Kappa Apex 3 instruction manual

Version 1.0
CuKα Prospector Edition

Procedure

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Introduction:

This short set of instructions is meant to serve as a guide for "routine" data collection on the APEX3 diffractometers. Please read through this guide and acquaint yourself with the diffractomer. If during the course of using the CCD, something happens that you don't understand, STOP, GET HELP. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable.

Assumptions:

In describing the steps involved in collecting a data set, several assumptions have been made regarding the status and conditions of the instrument.

1) The diffractometer is not in use.
   a) Check the lights inside the enclosure (should be green)
2) The generator is up and running at a normal levels (full power is 45kV 0.65mA)
3) You have mounted a crystal on a goniometer head.
   a) Plane polarized microscopes are available in IMSERC to examine your crystals.
   b) The use of magnetic heads is highly recommended.

Adjusting Temperature:

Most crystals should be measured at lower temperatures to avoid extensive thermal motion of the atoms, to prevent solvent loss or decay from the atmosphere and to obtain higher angle diffraction data.

1) Press the start button on the temperature control box. Wait for the instrument to go through its start-up routine.
2) Display will read "Cool to 100k' when it is done. Press the start button again to cool down to 100K, takes approximately 20 minutes.
3) Temperature will be noted on the control box.
4) When at temperature turbo is off (lights are down to 3 lights).

The Cryo... ...ument.
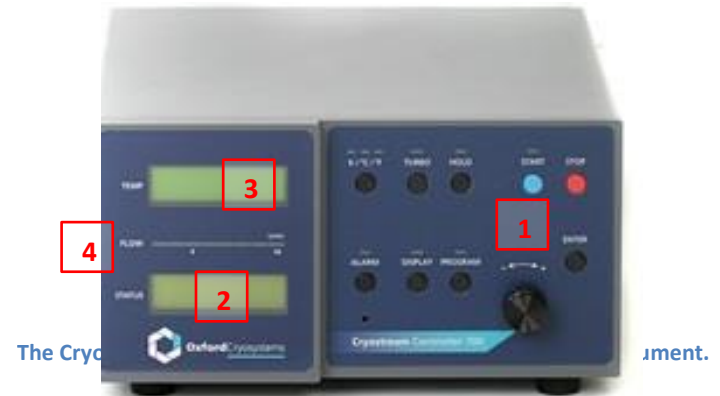
New Project

On the data control computer:

1) Make sure BIS is open (Bruker Instrument Service)
   a) BIS controls the instrument. All other programs communicate with BIS.
   b) BIS is an icon on the desktop. Double click to open.
   c) Wait until "instrument ready" is displayed to continue.
   d) Minimize BIS- you do no need to adjust anything from the BIS gui.
2) Start APEX3
   a) APEX3 is an Icon on the desktop.
   b) Double click to open.
3) Under **Sample** click on <New >. Fill in your sample name and save the last sample, when prompted
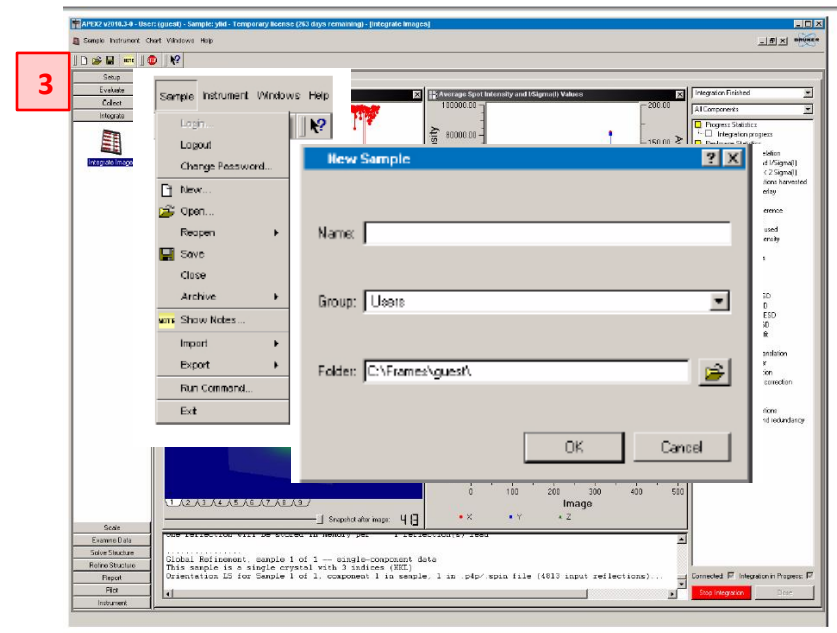
Centering your crystal

1) Ensure that the low temperature device is at your desired temperature (100K is standard).

