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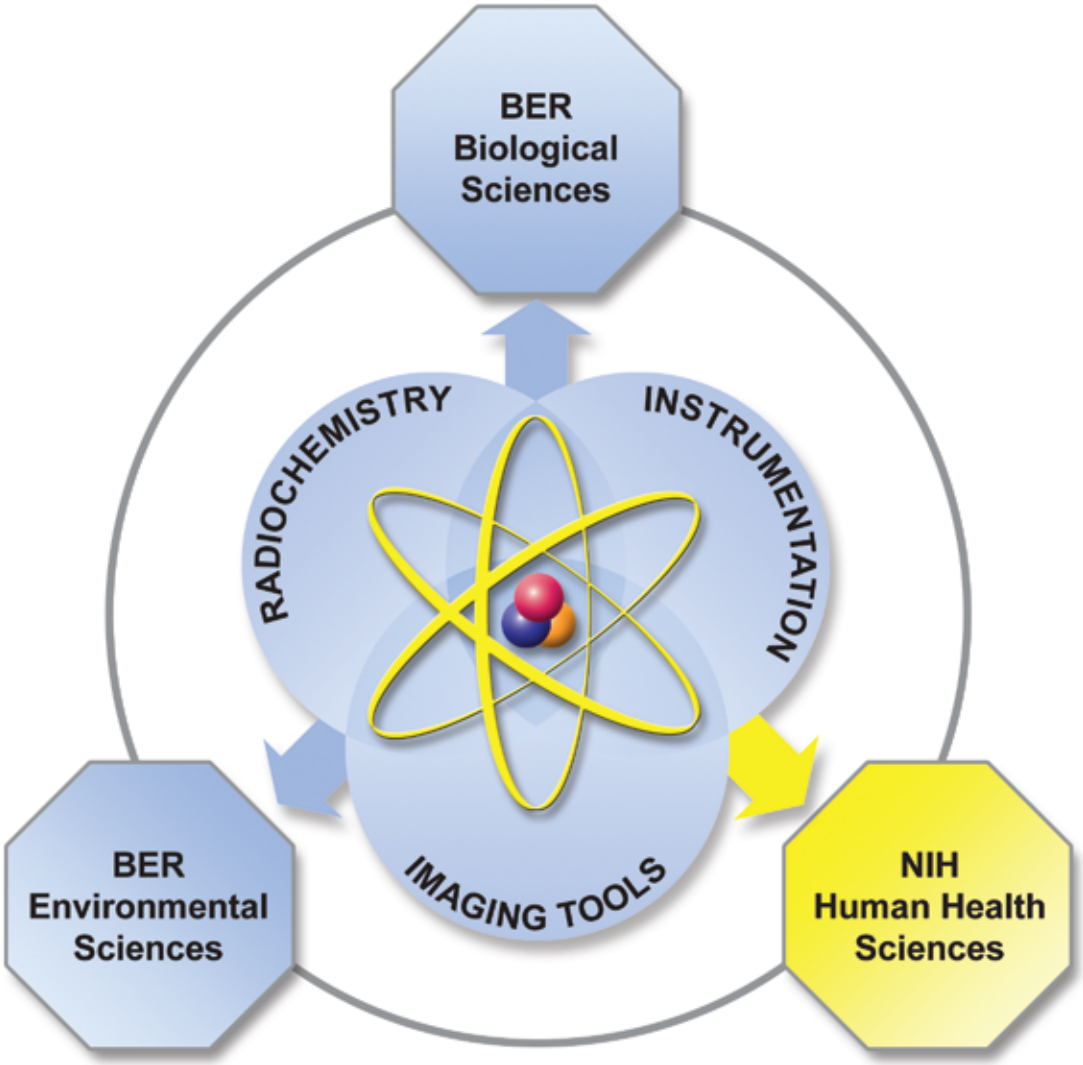
New Frontiers of Science in Radiochemistry and Instrumentation for Radionuclide Imaging

Creating the Tools for Research Advances in Biology, Environmental Sciences, and Nuclear Medicine

Report from the November 4–5, 2008 Workshop



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This report is based on a workshop designed under the leadership of Dr. Prem Srivastava and was developed from the interaction of participants listed in Appendix B. They were divided into working groups whose leadership authored the contents. Authors are:

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Preface

For over six decades, the Department of Energy's (DOE) Office of Biological and Environmental Research (BER) and its predecessor programs have supported research in the application of radionuclides for transformational new technologies for imaging. In 1961, Melvin Calvin, a Berkeley chemist, received the Nobel Prize for his elucidation of photosynthesis, a process used by plants to convert carbon dioxide, water, and sunlight into metabolic energy. This landmark advance depended on radiotracer studies of metabolic pathways. Concurrent development of the imaging technologies (such as SPECT and PET) and compatible new radiotracers made it possible to obtain images of metabolic processes in living organisms and to measure substrate utilization as unique indicators of metabolic function in real time. These landmark achievements facilitated the application of the tracer principle to research in biology and medicine and laid the foundation of modern nuclear medicine.

New techniques in radiochemistry and radionuclide imaging will result in major advances in our understanding of developmental biology. Fundamental technological advances growing out of this program will be transferable to broader research applications in the biological and environmental sciences as well as in nuclear medicine by public and private sectors.

For this workshop, BER brought together experts from nuclear medicine, biology, and the environmental sciences to discuss new paradigms for its Radiochemistry and Radionuclide Imaging Instrumentation program. The workshop explored how BER could best support fundamental research that would both advance DOE's missions in biology and the environmental sciences and be useful for medical applications pursued by other agencies and industry. This workshop represented the first step in exploring the potential of radiotracer imaging to solve biological problems in energy and environmentally-responsive contexts.

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Executive Summary

As research on optimization of plants and microbes for the production of biofuels progresses and programs are developed to engineer decontamination of the environment, there is an increasing need to map biological processes on different size and time scales—from local cellular trafficking to global biochemical pathways, and from minutes to days and weeks. Although optical imaging and autoradiography are the imaging tools most commonly used in biological applications on plants and microbes, they generally can be applied only to small objects and have limited depth of field. Fortunately, the penetrating nature of gamma rays makes radionuclide imaging methods currently used in human and small-animal imaging ideal for the wider application to plant, microbial, and environmental research. As the biofuels and bioremediation research areas progress, researchers will need to image increasingly larger model systems, and radionuclide techniques will become increasingly important. Therefore, it is crucial to make available, and where necessary, to modify radiotracer imaging devices and radiotracer probes and techniques so that they can be truly effective in biofuel production optimization and environmental research.

In the broader context of biological research, radionuclide imaging continues to stand out as a singular tool for studying living organisms in a manner that is highly quantitative, three dimensional, temporally dynamic, and non-perturbative of the natural biochemical processes under study. Radionuclide imaging methods have a well-known value for medical science and nuclear medicine applications, but their use in studying microbial and plant metabolism and for tracing dynamic processes in the environment is underdeveloped and provides a myriad of new opportunities. Some examples of these opportunities for radiotracer applications include tracking nutrient and photosynthetic metabolite flux in plants to improve biomass production; monitoring the partitioning of carbon intermediates in microbial systems to enhance production of end products of value such as biofuels (e.g., terpenes and lipids); examining large volumes of air, soil, or water to monitor and predict transport of contaminants including radioactive waste; optimizing genetically modified plants to enable economical fuel extraction; and monitoring the effects of temperature, moisture, nutrients, and metals on plant and microbial communities. Capitalizing on these opportunities, however, will require efforts in a number of related areas:

- Improvements in radiosynthetic chemical methods that will expand the range of radiotracers available will make them easier to prepare through automation and kit-like formulations, and thus will make them more available to a wider community of researchers. These advances will need to be based on a more sound understanding of the fundamentals of how chemistry operates at the tracer-scale level so that synthetic methods can be made more general and more reliable.
- New developments in radiotracer design will include new approaches to the labeling of macromolecules and nanoparticles at high specific activity. Other important developments include multimodality tracers, with appropriate considerations of imaging sensitivity and tolerable dose, and tracers for *in vivo* biological processes such as signal transduction, transcription, translation, cell division, and apoptosis.
- Improvements in the availability of radionuclides for radiotracer synthesis, such as new generator systems or compact and portable devices for the production of short-lived nuclides, will

allow radiotracers to be brought to the problem rather than having the problem brought to the production site.

- Tradeoffs among resolution, efficiency, imaging volume, and cost are different for plants than for patients, and these differences require improvements in spatial resolution by an order of magnitude or more, approaching the cell size in plants, as well as device geometry innovations to accommodate the imaging problems.
- New imaging devices will need to be designed that have dual-modality capability and are optimized to address problems of imaging resolution, imaging-object size, required sensitivity and time scale, and operation in a wide range of diverse and more field-like environments.

The advances noted above would begin to capture major opportunities for applying radiotracer imaging and analysis methods to wider problems in microbial, plant, and environmental systems, potentially reaping real benefits in enhanced production of biomass and biofuels, and improving efforts at bioremediation and monitoring of the environment.

Realization of these major opportunities for expanding radiotracer and radioimaging into new priority areas depends on the continued training of radiochemists and nuclear chemists. Also needed is the training of plant, microbial, and environmental scientists in the range of problems that can be effectively addressed by using radiotracers and associated data analysis. These training needs may be met by various mechanisms including graduate fellowships, sponsored visiting scientist programs, and postdoctoral grants.

Methods involving stimulated emissions at the microscopic level, such as fluorescence and secondary ion mass spectroscopy, are recognized as techniques with resolutions as much as four orders of magnitude finer than the *in vivo* radionuclide methods. The workshop participants demonstrated the power of these microscopic methods, but the focus of this report is on what can be achieved with radiotracers and contemporary imaging of nuclear emissions, where the spatial extent and depth of penetration are limitations for fluorescence and secondary ion mass spectroscopy imaging.

An additional benefit would be an active synergy between the developments in radiotracer imaging applied to microbial, plant, and environmental systems and further improvements in the technology for biomedical imaging with respect to radiochemistry, radiotracer development, isotope availability, and optimized imaging devices.

In closing, we note that biological end-users are not generally aware of what radionuclide imaging has to offer, and the PET and SPECT instrumentation developers and radiochemists are just beginning to learn about the needs of the plant, microbial, and environmental biology communities. While this workshop was an excellent start, the work of ensuring rapprochement between these two communities will require sustained dialogue focused on specific scientific or technical problems, with careful attention given to the selection of scientists who are best able to match the capabilities of radiotracer, chemical, instrumentation, and analytical methods with the many important problems in plant, microbial, and environmental biological sciences.

