Final Scientific/Technical Report

Principal Investigator: Michael E. Phelps

Institution: University of California, Los Angeles

Project Title: Microfluidic Technology Platforms for Synthesizing, Labeling and Measuring the Kinetics of Transport and Biochemical Reactions for Developing Molecular Imaging Probes

Project Award Number: DE-SC0001249

Project Period: 09/01/2009 - 08/31/2014

Distribution Limitation: Unlimited

Microfluidic Technology Platforms for Synthesizing, Labeling and Measuring the Kinetics of Transport and Biochemical Reactions for Developing Molecular Imaging Probes

I. Project 1: Synthesizer Technologies for Radiosynthesis

A. Executive Summary

Several trainees worked on projects at the cutting edge of technologies for radiosynthesis as the hands-on component of their training. Projects included: (i) development of a modular approach to radiosynthesis with the aim of providing flexibility for different synthetic protocols while simultaneously simplifying the process of programming the synthesizer; (ii) development of methods to rapidly and efficiently activate (dry) [F-18]fluoride for ultra-fast synthesis, and methods to concentrate [F-18]fluoride for increasing the amount of radioactivity that can be loaded into microfluidic synthesis chips; (iii) development of a microreactor technology compatible with high temperatures and pressures; (iv) exploration of the feasibility of an electronic microfluidic chip (electrowetting-on-dielectric, EWOD) for carrying out radiosynthesis in an ultra-small footprint that could enable benchtop radiosynthesizers that do not need to be housed in a hot cell; and (v) development of interfaces for automated reagent delivery for carrying out syntheses on digital microfluidic chips.

Trainees published numerous papers on the results of these research efforts. In addition, several of these technologies have been, or are being, commercialized to enable their dissemination to the broader research and radiochemistry community.

B. Goals and Accomplishments

The goals of this project were as follows:

1. Design and construction of a modular systems based on “unit operations”.
2. Design and construction of a module for [18F]Fluoride drying and concentration.
3. Design and construction of a microfluidic reactor module.
4. Investigation of “digital microfluidics” for radiochemical operations.
5. Design and construction of a microfluidic radiosynthesis platform.

During the project period these aims were successfully accomplished. A detailed description of accomplishments for each sub-project is listed below.

The trainees involved in the following projects were all new to radiochemistry and learned fundamentals of F-18 radiochemistry, analytical chemistry, chemistry automation, and microfluidics through development of these technologies.

Design and construction of a modular system based on “unit operations”.

We designed and built a first-generation prototype robotic reaction module for radiochemical reactions that require high temperatures and pressures. It can reliably hold above 150 psi to contain superheated solvents and corrosive reagents at up to 200°C, substantially expanding the range of reaction conditions beyond that of commercial systems. In addition to being in a sealed-reaction state, the reaction vial can be moved among other positions to perform functions such as addition of reagents, evaporations, and transfers out of the reactor. We verified basic engineering functionality of the system (motion, heating, cooling, stirring, pressurized transfers, etc.), and have demonstrated reliable synthesis of 1-[F-18]fluoro-4-nitrobenzene, [F-18]FTAG, and [F-18]FDG by connecting the reaction module to our previously-developed reagent delivery and cartridge purification modules. The reaction module measures approximately 4" wide and 11" tall so that several can fit side-by-side in a mini-cell.

To make the system more user-friendly, and to minimize the amount of wiring and tubing that is required to run between the outside and inside of the hot cell, we designed and built a second-generation control system. Reactors or other modules are controlled by sending simple commands via the 'mojobus' protocol across a serial communication bus. Software was developed to control the modules via a graphical interface, and to provide the capability of recording and playback of event sequences. Based on the new design, an additional two reactor modules have been built, bringing the total to three, enabling the synthesis of 1-(2'-deoxy-2'-[18F]fluoroarabinofuranosyl)cytosine ([18F]FAC), a probe of dCK enzyme activity with potential applications in immunology and oncology. We developed a robust synthesis protocol for the 4-step, 3-pot synthesis involving pressures up to 120 psi and consistently produce batches with RCY of 20-30% (decay-corrected) that are sufficient for multiple preclinical studies. A paper was published on the design and validation of this 'plug-and-play' modular system. Temperature control in the reaction module is provided via a metal heating jacket around the vial. The temperature of the metal jacket is measured for closed-loop feedback control. The internal temperature of the liquid inside the vial has been extensively studied for the purposes of calibration since temperature probes cannot be placed inside the liquid during actual radiosyntheses. Our data reveal a close relationship between the internal and jacket temperatures when using solvents heated below their boiling point, but a growing disparity at temperatures above the boiling point. We have observed the same profiles inside a vial heated by oil bath. We are exploring the relationship of the parameters of this phenomenon and will attempt to create a model of this process and then finalize and submit a manuscript on the reactor heating.

To increase the level of automation and to facilitate translation of this technology into other research labs and potentially the commercial marketplace, we designed and built a third-generation prototype. It has 3 high-pressure reactors, a liquid cooling system, an integrated HPLC injection system, and a simple reagent handling robot. These improvements enable nearly complete automation of the complicated [18F]FAC synthesis process. A further feature of the design is a disposable fluid path, comprising nearly all wetted components of the system to facilitate quick setup and operation with no cleaning required, a feature that is desired for

translation to clinical settings. Three papers were published on this system: one on the design, one on the software approach, and one on validation with many different tracers, all produced without any hardware reconfiguration. The system is currently being used in other research projects and to supply PET tracers for studies in the Crump Preclinical Imaging Technology Center.

Microfluidic Technologies for F-18 Drying and Concentration:

Due to high F-ion solvation energy in water, aqueous fluoride is relatively inactive unless it is released from its aqueous surrounding. This is usually achieved by mixing aqueous fluoride with a base and a phase transfer agent and azeotropically drying the resulting solution with acetonitrile. The dried active complex is then used for radiolabelling. Depending on the technical method of fluoride activation, this procedure may take up to 20-30 minutes and thus reduce the overall yield considerably.

We explored the use of an electrochemical cell to separate F-18 from [O-18]water as the contents of the cyclotron target are passed through the cell. An electric field is applied to trap the [F-18]fluoride ions on the anode, the cell is then dried with solvent and gas, and the [F-18]fluoride is released into anhydrous solvent. To systematically study the parameters that can impact the ion kinetics (e.g. temperature, flow rate, trapping voltage, release voltage, AC frequency, current, and residence time), we designed and built a remotely-controlled electrochemical system within a mini-cell. We designed the cell such that electrode materials could be interchanged to enable study the effect of material on fluoride trapping and release, and attempt to improve the overall efficiency of this approach and avoid clogging issues that have been observed due to erosion of carbon electrodes. Using analytical techniques such as X-ray photoelectron spectroscopy (XPS), we were able to elucidate some aspects of the mechanism of F-18 trapping. The trapping (and release) performance depend strongly on the electrode material, depth of intercalation and the nature of chemical bonds formed on the surface. These studies enabled selection of an optimal combination of materials (platinum and brass). We then optimized several critical operating parameters in this system and achieved reproducible trapping and release with overall efficiency of 84 ± 5% (n=7). To demonstrate the reactivity of the released [F-18]fluoride, the cell was coupled to a custom-built flow-through reactor (with cartridge-based hydrolysis) and automated synthesis of [F-18]FDG with a repeatable decay-corrected yield of 56 ± 4% (n=4) was completed in < 15 min. A paper was published on the design, optimization, and validation of this approach. The automated system could be applied to synthesize additional tracers.

