Apex 2/Apex Duo Quick Guide

Login

Sample → Login

Enter in Username (group name) and Password

Create New Sample

Sample → New

Enter in sample name, be sure to check white board or cards to establish next number

Directory under sample's name will be created in the folder C:\frames*Username**SampleName*

Center Crystal

Note: Take care when opening and closing the doors. **DO NOT SLAM THE DOORS**. When opening the doors, an audible *click* should be heard when the green Open Doors button is pressed along with a red Alarm light flashing below the button. If you press the Open Door button and don't hear the *click* but the Alarm light is flashing, the latch mechanism is stuck. Gently push in the door handles until you hear the *click* then proceed to open the doors. If the doors still won't open or lock, contact the crystallographer.

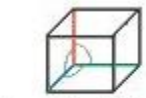
Use the APEXServer program and camera on the monitor inside the instrument. Ensure the sample is oriented for centering by clicking the Center button.

Using the goniometer tool, first adjust the crystal up-to-down in the middle of the crosshairs before adjusting left-to-right

Use the Phi 90 and Phi 180 buttons to rotate the crystal and continue to adjust the position of the crystal. Ensure that the central point of the crystal remains in the same spot on the screen during rotation

Determine Unit Cell

Select Evaluate Menu in left column, select Determine Unit Cell


Determine Unit Cell

Use the "Manual Mode" list on the right side of the screen

I. Collect Data
   A. Select Collect Data in list on the right side of the screen
   B. Select Collect in the lower right corner

Note: Pay attention to the frames as the data is collected. Spots should be well-defined circles and should not overlap. Samples that are twins or multi-crystals may have spots that are smeared and have spots very close to each other and possibly overlapping. If the sample is stable, it is worth trying to find other crystals that may give better diffraction patterns.

II. Harvest Spots
   A. Program will automatically select matrix frames from previous step and select Number of Runs and Images per Run from matrix files
   B. Adjust the Min I./sigma(I) value to 5

i. Values can be adjusted by manually entering number in the box or by moving the slider

C. Click Harvest button at the bottom of the page

Note: Take a look at the Expected Resolution table in the lower right corner. Crystals that give an expected resolution higher than 1 Å for 20 s/° exposure time are generally too weak to give a good enough structure for publication. If possible, other crystals should be tried.

III. Calculating the Unit Cell

The unit cell can be calculated by two different methods. Index is built into the Apex software and works well for well-defined single crystals but will have issues with twins and multi-crystalline samples. Cell Now is an external program that can be accessed using the command prompt and will calculate possible unit cells and help determine the orientation of the additional components for twinned or multi-crystalline samples. It is best to use Index first and resort to Cell Now if Index fails.

A. Index
   i. Use default Methods selected by program (Difference Vectors and Fast Fourier Transform)
   ii. Click Index button at the bottom of the page
   iii. Program will select “best” unit cell based upon calculated score. Click Accept button at the bottom of the page

Note: Pay attention and compare the two calculated unit cells. Did one method fail? Do both methods give the same unit cell? Are the scores comparable? Are the HKL histograms comparable? Are the HKL histograms all around or above 90%? Do the calculated spot circles overlay on a large number of observed spots? If a method failed, two different calculated unit cells were found, there is one very long cell length, and/or there appear to be a large number of overlapping spots, use Cell Now to calculate unit cell (see below)

   iv. Refine the unit cell parameters
      a. Adjust the Tolerance slider all the way to the left (selects all of the reflections for refinement)
      b. Click the Refine button on the lower half of the page
         i. The values and errors for the unit cell parameters will adjust upon refinement
         ii. Pay attention to RMS angle values, values around or below 1 are a good sign
      c. Click the Refine button a couple times until unit cell parameters and RMS angle values remain relatively consistent between refinements

Note: Pay attention to the overlay of calculated spots versus observed spots. Are there a large number of bright, observed spots that are not included in the calculated unit cell?

   i. Determine Lattice type
      a. Select Bravais on right side of the screen
      b. Program will calculate the lattice type. Green text suggests possible lattice, red text suggests discouraged lattice
      c. Select Triclinic P lattice and click Accept button at the bottom of the page

Note: Often the correct lattice type cannot be accurately determined from Bravais especially when the data gives weak reflections, or poorly-defined spots. Even well-diffracting samples

may appear to be higher symmetry than they actually are. It is best to collect as Triclinic to ensure plenty of data is collected that a good structure solution can be determined. If the initial data diffracted strongly and Bravais gives a fairly clear indication as to the lattice, or you are performing a quick screening of the crystal for a preliminary structure solution, higher symmetry than Triclinic can be selected.