2) Under the <**Setup**> tab on the left hand side open up the <*Center Crystal*> menu.

   a) Put the instrument in the "center" position by clicking <Center>. DO NOT LEAVE SAMPLE IN THE "MOUNT" POSITION, THIS LEADS TO FREEZING OF THE GONIOMETER HEAD

   b) Open the doors (door open button on instrument) and attach the goniometer head with the crystal mounted on it.

      i) If using the magnetic heads, just click it on to the goniometer head already on the instrument.

      ii) Otherwise the goniometer head is keyed to the goniometer. There is a little notch on the underside of the goniometer head that is aligned to the key on the goniometer. DO NOT OVER TIGHTEN.
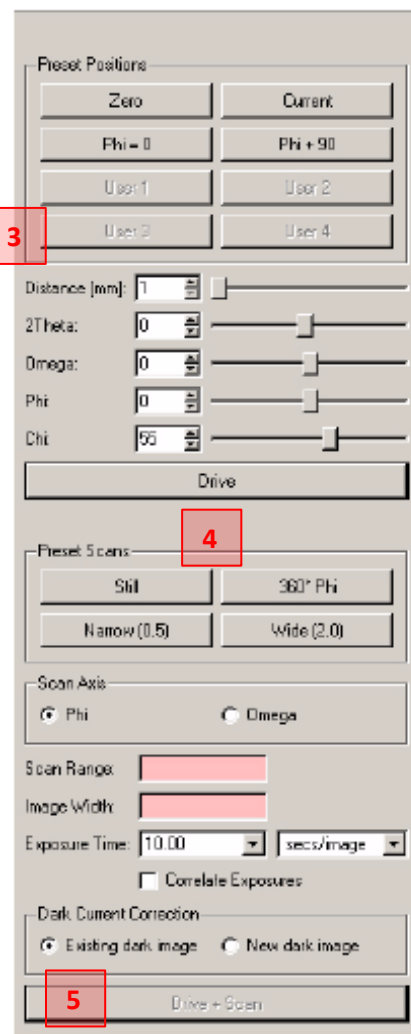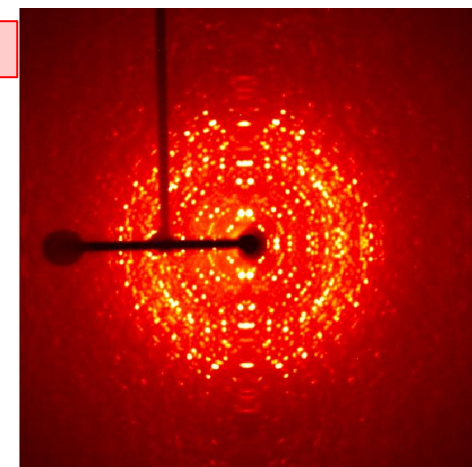
3) Center the crystal such that it rotates about its center of mass

   a) This is done by adjusting the adjustment screw pointing toward you as you face the mounted goniometer head.

   b) Adjust crystal in vertical position first and then horizontal

4) Rotate the crystal by clicking <Spin φ 90> and <Spin φ 180>.

5) Switch between the <Left> and <Right> position in order to ensure the z-axis of the crystal is correctly aligned.

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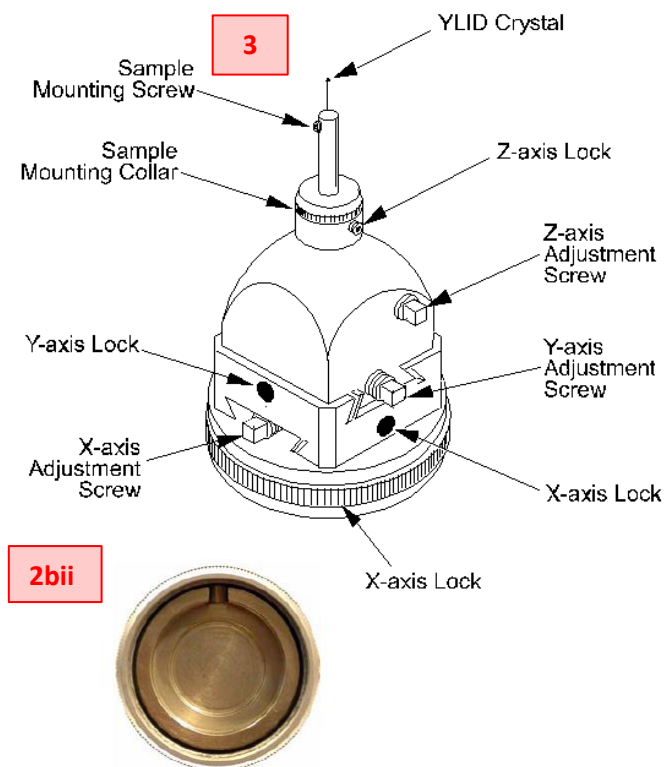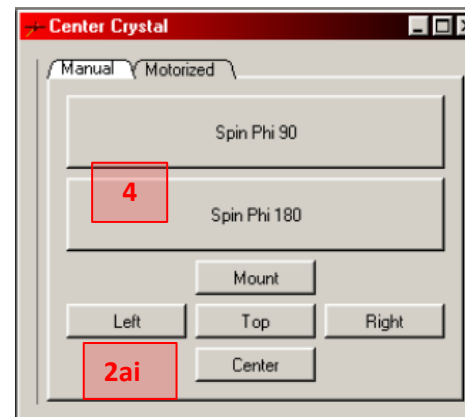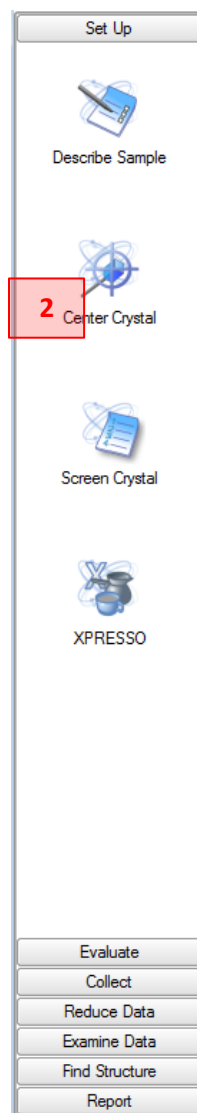
Initial Evaluation

