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Determination of crystal orientation from micrographs using a MATLAB program

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Crys.m is a MATLAB routine that combines a micrograph of a crystal with a scaleable, rotatable three-dimensional cage structure to determine the orientation of the crystal axes. The example presented here uses the morphology of tetragonal lysozyme. Rotation of the cage until it aligns with the crystal in the image yields the orientation of the *c* axis of the crystal relative to the image normal. This analysis can be used for quantitative determination of crystal orientation effects induced by electric, magnetic and/or gravitational fields.

1. Introduction

There has been significant interest in recent years in the growth of protein crystals in the presence of external fields (*e.g.* Ataka & Wakayama, 2002). In particular, significant alignment of lysozyme with electric and magnetic fields has been reported (Nanev & Penkova, 2002; Sakurazawa *et al.*, 1999; Yin *et al.*, 2003). Recent development of an electric-field growth apparatus (Charron *et al.*, 2003) will encourage further studies of field effects.

In the literature, optical micrographs of grown crystals have been used to determine the alignment with the applied field, but in order to maximize the field strength, it is desirable to have a small distance between the field poles during growth. This makes subsequent imaging easiest in the direction perpendicular to the pole faces, and hence parallel to the applied field. Determination of the degree of alignment in any direction requires a three-dimensional interpretation of a two-dimensional image; in the case above, this is particularly difficult. Because of the challenge of this analysis, many papers have reported the number or percentage of crystals 'aligned', rather than information on the angle of the crystallographic axes relative to the field, which would be a more sensitive indicator of field effects. The program we have written facilitates this analysis. It is written in MATLAB, using tetragonal lysozyme morphology as an example. The graphical user interface (GUI) permits a direct comparison of the photomicrograph and a computer-generated cage structure (based on the crystal morphology) to analyze the orientation of the *c* axis of the crystal. MATLAB permits ready modification of the parameters so that the program could encompass other crystals, and the open code permits modification by users interested in other orientation information. The images generated by the program are similar to the output of programs such as ESM's *SHAPE* (ESM Software, Hamilton, OH, USA) but the transparent cage and the interactive interface, combined with the background images, allow their use for crystal orientation analysis.

2. Key features

The input required for normal operation is an image (black and white or color) of the experimentally observed crystals in one of the common image formats such as tif, gif, bmp or jpg, recognized by MATLAB. The control file is `crys.m`, and the user will also need to have `cryst0.m` and `rotxtal.m` in the current directory in MATLAB, as these are called by the main program. The crystal model coordinates that describe the morphology are explicitly defined in the file `cryst0.m`, and can be changed by the user, if desired. The cage is drawn by connecting a set of vertices that are generated in the 'draw crystal model' section of the code. Replacing the provided tetragonal coordinates with those appropriate to a different crystal morphology requires substitution of the corner coordinates directly within the code.

The output of the program is the orientation of the *c* axis with respect to the image plane with which the cage is being compared, as well as the MATLAB image of the aligned cage, which can either be saved in a MATLAB format, or be exported as an image file. The individual axial rotations required to reach the final alignment are not saved, as they are not unique.

3. Interface

The program uses two control windows. The first (input) window is used to select the image file for analysis, specify whether or not it is in color, choose the initial size of the crystal model, and input information on the ratios of the face widths for the tetragonal model assumed. Once the user selects 'Run', the program opens a second window (image), with an interactive GUI interface, for matching the crystal model to the underlying image.

3.1. Input window

The image to be used for analysis is selected by push-button access to a browse function. Double clicking on the file in a folder loads it into the program, using typical Windows functionality. The user must manually select whether this is a color image file, since the difference in data storage structures for the two image types cannot be interpreted by MATLAB. Failure to identify the image type correctly, typically results in the absence of either the micrograph or the cage in the image screen. The user may select an initial scale factor to match the cage size to the crystal of interest. In addition, the user can input ratios of the lengths of the crystal model axes.

For lysozyme, the default value of *c*/*a* should not generally be changed as it is only a gentle function of conditions (Dobrianov *et al.*, 2001), but in the interest of generality, this capability is offered.

Changing this ratio would change the angles between the *c* axis and the [011] family of directions (the 'steepness' of the 'roof') of the crystal (see Fig. 1). The *b*/*a* input controls the ratio of the widths of the hexagonal faces in the tetragonal crystals (along the [110] *versus* [1$\bar{1}$0] directions). It does not change the underlying basis vectors, since the crystal is assumed to be tetragonal, but it extends growth of the (110) face relative to the (1$\bar{1}$0) face. We have not grown crystals in our laboratory that display this behavior, but have encountered images on the Web that have this property (see *e.g.* http://www.cir.tohoku.ac.jp/sazaki-p/).

