The following cookbook tutorial is crafted to guide you through your first processing of MicroED data using DIALS. DIALS is a powerful tool in data reduction. This tutorial is by no means a comprehensive guide to all that DIALS can do. The guide is meant to get you to process some data, get comfortable with the workflow, various tools, and produce .ins and .hkl files needed for structure solution and refinement.

The guide assumes that you have installed DIALS properly.

Start:

Download the data from here

To unzip the files from a terminal window navigate to the folder where the zip file is installed then use the following command insert the following.

```
unzip Tutorial-selected.zip
```

Navigate to the G4H4 folder

```
cd G4H3
```

Next create a folder to work in

```
mkdir dials
```

Move into this new folder

```
cd dials
```

The first thing is import the tilt series into DIALS. To do this we use the dials.import command but because we are in a different directory we need to use the following text that includes the ../ to start

```
dials.import ../*.img
```

The star indicates to look for all the files that are .img files. If you do this with the downloaded data, you will encounter an error such as below

```
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3$ dials.import *.img
DIALS (2018) Acta Cryst. D74, 85-97. https://doi.org/10.1107/S2059798317017235
DIALS 3.25.0-g16058fb4f-release
100%|                                                                                | 40/40 [00:00<00:00, 209.80it/s]
The following parameters have been modified:

input {
  experiments = <image files>
}

Sorry:
          Imageset contains no beam or detector model. This means you will be
          unable to process your data.

          Possible causes of this error are:
             - A problem reading the images with one of the dxtbx format classes
             - A lack of header information in the file itself.

          You can override this by specifying the metadata as geometry parameters

griz@Giovanna:~/snap/firefox/common/Downloads/G4H3$
```

With EPUD if you have taken screening images there will be an additional image in the folder with that follows the naming scheme of name_of_file_preview.img.

```
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3$ ls
dials             G4H3_0005.img  G4H3_0012.img  G4H3_0019.img  G4H3_0026.img  G4H3_0033.img     G4H3.xml
dials.import.log  G4H3_0006.img  G4H3_0013.img  G4H3_0020.img  G4H3_0027.img  G4H3_0034.img     imported.expt
G4H3_0000.img     G4H3_0007.img  G4H3_0014.img  G4H3_0021.img  G4H3_0028.img  G4H3_0035.img     Screening
G4H3_0001.img     G4H3_0008.img  G4H3_0015.img  G4H3_0022.img  G4H3_0029.img  G4H3_0036.img     test
G4H3_0002.img     G4H3_0009.img  G4H3_0016.img  G4H3_0023.img  G4H3_0030.img  G4H3_0037.img
G4H3_0003.img     G4H3_0010.img  G4H3_0017.img  G4H3_0024.img  G4H3_0031.img  G4H3_0038.img
G4H3_0004.img     G4H3_0011.img  G4H3_0018.img  G4H3_0025.img  G4H3_0032.img  G4H3_preview.img
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3$
```

There are a few ways to instruct dials to look at just the tilt series images. We recommend

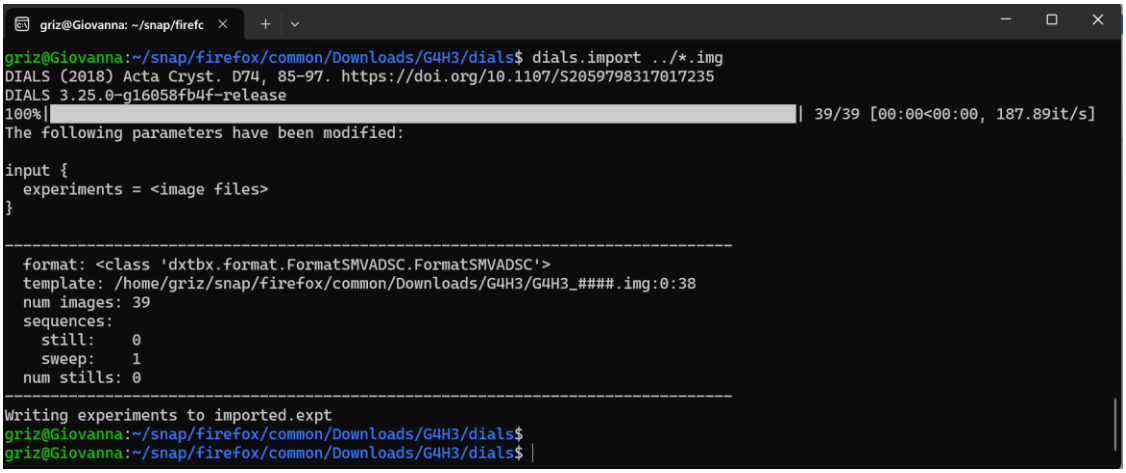
```
dials.import ../G4H3_????.img
```

Alternatively, you can just delete the preview image with

```
rm ../G4H3_preview.img
```

The proceed with the `dials.import ../*.img`

Once you import the images you will get a redout that looks like the following image.



We note here that we have 39 images and 1 sweep and an experiment file has been written to imported.expt

