2017 CMBEC40 Conference
Winnipeg MB
May 23–26, 2017

LIFE SCIENCE PROGRAMS AT THE CANADIAN LIGHT SOURCE: OVERVIEW OF THE BIO-MEDICAL STUDIES AT BMIT

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INTRODUCTION

The Canadian Light Source Inc. (CLS) is a mid-size 3-rd generation 2.9 GeV synchrotron located on the campus of the University of Saskatchewan in Saskatoon (www.lightsource.ca). Currently there are 15 beamlines operating at the facility. Four laboratories: Canadian Macromolecular Crystallography Facility (CMCF), Mid Infrared Spectromicroscopy (Mid-IR), Bio-Medical Imaging and Therapy (BMIT) and Biological X-ray Absorption Spectroscopy (BioXAS) are dedicated to study biological objects ranging from atomic resolution (CMCF and BioXAS) to cells and tissues (BioXAS and Mid-IR) through to larger samples such as organs, live animals and plants (BioXAS and BMIT) - Fig.1 [1].

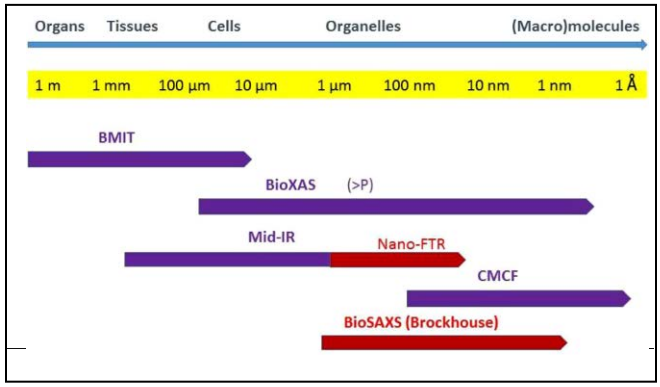

Figure 1: Complementarity of the biological and life sciences beamlines at CLS. Potential future developments: infrared imaging beyond the diffraction limit (Nano-FTR) and BioSAXS beamline. [1]

The Bio-Medical Imaging and Therapy (BMIT) facility provides synchrotron-specific imaging and radiation therapy capabilities [2-6]. There are two separate end-stations used for experiments: the Bending Magnet (BM-POE-2) that was opened for general user program in 2011 and the Insertion Device (ID-SOE-1) that started general user program in 2015. The two beamlines are devoted to advanced X-ray imaging and X-ray therapy techniques. Ongoing core research programs include; bone and cartilage growth and deterioration, cardiovascular and lung imaging and disease, human and animal reproduction, cancer imaging and therapy, spinal cord injury and repair, medical device and scaffold imaging, developmental biology, as well as the introduction of new imaging methods.

BMIT FACILITY

The bending magnet beamline 05B1-1 (See Fig. 2) is designed for X-ray imaging and irradiation experiments primarily in animals ranging in size from insects to mice to piglets, as well as tissue specimens including plants.

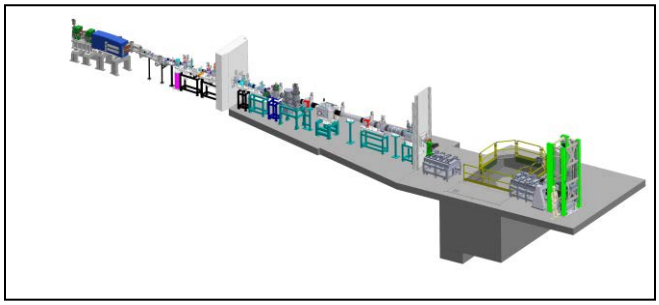

Figure 2: Model of the 05B1-1 Beamline – total length (source to detector) ~29 m [4].

The monochromatic spectral range spans 15–40 keV, and the beam is more than 200 mm wide in the experimental hutch. In addition users have access to filtered white beam which can be used for imaging, irradiation, and X-ray instrumentation development experiments. Experimental geometry can be varied as needed, including sample-to-detector distance up to 6 meters.

The insertion device beamline 05ID-2, is designed for imaging and therapy research in animals, with the current focus on medium size animals such as piglets, rabbits, dogs and sheep. The monochromatic spectral range spans 25 to 140 keV, and the beam is more than 220 mm wide in the SOE-1 experimental hutch. The second end-station (POE-2 hutch) of the ID beamline will provide in the future access to white beam for therapy and irradiation experiments.

Specifications of BMIT beamlines are shown in Table 1. The following techniques are available at BMIT; conventional absorption imaging – Fig. 3, K-edge subtraction imaging (KES), in-line phase contrast imaging (also known as propagation based imaging, PBI) and Diffraction Enhanced Imaging/Analyzer Based Imaging (DEI/ABI), all in either projection or Computed Tomography (CT) modes [7]. The research conducted at BMIT over the last 6 years has been described in 93 publications and contributed to 11 doctoral and 17 master's theses.

Table 1: BMIT beamlines specifications [1].

