Prediction of Protein X-ray Crystallisation Trial Image Time-courses

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Abstract: This paper presents an algorithm to predict the outcome of a protein x-ray crystallisation trial. Results obtained from classification of individual images in a time-course are used, along with random forests, to make a prediction of the time-course outcome. Experiments on multiple datasets show that the first 8 frames of each time-course are quite sufficient to predict the final outcome.

1 INTRODUCTION

X-ray crystallography is widely used to determine the three-dimensional atomic structure of biological macromolecules, and provides the ability to gain unique understanding about the function of a protein (Newman et al., 2007). Without hyperbole, X-ray crystal structures have transformed biology, being the most successful way of determining the fundamental structure of macromolecules; well over 80% of entries in the Protein Data Bank (rcsb-PDB) have been determined using X-ray crystallography. Understanding the structure / function relationship, as revealed by crystallographic analyses, is also one of the most important tools for rational drug design (Dessau and Modis, 2011).

The technique of X-ray crystallography uses diffraction patterns generated by irradiating a crystalline sample of the molecule of interest with X-rays, thus the production of diffraction quality crystals is mandatory for this process. To date, the production of crystals requires triaging through an enormous chemical space to find conditions that preserve the delicate tertiary structure of the protein molecules whilst enabling the molecules to form a crystal lattice. The limiting factor is most often production of the pure protein sample required for the crystallization trials. Thousands of crystallisation experiments may be carried out in a single structural biology laboratory every day. As crystal growth is time dependent, each experiment is observed over time, with the normal timespan being weeks to months. Only since the turn of the century have robotic imagers become available, and these instruments automatically collect photographic images of a crystallisation trial over the course of the experiment. As each trial is imaged over time, multiple images are collected of the same trial at different time points; a set of images that belong to a single trial is called a time-course.

Currently, analysis and classification of the crystallisation image data are performed manually. As most crystallisation trials do not produce crystals, the process of manual observation and annotation is tedious, with numbers from the CSIRO C3 crystallisation laboratory suggesting that less than 2% of the 10-20K images collected each day are annotated, and there is simply no way of estimating how many of the images have actually been examined. Automating the classification process may aid the goal of labelling all the images collected as individual frames, but also may well allow the assignment of a common label for a whole time-course using sequence classification. This would both save significant time and effort for crystallographers, as well as providing coverage for all the images produced. The final goal would be to be able to predict from the early images in a time-course what the eventual outcome might be.

As significant numbers of crystallisation time-courses may be produced in a single laboratory (in its ten years of operation, the C3 has produced over 3 million time-courses), a large number of interesting time-courses may get ignored due to lack of resources to analyse each one manually. Moreover, large

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amounts of memory are also occupied by the huge datasets collected over time. Even more compelling, crystallographers are interested in finding and interrogating crystals as soon as possible in order to minimise the time until a protein structure is available. Thus, it is important to identify interesting conditions that produce crystals as early as possible.

This paper focuses on the prediction of protein crystallisation trial image time-courses by developing sequence classification techniques. The next section provides a brief overview of related techniques. Our approach for the prediction of crystallisation sample time-courses is presented next, followed by the results obtained. The final section discusses our conclusions and potential future work.

2 RELATED WORK

Image processing on protein crystallisation trial images is a relatively unexplored research area. So far the experiments carried out on this data have focussed on classification of single frames without consideration of the time-course context of each image (Buchala and Wilson, 2008, Cumbaa and Jurisica, 2005, Kotseruba Y et al., 2012, Lekamge et al., 2013, Walker et al., 2007, Watts et al., 2008, Wilson and Wilson, 2006, Yang et al., 2006). Prediction of the final outcome of a protein crystallisation trial time-course is a new and orthogonal approach to the problem of classifying images of crystallisation trials.

Random Forests has been used to predict the amino acids in a protein sequence that may be involved in mediating protein - protein interactions. Šikić et al (2009) used a combination of sliding windows and Random Forests to predict the protein - protein interaction sites in sequences. First the system classifies the data using the sliding windows method and then Random Forests is applied on a weighted class system for prediction of protein - protein interaction sites.

Some techniques that have been used for sequence classification and prediction in machine learning include sliding windows (Babcock et al., 2002, Gama et al., 2013, Li et al., 2005) and Hidden Markov Models (Dietterich, 2002). Although these techniques provide good results for regular sequence learning problems, they can be challenging to apply to our data set as the time gap between frames in a single time-course varies considerably.

2.1 Contributions

To our knowledge, predicting the outcome of protein x-ray crystallisation trial time-courses has not yet been studied. This paper provides a new tool to the field of crystallography by proposing and testing a method for the prediction of the eventual outcome of a crystallisation trial. It utilizes pre-processing and single frame classification techniques already developed in our group (Lekamge et al., 2013, Lekamge et al., 2016, Mele et al., 2013), and extends them to time-course classification.

