SIMPLE ROBOTIC IMAGER FOR CRYSTALS

User Manual

ABSTRACT
We describe here a procedure for using the IMB designed home-built Simple Robotic Imager For Crystals to collect images from a 96-well crystallization plate containing screen solutions and a macromolecule.

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User Manual
Simple Robotic Imager For Crystals: Image acquisition and visualization

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Introduction

In 2014, IMB acquired ARI Crystal Gryphon, an instrument for setting up crystal plates. This automation eliminated the manual setting of crystallization screens. It also increased the throughput since we went from 24 well VDX plates to 288 well ARI Intelliplates. The increase in throughput directly increased the number of wells to be examined. So manual inspection of ~1mm diameter 288 wells became tedious and error-prone.

So, in late 2014 and early, I have designed and built an imager named Simple Robotic Imager (SRI) to automate the image acquisition. You can learn more about the Simple Robotic Imager and its capabilities.

We describe here a simple procedure for using the IMB designed home-built Simple Robotic Imager for collecting images from an ARI IntelliPlate containing screen solutions and a macromolecule you are crystallizing. The acquired images will be stored in a directory name with the prefix “images-“ followed by time stamp of data collection. Alternatively, you can specify the name of the image directory. All these steps are done in prism.biophysics.fsu.edu, a Linux computer that is connected to the robot. Ensure that you have a Linux account in prism; otherwise, talk to Michael Z. and get an account before proceeding.
Preparations

Now make sure the following conditions are met:

- The power cable for Arduino is plugged into power strip and switched on.
- The Arduino USB cable is connected to both the Arduino and prism computer.
- The microscope USB cable is connected to the microscope and to prism computer.
- Decide whether you are collecting 96-well-3-reservoir or 2-reservoir plate.

Upon completing these steps, one can proceed to setting up the plate for imaging.

Hardware Set-up for Imaging

Intelliplates have eight rows and twelve columns. The rows are labelled A to H (from top to bottom). The columns are labelled 1 to 12 (from left to right). Depending upon the catalog number, Intelliplates can have 2-wells, 3-wells. By default, Intelliplate’s A1 corner (top left) is designated as reference point.

Match Intelliplate’s A1 corner with Robot’s A1 corner. Make sure NOT to touch the USB Microscope, disturbing the microscope position makes it hard to get proper alignment of the robot. Place the 96-well-3-reservoir or 2-reservoir plate with A1 corner on the top left hand side on the IMB Robotic Imager’s X-Y stage and secure it the holding clips, making sure NOT to disturb the microscope. It is okay to move the microscope up or down using the knurled knob but avoid touching the microscope itself.

The picture on the left shows the plate before it is secured (microscope is way out in H1 corner and above). The picture on the right shows the plate after it has been secured with the holding clips. Note that now the ARI Plate Edge and X-Y Stage Edge are closer together.
Now, bring the USB camera down using the focusing knob to about 15 mm above the top of the plate. There is no need (or way) to focus it now. We will do it bit later.

**Software Set-up for Imaging**

Now create (`mkdir`) and move (`cd`) to the directory where you want to store your images. Then copy all the files from the following directory to the directory you just now created using the command below:

```
    cp -p /home/soma/Robot/RobotFiles/* .
```

Once all the files are copied, look for (using the command, `ls`) the following files: `preview.py`, `collect96s3`, `collect96s2`, `plateview.sh`. If you do not see these files then make sure, your copy command above worked correctly or consult Soma for further instructions. If you have all the files, you are good to go.

**Preview and focusing**

Depending upon how the last data collection ended, the microscope could be in one of the several positions. However, we need to focus it before we begin the current data collection. So issue the following command in the current working directory:

```
./preview.py
```

This will open a new window in the computer named “racspi preview” and the user will see an out of focus image on the computer screen from one of the wells (see the Figure below on the first row on the left). Now slowly bring the microscope down (to ~5mm above the plate surface) using the focus knob until the image on the computer screen becomes sharp (see the Figure below on the second row on the right). Close the preview window, by clicking the “X”
<table>
<thead>
<tr>
<th>Out of focus image on screen</th>
<th>Microscope out of focus (&gt;18mm gap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope in focus (~5mm gap)</td>
<td>In focus image on screen</td>
</tr>
</tbody>
</table>

This completes the previewing and focusing of the microscope and we are ready for the actual data collection.
**Image Data Collection**

Now we are ready to collect the image data. So, depending upon the 96-well plate (either a 3-reservoir or a 2-reservoir plate) issue the command below (here we are assuming the plate is 96-well 3-reservoir plate):

```
./collect96s3 [or ./collect96s2, if 96-well 2-reservoir plate]
```

The images will be written into a default directory with `images-YYYY-MM-DD-HH-MM-SS` name format.