I. Introduction

Applications of radiotracer methods, nuclear chemistry, and radiation detection instrumentation to studies of the physiology and metabolism of microbes, plants, animals, and human beings have been underway for over 80 years. The application of radioactive isotopes to follow kinetic processes in living systems was pioneered by Georg de Hevesy in 1923 in his studies of the movement of lead-210 and lead-212 in plants.[2] The first uses of short-lived radiotracers (e.g., carbon-11 and nitrogen-13) dated from 1939 and 1940, respectively (see Figure 1).[2] The 20-minute carbon-11 radionuclide produced in a 37-inch cyclotron on the Berkeley campus informed researchers of the cyclic biochemistry associated with photosynthesis, and this was the beginning of knowledge about the path of carbon utilization in photosynthesis. Carbon-14 and sulfur-35 were introduced to assist in unraveling plant and algal biochemistry, including the use of 14-inch by 17 inch X-ray film to perform autoradiography on two-dimensional chromatograms—the first radiotracer imaging studies, albeit *in vitro*.

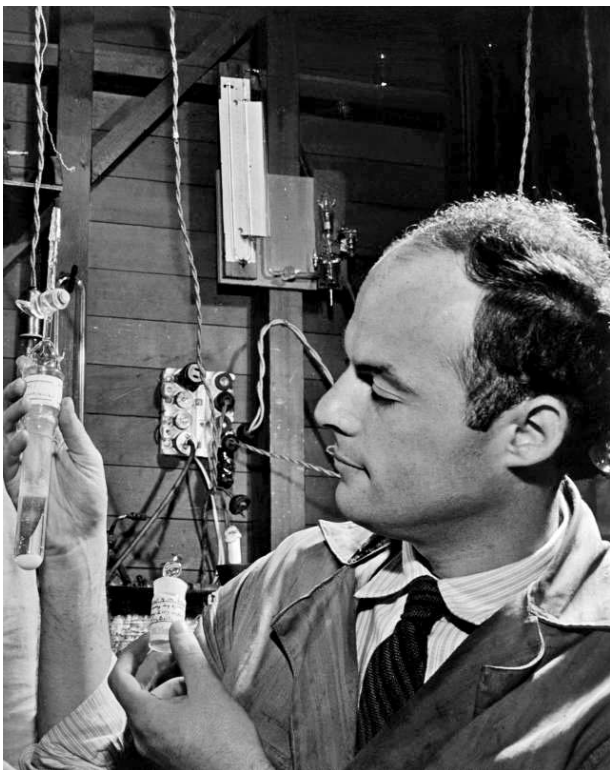

Figure 1. Sam Ruben with the world's supply of carbon-14 as sodium carbonate being used to trace plant metabolism in 1939.[1] Image Credit: Lawrence Berkeley National Laboratory.

Although X-ray imaging, which allowed internal anatomy to be viewed externally, revolutionized medical diagnosis, the use of radiotracers has revolutionized our understanding of the mechanisms and metabolic fluxes that underlie life processes themselves. The seminal discoveries of the 1940s were followed by vigorous progress in tracer methodology, namely, the development of new methods to produce a wide variety of radionuclides, the development of chemical methods to prepare from them an expanding array of specific radiotracers, and the development of new devices for detection and imaging of radiotracer distribution.

These advances opened up new opportunities to perform fundamental studies *in vitro* and for physiological studies and diagnosis of disease processes *in vivo*. In the most recent 25 years, the uses of radiotracers and modern imaging methods, including positron tomography and synchrotron radiation beams, have broadened to include the evaluation of effects of herbicides, heat, cold, light, moisture, pH, oxygen, carbon dioxide, nitrogen, and nutrients on plants including rice, barley, peas, sunflower, moonflower, and tobacco cells as well as bacteria (see Appendix A). The

[1]Benson, A. A., and Calvin, M. 1947. *Science* **105**: 648–649; Krohn, K., and Mathis, C. 1981. *Short-lived Radionuclides in Chemistry and Biology*, Advances in Chemistry Series, American Chemical Society **197**: 235–249.

[2]Hevesy, G. 1923. *Biochemical Journal* **17**: 439–445.

radioisotopes used have ranged from the long-lived carbon-14 to the short half-life positron emitters: carbon-11 (carbon dioxide), nitrogen-13 (gas, nitrate, and ammonia), oxygen-15, fluorine-18, iron-52, manganese-52, zinc-62, and cadmium-107. Far from being a mature field, the utility of radiotracers to study life processes, energy production, and the environment, broadly conceived, is still in a state of infancy. Many examples of problems yet to be explored by the deployment of metabolic and environmental tracers abound; these include studies of lignin synthesis and degradation, algal metabolism, resource allocation in plants, bacterial community dynamics, metabolism of environmental toxins, and the effects of increased concentrations of atmospheric gases, changes in temperature, light, soil concentrations of metals, and other environmental factors.

This workshop was convened to explore the potential applications of radiotracer techniques—now in widespread use as sophisticated methods and tools for medical science discoveries—to microbial, plant, and environmental sciences. New applications of these techniques are likely to enhance biofuel production, improve waste site bioremediation, boost agricultural production, monitor atmospheric ozone depletion, probe plant and microbial resistance to environmental stresses, monitor environmental pollutants, and probe plant-pathogen and plant-herbivore interactions.

The selection of and improvement in technologies and instrumentation for research, as we endeavor to do, is best done by first asking, "What is it that we want to measure?" Frequently, in fact, we do not have good answers to this question, because we have little knowledge of what can be or what should be measured. Thus, the workshop brought together plant, microbial, and environmental scientists who are searching for methods to answer challenging questions (e.g., How can we evolve plants with easily digestible lignin? How can we monitor perturbations in pathways of microbial metabolism that will lead to more efficient biofuel production? How can we follow the movement of radioactive waste in air, water, earth, and plant life?) with chemists, physicists, biochemists, and molecular biologists who can develop strategies and technologies to answer these questions.

It is useful to consider how diverse scientific queries, as exemplified by those above, can be related to the various alternate methods for imaging structure and function (shown in Table 1). Such correlations should be based on how the needs for sensitivity, specificity, speed, and resolution that are inherent in solving the problem can be best matched with the performance characteristics offered by the different imaging methods. The sensitivity of modern imaging methods relative to the range of object sizes is shown in Figure 2.

The emphasis of this study of the applications of radiotracers and detection instrumentation to plant, microbes, and the environment is on emission imaging from radioactive isotopes. The thickness and spatial extent of objects that these techniques can study are far greater than most optical and microscopic techniques; however, the microscopic methods involving stimulated emissions at the microscopic level, such as fluorescence and secondary ion mass spectroscopy, are recognized as methods that have resolutions as much as four orders of magnitude better than the *in vivo* methods using radioactive tracers. The workshop participants demonstrated the power of these microscopic methods, but the focus of this report is on what can be achieved with radiotracers and contemporary imaging of nuclear emissions.

Table 1. Imaging methods for structure and function approximately ordered by spatial resolution, one of several key performance characteristics

Lower Resolution
Positron Emission Tomography (PET)
Single Photon Emission Computed Tomography (SPECT)
Magnetic Resonance Imaging/Spectroscopy
Ultrasound and Photo/Thermo (optoacoustics)
Bioluminescence
Fluorescence Imaging
Light Microscopy (confocal, phase, Nomarski)
Fluorescence Microscopy (including two-photon excitation)
Synchrotron Light Source Spectroscopic Imaging
Autoradiography (emission tomography)
Secondary Ion Mass Spectroscopy
Quantum Dot and Nanoparticles
Förster Resonance Energy Transfer (FRET)
Stochastic Optical Reconstruction Microscopy (STORM)
Atomic Force Microscopy
Electron Microscopy
Higher Resolution

In considering what new things might be needed for the expanded use of radiotracers and imaging methodologies in plant, microbial, and environmental systems, it is worth examining how tracer and imaging methodologies, including analytical and statistical methods, are currently used in the service of medical science, as illustrated in Figure 3.

The radiotracer techniques used in medicine, however, represent only a small part of the imaging techniques available to plant, microbial, and environmental researchers, as shown by the list in Table 2. Thus, for radiotracer imaging applications in plants, microbes, and the environment, the time scales, sensitivity and specificity needs, size and shape of the objects to be measured, and the location where measurements are to be made will be different and very diverse. This will necessitate new considerations for enhancing the availability and use of tracer nuclides and for improving the design of tracer molecules and the performance of the imaging devices.

The participants in this workshop understood the need to define the challenges that derive from the many new types of problems to be addressed, as well as to recognize the broad opportunities for enhancing the research that come from greatly expanding the arena of radiotracer and imaging methods from medicine to plant, microbial, and environmental systems. As part of our efforts, we attempted to make some comparisons and give a realistic assessment of the strengths and weaknesses of current radiotracer and imaging methods relative to powerful optical methods (fluorescent techniques). Our goal was to emphasize where and how improved avail-