B. Cell Now
    i. Export harvested spots into formatted P4P file
        a. **Sample → Export → P4P file**
        b. Automatic file name of *SampleName*.p4p, file name can be edited
        c. Select CELL_NOW for export file format
        d. Click OK button at bottom of window
    ii. Open Command Prompt
        a. **Sample → Run Command**
        b. Type “cell_now” <enter>
        c. Type in file name, default of “*SampleName*.p4p” <enter>
        d. Enter file name of Cell Now file. Typing <enter> accepts default name [*SampleName*._cn]
        e. To calculate unit cell, press <enter> to begin search
        f. Accept default for superlattice threshold, <enter>
        g. Adjust minimum and maximum cell lengths if longer than default of 5-45, otherwise press <enter> to initial unit cell calculation
            i. A table of possible unit cells will be generated and ordered by calculated Figure of Merit (FOM)
            ii. Many unit cells will be mathematically related with very similar cell lengths and cell angles. All that are mathematically related would Bravais to the same cell
        h. The top unit cell calculation is selected by default. Press <enter>.
        i. If a different cell is wanting to be selected, type in the number from the list, otherwise accept the current cell by pressing <enter>
        j. Write file name for P4P: “cell#a.p4p” <enter>
        k. If a large number of observed spots does not fit into the calculated unit cell (second column in table), can search for reorientation of selected unit cell if the sample is a multicrystal or non-merohedral twin. Save resulting orientation as “cell#b.p4p”
    iii. Import resulting P4P file into APEX
        a. **Sample → Import → P4P/SPIN file**
        b. Select appropriate P4P file created from Cell Now. Click Open button
        c. Click OK button

Note: The lattice type from Cell Now (P, C, etc.) is not transferred when importing the P4P file. If Bravais does not recognize the unit cell as having the same lattice type as found in Cell Now (often Monoclinic P vs Monoclinic C), this can be changed by selecting the Edit button next to the Unit Cell Box, and selecting the appropriate Bravais lattice type. You can skip the Bravais step if this is the case.

IV. Refine
    A. Select Refine on the right side of the screen

B. Adjust the Tolerance slider all the way to the left to select all of the reflections for refinement
C. Refine the unit cell a couple times until the unit cell parameters and RMS angle stay consistent
D. Click Accept button at the bottom of the page

V. Determine Exposure Time
A. At the bottom of the page, the Expected Resolution table lists expected resolution of the data for set exposure times. These values are to be taken into consideration when setting up the data collection in the next step

Note: The expected resolution values are determined from the Min I./sigma(I) value used when harvesting spots earlier. These suggested exposure times should be used as a guideline when creating your data collection strategy. When in doubt, use a longer exposure time.

VI. Examine your Unit Cell
A. Check your unit cell versus known materials
i. Check previous samples on the X-ray facility website
ii. Check unit cell versus published structures in Cambridge Database
B. Calculate the density and Z value for your sample
i. Use formula $density = \frac{MW}{V} * 1.66 * Z$ where MW = molecular weight (g/mol) and V = unit cell volume (Å^3). Reasonable density should be 1.2 – 1.8 g/cm^3.

Note: Use the following list of Z values for common Lattice types:
Triclinic P: Z = 2, 1 (if P1 or molecule on inversion center)
Monoclinic P: Z = 4, 2 (if chiral space group or on special position)
Monoclinic C: Z = 8, 4, 2
Orthorhombic P: Z = 8, 4, 2

<u>Calculate Collection Strategy</u>

Select Collect Menu in left column. Two strategy calculation programs can be used.

