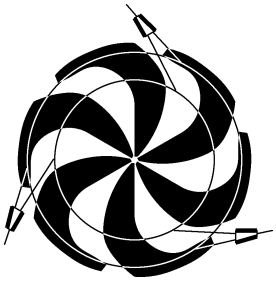
TRIUMF



ANNUAL REPORT
SCIENTIFIC ACTIVITIES
1997

CANADA'S NATIONAL MESON FACILITY
OPERATED AS A JOINT VENTURE BY:

UNIVERSITY OF ALBERTA
SIMON FRASER UNIVERSITY
UNIVERSITY OF VICTORIA
UNIVERSITY OF BRITISH COLUMBIA

ASSOCIATE MEMBERS:

UNIVERSITY OF MANITOBA
UNIVERSITÉ DE MONTRÉAL
UNIVERSITY OF REGINA
UNIVERSITY OF TORONTO

UNDER A CONTRIBUTION FROM THE
NATIONAL RESEARCH COUNCIL OF CANADA

APRIL 1998

The contributions on individual experiments in this report are outlines intended to demonstrate the extent of scientific activity at TRIUMF during the past year. The outlines are not publications and often contain preliminary results not intended, or not yet ready, for publication. Material from these reports should not be reproduced or quoted without permission from the authors.

LIFE SCIENCES

Introduction

The Life Sciences Progam at TRIUMF is involved in biomedical research, making use of the intellect, concepts and equipment associated with the basic program. For example the production of radiotracers relies on the techniques of the cyclotrons and their targetry. In the following sections, progess during the past year is discussed for the primary projects. The longest standing project and the largest is the PET program. Details of the multidisciplinary aspects of this project illustrate the interplay of resources for nuclear science at TRIUMF. The TRIM program has made significant progress while still being hampered by the lack of laboratory space in which to perform chemical separations. Progress continues in the effort to label compounds that will be incorporated into DNA. Success in this project will have wide reaching impacts for the biomedical community. Research into detector response through computer simulation was concluded with a software package by year's end after more than a decade of effort in detector design, construction and simulation measurements.

PET program – UBC site

(T.J. Ruth, UBC)

The progress report for the PET program at the Univ. of British Columbia is presented in two basic parts, the physics program and the human studies. The radiopharmaceutical development and cyclotron operations are discussed in separate sections.

Tomograph physics

The following projects have been the focus of the PET physics program:

Quantitative comparison of 3D and 2D PET with human brain studies

A comparison between the quantitative, biologically meaningful results obtained from 2D and 3D scans of the same patients was performed on four subjects using ^{11}C-Schering(Sch), on four different subjects using 11C-Dihydrotetrabenazine (DTBZ) and three different subjects using 18F-Fluorodeoxyglucose (FDG) as tracers. Distribution volume (1), distribution volume ratios (2), striatum-to-occipital cortex and striatum-to-cerebellum ratios were used as analysis figures of merit for the neuroligand studies, while LMRGlu was used as analysis figure of merit for the FDG study. In the case of the neuroreceptor studies, 3D data tended to yield approximately 5% higher values compared to the 2D data. Although statistical analysis performed on the results of these neuroreceptor studies did not show this difference to be significant, except in a few isolated cases, reliability analysis indicated that comparing directly results from 2D and 3D acquisition might introduce some inaccuracies. Good agreement between 2D and 3D data was confirmed by the LMRGlu applied on the FDG studies. This work was submitted to the Journal of Nuclear Medicine and presented at the 1997 IEEE/MIC conference in Albuquerque.