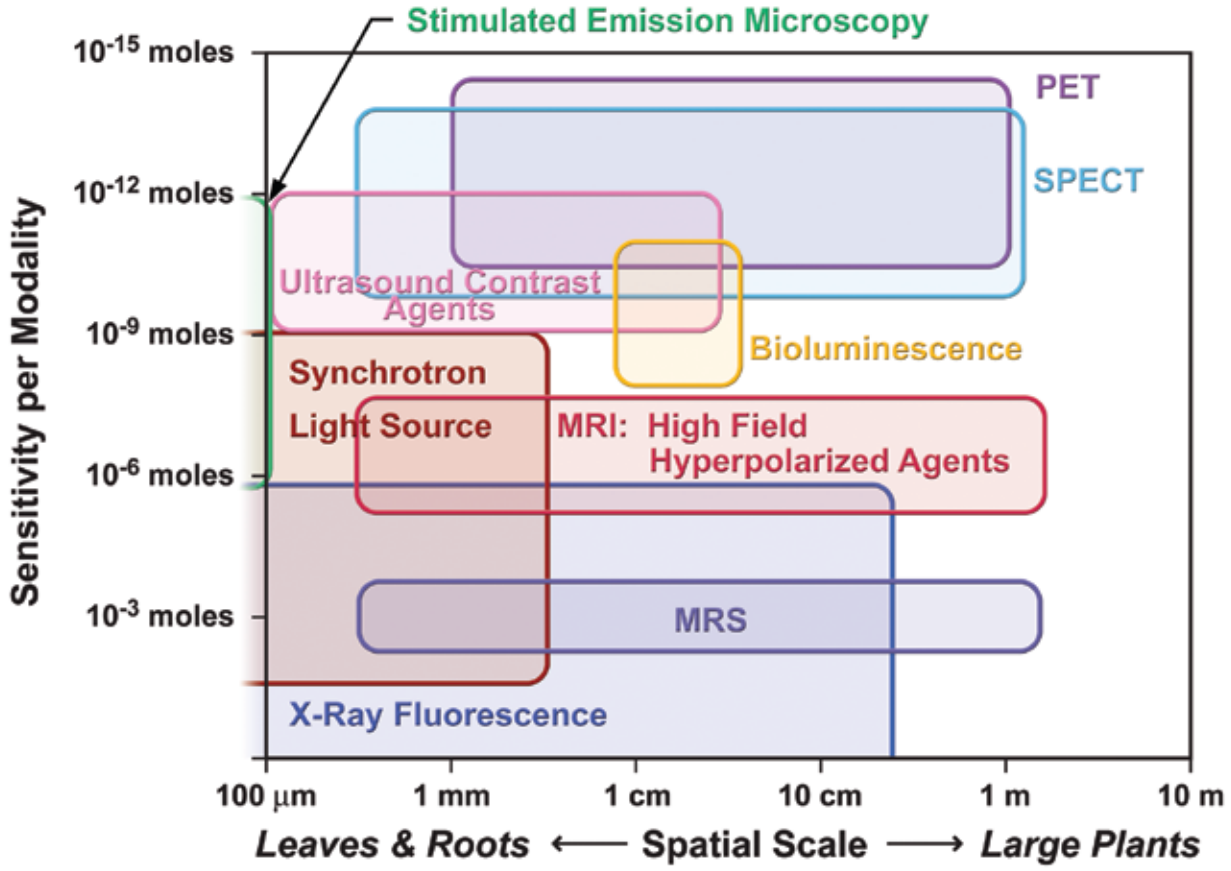

Figure 2. The sensitivity and image size range for the major imaging methods applicable to studies of plants and microbes.[3] Optical methods and stimulated emissions shown in green have resolution capabilities orders of magnitude better than techniques discussed in this report but lack depth penetration. The bioluminescence box refers to fluorescence from cells in animals after gene transfection (e.g., luciferase, green fluorescent protein), where depth limits resolution. For plant studies, the resolution capability is <1 mm.

ability of radionuclides, better methods for the chemical synthesis of labeled compounds, and the development of imaging techniques with better resolution, broader applicability, more field robustness, and other improvements can best serve the needs and solve important problems in plant and microbial biology and in environmental sciences. The next three sections of this report give summaries and a list of opportunities from reports produced during a two-day workshop:

- Challenges and Opportunities in Radiotracer Chemistry
- Challenges and Opportunities in Radionuclide and Hybrid Instrumentation Development
- Radioisotope Methodologies and Synergistic Technologies for Probing Plants, Microbes, and the Environment and Applications to Nuclear Medicine

[3]Modified from Meikle, S. R., et al. 2005. *Physics in Medicine and Biology* **50**: R45–R61; and Tsien, R. Y. 2003. *Nature Reviews Molecular Cell Biology* **4**(Suppl): SS16–21.

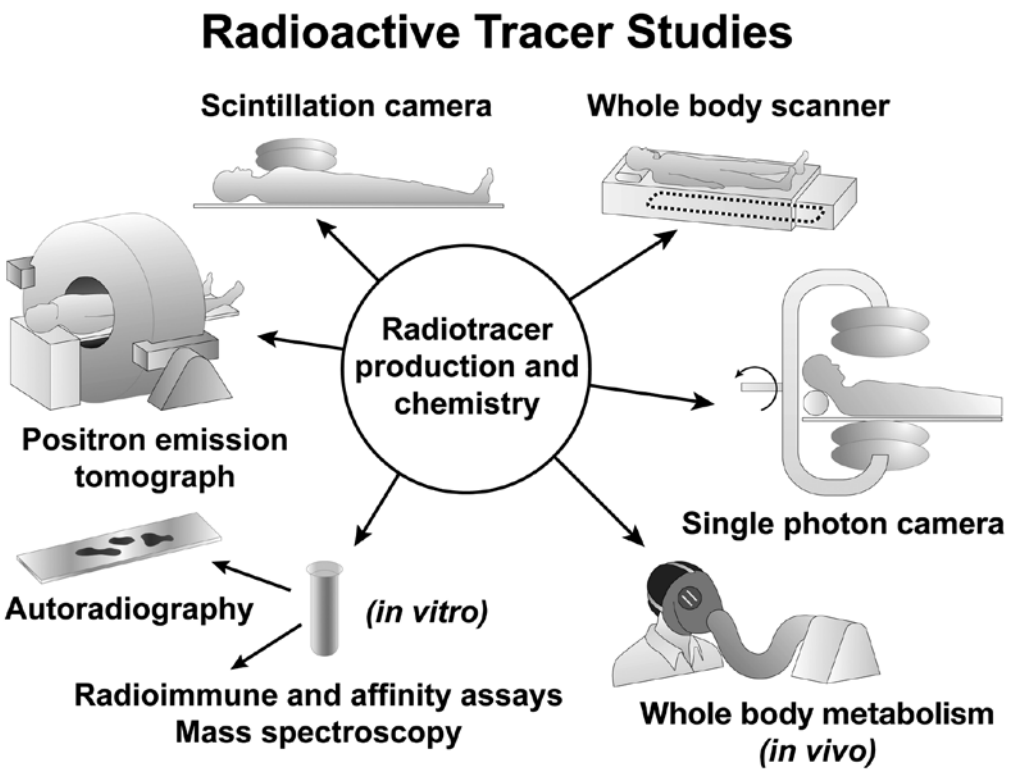

Figure 3. Multiple approaches to measurements using radiotracers in medicine.

Table 2. Information from different imaging techniques

Imaging techniques	Information available
Electron microscopy, X-ray, MRI	Anatomy (structure)
Fluorescent and radiotracer ligands, nanodots, bubbles	Detection of specific molecules
Spectroscopy: NMR, electron, X-ray, synchrotron, secondary ion mass spectroscopy	Chemical composition
Spectroscopy, FRET, tracer dynamics, fluid and organ motion	Metabolic/physiologic function

An executive summary, found at the beginning of this report, presents a succinct set of observations, opportunities, and conclusions. An appendix of 52 citations is given to represent important research contributions in the use of radiotracers and nuclear instrumentation in the study of plants and microbes but is not intended to be an exhaustive literature survey (Appendix A).

II. Challenges and Opportunities in Radiotracer Chemistry

Background

The enabling technology that underlies all forms of radiotracer imaging is radiotracer chemistry, because it is required for the development of radiotracer probe molecules (both small molecules and macromolecules). The diversity of applications of such radiotracer probes in imaging is illustrated in Figure 4.

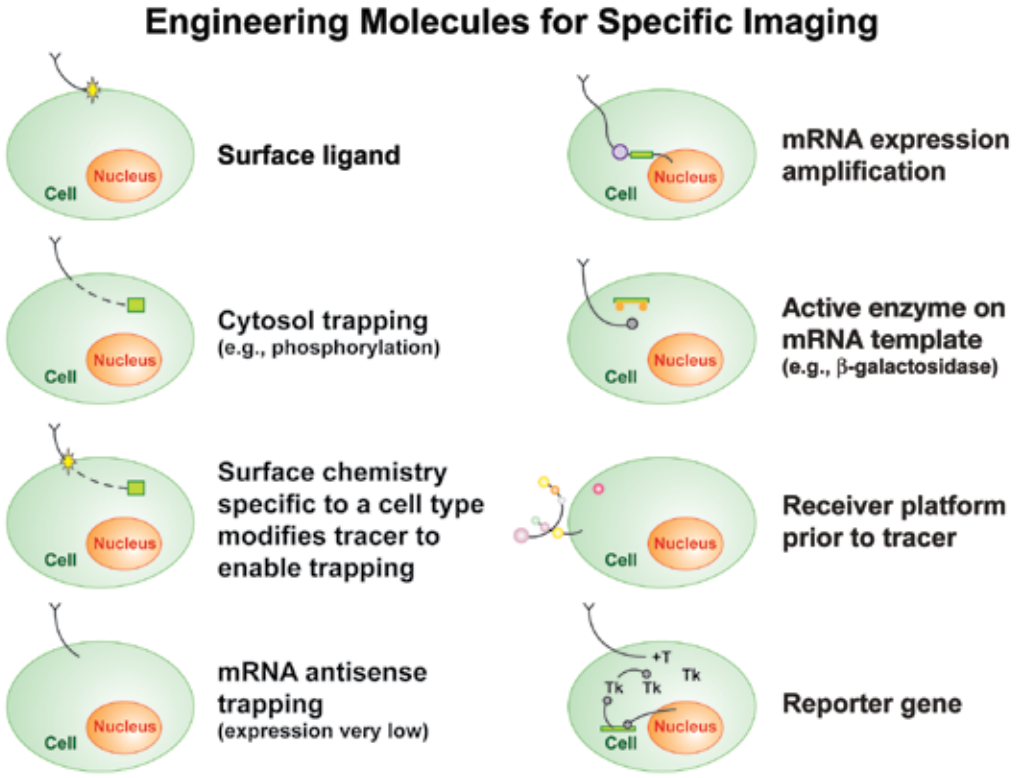

Figure 4. Eight mechanisms whereby radiosynthesized specific molecules facilitate measurements of physiological processes.

Radiotracer chemistry is more than a subdivision of synthetic chemistry and represents a unique discipline that demands special reactions, equipment, techniques, understanding, and training. Its distinctiveness stems from the following:

- large number of different elements whose radioisotopes are of interest (halogens, metals, carbon, and main group elements),
- unique behavior of chemical reactions conducted at the tracer-level scale, without carrier addition, to obtain products of very high specific activity,
- need for efficiency and speed associated with performing chemical transformations, isolations, and product purifications on materials labeled with short half-life radionuclides,

- knowledge of both inorganic and organic synthetic chemistry, and
- hazards of working with ionizing radiation.

Radionuclides: Decay, Modes of Imaging, Half Lives, and Availability

Radiotracers or radionuclides used in imaging studies can be divided into two groups, depending on how the detected photons (gamma rays) are generated (see Table 3). Members of the first group, the positron emitters, decay by emission of a positron, an antimatter particle that undergoes annihilation with a nearby electron, giving rise to the simultaneous emission of two gamma rays 180 degrees from each other. The coincident and transaxial nature of this emission enables imaging by positron emission tomography (PET). Positron emitters include radioisotopes of the common first-row elements—carbon, nitrogen, oxygen, and fluorine. Members of the second class of radionuclides, which include important elements, such as iodine, iron, and other metals, give rise to single photons of one predominant energy, and they can be imaged using single photon emission computed tomography (SPECT). Thus, the nature of the radiation from positron emitters vs. single photon emitters determines the nature of the instrumentation that is best suited for imaging. An advantage of PET is high sensitivity that allows spatial localization of very small amounts of a positron-emitting radiotracer. An advantage of SPECT is its ability to independently track two or more tracers simultaneously, provided their gamma emissions have distinct energies.

