

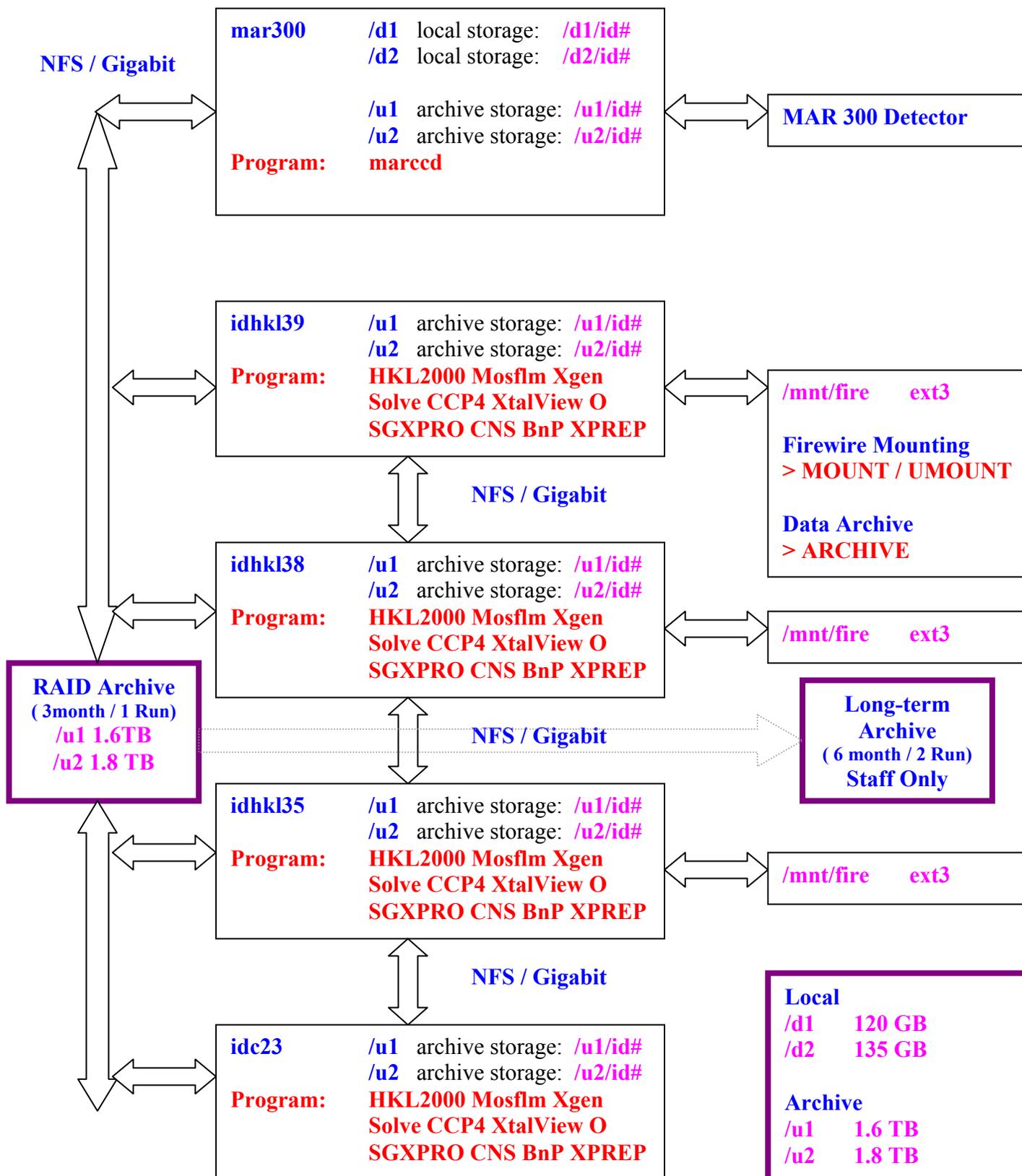


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Computer Disk Structure



Experimental Procedure

1. Log into MAR computer **mar300** (IP: 164.54.208.63) with assigned username and password.
2. Start MAR program. Type: **marccd**
3. Create user main experimental data storage directory in both local drives (**/d1/id# or /d2/id#**) and archive drives (**/u1/id# or /d2/id#**) with following example format:
Local data: **/d1/id1/09_17_04_SERCAT**
Archive data: **/u1/id1/09_17_04_SERCAT**
Note: make sure you have enough disk space in local drives
4. Mount user firewire drive on beamline computers by following **Firewire Drive Mounting Procedure**.
 - a. **ext3: idhkl35, idhkl38, idhkl39**
 - b. **Staff can help reformatting user's vfat drive to ext3 format.**
5. Start automatic data archive script from the machine with firewire drive mounted by following **Data Archive Procedure**.
6. Change beam energy/wavelength and align beamline by following **Energy Change and Beam Alignment Procedure**.
7. User should log into any computer with the same assigned user name and password.
8. Any individual user can access only the directories with the same ownership in all the disks. For example, user ID1 can only access **/d1/id1, /d2/id1 and /u1/id1, /u2/id1** for data storage. User should set **/d1/id# or /d2/id#** as **data** directory, and set **/u2/id#** as **archive** directory in data collection setup (**marccd** program)
9. After finish experiment and data processing:
 - a. Stop data archive script with CTRL^C by following **Data Archive Procedure**.
 - b. UMount firewire drive by following **Firewire Drive Mounting Procedure**.
10. User's data will be kept in **/u1 or /u2** RAID archive disk for about **3 months (1 run)**. At the same time, the same data will be archived by SERCAT staff to the **long-term storage** disk (staff access only) for about **6 months (2 runs)**.
11. User with current beamtime has the priority for using all the computers on the experimental floor.
12. **User should NOT install any programs, change computer settings, delete other user's data, or reboot computers without staff permissions.**

Firewire Drive Mounting Procedure

1. Four beamline data processing computers (**idhkl38, idhkl39, idhkl34, idhkl35**) have the capability for firewire drive mounting. The Firewire drive mounting file systems were preconfigured and are displayed in login window of each machine. User should **ONLY** mount correctly configured firewire drive to the corresponding machines.

2. From beamline staff and users' experience, we found out that Firewire drive with **vfat** file system is very unreliable and easy to corrupt.