1) Under the **<Setup>** tab Click *<Screen Crystal>*
2) Determine if the crystal is worth collecting by using a 360 degree rotation scan and/or a 0.5 degree rotation scan
3) Click <User1> to set the goniometer in a good position for taking a simple scan.
4) Choose either <360° Phi> or <Narrow (0.5)>
   a) Exposure Time for 360° Phi should be 60 seconds
   b) Exposure Time for anything else should be between 5-60 seconds
5) Click <Drive and Scan>
6) Spots should look single and round. A rotation scan should have 2 intersecting mirror planes of single looking spots.
7) If the crystal is worth collecting, then move on to the next section, otherwise choose another crystal and try again. If you are unsure, consult an expert.

A good looking 360 rotation image

Crystal and compound description

1) Under <**Setup**>, go to <*Describe*>
2) Fill in all values that apply. Always put in the formula, appearance, color and crystal shape. These will be carried over to your .p4p file. The crystal dimensions will be filled in after indexing the faces.
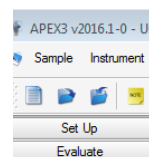
Unit Cell Determination

There are two procedures: An automatic mode that will try to get a unit cell without much input and a manual mode where one can tweak things.   The default settings are 30 frames for 10 seconds a frame.

1) Under <**Evaluate**> click on <*Determine Unit Cell*>
   a) In Automatic Mode click <run>
      i) Wait for system to finish.
      ii) If the cell is unreasonable or no cell is found skip to Manual Mode below.
      iii) If the cell is reasonable, skip to Viewing Images below
   b) Or start with Manual Mode (described below)
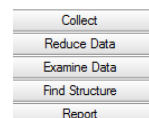
In Manual Mode (or when auto mode fails)

1) To start manual mode click on <Collect Data> (see Fig. pg. 6)
   a) Image location is where collected frames will be saved, should be your working directory.
   b) Image base name is the text string that is appended to each frame's filename and used to identify frames by their filenames.
   c) Choose which run number will be the first run. It's useful for adding runs without overwriting previous ones.
   d) Detector Distance[mm] (usually 50)
   e) Choose an exposure time in seconds/frame (usually 10 sec.).
   f) Image width in degrees that the scan axis travels (usually 0.5°).
   g) Click <Collect>

2) When finished click on <Harvest Spots>
   a) Select the first image in the group of images to be examined for the spots.
   b) Select number of runs to be examined and the number of images to be examined in each run. The software will warn you if the runs do not exist.
   c) Select an I/sigma cut off value for the diffraction spots to be used. Suggestion are I/sig > 3 for observed data or I/sig = 1.75 if you are looking for a twin law.
      i)   Look at the frame to the left to see which spots will be used, covered in green circles.
      ii) Click on <Harvest>

3) Go to <Index> and click on <Index>.
   a) If the unit cell looks reasonable (over ~80% reflections used for matrix) click on <Accept>.
   b) If you cannot find a reasonable cell, run Cell Now, explained below.

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4) Refine the matrix with <Refine>.

4a

a) Check to see that the overlay of the lattice is on top of most of the reflections. You can adjust the mosaicity (how wide the peaks are) of the overlay. Right-click on frames window and adjust the size of the mosaicity.

b) Look at the <Histogram> to make sure most of the HKLs are integers.

c) Refine until you are happy with the errors and histogram.

i) You may adjust the slide bar tolerance on how many reflections you use.

ii) You may delete reflections from the histogram by right clicking on the outliers.