Another project explored a method for concentrating [F-18]fluoride ion, for the preparation of small volumes (e.g. a few µL) containing sufficient amount of radioactivity for production of tracers for preclinical (and potentially clinical) imaging in microfluidic radiosynthesis devices. The method involves evaporation of solvent through a gas-permeable membrane while the solutes (i.e., [K/K2.2.2]+18F- complex) are retained in a microchannel. The dried residue is eluted with an anhydrous solvent. We have developed several generations of PDMS

microfluidic chips to study this process. The latest generation was optimized for maximum evaporation rate by: (i) minimizing the thickness of the gas-permeable membrane (~20 micron); (ii) incorporating a vacuum channel to maximize the pressure gradient across the membrane; (iii) using a high-permeability membrane (polydimethylsiloxane, PDMS); and (iv) having perpendicular serpentine evaporation and vacuum channels to provide large overall surface area for vapor transport. A prototype, remote-controlled drying system was built inside a mini-cell. Quantitative measurements were made after each step of the process (loading initial [F-18]fluoride solution, evaporation and elution) using the Cerenkov imaging technique. Imaging the chips in this manner revealed several important results. (i) There is a time- and temperature-dependent loss of radioactivity from the chamber that begins after all the liquid has been eliminated. (ii) There are two qualitatively-distinct spatio-temporal evaporation patterns that can occur: a rapid "burst" pattern and a slower "directional" pattern. Optimized operation exhibits high reliability and repeatability, low loss during evaporation (< 10%), and effective elution of [F-18]fluoride with MeCN (<10% residual activity remaining in the chip). A journal paper on this research is expected to be submitted shortly. In addition to drying, we examined the possibility to perform on-chip concentration by controlled partial evaporation of solvent using the "directional" evaporation pattern. Solvent is removed by vacuum evaporation (and heating) through a gas-permeable membrane. The F-18 is observed to remain in solution throughout this process, even as the solvent volume decreases. About 20x concentration has been achieved in-chip with a potential up to about 32-40x based on solubility measurements. Eluting concentrated [F-18]fluoride out of the chip has been demonstrated for a 50-90% reduction in solvent volume. These preliminary results formed the basis for a new project to use microfluidic concentration to replace rotary evaporation after HPLC purification of PET tracers.

Design and construction of a microfluidic reactor module:

Though several microfluidic batch reaction platforms have been reported for radiosynthesis, they tend to be limited to more mild reaction conditions (i.e., lower temperatures and pressures) than their continuous flow counterparts. Handling high pressures is important, however. To ensure fast kinetics and high yields, radiosynthesis is often performed in a sealed vessel under super-heated conditions, resulting in the generation of significant solvent vapor pressure. For example, the probe 1-(2′-deoxy-2′-[F-18]fluoroarabinofuranosyl)cytosine ([F-18]FAC) and its analogs require the containment of the vapor pressures of solvents well in excess of 100 psi. To combine the advantages of continuous flow microreactors (wide range of reaction conditions) with those of batch microreactors (small overall volume), we develop a batch microreactor capable of withstanding high pressures. The approach uses "phase-change" microvalves, in which the fluid in a channel is reversibly converted to solid (frozen or melted) to close or open the fluid path. Impressive reports demonstrate the capability of to withstand up to 1450 psi with this technique without the need for complex fabrication or moving parts. Using a new phase-change material, dimethyl sulfoxide (DMSO), we extend the application of phase-change valves to high-pressure organic chemistry in batch microreactors to address the limitation of current phase-change

valves. In the field of radiochemistry, DMSO is a commonly used solvent and is thus intrinsically favorable as a phase-change material. The solvent compatibility allows direct translation of a known chemical reaction onto a microfluidic platform, without the need to change the chemistry to avoid the risk obtaining suboptimal yield due to contamination. Physically, DMSO has a relatively high freezing point (18.9 °C) making it easier to freeze than water and many other organic solvents. The microreactor was implemented using microbore tubing thermally coupled to heating and cooling blocks. After introduction of the reagents, the cooling block froze a small volume of liquid to close the two ends of the tubing. The contents between these closed valves was then heated to perform the reaction. As an example reaction, we successfully demonstrated the radiofluorination step in the multi-step synthesis of [F-18]FAC. The synthesis was performed with high conversion efficiency comparable to what can be typically achieved under sealed, pressurized conditions at the macroscale. This work was published in a leading microfluidics journal and became the basis for a portion of the PhD dissertation of one of our trainees.

Development of Digital Microfluidics (EWOD) for Radiosynthesis:

The EWOD radiosynthesizer project made significant overall progress towards reliable multistep synthesis of F-18 labeled PET tracers using 2-fluoro-2-deoxy-D-glucose ([F-18]FDG) as a model system. We successfully demonstrated the synthesis [F-18]FDG with high fluorination efficiency (88±7%, n=11) and quantitative hydrolysis. We performed high throughput optimization processes to establish optimal radiosynthesis conditions in microdroplets. We found that the fluorination reaction was most effective in DMSO, mainly due to its ability to solvate the reaction droplet at an elevated temperature throughout the fluorination step, without the need to replenish with additional solvent droplets. Due to the minute amount of DMSO used on the EWOD chip, the residual DMSO did not affect the subsequent hydrolysis reaction. In addition to the chemistry modification, an improved EWOD chip with larger reaction heater design (diameter: 12 mm vs 1 mm) enabled several mCi of radioactivity to be loaded onto the EWOD chip, which is sufficient to synthesize several mCi of [F-18]FDG. To purify the sub-microliter volumes of crude product synthesized on EWOD, we designed a miniaturized custom-made cartridge to achieve high recovery of [F-18]FDG with >99% chemical purity. We also confirmed that [F-18]FDG synthesized on EWOD passes the standard quality control analysis determined by the United States Pharmacopeia (USP). During the development of [F-18]FDG synthesis on the EWOD chip, a dedicated Cerenkov and bright field imaging system was set-up to determine the real-time distribution of radioactivity at every step of the [F-18]FDG synthesis. Compared to the Cerenkov-PDMS microfluidic imaging system described in the original application, we have further optimized both the Cerenkov and video rate bright field imaging modalities by utilizing a rotatable mirror to switch between the two cameras. Cerenkov images were acquired at every step including K+/[18F]F-/K2.2.2 loading, drying, fluorination and hydrolysis, and the spatial distributions of radioactivity were determined for each. Based on these kinetic studies, an optimized methodology was developed to maximize the utilization of radioactivity and to

achieve high synthetic reproducibility on EWOD chip. Papers were published on the reliable synthesis of [F-18]FDG on EWOD and on the use of Cerenkov imaging to perform optimization of processes on the EWOD chip.

Design and construction of a microfluidic radiosynthesis platform:

We developed a first-generation prototype of a reagent storage and pumping system to interface with digital microfluidic (EWOD) chips, comprising a fixture to precisely position needles at loading sites for delivery of reagents on demand. A paper was published on the design and characterization of this system and the demonstration of the synthesis of [F-18]FDG. While this system provides a practical means to accurately deliver precise volumes of volatile reagents to the chip, the need for multiple syringe pumps (one for reach reagent) does not scale well as the synthesis complexity increases. To overcome this limitation, we developed a second-generation approach that achieves on-demand loading of volatile reagents using a much simpler mechanism. The reagent is propelled by gas pressure from a vial up toward the chip. When the reagent arrives at the chip, the pressure is vented and the gravitational force on the liquid in the tubing retracts the liquid so that excess liquid does not “flood” into the chip. This approach easily scales to the number of reagents needed for [F-18]FDG and beyond and relies on primarily disposable components and thus eliminates many of the problems associated with the first-generation approach. We have characterized the performance of a prototype of this system for a variety of solvents and reagents relevant to F-18 radiochemistry, and designed a new EWOD chip to interface with multiple, independent reagent inlets. A paper was published on this improved reagent-delivery approach. Both techniques can be applied in other applications of digital microfluidic chips.

Detection of liquid arrival at the chip was achieved with impedance sensing. We developed a simple modification to the electrode control circuitry for monitoring the AC current from the driving electrode on one of the chip substrates, through the gap in the chip (air or droplet) and through the ground electrode on the second chip substrate. The contents of the gap has a tremendous influence on this current, and the current can be monitored to determine the volume of liquid (if the liquid is known), or to distinguish different liquids and concentrations. A paper was published on this technique. One of our trainees extended this fundamental approach to explore the feasibility of monitoring reaction progress directly on chip. A new measurement algorithm, based on subtraction of the signal from a “standard” droplet, enabled decoupling of the impedance of the sample from the intrinsic impedance of the chip, resulting in a tremendous improvement in sensitivity and dynamic range. Using the improved approach, monitoring of a titration reaction was successfully observed. This could be used to monitor reactions such as hydrolysis (where reagents undergo conductivity change) or the process of neutralization. A manuscript on this technique is in preparation.