To protect user's precious data, we will only support user's Firewire drives with file system **ext3.**

3. **idhkl35, idhkl38, and idhkl39** are configured to mount firewire drive **ONLY** with file system **ext3**.

Mounting point: **/mnt/fire**

Mounting steps:

- a. To mount: **\$ MOUNT** (Notice: Upper case cmd and follow instruction)
- b. To umount: **\$ UMount**

4. **Procedure to reformat user's firewire drive to ext3.**

- 1) Login as **root**.
- 2) Connect firewire drive to power supply and wait for the front light turn to green.
- 3) Connect firewire cable to the computer.
- 4) **% rescan-scsi-bus -r** # clean up the scsi bus
- 5) **% /sbin/fdisk /dev/sda** # reformat the drive
 - d** # delete old partition
 - n** # ask for a new partition
 - p** # specify a primary partition
 - 1** # pick cylinder 1 to begin with
 - w** # write out the new partition table
- 6) **% /sbin/mke2fs -j -m0 -LNAME /dev/sda1** # make a filesystem

Experimental Setup

1. Goniostat mounting stage **Z-translation** range is about **15 mm**. Hampton **CrystalCap Copper Magnetic** cap is recommended. The pin length should be **12 ~ 21 mm**. For details, please refer to **Requirement of Cryo-pins**.
2. All the Cryo Tools and microscopes should **NOT** be removed from beamline.
3. SERCAT provides member users the necessary **Hampton Research Heavy Atom Kits** for basic heavy atom soaking. It is strongly recommended that user pre-freeze derivatized crystal in user's home laboratory.
4. User should handle all the heavy atom reagents with the most caution and strictly follow **Standard Operating Procedure for Preparation of Heavy-atom Reagents in the SER-CAT Laboratory B030**. Please contact staff if user needs any assistance.
5. Cryostream / Cryojet system has been set up for automatic LN fill up and described in **Cryosystem Operation**.
6. Detector distance is set from between 90 mm to 1200 mm with collision switches to protect CCD detector. User should not try to change the setting of collision switches. To move detector distance less than 80 mm for high resolution data, please contact CAT staff.
7. To setup experiment for **Sulfur Phasing** at 2 Å wavelength, Helium path is very helpful to reduce absorption and yield better data quality. Please contact CAT staff for Helium path setup.
8. By default, the beam size (or slit size) has been set as 100 x 100 μm, which should be appropriate for most experiments and crystal samples. In some cases, user might want to reduce background for small samples by reducing slit size. User should take precaution to align the beam, goniostat and crystal carefully.
9. For MAD experiment or changing wavelength, please follow **Energy Change and Beam Alignment Procedure**.