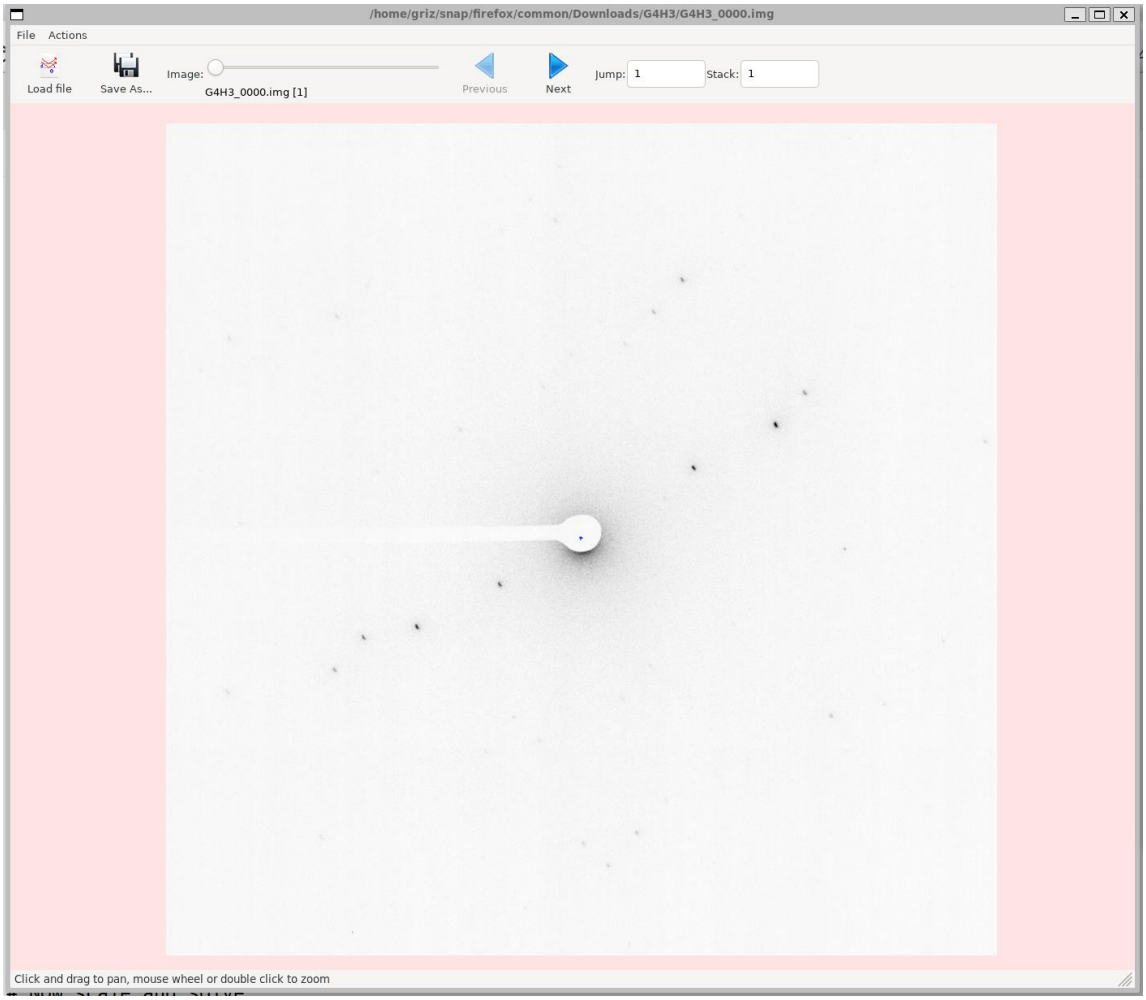
We will use this file to do a few things.

- Find beam center
- Set a mask

To evaluate the diffraction images of the tilt series, pull up the image viewer.

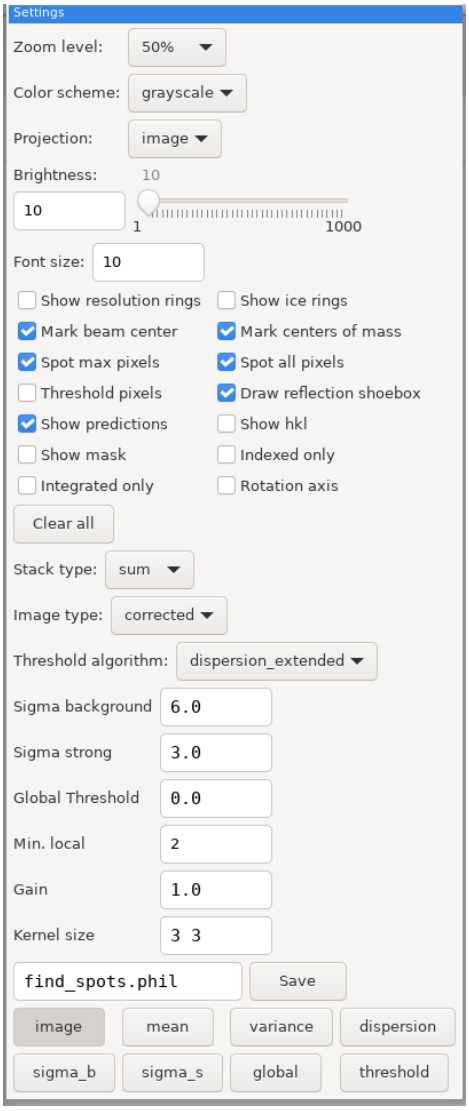
```
dials.image_viewer imported.expt
```

- Two windows will then pop up.



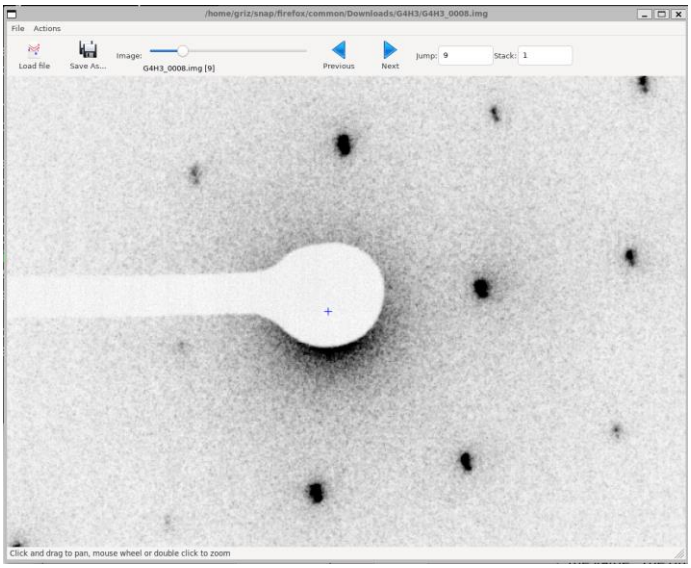
The first is the image viewer.

- The scroll wheel will change the zoom level. Left click and movement of the mouse will change the positioning of the image. Mousing over the image will let you know a few parameters. Including fast and slow beam center, which will be important for setting beam center.