3 APPROACH

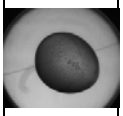
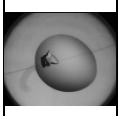
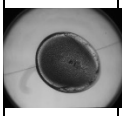
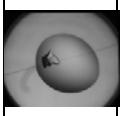
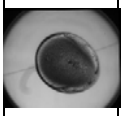
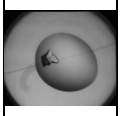
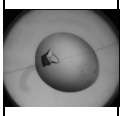
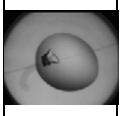
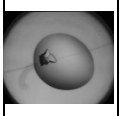
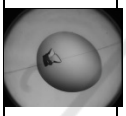
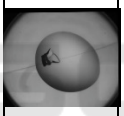
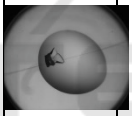
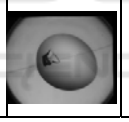
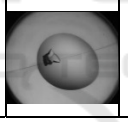
Processing of the protein crystallisation trial time-course is different from normal video processing. In a normal video, there are usually a large number of frames, whereas in the crystallisation dataset the number of frames is very small, varying between 9 and 15 frames. Moreover, in a normal video, the frame rate is stable whereas in the crystallography data the time gap between the frames is variable and very large. An example of a protein crystallisation trial image time-course is presented in Table 1.

The proposed method to predict time-course labels consists of several steps. First each image is pre-processed to find the area around the droplet and align each image according to the time-course. Next the difference images are obtained by obtaining the difference between the first image in a time-course and the rest. Then single frame classification is carried out using both original and difference images, and the results obtained from single frame classification are used for time-course label prediction. An overview of the approach is presented in Figure 1. This paper mainly concentrates on the last step, namely time-course prediction.

3.1 Data

The data for this work was acquired from C3 (Collaborative Crystallisation Centre), CSIRO, Melbourne, Australia. Each image data set is the result of observing crystallisation experiments over a period of time, from one hour to 10 weeks after the start of the experiment. The set of images belonging to one experiment is called a time-course (Table 1). The images are gray scale and each provides a snapshot of changes inside the experimental droplet at that point in time.

Table 1: A crystal producing time-course with time stamps.

Frame number	Frame	Time stamp	Frame number	Frame	Time stamp
1		1 hour	8		2 weeks
2		5 hours	9		3 weeks
3		10 hours	10		4 weeks
4		1day	11		5 weeks
5		2 days	12		6 weeks
6		5 days	13		7 weeks
7		1 week	14		8 weeks

3.2 Pre-processing and Frame Classification

Images belonging to a time-course are first arranged according to acquisition time (step 2, Figure 1), and the area of the droplet (Figure 2) is identified using the droplIt algorithm (Vallotton et al., 2010). Then the difference images are computed (step 3, Figure 1). This has been explained in more detail elsewhere (Mele et al., 2013). Next feature extraction (step 4, Figure 1) is carried out (Lekamge et al., 2013).

After pre-processing, classification of single frames is carried out using multi-view learning, with random forests as the classifier for each view (Lekamge et al., 2016) (step 5, Figure 1) The MVL-based algorithm for single frame classification will be termed as Crys_MVL_RF hereafter.

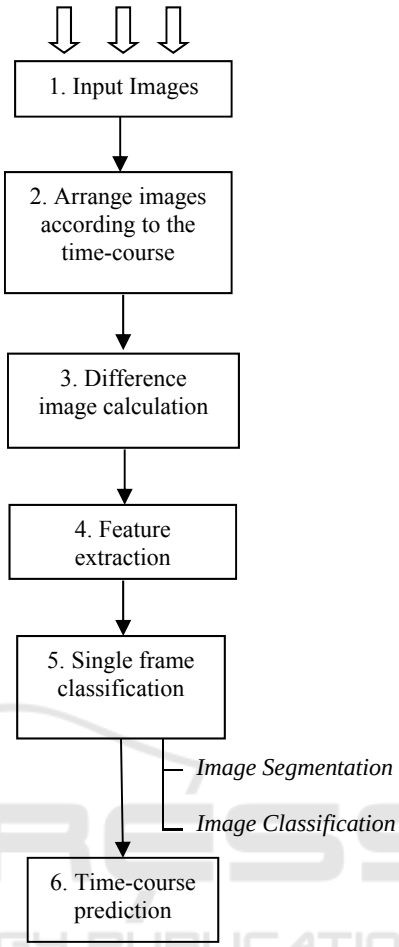


Figure 1: System overview.