C. Papers and other products delivered

Papers

Radiochemical Synthesizers:

1. Huijiang Ding, Saman Sadeghi, Gaurav J. Shah, Supin Chen, Pei Yuin Keng, Chang-Jin "CJ" Kim, R. Michael van Dam. Accurate dispensing of volatile reagents on demand for chemical reactions in EWOD chips. Lab on a Chip 12: 3331-3340, 2012.
2. Pei Yuin Keng, Supin Chen, Huijiang Ding, Saman Sadeghi, Gaurav J. Shah, Alex Dooraghi, Michael E. Phelps, Nagichettiar Satyamurthy, Arion F. Chatziioannou, Chang-Jin "CJ" Kim, R. Michael van Dam. Micro-chemical synthesis on a digital microfluidic device. PNAS 109(3): 690-695, 2012. [PMCID: PMC3271918]
3. Saman Sadeghi, Huijiang Ding, Gaurav J. Shah, Supin Chen, Pei Yuin Keng, Chang-Jin "CJ" Kim, R. Michael van Dam. On-chip droplet characterization: A practical, high-sensitivity measurement of droplet impedance in digital microfluidics. Analytical Chemistry 84(4): 1915-1923, 2012. [PubMed – in process]
4. Saman Sadeghi, Vincent Liang, Shilin Cheung, Suh Woo, Curtis Wu, Jimmy Ly, Yuliang Deng, Mark Eddings, R. Michael van Dam. Reusable electrochemical cell for rapid separation of [18F]fluoride from [18O]water for flow-through synthesis of 18F-labeled tracers. Applied Radiation and Isotopes 75: 85-94, 2013.
5. Gaurav J. Shah, Huijiang Ding, Saman Sadeghi, Supin Chen, Chang-Jin "CJ" Kim, R. Michael van Dam. On-demand droplet loading for automated organic chemistry on digital microfluidics. Lab on a Chip 13: 2785-2795, 2013.
6. Bernard Amarasekera, Phillip D. Marchis, Krzysztof P. Bobinski, Caius G. Radu, Johannes Czernin, Jorge R. Barrio, R. Michael van Dam. High-pressure, compact, modular radiosynthesizer for production of positron emitting biomarkers. Applied Radiation and Isotopes 78: 88-101, 2013.
7. Henry Herman, Graciela Flores, Kevin Quinn, Mark Eddings, Sebastian Olma, Melissa D. Moore, Huijiang Ding, Krzysztof P. Bobinski, Mingwei Wang, Dirk Williams, Darin Williams, Clifton Kwang-Fu Shen, Michael E. Phelps, R. Michael van Dam. "Plug-and-play modules for flexible radiosynthesis." Applied Radiation and Isotopes 78: 113-124, 2013.
8. Mark Lazari, Kevin Quinn, Shane Claggett, Jeffrey Collins, Gaurav J Shah, Henry Herman, Brandon Maraglia, Michael E Phelps, Melissa Moore and R. Michael van Dam. ELIXYS - A fully automated, three-reactor high-pressure radiosynthesizer for development and routine production of diverse PET tracers. European Journal of Nuclear Medicine and Medical Imaging (EJNMMI) Research 3: 52, 2013.
9. Shane B Claggett, Kevin M Quinn, Mark Lazari, Melissa Moore and R. Michael van Dam. Simplified programming and control of automated radiosynthesizers through unit operations. European Journal of Nuclear Medicine and Medical Imaging (EJNMMI) Research 3: 53, 2013.
10. Xiaoxiao Ma, Wei-Yu Tseng, Mark Eddings, Pei Yuin Keng, R. Michael van Dam. Microreactor with phase-change microvalves for batch chemical synthesis at high temperatures and pressures. Lab on a Chip 14(2): 280-285, 2013.
11. Shilin Cheung, Jimmy Ly, Mark Lazari, Saman Sadeghi, Pei Yuin Keng, R. Michael van Dam. The separation and detection of PET tracers via capillary electrophoresis for chemical identity and purity analysis. Journal of Pharmaceutical and Biomedical Analysis 94: 12-18, 2014.

12. Gaurav J. Shah, Uday Tata, R. Michael van Dam. Automation and interfaces for chemistry and biochemistry in digital microfluidics. Technology 2(2): 83-100, 2014.
13. Mark Lazari, Jeffrey Collins, Bin Shen, Mohammed Farhoud, Daniel Yeh, Brandon Maraglia, Frederick T. Chin, David A. Nathanson, Melissa Moore, R. Michael van Dam. Fully automated production of diverse 18F-labeled PET tracers on the ELIXYS multi-reactor radiosynthesizer without hardware modification. Journal of Nuclear Medicine Technology 42(3): 203-210, 2014.

New methods of [F-18]fluorination:

1. Maxim E. Sergeev, Federica Morgia, Muhammad R. Javed, Mami Doi, Pei Y. Keng. Enzymatic radiofluorination: Fluorinase accepts methylaza-analog of SAM as substrate for FDA synthesis. Journal of Molecular Catalysis B: Enzymatic 97: 74-79, 2013.
2. Maxim E. Sergeev, Federica Morgia, Muhammad R. Javed, Mami Doi, Pei Y. Keng. Polymer-immobilized fluorinase: Recyclable catalyst for fluorination reactions. Journal of Molecular Catalysis B: Enzymatic 92: 51-56, 2013.
3. Qinggang He, Ying Wang, Ines Alfeazi, Saman Sadeghi. Electrochemical nucleophilic synthesis of di-tert-butyl-(4-[18F]fluoro-1,2-phenylene)-dicarbonate. Appl Radiat Isot. (92): 52–57, 2014. PMID: 25000498
4. Qinggang He, Saman Sadeghi. Carrier-free electrochemical nucleophilic radiofluorination of aromatics Journal of Radioanalytical and Nuclear Chemistry. 2014 (In press)

Conference Presentations:

1. Chang-Jin “CJ” Kim, R. Michael van Dam, Supin Chen, Huijiang Ding. Radiosynthesis of Positron Emission Tomography (PET) Imaging Probes on Electrowetting-on-Dielectric (EWOD) Chip.7th International Meeting on Electrowetting (EW2010), Pohang, South Korea, June 24-26, 2010.
2. Pei Yuin Keng, Huijiang Ding, Supin Chen, Saman Sadeghi, Gaurav J. Shah, Michael E. Phelps, Nagichettiar Satyamurthy, Chang-Jin “CJ” Kim, R. Michael van Dam. Digital microfluidics: Toward a bench-top platform for on-demand radiosynthesis of diverse PET tracers. 240th ACS National Meeting, Boston, MA, Aug 22-26, 2010.
3. Pei Yuin Keng, Supin Chen, Huijiang Ding, Saman Sadeghi, Michael E. Phelps, Nagichettiar Satyamurthy, Chang-Jin “CJ” Kim, R. Michael van Dam. All-Electronic Microfluidic Chips for Radiosynthesis of Diverse Molecular Probes. Poster Presentation. World Molecular Imaging Congress, Kyoto, Japan, Sept. 8-11, 2010.
4. Saman Sadeghi, Jimmy Ly, Yuliang Deng, R. Michael van Dam. A Robust Platinum-Based Electrochemical Micro Flow Cell for Drying of [18F]Fluoride for PET Tracer Synthesis. Proceedings of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 318-320, Groningen, The Netherlands, Oct 3-7, 2010.
5. W-Y Tseng, J. Cho, X. Ma, K. Mahal, A. Chatziioannou, R. M. van Dam. PDMS Evaporation Chip to Concentration [18F]Fluoride for Synthesis of PET Tracers in Microfluidics. Proceedings of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 1010-1012, Groningen, The Netherlands, Oct 3-7, 2010.

