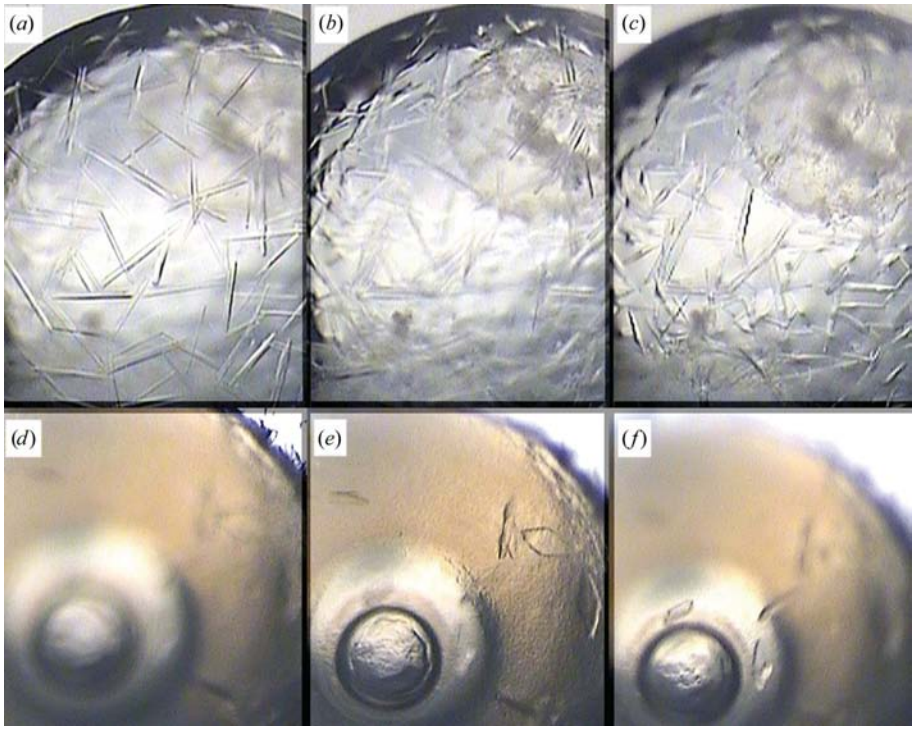


Figure 6
Two examples of the slicing function. (*a*), (*b*) and (*c*) show three images displaying part of the same hanging drop collected with the slicing option on and a focus change of 188 µm between images. The first image (*a*) shows the crystals growing close to the glass in the top of the drop. (*b*) shows crystals growing on the side surface of the drop and that there are far fewer crystals in the middle of the drop. (*c*) shows crystals at the bottom surface of the drop. (*d*), (*e*) amd (*f*) show three images displaying part of the same sitting drop, collected with the slicing option on and a focus change of 313 µm between images. (*d*) is focused on the top of the sitting-drop well, above the actual drop. (*e*) clearly shows crystals growing on the side of the drop well and the bubble in the bottom. (*f*) displays crystals in the bottom of the drop well.

3.3. Outlook and improvements

The robotic system has been developed to be low-cost and robust. Obvious improvements could be a low-noise higher resolution CCD camera and higher quality mono optics. The system has now been running for a testing period as an integrated part of our laboratory. We have found the greatest limitation to be that the microplates need to be exchanged manually, forcing users to be in the vicinity of the robot while scanning the microplates. An automated microplate-exchange system with simple microplate storage would greatly increase the system's performance.

Currently, the user inputs the microplate ID by hand while starting the automatic image collection. Typing errors could prevent the user from accessing the collected images through the image-management system. Integrating a barcode reader and printer into the system would reduce human error and enhance system performance.

The image-management system's performance is greatly dependent on the speed of the network it is accessed through. A choice of viewing the collected images at a lower resolution would make it more convenient for a user to browse images with a low-speed connection.

The web interface for microplate registration and image evaluation could be made more advanced. If it incorporated functions for users to select or input all crystallization conditions in a simple way for the whole plate, the

database could also harvest more detailed information about negative crystallization results, possibly helping to optimize crystallization screens (Rupp & Wang, 2004; Page & Stevens, 2004).

4. Conclusions

An in-house automated imaging system has been designed and built using mostly commercially available components. It is a low-cost solution that has been integrated into a university genomics platform (Su *et al.*, 2006). Images from a standard 96-well SBS microplate can be collected in 2–6 min, depending on the selected collection strategy. The collected images are automatically transferred to an imaging server. The image-management system is reached through the in-house-developed LIMS, which quickly made it an integrated part of the laboratory environment. The image-management system allows users to follow, evaluate and summarize crystallization experiments through any modern web browser. The system has greatly reduced information loss from crystallization experiments in a high-efficiency structural genomics laboratory environment. With an added automated plate-exchange system, it should also greatly increase the throughput of crystallization experiment evaluations.

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