Supplement 2: Repeatability Results with Cryo and RT

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Abstract This supplement is provided to show repeatability of results for room temperature (RT) and cryo data. Overall, for cryo conditions the results were very repeatable. For room temperature an improved method to fix the crystal to the goniometer is needed, as well, as adjustments to the motion control system due to the factor of 10 increase in required precision. This increase comes mainly from the sampling step size used to record the data. For cryo, typical reflection profiles can be accurately recorded with a step size of 0.01º while the crystal is frozen in place and therefore cannot move. For room temperature typical reflection profiles can be accurately recorded with a step size of 0.001º but the crystal can slip in the, commonly used, capillary mount.

1. Introduction

We used fine ϕ slicing to measure reproducibility in oscillation images. Besides the crystal radiation damage many other aspects of the experiment can affect reproducibility. If the mount used to fix the crystal is not sufficiently stable or the mechanical drive system cannot reliably position at precise locations then it will be impossible to obtain reproducible results.

2. Method

For each crystal a coarse data set was collected to determine the orientation matrix of this crystal. A range within the coarse data set was collected using the super fine ϕ method described previously. Unlike previous studies the same range was collected multiple times and differences between the profile data were analysed. In the case of cryo data collection, five 1-deg oscillations (coarse images) were recorded followed by ~300 (0.01º) fine images inside the range of the coarse data. The fine slicing sequence was repeated once for one crystal and twice for another crystal. For room temperature data collection, again five coarse images were taken followed by ~250 images at 0.001deg oscillation angle inside the range of the coarse data. The cryo data of the fine slicing sequence was repeated once.

The data is plotted as the positional difference in degrees between identical reflections as mapped over the surface of the detector. Areas where no reflections where recorded have been filled by taking a Delaunay triangulation of the available reflections and then using linear interpolation to fill in the missing data. The result provides an angular map of the repeatability reflection data as it might appear on the surface of the detector if reflections were everywhere. Since Delaunay triangulation can only be used when the location to interpolate is bounded by three points, only points within an area bounded by all the reflections have values.

3. Cryo Results

Since the crystal is frozen in a loop, which is fixed to the goniometer, the only variance in the system should be determined by the mechanical precision. Crystals at cryo conditions tend to have a broader mosaicity then at room temperature and therefore the resolution needed to record an accurate representation of the profile is typically 0.01º and the mosaicity is normally 0.50º or higher. This leads to nearly a hundred measurements or so for each profile. If the profile shifts by ±0.05º then it will still be in almost the same position (only a few frames either way for the maximum). To test this condition, two fine ϕ runs were conducted over the exact same angular range. Good profiles recorded for each run were compared for the location of the maximum of the profile from one run to the next. The comparison yielded an average angular position deviation of -0.001º with a standard deviation of 0.0195º. Since the profiles were measured in 0.01º increments and most of the differences fall into a range that is less than two measurement points in either direction and the average difference is basically zero it is reasonable to say that for cryo data with a step size of 0.01º the experimental results are very repeatable. The worst drift was only eight samples compared to the previous locations and they are all in the Lorentz region. The data is shown graphically in Fig. 1. The data is plotted across the surface of the detector (here: a marCCD-165 with 2048x2048 pixels).