Investigation of an optimum detector normalization source and detector geometric parameters

Detector normalization corrects for a non-uniform detection efficiency of the tomograph crystals. The correction has two components, one related to the geometric properties of the crystal rings and the other to the individual crystal efficiencies. An optimum number of geometric degrees of freedom was identified which include the radial and axial crystal position and the crystal position in the block. A scatterless source was identified as being the optimum source for the determination of the geometric part of the normalization correction factors. The shape and size of the source to be used for the determination of the intrinsic crystal efficiency correction factors was found not to be very important. The quality of the detector efficiency correction algorithm was tested by quantifying the radial and axial uniformity of a uniform phantom image. Both were comparable to those achieved from data acquired with the 2D acquisition mode, which is considered as "gold standard". This work was accepted by the Journal of Physics in Medicine and Biology, was submitted to the IEEE TMI and presented at the 1997 International meeting on fully three-dimensional image reconstruction in radiology and nuclear medicine in Pittsburgh and the 1997 IEEE/MIC conference in Albuquerque.

A gaseous planar source for normalization of the PET scanner is also under investigation. This source will be short-lived, very uniform, and will produce fewer scattered gamma rays than any other plane-source currently available.

Practical implementation of 3D data processing algorithms

The 3D data processing protocol is still a fairly lengthy process: data need first to be copied from the acquisition front end disk to a SUN disk (approximately 2 min/frame), scatter corrected with an iterative scatter correction method (approximately 3 min/frame on an ULTRA SPARC 1) and corrected for detector normalization (approximately 2 min/frame on an ULTRA SPARC 1) and finally attenuation corrected and reconstructed on a Supercard i860 (approximately 20 min/frame). We are at present perform-

ing up to four 16 frame studies a day, thus requiring very efficient data processing protocols. To facilitate this task a graphical user interface was built, that uses a system of control locks to allow for streamlined data processing with minimum operator intervention. To make all of this possible for the routine use of 3D scanning a 2.1 GB hard disk has been installed in the tomograph acquisition system.

Evaluation of Fourier rebinning of the 3D data followed by iterative reconstruction algorithms

The Fourier rebinning algorithm (FORE), the ordered subsets expectation maximization algorithm (OSEM) and the space alternating generalized EM-algorithm (SAGE) have been implemented. The following reconstruction algorithms have been compared: 1) 3D filtered back projection (3DFBP), 2) FORE + 2DFBP, 3) FORE + OSEM, and 4) FORE + SAGE. The following figures of merit have been used: 1) resolution, 2) image noise, 3) image axial and radial uniformity, 4) contrast recovery, 5) signal-to-noise ratio, and 6) quantification accuracy. Preliminary results indicate that FORE followed by an iterative rebinning algorithm has better signal-to-noise ratio performance compared to the algorithms that use FBP techniques for the same reconstructed image resolution.

Uniformity performance is comparable, while quantitation accuracy studies are under way.

Emission and transmission registration using a post-injection transmission scan

Movement between emission and transmission data can cause artifacts in the image. Such movement is even more likely when long dynamic scans are performed on an affected subject. A movement correction method has been developed where no motion between the transmission scan and the temporally closest emission scan must be assumed. We eliminated this requirement by demonstrating the feasibility of using the emission part of a simultaneously acquired emission and transmission post-injection scan as alignment reference for all other emission scans. This work was presented at the Brainpet 97 meeting in Bethesda and accepted for a book chapter in Quantitative Functional Brain Imaging With Positron Emission Tomography [Academic, in press].

Development of an ROI based image analysis method

We developed a program to facilitate the extraction of physiological PET data using ROIs placed on a coregistered MRI image. The ROI storage system permits a hierarchical approach to data analysis, so that a large anatomical region can be specified by smaller components. This program is used by the PET group as well as the Psychiatry Department for data analysis.

Development of quantitative blood flow measurements

We are implementing quantitative blood flow measurements in a porcine model, which will be used as a "gold standard" to validate a completely non-invasive blood flow measurement technique using infrared (IR) light. The PET blood flow effort requires production of the tracer ([^{15}O]-H$-$2O) at TRIUMF, implementation of current measurement techniques, and development of a data analysis method.