Energy Change and Beam Alignment Procedure

[Any energy change between 6.2 Kev (2 A) and 13.5 Kev (0.92 A)]

- 1) Change energy from **sergui.py** window.
- 2) Enable the experimental hutch and open safety shutter.
- 3) From **sergui.py** > **hutch** tab, run following optimizations.

Optimize Tune

Program will automatically run scans until the Gaussian peak is found.
Program will automatically move to optimized peak.
No user interaction is necessary.

Optimize Horizontal Slit

Program will automatically run scans until the Gaussian peak is found.
Program will automatically move to optimized peak.
No user interaction is necessary.

Optimize Vertical Slit

Program will automatically run scans until the Gaussian peak is found.
Program will automatically move to optimized peak.
No user interaction is necessary.

If user wants to do large energy change, for example, from 12 keV to 6 keV, user may continue

- 4) Put on the phosphor ball provided by CAT staff and align the ball to the center.
- 5) Tweak the goniostat position to bring the spindle center to beam center
 - i. Reduce slit size to 20x20 and put in some filters to have better view of the beam center profile.
 - ii. From **motor**> window, type command

```
motor> mrel g_hor 10 (+/- 10 to +/- 100 movement)
motor> mrel g_vert 10 (+/- 10 to +/- 100 movement)
```
 - iii. After align the beam center to the center of cross box, close the timing shutter, increase slit size to normal 100, and set filter to desired number

MAD Experiment

1. Change the energy to the desired heavy atom absorption edge (K_{α} or L_3) energy, which must be between **5 Kev and 20 Kev** and align the beam by following **Energy Change and Beam Alignment Procedure**.
- 6) Put on sample crystal and align it.
- 7) Make sure the Rontec fluorescence detector is aligned (by default) and power supply is **ON**.
- 8) Run fluorescence scan:
 - 1). Change window to **sergui.py>MAD** tab.
 - 2). From **Periodic Table**, choose the desired heavy atom and correct edge.
 - 3). Change the foil number to 25.
 - 4). Click “**Start Test**” to test detector scale. (Notice: this step will open the timing shutter automatically and sample crystal will be exposed by X-ray attenuated by 25 foils.)
 - 5). Change the foil number to make the real time reading of detector on text display of **MAD** window stay under 60,000. Then click “**Stop Test**”.
 - 6). Click “**Run Scan**” and wait until the scan is done (about 1 minute) and the scan plot is displayed. Timing shutter will be automatically closed after scan.
 - 7). Click “**Analysis**” to run **Benny-Chooch** programs.
 - 8). **Benny-Chooch** window will start automatically to calculate f' and f'' . f' and f'' plots will be displayed for the scan.
 - 9). Close the plot window and “**Hit return to Close**” the **Benny** window. The calculated inflection wavelength, peak wavelength and their f' and f'' will be automatically updated and displayed to the **sergui** window. Type in remote energy about 50 – 100 eV beyond the edge energy in the window to calculate its f' and f'' .
- 9) Change the foil number to desired number for real data collection.
- 10) Setup data collection with calculated inflection, peak and remote energies.
7. After data collection and processing, use the program **XPREP** to calculate Patterson map by following **Patterson Map Calculation with Program XPREP**.
8. To search multiple heavy atom sites, user may use following programs by **Crystallographic Software**.

sgxpro
BnP

run either solve or shelxd to search heavy atom.
run PHASES and SnB packages GUI

Data Collection

1. Run **marccd** program from **mar300**, user ID1 should set up data storage as following format:

Main data storage: `/d1/id1/09_15_03_UserInstitutionName/xtal` or
`/d2/id1/09_15_03_UserInstitutionName/xtal`

Archive: `cp %s /u1/id1/09_15_03_UserInstitutionName/xtal` or
`cp %s /u2/id1/09_15_03_UserInstitutionName/xtal`