6. Pei Yuin Keng, Supin Chen, Huijiang Ding, Sam Sadeghi, Michael E. Phelps, N. Satyamurthy, Chang-Jin "CJ" Kim, R. Michael van Dam. Optimization of Radiosynthesis of Molecular Tracers in EWOD Microfluidic Chip. Proceedings of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 668-670, Groningen, The Netherlands, Oct 3-7, 2010.
7. Saman Sadeghi, Jimmy Ly, Yuliang Deng, Nagichettiar Satyamurthy, R. Michael van Dam. Electrochemical micro flow cell for rapid PET tracer synthesis. Society of Nuclear Medicine Annual Conference, San Antonio, TX, June 4 -8, 2011.
8. Pei Yuin Keng, Supin Chen, Michael E. Phelps, Nagichettiar Satyamurthy, Chang-Jin "CJ" Kim, R. Michael van Dam. Toward on-demand production of diverse PET tracers: Repeatable, multi-step synthesis of [18F]FDG on a compact, electronically-actuated droplet-based microfluidic platform. Society of Nuclear Medicine Annual Conference, San Antonio, TX, June 4 -8, 2011.
9. Henry Herman, Graciela Flores, Kevin Quinn, Melissa Esterby, Gaurav J. Shah, Michael E. Phelps, Nagichettiar Satyamurthy, R. Michael van Dam. Multi-pot radiosynthesizer capable of high-pressure reactions for production of [18F]FAC and analogs. Society of Nuclear Medicine Annual Conference, San Antonio, TX, June 4 -8, 2011.
10. W.C. Nelson, M. Yen, P.Y. Keng, R. M. van Dam, C-J Kim. High pressure EWOD digital microfluidics. Proceedings of the 16th IEEE International Solid-State Sensors, Actuators and Microsystems Conference (Transducers), Beijing, China, June 5-9, 2011.
11. G.J. Shah, H-J Ding, S. Sadeghi, S. Chen, C-J Kim, R.M. van Dam. Milliliter-to-microliter platform for on-demand loading of aqueous and non-aqueous droplets to digital microfluidics. Proceedings of the 16th IEEE International Solid-State Sensors, Actuators and Microsystems Conference (Transducers), Beijing, China, June 5-9, 2011.
12. Pei Yuin Keng, Supin Chen, Huijiang Ding, Saman Sadeghi, Michael E. Phelps, Nagichettiar Satyamurthy, Chang-Jin "CJ" Kim, R. Michael van Dam. Electrowetting on dielectric digital microfluidic chips for multistep radiosynthesis of PET tracers. 19th International Symposium on Radiopharmaceutical Sciences, Amsterdam, The Netherlands, Aug 28 – Sept 2, 2011.
13. Henry Herman, Kevin Quinn, Melissa Esterby, Gaurav J. Shah, Graciela Flores, Michael E. Phelps, Nagichettiar Satyamurthy, R. Michael van Dam. Flexible radiosynthesizer capable of multi-pot high temperature and pressure reactions. 19th International Symposium on Radiopharmaceutical Sciences, Amsterdam, The Netherlands, Aug 28 – Sept 2, 2011.
14. Saman Sadeghi, Jimmy Ly, Nagichettiar Satyamurthy, R. Michael van Dam. High-yield electrochemical separation of [18F]fluoride from [18O]water in a transition metal flow cell. 19th International Symposium on Radiopharmaceutical Sciences, Amsterdam, The Netherlands, Aug 28 – Sept 2, 2011.
15. Gaurav J. Shah, Supin Chen, Pei Yuin Keng, Saman Sadeghi, Chang-Jin Kim, R. Michael van Dam. Automation of a digital microfluidic system for repeatable radiosynthesis of 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG) and other tracers. World Molecular Imaging Congress, San Diego, CA, USA, Sept 7-10, 2011.
16. Kevin Quinn, Melissa Esterby, Gaurav J. Shah, Graciela Flores, Shane Claggett, Mark Lazari, Henry Herman, Michael E. Phelps, Nagichettiar Satyamurthy, R. Michael van Dam. Synthesis of [18F]FAC on a new, cassette-based radiosynthesizer capable of multi-

pot high temperature and pressure reactions. World Molecular Imaging Congress, San Diego, CA, USA, Sept 7-10, 2011.

17. Huijiang Ding, Saman Sadeghi, Pei Yuin Keng, Supin Chen, Gaurav J. Shah, Chang-Jin "CJ" Kim, and R. Michael van Dam. Accurate dispensing of volatile reagents on demand for EWOD chips. Proceedings of the 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 227-229, Seattle, WA, USA, Oct 2-6, 2011.
18. Gaurav J. Shah, Saman Sadeghi, Huijiang Ding, Supin Chen, R. Michael van Dam. Impedance-based droplet volume and concentration measurement in digital microfluidics. Proceedings of the 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 1308-1310, Seattle, WA, USA, Oct 2-6, 2011.
19. Melissa D. Moore, Kevin Quinn, Shane Claggett, Mark Lazari, Gaurav J. Shah, Nagichettiar Satyamurthy, Michael E. Phelps, R. Michael van Dam. ARC-PHS+: A versatile radiosynthesizer for the production of PET tracers. American Association for Cancer Research (AACR) Annual Meeting, Chicago, IL, USA, Mar 31–Apr 4, 2012.
20. Pei Yuin Keng, Supin Chen, Alex Dooraghi, Mark Lazari, Muhammad Rashed Javed, Arion F. Chatziioannou, Chang-Jin "CJ" Kim, R. Michael van Dam. In situ Cerenkov imaging for optimization of radiochemistry on chip. 4th International Symposium on Microchemistry and Microsystems, Hsinchu, Taiwan, Jun 10-13, 2012.
21. Shane Claggett, Kevin Quinn, Mark Lazari, Jeff Esterby, Melissa Esterby, Michael van Dam. A new paradigm for programming and controlling automated radiosynthesizers. Society of Nuclear Medicine Annual Meeting, Miami Beach, FL, June 9-13, 2012.
22. Mark Lazari, Jeffrey Collins, Kevin Quinn, Shane Claggett, Pei Yuin Keng, Michael E. Phelps, Nagichettiar Satyamurthy, Melissa Esterby, R. Michael van Dam. Diverse nucleophilic syntheses on the ELIXYS multi-pot, cassette-based radiosynthesizer. Society of Nuclear Medicine Annual Meeting, Miami Beach, FL, June 9-13, 2012.
23. Gaurav J Shah, Muhammad Rashed Javed, Anqi Yang, Pei Yuin Keng, R. Michael van Dam. Integration of [F-18]fluoride pre-concentration into a digital microfluidics-based radiosynthesizer for the benchtop. Society of Nuclear Medicine Annual Meeting, Miami Beach, FL, June 9-13, 2012.
24. Xiaoxiao Ma, Wei-Yu Tseng, Mark A. Eddings, R. Michael van Dam. Microreactor with phase-change microvalves for high-pressure batch synthesis of radiotracers. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.
25. Mark S. Lazari, Shane Claggett, Kevin Quinn, Melissa Esterby, R. Michael van Dam. Sequential synthesis of three different 18F-labeled PET tracers on the ELIXYS radiosynthesizer. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.
26. Kevin Quinn, Shane Claggett, Mark S. Lazari, Jeff Esterby, Melissa Esterby, R. Michael van Dam. Radiosynthesizer software applied to mobile devices for the rapid communication of PET probe synthesis outcomes. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.
27. Vincent Liang, Suh Woo, Curtis Wu, Shilin Cheung, Saman Sadeghi, R. Michael van Dam. A reusable electrochemical cell for rapid microfluidic synthesis of 18F-labeled radiotracers. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.
28. W.-Y. Tseng, J.S. Cho, A. Chatziioannou, R. M. van Dam. Interdigitated evaporation chip for efficient solvent exchange in microchannels. Proceedings of the 16th

International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Okinawa, Japan, Oct 28 – Nov 1, 2012.