I. Knight (Fig. 1) 

A. Adjust resolution **(1)**
i. If light atom structure (nothing heavier than S or Si), enter 0.83 Å
ii. If heavy atom structure (includes S, Si, or heavier), enter 0.77 Å
B. Detector Distance **(2)**
i. Detector distance will automatically adjust for unit cells with long cell axis
ii. If possible twin or multi-crystal, increase the detector distance to 50 mm or 60 mm
C. Adjust Laue Class and Laue Type **(3)**
i. Match class and type to that of Bravais lattice from calculated unit cell
a. Recommended to use Centrosymmetric (-1)
b. If compound is known to be chiral and is triclinic select Chiral (1)
D. Exposure Time **(4)**
i. Enter time in the first box and click Same button to adjust exposure time for all boxes

ii. Recommended maximum exposure time of 30 seconds. Collecting higher multiplicity can help with weaker diffractions more than longer exposure times.

E. Set Redundancy
   i. Check box for Even Out Redundancy (**5**)
   ii. Enter target value for redundancy (**6**)
      a. For likely Triclinc space group, use a minimum redundancy of 6
      b. For likely higher symmetry space group, use a minimum redundancy of 4

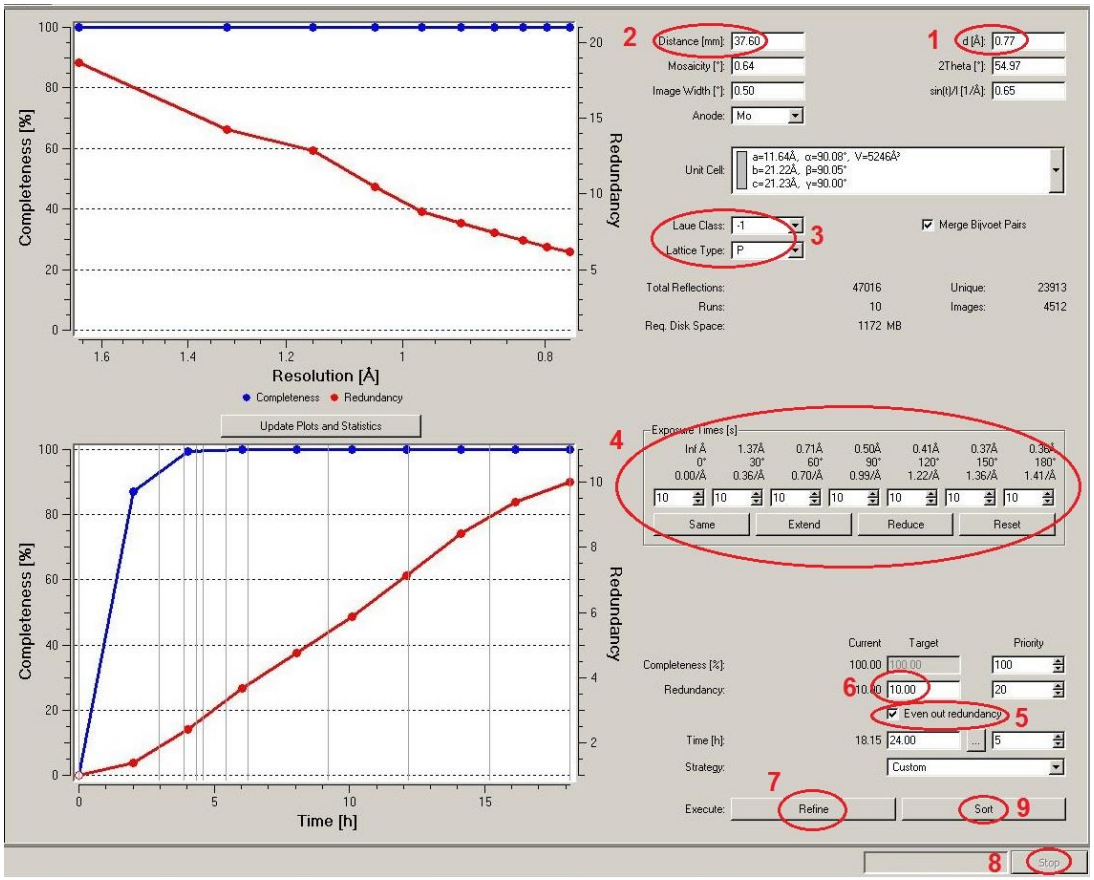
F. Click Refine button in lower right corner (**7**)

Data collection strategy determination will begin. Predicted completeness, redundancy, and experiment time will continually adjust. Progress bar in lower right corner will show "completion" of strategy determination. It is not required to let the strategy determination to go until completion. Manually stop strategy determination by clicking Stop button (**8**) next to progress bar.

