Australian Synchrotron Development Plan
Project Submission Form

Section A: Summary and Proponent Details

Project Title

Microscopy Enabling Facilities and Coordination (MFU)

Spokesperson

Name	Hugh Harris
Institution	The University of Adelaide
Email	hugh.harris@adelaide.edu.au
Phone	08 8303 5060

Executive Summary (approx. 100 words)

Microscopy will form a major part of the scientific endeavour at the Australian Synchrotron in the long term. This proposal seeks to provide a range of onsite facilities to enable new approaches to microscopy experiments, and place Australasian scientists at the forefront of research in this area. Experimenters frequently encounter issues associated with sample preparation and transport that limit the extent of information available from their samples; the ability to prepare samples onsite will overcome these issues in a large number of cases. The coordination of sample mounting and handling across beamlines and energy regimes will extend these microscopy capabilities, providing further advantage.

Other proponents (add more rows if necessary)

Name	Institution	Email address
Peter Lay	The University of Sydney	p.lay@chem.usyd.edu.au
Jade Aitken	The University of Sydney	j.aitken@chem.usyd.edu.au
Paul Witting	The University of Sydney	pwitting@med.usyd.edu.au
Enzo Lombi	The University of SA	Enzo.Lombi@unisa.edu.au
Michael Mclaughlin	The University of Adelaide	Mike.Mclaughlin@csiro.au
Chris Ryan	CSIRO	chris.ryan@csiro.au
David Paterson	Australian Synchrotron	David.Paterson@synchrotron.org.au
Martin de Jonge	Australian Synchrotron	Martin.deJonge@synchrotron.org.au
Chris Glover	Australian Synchrotron	glover.cj@gmail.com
Mark Tobin	Australian Synchrotron	mark.tobin@synchrotron.org.au
Garry Foran	Australian Synchrotron	Garry.Foran@synchrotron.org.au

Michael Parker	St. Vincents Institute	mparker@svi.edu.au
Greg Giles	The University of Otago	gregory.giles@otago.ac.nz
Frank Reith	The University of Adelaide	Frank.Reith@csiro.au
Peter Barnard	La Trobe University	p.barnard@latrobe.edu.au
Stuart Ramsay	James Cook University	stuart.ramsay1@jcu.edu.au
Carolyn Dillon	Wollongong University	carolynd@uow.edu.au
Sola Ajiboye	The University of Adelaide	sola.ajiboye@adelaide.edu.au
Balwant Singh	The University of Sydney	B.Singh@usyd.edu.au
Andrea Gerson	The University of SA	Andrea.Gerson@unisa.edu.au
David Parsons	Women's and Children's Hospital	david.parsons@cywhs.sa.gov.au
Louis Rendina	The University of Sydney	rendina@chem.usyd.edu.au
Giuseppe Ciccotosto	The University of Melbourne	j.ciccotosto@unimelb.edu.au
William Skinner	The University of SA	William.Skinner@unisa.edu.au
Joel Brugger	The University of Adelaide	Joel.Brugger@samuseum.sa.gov.au
Barbara Etschmann	The University of Adelaide	barbara.etschmann@adelaide.edu.au
Damian Myers	Melbourne University	damianem@unimelb.edu.au
Andrew Peele	La Trobe University	A.Peele@latrobe.edu.au
Daryl Howard	Australian Synchrotron	Daryl.Howard@synchrotron.org.au
Antony Cooper	Garvan Institute	a.cooper@garvan.org.au
Craig Marshall	Universirty of Kansas	cpmarshall@ku.edu
Farideh Jalilehvand	University of Calgary	faridehj@ucalgary.ca

Section B: Detailed Description

B1: Description of Proposed Beamline/Development Project

A major barrier to the sophistication of microscopy experiments performed at synchrotron facilities, and the improvement of the resultant scientific outcomes, is the existing paradigm of external sample preparation followed by transport to the facility and subsequent measurement. A second potential barrier to improving scientific output, particularly in terms of efficiency, is the lack of coordination of equipment access between beamlines. The Australian Synchrotron and its user base must achieve the removal of these barriers if they are to compete with leading international synchrotron facilities. This proposal aims to remove these barriers with the implementation of facilities described below. These facilities should be accessible for all AS users so that their benefits can be realised for any experiment undertaken at the synchrotron.