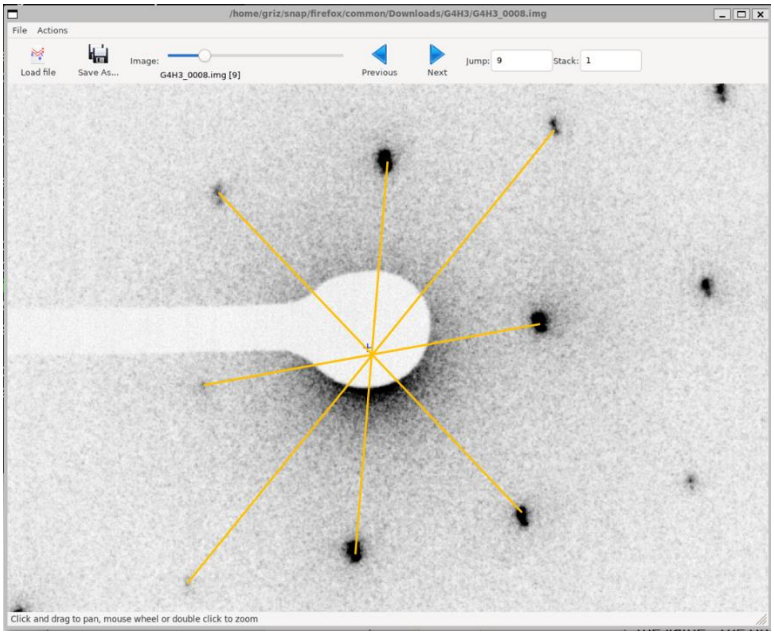


The second window is the settings window. Here there are a number of things that we can change. The parameter we will concern ourselves with right now is the brightness. Play with the slider. Then use the text box next to the slider to use 25 and press the enter key to set the value. The other box to be aware of here is the box "Mark beam center". Check this on and off and note the blue cross that shows up and leaves. If you hover over it and look at the bottom of the image you will see fast=1024 / slow=1024. Think of these as x,y coordinates.

Use the image window and navigate to image 9 (G4H3_0008). Use the right/left .

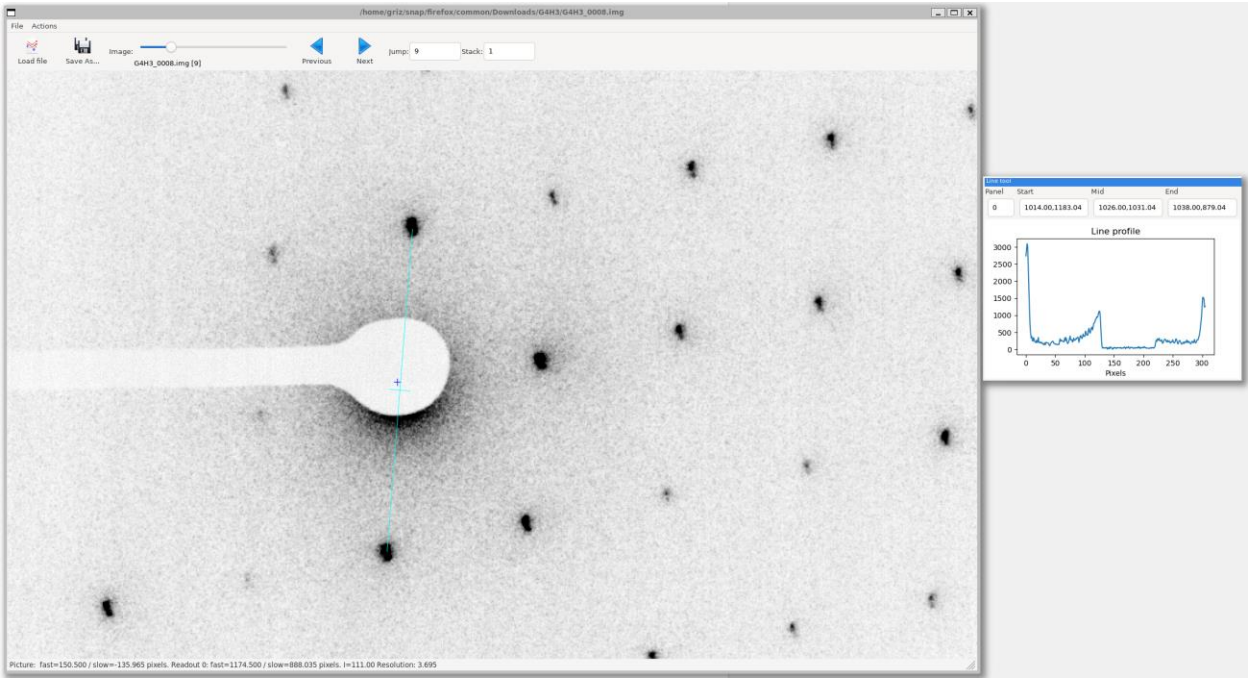


If we look closely at this image the beam center is slightly off. This is highlighted by drawing lines between Friedel pairs and draw lines between them. The beam center should be in the center of Friedel pairs. See below image. Once you get experience looking at these images you likely will not have to draw lines.



You can find the midway point between the Fridel pairs by using the line tool in from the Dials Image viewer. Click on the action button in the upper left-hand corner and select “Show line tool”

A new pop-up window will appear. Click on the center of one of the reflections then click on the center of its pair.



Use your cursor to hover over the center of the line as indicated by the cross in the line to obtain new coordinates. It may be beneficial to do this several times and come up with some sort of average integer value.

With these coordinates we will need to re-import the images but with an additional text to define the beam center. The value used here is 1026,1029.