Note: Take note of the expected run time listed at the bottom of the page. Adjust multiplicity and exposure time for experiment to end at a reasonable hour such that a new sample can be readily collected.

G. Click Sort button in lower right corner (**9**)

Figure 1. Calculate Data Collection window using Knight.

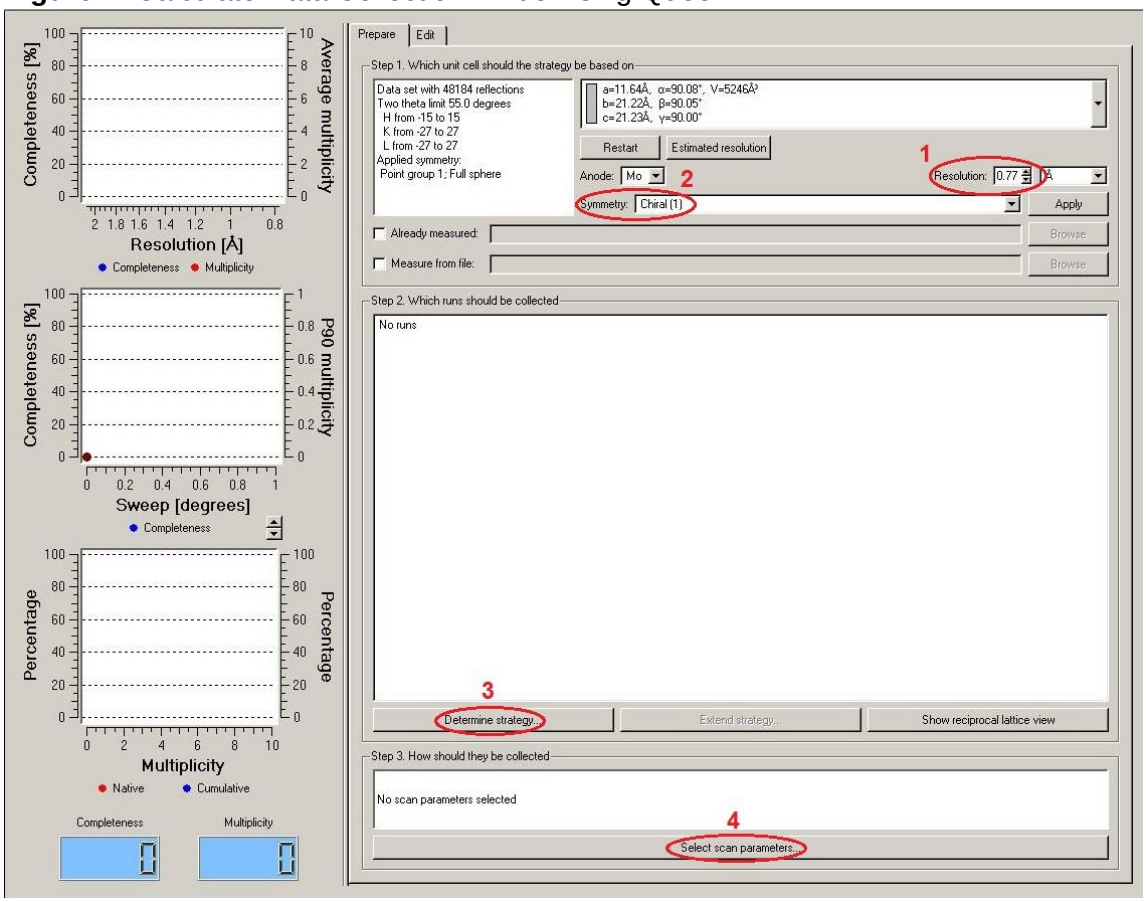


II. Queen (Fig. 2)

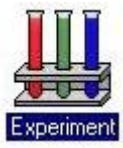
A. Adjust resolution **(1)**
   i. If light atom structure (nothing heavier than S or Si), enter 0.83 Å
   ii. If heavy atom structure (includes S, Si, or heavier), enter 0.77 Å
B. Adjust symmetry **(2)**
   i. Match symmetry to that of Bravais lattice from calculated unit cell
      a. Recommended to use Centrosymmetric (-1)
      b. If compound is known to be chiral and is triclinic select Chiral (1)
C. Click Determine Strategy button **(3)**
   i. Adjust detector distance
      a. Detector distance will automatically adjust for unit cells with long cell axis
      b. If possible twin or multi-crystal, increase the detector distance to 50 mm or 60 mm
   ii. Adjust multiplicity, recommended of 1.00
D. Click Scan Parameter button **(4)**