Table 3. Radioisotopes used as tracers in plant biology or of potential use for environmental studies or as tags on molecules (e.g., herbicides)

Positron emitters		Single photon emitters	
Carbon-11	20 min	Sodium-24	15 hours
Nitrogen-13	10 min	Potassium-43	22.3 hours
Oxygen-13	2 min	Chromium-51	26.6 days
Fluorine-18	110 min	Gallium-67	78 hours
Sodium-22	2.6 years	Technetium-99m*	6 hours
Manganese-52	5.7 hours	Iodine-123	13 hours
Iron-52	8.3 hours	Iodine-131	8 days
Zinc-62	9 hours	Iodine-125	60.1 days
Copper-62*	9.7 min	Thallium-201	72.5 hours
Copper-64	12.7 hours	*Tracers for Kinetics and Autoradiography*	
Gallium-68*	68 min		
Arsenic-74	17.8 days	Tritium	12.3 years
Rubidium-82*	1.2 min	Carbon-14	5730 years
Cadmium-107	6.5 hours	Phosphorus-32	14.3 days
Iodine-122*	3.6 min	Phosphorus-33	25 days
Iodine-124	4.3 days	Sulfur-35	86.7 days

*Generator Produced

The half life of a radionuclide has important implications for chemical syntheses, the physiologic and biochemical processes that can be studied, and the radiation dose to the plant, microbe, environment, or animal being studied. As exemplified in Table 3, half lives vary widely and are particularly short for some of the most important radionuclides (e.g., carbon-11, nitrogen-13, oxygen-15, and fluorine-18). For these isotopes, this means that the site of their generation needs to be near the site where they are to be used, and chemical synthesis methods must be fast.

Some useful radionuclides are not naturally found in living systems but have been generated because they are convenient for labeling compounds of importance to physiologic and other biotracer studies. The best-known radionuclide of this type is technetium-99m. It is particularly useful because it has a convenient half life (6 h) and a gamma emission energy in a good range (140 KeV), and it can be obtained by decay of a long-lived parent nuclide (molybdenum-99, $t_{1/2}$ = 66 h) from a generator system that can be shipped from the production site such as a reactor located many miles from the research site. This and other radioisotopes are listed in Table 3.

The maximum time duration of an experiment involving radionuclide imaging or detection is a function of the half life of the particular radionuclide (Table 3). Radionuclide-labeled substrates or tracers cannot probe time scales that are significantly longer than three to four half lives. For example, processes wherein $[^{15}O]O_2$ or $H_2{}^{15}O$ ($t_{1/2}$ = 2 min) are being traced cannot be studied for more than 8 minutes after injection, although methods of constant infusion allow oxygen metabolism and water perfusion to be followed for longer periods. Another example is the use of nitrogen-13 labeled compounds ($t_{1/2}$ = 10 min) wherein the movement of the label can be followed for as long as 40 minutes; in some applications where imaging statistics are not required, the radionuclide activity can be detected for more than one hour. Fluorine-18 has a half life of about two hours, and the fate of F-18 labeled sugars, peptides, and hormones can be followed for as long as six to eight hours.

Availability of Radionuclides

Radionuclides used as radiotracers, either as ions or as part of synthesized molecules, are obtained from three primary sources: neutron reactors, linear accelerators (particle and photons), and cyclotrons. Useful radionuclides can also be obtained from generators (noted above) that produce radioisotopes from the decay of long-lived precursors, themselves made by one of the three primary sources.

The technologies employed in the three primary radioisotope sources are generally old, and until recently, the motivation for innovations has been only to provide commercially available radionuclides for medical applications. Most researchers whose primary tools are radionuclides have acquired their own cyclotrons or have access to one. For example, seminal studies on plant metabolism in New Zealand and the United States used van de Graaf accelerators in physics facilities.[4] Nevertheless, the capital expense and annual facility support costs, including radiation protection, have placed severe practical limits on the use of the short-lived radionuclides, such as carbon-11 and nitrogen-13. Consequently, there is a need to improve accessibility of these short-lived nuclides by considering initiatives to develop table-top generators, an aspect that is outside

[4]McCallum, G. J., et al. 1981. *Nuclear Science Applications* **1**: 163–190.

the purview of this report.

Three generator systems are available commercially: Gallium-68 from the long-lived germanium-68; rubidium-82 from the long-lived strontium-82; and technetium-99m from molybdenum-99 (the last, detailed above). Examples of other generators that plant and environmental scientists would find useful are copper-62 from zinc-62 and iodine-122 from xenon-122. However, no long-lived radionuclide precursors for the major elements are found in organic compounds in nature (i.e., carbon, nitrogen, oxygen, sulfur, phosphorus); so, the only known method for producing these elements as radionuclides is through high-energy manipulation of the nucleus of stable isotopes. The technologies to achieve new methods for, say, carbon-11 production include superconducting cyclotron innovations, induction linear accelerators, modern laser-based stimulations, and other accelerator design concepts. These might be incorporated in more portable and less expensive devices than current proton linacs and cyclotrons.

Radiotracer Synthesis

While there are many radiosynthetic methods that are widely used to prepare radiotracers, this area is underexplored in many ways. Further work is needed to expand the scope of radiotracers available and the ease, efficiency, and reliability of their production.

The demands for high specific activity and tracer-scale synthesis present special challenges. One of the major strengths of radiotracer technology is the ability to apply the tracer method, which allows the interrogation of a biological system externally without perturbing or saturating the system. High specific radioactivity (i.e., a high level of radioactivity per mole of compound) is essential for the tracer method; however, the synthesis of high specific radioactivity compounds has been, and remains, a key challenge in radiotracer chemistry.

In a typical radiotracer synthesis, performed at the tracer-level scale (i.e., without addition of carrier), the radionuclide is the limiting reagent, and it is reacted with a vast excess of the substrate (the precursor to be labeled). The excess substrate is necessary to achieve reasonable reaction rates, given that the concentration of the radionuclidic portion is often sub-nanomolar. Under these conditions, reactions are driven to completion relative to the radioactive reagent, and only a small fraction of the substrate is consumed in the desired reaction. Incorporation of the radioactive reagent and degradation or competing side reactions of the precursor are often very different under these unusual mass and concentration conditions when compared to normal synthetic chemistry (i.e., with reagent in excess over substrate). These differences are often not well understood and are difficult to model with non-radioactive chemistry. Our lack of understanding in this area severely impedes the translation of reaction methodologies from synthetic chemistry to radiotracer chemistry. Thus, more rigorous application of analytical and physical chemistry principles to tracer synthesis is needed to drive the development of new methodologies in both synthesis and purifications.

The scope and generality of radiolabeling methods are limited. Currently, most molecules cannot be labeled with a radionuclide, and those that can be labeled need to be prepared by radiosynthetic research experts. This deficiency is due to the limited scope of labeled precursors and the general inefficiency of the reactions available to radiotracer chemists. The development of fundamental chemistry for radionuclide labeling will greatly expand the types of molecules, entities, and functionalities that can be radiolabeled and used as probes in biological systems. However,

new reactions should be developed with broad applicability in mind so that they are easily adapted to a variety of compounds within a functional group class. Ideally, new reactions will be easy to reproduce, without the need for highly specialized equipment or exotic reagents, so that translation of the synthesis from researchers to technicians and from one laboratory to another can readily occur.

The availability of new radioisotopes needed for specific imaging applications will require development of new radiosynthetic methods. As additional radionuclides become available by new production schemes or the development of generator systems, appropriate radiotracer chemical methods need to be developed to put these radionuclides into use in diverse applications.

Radiosynthesis Kits and Automation

Radiosynthesis protocols are generally complicated and typically need to be performed by expertly trained research-level synthetic chemists. To facilitate the availability of radiotracers to researchers not trained in radiotracer chemistry, kit-like radiosynthetic methods are needed. Included in these could be synthesis expedited using solid-phase reagents or reactants and solid-phase expedited purifications. The kit concept has been very successful and transformative in other fields such as molecular biology, where once complicated procedures like mutagenesis can now be employed on a routine basis, even at the high school level, because of the availability of well-designed, highly reliable kits. Within radiotracer chemistry, kits have also had an impact, notably for radiometals such as technetium-99m, but thus far, these developments have been limited to a few radionuclides; they should be expanded to nonmetal nuclides, such as fluorine-18 and carbon-11. Moreover, implicit in the development of kit-like methods is the standardization of radiolabeling procedures, which will improve reproducibility among experiments, researchers, and laboratories.

The use of automation in radiotracer chemistry increases the productivity, safety, and reproducibility of radiolabeling. Automation can also be an enabling technology that allows radiotracer synthesis to progress from the bench to routine production facilities. There is a need to develop automated, potentially microfluidic systems that are flexible, modular, and convenient for performing a wide range of radiochemical procedures. Separate application-specific systems create redundancies that occupy space and require additional time for training and maintenance. As automation becomes more versatile and modular, automated systems will also become more widely used, which will help establish standardized protocols to improve the reliability, reproducibility, and safety of radiotracer production at multiple institutions and by individuals with only technical training. Opportunities to capitalize on commercial-academic partnerships for developing standardized protocols and automated instrumentation should be explored.

Radiotracer Purification and Analysis

Isolation of a radiolabeled compound after synthesis is often as demanding and more time consuming than the radiosynthesis itself. The most common method of purification, high performance liquid chromatography (HPLC), requires distinct methods development for each labeled compound. This effort is often wasted if a labeled compound does not function appropriately as a radiotracer and a different compound must be labeled and pursued as a substitute. Modular purification strategies using affinity tags and scavenger resins could increase the throughput of compounds that can

be evaluated as potential radiotracers. Ideally, these methods could also be carried to the "field," where purification by HPLC may not be practical.

Once a tracer is introduced into a biological system, it is often metabolized or modified by enzymes. Therefore, identification of the chemical species producing the image, typically by radio-HPLC analysis, becomes paramount (often the point of the research itself). In some cases, the chemical species giving rise to the image can be interrogated externally by labeling the same molecule in different positions or by double labeling (stable isotope + radiolabel). Highly efficient and sensitive methods such as liquid chromatography multidimensional mass spectrometry methods will need to be employed in parallel with radioactivity monitoring to fully characterize and understand what is producing the signal within the system that underlies the image.