	BMIT beamlines specifications.	
	05B1-1	***05ID-2***
Photon energy range (keV)	15–40	25–150
Flux (photons/s)	$5x10^{13}$ @ 15 keV, white beam	$2x10^{15}$ @ 25 keV, white beam
Beam size on sample (HxV)	240 mm x 7 mm @ 25 m	220 mm x 11 mm @ 55 m

IMAGING WITH X-RAYS

BMIT provides both detectors and stages for imaging samples ranging from millimeter to meter in size with a resolution from 4 to 200 microns, with energy spanning 15-140 keV. With the high energy and high flux available good quality, high resolution radiographic images can be obtained – Fig. 3.

Quasi-coherent and monochromatic light provides unique options for K-Edge Subtraction techniques (to visualize bone growth and development), Diffraction-Enhanced Imaging/Analyzer-Based Imaging techniques (to visualize tissue engineering samples and low contrast tissue samples) and In-Line Phase Contrast Imaging techniques (to visualize lungs and Cystic Fibrosis).

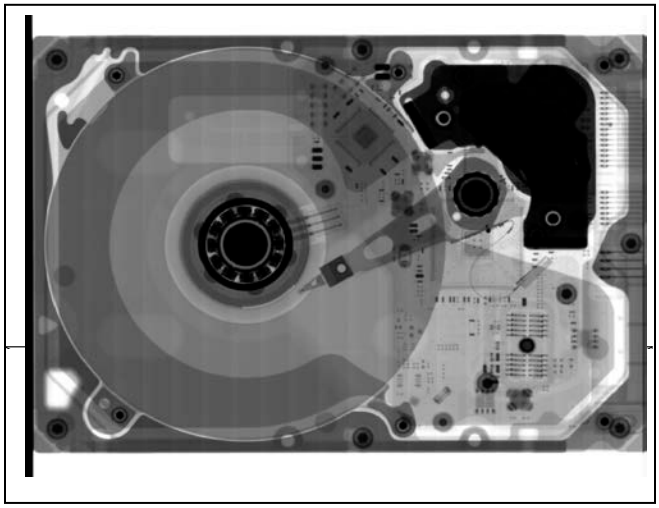

Figure 3: High resolution radiograph of the hard-drive.

Phase-sensitive imaging methods exploit differences in the refractive index of tissue and imaging can be done at higher energies, where the absorbed radiation dose can be less, thereby reducing potential damage to tissues. Phase-sensitive imaging techniques offer improved contrast sensitivity, especially when imaging weakly absorbing samples (low Z materials) [8].

These techniques prove useful in biological and medical studies because the phase contrast falls off less quickly at higher energies than absorption contrast. The high energy X-rays used at BMIT ensure low radiation dose for the animals, and the high brightness of the X-rays reduces the exposure time required, which is extremely important for live animal experiments and longitudinal studies [8].

A recent example of research done at BMIT is related to arthritis. To better understand the causes of osteoarthritis, investigators introduced a non-radioactive strontium tracer into joints developing osteoarthritis and spatially mapped the temporal changes in bone, using K-edge subtraction synchrotron micro-CT [9].

3D imaging indicated clear differences between healthy bone, which showed a uniform distribution of strontium, and subjects developing osteoarthritis, which showed

pathological changes occurring in the bone microstructure. In particular, tracer was found beneath the cartilage and at bone margins, which eventually develop into bone spurs and limit normal joint movement.

With the substantial progress recently made in the development of complex tissue engineered structures, imaging techniques for three dimensional (3D) and non-invasive analyses have been identified as strategic priorities in Tissue Engineering and Regenerative Medicine (TERM) research and are required to accelerate the progress in this field. In-line phase contrast imaging available at BMIT provides high resolution, non-invasive 3D X-ray imaging technique that has become more attractive than ever to TERM researchers [8].

New X-ray optical instrumentation projects are being pursued at the BMIT beamlines to address the needs for various imaging programs, for example, an expander for dynamic real life imaging which allows the imaging of larger objects using a single frame, development of the new X-ray monochromators and others.

THERAPY WITH X-RAYS

There are two main programs that use X-rays for cancer treatment trials at the synchrotrons: Synchrotron Stereotactic Radiation Therapy (SSRT), which is based on a local drug uptake of high-Z elements in tumors, followed by stereotactic irradiation with the monochromatic beam and micro-beam (MRT) radiation therapy, based on the spatial fractionation of X-rays.

The micro-beams typically have a width of tens of micrometers and are separated by regions of low dose, ≤ 20 Gy. This arrangement spares the healthy tissue in the low dose volume, which can quickly regenerate the irradiated cells, while preferentially affecting the tumor. The skin is one of the dose-limiting organs in radiation therapy and this tissue sparing method can dramatically reduce the risk of acute radiation dermatitis.

monochromatic MRT

In 2016 [10] a pilot program was implemented at BMIT to assess the feasibility of the monochromatic micro-beam therapy (m-MRT) technique, a radiotherapy concept that uses high brilliance, monochromatic X-ray micro-beams smaller than 200 microns, applied to irradiate cell samples and to treat breast cancer tissue. The light source for the 05ID-2 beamline is a multi-pole superconducting 4.3 T wiggler. The high field gives a critical energy of over 20 keV. The optics in the POE-1 and POE-3 hutches prepare a beam. The double crystal bent-Laue monochromator provides an energy range appropriate for therapy studies in animals (50-150 keV).