d) Click on <Accept>.
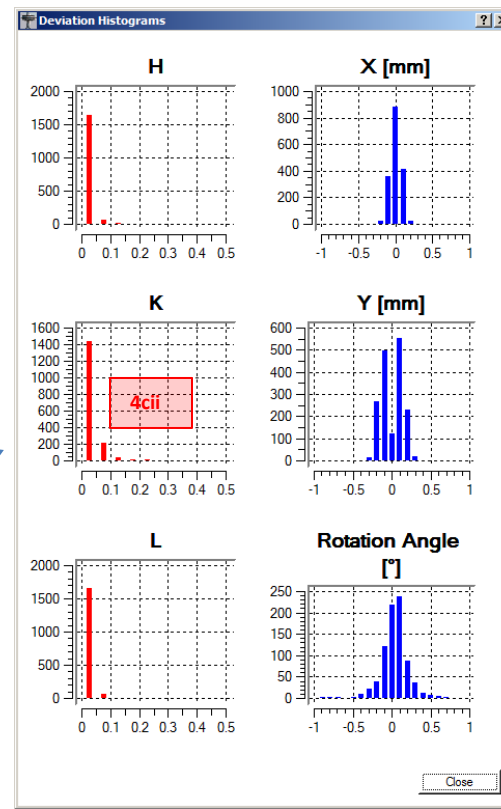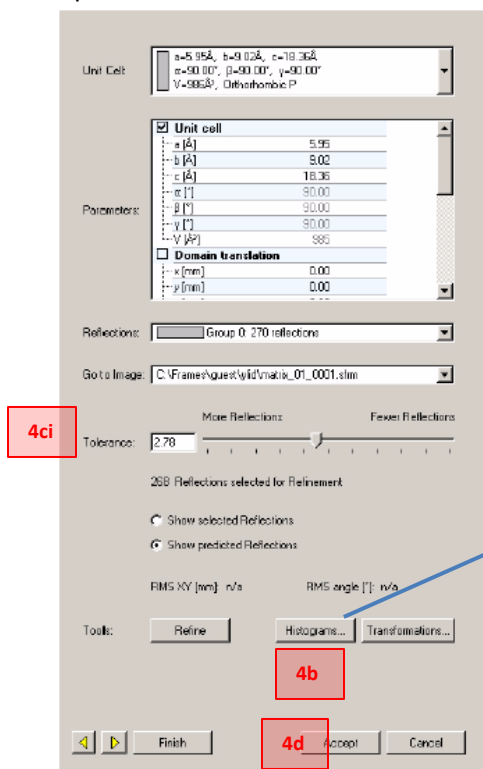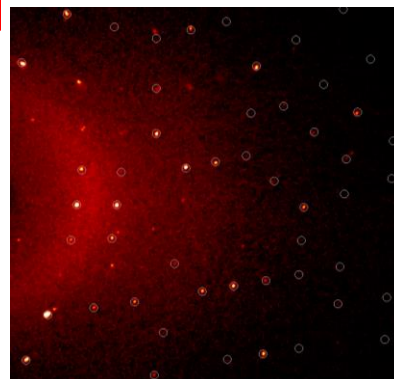
5) Go to <Bravais> and click on <Bravais>. A list of possible Bravais lattices will be displayed with APEX2 picking what it thinks is the most reasonable.

Initial Unit Cell:
a=12.47Å, α=90.00°, V=3961Å³
b=16.17Å, β=90.00°, Orthorhombic P
c=19.65Å, γ=90.00°

a) Check the Figure of Merit, the range is from 0 to 1.
b) Bravais lattices that are in agreement with the unit cell are displayed in green, those not are displayed in red.
c) Most likely lattice is chosen automatically, you may need to override this decision. When in doubt, choose triclinic.
d) Click on <Accept>

| Bravais Lattice	FOM	a [Å]	b [Å]	c [Å]	α [°]	β [°]	γ [°]
Cubic F	0.01	28.34	28.34	28.34	110.42	92.19	127.80
Cubic I	0.01	23.27	20.42	25.45	59.79	49.30	70.91
Cubic P	0.01	12.47	16.17	19.65	90.00	90.00	90.00
Hexagonal P	0.01	12.47	16.17	19.65	90.00	90.00	90.00
Rhombohedral R	0.02	20.42	23.27	28.34	110.48	79.45	109.09
Tetragonal I	0.01	12.47	16.17	44.29	68.59	73.65	90.00
Tetragonal P	0.02	12.47	16.17	19.65	90.00	90.00	90.00
Orthorhombic F	0.01	12.47	34.66	41.23	83.76	107.60	111.08
Orthorhombic I	0.01	12.47	16.17	44.29	111.41	106.35	90.00
Orthorhombic C	0.02	20.42	20.42	19.65	90.00	90.00	75.27
Orthorhombic P	1.00	12.47	16.17	19.65	90.00	90.00	90.00
Monoclinic C	0.02	20.42	20.42	19.65	90.00	90.00	104.73
Monoclinic P	1.00	16.17	12.47	19.65	90.00	90.00	90.00
Triclinic P	1.00	12.47	16.17	19.65	90.00	90.00	90.00

6) Repeat the cell refinement as in Step 4 above.
a) Go to <Refine>
b) Click on <refine>
c) Refine again until nothing changes
d) Click on <Accept>