Dual-Modality Probes

Multimodality imaging (e.g., an agent bearing both radiotracer and fluorophore probes) can expand the potential impact of imaging by enlarging the range of size, time scales, resolution, sensitivity, and specificity of detection that can be achieved (see Figure 2). However, the development of multimodality imaging agents is challenging because the attachment of multiple detection agents (i.e., attachment of a separate agent for each modality) can interfere with the localizing or targeting behavior of the imaging agent. Thus, consideration of potential benefits from dual-labeling approaches to multimodality imaging must be preceded by a clear understanding of the following:

- relative sensitivities of the different modalities and how the sensitivity of the detection method and the capacity of the target of imaging need to be matched with the administered mass of the radiotracer,
- different types of information that are obtained from the different imaging modalities, and
- differences in the rates and capacity for uptake and clearance in various "species" (animals vs. plants vs. microbes).

The drawbacks and benefits of a dual-labeled probe must be carefully weighed against the coadministration of two individual probes, each of which might be more fully optimized for a single imaging modality.

Specific Challenges in Labeling Macromolecules: Proteins and Nanoparticles

It is well understood that proteins, being large polypeptides of defined composition and structure, have relatively limited tolerance to harsh chemical and physical conditions. Therefore, methods for protein radiolabeling and purification need to be gentle and efficient. By contrast, the term "nanoparticle" comprises a broad array of materials that have very specific and unique features based on their size, composition, morphology, and surface coating. Multiple steps are often required for the fabrication of nanoparticles, each of which may or may not be altered by the incorporation of targeting or probe moieties. The diversity in the structure and synthesis of nanoparticles requires that multiple approaches be developed for labeling. New methods for nanoparticle labeling should strive to

- use mild reagents and conditions to maintain structural and functional integrity, and

- achieve high labeling efficiency under stoichiometric concentrations to afford optimal detection sensitivity and/or high specific activity (i.e., most particles should be labeled by the process; see below).

Perhaps the biggest challenge in labeling macromolecules, be they proteins or nanoparticles, is that the labeled and unlabeled materials cannot be separated, because the addition of the tracer unit, be it a radionuclide or a contrast agent, is such a small modification relative to the overall characteristics of the macromolecule. The development of new methods to separate labeled particles from unlabeled particles will become critical to their usefulness in imaging.

Training of Scientists in Radiotracer Methodologies

There is great concern that the acute and growing shortage of synthetic and analytical chemists who have solid training in radioisotopic labeling will limit the application of radiotracer and imaging methods to studies in new areas of biology. Thus, the training of radiochemists and imaging scientists is recognized as an opportunity to address this unmet need. Training of plant, microbial, and environmental scientists in the use of radiotracers and associated data analysis also is needed. These goals can be accomplished by mechanisms such as graduate fellowships, sponsored visiting scientist programs, and postdoctoral grants.

Opportunities in Radiotracer Chemistry

- *Development of new chemical reactions that meet the demands and synthetic constraints of working with radioisotopes at high specific activity.* All aspects of radiotracer imaging will benefit from an increased number and improved set of chemical reactions that can be used to label molecules of interest with a radioisotope. Radiotracer chemistry methods that are versatile and easy to translate from molecule to molecule will catalyze the use of radiotracer technology across "species."
- *Utilization of physical chemistry methods to develop models that can predict and explain reactivity at the tracer mass scale*. By increasing our understanding of critical parameters of chemical reactions at tracer scale, optimization of reaction methodologies and labeling protocols will be accelerated.
- *Construction of nanoparticle platforms that can incorporate one or more imaging agents and targeting moieties.* Such particles may be ideal for dual-modality applications. There is a significant opportunity for the development of methods for highly efficient radiolabeling and purification techniques able to separate labeled and unlabeled macromolecules, proteins, and nanoparticles, thus increasing their specific activity.
- *Creation or improvement of automation technology for radiotracer synthesis that is adaptable in a variety of reaction scenarios through modular or kit-like construction.* Reliable kit-like labeling and purification protocols and versatile automated systems can potentially bridge the gap that exists in translating radiotracer technologies into new research areas.

III. Challenges and Opportunities in Radionuclide and Hybrid Instrumentation Development

Background

Several DOE mission areas, notably biofuels and bioremediation, apply various forms of imaging for biological applications in plants and microbes. Optical imaging is already a mainstay in this area, and further refinement of this modality will likely be of great benefit [e.g., Förster resonance energy transfer (FRET), optical diffusion fluorescence, laser speckle]. While these methods have excellent (subcellular) spatial resolution, they generally can only be applied to small objects and have limited depth of field. As the biofuels and bioremediation fields mature, there are likely to be many questions that cannot (or do not necessarily need to) be answered at the single-cell level, but require imaging at size scales of an intact plant and larger or intact soil systems. While this can be difficult to implement with optical techniques, the penetrating nature of gamma rays implies that the radionuclide imaging methods currently used in human and small-animal imaging have exceptional promise for situations where optical methods are not possible due to limitations in depth of penetration. Thus, optical and radiotracer methods can be viewed as complementary. Radionuclide modalities have poorer spatial resolution (compared to optical), but typically provide higher sensitivity, fully three-dimensional imaging capabilities over larger fields of view (including deep tissues), highly quantitative results, and—when performed at the tracer level—minimization of biological disturbance from the imaging process, thus enabling repeat studies.

The main radionuclide imaging methods are positron emission tomography (PET), single photon emission computed tomography (SPECT), and autoradiography. PET involves a method of detection that does not require a lead collimator or aperture such as is required for SPECT; thus, PET is many times more sensitive than SPECT, but SPECT uses radionuclides that have generally longer half lives and represent important elements in the study of plants (see Table 3). Like optical imaging, autoradiography is in common use by biological researchers. Autoradiography is capable of subcellular spatial resolution and can be applied to small specimens. Some previous work has been done using both PET and SPECT techniques and non-imaging variants using non-imaging radiation detectors (see Appendix A), but the techniques themselves are not widely adopted by the plant and microbial biology communities, despite the great opportunities they afford.

PET and SPECT are not the only non-invasive imaging methods that could be applied (see Figure 2). Techniques such as magnetic resonance imaging (MRI) at high or low fields, MR spectroscopy, ultrasound, and electron microscopy all have potential uses, although each method has its own distinct range of sensitivity, specificity, and resolution. X-ray fluorescence imaging can achieve sub-micron resolution, although at prohibitively high radiation doses for repeated studies on living systems. The associated particle technique (APT) uses neutron activation to provide non-invasive imaging of chemical composition (albeit on a relatively coarse spatial scale). As it has many parallels to PET (especially time-of-flight PET), it would likely benefit through input from PET physics research in terms of its application to biological problems. Simplified and less expen-

sive instrumentation to produce short-lived radioisotopes and make them more readily available to biological researchers is important, as was discussed in Section II.

Finally, there may also be significant merit to combining imaging modalities for more complete information. For example, MRI and X-ray computed tomography can provide excellent contrast between different tissue types at high resolution; this is an excellent complement to radionuclide imaging, which often lacks sufficient anatomical information, especially for highly specific tracers. However, the focus of this workshop was on radionuclide imaging, so the remaining discussion will center on this modality.

Imaging Scales: Time, Size, and Resolution

As Figure 5 shows, the processes involved in plant metabolism span a wide range of scales. Size scales range from the macromolecular and single-cell dimension to the tissue level, to the entire plant, and to a natural setting, such as whole microbial communities in the oceans. The resolution capabilities of PET and SPECT have improved over the last 50 years as shown in Figure 6. Time scales range from milliseconds to minutes to days. The maximum time scale for radionuclide imaging is set by the half life of the radionuclide involved; thus, imaging cannot probe scales that are significantly longer than a few multiples of the half life. In broadening the scope of radionuclide imaging to these new applications, it is clear that the imaging requirements and constraints can be significantly different from those involved in nuclear medical imaging, and so new approaches to instrumentation development will be required.

With regard to size scale, current techniques for biological imaging of plants and microbes (such as optical methods) tend to image very small fields of view with very high spatial resolution, ideally at the micron level to observe subcellular function. Currently, the best spatial resolution achievable for SPECT is ~300 microns using pinhole collimators and for PET is ~1 mm. These values have largely been determined by a tradeoff between spatial resolution and sensitivity in these approaches, and sensitivity is at a premium for medical imaging because of the need to follow relatively fast dynamics. The dynamics of tracer movement in other biological systems is typically much slower, which allows the tradeoff to be shifted in the direction of higher resolution, because detection efficiency can potentially be sacrificed. With infinite statistics, fundamental limiting effects on the spatial resolution in PET are the gamma non-collinearity and the positron range (i.e., the variable distance a positron travels from its parent radionuclide before it signals its location with annihilation radiation), while the limit for SPECT is set by collimator penetration. However, the blurring due to all these effects can be accurately modeled. This suggests that these effects can be deconvolved and that higher resolution for both PET and SPECT can be obtained, provided a sufficient number of counts can be acquired. Using this approach, it may be possible to improve the existing spatial resolution by up to an order of magnitude, which approaches cellular level imaging. It is also noted that the positron range can be reduced, at least in two dimensions, using a strong magnetic field.

The maximum size scale is set by the attenuation length of the emitted gamma radiation, which is ~10 cm in water (which approximates the density of both plant tissue and soil reasonably well). Thus,