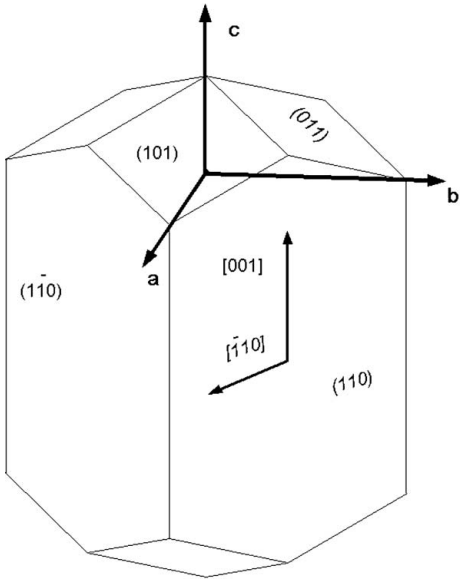

Figure 1
Crystallographic faces and directions of lysozyme (after Li *et al.*, 1999).

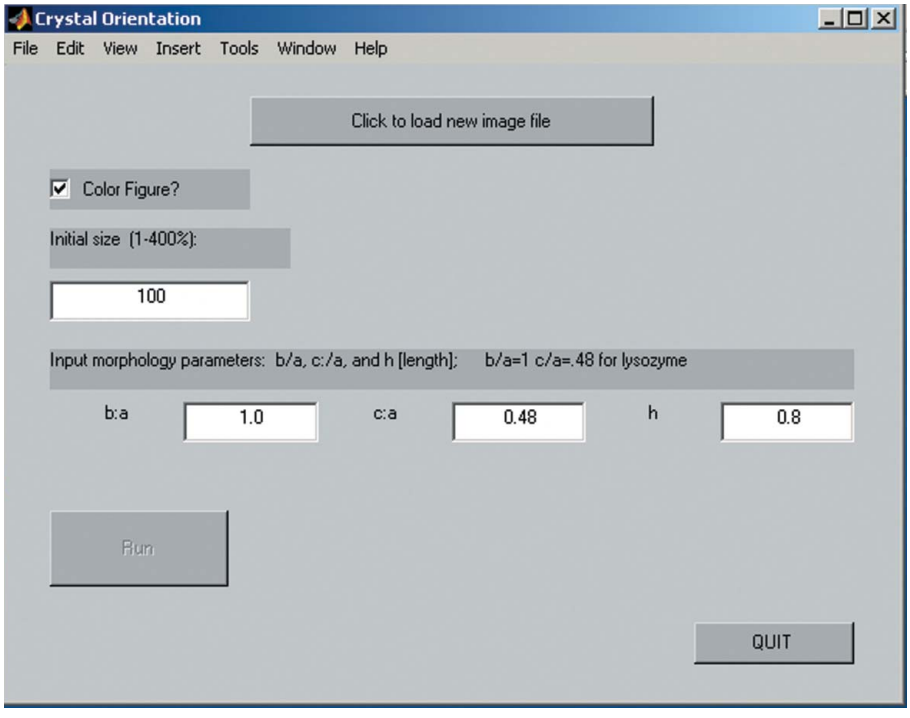

Figure 2
The input screen as it appears before loading a file. The color box is checked as the default, due to the prevalence of color images. The Run button is inactive until a file is chosen.

The other parameter, *h*, allows the user to adjust the length of the crystal in the [001] direction relative to the face width in the [110] direction. In the laboratory, this elongation is typically a function of pH relative to the pK_a of the buffer used for growth (Gibson *et al.*, 2000), with lower pH and smaller crystal size associated with more needle-like crystals.

Fig. 2 is a screen shot of the input screen before file selection; the 'Run' button is inactive until a file is loaded. The path and file name appear below the file input button once the user makes a selection.

3.2. Image window

After choosing the morphology parameters and the image file, the user clicks the 'Run' button to begin the real-time rotation of the cage to match the underlying image. The cage is rendered as a flattened three-dimensional image, assuming a perspective angle of 7° (the MATLAB default). Fig. 3 shows the image window, with the default values implemented. Rotation around the *X* and *Y* axes [defined relative to the normals to the (110) crystal faces], and the *Z* axis (*c* axis), respectively, give the user a straightforward way of manipulating the cage. We have found that normals to the principle faces are more intuitively attractive than the basis vectors of the crystal lattice. The slider controls allow incremental rotations of 1, 2 or 10°, obtained by clicking the arrows, clicking the bar, or sliding the control all the way to the end, respectively. After each input, the most recent increment appears under the slider.