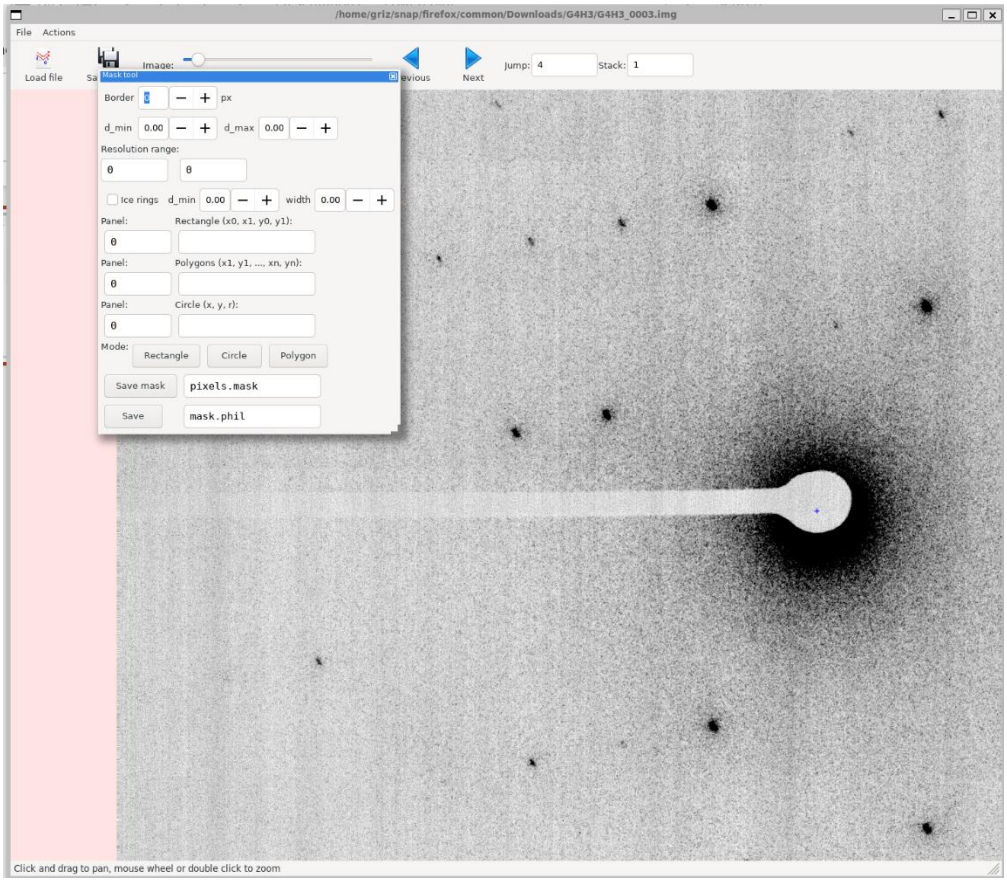
```
dials.import ../G4H3_????.img fast_slow_beam_centre=1026,1029
```

Doing this will overwrite the old imported.expt file.

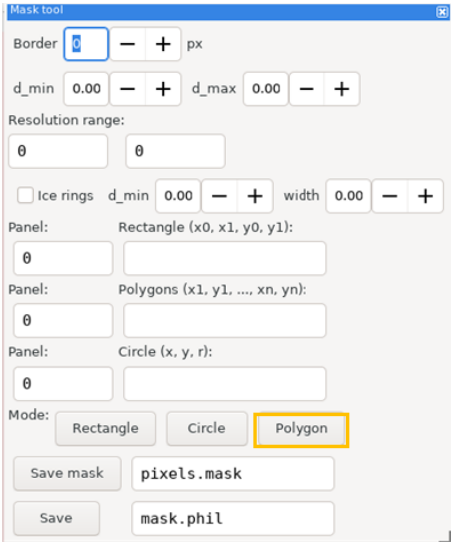
If you want, you can then check how the beam center changed in the image viewer.

Next we want to generate a beamstop mask. Which will also require the image viewer. We will use the dials mask tool.

In the Image window, navigate to the upper right hand corner this is a menu tab. Select on “Show mask tool” doing so will then give you a new window as shown below.

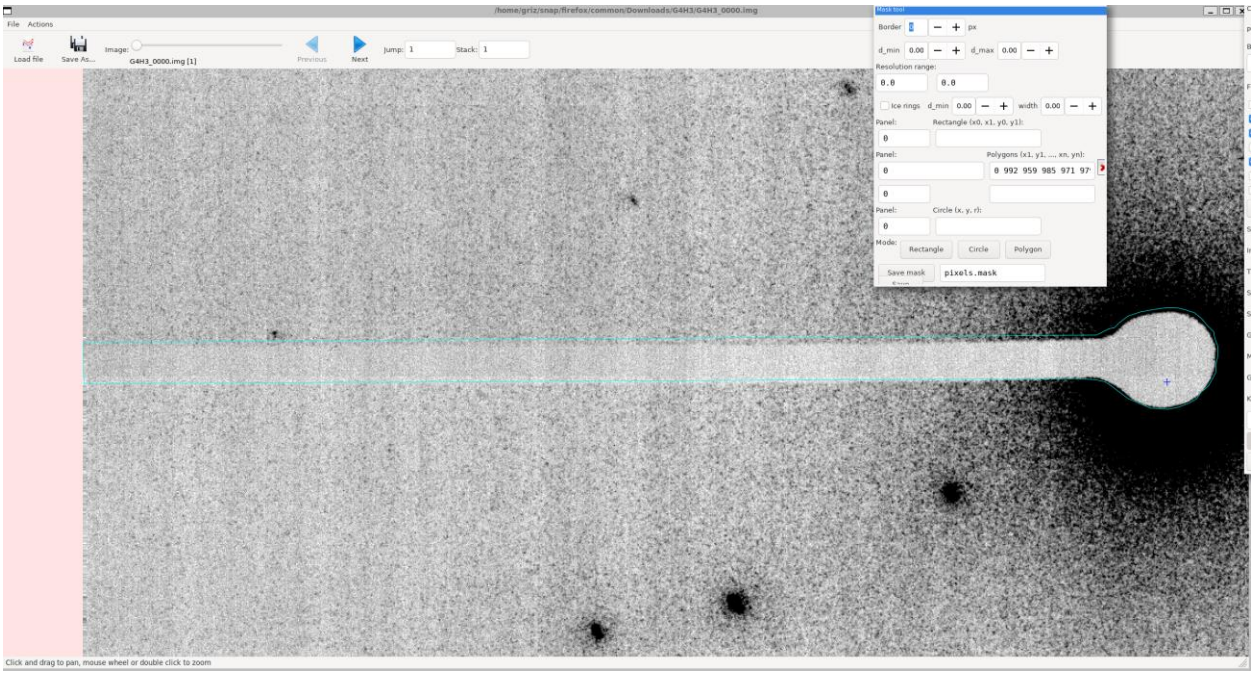


Shown to the right is the mask tool shown larger. Adjust the brightness again to 80 to better highlight the outline of the beamstop. Now click on the “Polygon” button.