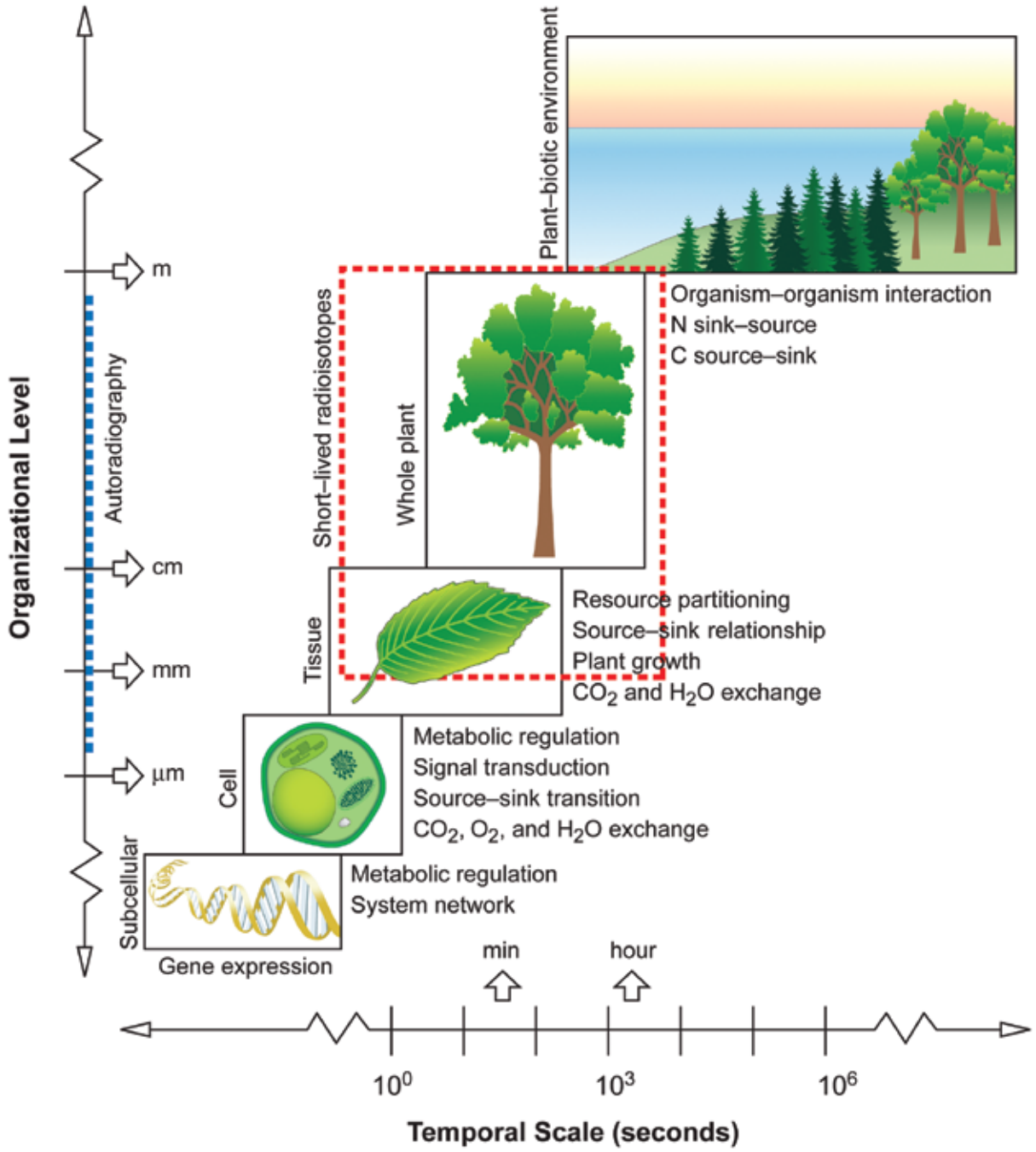

Figure 5. Temporal and spatial scales involved in photosynthesis.[5]

[5]Original concept from Osmond, C. B., and Chow, W. S. 1988. *Australian Journal of Plant Physiology* **15**: 1–9. Modified after Kiser, M. R., et al. 2008. *HFSP Journal* **2**: 189–204.

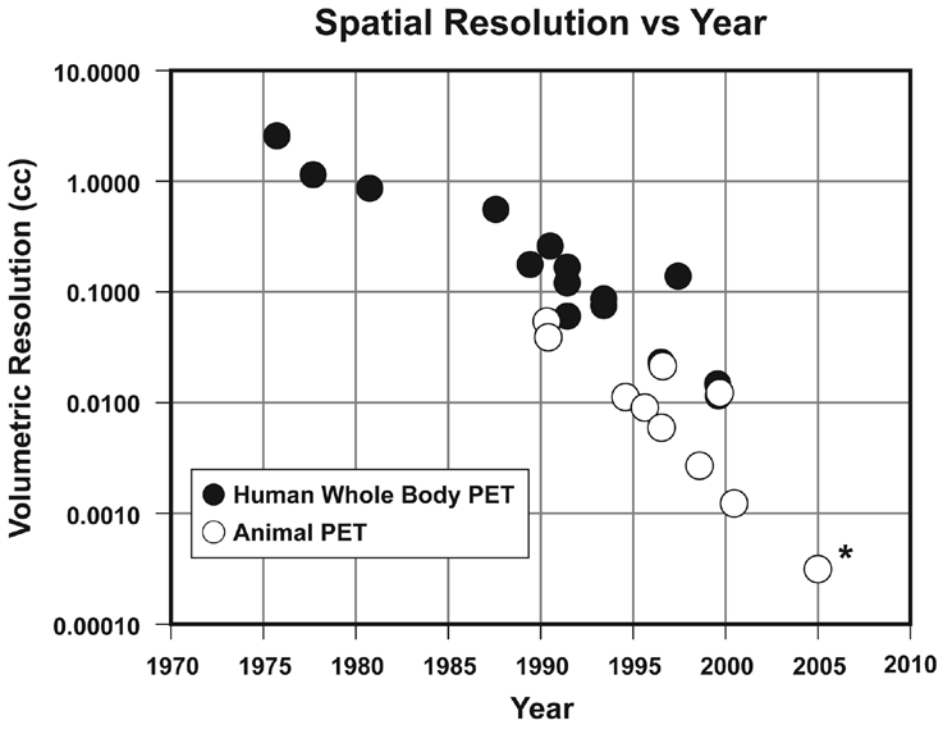

Figure 6. Thirty-year history of volumetric resolution based on in-plane area times slice thickness. (*A wire chamber system limited to sample sizes of 100 cc.)[6]

it will be difficult to image solid objects with sizes greater than ~0.5 meters. However, most of the plant systems that require study are not solid objects, but have a significant amount of air interspersed within the plant tissue (e.g., a 1-meter volume containing the foliage of a plant is likely to contain more than 90% air). Thus, it is reasonable to consider size scales up to meters in size, basically limited by tradeoffs between the detection efficiency and the cost of the imaging instrument. This size scale is applicable to samples from oceans, soil, and plants, where over a cubic meter can be evaluated for nutrient flow, elemental diffusion, and microbial metabolism in contained experimental chambers.

Imaging Geometries

Another difference between imaging plants and imaging animals is the scanner geometry. For basic studies of carbon flow throughout a living plant, high resolution may not be needed, and designing practical systems that can be used on a larger scale may be more important. On the other hand, cylindrical scanners like those typical in medicine may be useful in plant imaging, particularly when whole plants are to be imaged, for example, to follow the dynamics of signaling processes between leaf and root. This geometry is also the most flexible for imaging a variety of plant types and sizes and can easily be scaled up to cover larger specimens.

[6]Data from Simon Sherry (University of California, Davis) and Michael Schafers (University of Muenster).

However, one can take advantage of the relatively two-dimensional nature of the leaf to improve imaging performance significantly for this case. The best geometry for leaves with radionuclide imaging would likely be two parallel plates with variable separation that could be brought close to the leaf without damaging it. This would provide very high solid angle, an important consideration to provide the increased counting statistics needed for high resolution and fast processing. An important feature of this geometry to achieve high spatial resolution would be depth-of-interaction measurement capability in the detectors, since gamma rays would be impinging at a large range of angles. This aspect of detection instrumentation has shared importance for human and plant imaging.

Opportunities in Radionuclide and Hybrid Instrumentation Development

Although the problems to be solved by plant, microbial, and environmental scientists have not yet been fully articulated, some general directions or opportunities in the area of imaging instrumentation can be offered at this point.

- *Explore new scanner geometries to match the diversity of new uses and size scales to be imaged.* While existing nuclear medical imaging apparatus can be used for pilot studies, these systems are not likely to be optimal, so task-specific, optimized systems will need to be developed. The variety of shapes and sizes of objects in the plant community is far greater than in the human community, so a variety of devices with very different dimensions and geometries are likely to be necessary. It is likely that small field-of-view devices with high spatial resolution in the range of 100 μm will be needed; yet higher resolutions might be needed for some applications in microbial systems. On the other end, systems capable of imaging cubic-meter volumes (e.g., measuring the relative amounts of radiotracer in the foliage, stem, and roots of a small community of plants), and most size scales in between will be required.
- *Work for higher spatial resolution by development of PET detector systems resulting in a dramatic improvement in spatial resolution, sensitivity, and temporal resolution.* Currently available detector systems are not adequate for the study of microbial research since sub-millimeter resolution will be required. Given the current importance of optical imaging at the cellular level in plant studies and the potentially profound implications of the ability to apply radionuclide imaging at this level, it is of great interest to explore the fundamental limits of spatial resolution for the radionuclide methods. The tradeoffs between resolution, efficiency, imaging volume, and cost will be much different for plants than for patients, and it is reasonable to envision that these differences will enable an improvement in spatial resolution by an order of magnitude or more, approaching the cell size in plants.
- *Explore benefits of dual-modality imaging methods.* Dual-modality imaging has provided great benefits in many fields, and it is likely that adding complementary imaging modalities (e.g., optical, X-ray computed tomography, X-ray fluorescence, MRI, MRS) would enhance the radiotracer techniques in applications to studies in plants and microbial systems.

- *Develop imaging devices capable of operating in diverse environments.* These instruments may need to function in a laboratory, greenhouse, or field environment (as opposed to a hospital), which provides further challenges.

Finally, it is notable that these directions of study have a high probability of feeding back into improvement in medical imaging applications, because the tools and techniques that could be developed have potential to become incorporated into instruments optimized for human or small-animal imaging. For example, techniques to measure and correct for depth of interaction (i.e., detector astigmatism) would be valuable in whole-body human, disease-specific human, and small-animal imaging systems (see Section IV).

IV. Radioisotope Methodologies and Synergistic Technologies for Probing Plants, Microbes, and the Environment and Applications to Nuclear Medicine

Background

Radiotracer technology, including probes and instrumentation, is well suited for exploring metabolic pathways and the physical distribution or movement of chemical species in living organisms. The application of radioactive isotopes to follow kinetic processes in living systems was pioneered by Georg de Hevesy in 1923 in his studies of the movement of lead radioisotopes in plants. In the 1940s, Ruben and Kamen studied carbon pathways in living systems using short-lived carbon-11 and nitrogen-13, as well as the longer lived carbon-14. These tracer methods were then applied to studies in mammalian species. Many early radiotracer technologies and methods of detection, perfected in non-human systems, were later incorporated into nuclear medicine. Subsequently, radiochemistry and instrumentation developed for nuclear medicine have been employed to evaluate function in many organisms. Thus, a synergistic relationship exists for the development and application of enabling radiotracer techniques in both nuclear medicine and plant and environmental science, regardless of their original intended use.

Examples are presented below that demonstrate the potential for the application of radiotracers and detection instrumentation, broadly based, in biological and environmental studies.