B2: Applications and Potential Outcomes to Australian Scientific Community

B2.1 Cell Culture Facilities

Several Australian groups have pioneered the international effort into spectroscopy and imaging of single mammalian and bacterial cells using synchrotron radiation. Until recently these experiments have involved treatment and preservation of samples followed by lengthy travel to overseas facilities. This has limited the scope of experiments that could be performed and resulted in degradation of the structural and chemical integrity of some samples. A local facility provides the opportunity to prepare cell samples immediately prior to measurement and overcome these limitations. Examples of previously impossible experimental approaches that would be enabled by culture facilities on site are: single cell imaging of live or hydrated flash-frozen cells on IR and X-ray beamlines; differentiated and long-term stable respiratory-cell cultures of rodent, sheep, non-human primate and human origin – these will provide crucial and revealing data about respiratory disease and treatment issues in pre-clinical studies that prevent the need for excessive use of live animals; XRF flow cytometry at microprobe lines to study pharmacokinetics of metal-based drugs; instant feedback of results into experimental design – researchers could design and carry-out new treatments during one beamtime allocation in response to observations at the beamline rather than wait six months or more for their next beamtime allocation. Culture facilities would benefit biological users of other beamlines in a similar manner.

B2.2 I&M animal handling access

There is strong potential demand for access to animal handling and surgery facilities, currently located at the I&M beamline, for experiments performed at beamlines other than the I&M line, for reasons similar to those outlined in 2.1. This access would expand the range of experiments possible on animal tissues and live animals at microscopy lines. An example experiment that could be enabled by such access is XRF/XAS monitoring of blood components in live animals under treatment (arterial/veinal bypass with inline measurement cell) and many other experiments are feasible.

B2.3 Plant handling/treatment facility

An onsite greenhouse would allow the significant botanical user group to expand the range of microscopy experiments that are feasible. For example, studies on live heavy metal phytoremediating plants require the plant to be growing in an environment containing the toxin immediately before the experiment. At present this is not feasible and interstate transport of plants will result in a loss of chemical and structural integrity of the sample. An onsite plant

facility would also alleviate issues associated with transport of agriculturally sensitive plants across state quarantine boundaries.

B2.4 Beamline portable Raman microprobe for simultaneous complementary imaging
The successful implementation of coaxial beam Raman and x-ray microprobes allowing simultaneous multiregime measurements has been demonstrated (Davies et al. Appl Phys Lett **87**, 264105, 2005). A similar Raman instrument (with data acquisition times and beam spot size similar to current XRF capabilities) that could be installed on x-ray microscopy beamlines at the AS would allow users across all disciplines to attain complementary structural information simultaneously on the one sample; for example, the distribution of heavy elements at the same time as the distribution of biomolecules such as lipids or DNA in biological samples.