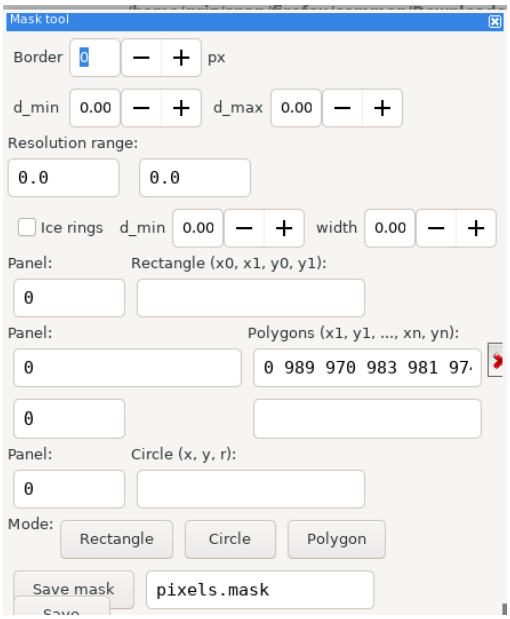


Take your cursor on the image and click near the left edge of the image (don’t click outside of it) where the top left corner of the beamstop begins. Second click will be near where the beamstop becomes a circle. Then click several times around the rounded portion of the beamstop till you get all the way around to the bottom horizontal flat portion. The final click will be near the left edge of the image below your start mark. **When you are finished click the polygon button again.** This will complete the polygon making a final connection between your first click and the last click. The mask tool window will now have numbers populating the polygon field. The image window should look something like the image at the bottom of the page.

Please note. If you make a mistake there is no “undo”. Close the window and start again. Good news is that saving the file at the end should permit you to use the same mask along all the data sets you collect, as we expect the beamstop to not shift very much between data sets on a particular instrument.



Here is the mask tool window magnified.



Click on 'save' (not 'save mask') Then close the image viewer. The terminal should show the text “Saving parameters to mask.phil”.

Next we need to generate the mask from the mask file.

```
dials.generate_mask imported.expt mask.phil
```

This will generate a pixels.mask file

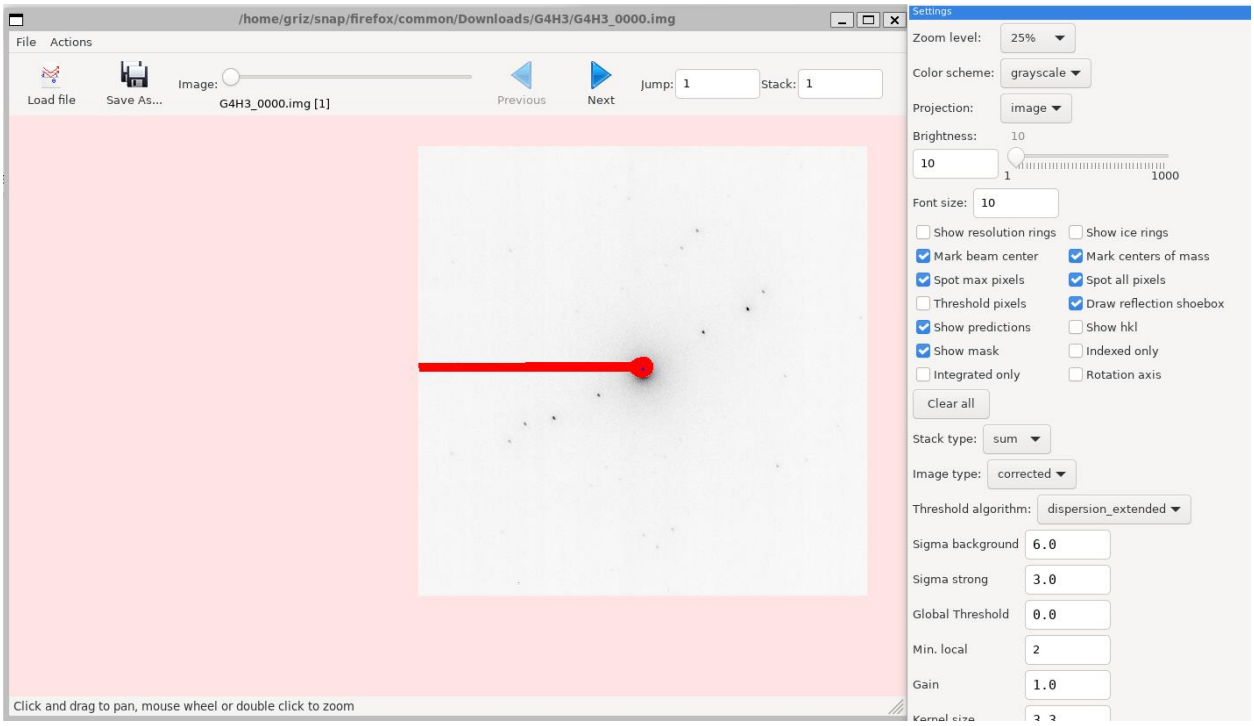
Next we will apply this file to the imported experiment.

```
dials.apply_mask imported.expt mask=pixels.mask
```

This will then generate a new masked.expt file.

To confirm that this mask has been applied. Open up the image viewer (be sure to use masked.expt) and check the “show mask” box. See example below.