Viewing Images

1) Under <Evaluate> click <*View images*> to check how the expected

   View Images

   reflections fit with your data.
a) Choose the box to put over the reflection and right click inside the box.
b) Choose <Rocking Curve>
c) Look at the width at half height.
   i) For a nice crystal this should be a sharp peak.
   ii) Split peaks are a sign of twinning and you should try a different crystal or try [Cell Now] to get a twin law. If you need help, consult an expert.
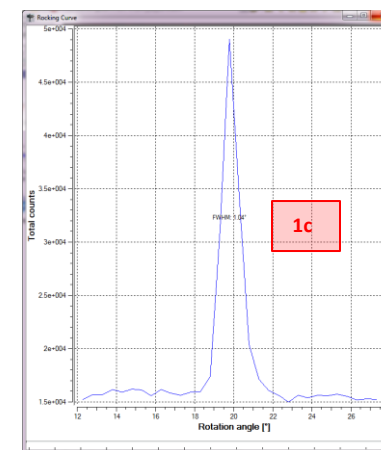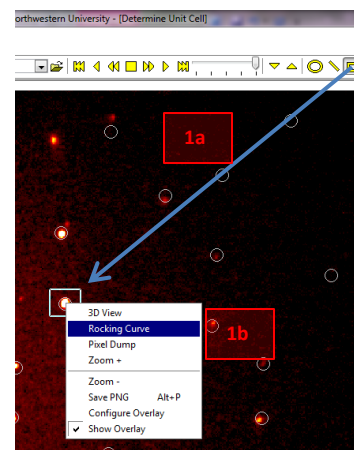
Cell Now

If the histogram is irregular or there are spots that aren't predicted you might have a twinned crystal. Or if you are having trouble finding a unit cell you might succeed by taking your data to Cell Now.

1) Export your data to a .p4p file using **Sample → <Export>**. Choose the Cell Now option in the pop-up box.
2) Start a command window (dos prompt): **Sample → <Run Command>**
3) Type "cell_now –t" and follow through with Cell Now – the defaults are usually correct.
   a) If you have a larger or smaller cell axis, you may have to change the defaults [5 and 45].
   b) If you are searching for a known unit cell, you can enter this in at the appropriate time by entering "S" and then giving a range for your known unit cell.
4) If you get a unit cell where a large percentage of the spots do not fit the cell you may have a twin.
   a) After finding the first domain, Cell Now will look for a twin domain.
   b) Ideally you will find a twin law with a near 180° rotation about a direct or reciprocal lattice axis but other rotations are acceptable.
   c) If you find multiple twin domains with only a small percentage of unique spots, and rotated about strange angles or axes, find a new sample or consult an expert.
   d) Write out a new .p4p file so you can import it back to APEX2.
5) If you get a unit cell where nearly all the spots fit, it is likely that you don't have a twin at all.
   a) Write a new .p4p file to import to APEX2.
6) Back in APEX2, import your new .p4p file: **Sample → <Import>**. Make sure to import all, so your twin flagged reflections will be read in properly.
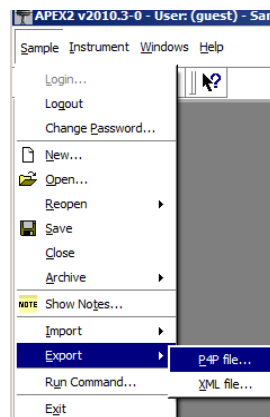
```
Cell for domain  1:    13.373    25.466    18.293     90.12    103.28     89.99
 Figure of merit: 0.741    %(0.1):  60.6    %(0.2):  63.8    %(0.3):  69.6


 Orientation matrix: -0.07139324 -0.01062766 -0.02578462
                      0.01731679  0.00492689 -0.04989317
                      0.02249989 -0.03748066  0.00062439


Percentages of reflections in this domain not consistent with lattice
types:
 A: 48.8,   B: 50.1,   C: 51.7,   I: 47.2,   F: 75.3,   O: 67.5  and  R: 67.0%


Percentages of reflections in this domain that do not have:
h=2n: 49.3, k=2n: 52.4, l=2n: 51.7, h=3n: 60.4, k=3n: 67.4, l=3n: 68.3%


   687 reflections within tolerance assigned to domain  1,
   687 of them exclusively;   431 reflections not yet assigned to a domain
-----------------------------------------------------------------------
 Cell for domain  2:    13.373    25.466    18.293     90.12    103.28     89.99
 Figure of merit: 0.690    %(0.1):  59.4    %(0.2):  60.3    %(0.3):  65.8


 Orientation matrix:  0.05414281  0.01027669 -0.02693450
                     -0.05002801 -0.00514523 -0.04928022
                     -0.02165335  0.03754913  0.00074700
```

[4]

```
 Rotated from first domain by 179.7 degrees about
 reciprocal axis  0.009  0.003  1.000  and real axis  0.329  0.003  1.000


 Twin law to convert hkl from first to     -0.994  -0.003    0.017
 this domain (SHELXL TWIN matrix):          0.013  -1.000    0.007
                                            0.657   0.007    0.994
```

[5]

```
   551 reflections within tolerance assigned to domain  2,
   421 of them exclusively;   10 reflections not yet assigned to a domain
```

Indexing the Faces of your crystal

Make sure you do this before data collection. If you change cell settings after data collection, you may need to reindex.