There are sliders for horizontal and vertical translations (arbitrary units) so that the cage can be placed atop the relevant part of the image for a best match of the crystal faces and angles. These sliders move the cage approximately 4% of the image size for a bar click, and 0.4% of the image size for an arrow click, varying somewhat with the dimensions of the input image.

In addition, the size of the cage can be interactively adjusted to obtain a good fit. The size button redefines the cage vertices, and updates the lines using the color lookup table for the surface. If the user has rotated the cage significantly, this will revise the cage so that lines which are 'closer' (larger *z* values) will be white in the image, and the 'deeper' ones black (using the default color map). Clicking on the center of the slider without moving it can be used to update the black/white values for the cage without a size change.

In order best to adjust the cage to the underlying image, the user needs to rotate the cage so that the perspective view is reproduced by the cage. For tetragonal lysozyme, foreshortening of the (101) family of faces that are turned away from the image normal is a key feature for making an accurate alignment. The user needs to match the apparent shapes of these faces, and assure that the [001] direction stays aligned with the appropriate crystal edges. Fig. 4 uses the same underlying image as Fig. 3, but shows the crystal cage rotated and sized to match one of the crystals. Note that the crystal in this instance grew attached to the base of the crystallization plate, so that the top faces of the crystal are used to determine the proper alignment. For crystals of

lower quality than those illustrated here, the length of well defined edges is the primary determinant of the quality of the fit. In some cases, using an image filter before fitting to sharpen edges, or going to a black and white rendering of the image, can give improved clarity of the boundaries that are to be used.

It is useful to note that the edit functions of MATLAB figures are still available while using this program (*via* the Tools...Edit Plot menu in the figure window). This means that the user can draw lines parallel to crystal edges to highlight them during analysis, add labels, or change the color lookup table for the cage to improve the contrast between the image and the cage. Because the size button redraws the object, some property changes applied to the object may be lost when the size is changed. Line color and style, the most likely to be modified by the user, are retained.

Advanced users can change the amount of perspective through the CameraViewAngle command to match the f-number of the imaging system. Unfortunately, the grab and rotate (rotate3d) function native to MATLAB cannot be used to simulate the cage for many crystals, since that routine changes the viewing angle of the cage rather than the orientation of the cage itself, and the view is limited to the upper half-plane. This makes matching some crystals impossible.

The 'Reset' button has the expected function, except the program retains the line color and style through this operation.

The image window can be closed to initiate analysis with different structural parameters on the same image file, using the 'Close this window' button. Changing the parameters and clicking 'Run' again will reload the same image, but with a modified crystal cage. This is the required procedure for resetting the length ratio, or the *b*/*a* ratio of the crystals.

Clicking on the 'Load new image' bar in the input window allows a change of image files without exiting the program.

The user can also return to the input window by clicking on it. Multiple image windows can then be displayed; however, the GUI will only be active in the most recently opened image file.

The purpose of the program is to determine the angle between the *c* axis and the image normal (the assumed field direction). This angle is displayed on the GUI just above the reset button, and is updated after each rotational step. This angle was chosen as the output based on our studies of crystals grown with the electric field parallel to gravity (and hence perpendicular to the easiest imaging axis). Investigators interested in other alignment metrics can modify the code to return other angles. Using this program to align a cage with each crystal in an image, it is