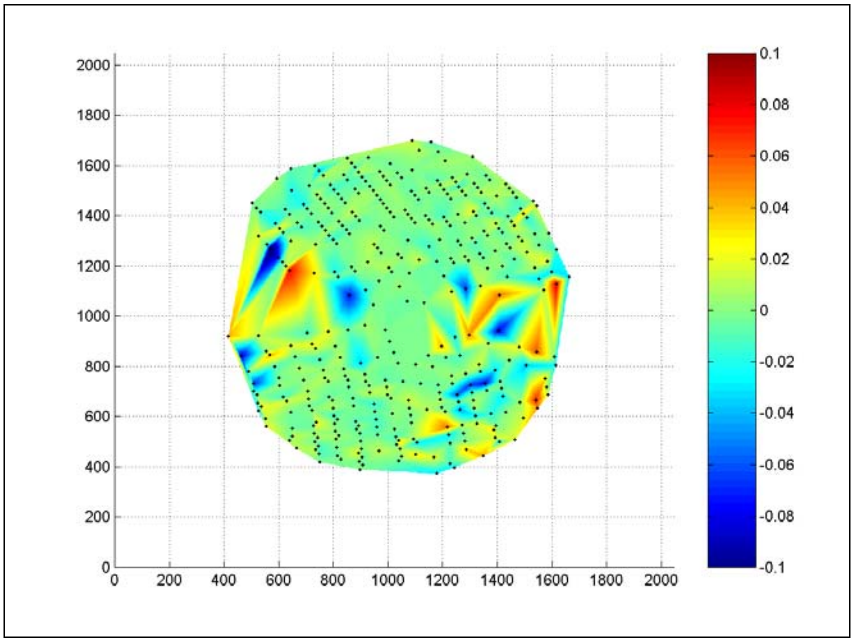

Figure 1 Cryo crystal 1 repeatability results. Data points for profiles were recorded at 0.01º intervals. The graph shows the positional change in degrees for the same reflection. Light green indicates very little motion. Blue indicates the reflection appeared earlier in the oscillation than the first time and red indicates later in the oscillation than the first time.

4. RT Results

During room temperature data collection the crystal can slip in the capillary. Additionally, there can be a large accumulation of radiation damage, which may also change the crystal diffraction properties. Two different crystals were used for this repeatability study. Data from the first crystal was collected at 0.001º slices over the same area three consecutive times. Comparing the first to the second run, the average difference was -0.00037º with standard deviation of 0.0020º. Comparing the second run to the third run, the average difference was 0.00068º with standard deviation 0.0024º. Comparing the first run to the third run, the average difference was 0.00027º and the standard deviation was 0.0025º. For the second crystal two runs were collected. Comparing the first run to the second run, the average difference was 0.0020º and the standard deviation was 0.0105º. The measurements are shown graphically in Figs. 2-5 with Figs. 2-4 for the first crystal and Fig. 5 for the second.

For the first crystal the average and standard deviation are good with respect to the sampling frequency. There does appear to be some structure to the variations, which may indicate some crystal slippage or due to some mechanical positioning problems. For the second crystal the average looks good but the standard deviation is approaching that of the cryo crystal. This makes it difficult to locate a reflection because the reflections can be moving by distances greater than the width of the reflection profiles (only 0.005º-0.008º for RT crystals). Judging the reflection profiles it is most likely that the crystals “slipped”, *i.e.* crystals could have moved during the oscillation as they were not “locked” (frozen) into position. Overall this level of repeatability would be sufficient for conventional crystallographic data collection.

For further topographic and mosaic studies the crystals need to be mounted in a more sturdy fashion and the motion control systems need to be fine tuned for very small angular motion. The best way to limit motion control issues would be to use stills for the data collection and making sure the positional resolution is much smaller than the step size of motion that is intended to be used. To prevent slipping the crystals could be encased in glue (Knapp *et al.*, 2004) and held in place using a pin and chuck or other rigid assembly.