1) Select the <**Scale**> tab.
2) Select < *Index Crystal Faces* >.
3) Click <Acquire new> and <Save> in the resulting pop-up box. This will have the goniometer take a movie of the crystal rotating around 360°.
4) Once the crystal view appears in APEX 3 scroll with the mouse wheel to play through the "movie". Line up the straight-edge along an edge of the crystal and right-click to add the face.
5) The face list displays the faces you have defined for the crystal with the distance to the center.
6) If you don't agree with a face you can right click the HKL in the display and remove it.
7) You should be able to make a box around the crystal the shape of the crystal.
8) The Max Miller index area shows the highest Miller index as face normals in the overlay, try to use lower indices such as 1 and 2.
9) <Remove Invisible Faces> will take out an obscured HKL from the face list. Do this now to avoid trouble in XPREP.
10) Make sure the Display says that the faces define a complete volume by the closed button saying yes.
11) The size dimensions will be forwarded to the .p4p file.
12) If you are having trouble seeing the faces you may want to change the display colors and also the centering of the microscope.
    a) Right click in the image display area and choose "configure Overlay".
    b) Click a color you want to change to choose a better color for the faces.
    c) You can change the x and y centering by adjusting with the up and down arrows.
    d) When you are happy with your changes click <ok>.
13) There is no button to exit this screen. Simply move on when you are done.

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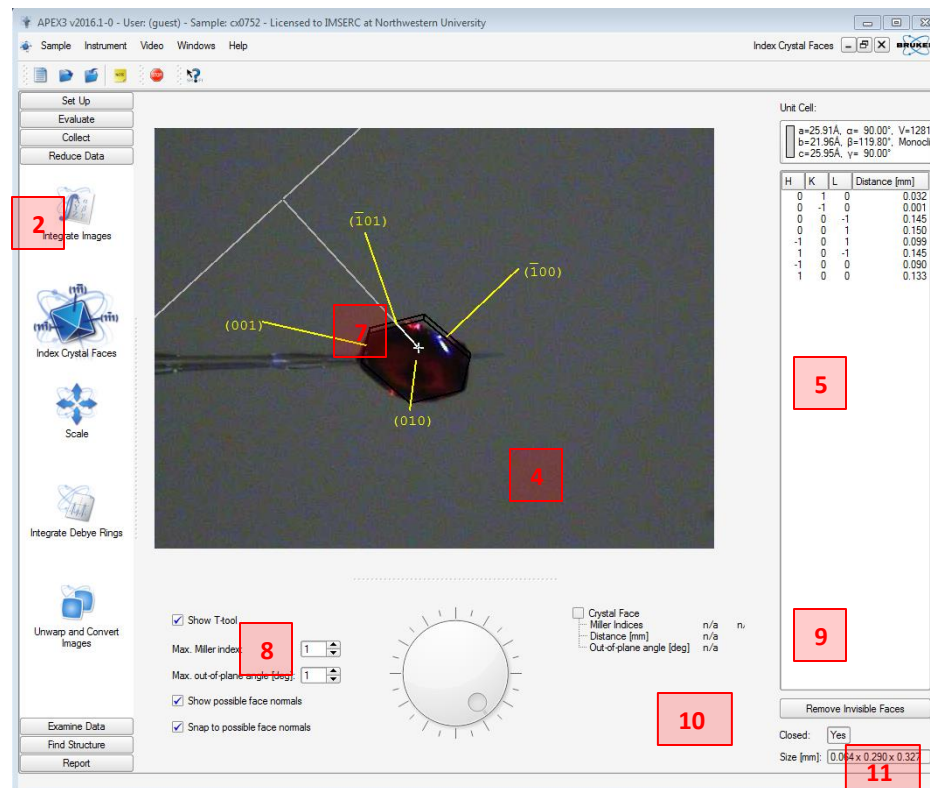
Data collection Strategy

You can either choose the Knight or the Bishop. (We will not describe the Bishop here). Try to get a complete data set with plenty of redundant data.

Each time a value is changed the data collection strategy is recalculated. <u>**Be Patient.**</u>

1) Under <**Collect**> choose the Knight <*Data Collection Strategy*>
2) Change the resolution to no lower than 0.83. The assumptions by the software are not always correct. It is best to get a sense of the maximum resolution from your Matrix frames.
3) Change the distance to "40". **Never put the distance closer than 40 due to collisions and overlap of spots, no matter what the default value is!**
4) Check that the appropriate Laue symmetry is shown.
5) Un-check <merge Bijvoet pairs> if you have a chiral compound.
6) Change the time to a suitable exposure time depending on how your crystal diffracts. A rough guide can be found in a table on the unit cell window. Click <Same> to have the same time over all ranges.
7) Change the Strategy to <Best in X hours> make sure the X is close to the end of your reservation time.
8) Check the box to <Even out Redundancy>
9) Click on the ellipsis [...] to choose the exact time you want the data collection to end.
10) Click <Refine>
    a) The strategy begins to refine approaching the target completeness, redundancy and time in the priority order.
    b) Click <Stop> when you are satisfied. You do not have to wait for it to end.
11) Click <Sort> so that the non-redundant data gets collected first.