Human studies

Completed human studies

Studies on the effects of ageing on dopamine D1 receptors, and on reproducibility: ^{11}C-methylphenidate (MP) and ^{11}C-dihydrotetrabenazine (DTBZ) have been completed. We have also completed studies of pre- and post-synaptic function in Rett syndrome in collaboration with Dr. H. Dunn of Children's Hospital. This study involved the first substantive effort to implement MRI-PET co-registration here at UBC which turned out to be more difficult than the literature would imply.

Origins and progession of Parkinson's disease

In an effort to understand the origins of Parkinson's disease, we are beginning studies of familial Parkinsonism in collaboration with Dr. Z. Wszolek of the Univ. of Nebraska who has a set of clearly defined family members to study. Based on the successful implementation of the 3D protocols outlined in the section above, we have introduced the routine use of 3D acquisition for all human studies. We have initiated and made substantive progress on an asymmetry study resulting in 1 abstract presented and 1 submitted. The asymmetry study is aimed at trying to understand the progression of Parkinson's disease by studying a cohort of early and long term sufferers of the disease that present as having effects dominantly on one side. We are using the 3 presynaptic tracers in this study. We have also initiated studies to estimate synaptic dopamine concentration, using displacement of [^{11}C]raclopride – a novel undertaking for UBC.

Therapeutic efforts

We have an ongoing multicentre NIH collaboration studying the efficacy of fetal nigral transplants for Parkinson's disease. In a similar way we have initiated a series of studies on fetal striatal transplantation for Huntington's disease in collaboration with Dr. T. Freeman and Dr. R. Hauser of the Univ. of South Florida.

PET facilities

(K.R. Buckley, TRIUMF)

The PET facilities comprise the TR13 13 MeV H$^-$ cyclotron, the ECAT 953B/31 tomograph, and ancillary equipment such as counting and data acquisition

systems. This progress report summarizes the status of this equipment as of December 8, 1997.

TR13 cyclotron

The TR13 cyclotron continues to be the backbone of the PET program, conveniently and reliably supplying isotopes throughout the day for radiochemical synthesis. Usage of the cyclotron increased 6% (by delivered beam) this year over 1996, reflecting an increased demand for PET radiopharmaceuticals (LS 3). The number of runs performed actually dropped, but this reflects the lack of pre-runs this past year. Prior to implementing the gas phase methyl-iodide system, 5 minute irradiations were performed on the ^{11}C-CO_2 target and dumped, just prior to the production run. We have found the yield of ^{11}C-CH_4 to be unaffected by this pre-run and thus it is no longer performed.

Downtime this year was caused primarily by the cyropumping system and target window failures. Both systems have been addressed and are expected to pose less of a risk in the future.

The cyropump troubles manifested themselves in two forms. The first was a continuation of troubles with the compressor intermittently shutting down. This was eventually determined to be a problem with the compressor electronic controls. The second form of trouble proved to be more pernicious. It presented itself as an inability to cool down one cyropump; this typically indicates a need for a seal replacement/rebuild in the pump head which was carried out. The pump operated normally for a short time and then failed again. After more testing it was determined that a bearing had failed in the stepper motor for the pump head. The stepper motor was ultimately rebuilt after a demonstration of why stepper motors should not be disassembled.

The ion source filament was replaced once this year when a feedthrough developed a water leak. In addition to this, the ion source was opened and cleaned a few times following failure of the ^{18}F-F_2 target during the Ar/F_2 recovery run. It was found that F_2 contamination in the ion source severely affected performance. The fix was as simple as opening the ion source and cleaning the surfaces lightly with scotchbrite followed by acetone.

We performed several extraction foil changes, mostly as a result of target window failures. An error reassembling the helium cooling window chamber resulted in a few more broken target foils before this was detected and corrected. Through this time the target selector carbon baffle was shattered when a gas target failed, sending carbon pieces into the cyclotron tank. This prompted us to open the cyclotron tank for the first time in over two years. Inspection of the inflectors and dees and low radiation field measurements demonstrated that nothing unexpected is occurring inside the tank.

