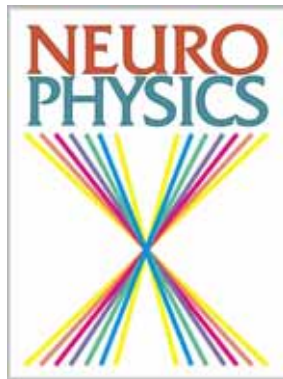


TUTORIAL MEMORANDUM ON MOLECULAR
IMAGING IN DRUG DISCOVERY AND
DEVELOPMENT USING ANIMAL MODELS



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Background

The interior workings of laboratory animals can be imaged in two ways. One is called anatomical or structural and the other is called molecular or functional.

Anatomical imaging is done with CT (x-ray) and MRI machines. CT images the density of electrons in tissue and therefore reveals bone with high contrast. Since the "soft tissues" that make up the rest of the body have nearly the same electron densities they are poorly, if at all, separated in CT images. On the other hand, MRI machines are sensitive to proton (hydrogen atom) density which does vary between organs and other soft tissue. They can produce highly detailed images of the animal's interior structures. Neither are useful for identifying the early stages of pathology or other active processes. Only when very large numbers of cells become involved will the effect on structure be seen. Nor can such imaging tell us how well an organ is functioning — an anatomical image of a freshly dead animal is no different from when it was alive!

The new field of molecular or functional imaging can do much more. It can actually see what is going on inside the animal at the *molecular* level as a function of time — and do so *noninvasively*. The consequences for drug discovery and development are enormous.

Target specific imaging is experiencing very rapid growth. Methods originally developed for studying biochemical systems in solutions or isolated cells (*in vitro*) have been translated into laboratory animals (*in vivo*) allowing testing of potential drugs in the context of a living organism.

Modern drugs are designed to interact with a well-characterized molecular target and their development has become both time-consuming and very expensive (~\$800 million).² Molecular imaging of laboratory animals can quickly provide direct proof that a therapeutic concept is valid (or not). Note that shortening the development time will not only reduce the cost but also extend the proprietary phase of the market.

Molecular imaging will soon provide an indispensable tool for drug discovery and development.

Using Radioactive Atoms for Drug Discovery and Development

Functional imaging at the molecular level is the three-dimensional mapping of the concentrations of selected biological molecules within the body of a live laboratory animal — usually as a function of some test parameter. One way to do this is to attach (tag) radioactive atoms to the selected molecules and inject or otherwise deliver them into the animal. Much like tiny radio transmitters, the radioactive atoms emit a signal that can be detected externally by camera-like devices that provide images of the localization and concentration of the tagged molecules.

¹ HF Stoddart, 12 April 2007

² Some of this material came from Markus Rudin, University of Zürich

Learning where molecules go and in what concentration is extremely useful in drug discovery and development. For example, tagged molecules that go to a tumor can be used to see if the tumor shrinks in time due to some new drug under development. Another example is following the concentration of synapses that are compromised in Parkinson's disease as a function of administering new drugs to treat the disease.

The radioactive "transmitters" must produce a signal that has sufficient energy to enable it to get out of the animal's body without being absorbed or scattered.³ Three such signaling radiations are available: annihilation radiation, gamma rays, and x-rays. All, like light, are electromagnetic photons, uncharged and traveling at the speed of light. While the energy of light photons is only a few electron volts (eV), the energy of x-ray photons is about 10^4 eV, gamma rays 10^5 eV, and annihilation photons nearly 10^6 eV.

Molecular imaging modalities are classified in part by the kind of radiation emitted by the radioactive "transmitters" and in part by the imaging instrumentation that is used.

Autoradiography

The earliest form of molecular imaging of internal concentrations of radioactive atoms in live animals using radioactivity, and still widely used, is called "autoradiography." As with all modalities described here, the animal is administered molecules of interest tagged with radioactive atoms. But then, the animal is euthanized, frozen, and sliced into many thin sections. The sections are laid out on photographic film (in the dark) and left for a day or more while the radiation from the distributions of radioactivity exposed the film. When the film is developed the distribution of radioactivity becomes evident. The slices are similar to the ensemble of slices produced by the MollyQ — which has been called in vivo autoradiography. The advantage of the MollyQ is that the mouse is still alive and available for longitudinal or other studies. The wide choice of "off-the-shelf" tagged molecules uniquely available to the MollyQ is the result of their applications in autoradiography!

PET Scanners Use Coincidences in Rings of Detectors

When annihilation radiation from positron emitting isotopes is imaged, the modality is called "PET scanning." The isotopes used for tagging molecules emit fast-moving *positrons*. These are electrons that carry a positive charge in place of the usual negative charge. After it slows down (perhaps several millimeters from the point of origin) the positron combines with an ordinary electron and the two destroy each other converting their combined mass to *two* very high energy (511 keV) photons which go off, back-to-back, in opposite directions.