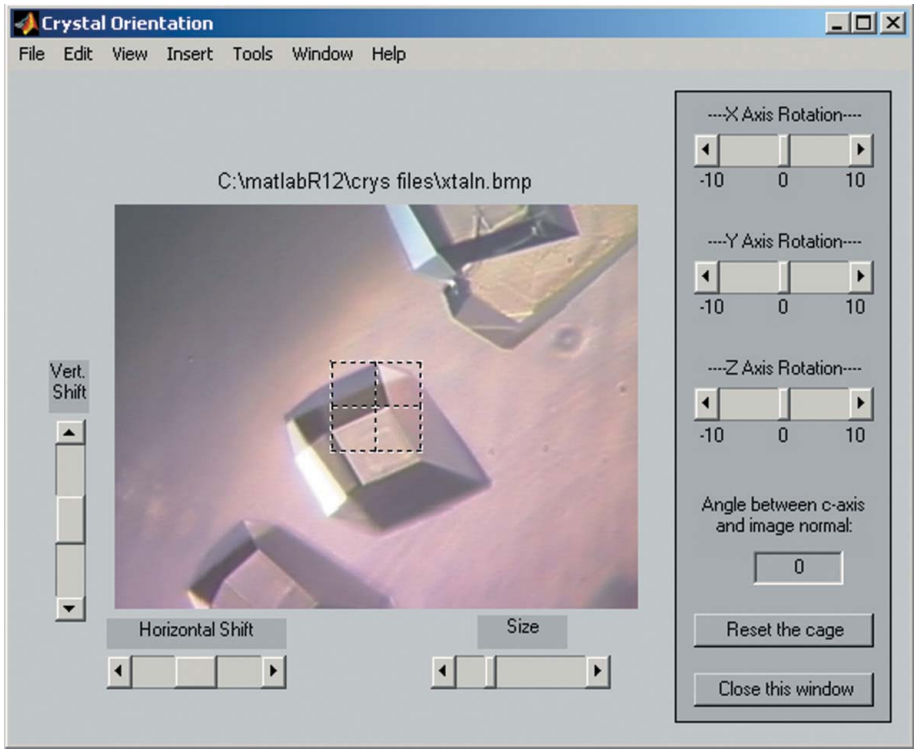

Figure 3
The GUI image screen, as it first appears, with the crystal cage in its default position.

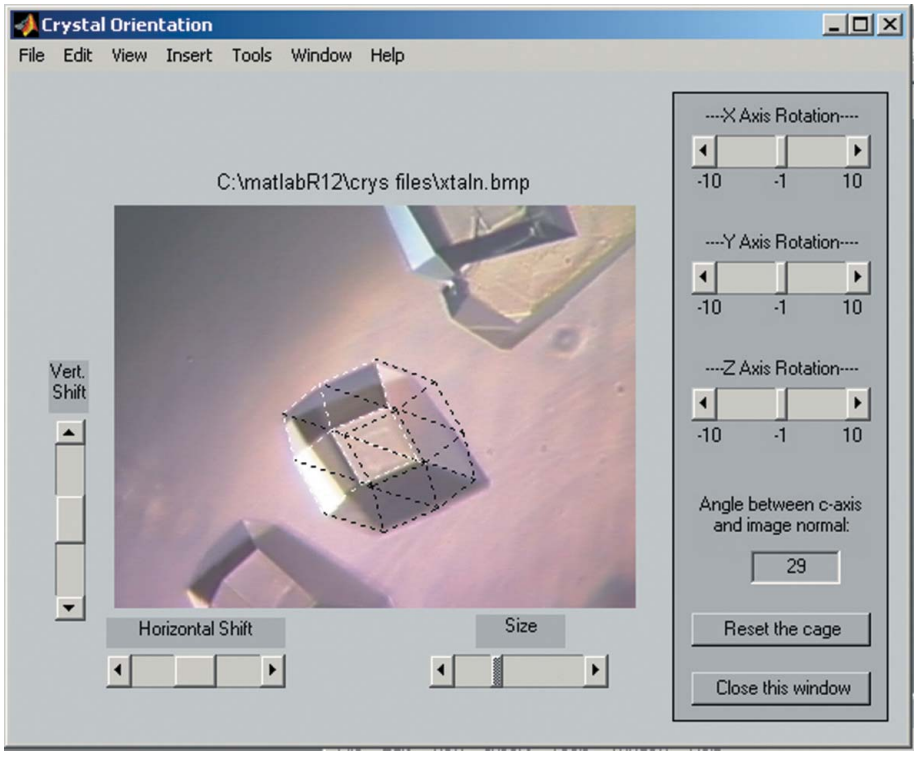

Figure 4
Screen shot of the image window, after cage alignment. (In this image, the crystal has grown attached to the bottom of the well, so the crystal is truncated relative to the computer-constructed cage.)

possible to extend existing metrics of 'percent of aligned crystals' to more meaningful angular distributions as a function of growth conditions. In repeated trials, the orientation angle could be determined to $\pm 1.5^\circ$ in less than 2 min per alignment procedure, by users familiar with the interface.

4. Distribution

This program is freely available from http://www.dartmouth.edu/~ujg/downloads/matlab.html.

5. Summary

We have written a MATLAB program that permits alignment of a transparent crystal model on top of an image file. In its present form, the model uses the morphology of tetragonal lysozyme crystals, but due to the flexibility of MATLAB, this program can be readily modified by the user to accommodate other structures. The program has a graphical user interface designed to permit rapid determination of the orientation of the crystal to within a couple of degrees. This information can be used to determine crystal alignment with external fields in cases where partial alignment has occurred; this regime has been difficult to address with previously available methods.

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