2. Check available space in both **/d1 (or /d2) and /u1 (or /u2)** and make sure you have enough space for your data.
3. Before user (**ID1**) start data collection, **create all the directories** you plan to store your data in both **/d1/id1/09_15_03_UserInstitutionName/xtal (or under /d2/id1)** and archive disk **/u1/id1/09_15_03_UserInstitutionName/xtal**.
4. If you don't have enough space in both drives and data does not belong to you, contact beamline staff.
5. If you have more than one day beamtime, you may fill up those drives during your experiment. It is your responsibility to backup your data from those drives and clean up the drives (**/d1 and /d2**) for further data collection. Your archive data will stay in **/u2**.
6. For MAD experiment, make sure the three wavelengths were set correctly for data run.
7. For MAD experiment, set data collection sequence in the order of **Peak, Peak with inverse beam, Inflection, Inflection with inverse beam, Remote**
8. If the beam is dumped during normal data collection,
 - 1). Click "STOP" and pick "Stop after current frame..".
 - 2). After beam current is restored, open the A shutter on PSS manually.
 - 3) Set the correct next frame number and click "Start" to continue data collection.

Example:

 - If beam was dumped at frame #90, user noticed and stopped data collection at frame #94, then next frame number would be #95.
 - Frame 90 to frame 94 are empty frames.
 - After beam is restored, set the next frame number to 90 to start from the first empty frame. User should not change other Phi, Omega, Kappa values.
 - After click "Start", there will be two error messages shown up, click "Continue anyway...." to start.

Data Reduction

XGEN, HKL2000, MOSFLM were installed in data processing computers **idhkl35, idhkl38, idhkl39, idc23**

Run XGEN:

From data processing computer:

```
bash> cd data-directory           # change into data directory
```

```
bash> xgenproc [-dns] [-cfhipxy<val>] [dirn]
```

i.e. d,n,s are Booleans and the other - options all take values

and an option that doesn't begin with a minus is a directory name

```
-c*)    # specify the number of spots to use
-d*)    # compress images after processing
-f*)    # specify a sample image name
-h*)    # specify the last image number to use
-i*)    # specify an International Tables #
-n*)    # means do NOT recenter after indexing
-p*)    # specify an image prefix
-s*)    # flip the sign of the stepsize
-x*)    # specify the beam Xcenter
-y*)    # specify the beam Ycenter
*)      # specify the data directory
```

for example:

```
bash> xgenproc -x112.5 -y113           # reset beam center to (112.5, 113)
```

1. XGEN will create directory **xgena** (or **xgenb, ... xgenz**) under data directory.
2. XGEN will automatically run through

Peak search
Autoindexing
Integration
Scaling

3. Even though XGEN can autoindex single image frame as what HKL2000 does, it will work better with multiple data frames (consecutive or separated).
4. XGEN is sensitive to the beam center for successful autoindex. User may change beam center to correct position with parameters.
5. XGEN will create log file **datafilename.xlg** under **xgena**
6. XGEN will create **datafilename.sca** output data as **.sca** format
7. XGEN will create **datafilename.asc** output data with anomalous ON as **.sca** format

Data Reduction

Run HKL2000:

From data processing computer:

```
bash> cd data-directory # change into data directory
```

```
bash> HKL2000
```

If user wants to use special scalepack for different use, please contact staff

```
scalepack16m  
scalepack8m  
scalepackmanyframes  
scalepackribo  
scalepackvirus
```

for example:

scalepack for Virus project

```
bash> cp /usr/local/bin/scalepack /usr/local/bin/scalepacknorm  
bash> cp /usr/local/bin/scalepackvirus /usr/local/bin/scalepack
```

Run Mosfilm:

From data processing computer:

```
bash> cd data-directory # change into data directory
```

For ID beamline data from mar300 detector:

```
bash> ipmosflm # start MOSFLM program  
> detector marccd  
> limits xmin 0 xmax 150 ymin 0 ymax 150 xscan 150 yscan 150  
> template yourdataset.####  
> image 1  
> go
```

For BM beamline data from mar225 detector:

```
bash> ipmosflm # start MOSFLM program  
> detector marccd reversephi # reverse phi convention for BM  
> limits xmin 0 xmax 150 ymin 0 ymax 150 xscan 150 yscan 150  
> template yourdataset.####  
> image 1  
> go
```

Heavy Atom Search, Phasing, Maps

Run SGXPRO:

SGXPRO is an application software suite, developed at University of Georgia, for solving X-ray crystallographic structures of biological macro-molecules.