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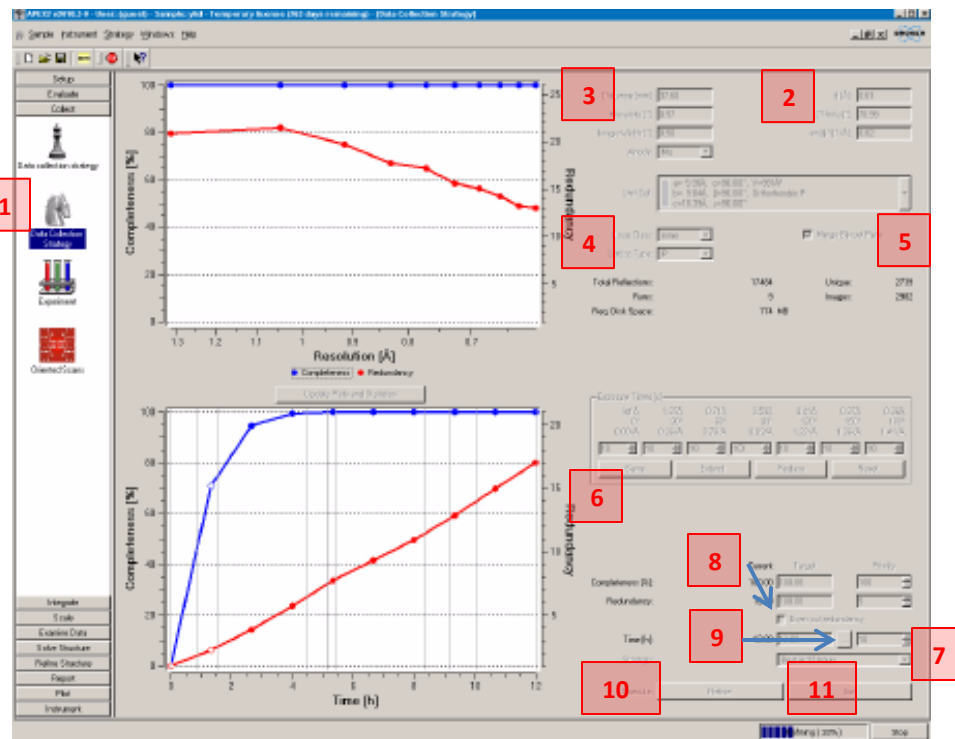
Data Collection

1) Under the <**Collect**> tab choose <*Experiment*>. Run Experiment
2) Click <Append Strategy>.
   a) The runs from the strategy will appear on the operation window.
3) If you have a table already with a strategy you want to collect you can choose <load table>.
4) If you are running over the weekend and no one is going on after you please turn off the low temperature so we do not waste nitrogen.
   a) In a row after your data collection runs click "No Operation" and change it to "Thermostat".
      i) Ramp to room temperature (298K).
   b) In the next row click "No Operation"' and change it to still.
      i) Change the chi value to somewhere around "50".
   c) In the next row click "No Operation"and change it to "Thermostat off"
5) Click Validate to be sure there are no collisions in your strategy. If there are you can:
   a) Delete the run if you have a high enough redundancy
   b) Try to modify the run to make it work.
   c) Consult an expert.
6) Click <Execute> to start the collection.

The instrument is now collecting your dataset. You can check BIS or Instrument Status to see when the dataset will end.