Tracing Pathways of Hydrocarbon Production in Microalgae

Photosynthesis in microalgae may be diverted away from biomass production to hydrocarbon production. The microalgae enzymatic pathways for the production of fatty acids, terpenoids, and sugars are shown in Figure 7. To derive more hydrocarbon mass from the process, it will be important to determine the mechanism nature uses to divert the flow of photosynthetic carbon from the synthesis of low energy-content sugars into the alternate pathways leading to high energy-content terpenoids or fatty acids. Carbon-11 or fluorine-18, currently available from small medical cyclotron facilities or physics van de Graaf machines, may be used for the production of labeled tracers (shown in Figure 7) that may be employed to interrogate the carbon flux pathways proceeding to terpenoid and alkane synthesis. Some tracers listed in Figure 7 already exist for mammalian metabolic pathway imaging. The recent significant effort to support lignin biosynthesis research relevant to cellulose production may also benefit from available or new radiotracers. These tracers may be introduced into the microalgae or plant environment with the metabolites assessed by liquid chromatography.

Lignin Digestion

A major problem in the use of biomass for the production of hydrocarbon-based fuels is the digestion of lignin—the structural matrix of plants. Lignin comprises between 3 and 30 percent of the

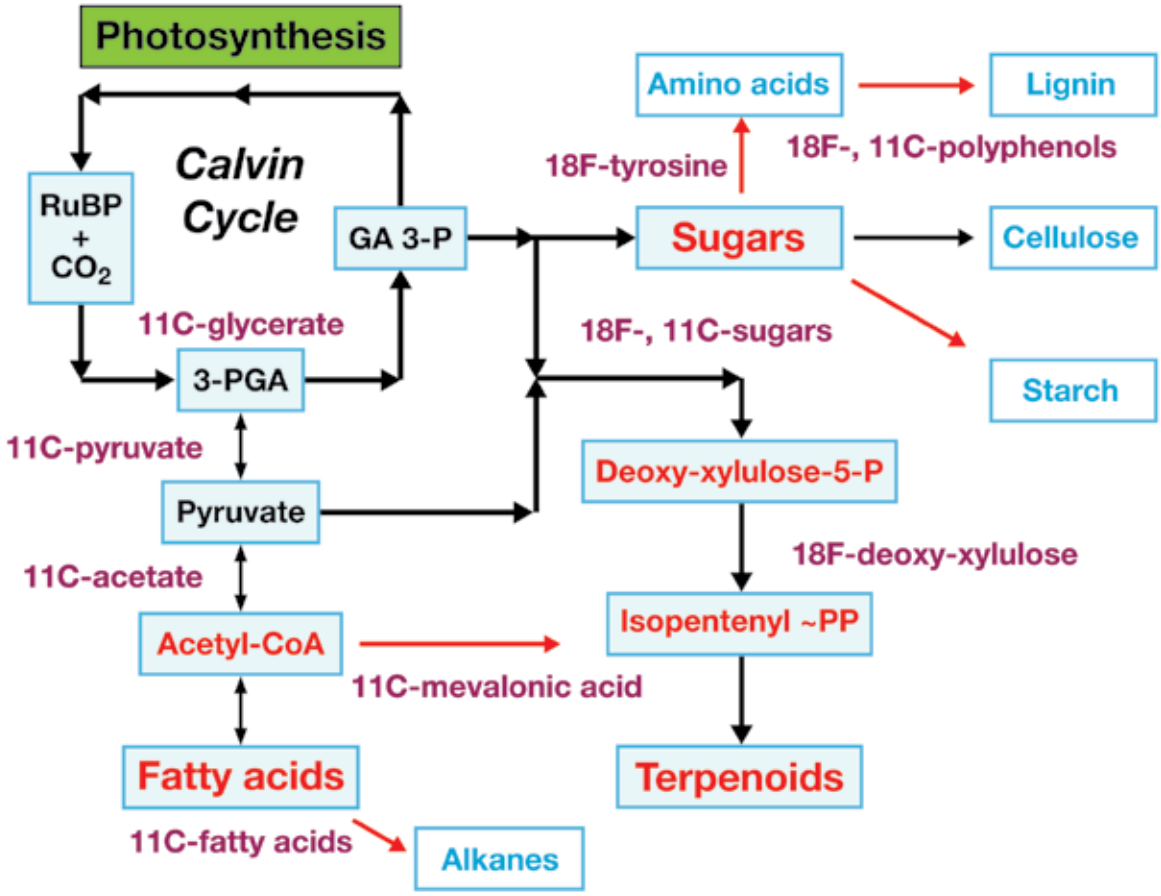

Figure 7. Major pathways of photosynthesis and plant metabolism.[7]

biomass and varies widely with the biomass source (see Figure 8, *right*). Pretreatment of lignocellulosic biomass can disrupt cellulose crystallinity and circumvent the lignin barrier; however, when done by physical or chemical means (see Figure 8, *left panel, upper reaction*), this transformation is the most costly step in bioethanol production from woody biomass. Research seeks to determine how lignocellulose-degrading microbes are able to achieve the same result under milder biological conditions (see Figure 8, *left panel, lower reaction*). Imaging and tracer methods have a role in the development of efficient and economic digestion systems or the selection of genetically modified plants that are more easily digestible than the current species.

Steroid Hormones

Radiolabeled steroids have been used to assess steroid receptor expression in tumors, especially breast and prostate cancer. Similarly labeled steroids are being employed to evaluate the effects of steroid hormone replacement therapy on the brain and to predict the outcome and to monitor

[7]Created by Scott Taylor (Lawrence Berkeley National Laboratory) and Tasios Melis (University of California, Berkeley), Nov. 2008.

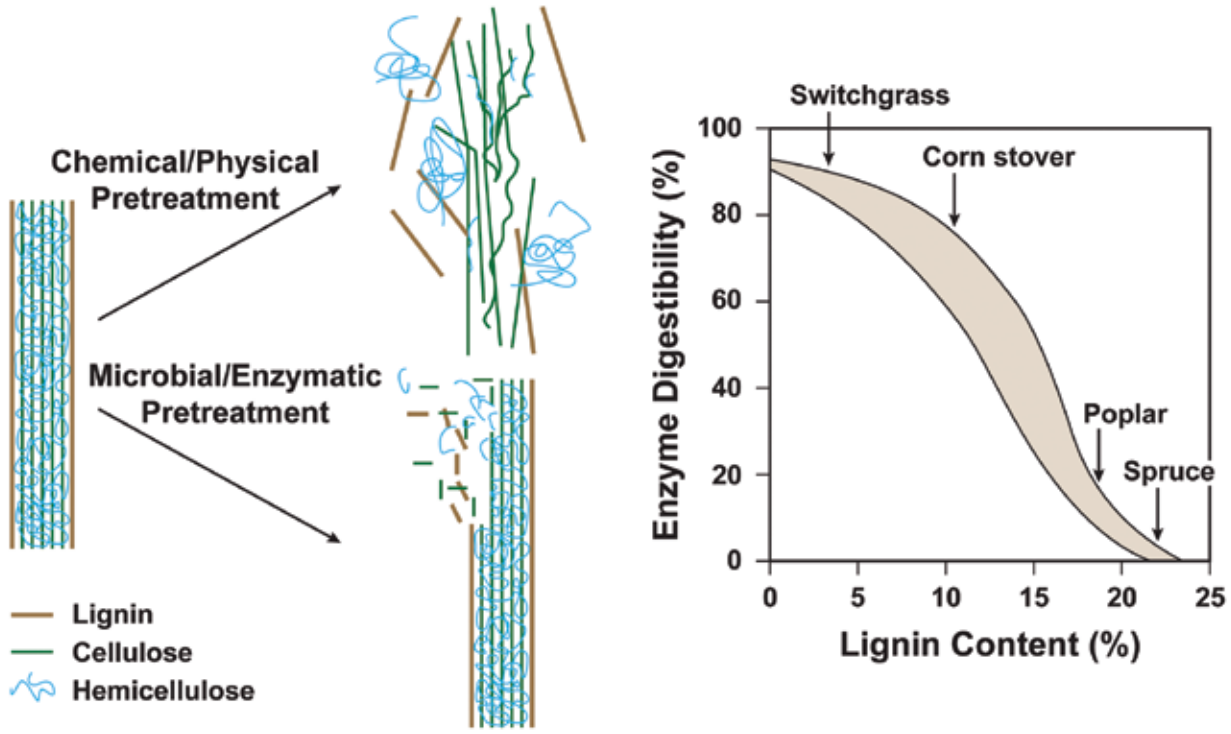

Figure 8. ***(left)*** **Methods for pretreatment of lignocellulosic biomass to disrupt cellulose crystallinity and circumvent the lignin barrier.** ***(right)*** **The digestibility of lignocellulosic material is inversely proportional to the lignin content, which varies widely with source.** With no lignin content, most if not all of the polysaccharides are accessible to hydrolytic enzymes, but in biomass greater than 25% lignin, only lignin-degrading microbes can gain access to the carbohydrates of wood.[8]

the effect of the treatment of hormone-dependent cancers. There are a number of steroids and steroid-like compounds produced in plants. These include brassinosteroids (crucial for cell elongation and plant growth), phytosterols (the plant wall equivalent of cholesterol in mammalian cell membranes), and phytoestrogens (natural herbicides that interfere with the reproductive system of herbivores). Development of labeled versions of these steroidal-type compounds can be used to evaluate biosynthetic pathways in plants and assess changes in receptor density in response to endogenous and environmental change.

PET Isotopes and Radiotracers: Applications in Plants

Imaging studies using short-lived isotopes such as carbon-11 and nitrogen-13 in plants have not received as much attention as research studies in humans and animal models of human disease. However, there has been a steady level of activity in radiotracer imaging studies in plants, largely involving the use of low molecular weight precursors and metabolic substrates such as (a) [^{11}C]

[8] *(left)* Mosier, N., et al. 2005. *Biosource Technology*. **96**: 673–686; *(right)* Data from Baker, A. J. 1973. *Journal of Animal Science* **36**: 768–771.

carbon dioxide to measure the process of carbon flow dynamics in different plant species, (b) nitrogen-13 in the form of ammonia to track amino acid biosynthesis, (c) [^{13}N]nitrate which follows nitrogen uptake and fixation pathways, (d) fluorine-18 as fluoride ion to trace water movement and [^{18}F]fluorodeoxyglucose to highlight carbohydrate synthesis and transport, and (e) oxygen-15 to study water transport. These studies have again given important information on how plants respond to heat, cold, carbon dioxide levels, nitrogen partial pressure, moisture, pH, and toxins (see Appendix A for a sample of the relevant literature).

The evolution of instrumentation for the production of PET radionuclides and the development of three-dimensional PET/computed tomographs and microPET scanners have been driven by both the rapid expansion of clinical PET and the emergence of imaging studies in animal models of disease. However, commercially available PET and microPET scanners are not optimal for imaging studies focused on plant and microbial research. Specialized detection equipment has been assembled to measure the distribution of the isotopes and tracers in the roots, shoots, and leaves of the plants. Kinetic analysis tools developed for nuclear medicine have been applied to quantify tracer movements with time. From these studies, one can measure the functional consequences of the environment on the various metabolic pathways that control plant behavior. However, many of the imaging systems currently used in this field of research rely on outdated detector technologies that lack the spatial resolution, temporal resolution, and sensitivity of those currently available in commercial PET and microPET scanners.