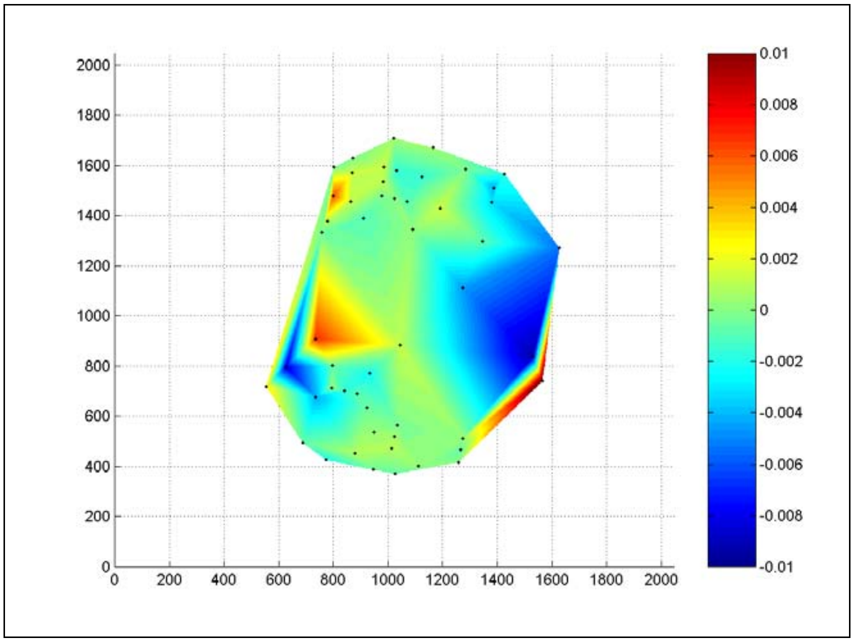

Figure 2 RT crystal 1 repeatability results for first and second run. Data points for profiles were recorded at 0.001º intervals. The graph shows the positional change in degrees for the same reflection. Light green indicates very little motion. Blue indicates the reflection appeared earlier in the oscillation than the first time and red indicates later in the oscillation than the first time.

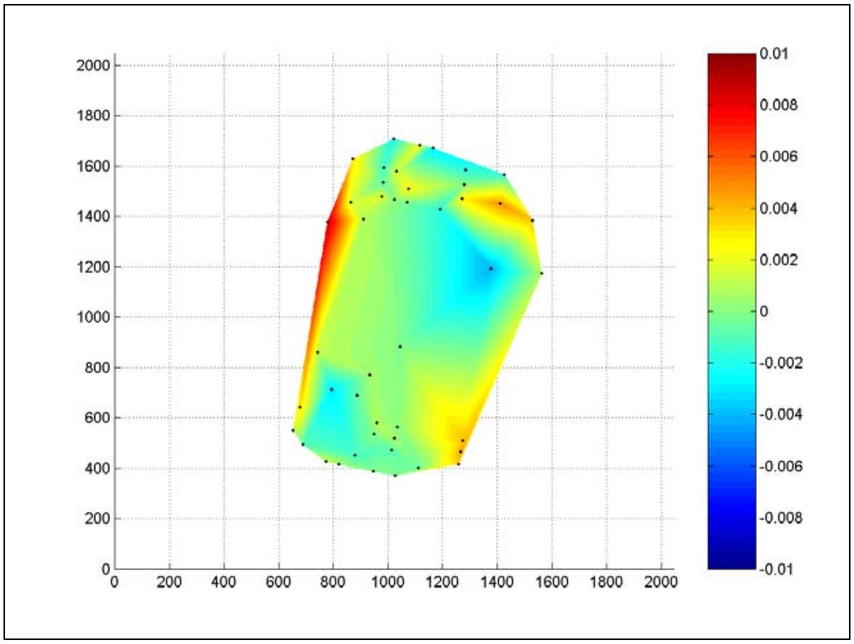

Figure 3 RT crystal 1 repeatability results for second and third run. Data points for profiles were recorded at 0.001º intervals. The graph shows the positional change in degrees for the same reflection. Light green indicates very little motion. Blue indicates the reflection appeared earlier in the oscillation than the first time and red indicates later in the oscillation than the first time.