Concrete blocks have been designed and are in the process of being detailed for construction by the end of the year or early next year. These blocks will form a contiguous shield over the ion source/injection line area of the cyclotron replacing the water tanks presently there. The blocks will be removable by the overhead crane.

TR13 targetry

Presently there are four target locations occupied of the available eight. These consist of

- one ^{18}O-O_2 gas target
- two ^{18}O water targets
- one CH_4 gas target

In addition a lithium target for the production of ^{7}Be has been installed and removed many times over the year for tests and has been demonstrated to operate reliably at 50 μA.

O_2 system: We have installed a new ^{18}O-O_2 gas handling system which utilizes simple commercial components for containment, LN_2 cooling, and valving. This system traps and releases the O_2 much faster than our original system allowing for back-to-back ^{18}O-O_2 irradiation and ^{18}F-F_2 recovery sequences (i.e. ^{18}O irradiation, ^{18}F recovery, ^{18}O irradiation, ^{18}F recovery, etc). An important feature of this system is that in the default state the target can be loaded immediately with no cooling or pre-trapping required. We recover approximately 70 mCi/μA (saturation) on the first recovery shot and approximately 28 mCi/μA (saturation) on a second recovery shot.

F^- system: We installed a local loading panel for the F^- target adjacent to the cyclotron which allows the bolus to be loaded within a couple of metres of the target and recovered in the lab 50 m away subsequent to irradiation. This system has improved target reliability considerably. Recent tests of yield resulted in a saturated yield of 115 mCi/μA for a 2 hour irradiation at approximately 18μA.

^{11}C-CH_4 system: Developments in the production of ^{11}C methyl iodide, ^{11}C-CH_3I, via a gas phase reaction reported by both Larsen *et al.* and Link *et al.* at the last International Symposium on Radiopharmaceutical Chemistry and Workshop on Targetry and Target Chemistry meetings prompted us to look at the production of methane, CH_4, from the irradiation of a gas mixture of N_2 and H_2. We felt the direct production of ^{11}C-CH_4 in target was the most elegant implementation of the gas phase reaction for routine use of ^{11}C-CH_3I. The system has been built and in

operation through much of 1997 resulting ^{11}C-labelled radiopharmaceuticals with specific activities (SA) increased as much as an order of magnitude. While the methyl iodide system is working well, the production of ^{11}C-methane has not been optimized. Studies continue on improving the yields from the target.

ECAT tomograph

The ECAT has had a typical year with several detector block failures. Follow up with CTI indicates that these failures have been caused by PMT faults or separation of the PMT from the crystal.

A 2.1 GB hard disk was installed in the ECAT acquisition system to accommodate the ubiquitous 3D sinograms resulting from our implementation of routine 3D scanning.

Statistics (to December 8, 1997)

Table III. TR13 run data.

Total runs conducted	671	
Total runs lost	13	
Integrated charge delivered	291321	μA-mins
Delivered to - LS 3	222907	
- LS 4	34683	
- LS 7	343	
- LS 8	8890	
- LS 13	19975	
- LS 24	4523	

Table IV. ECAT run data.

Total scans conducted	390
Total scans lost	39
Lost to - patient	10
- cyclotron	13
- chemistry	4
- tomograph	6
- sick days	6

PET radiopharmaceuticals

(M.J. Adam, TRIUMF)