Integration

1) Under < **Reduce Data** > click <*Integrate Images*>
2) You can start this while the data set is still collecting.
3) Change the resolution limit to what you set in step 1 above.
4) Click on <Import runs from experiment> or <Find Runs>
5) In <Refinement Options> you might want to increase your intial XYZ box sizes to about "0.6" depending on your peak width.
6) If you have a twinned crystal uncheck "Enable Box Size Refinement"
7) Under< Integration Options> you might want to lower you intensity/sigma to 5 and 4 if you have a weakly diffracting crystal.
8) Click on <Start Integration>. This will start the integration. It will take several minutes to finish, or run concurrent with your data collection.
9) Check GOF and see if you should reintegrate your data (should be low).
10) If need be click on **Sample** → <Import> and import the merged .p4p file and reintegrate.
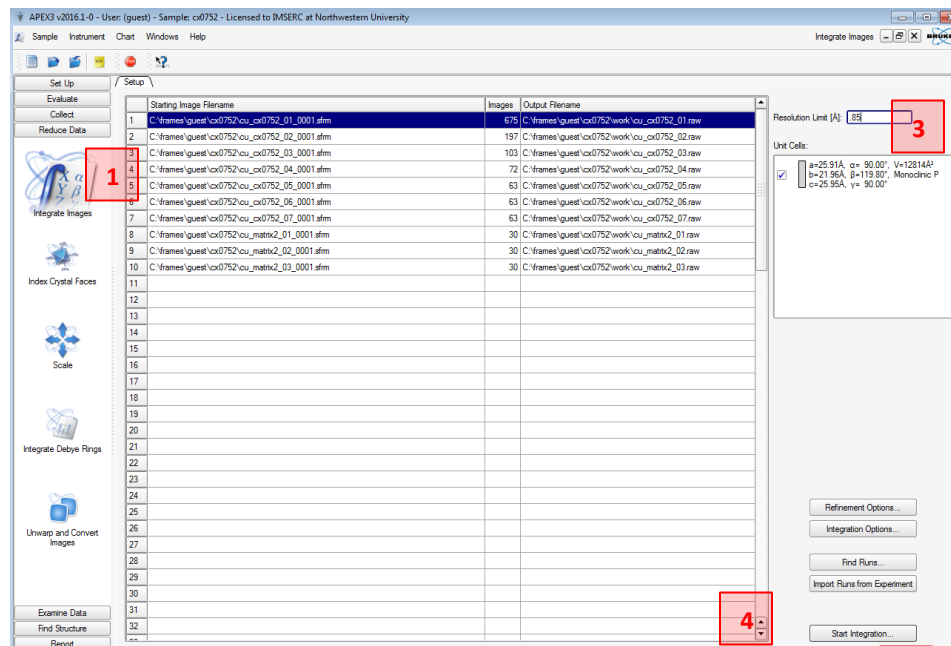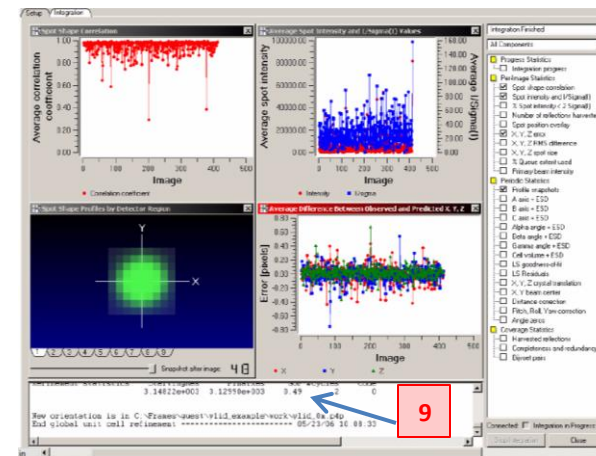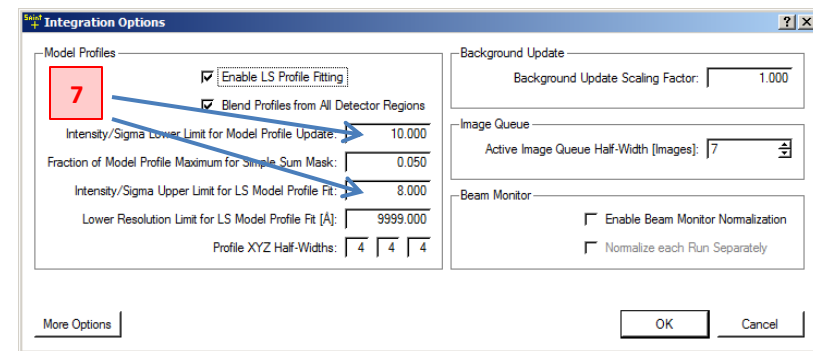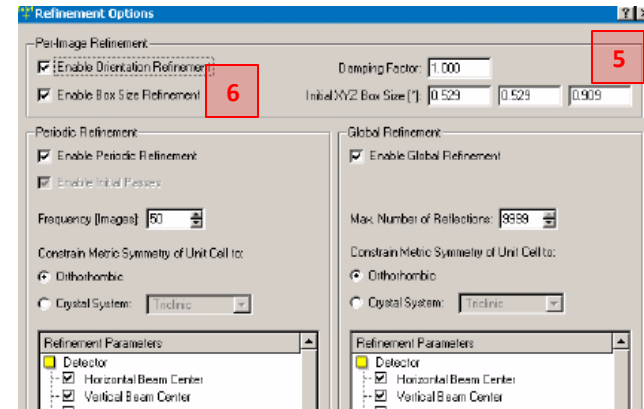
What the integration of a good crystal should look like.

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Scale

To do a 'SADABS', Multiscan absorption correction:

1) Under <**Scale**> Click on <*Scale*>

2) The merged batch from integration should be loaded. Otherwise click on the file icon and select the files you want to scale (open all with base)

3) Check the point group and if you know the sample is acentric make sure you uncheck "use only centrosymmetric point groups". Select the proper point group.

4) Click <Next>

5) On the next page click <Refine>. If it does not converge (the black, red and blue lines should end being horizontal) change the number of refinement cycles to 500 and click <Refine> again. When you are done, click <next>.

6) On the Error Model page click <Determine Error Model>. Check the R(int) values. A good R(int) should be under 0.10 but depends on crystal quality.
   a) If all are high try a lower symmetry point group.
   b) If one is bad, uncheck it and repeat parameter refinement.

7) When you are happy, click <Finish>.