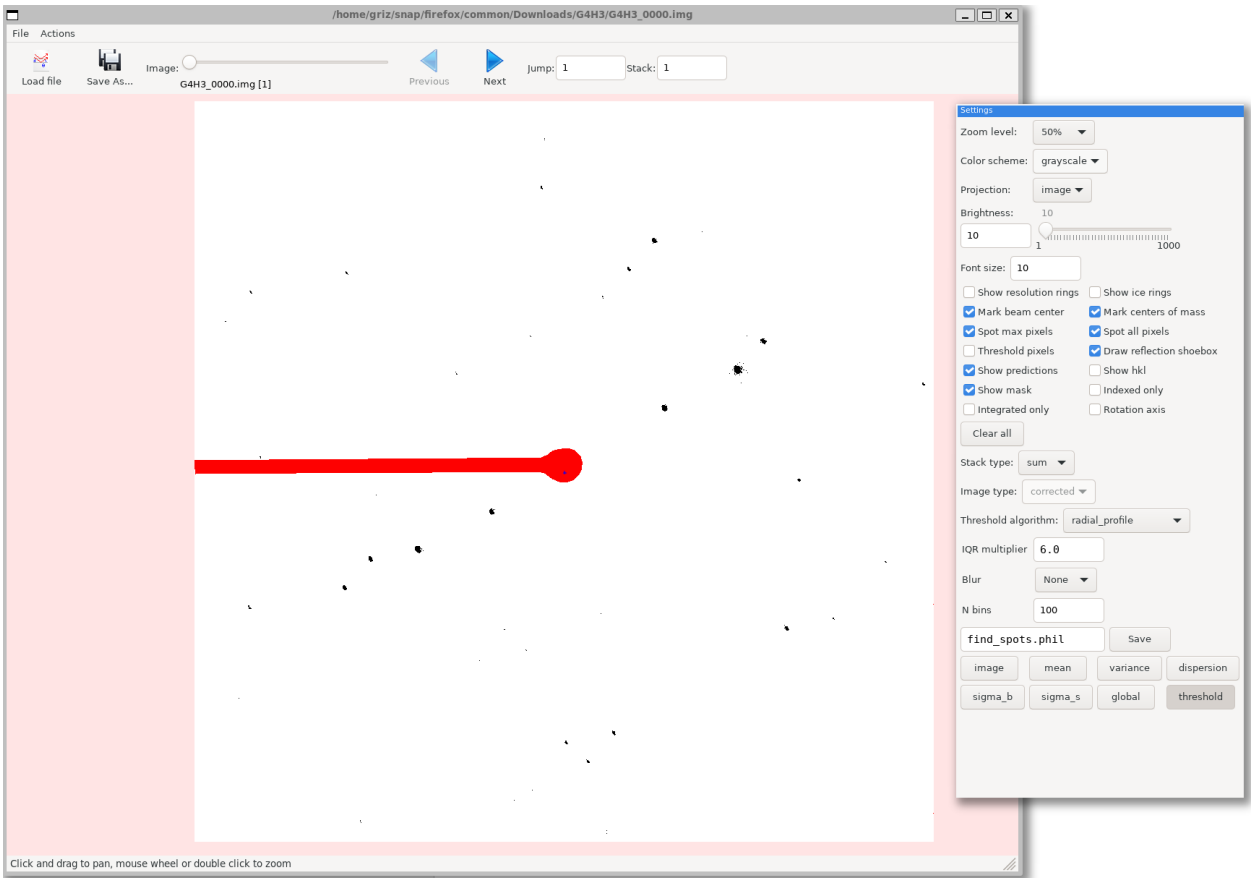
```
dials.image_viewer masked.expt
```



Next we need to identify diffraction spots that we want to use for indexing. This on occasion can take some time and with data collected. However, when using Ceta-D camera and EPU-D it seems that the radial profile algorithm does the best.

While the image viewer is up, in the settings window, you can take a look at this by selecting “radial_profile” in the dropdown menu under “Threshold algorithm”. After selecting this then click on the threshold button at the bottom of the settings window. You can also adjust other parameters as well such as *IQR multipler, Blur, N bins*.

The goal is to have enough spots corresponding to the lattice of interest to index your data and not include spurious spots. For this particular tutorial, leave the defaults, but change blur to wide. Using the threshold button the image viewer will look like below.



Close the image viewer.

In the terminal type

```
dials.find_spots masked.expt threshold.algorithm=radial_profile
blur=wide
```

The terminal will the look like the following image.

```
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3/dials$ dials.find_spots masked.expt threshold.algorithm=radial_profile blur=wide
DIALS (2018) Acta Cryst. D74, 85-97. https://doi.org/10.1107/S2059798317017235
DIALS 3.25.0-g16058fb4f-release
The following parameters have been modified:

spotfinder {
  threshold {
    algorithm = dispersion dispersion_extended *radial_profile
    radial_profile {
      blur = narrow *wide
    }
  }
}
input {
  experiments = masked.expt
}

Setting spotfinder.filter.min_spot_size=6
Configuring spot finder from input parameters
--------------------------------------------------------------------------------
Finding strong spots in imageset 0
--------------------------------------------------------------------------------

Finding spots in image 0 to 38...
Setting nproc=16
Setting chunksize=3
Extracting strong pixels from images
 Using multiprocessing with 16 parallel job(s)

Found 4049 strong pixels on image 0
Found 3715 strong pixels on image 1
```

And it ends with the following

```
Found 7 strong pixels on image 37
Found 32 strong pixels on image 38

Extracted 2096 spots
Removed 579 spots with size < 6 pixels
Removed 16 spots with size > 1000 pixels
Calculated 1501 spot centroids
Calculated 1501 spot intensities
Filtered 1100 of 1501 spots by peak-centroid distance

Histogram of per-image spot count for imageset 0:
1100 spots found on 39 images (max 56 / bin)
                        ***
                      * ***
                   ********* *
*                * ********* * *
*              * ************ * *
*            * * ************ * ***
**** **** ************************
********* ************************
**********************************
**********************************
0                image                38

--------------------------------------------------------------------------------
Saved 1100 reflections to strong.refl
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3/dials$
```