From 1995 to the present day we continue to use both the TR13 and CP42 cyclotrons to prepare the radioisotopes used in the production of the positron labelled radiopharmaceuticals used in the PET program. However, most of these production and research runs are now carried out on the TR13 cyclotron. During this period we have brought on line three new radiopharmaceuticals for a total of seven radiopharmaceuticals in routine production, three labelled with ^{18}F (FDG, FDOPA, and Setoperone) and four labelled with ^{11}C (SCH23390, Raclopride, Dihydrotetrabenazine, and Methylphenidate). The total number of production runs for each radiopharmaceutical for the calendar year is: FDG 45, FDOPA 87, Setoperone 43, SCH23390 33, Raclopride 88, Dihydrotetrabenazine 82, Methylphenidate 65, and Fluoro-metatyrosine 2 (total runs = 419). These production numbers are approximately 36% higher than in 1996 (total = 315) and approximately twice as many as at the beginning of 1995. Most of the compounds with the exception of FDG and Setoperone are used to probe the dopaminergic system for the study of movement disorders such as Parkinson's disease. FDG and Setoperone are used by the Psychiatry group headed by Dr. Little. Automated chemistry systems continue to be developed and play a major role in the production of our radiopharmaceuticals. Many improvements have been made to the ^{11}C production systems that improve efficiency of the chemistry and reduce the dose to the operator. During this period we have developed and implemented a new gas phase ^{11}C-methyl iodide system for the sequential production of the ^{11}C-receptor binding drugs. Specific activities of the ^{11}C

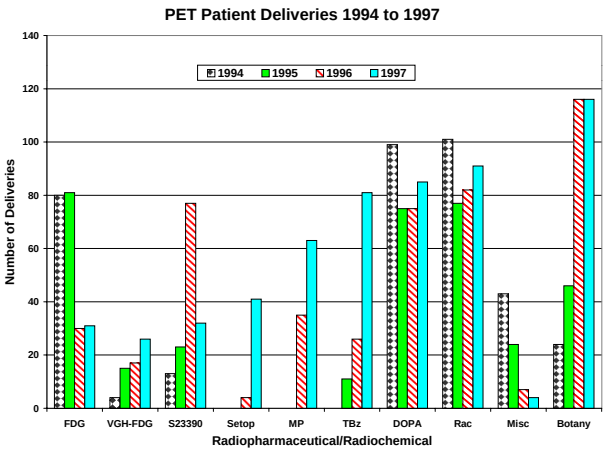


Fig. 81. PET patient deliveries.

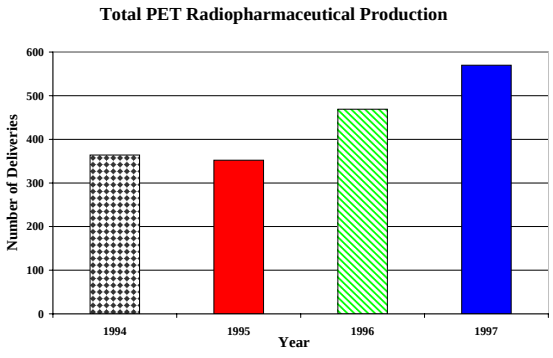


Fig. 82. Total PET radiopharmaceutical production.

compounds continue to rise to a level of 2000–4000 Ci/mmol. Also, a new $^{18}O_2$ system on the TR13 cyclotron has been installed which allows for a much more rapid "double shoot" irradiation for the production of $^{18}F_2$.

A collaborative project with Dr. S. Withers of the UBC Chemistry Department was also initiated during this time. This project was designed to develop ^{18}F-fluorinated glycosidase inhibitors for the study of lysosomal storage diseases such as Tay-Sachs disease with PET. This project has resulted in several publications and presentations at international meetings.

During this period we also began to send, on a regular basis, batches of ^{18}F-FDG (total 25/year) to the Department of Nuclear Medicine at Vancouver General Hospital for imaging lung nodules as well as the heart. This has resulted in one publication and two presentations at international meetings. We have also sent our first batch of FDG to Lions Gate Hospital for breast cancer research during 1997.

Student training

Since 1995 this program has graduated two Ph.D. students through the collaborative projects with the Chemistry Department and has trained 8 chemistry co-op students in radiochemistry and synthetic organic chemistry. The two Ph.D. students, Julius Balatoni and John McCarter, have gone on to postdoctoral fellowships. Dr. Balatoni is at Memorial Sloan Kettering Cancer Institute in New York and Dr. McCarter is an MRC fellow in the laboratory of Prof. Jack Kirsch at the University of California, Berkeley. Dr. McCarter was recently awarded the Bio-Mega/Boehringer Ingelheim Award for outstanding doctoral research.

