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Ripple: a program to collect and analyze digital topographic sequences

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A Microsoft Windows-based system called *Ripple* has been developed to drive a digital topography camera and to collect and analyze a sequence of topographic images. It has a graphical user interface, is easy to use and can be parasitically installed at existing beamlines. The modular design allows for the simple addition of detectors, detector image file formats, triggers and motion controllers.

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1. The crystallographic problem

Topography is the high spatial resolution imaging of an individual reflection (Stojanoff & Siddons, 1996; Stojanoff *et al.*, 1996, 1997; Dobrianov *et al.*, 1998; Otálora *et al.*, 1999; Boggon *et al.*, 2000). Topography is the only technique that provides a detailed picture of the internal mosaic domain structure of a protein crystal. This information can be used to understand better how various processes, such as cryo-cooling, annealing and radiation damage, affect protein crystals. Conventional film and nuclear emulsion topography methods have many drawbacks that can be overcome by using a CCD detector with a small pixel size (~8 µm) (Ludwig *et al.*, 2001; Lovelace *et al.*, 2004; Lübbert *et al.*, 2004). Such high-resolution CCDs generally cover only a very small active area as compared with the large CCD-based area detectors commonly used in protein crystallography and are not practical for the collection of structural data. There was a need to collect topographic data rapidly at a typical protein crystallography beamline with limited disruption to normal beamline function. The *Ripple* software described here, coupled with a high-resolution CCD-based detector system, implements this type of experiment so that topography data can be collected to supplement conventional structural diffraction data.

2. Method of solution

We use a large area detector to identify target reflections and then use this information to position the small topography detector mechanically. This novel method allows the topography detector to be quickly and parasitically installed at many existing beamlines, as has been demonstrated previously (Lovelace *et al.*, 2004, 2005). *Ripple* software was created to implement and test this dual detector approach by providing control of the topographic data collection and allowing for basic analysis of the topographs in real time (Lovelace *et al.*, 2006).

Ripple uses a system based on modules to interact with external components. For example, the internal image display engine uses a module to read an image file and convert it into a consistent internal format. Using this approach it is possible to create a new module for a previously unrecognized detector image file format without making any changes to the core *Ripple* executable. The modules are encapsulated in dynamic linking library (DLL) files. When *Ripple* starts it scans the DLL files in its module directory and dynamically loads the modules it finds. Currently, *Ripple* supports four types of modules:

motion controllers, detectors, triggers and detector image decoders. The motion control module allows the software to position a topography detector. At present, *Ripple* includes one motion control module that is designed to drive a BiSlide (Velmex, Bloomfield, NY, USA) positioning system. Detector modules allow *Ripple* to capture frames of data from a detector. The software has one detector module which drives an EDC-2000S camera (Electrim, Princeton, NJ, USA). This module outputs the images as 16-bit greyscale TIFF files (Aldus Corporation, 1986) with an integrated text header similar to the header used by ADSC CCD (Area Detector Systems Corporation, Poway, CA, USA) and SBC CCD (Structural Biology CAT, Chicago, IL, USA) detectors. This allows the image header data to be read with a text-based program and the images to be read with image software that supports TIFF files. Trigger modules allow detector exposures to be synchronized with external events. The software provides three trigger modules. The manual trigger allows the user to begin and end an exposure by pressing a GUI-based button to start the exposure and a second time to stop the exposure. The timed trigger allows the user to enter an exposure time and when a button is pressed the camera will expose for that amount of time. The UDP (user datagram protocol)-based trigger allows the system to synchronize camera triggering with beamline hardware using ethernet. UDP triggering has been extensively tested at BioCARS beamline 14-BMD at the APS. Detector image decoder modules provide a mechanism for *Ripple* to display various detector formats. The image formats supported are ADSC CCDs, MarCCD (Mar Research, Norderstedt, Germany) and the 16-bit TIFF format created by the EDC-2000S detector module.

Ripple stores all configuration and project files in extensible markup language (XML) (Bray & Paoli, 2004). XML format is human readable and can be displayed with modern web browsing software or other software that supports the XML format. XML allows for the easy inclusion of new features while keeping the file backward-compatible with previous versions.

