

# The GCI Multi-target X-Ray Microprobe: Imaging, Control and Software Automation

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## *Introduction*

A new X-ray microprobe is being developed at the Gray Cancer Institute and will operate alongside two existing micro-irradiation facilities; a charged-particle microbeam and a prototype X-ray microprobe optimised for focussed  $C_K$  X-rays. It generates X-rays through bombardment of carbon, aluminium or titanium targets by a focused electron at acceleration energies of up to 15 kV (see Folkard *et al.*, these Proceedings). The new facility takes advantage of advances in technology that have arisen since the original microprobe was commissioned some ten years ago. Specifically, dramatic improvements in the processing power of desktop computers and the availability of affordable, sensitive CCD cameras have enabled much improved automated procedures for cell recognition, positioning and irradiation to be implemented.

Dose delivery is controlled either by presetting irradiation times or through integration of electron beam current: when the required counts are reached, a shutter in the X-ray path is activated. All aspects of the source can be computer-controlled or overridden using manual controls.

## *Automated Micro-Irradiation and Microscopy*

A micro-irradiation experiment requires a high degree of hardware automation to fulfil the requirements for speed, reliability and reproducibility, particularly when large numbers of cells ( $10^2$ - $10^4$ ) are involved. The control of the radiation source and dose delivery is essential alongside the control of a cell positioning system as well as some means of cell detection or imaging, usually based on standard microscopy techniques. A convenient way to control, in a flexible manner, such disparate hardware is through the use of a desktop computer and appropriate software. This approach also allows 'intelligent' experiments to be performed, during which decisions about 'where' to irradiate can be made automatically. For example, the cellular nucleus or cytoplasm can be targeted through the use of appropriate image processing algorithms. The recent construction of the micro-focus X-ray source has led to a push to develop a faster and more accurate software-driven system, as described below. This approach is equally applicable for performing fast and complex irradiations with particle microbeams and addresses current and future experimental needs.

This software control system uses a modular approach, whereby individual programs are developed to control each specific hardware subsystem. These modules are combined to form the final program but, in order that they remain self-contained, message passing is used to allow the modules to communicate. Self-containment is important as debugging is simpler and it allows code re-use between projects. Just as importantly, it forces the code developer to consider this aspect. Furthermore, this approach aids hardware testing and complements the design of the hardware control systems. We implement hardware control using an expandable, daisy-chain bus approach, based on the use of the I<sup>2</sup>C bus. In turn, this is controlled through a single, standard USB link to the host PC. This allows us to readily add devices perhaps not envisaged at the design stage.

Modern computers are able to run more than one program at once, and true parallel processing can be achieved by installing multiple processors, or by using 'multiple core' processors that are now available. We can make effective use of these by implementing multi-threaded programs; this has been extensively used not only to speed up processor-intensive tasks but also to add a degree of system adaptability during automated, repetitive tasks. For example, differences in dose delivery or camera exposure times will exist between experiments; an adaptable automated system easily copes with this.

### *Image processing and performance*

There are two aspects to a 'typical' micro-irradiation experiment. These are (1) cell finding i.e. XYZ coordinate mapping and target identification and (2) individual target re-visiting and irradiation. Many variations on how these are sequenced can be envisaged e.g. all targets within a field of view identified, followed by their irradiation or all targets within a dish region identified then irradiated. The most common imaging method is based on widefield steady-state fluorescence microscopy, usually performed with a 40x water-dipping objective (0.9 na) which restricts the field of view to around 220 x 290  $\mu\text{m}$  but provides diffraction-limited resolution (215 nm/pixel) and high sensitivity. Some  $10^4$  cells/hour can be processed under these circumstances; using interpolated 3-point focusing (i.e. where a focus plane is defined, image stitching is used to provide a true dish map of cells and targets along with classification of different object 'types', i.e. nuclei, debris, overlapping/binucleated cells etc. A range of image processing functions is performed during automated experiments, ranging from image corrections for variations in fluorescence excitation illumination through to cell finding and the delineation of the nucleus. The more sophisticated of algorithms use a Compact Hough Transform And Radial Map approach (CHARM), whereby the centres of objects of approximately circular shape are found by a modified Hough Transform and their outlines are found by search outwards from these centres to form radial maps. Importantly, fast shape processing can be performed on these radial maps to improve outlines and resolve conflicts in overlapping shapes or cells in contact. In particular, our algorithm is rugged and insensitive to minor variations in focus where features may not be perfectly delineated, with much enhanced performance over the more usual watershed- or threshold-based approaches. A range of morphological and intensity features are logged to aid cell-cycle related studies. By searching outwards from the 'found' object, we make use of edge strength, edge completeness and mean radius measures to aid the classification process. Live overlays are extensively used to 'mark' objects, targets and source beam positions.

### *System hardware*

The imaging hardware is implemented from 'standard' Nikon components and accessories, modified to allow other imaging modes (e.g. polarised light epi-illumination to aid alignment of the X-ray source zone-plate and order-selecting aperture). Other imaging modalities (e.g. time-resolved fluorescence detection) can be readily added. The assembly is integral to the table-top source (see Folkard *et al.*, these Proceedings) and uses a closed-loop motorised sample positioning stage (x,y axes) complemented by two z-axis motorised drives, one controlling optical focus, the other X-ray source focus, as well as other motorised optical components. Images are stored using the Imaging Cytometry Standard (ICS, <http://libics.sourceforge.net/>) and all operating conditions (e.g. objective, filter cubes, camera set up) are monitored and logged as metadata in these files.

The host computer uses dual hyperthreading Intel P4 processors and modular experiment user interfaces are available on dual graphics screens. The software is developed in the C and C# programming languages and runs under the National Instruments CVI LabWindows environment.

The X-ray source hardware is similarly controlled and its performance continuously monitored, displayed and logged. In this instance, in-house developed hardware is utilised, the main components of which are electron gun filament and grid supplies, an electron beam acceleration supply (15 kV, 100 W max.), magnetic beam deflection supplies and a range of shutter and status control and monitoring systems. This integrated approach allows to easily monitor and control the source vacuum system, to sequence the various power supplies, to condition the safe starting, stabilisation and shutdown of the source as well as to implement specific sequences e.g. during conditioning of a new filament/cathode assembly. Once aligned, the intensity of the electron beam may be stabilised through control of the grid voltage, using a hardware-implemented feedback loop which compares the electron beam current collected at the source target with a set-point value, up to currents in excess of 5 mA.

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