29. Shilin Cheung, Jimmy Ly, R. Michael van Dam. Capillary electrophoresis separation of 18F-labeled PET tracers from impurities: towards miniaturized quality control. 20th International Symposium on Radiopharmaceutical Sciences, Jeju, South Korea, May 12 – 17, 2013.
30. Shilin Cheung, Jimmy Ly, R. Michael van Dam. Towards miniaturized quality control of 18F-labeled PET tracers: separation of D-FAC alpha and beta anomers via capillary electrophoresis. Society of Nuclear Medicine and Molecular Imaging Annual Meeting, Vancouver, BC, Canada, June 8 – 12, 2013.
31. Mark Lazari, Shuang Zhang, R. Michael van Dam. Solvent temperature during short radiochemical reactions. World Molecular Imaging Congress, Savannah, GA, Sept. 18-21, 2013.
32. Gaurav J. Shah, Andres Saucedo, R. Michael van Dam. Investigation of bursting of heated droplets for chemistry applications in digital microfluidics. Proceedings of the 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Freiburg, Germany, Oct 27 – 31, 2013.
33. A.A. Dooraghi, P.Y. Keng, S. Chen, M.R. Javed, C.-J. Kim, R.M. van Dam, A.F. Chatziioannou. Cerenkov imaging as a quantitative tool for PET probe synthesis on the EWOD microfluidic platform. Proceedings of IEEE Nuclear Science Symposium and Medical Imaging Conference, Seoul, Korea, Oct. 27 – Nov. 2, 2013.
34. Xiaoxiao Ma, Supin Chen, Chang-Jin "CJ" Kim, R. Michael van Dam. Towards on-chip chemical reaction monitoring by EWOD impedance measurement. Proceedings of the 27th International Conference on Micro Electro Mechanical Systems (MEMS), pg. 338-341, San Francisco, CA, Jan. 26-30, 2014.
35. J. Ly, S. Cheung, R. M. van Dam. Micromolar detection of analytes via sub-300 nm UV absorbance in a PDMS microchip capillary electrophoresis device. Proceedings of the 6th International Symposium on Microchemistry and Microsystems (ISMM), pg. 222-223, Singapore, July 30 – Aug 1, 2014.
36. Xiaoxiao Ma, Supin Chen, Chang-Jin "CJ" Kim, R. Michael van Dam. High-sensitivity electrical conductivity measurement of droplets in digital microfluidics. Proceedings of the 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), San Antonio, TX, Oct. 26-30, 2014.

Patent applications:

1. Van Dam, R. Michael, Kim, Chang-Jin, Chen, Supin, Ding, Huijiang, Shah, Gaurav, Keng, Pei Yuin. Digital Microfluidic Platform for Radiochemistry. WO/2011/046615 (PCT/US2010/002756) filed Oct 15, 2010. [Priority Data: 61/252,095, filed Oct 15, 2009]
2. G.J. Shah, Pei Yuin Keng, R. Michael van Dam. Disposable world-to-chip interface for digital microfluidics. (UCLA Case No. 2012-816) (U.S. provisional 61/657570 filed Jun 8, 2012)
3. R. Michael van Dam, Melissa Moore, Kevin Quinn, Shane Claggett, Henry Herman, Mark Lazari. An automated, multi-pot high-pressure radio-synthesizer for production of

PET tracers. (UCLA Case No. 2013-366) (U.S. provisional 61/805,411 filed Mar 26, 2013)
4. Shane Claggett, Kevin Quinn, Mark Lazari, Melissa Moore, R. Michael van Dam. Simplified programming and control of automated radiosynthesizers through unit operations. (UCLA Case No. 2013-365) (U.S. provisional 61/805,879 filed Mar 27, 2013)
5. Qinggang He, Artem Lebedev, Saman Sadeghi. Synthesis of Fluorinated Radiopharmaceuticals via Electrochemical Fluorination. U.S. provisional patent application, 2013.

PhD Theses:

1. Ma, Xiaoxiao. Expanding the capabilities of microfluidic systems for positron emission tomography (PET) tracer synthesis and analysis. PhD Thesis, University of California Los Angeles, 2014.

Other products:

Radiosynthesis technologies:

1. First-generation modular radiosynthesizer for semi-automated synthesis of the [18F]FAC family of tracers (or tracer development/optimization), comprising three reaction vessels compatible with high-pressure (150psi), high-temperature (200°C) reactions.
2. Two second-generation modular radiosynthesizers with reagent delivery modules, high-pressure reaction modules, and cartridge purification modules.
3. Software to control a modular radiosynthesizer.
4. Third-generation radiosynthesizer with three integrated high-temperature, high-pressure reactors, recirculating liquid cooling system for rapid control of reactor temperature, and user-friendly software interface.
5. Microfluidic radiosynthesizer based on electrochemical flow-cell.
6. Base platform for fluoride-concentration and reagent delivery to digital microfluidic radiochemistry

Technology transfer:

Some technologies supported in part by this award and developed by trainees have been or are being commercialized to enable access to these technologies by the broader scientific community. These technologies include:

- The ELIXYS radiosynthesizer, now manufactured and sold by Sofie Biosciences, Inc. was based on the third-generation radiosynthesizer with high-temperature and high-pressure capabilities. It is also undergoing ongoing improvement and development via collaboration between Sofie Biosciences and UCLA.
- The PHENYX benchtop radiosynthesizer is based on the proof-of-concept digital microfluidic synthesis technology. A commercial prototype is being developed in a collaboration between Sofie Biosciences and UCLA.

II. Project 2: Development of Quantitative Radioactivity Imaging Methodologies

A. Executive Summary

The range of signal intensity involved in the generation and usage of radiolabeled probes that was investigated in this work spans approximately twelve orders of magnitude (10^{12}). For example, the activity taken up by individual cells is on the order of a few picoCi/cell (10^{-12}) pCi/cell, while the total activity incorporated in a microfluidic synthesizer for clinical synthesis levels is on the order of a few Ci. This tremendous range is impossible to cover with any single technology. Therefore, two separate technologies are necessary to completely and reliably cover this signal range. One technology at the high sensitivity end, where small populations of cells or individual cells are involved and a very different technology at the high intensity end, where clinical synthesis levels of activity are generated. The high sensitivity end was covered successfully by the solid state **Position Sensitive Avalanche Photodiode Detector**, integrated into the **betaBOX** system, while the high intensity end was covered by the **Cerenkov** imaging system. Both these systems were developed in this project for the detection of the presence of energetic charged particles in microfluidic solutions and therefore some similarities between the two systems exist, especially regarding the methodologies for Monte Carlo simulations of the physical processes involved as well as the goals of the calibration. At the end though, despite the fact that these two systems are both capable of imaging quantitatively the distribution of charged particles in microfluidic devices, they are very different from each other. Therefore a number of different projects that address the performance of each of these two systems were developed, leading to the publications listed below. In the initial submission of the proposal, these two opposing sides of the range were presented sequentially (section 3.C.1.a for synthesis levels, and 3.C.1.b for cell culture levels), but the Cerenkov and the PSAPD imaging systems were also featured in sections throughout the proposal, such as 3.A.2.a, 3.A.4, and 3.D.1. Today, both of these systems have been developed and have been used in applications ranging from the optimization of synthesis of diverse F-18 labeled probes, to the investigation of the response of different cell types to interventions. In order to present a more cohesive picture of the development of these two systems as was necessary for an integrated solution to the measurement of radioactive solutions in microfluidics, we include below the steps we took to develop both the PSAPD as well as the Cerenkov imaging system.

B. Goals and Accomplishments

The goals of this project were as follows:

1. Development of detector electronics.
2. PSAPD feedback voltage control.
3. CCD image integration and software interface.

During the project period these aims were successfully accomplished. A detailed description of accomplishments for each sub-project is listed below.