One can also issue the same command with specific image directory. In this case, the images will all be written into this directory rather than default directory `images-YYYY-MM-DD-HH-MM-SS` format.

```
./collect96s3 TS-CCS01-day00 [here image_directory is specified]
```

The X- and Y-axis motors will engage and will move the plate’s A1 corner right underneath the microscope, if needed, at this point quickly recheck the focus. We should begin the data collection now. However, if you hear a noise from the motors, immediately disconnect the power to Arduino and contact Soma. Note that this is IMB designed and home-built machine and will occasionally misbehave. If everything works okay, the user should see images being acquired one column at a time and the plate moving in a snake like pattern (down, side-ways, up, side-ways, down, etc.). The position names of the reservoirs will be annotated on top of the image, like A01S1, A01S2, A01S3, A02S1, A02S2, etc. After about 11 minutes (for is 96-well 3-reservoir plate), all the 96x3=288 well images would have been acquired and the user will see the “done collecting” message on the screen and microscope should turn off.
Viewing Images

Upon completion of the data collection, simply move the microscope up and away from the plate, remove the plate, and store it. Now, we are ready to view the collected images. The easiest way is to view all the images with layout similar to how the plate has been set-up. In order to do it, issue the command shown below replacing {directory name} with your directory:

```
./plateview.sh {directory name}
```

This will open a new window with a name “feh [thumbnail mode]” and the user will see all the images collected during the data collection run. Clicking any individual image will open another window with zoomed up image of that individual image with the reservoir name as the window name, say A01S1.

The Full view can be manipulated the following ways:

- Using the computer’s Number Pad
  - Up (#8) and Down (#2) arrows will scroll the images up and down. Left (#4) and Right (#6) will scroll the images left and right.

- Using the computer’s Up and Down Arrows (not the one in the Number Pad)
  - Zoom up and zoom down the images

Special Collection

If you want to collect full-plate of images repeatedly, say every one-hour and you want to do it for 12 hours; you can issue the following command:

```
./collect-loop 60 12 [it will collect every 60 minutes 12 times]
```
Customized Views

Intelliplates have eight rows and twelve columns. The rows are labelled A to H (from top to bottom). The columns are labelled 1 to 12 (from left to right). Depending upon the catalog number Intelliplates can have 2-wells, 3-wells. Intelliplates have

![Intelliplate numbering scheme. Rows: From A to H; Columns: 1 to 12](image)

Intelliplate 3-well  |  Intelliplate 2-well

If you want to see only **Row A** all **sub-wells S1**, issue the following command (this will be top row from left to right):

```
feh --thumbnails --thumb-width 100 --thumb-height 75 -W 1200 images-directory/A*S1.png
```

![All Row A all sub-wells S1 (top row left to right)](image)

As the image above shows, it will display all S1 wells from left to right, 12 of them in a row similar to plate layout.
If, however, you want to see only **Row A to H** all **sub wells S1**, issue the following command (this will be left most column from top to bottom):

```
feh --thumbnails --thumb-width 100 --thumb-height 75 -H 800 images-directory/*01S1.png
```

![All Column all sub-wells S1 (Left column top to bottom)](image)

As the image above shows, it will display all S1 wells from top to bottom all 8 of them in a column similar to plate layout.

If, however, you want to compare two sets of images collected in two different times, and assuming you want to compare **only Row A** all **sub-wells S1** from **day 1** and **day 2**, issue the following command (here we are assuming image directories varies by the “date”):

```
feh --thumbnails --thumb-width 100 --thumb-height 75 -W 1200 images-2017-04-2*/A*S1.png
```

![All Row A all sub-wells S1 (top row left to right) (day 1 and day 2)](image)

Optionally, if you want to write an image of the files you are viewing you could add **--output file_name.png** to create a file.

```
feh --thumbnails --thumb-width 100 --thumb-height 75 -H 800 --output ColA-H_Sub1.png images-directory/*01S1.png
```

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