   i. Adjust Frame Angle to 0.50
   ii. Adjust Frame Time (exposure time) accordingly. Maximum recommended exposure time of 30 seconds.
E. Adjust Multiplicity
   i. In Determine Strategy, increase the multiplicity such that the calculated average multiplicity is between 4-6 for well diffracting crystals
   ii. Weak crystals are recommended to collect with high multiplicity over exposure times greater than 45 seconds

Note: Take note of the expected end time listed at the bottom of the page (time listed in 24 hour time). Adjust multiplicity and exposure time for experiment to end at a reasonable hour such that a new sample can be readily collected.

Figure 2. Calculate Data Collection window sing Queen.



<u>Run Experiment</u>



I. Click Experiment Icon in the left menu (Fig 3.)

II. Select Append Strategy button in lower left corner (**1**)

III. If scans have Chi values of 90°±10° or –90°±10°, cut the scan and paste at the end of the scan list. Alternate 90° and –90° to help knock off any ice formation. (**2**)

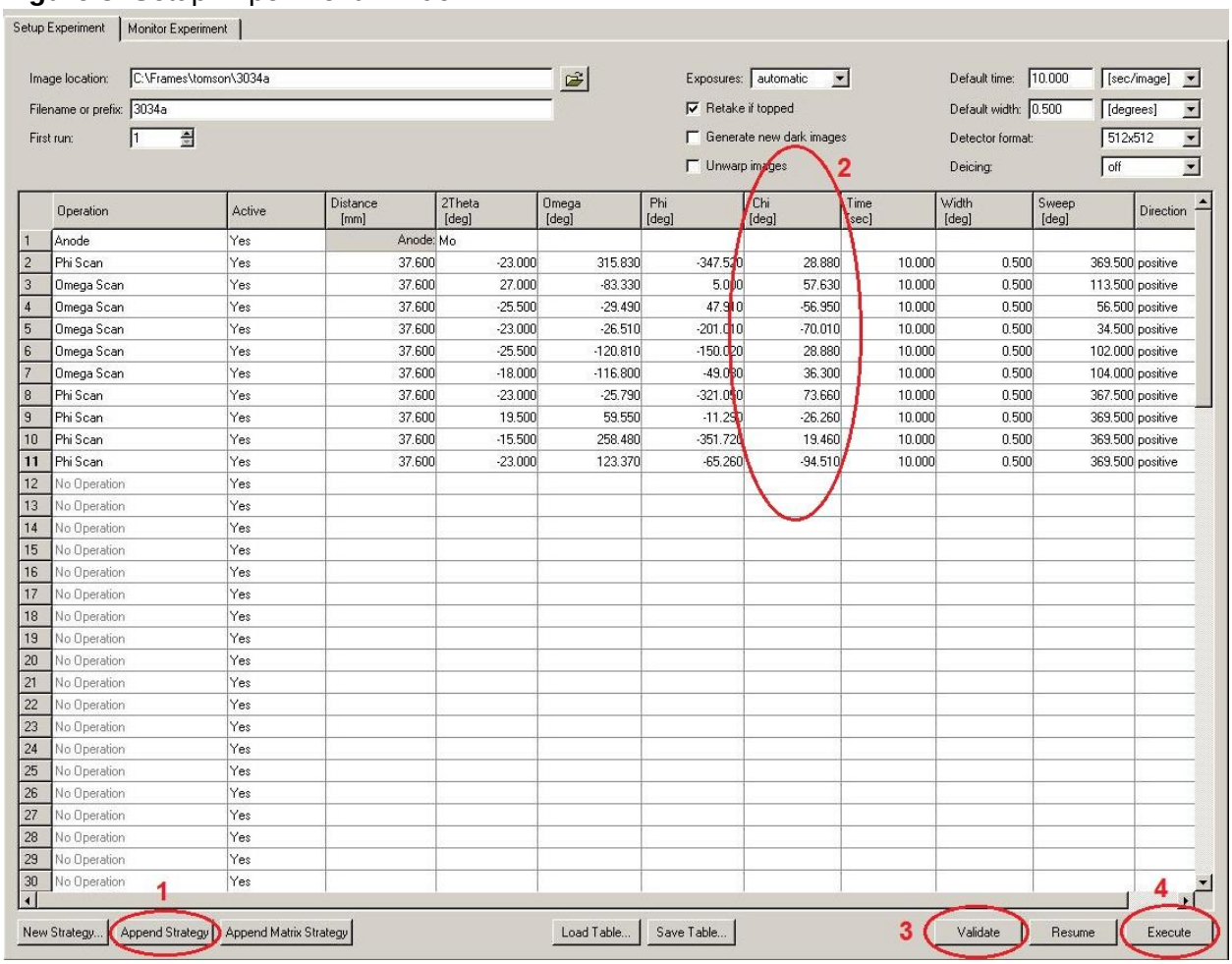
Note: Scans with Chi values around ±90° are prone to developing ice around the crystal.