The first commercial 2-D brain scanner based on annihilation radiation coincidence from positron emitters was designed by HF Stoddart in 1957 and a dozen were produced and sold by the Atomic Instrument Company.

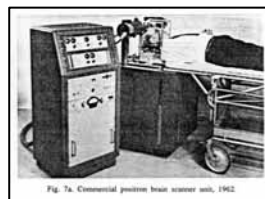


Fig. 7A. Commercial positron brain scanner unit, 1962.

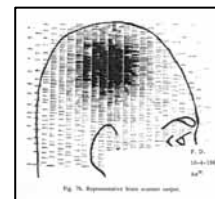


Fig. 7B. Representative brain scanner output.

³ Optical imaging systems using fluorescence do not meet the transmission requirement and are only useful for molecular concentrations located in shallow depths such as the skin.

Coincidences in a cylindrical ring of detectors about the subject enable the reconstruction of 3-D images of isotope concentration in the subject.⁴ The difference between the location of the emitting atom and the point of disintegration limits the resolution of PET scanners. While not important for clinical scanning, this is a fundamental limitation on resolution for small animals.

A major advantage of PET scanners for animals is that there are isotopes of elements that are common to biological molecules such as carbon, nitrogen and oxygen that are positron emitters. While this makes the tagging to organic molecules easier, the very short half lives of these isotopes require a nearby cyclotron and very rapid hot-lab chemistry that makes the necessary infrastructure very expensive.

SPECT Cameras Use Rotating Gammas Equipped with Pinholes

Unlike *two-photon* positron emitting isotopes, gamma ray emitters generally emit one useable photon at a time. In order to discriminate this modality from PET it was arbitrarily given the acronym "SPECT," for single photon emission tomography.⁵ While PET uses a cylindrical ring of detectors wired to detect coincidences, SPECT uses entirely different instrumentation consisting of gamma cameras fitted with pinholes and rotated about the longitudinal axis of the animal building an image from projections of gamma-ray emitting isotopes.⁶ While very small diameter pinholes are necessary to obtain high spatial resolution, they seriously limit the sensitivity since gamma rays that do not make it through the pinholes go off and are lost and gone forever.

Gamma cameras are fussy devices that require frequent adjustment and are prone to artifacts that can corrupt images.

The New Scanning Focal-Point Microscope⁷

Code-named the MollyQ™, NeuroPhysics Corporation has pioneered a very high resolution scanner using technology similar to scanning optical microscopes used to obtain detailed, three-dimensional images of thin biological tissue samples. In such microscopes, large solid angle lenses focus light to a spot that is moved about to sample the volume uniformly. The returning light signal is used to reconstruct high-resolution, tomographic images of tissue.

⁴ The ability to produce 3-D images is called "tomography." It is from the Greek "tomos" meaning slice. It first came into wide use with the invention of computed tomography (CT) scanners for clinical anatomical imaging using an x-ray tube rotating about the body. These scanners developed 3-D images from a contiguous sequence of 2-D "slices." "PET" is the acronym for positron emission computed tomography.

⁵ In Europe this is often abbreviated to "SPET."

⁶ The gamma camera was invented in 1957 by Hal O. Anger and is sometimes called the "Anger Camera." It quickly became widely used in clinical medicine.

⁷ Patent applied for.

In order to see deeper into laboratory animals, the MollyQ™ uses the more penetrating x-ray emitting radioactive isotopes. Because x-rays emitted by the biomarkers cannot be focused by conventional optics, eight special x-ray “lenses” are used for this purpose. These lenses consist of long-bore collimators each having 10,042 highly focused conical channels with 343 μm diameter entry apertures.⁸ Each collimator is coupled to an array of six scintillating crystals and photomultipliers.⁹

The huge acceptance angle of these collimators provides greatly enhanced sensitivity over anything previously available. They have been specifically developed to measure radioactive iodine-125 for which every five disintegrations yield seven 27 keV photons. Amounts of activity as low as 10^{-7} Curies can be imaged with resolutions of 2×10^{-7} liters!

Because the MollyQ™ concept is so very different from the other two modalities, we occasionally abandon the acronym “SPECT” to avoid confusion with pinhole based gamma cameras.

The Supporting Role for Anatomical Imaging

It is often very useful and even necessary to have a congruent (coregistered) anatomical image. While some information can be obtained from CT, only MRI provides details of the organs and soft tissue. This is especially true for identifying the complex structures of the animal brain and superimposing the radioisotope concentration. A number of organizations have developed MRI “atlases” of common laboratory animals. While x-ray CT is often provided with other modalities (PET/CT and SPECT/CT), only NeuroPhysics provides for seamless coregistration with MRI using proprietary animal cradles, coils, and associated electronics and software.