B2.5 High quality visible light microscope facility and coordinated sample positioning
Publication of single cell and tissue section images from synchrotron-based techniques in high impact journals now routinely requires matching visible light micrographs. The current visible light microscopic facilities at the Australian Synchrotron are inadequate for this purpose and are greatly inferior to facilities at other synchrotrons such as the APS. This means that visible light micrographs must be attained away from the AS, either prior to, or after the synchrotron experiment, which is incompatible with the flexible and enabling approach described in 2.1. In addition, locating samples in the current x-ray microscope wastes beamtime. A coordinated sample positioning system between the x-ray, IR and other beamline microscopes, correlated with the visible light microscopes via the use of a kinematic mounting system, would significantly improve throughput for the microscopy user base. A universal sample mounting and positioning system across beamlines would also enable and encourage correlated multi-regime measurements on samples, including co-registration of images obtained from the beamline and optical imaging, improving the scientific impact of the results obtained.
The opportunity exists to expand the scope of correlated imaging by implementation of optical fluorescence and multi-channel laser scanning confocal microscopy facilities on site. These facilities would allow researchers to combine information from synchrotron-based measurements with that from established optical microscopy methods including immunofluorescence labeling, staining and histology, and importantly, feed this information back into the design of new experiments during one beamtime allocation.
A high frequency-resolution multispectral fluorescence detection system (e.g. CRI Nuance – Quantum Scientific) would enable high specificity multicolour fluorescence and brightfield imaging, providing exquisite spatial fluorescence resolution in biological samples being examined using fluorescence as well as immunohistochemistry. These systems also essentially eliminate autofluroescence in biological sample imaging. The imaging capture system mounts to standard microscope viewing ports and so is multi-user at the AS.

B2.6 A pool of beamline portable fluorescence detectors
XFM/XAS imaging over a range of length scales, from tens of nanometers to hundreds of microns, will be performed at a number of current and proposed beamlines at the AS (XFM, XASID12, HCNP, BendXAS, μ-XRD/XFM etc.). User requirements for fluorescence detectors vary considerably depending on the specifics of the samples, and several beamline-detector combinations are imaginable to cover the range of experiments. The transport of detectors between beamlines and associated scheduling coordination would allow flexibility in these combinations and improved output from all beamlines involved. This approach has been

implemented at SSRL amongst the XAS and imaging lines and has resulted in more efficient utilization of available beamtime. In addition, the pool of detectors available should be enhanced over time to match demand.

B2.7 Cryogenic sample preparation and mounting

The push across all disciplines to gain information about species of interest at ever-lower concentrations has resulted in the development of higher flux beamlines, with a concomitant increase in the likelihood of photodamage to samples. Continued progress in this direction cannot be achieved unless measurements are performed under cryogenic conditions to alleviate potential photodamage; as such the development of cryostage implementations on microscopy beamlines is a crucial priority. In addition, on site cryogenic sample preparation facilities (safe flash freezer, freezedriers) are required to allow users to trap samples under particular conditions prior to measurement.

Demand for sectioning equipment from the microscopy user base is strong; this includes supporting equipment to deal with sample embedding, microtomes, vibrotomes to allow unsupported sectioning, and importantly a cryogenic sectioning capability.

The sample preparation equipment would be ideally colocated with the optical microscopy and culture facilities in a dedicated sample preparation laboratory that should also house long-term cryogenic storage facilities and be outfitted according to PC2 and QC2 standards to allow import of foreign organisms.

B2.8 Equipment for improved XRF measurements

The accurate determination of elemental concentrations by XRF measurements requires the use of certified calibration standards, such as those available at the APS (XOR). Such standards are not currently available to XFM users and this shortcoming will extend to other beamlines where XRF experiments are to be conducted. In addition, XRF mapping of light elements ($14 \leq Z \leq 20$) at the XFM line is currently severely hampered by air (Ar) attenuation of fluorescence and peak overlap due to the lack of a He-purged sample environment. Elemental calibration standards and He-purged environments are required for beamlines where XRF experiments are to be conducted.

B3: Match to Selection Criteria

1. The proposal is primarily directed at enabling new experimental approaches for the existing and potential microscopy user bases.
2. Contemporary synchrotron microscopy leverages flux and focusing characteristics of third generation sources.
3. Open user access to facilities similar to those outlined above is rare at other synchrotrons; hence such access will provide significant scientific advantage to AS users.
4. Predominantly off the shelf equipment is required, kinematic mounts and cryostages will require development but are feasible in a three year timeframe.

B4: Potential Users – addressed at B2.