Development of detector electronics

The original electronics for the PSAPD based detector system were based on charge sensitive preamplifiers paired with conventional Nuclear Instrumentation Modules (NIM). These amplifiers are typically required when solid state detectors with low signal gain are used. The benefit of such charge sensitive amplifiers is the low noise characteristics, which is very important when the raw signal is composed of only a few electrons that need to be detected across a macroscopic device with non-zero capacitance. The use of these amplifiers though severely restricts the bandwidth of the detection system, and results the introduction of potentially harmful detector ***dead time*** inserted after the identification of each valid event. As the event rate increases, this dead time has two effects. First, it generates loss of signal (that is new events that fall in the system dead time are lost and not recorded), and secondly, it also generates signal pile-up, where subsequent events are distorted and thereby create artifacts in the acquired image. Both these effects are deleterious, and can result images that are not quantitatively accurate, and do not show an accurate representation of the activity in the imaged distribution. Therefore our first task was to investigate the use of different technology, such as transimpedance amplifiers, such that we could improve the system count rate. This work was developed and successfully presented by Alex Dooraghi, one of the START program trainees at the IEEE conference in Valencia Spain, in 2011.

PSAPD feedback voltage control

While some of the original work on temperature dependence of the PSAPD was done with the conventional bulky NIM electronics, the optimization of the readout electronics through the development of the new transimpedance amplifiers opened up new paths for the system integration. To this end, we developed a Printed Circuit Board (PCB) version of these optimized electronics, such that the PSAPD could be used without the very bulky NIM crate and the associated power supplies. The result was that the entire PSAPD imaging system, including its readout electronics and temperature control could now be encapsulated in a small box, measuring 8x7x4”. This box, instead of a tangle of coaxial and power cables, has now a USB readout connection and can be operated with a standard laptop, anywhere inside a biology lab. This technical advance has enabled the duplication of the betaBOX system, and its use in temperature controlled environments, with a backup system. The calibration protocol and Standard Operating Procedures that we developed for its use has proven to be robust, and does not require a tight regulation of the PSAPD temperature. The two prototype betaBOX systems we created have been in use quite successfully over the past few years.

CCD image integration and software interface

The system integration described above, allowed us to also integrate a USB based CCD camera, with a close focus zoom lens for (a) real time monitoring of the flow of solutions on the chip (b) providing linearity corrections and (c) for co-registration with the radionuclide image. This work had also similarities with the work on the integration of a slow scan scientific CCD camera, with a real time video camera, for the Cerenkov imaging system that is described below. The hardware and software for the integration of the visual image, with the radioactivity image was developed in Matlab (MathWorks, Natick, Massachusetts, US). This software runs on the laptop that is used for the acquisition of the radioactivity data, and allows the control of acquisition starting and stopping, definitions of time frames, streaming of listmode data, generation of radioactivity images, corrections for spatial distortions and isotope decay, and Region Of Interest analysis of static and kinetic data.

Cerenkov imaging system calibration

The signal level in Cerenkov images is dependent on the number of optical photons reaching the slow scan scientific CCD camera, that are generated from the Cerenkov effect inside the microfluidic synthesizer chips. This light is of course dependent on the transmission of these optical photons through the optical chain, which can be relatively easily calibrated. Besides the optical chain though, the generation of these photons is not entirely straight forward. The level of Cerenkov light depends on the energy of the initial charged particles, the index of refraction of the microfluidic chip and the solution, the density of the chip and the solvent, as well as the geometry of the microfluidic chip and the droplet solution. This rather complicated phenomenon required a detailed investigation of the signal chain, starting from the generation of Cerenkov light, all the way to its detection in the CCD. To achieve this, Monte Carlo simulations were generated starting with first principles, to evaluate the level of Cerenkov light generated at different wavelengths, for different geometries of solvent solutions, solvent materials and synthesizer chips. Given all these parameters, an absolute calibration would be elusive, as the solvent density, geometry and index of refraction changes over time, due to the cycles of solvent evaporation and heating during synthesis. Despite that, the Monte Carlo simulations demonstrated that for the typical EWOD microfluidic chip geometry, the overwhelming majority of Cerenkov light is produced in the glass substrate and the glass coverslip, instead of the solvent geometry. That is due to the very close geometry of the cover slip with the supporting glass, which generates solvent droplets that are very flat in shape and have large diameters and shallow heights. Therefore, to a first approximation, the geometry, density and index of refraction of the solvent are not significant factors in the signal intensity, allowing a single calibration to provide the correlation between the image value and the activity in the microfluidic chip. As a result, this method was proven useful to identify losses of activity and led to optimization of the radioactivity decay corrected yield in synthesis experiments.

Cerenkov imaging system integration

Although the Cerenkov image provided a relatively fast feedback of the status of the reaction, on the order of 5-30 seconds, the slow scan nature of the scientific grade CCD used for the detection of the weak Cerenkov signal, did not allow for a real time imaging. This was achieved with the introduction of a real time color video camera that could be used to monitor the flow of solvents in the microfluidic chip. A front surface mirror was used to select which of the two imaging systems was used, and software image registration was developed in Matlab, in a similar fashion as for the betaBOX system above. The additional significant advantage of this approach was that now, lead shielding could be introduced in the direct line of sight between the CCD sensor and the radioactive solution, greatly reducing the number of direct hits to the CCD from interactions of annihilation photons with the Si material of the photodiodes. This way, the background to the CCD was greatly reduced and the quantitative accuracy of the measurement was improved, enabling shorter acquisition times in Cerenkov mode. Furthermore, this method also provided information regarding the transparency of the solvent through the synthesis, changes of which could have significant effect to the quantitative accuracy of the Cerenkov images.

C. Papers and other products delivered

Papers

1. NT Vu, ZT Yu, B Comin-Anduix, JN Søndergaard, C Chang, A Ribas, HR Tseng, **AF Chatziioannou**, "A microfluidic beta camera for real-time radioassay imaging of glycolysis in small cell populations", Journal of Nuclear Medicine, 52:815–821, 2011. ***PMC3270819.***

2. PY Keng, S Chen, H Ding, S Sadeghi, GJ Shah, A Dooraghi, ME Phelps, N Satyamurthy, **A Chatziioannou**, CJ Kim, RM van Dam, "Micro-chemical synthesis of molecular probes on an electronic microfluidic device", PNAS, 109: 609-695, 2012. ***PMC3271918***.

3. J Wang, K Hwang, D Brass, A Dooraghi, D Nathanson, D Campbell, J Gu , T Sandberg1, P Mischel, C Radu, **AF Chatziioannou**, ME Phelps, H Christofk, JR Heath, "Fast Metabolic Response to Drug Intervention through Analysis on a Miniaturized, Highly Integrated Molecular Imaging System", doi:10.2967/jnumed.112.118497. JNM, 2013. ***PMID: 23978446***

4. AA Dooraghi, NT Vu, RW Silverman, R Farrell, KS Shah, and **AF Chatziioannou**, "Betabox: a beta particle imaging system based on a position sensitive avalanche photodiode", Physics in Medicine and Biology, 58: 3739-3753, 2013. ***PMC3706465***

5. AA Dooraghi, PY Keng, S Chen, MR Javed, CJ Kim, **AF Chatziioannou** and RM van Dam, "Optimization of microfluidic PET tracer synthesis with Cerenkov imaging", Analyst, 138 (19): 5654 – 5664, 2013. ***PMID: 23928799***

Conference Presentations

1. AA Dooraghi, RW Silverman, DL Prout, R Tashereau, NT Vu, AF Chatziioannou, "Evaluation of transimpedance amplifiers for readout of a position sensitive avalanche photodiode" IEEE Nuclear Sciences Symposium and Medical Imaging Conference, 2011 Valencia, Spain.
2. S Chen, AA Dooraghi, M Lazari, RM van Dam, AF Chatziioannou, CJ Kim "On-Chip Product Purification For Complete Microfluidic Radiotracer Synthesis", Micro Electro Mechanical Systems (MEMS), 2014 IEEE 27th International Conference.

PhD Theses:

1. Dooraghi, Alex A. Applications of Beta Particle Detection for Synthesis and Usage of Radiotracers Developed for Positron Emission Tomography. PhD Thesis, University of California Los Angeles, 2014.

Cerenkov and Betabox:

1. First-generation Cerenkov imaging system for quantitatively monitoring microfluidic chips
2. Second-generation Cerenkov imaging system for quantitatively monitoring microfluidic chips. Incorporates mirror to shield CCD from direct gamma rays.