The 8 co-op students, Jessie Leighton, Poul Rosen, Angela Schlak, Ron Neufeld, Cecilia Stevens, Chris Carmichael, Mario Magon and John Gordon, were all from the University of Victoria Chemistry Co-op program. Since graduating, John Gordon is now working at Forintek as a chemistry technician, Mario Magon is at Stanford Research Inst. in California in the Biology division doing pharmaceutical research, both Chris Carmichael and Cecilia Stevens are in their last term in Honours Chemistry and will be attending Grad School after graduation and the others are still finishing their degrees. Several of the co-op students who worked in the PET Chemistry group prior to 1995 have gone on to obtain Ph.D.'s in chemistry while others have gone to work at companies such as Blue Star and Ballard Batteries.

Aptamer imaging agents

(H. Dougan, TRIUMF)

Research is progressing with radiolabelled ^{123}I short DNA molecules (aptamers) with an affinity for blood clot components. The goal is to master the use of DNA radiopharmaceuticals and obtain an imaging agent for thrombus. A highlight in 1997 was a publication on organotin/radioiodination procedure for short DNA molecules. The reaction has also been extended to very high specific activity ^{123}I DNA, to phosphorothioate DNA, and to alternative aptamer sequences (up to 38 bases in length to date). A preliminary abstract reports the extensions. Biochemical testing of the first aptamer sequence showed that it bound to thrombin, and inhibited coagulation, but it did not bind to clot. Apparently, clot fibrin binds to thrombin at the same site as this aptamer. In mice the initial aptamer was removed from the blood very rapidly; there would be little time to bind a clot *in vivo*. Work continues on new aptamer sequences and nucleic acid chemistry to address the biological problems.

TRIM

(J.S. Vincent, TRIUMF)

TRIM activities were limited again in 1997 by the absence of radiochemical facilities at TRIUMF. However, LS18, the development of ^{82}St-Rb generators for human use in Canada, was terminated successfully. A final report listing the publications and progress of this work was written with the Ottawa Heart Institute and it is available from the Science Division office. The second project, LS14, ^{127}Xe from metallic cesium received about two days for beam development. Beam currents up to 20 μA at 105 MeV were run successfully with the target at equilibrium. As the year closed the xenon extraction equipment has been commissioned and the xenon extraction efficiency 78% of production has been realized. Some modification of the beam collimators and halo monitor is in progress and this should permit operation of the target at higher currents. The third project to measure the production cross sections of ^{103}Pd from natural silver was completed with INR-Troitsk. Excitation functions from 50 to 145 MeV have been measured for the isotope of interest and the significant contaminating species. The final separation chemistry remains to be developed. A proposal has been submitted, LS31, auger emitters for therapy, which will cover this work.

A platform for the design of position encoding multicrystal detectors

(C. Moisan, TRIUMF)

Summary

The goals of this project include the feasibility studies of novel position encoding detectors based on fast inorganic scintillators, the development of a simulation allowing easy design specifications of such detectors by the user, and, the validation of the predictions of the simulation against measured performances.

Research and development for detector simulation

Publication of models of LSO HR+ block and scanner

Our group has brought to publication its studies of the impact of LSO on the performances of PET camera and block detector of the HR+ generation. These studies are reported in Moisan *et al.* [IEEE Trans. Nucl. Sci. **NS44**, 1219 (1997)] and Moisan *et al.* ["Simulating the performances of an LSO based position encoding detector for PET", IEEE Trans. Nucl. Sci. (in press)] respectively.