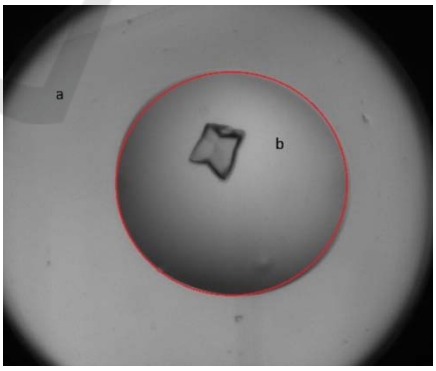


Figure 2: Droplet in a well (a)Well area (b) Droplet area.

3.3 Prediction of Time-course Labels

To predict the final outcome of a protein crystallisation experiment, named time-course prediction hereinafter, the single frame classification results obtained using Crys_MVL_RF are used as the starting point. During single frame classification,

each image is labelled as belonging to one of the following classes:

i. Crystal
ii. Precipitation
iii. Skin
iv. Clear and
v. Other

Some examples of these classes are presented in Figure 3.

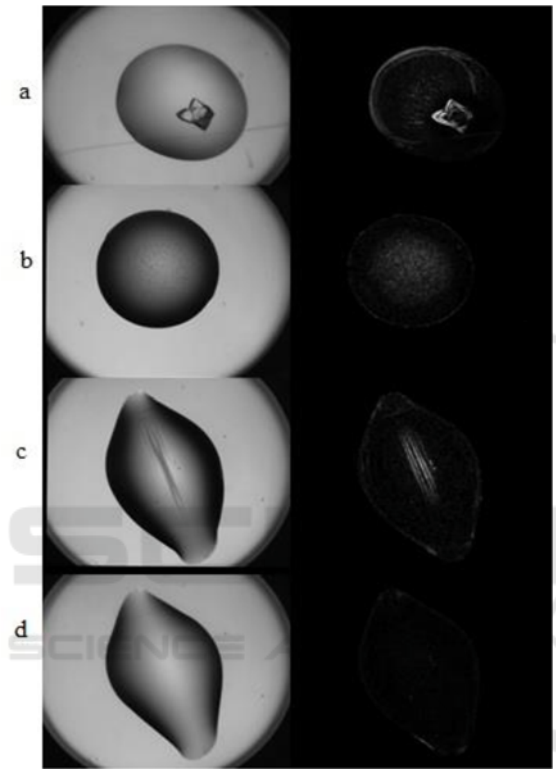


Figure 3: Classification examples. The original and respective difference images (a) crystal (b) precipitation (c) skin and (d) clear (Lekamge et al., 2016).

For the time-course prediction experiments, only four classes were used; the skin class was again re-classified into one of the following classes.

i. Crystal
ii. Precipitation
iii. Clear
iv. Other

Random Forests was used, along with the frame classification results, to predict the whole time-course label. The number of frames used in prediction was varied systematically and the corresponding prediction accuracies were computed. The least number of frames required to make an accurate prediction of the final outcome of a protein crystal experiment was then picked out.

The purpose of predicting the final outcome of a crystallography time-course is to identify at the earliest the experiments that are most likely to produce interesting results. In turn this would allow discontinuation of those experiments that are unlikely to have interesting outcomes. The images produced by the experiments were grouped together into a dataset based on the protein sample, as the number of frames in a crystallography experiment depends on the settings chosen for imaging purposes, and these remain constant for a specific protein. These datasets were obtained during separate experiment rounds.

For each dataset, Random Forests was used as the prediction algorithm and the single frame results obtained using Crys_MVL_RF were reused as the input. The experiments were repeated for different numbers of training frames in each round per time-course, and the prediction outcomes were computed in each round. The number of frames was increased by one in every iteration of these experiments.

4 RESULTS

The results are presented below on a per-protein basis, as that is the basis for grouping into a dataset.

MA003389 Dataset: This dataset has 15 frames in each time-course, and at every round of training an extra frame was added to the training set until 14 frames were used for training. The prediction of the final frame was recorded at each round. This dataset has 96 time-courses. For classification, Random Forests was used with 10 times 10-fold cross validation. The results obtained are illustrated in Fig. 4 (blue line).

From the results, it is clear that the prediction accuracy increases rapidly up to the eighth frame, thereafter the accuracy increases much more slowly and tails off after about 10 frames. Therefore, the crystallographer could choose to terminate the experiments after 8-10 timeframes, depending on the urgency and need to conserve resources.

MA100420 Dataset: This dataset has only 9 frames in each time-course and at every round an extra frame was added to the training set until upto 8 frames. The prediction of the final outcome was recorded for each round as before. This dataset also has 96 time-courses. Random Forests was used for prediction and the results obtained are illustrated in Fig. 4 (red line)

On analysing the results, it can be seen that even though the prediction accuracy keeps increasing as expected, the highest prediction accuracy achieved is lower compared to the MA003389 dataset. It appears

that a total of 9 frames is insufficient and extending the crystallography experiments for a longer period of time might help. More experimental data is needed to verify the trend and evaluate the utility of extending the time period.