III. Project 3: Tracer Kinetic Assays on a Chip

A. Executive Summary

The development of the betabox, a combination of a miniature cell culture environment with exquisite control of the culture parameters, (such as input/output solutions over time, numbers of cells being cultured etc), and an imaging sensor with unprecedented sensitivity and low noise, created a new platform with unique capabilities. This platform, can be used for the evaluation of the uptake of various radiolabeled probes on cells or other biologically active reagent solutions (eg protein binding) over time as well as the effects that perturbations can have on these cells and the uptake of the probes. Due to the nature of the measurement mechanism with counting of individual radiation interaction events, the temporal resolution of the betabox is very high, as it can measure changes in sub second time intervals. Therefore this system has the potential to provide answers that are not easy to obtain with other means, and certainly not with small populations of cells. One limitation of the system is its spatial resolution, which does not allow the distinction of individual cells in the radioactivity image. The reasons for this are multiple, with most important being the distance (10-100 μicrons) separating the cell culture chip from the PSAPD detector, in combination with the range of the charged particles (positrons in this case). Nevertheless, the goals of this project have been to develop methodologies that will provide accurate parameters of kinetic models with sufficient confidence, capitalizing on the system's temporal resolution and sensitivity. To achieve that, given the limited spatial resolution of the

device, each cell culture chamber needs to be occupied by a known number of cells that have the same phenotype. Solutions with a radioactive probe are then inserted in the chamber, and the retention of these probes in the biologically active cell culture needs to be quantified on a per cell basis, once the cells are counted in a microscope. To achieve adequate contrast, that is a separation of the input function of radioactivity in the solution, from the activity retained in the cells, requires that the signal in the solution can be subtracted at any given time from the activity in the entire cell culture chamber. The fractional volume of the cells in the culture chamber is a very small fraction of the entire chamber volume (<5%), and as a result noise in the measurements of the solution under most scenarios overwhelms the measurement of activity in the cells. A series of methods were developed for switching the input function, with cycling the cell culture media between solutions with and without radiolabeled probe. These methods were developed as a part of an algorithm that was used for establishing the kinetic parameters and are described below.

B. Goals and Accomplishments

During the project period the primary aim was to optimize the time course of the tracer exposure and the measurement cycle, using a simulated annealing algorithm that has been validated before for optimizing tracer delivery schedule for estimating model parameters in human and animal PET studies. The optimization criterion was the calculated variability of the estimated model parameters. The practical limitation of preferring smaller numbers of cycles and/or requiring each interval period to have a minimal length were incorporated as penalty functions in the optimization criterion. The sensitivity of the optimized strategy to the transport and the biochemical rate constants was evaluated. We aimed to select a time course strategy that was applicable to various cases with a wide range of parameter values, so it could be used for many different types of tracers and biological conditions.

Algorithm optimization

Using a 12-chamber PDMS microfluidic chip coupled to a PSAPD detector, we showed that FDG transport and phosphorylation constants in CaP8 cells (a prostate cancer cell) can be estimated using a switching strategy that consisted of alternating tracer-incubation (TI) and background removal (BR) periods. Furthermore, the switching schedule could be optimized to increase the reliability of the estimates without increasing the FDG concentration in the incubation medium. The optimization schedule (OS) was shown with computer simulations to give superior performance compared to constant infusion (CI) or equal-cycle switching (ES) strategy. Subsequently, we performed a set of FDG kinetic experiments on the 12-chamber PDMS microfluidic-PSAPD system, using CaP8 cells and with three different tracer infusion strategies (OS, ES, CI) on 3 consecutive days. Multiple measurements under the same condition were obtained from different micro-chambers in the microfluidic chip. We estimated the transport and phosphorylation rate constants of FDG in CaP8 cells by model fitting and calculated the variability of the rate constants under the same conditions.

For the case of high glucose level (200mg/dL) in the medium, the CV% of the estimated Ki was, 29%, 28% and 20% for CI, ESS and OSS, respectively. A larger decrease of the variability of the estimates of K1, k2 and k3 were also found for OSS as compared to those of CI, but the amount of improvement in the CV % of the estimates seemed to be dependent on the glucose level in the medium (largest at highest glucose level). Overall, the amount of improvement due to OS that was shown with the real experimental data was not as large as predicted by computer simulation studies. The most likely explanation for this finding is that experimental conditions deviate from the ideal situation.

C. Papers and other products delivered

Papers:

1. C Fang, Y Wang, NT Vu, W Lin, Y Hsieh, L Rubbi, ME Phelps, M Muschen, Y Kim, AF Chatziioannou, HR Tseng, TG Graeber, "A kinase activity radio assay for minute patient cancer samples using an integrated microfluidic and solid- state beta camera platform", DOI: 10.1158/0008-5472.CAN-10-0851, Cancer Research, 70 (21):8299-8308, 2010.

Conference Presentations:

1. W. Sha, Zeta T.F. Yu, Nam T. Vu, Michael E. Phelps, Arion Chatziioannou, Hsian-Rong Tseng and Henry S.C. Huang. New kinetic strategy for extracting FDG transport and uptake information in microfluidic multi-chamber cell culture chip coupled with PSAPD. J Nucl Med. 2009; 50 (Supplement 2):474

2. W. Sha, Z. Yu, N. Vu, A.F. Chatziioannou, H-R. Tseng, M.E. Phelps, S-C. Huang. Optimal design of a new kinetic strategy for extracting FDG transport and uptake information in microfluidic multi-chamber cell culture chip coupled with PSAPD camera. 2009 IEEE Nuclear Science Symposium Conference Record.

3. Sha, W., Yu, Z., Vu, N., Chatziioannou, AF, Tseng, H-R, Phelps, ME, Huang, S-C, New strategy for extracting FDG transport and uptake information in microfluidic multi-well cell culture chip coupled with PSAPD, 56th Annual Meeting of the Society of Nucl. Medicine, Toronto, Canada, June 13-17, 2009, J. Nucl. Med. 50(supp2):124P, 2009

4. Sha, W., Z. Yu, N. Vu, A.F. Chatziioannou, H-R. Tseng, M.E. Phelps, S.-C. Huang, Measurement of FDG transport and uptake rate constants in cell culture: assay with a microfluidic cell culture array coupled with a PSAPD camera, 2009 World Molecular Imaging Congress, Montreal, Canada, September 23-26, 2009.

5. Sha, W., Z. Yu, N. Vu, A.F. Chatziioannou, H-R. Tseng, M.E. Phelps, S.-C. Huang, Optimal design of a new kinetic strategy for extracting FDG transport and

phosphorylation information in a microfluidic multi-well cell culture chip coupled with PSAPD camera, IEEE MIC Symposium, Orlando, FL, Oct. 28-Nov 1 , 2009

IV. Training

A. Executive Summary

This program has trained graduate students and postdoctoral scholars at the interface of radiochemistry and clinical imaging to prepare them for careers in academia and the clinic in the areas of (1) PET probe design and development, (2) Synthesis automation and optimization, (3) preclinical and clinical imaging, and (4) instrumentation and technologies for radiochemistry. Particular emphasis was placed on the three step process of research, development and evaluation related to translation of novel molecular imaging probes. As an additional training component, the Scholars Trained in Advanced Radiochemistry Technologies (START) program was designed to transition talented chemists, engineers and physicists into fields of radiochemistry and molecular imaging. A major goal of the program was to address the growing shortage of young people trained in radiochemistry and nuclear medicine technologies in the United States. Two high caliber START fellows were admitted to the program. Shilin Cheung was admitted into the laboratory of Dr. Michael van Dam and Maxim Sergeev joined the laboratory of Dr. Pei Keng.

Training consisted of a combination of coursework as well as cutting-edge hands-on research in molecular imaging in the state-of-the-art facilities of the Crump Institute for Molecular Imaging at UCLA and in the Ahmanson Translation Imaging Division. Examples of research projects include:

- Microfluidic-based synthesizers
- Microwave synthesis systems
- New synthetic methodologies
- Radiochemistry automation
- Principles of molecular imaging
- Modeling tracer kinetics
- Molecular imaging probe design for assays
- Probe synthesis and optimization

Coursework included seminars by leading scientists and several hands-on laboratory sessions to prepare the trainees for their research projects.