Finally, the evolution of medical cyclotrons has occurred to facilitate the production of PET radionuclides in high yield (Ci levels) and high specific activities (Ci/mmol) that are clearly in excess of what is required for plant and microbial research, because lower levels of radioactivity are generally used in plant research. Therefore, the development of simpler and less costly lower-energy particle accelerators for the production of positron-emitting radionuclides in levels consistent with the requirements for plant and microbial research will be needed to expand this avenue of PET imaging research.

Environmental Imaging

Environmental tracers can be used in a variety of applications, including investigations of atmospheric migrations and transformations of chemical entities such as volatile organic compounds, and studies of the transport and fate of nanoparticles through ground water and soils. In addition, radiotracer and chemical detection techniques can be applied to large-scale studies in the field, such as evaluation of the nitrogen cycle in the oceans and biomass of forests (see Figure 9). Radionuclides have also entered the environment through the leakage of storage tanks involved in nuclear weapons and reactor research. Studies of the uptake of these radionuclides or their metabolism by plants or microbes may play an important role in understanding their effect on organisms higher in the food chain or provide insight on how to immobilize radionuclides that have entered the environment. Perhaps a combination of microbial and plant activity and the involvement of minerals in the environment may lead to a solution for immobilizing these radionuclides.

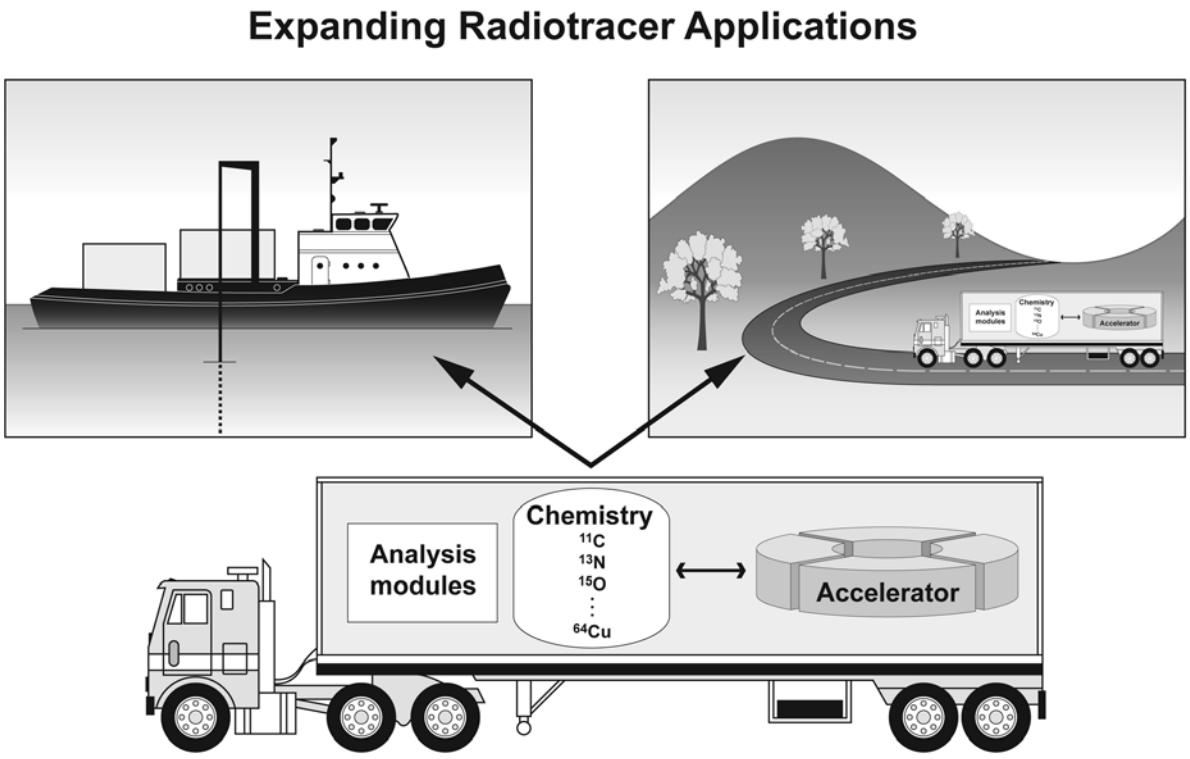

Figure 9. For some applications the use of short-lived radionuclides is needed and these would require production of the radiotracer and associated chemistry and imaging in the field.

Metabolic Tracers

For both plants and microbes, understanding the partitioning of carbon between metabolic pathways is important. For example, in microalgae the ability to shunt carbon precursors to terpenoid or fatty acid metabolism rather than to gluconeogenesis will help to develop algae that can produce more hydrocarbons for fuels. Radiotracers that can be used to track this carbon partitioning in microbes will enable a better understanding of the fundamental principles that guide carbon distribution between metabolic pathways. Other means of distinguishing metabolic pathways, such as the use of deuterated substrates with specific carbons deuterated or labeling compounds in different positions, could also be useful.

Many cellular functions, such as signal transduction, regulation of gene expression, and protein interactions, contribute to regulating metabolic events. The use of fluorescence methods has helped our understanding of metabolism regulation and many other cellular events such as intercellular communication and signal transduction responses to activation of cell surface receptors. Metabolism on the organismal level, including microbial communities, might be studied using radiotracer imaging methods as they allow evaluation of metabolism and its regulation when imaging is integrated with analytical systems capable of identifying the radiolabeled molecule.

Real-time imaging in living cells

Many biological questions are best answered if living cells or organisms can be imaged continuously over a defined time period. Real-time imaging is not yet available for many cellular events, especially gene expression events that include transcription, RNA splicing, and localization and quantification of mRNAs and other important cellular RNAs such as microRNAs. Although fluorescence spectroscopy is used to image proteins in real time—for example, tracking intracellular protein interactions and movements—this technology suffers from the limitation that the fluorescent proteins used to image the protein function can also perturb the system. Alternate radio-imaging approaches that either do not perturb the system or interact with the system by mechanisms that differ from those of fluorescent fusion proteins would help to clarify our understanding of cellular events and how they are regulated in the context of the multicellular organisms or microbial community.

Sensitivity of imaging

Radioisotopes offer the advantage of extreme sensitivity and a large dynamic range. This is important for expanding the options for imaging applications because many regulatory events in cells involve small numbers of molecules and some of the changes in activity or function of these molecules are large.

Opportunities for Synergistic Technologies for Probing Plants, Microbes, and the Environment and Applications to Nuclear Medicine

As demonstrated in these few examples, many biological and biochemical processes are relevant and shared across species. Many of the tracers, specialized instruments, and software that have been developed for medical applications may be translated to and/or modified for the study of plants, microbial systems, and the environment. Likewise, new chemistry, instrumentation, and data-handling advances made while investigating plant pathways may have relevance to applications in mammalian species such as diagnostic imaging probes or tools for developing drugs or monitoring therapeutic interventions. Broadening the perspective of radiotracer use will drive the technological advances that in turn will benefit a spectrum of applications.

- *Develop a low energy particle accelerator (possibly using ^{3}He or a tandem cascade proton/deuteron accelerator), having a reduced power demand, low neutron flux, and small footprint.* Such user-friendly, short-lived radioisotope production units will be needed to facilitate PET imaging studies in laboratories focused on plant and microbial research. The successful development of a particle accelerator possessing these properties will also be of value in clinical nuclear medicine imaging applications due to the current need for the development of lower energy particle accelerators for single dose productions of short half-life radiotracers such as carbon-11 as [^{11}C]CO or [^{11}C]CO_2; nitrogen-13 as [^{13}N]ammonia, nitrate, and nitrogen gas; and oxygen-15 as [^{15}O]water or gas.

- *Development of high spatial resolution imaging and high sensitivity detection systems needed for new plant and microbial imaging tools (emphasized in Section III of this report) will provide synergistic benefits to nuclear medicine research and applications.* Examples of this include (a) imaging of micro metastases in the sentinel lymph node of breast cancer patients; (b) imaging the shell vs. the core of the nucleus accumbens in substance abusers; and (c) functional separation of the hippocampus into the dentate gyrus, CA1, and CA3 regions in subjects diagnosed with depression or age-related cognitive impairment.
- *Combination of radiotracer techniques with ^{14}C mass spectroscopy and ^{13}C-MR spectroscopy techniques, possibly using hyperpolarized ^{13}C*. A disadvantage of the ^{11}C-carbon flow dynamic studies in plants is the inability to non-invasively identify the molecular form of the radionuclide in various structures within the plant (leaf, shoot, root system). Therefore, the development of ^{13}C-MR spectroscopy (mass and hyperpolarized ^{13}C NMR) to study the metabolic fate of PET radiotracers will likely be needed in the kinetic analysis of PET imaging studies in plants and microbes.
- *Development of detector systems that directly measure charged ions (b+, b-, Auger electrons)*. Such an instrument would take advantage of more diverse forms of radioactive decay besides gamma emission and potentially serve research needs in microbial sciences.

V. Summary

On November 4 and 5, 2008, a workshop, organized by the Office of Biological and Environmental Research (OBER) of the Department of Energy (DOE), brought together 43 scientists from plant, microbial, and environmental biology with chemists, physicists, and engineers from the nuclear medicine technology research community. These scientists came from academia, industry, NIH, and DOE National Laboratories. Their charge was to jointly ascertain how radiotracers and related detection instrumentation and analytical techniques could be used to benefit diverse aspects of basic research in microbial and plant metabolism relevant to biofuel and energy production, and to advance novel strategies for environment decontamination and mitigation. Workshop seminar presentations and discussions on the first day were followed by breakout working group panel discussions and draft consensus reports on the second day. With the help of the workshop participants and working group leaders, these ideas have been developed into this report.

This report provides an analysis of the current state of radiotracer chemistry, radioanalytical methodology, and imaging instrumentation and then presents a series of new opportunities for DOE developments in these areas that could provide major benefits to fundamental research in alternative energy production and environmental sciences. It was recognized, however, that this effort was only a beginning. With a clearer recognition of the capabilities that basic nuclear medicine technologies can provide to biologists and environmental scientists and a better understanding of the problems being tackled in plant biology by the chemists, physicists, and engineers, this merger of talent has great potential for advancing current missions of DOE.

Appendix A: Selected Literature

Selected References on Radiotracer Applications to Plant and Microbe Research

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Appendix B: List of Participants

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