MC006299 Dataset: This dataset has 14 frames in each time-course and in every round an extra frame was added to the training set until upto 13 frames, and the prediction of the final frame label was recorded for each round. This dataset has 192 time-courses. Random Forests was used again for these experiments. The results obtained for this dataset are illustrated in Fig. 4 (green line).

On analysing the results for the MC006299 dataset, it is clear that the prediction accuracy follows a similar pattern to MA003389, with the prediction accuracy increasing steadily until the eighth frame, then tailing off. In this case, the recommendation to terminate the crystallography experiments after 8 frames is very clear and easy to make.

MC007204 Dataset: This dataset again has 14 frames in each time-course and in every round an extra frame was added to the training set until up to 13 frames, and the prediction of the final frame label was recorded for each iteration. This dataset has 192 time-courses as well. Random Forests was used again in these experiments. The results obtained for this dataset are illustrated in Fig. 4 (yellow line).

On analysing these results, it can be seen that the results obtained by this dataset are similar to both MA003389 and MC006299 datasets. The results also show a steady increase in prediction accuracy until the eighth round, then more modest increase. The choice to terminate after 8 frames is available, if desired.

By analysing the results for all the datasets, it can be seen that the highest prediction accuracy obtained is 88.75%. However, this accuracy is attained only when all the frames before the final frame are used for training, and therefore is quite expensive.

To estimate the minimum number of frames necessary for prediction of a time-course label, the best accuracy ratio per number of frames used is computed. The formula is presented below:

$$Prediction\ accuracy\ ratio = \{(prediction\ accuracy\ obtained / number\ of\ frames\ used\ for\ training)\} * total\ number\ of\ frames\ available \quad (1)$$

The prediction accuracy ratio was computed for all the datasets and is presented in Fig 5.

On analysing the values and trends for the prediction accuracy ratio, it can be seen that except for the dataset MC006299 all the datasets attain the

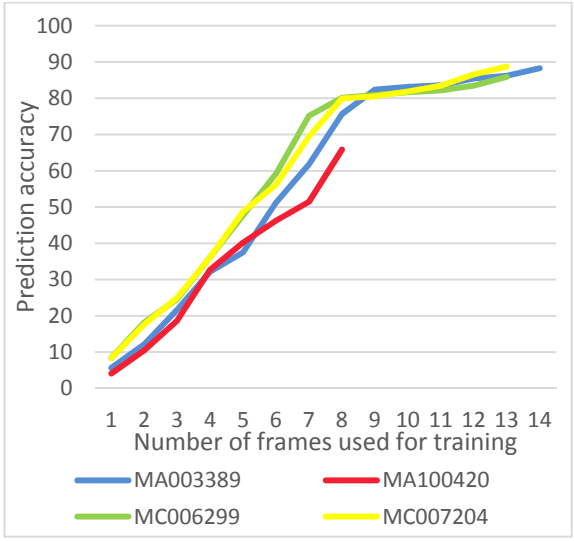


Figure 4: Prediction results for all datasets.

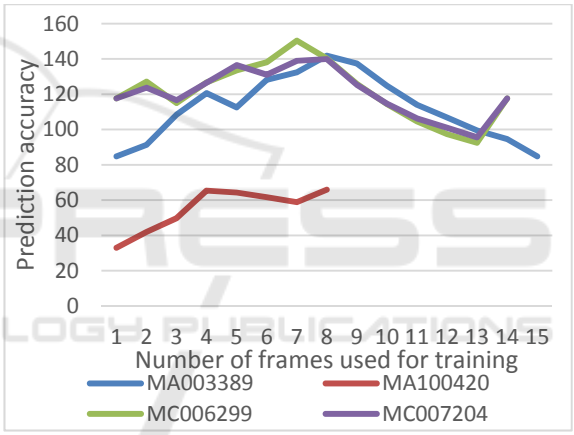


Figure 5: Prediction accuracy ratio for all datasets.

peak value of the prediction accuracy ratio on frame 8, with the MC006299 dataset attaining its peak at frame 7. For dataset MA100420 (represented by the red line graph), the peak value is impossible to declare as it has only 8 frames per time-course.

5 CONCLUSIONS

By analysing the values of the prediction accuracy ratios, it is possible to declare that the best possible number of frames for prediction is 8 frames as the increase in prediction accuracy is relatively low after that, and in fact the crystallography experiments may be terminated after 8 frames. These results also co-incide with the observations reported by Ng et al who observed the experiments carried out at the Oxford

site of the Structural Genomics Consortium (Ng et al., 2016). More experiments can be carried out using different protein solutions to confirm the number of frames required to accurately predict the final outcome of a time-course in the future. Moreover, details about the protein solutions also can be used along with the frame labels in order to confirm the prediction accuracy.

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