In the experimental hutch, the broad beam is divided into 4 mm wide and 50 μm high slices, with 400 microns c-to-c spacing, using tungsten collimator – see Fig. 4.

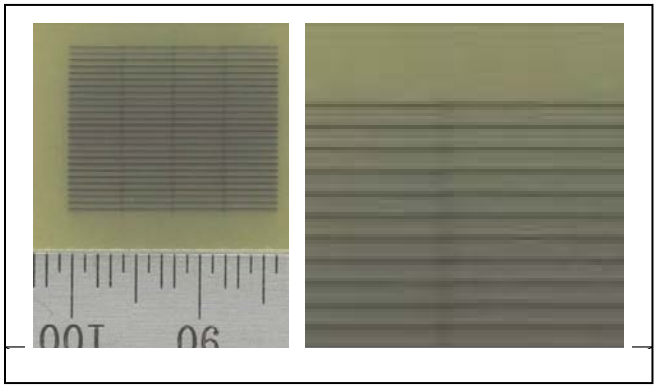

Figure 4: Radio-chromic film (EBT-3) record of the four adjacent micro-beams irradiations (left) (@100 keV) with the beam dose of 250 Gy, scale in mm. Right: zoom-in view.

Two different energies were used for those tests: 50 keV and 100 keV. In Phase I, the tumor fragments and cells samples were irradiated ex-situ and then analyzed to assess the damages induced by m-MRT irradiation and to define the radiotherapy threshold. In Phase II, eight patient derived xenografted (PDX) tumor fragments were irradiated and implanted in live NOD Severe Combined Immuno-deficient (SCID) gamma (NSG) mice to assess the effect of irradiation on tumor growth comparing to the control.

The pilot studies showed, that the m-MRT treatment of cancerous tissue slowed down the tumor growth in (NSG) mice as compared to untreated controls. The biomolecule analysis demonstrated that the irradiation induced

cancer cell apoptosis by triggering a stress response of the cells at radiation dose of 60 Gy or higher.

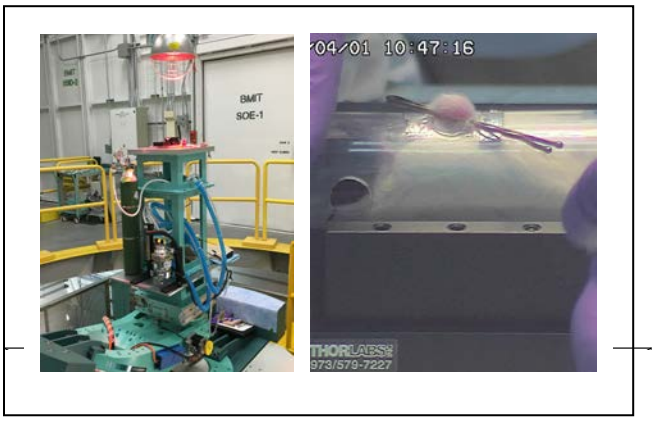

Figure 5: Experimental set-up (left) and close-up of the irradiated tumor (right) for in-vivo treatment of human breast tumor model in mice.

In phase III of the studies optimized irradiation treatment of tumor in vivo in live animals was performed with improving outcomes.

These findings help to understand the underlying processes of the irradiation radiology. Future studies will lead to obtaining more systematic data and to further optimize the treatment plan.

CONCLUSIONS

The CLS Department of Biological and Life Sciences contains four sets of facilities: the Canadian Macromolecular Crystallography Facility, Mid-Infrared Spectromicroscopy, Bio-Medical Imaging and Therapy and Biological X-ray Absorption Spectroscopy. This makes the department well equipped to study biological objects ranging from atomic resolution (CMCF and BioXAS) to cells and tissues (BioXAS and Mid-IR) through to larger samples such as organs, live animals and plants (BMIT).

BMIT facility provides unique in North America synchrotron-specific imaging and therapy capabilities that have proven to be powerful tools for visualization of soft tissue using phase contrast techniques as well as functional imaging of systems using K-edge subtraction. Several different X-ray detectors are available with resolutions ranging from 2 µm to 200 µm. The established imaging program extends from 15 keV to higher energies (up to 140 keV) and from small stages to a high capacity positioning system (up to 450 kg load capacity).

ACKNOWLEDGEMENTS

The Canadian Light Source Inc. is supported by the Canada Foundation for Innovation, Natural Sciences and Engineering Research Council of Canada, the University of Saskatchewan, the Government of Saskatchewan, Western Economic Diversification Canada, the National Research Council Canada, and the Canadian Institutes of Health Research.

The author would like to thank the CMCF, BMIT, Mid-IR and BioXAS beam-teams as well as CLS and beamlines staff for their contributions toward the respective projects.

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