IV. If data collection will end at night or over the weekend with no new sample to be mounted afterwards, add Thermostat Off at the end

V. Click Validate button in lower right corner (**3**)
   A. If error occurs, resolve error
   B. If all operations valid, click OK

VI. Click Execute button in lower right corner (**4**)

Figure 3. Setup Experiment Window.



Integration of Data

Note: Twinned and Multi-crystalline samples require extra steps when integrating. Consult an expert for integrating non-standard samples.

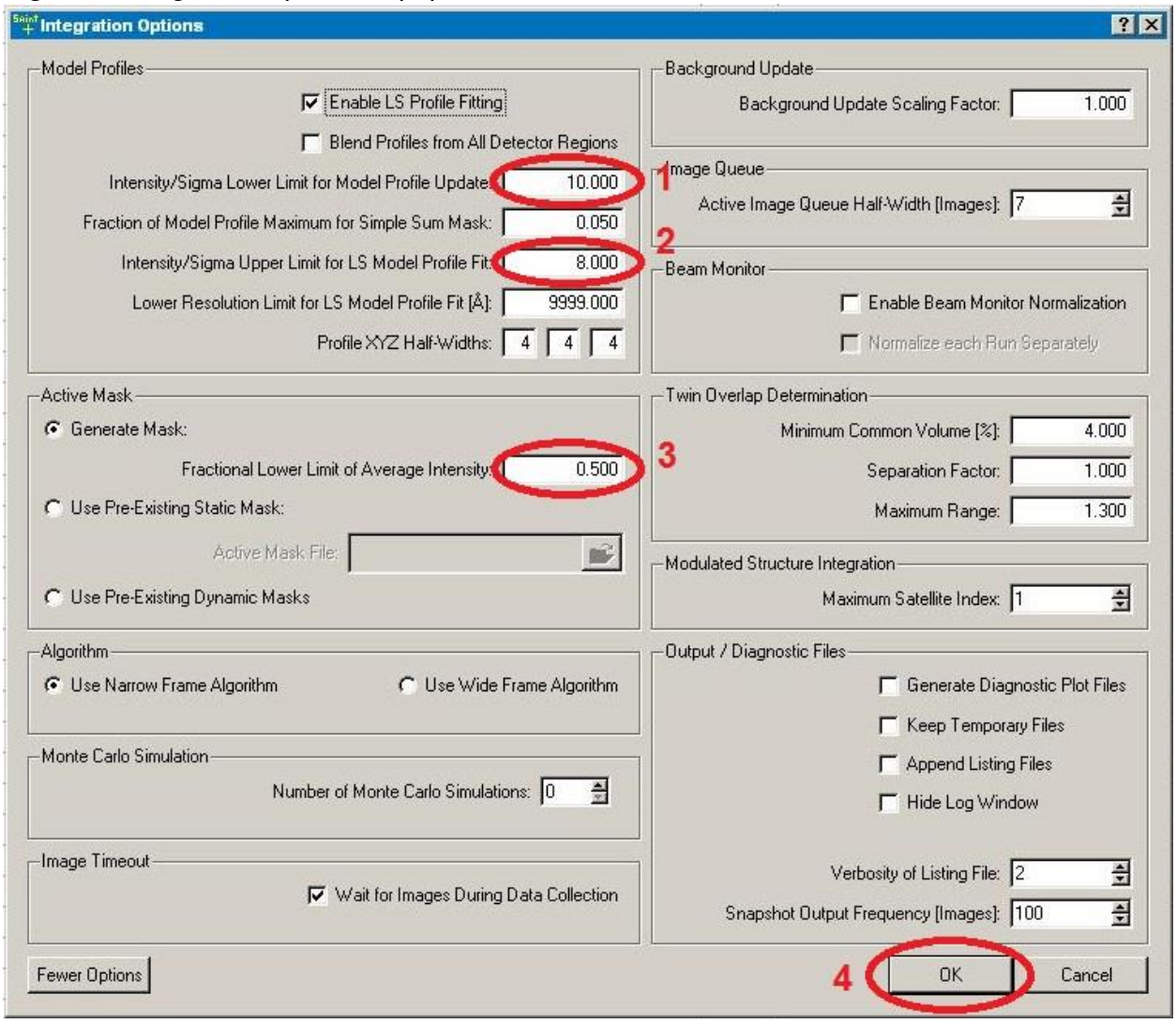
Select Integrate Menu in left column, select Integrate Image

I. Set Resolution Limit value located in upper, right corner of the screen
   A. For light atom structures, a value of 0.83 Å should be used
   B. For heavy atom structures, a value of 0.77 Å should be used
II. Integration Options and Refinement Options
   A. If spots are well defined and the sample is not twinned/multi-crystalline
      i. Refinement Options
         a. No adjustments are needed
      ii. Integration Options (Fig. 4)
         a. Click Integration Options button in lower right corner of the screen, a new window will appear

b. Change Intensity/Sigma Lower Limit for Model Profile Update to “5.00” (**1**)
c. Change Intensity/Sigma Upper Limit for LS Model Profile Fit to “4.00” (**2**)
d. Generate Mask
   i. Click More Options button at the lower left corner for the window to expand
   ii. Change Fractional Lower Limit of Average Intensity to “0.550” (**3**)
e. Click OK button in lower right corner of the window (**4**)

B. If reflections are smeary or the sample is likely twinned/multi-crystalline
   i. Refinement Options
      a. Unselect “Enable Box Size Refinement”
   ii. Integration Options
      a. Adjust Intensity/Sigma Lower Limit, Intensity/Sigma Upper Limit, and Generate Mask values as done above
      b. Select “Blend Profiles from All Detector Regions”

Figure 4. Integration Options Popup Window.



III. Select Runs for Integration
   A. Click Find Runs button in lower right corner of the screen, a new window will appear

B. If needed, change the file directory to D:\frames*Username**SampleName* to list the images
C. In the right column of the window, check the boxes for the runs you want to integrate. **Do Not** select the matrix files from the unit cell determination
D. Click the Choose button in the lower right corner of the window
E. The selected image files will be listed in the table