SGXPEO uses user-friendly GUI for parameter input and is able to run all the procedure as pipeline after user setup.

SGXPRO can be distributed to academic users free.

- 1). Heavy atom sites searching: **SHELXD** and/or **SOLVE**
- 2). Phasing (SAD or MAD): **ISAS** and/or **SOLVE**
- 3). Auto-tracing: **RESOLVE**

Start program: **bash> sgxpro**

Run BnP:

BnP is the Buffalo and Pittsburgh interface, which is combining **SnB** and **Phases** suite for complete protein phasing.

Start program: **bash> BnP**

Run CCP4:

Start program: **bash> ccp4i**

Run CNS:

Start program: **bash> cns_web**

Run O:

Start program: **bash> lin_ono**

Run XtalView:

Start program: **bash> xtalmgr**

Data Archive Procedure

Procedure

From data collection computer **mar300**, example:

```
% mkdir /d1/ID1/02_28_05_SERCAT # create data directory in local mar300 disk
% mkdir /u1/ID1/02_28_05_SERCAT # create data directory in archive disk
```

From data processing computer **idhkl35, idhkl38, idhkl39**

```
% MOUNT # start automatic MOUNT script to mount firewire drive
```

```
% ARCHIVE # start automatic ARCHIVE scrip to backup data
From directory: /u1/ID1/02_28_05_SERCAT # user input: from archive directory
To directory: /mnt/fire # user input: to mounting point
```

Notice: ARCHIVE script will keep running every 5 minutes, and will automatically backup user's data from /u1 or /u2 to firewire drive incrementally.

```
Ctrl^C # stop ARCHIVE script after finishing data backup
```

```
% UMOUNT # start automatic UMOUNT script to unmount drive
```

Policy

1. Before user leaves the site, it is user's responsibility to backup experimental data and to inform SERCAT staff of the whereabouts of archived data in **/u2 RAID** disk.
2. User should follow **Firewire Drive Mounting Procedure** to mount / unmount firewire drive.
3. Archived data will be kept in **/u1 or /u2 RAID** disk for about **3 months (1 run)**.
4. CAT staff will then archive data from **/u1 or /u2 RAID** disk to a long-term storage system (**staff access only**). The archived data will be kept in long-term storage for about **6 months (2 runs)**.
5. All user's data in **/d1, /d2** will be deleted immediately after user leaves the site.
6. Due to tightened APS firewall policy, user is not able to access data processing computers remotely. To retrieve archived data in the future, user should provide staff user's institution ftp server IP address, user name, password and large disk space.
7. Occasionally, user may borrow a loan Firewire drive (preformatted and 160 GB) from SERCAT. Please leave user name, institution name, and contact information on the assigned log book; and return the drive to staff as soon as possible.

Troubleshooting

1. MARCCD program is frozen.

- 1) From any shell window, type following command
bash> ps -ax | grep marccd
- 2) Kill the marccd program with shown PID from last command.
bash> kill -9 xxxxx
- 3) Restart marccd program

2. MARCCD computer is frozen (you can not even move mouse)

- 1) Hold the computer power button for at least 5 seconds to reboot.
- 2) After reboot, restart marccd program.
- 3) In marccd program, go to dropdown menu > Configure > Deterctor.
- 4) Click **Reboot** button to start detector parameter downloading and cooler restarting.

3. MARCCD computer data collection function could not drive detector distance

- 1) from MARCCD computer
bash> restartmx
- 2) Restart marccd program

4. Remote control box for Phi, Kappa and Z does not work.

- 1) Push down the Phi 0 90 180 270 four buttons at the same time
- 2) From marccd program, take a single exposure.
- 3) After exposure, the remote should be activated and ready to use.

5. Could not find data in archive directory set from marccd program.

- 1) Check the data collection setup, make sure the archive setup is shown like following format.
cp %s /u2/id5/SERCAT/
- 2) Before start the data collection, user must manually create all the directories from shell window. The marccd program can not create directory automatically.

6. Could not mount/umount user's firewire drive.

- 1) Please contact CAT staff.