DIALS writes a reflections file “strong.refl”

To see what the reflections look like go to the image viewer with

```
dials.image_viewer masked.expt strong.refl
```

So now we need to try and index and find a unit cell.

```
dials.index imported.expt strong.refl detector.fix=distance
```

```
RMSDs by experiment:
+-------+--------+----------+----------+------------+
|   Exp |   Nref |   RMSD_X |   RMSD_Y |     RMSD_Z |
|    id |        |     (px) |     (px) |   (images) |
|-------+--------+----------+----------+------------|
|     0 |    953 |   2.0131 |   1.6077 |    0.28024 |
+-------+--------+----------+----------+------------+

Refined crystal models:
model 1 (1000 reflections):
Crystal:
    Unit cell: 7.11256(15), 9.2397(3), 11.6892(6), 90.047(4), 82.043(3), 89.313(3)
    Space group: P 1
    U matrix:  {{ 0.651526, -0.758617, -0.003744},
                { 0.468979,  0.406645, -0.784027},
                { 0.596299,  0.509059,  0.620715}}
    B matrix:  {{ 0.140596,  0.000000,  0.000000},
                {-0.001686,  0.108236,  0.000000},
                {-0.019657,  0.000272,  0.086381}}
    A = UB:    {{ 0.092955, -0.082111, -0.000323},
                { 0.080663,  0.043801, -0.067725},
                { 0.070778,  0.055267,  0.053618}}
+------------+-------------+---------------+---------------+-------------+
|   Imageset |   # indexed |   # unindexed |   # unindexed |   % indexed |
|            |             |         total |       non-ice |             |
|------------+-------------+---------------+---------------+-------------|
|          0 |        1000 |            99 |            76 |          91 |
+------------+-------------+---------------+---------------+-------------+
Saving refined experiments to indexed.expt
Saving refined reflections to indexed.refl
```

A few points of note here.

- % indexed is 91% this is good. Don't be discouraged if on your own research samples is lower. Although sometimes low values can indicate issues with the experimental model.
- We note that two angles are close to 90. This can be suggestive of a monoclinic crystal system. We know the example here is Paracetamol (acetaminophen) and it is known that it does crystalize in a monoclinic space group. So to force indexing into a primitive monoclinic crystal system we can do this a few ways.

```
dials.index masked.expt strong.refl detector.fix=distance
space_group=P2
```

or

```
dials.refine_bravais_settings indexed.expt indexed.refl
detector.fix=distance
```

This is the output from the above command. Here we note that two .expt files are written.

```
Selected 955 / 1106 reflections for calculation
Chiral space groups corresponding to each Bravais lattice:
aP: P1
mP: P2 P21
+------------+--------------+--------+--------------+----------+-----------+-----------------------------------+----------+---------+
|   Solution |   Metric fit |   rmsd | min/max cc   |   #spots | lattice   | unit_cell                         |   volume | cb_op   |
|------------+--------------+--------+--------------+----------+-----------+-----------------------------------+----------+---------|
|   *      2 |       0.6781 |  0.094 | 0.578/0.578  |      919 | mP        | 7.06  9.23 11.71 90.00 97.52 90.00 |      756 | -a,-b,c |
|   *      1 |       0      |  0.07  | -/-          |      924 | aP        | 7.11  9.24 11.69 90.07 82.06 89.34 |      760 | a,b,c   |
+------------+--------------+--------+--------------+----------+-----------+-----------------------------------+----------+---------+
* = recommended solution

Saving summary as bravais_summary.json
Saving solution 2 as bravais_setting_2.expt
Saving solution 1 as bravais_setting_1.expt
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3/dials$
```

Please note that if you take bravais_setting_2.expt and move forward from here that you will have to re-index using that file and indexed.refl

```
dials.index bravais_setting_2.expt indexed.refl detector.fix=distance
```

After indexing we refine the unit cell using the following.

```
dials.refine indexed.expt indexed.refl detector.fix=distance
```

```
RMSDs by experiment:
+-------+--------+----------+----------+------------+
|   Exp |   Nref |   RMSD_X |   RMSD_Y |     RMSD_Z |
|    id |        |     (px) |     (px) |   (images) |
|-------+--------+----------+----------+------------|
|     0 |    806 |   1.5639 |   1.3177 |     0.2883 |
+-------+--------+----------+----------+------------+
Updating predictions for indexed reflections

Final refined crystal model:
Crystal:
    Unit cell: 7.042(2), 9.237(2), 11.700(3), 90.0, 97.43(3), 90.0
    Space group: P 1 2 1
    U matrix:  {{-0.652938,  0.757374,  0.007478},
                {-0.478563, -0.404881, -0.779133},
                {-0.587068, -0.512304,  0.626814}}
    B matrix:  {{ 0.142000,  0.000000,  0.000000},
                { 0.000000,  0.108262,  0.000000},
                { 0.018518,  0.000000,  0.086191}}
    A = UB:    {{-0.092579,  0.081995,  0.000644},
                {-0.082384, -0.043833, -0.067154},
                {-0.071757, -0.055463,  0.054025}}
    A sampled at 39 scan points
Saving refined experiments to refined.expt
Saving reflections with updated predictions to refined.refl
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3/dials$
```