IV. Click the Start Integration button in the lower right corner of the screen to begin integration
A. Pay Attention to:
i. Spot Shape Correlation
a. The average correlation coefficient values should remain high and consistent if a reasonable lattice symmetry has been selected
b. Small, random dips that last for a few frames do occur and should not raise any concern
c. Sudden and sustained drops in the correlation coefficient suggests something is wrong from the collected frames. Possible issues could be a change in the temperature of the cold stream or rings occurring from diffraction from the goniometer head has occurred. Inspect the images where the drop off occurred to determine the cause
ii. Spot Shape Profile
a. The spot should be well defined and ideally near spherical
b. If the spot is smeared in shape or has a dumbbell shape, integration without the box size being refined should be considered (see below)
B. Estimated time for integration can be observed by selecting the Integration Progress box in the column on the right side of the window
C. Integration process is finished when the Close button in the lower right corner is highlighted and can be clicked on

<u>Absorption Correction</u>

Note: Twinned and Multi-crystalline samples require extra steps for absorption corrections. Consult an expert for non-standard samples.

In Scale Menu in left column, select Scale 

I. Set Up
A. Select appropriate RAW files
i. Can use individual files (*SampleName*_01.raw, *SampleName*_02.raw, etc.) or single merged file (*SampleName*_0m.raw)
B. Select appropriate Laue Group
C. Click Next button in lower right corner

II. Parameter Refinement
A. Click Refine button in lower right corner
B. Examine display on left side of the screen
i. Mean Weight should plateau by the end of the cycles. Values around and above 0.90 are good
ii. R(%) values should level out by the end of the cycles. Lower R(%) are better.
iii. If values haven't converged, adjust number of refinement cycles if needed and rerun
a. Increase value in Number of Refinement Cycles, click Refine button to rerun refinement

C. Click Next button in lower right corner

III. Error Model
   A. Look at R(int) values for individual batches
      i. Single, high R(int) batches can be unselected. Click Repeat Parameter Refinement button
   B. Look at Number of Reflections bar graph
      i. Large number of rejected reflections suggest wrong space group or other underlying problem
   C. Click Finish button

IV. Diagnostics
   A. ABS file will be created
   B. Clicking on tabs at the bottom of the page allows for reviewing of previous steps
   C. Click Exit AXScale button

<u>Generate INS File</u>

In Examine Data Menu, select Space Group Determination

I. XPREP
   A. Select files
      i. Select P4P file
      ii. Select HKL (if absorption corrected) or RAW file (if not absorption corrected)
      iii. Click OK button
   B. In XPREP window
      i. Select default options
         a. Pay attention to R(sym) values for determining higher symmetry
         b. Pay attention to CFOM values as well as systematic absences values for space group determination
         c. Make note of Completeness, Redundancy, Mean I/s values, and Rmerge/Rsigma values when merging data sets
            i. Completeness should be above 95% for publication
         d. Enter formula as single line of text
            i. Formula is text-sensitive
            ii. Number pad on right side of keyboard cannot be used
            iii. Pay attention to Z value and calculated density
         e. Write output file name
            i. Default name is fine, can write custom name if testing multiple space groups
      ii. Press <enter> until program is quit and window closes

<u>Structure Solution</u>

Two different but related programs can be used to structure solution

I. Auto-Structure 

   A. Select appropriate INS file
   B. Click Start button in lower right corner

C. Adjust zoom slider so to see the entire structure. Shown structure will display symmetry related atoms if necessary

Note: Program will have difficulty if the wrong atom types are given or if there is heavy disorder.

II. Solve Structure

A. Select Appropriate HKL and INS files
B. Enter formula if not present
C. Select Method for solving the structure
    i. Intrinsic Methods
        a. Uses ShelXT, resulting RES file has all atoms isotropic and corresponding HKL file
        b. May generate more than one RES file, each solving the structure in a different, but related space group. Select the appropriate RES file based on both R values as well as Flack parameters (avoid space groups that give a Flack value of ~0.50). The LXT file will list the preliminary R values, Flack patameters and space groups of each of the generated RES files.
    ii. Direct Methods
        a. Uses XS, uses original space group determined in XPrep
        b. Generally only shows Q peaks and a few heavy atoms
    iii. Patterson Methods
        a. Uses original space group determined in XPrep
        b. Only assigns the heaviest atoms
        c. To be used if sample contains a transition metal or heavier
        d. Continued refinement and assigning of Q peaks will be required to determine the remaining atoms in the structure

Resulting RES and HKL files can be copied and transferred for use in crystal solving program of choice