

## **2dx - Automated 3D structure reconstruction from 2D crystal data**

B. Gipson, X. Zeng, and H. Stahlberg

Molecular & Cellular Biology, College of Biological Sciences, University of California at Davis,  
1 Shields Ave., Davis, CA 95616, USA

Membrane proteins are of central importance to health and disease. The structure of membrane proteins in the membrane-embedded state can be determined by cryo-transmission electron microscopy of two-dimensionally crystallized membrane protein crystals. Recorded images have typically a very low signal-to-noise ratio, so that extensive computer image processing is required to extract the signal from these images. Combination of a multitude of images from differently tilted 2D crystals can be used to produce a 3D reconstruction [1].

Software for this image processing task is available in the so-called MRC software suite [2], and has been used to determine the structure of 7 membrane proteins so far. While the different programs of the MRC package offers powerful tools for various tasks, their usage is difficult and labor intensive.

Here we present a new software system called *2dx*, which was originally based on the MRC programs. *2dx* now optionally allows fully automated 3D reconstruction from a number of 2D crystal images [3, 4]. Importantly, *2dx* can also be used to analyze and process noisy low-dose images of materials sciences specimen, as long as these show an underlying crystalline structure.

A number of key features have been added to *2dx* in recent releases. *2dx\_image* can fully automatically process one 2D crystal image, including determination of the defocus, tilt geometry and crystal lattice parameters [5]. We have added a merging and management program called *2dx\_merge*, which performs a 3D reconstruction from individually processed images. This 3D reconstruction can then be used by *2dx\_image* to create a synthetic reference that then allows improved 2D image processing, which in turn can result in a better 3D reconstruction. Such refinement can be applied iteratively, which in the case of smaller crystal images can further improve the resolution.

We have also implemented a Maximum-Likelihood (ML) component for *2dx\_image* and *2dx\_merge*, which allows for single-particle style 2D reconstructions, while allowing for crystalline boundaries and other problems specific to 2D crystal images [6]. We here show cases where ML routines can dramatically improve resolution for poorly ordered crystals as an alternative to MRC style unbending.

These features allow for continuous, automatic refinement of resulting volumes, even as new data are collected, incorporated and processed. Here we discuss and compare the application and results of automatic refinement via *2dx* to existing, high-resolution datasets with known structures, as examples for 3D reconstruction for general cases where structures are currently unknown.

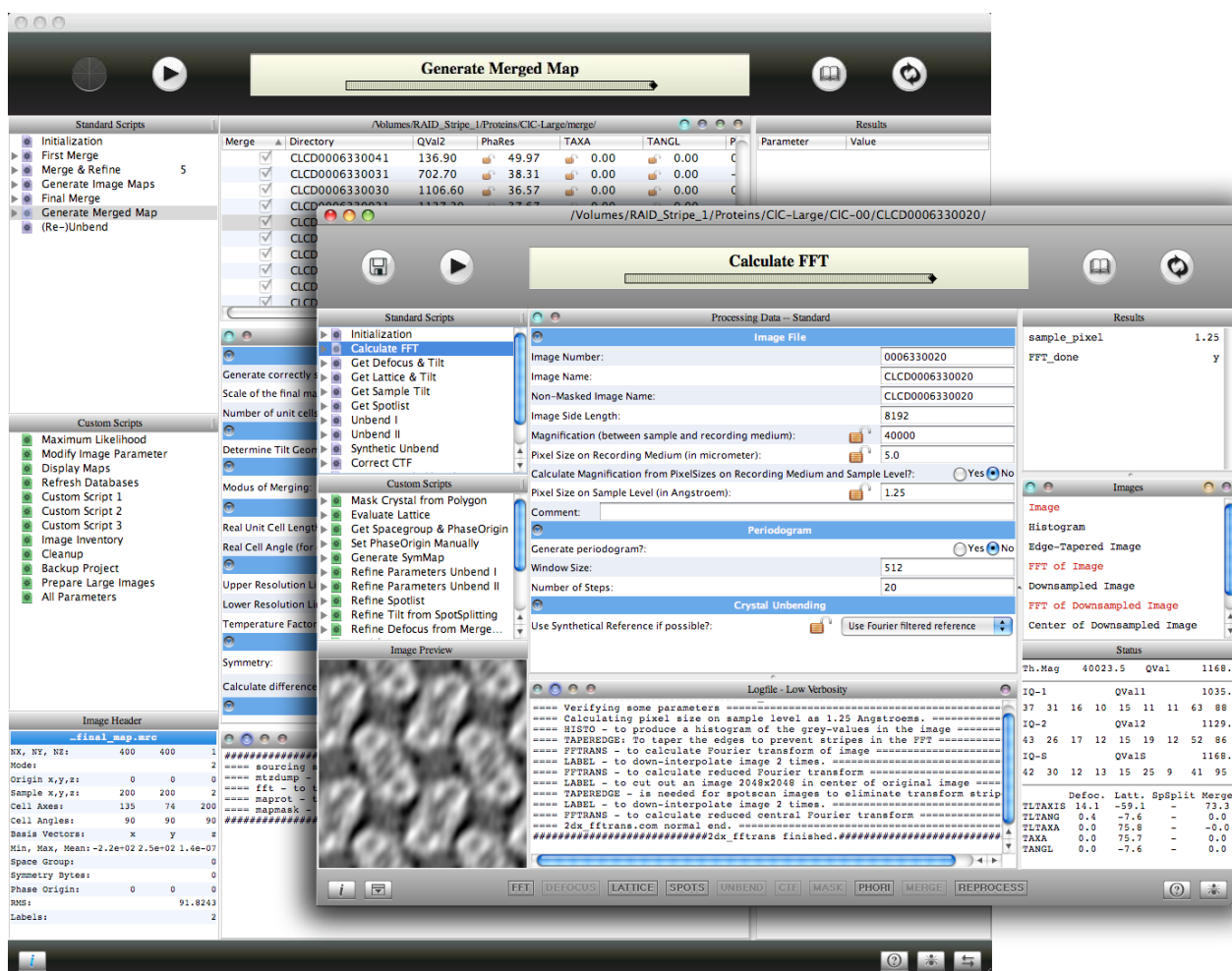


Figure 1: The Graphical User Interface of 2dx. 2dx features platform independence, user guidance, automation, software update, a bug-report and management system, and is ideally suited for the user-friendly implementation of new algorithms.

## References:

1. Renault, L., et al., *Milestones in electron crystallography*. J Comput Aided Mol Des, 2006. **20**(7-8): p. 519-27.
2. Crowther, R.A., R. Henderson, and J.M. Smith, *MRC image processing programs*. J Struct Biol, 1996. **116**(1): p. 9-16.
3. Gipson, B., X. Zeng, and H. Stahlberg, *2dx\_merge: Data management and merging for 2D crystal images*. J Struct Biol, 2007. **160**(3): p. 375-84.
4. Gipson, B., et al., *2dx--user-friendly image processing for 2D crystals*. J Struct Biol, 2007. **157**(1): p. 64-72.
5. Zeng, X., et al., *Automatic lattice determination for two-dimensional crystal images*. J Struct Biol, 2007. **160**(3): p. 353-61.
6. Zeng, X., H. Stahlberg, and N. Grigorieff, *A maximum-likelihood approach to two-dimensional crystals*. J. Struct. Biol., 2007. **160**(3): p. 362-374.