B. Goals and Accomplishments

As presented in the original proposal, our goal was to closely integrate the training program with the research performed at the participating faculty labs. In that way, while these trainees pursue their doctoral or post-doctoral education, they are directly applying their acquired skills to the

research projects described in the proposal. At the same time, these students and post-docs closely collaborate with other members of the Crump Institute, as well as other researchers at the UCLA Department of Molecular and Medical Pharmacology, the Jonsson Comprehensive Cancer Center and the Institute for Molecular Medicine. This open environment facilitates knowledge transfer, broadens the trainee's education to include related areas of biology and imaging, and educates other members of our overall UCLA program about radiochemistry and related instrumentation. The goals of the training program were to introduce individuals to the field of PET imaging with special emphasis on PET radiochemistry, PET probe development and technologies for PET probe synthesis. An introductory START course was designed for interested students from the Crump Institute for Molecular Imaging and the Department of Molecular and Medical Pharmacology as well as for the START postdoctoral fellows. The curriculum put together by faculty from the Crump Institute and the Ahmanson Translational Imaging Division within the department of Molecular and Medical Pharmacology provided graduate trainees and recent Ph.D. graduates hands-on experience, coursework, and lectures to provide a basic understanding of radiochemistry.

In addition to the primary research projects described in the original proposal and in this progress report, some trainees worked partly on additional exploratory projects at the cutting edge of the fields of radiochemistry and synthesis technology. These projects provided similar opportunities to receive hands on training in radiochemistry, analytical tools, synthesis technologies, etc.

- **Enzymatic fluorination**. The fluorinase enzyme, isolated from a species of bacteria, performs fluorination (and radiofluorination) reactions under conditions that are very mild compared to typical organic synthesis methods typically used. Such conditions reduce the stringency of requirements for automated equipment to perform synthesis, and also could enable the direct labeling of more labile biological molecules that typically must be labeled via preparation of a radiolabeled 'prosthetic group' and subsequent coupling of this group to the biomolecule under mild conditions. This project explored the breadth of the substrate scope of the fluorinase enzyme, and also techniques for solid-phase enzymatic fluorination as a means to recycle the enzyme (to ensure low overall synthesis cost). Two publications resulted from this.
- **Electrochemical fluorination**. This project focused on developing a new radiosynthesis platform to enable [F-18]fluoride anion to react with neutral molecules, or even molecules with partial negative charge. Electrochemical synthesis methods can enable efficient nucleophilic fluorination, by lowering the reaction activation energy, of molecules that are difficult to label by other approaches. This project explored the ability to electrochemically radiofluorinate aromatic rings and involved the development of a system for automated electrochemical fluorination. Two publications resulted from this work.
- **Capillary electrophoretic separation of PET tracers**. In this project, the feasibility of using capillary electrophoresis (CE) for separation and quantitation of PET tracers and impurities for purposes of quality control testing was explored. Compared to the use of high-performance liquid chromatography (HPLC) and gas chromatography (GC), which use expensive and bulky equipment, CE has the potential to be miniaturized into a low-cost microfluidic chip. This could enable the development of a compact platform for testing the safety of PET tracers – a critical technology to accompany the development of platforms for routine, benchtop production of PET tracers on demand. One publication

has been published showing the potential of this technique for analysis of [F-18]FLT and [F-18]FAC samples and another publication is expected to be submitted shortly.

C. Outcome of training program

A course syllabus and lecture materials were developed over several years. Trainees benefited from early versions of several lectures in the 2011-2012 academic year and the finalized course in its entirety was offered in the 2012-2013 academic year. It will be offered again in the the 2014-2015 academic year and we are planning to offer it on a continuing basis depending on interest of students and postdocs in the Crump Institute. (In the 2013-2014 academic year there was not a sufficient class size and the course was not offered.) The course was made available to the START fellows, all graduate student and postdoc trainees funded on this DOE grant and those interested in the lecture topics. Lectures were taught by Drs. David Stout, Pei Yuin Keng, Michael van Dam, Michael Phelps, Caius Radu, Johannes Czernin, Martin Allen-Auerbach, Gaurav Shah, and Jorge Barrio. The lecture materials are available online (http://www.crump.ucla.edu/start/training.aspx) to the public as a resource that could help further expand the radiochemistry workforce.

The coursework included the following topics with lectures from experts in each field:

(1) Radiation safety and cyclotron
(2) Fundamental of radiochemistry
(3) Fluorination strategies
(4) Biology of PET imaging
(5) PET tracers design
(6) Automated radiosynthesizer
(7) Instrumentation, quality control
(8) Microfluidic technologies for PET radiochemistry
(9) FDA regulation for radiopharmaceuticals
(10) PET in the clinic

Essential skills developed as part of the training program through lectures and research in participating faculty labs included:

1. Radiochemistry Lab Safety and Lab Management
   - Handling of radioisotopes, exposure limits, minimizing exposure
   - Radiochemistry lab protocols and procedures
   - Chemical safety, fume hoods, reagent/solvent storage and handling
   - Regulatory requirements for radiation workers and radiochemistry facilities
2. Overview of PET
   - Research and clinical applications of PET
   - Physics of positron-decay and annihilation, radiation detectors, image reconstruction, PET scanner
   - Biological mechanism of tracers, tracer design
   - PET isotopes, half-life, benefits and challenges of short half-life isotopes
   - Workflow of PET probe production and imaging
3. Fundamentals of PET radiochemistry

- Radioisotope production
- Synthesis with F-18, C-11, and other radioisotopes
- Fundamentals of chemical reactions, chemistry at trace concentrations
- Common practices using several 18F-labeled tracers as case studies
- Techniques for synthesis development and optimization
- Standard purification techniques, formulation, QC procedures to meet FDA requirements for human use

4. Tracer design and development
- Tracer biology, tracer kinetics, quantitative PET
- Constraints in new tracer development imposed by chemistry (e.g. location of radioisotope, number of synthesis steps, can it be synthesized?) as well as biology (e.g. specificity, metabolism of tracers / in vivo stability, etc.)

5. Fundamentals of radio-analytical chemistry
- Making measurements, calibration, noise, sources of error
- Common radiochemistry analytical instruments (radio-TLC, HPLC, activity measurement, GC-MS) – theory and practice
- Additional radiation detection techniques useful for instrumentation development (Cerenkov imaging, beta-box, autoradiography, microPET, …)

6. Instrumentation and technology in radiosynthesis
- Automation and systems engineering
- Basic familiarity with electronics and electronic instruments
- Macroscopic, microwave, microfluidic radiosynthesizers
- Techniques for characterization and validation of fluidic systems
- Data acquisition fundamentals
- Introduction to microfluidics and chemistry on small scales
- Microfluidic chip design and fabrication

7. Essential skills for scientists and future PIs
- Hypothesis-driven science, critical thinking, experiment design, problem-solving and troubleshooting
- Identifying funding opportunities, grant writing
- Preparing scientific presentations and manuscripts
- Mentoring, recruiting, effective collaboration

Graduate students trained:

- Xiaoxiao Ma (granted PhD degree during program)
- Jimmy Ly
- Mark Lazari
- Henry Herman (granted Master's degree during program)
- Anqi Yang
- Wei Sha (granted PhD degree during program)
- Alex Dooraghi (granted PhD degree during program)

- Zheng Gu
- Philip Chao
- Yanisley Valenciaga

Postdoctoral scholars trained:

- Saman Sadeghi (Transitioned to a faculty position after program; pursued radiochemistry-related research)
- Pei Yuin Keng (Transitioned to a faculty position after program; pursued radiochemistry-related research)
- Wei-Yu Tseng (Transitioned to Senior Engineer position in industry after program)
- Gaurav Shah (Transitioned to Director-level position in startup company after program; developing radiochemistry technologies)
- Shilin Cheung- START fellow
- Qinggang He
- Maxim Sergeev- START fellow

In addition, several undergraduate students participated in research projects.