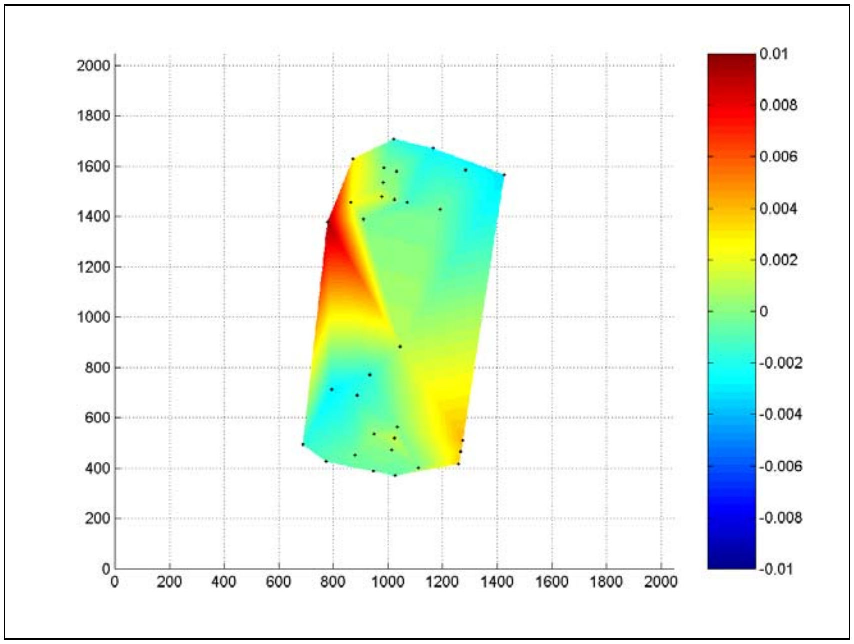

Figure 4 RT crystal 1 repeatability results for first and third run. Data points for profiles were recorded at 0.001º intervals. The graph shows the positional change in degrees for the same reflection. Light green indicates very little motion. Blue indicates the reflection appeared earlier in the oscillation than the first time and red indicates later in the oscillation than the first time.

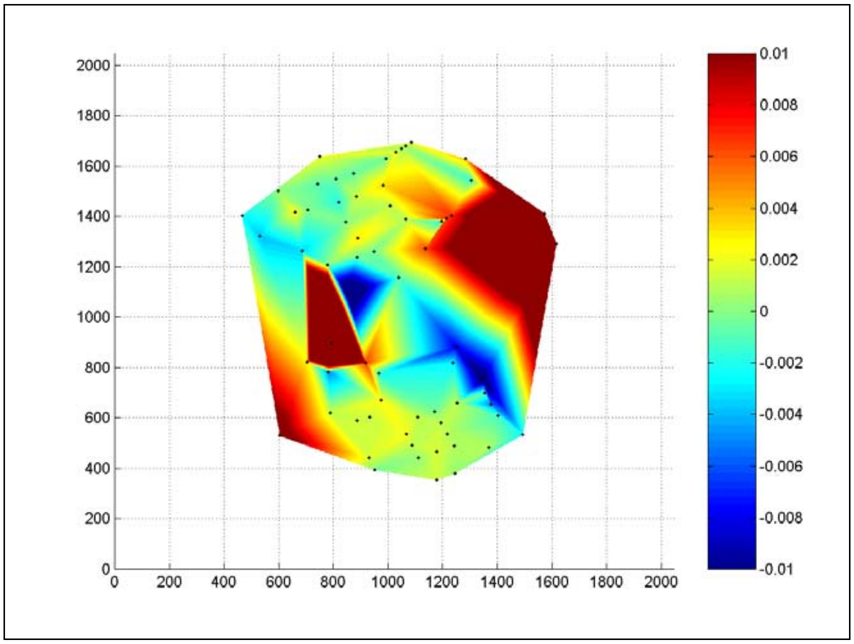

Figure 5 RT crystal 2 repeatability results for first and second run. Data points for profiles were recorded at 0.001º intervals. The graph shows the positional change in degrees for the same reflection. Light green indicates very little motion. Blue indicates the reflection appeared earlier in the oscillation than the first time and red indicates later in the oscillation than the first time.

References

Knapp, J. E., Šrajer, V., Pahl, R. & Royer, W. E. (2004). Micron 35, 107-108.