With the refined .expt and .refl file

From this we need to integrate these files using the following command

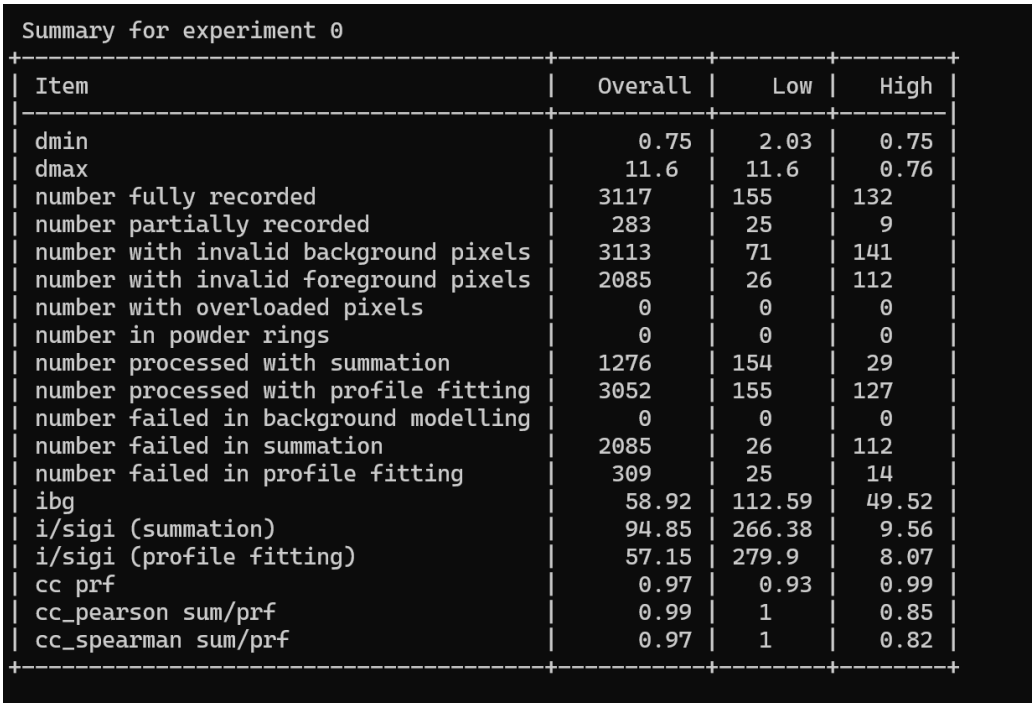
```
dials.integrate refined.expt refined.refl
```

Note on resolution cut: Determining where to make the resolution cut is a nuanced discussion. It is often stated that asking this question to two crystallographers will result in three different answers. So, we will avoid any recommendations here. However, it is important to note it is possible to make some rough determinations on early resolution cuts based on what we see from the raw diffraction images, as well as some of the integration statistics. It is also possible to do a resolution cut later when we scale. We also can do resolution cut during the final refinement of the structure as well.

For the walkthrough here, we will do use an arbitrary resolution cut at the integrations stage using the following

```
dials.integrate refined.expt refined.refl d_min=0.75
```

At the end of the integration, you should have a chart that looks like the below image.



Summary for experiment 0

Item	Overall	Low	High
dmin	0.75	2.03	0.75
dmax	11.6	11.6	0.76
number fully recorded	3117	155	132
number partially recorded	283	25	9
number with invalid background pixels	3113	71	141
number with invalid foreground pixels	2085	26	112
number with overloaded pixels	0	0	0
number in powder rings	0	0	0
number processed with summation	1276	154	29
number processed with profile fitting	3052	155	127
number failed in background modelling	0	0	0
number failed in summation	2085	26	112
number failed in profile fitting	309	25	14
ibg	58.92	112.59	49.52
i/sigi (summation)	94.85	266.38	9.56
i/sigi (profile fitting)	57.15	279.9	8.07
cc prf	0.97	0.93	0.99
cc_pearson sum/prf	0.99	1	0.85
cc_spearman sum/prf	0.97	1	0.82

We will use these integrated files to merge multiple datasets.

However, if wanted to look at statistics of the single dataset, we should then scale the single tilt series using the following command.

```
dials.scale integrated.refl integrated.expt
```

```
           -------------Summary of merging statistics--------------

                                          Overall    Low     High
High resolution limit                        0.75    2.02    0.75
Low resolution limit                         7.23    7.23    0.76
Completeness                                57.2    56.0    53.5
Multiplicity                                 2.1     2.0     1.8
I/sigma                                     35.0   165.2     4.5
Rmerge(I)                                  0.149   0.068   0.359
Rmerge(I+/-)                               0.153   0.082   0.487
Rmeas(I)                                   0.207   0.095   0.489
Rmeas(I+/-)                                0.216   0.115   0.689
Rpim(I)                                    0.143   0.067   0.329
Rpim(I+/-)                                 0.153   0.082   0.487
CC half                                    0.951   0.979   0.588
Anomalous completeness                      51.2    57.6    39.1
Anomalous multiplicity                       1.2     1.2     1.1
Anomalous correlation                     -0.296   0.000   0.000
Anomalous slope                            3.579
dF/F                                       0.244
dI/s(dI)                                   4.465
Total observations                          2390     132      98
Total unique                                1153      65      54

Writing html report to dials.scale.html
Saving the scaled experiments to scaled.expt
Saving the scaled reflections to scaled.refl
See dials.github.io/dials_scale_user_guide.html for more info on scaling options
griz@Giovanna:~/snap/firefox/common/Downloads/G4H3/dials$
```

The command writes and .expt and a .refl file. It also generates a report file dials.scale.html can be opened with a web browser and contains various statistical information.

At this point you can go and do the same thing with the other two data sets.

Please note generally you can copy and paste the mask.phil file to other experiments conducted on the same instrument. You can use this file to then apply the mask to the other experiments.

Once you are done with integrating the 2 other data sets. Then it is time to merge all three data sets, and convert the data into a format that we can use to solve and refine.

Merging the three data sets.

Make a new folder and transfer the three pairs of integrated.refl and integrated.expt to a new folder.

```
mkdir combine
```

```
cd combine
```

```
mkdir solution
```

```
cd solution
```

```
dials.two_theta_refine  ../*.refl ../*.expt
```

This command will look at the unit cells of three data sets and generate a refined unit cell from them. This then generates a single .expt file that we will use in the scaling steo.

```
 dials.scale refined_cell.expt ../*.refl filtering.method=deltacchalf deltacchalf.mode=image_group deltacchalf.group_size=2
```

This command scales and merges the 3 data sets into a single scaled.expt and scaled.refl file that will be used to look at stats as well as for exporting to an .ins and .hkl file that will be used to solve and refine.

```
dials.export scaled.expt scaled.refl format=shelx composition="C H N O"
```

This exports a necessary files for solving and refining a the structure.