Sub-4 mm DOI encoding block design studies

During the period extending from June 1996 to July 1997, the LS12 group has been experimenting with a method of effecting depth-of-interaction sensitivity in PET detectors. The approach exploits a significant difference in the index of refraction between adjacent scintillator segments and the compound optically coupling them to induce discrete and resolved photopeak pulse heights depending on the segment of interaction of γ-rays. This was put to an experimental test by manufacturing two prototypes with LSO crystals of dimensions $4\times4\times30$ mm and $2.5\times2.5\times20$ mm respectively, each comprising three segments along their longitudinal axis. Measurements of their absolute pulse height responses when irradiated by 511 keV photons were recently reported in Moisan *et al.* ["Segmented LSO crystals for depth-of-interaction encoding in PET", presented to 1997 IEEE Nucl. Sci. Symp., Albuquerque (submitted to IEEE Trans. Nucl. Sci.), TRI-PP-97-66]. The energy response of the segmented LSO crystals evidences resolved peaks for photoelectric interactions occurring in each longitudinal segment, which permits DOI encoding. The expected performances of would-be PET detector units comprising a plurality of such segmented LSO crystals were studied extensively with the TRIUMF simulation platform and were briefly discussed in Moisan *et al.* [*op. cit.*].

The technology has been protected by a disclosure presently filed as a US provisional patent. At the time of writing, a licensing agreement is being worked out between TRIUMF's Technology Transfer Office and a significant commercial partner. This will hopefully ensure the transfer of the technology while securing a reasonable return to TRIUMF.

Implementation of DETECT optical tracking into GEANT 4

The source code for DETECT as well as its best knowledge of the physics of scintillation photon tracking has been shared with the local spokesman for the GEANT 4 Collaboration. This represents a good transfer of the knowledge base developed at TRIUMF through the Detector project. In the long run, this action should certainly facilitate the development of this important sub-field in the modeling of radiation detection devices.

Surface model experimental validation

In Moisan *et al.* ["Testing scintillation transport models with photoelectron yields measured under different surface finishes", *ibid.*, TRI-PP-97-67] our group reported a first exhaustive test of the UNIFIED, POLISH, and GROUND surface models found in DETECT97. The models were tested for their capacity to predict the photoelectron yields measured with crystals of different surface finishes and reflective coats. Two BGO crystals of $5.6\times12.8\times29.7$ mm, with respectively a polished and a corrugated surface finish were considered. Stylus profilometer scans were first taken to quantify their surface roughness. The crystals were then prepared in three different surface coat configurations to subsequently measure the photoelectron yield collected when excited by a beam of 511 keV photons. These measurements were used to confront the models' predictions on absolute ground. The results indicate that the transport of scintillation photons internally trapped within the volume of a highly polished crystal is well accounted for. However, significant discrepancies remain between simulations and measurements when considering a corrugated finish or when the surface is coated by a diffuse reflector. Possible explanations are now well understood and will give ground to future investigations.

DETECT98: treatment of frequency dependent parameters

During the period extending April–September, DETECT97 has been completely re-engineered. The update was initiated in order to allow for the treatment of the dependence of the optical properties of active and passive components of scintillation counters upon the frequencies of emitted photons.

This substantially updated version of DETECT, DETECT98, will enable the user to model the impact on his detector's performances of, among others:

- the emission frequency spectrum of active scintillators;
- the dispersive nature of these scintillators' index of refraction and its impact on interactions at interfaces and transfer efficiency;
- the frequency dependence of the bulk diffusion and absorption lengths of the scintillators;
- the frequency response of readout photosensors;
- the frequency response of passive components such as glues and reflective paints;

- the frequency dependence of the absorption and re-emission processes in wavelength shifting components; and
- the frequency dependence of surface properties' specifications.

DETECT98 validation/documentation and release

At the time of writing, final tests to validate DETECT98 along with its documentation are being conducted. Public domain release of DETECT98 is expected in early 1998.

POP of a VRML based output GUI for DETECT

An interesting spin off of the re-engineering of DETECT, was the development of a proof-of-principle output graphical user interface (GUI) for DETECT. The interface is based on the virtual reality markup language (VRML) version 2 for which navigators, such as VRWEB, are available on numerous platforms. The development of a prototype VRML interface for DETECT is now being pursued.