A *Ripple* screenshot is provided in Fig. 1. It shows the main *Ripple* menu with tool bar (Fig. 1a), an image window (Fig. 1b), header window (Fig. 1g), a trigger status window (Fig. 1i) and a detector status window (Fig. 1h). The image window has three different tabs. The first tab (called 'Topography Image' in Fig. 1b) displays an image from the topography detector. The second tab (called 'Coarse Image' in Fig. 1b, corresponding image not shown) displays the image from the large area detector. The third tab (called 'Motion Overlay' in Fig. 1b, corresponding image not shown) shows the area covered by the

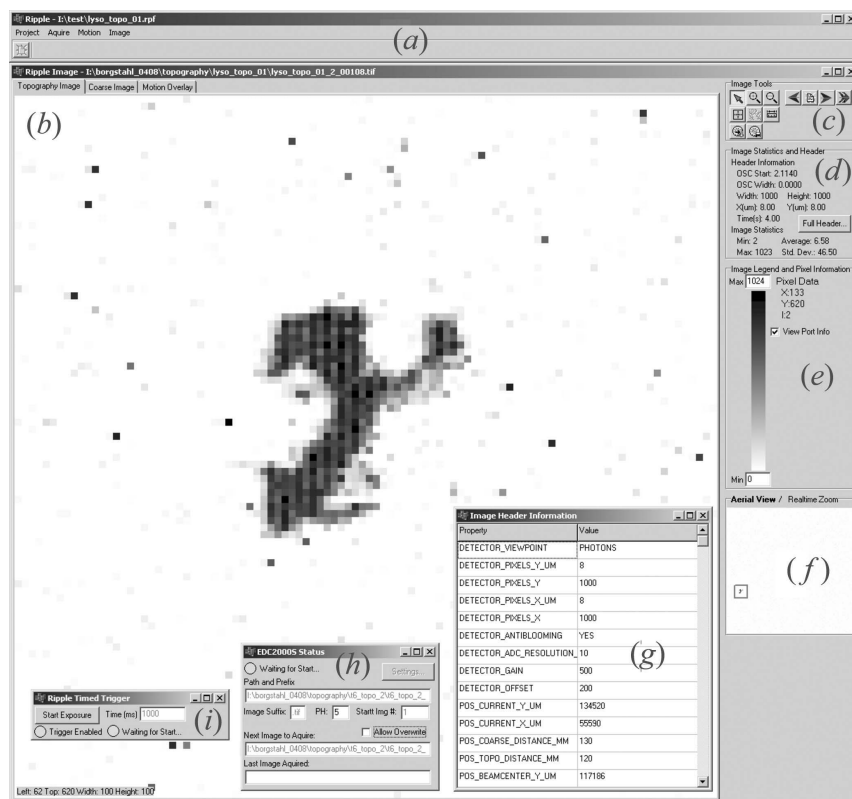


Figure 1

Ripple, a program to drive and display data for topography experiments: (a) main window; (b) an image window with a topography capture; (c) image window tools; (d) image statistics; (e) color bar and current pixel data; (f) real-time zoom/aerial view window, currently showing an aerial view with the box indicating the portion displayed in the image window; (g) header window showing current image header data; (h) detector status window; (i) trigger status window.

motion control system and the current location of the camera. Many useful image tools are available along the right side of the image. They include the ability to zoom in/out, advance to the next image, follow images as they are collected, determine intensity values and measure distances (Fig. 1c). The camera can be positioned by selecting a location on either the coarse image from the large area detector or in the motion overlay wide-field image (Fig. 1b tabs). The color bar along the left side (Fig. 1e) shows how colors are related to counts in the image and it shows location and count information for the area the mouse is currently over. There is also a real-time zoom/aerial view window (Fig. 1f). This window shows a real-time zoom of the image directly below the mouse when the image is displayed full screen and it shows an aerial view of the image with a box indicating the displayed portion of the image when the image is zoomed in.

From an analysis standpoint, the software allows the user to see the collected data in real time. The data can be shown as an animated sequence to visualize the different mosaic domains of the crystal. The user can also measure the size of features for comparison as well as look at the different count values. The program will also generate the necessary scripts to process the data further in *Matlab* (Mathworks, Natick, MA, USA). This program and related hardware allows for topography data to be collected easily along with conventional protein crystallography at regular protein crystallography beamlines

which can operate with a parallel beam. There is no other software-based system that offers similar functionality. An example of how *Ripple* and the digital topography detector system were used to study cryo-cooled protein crystals can be found elsewhere (Lovell *et al.*, 2005, 2006).

3. Software environment

Microsoft Windows 2000 or better is required for the program.

4. Hardware environment

Minimum requirements are a computer running Microsoft Windows 2000 with at least 128 Mbyte memory, a free PCI slot for the Electrim camera (if it is the CCD that will be capturing the topographs), an RS232 serial port (to control the Velmex BiSlide motion controller) and an ethernet connection (if ethernet will be providing the camera triggering). Other detector/trigger combinations may require more or less hardware.

5. Documentation and availability

Ripple, hardware and associated documentation can be obtained from the authors on a collaborative basis and are available 'as is'. HTML-based help and file format specifications are available with the program. New modules for decoding different detector formats, motion controllers and triggers will be created as needed.

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