⁸ Biomarkers located at the foci are “seen” by the entire area of the crystals while activity less than a millimeter away is blocked. The focal points of the collimators lie *inside* the field of view and, like the scanning microscope, are moved from side-to-side and in-and-out, to uniformly sample an entire transaxial slice. Translating the animal holder between scans enables the foci to sample the entire volume of the animal. High-resolution images are obtained from a three-dimensional maximum *a-posteriori* (MAP) reconstruction similar to that used for scanning microscopes.

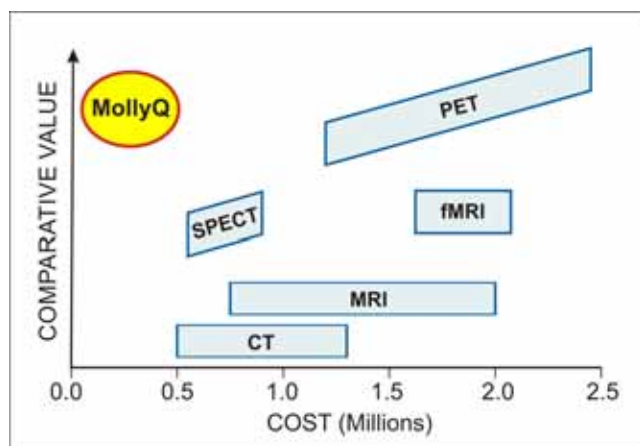
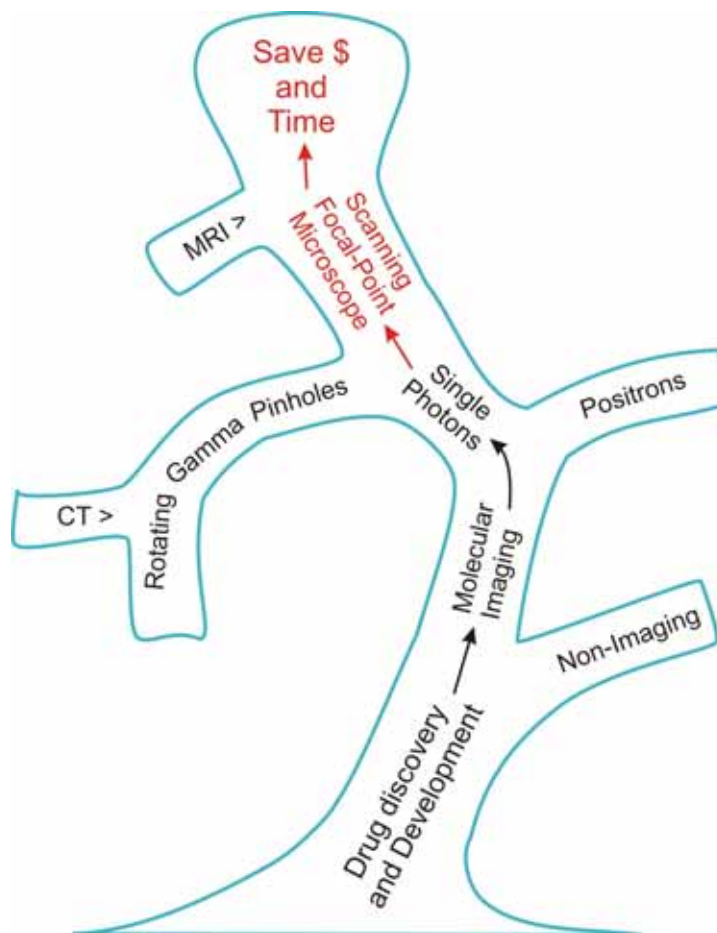
⁹ HF and HA Stoddart, the inventors and developers of scanning focal-point technology, recognized that detector rotation would be unnecessary as long as the ensemble of wide-angle collimators sampled all angles.

MOLECULAR IMAGING MODALITY COMPARISON			
	Positron Scanner	Rotating Pinhole Gamma Camera	Scanning Focal-Point Microscope
Instrumentation	Elaborate, requiring many detectors and very fast electronics	Old technology using gamma cameras invented in 1957	Unique, uses highly focused collimators and minimal parts for artifact-free images
Size	Large, requires room	Large, requires room	Small, sits on table.
Cost	~\$1,400,000 not including cyclotron	~\$700,000	~\$240,000
Emission of Radioactive Tag	511 keV Annihilation Photons	~140 keV Gamma Rays	~30 KeV X-Rays
Half Life	Minutes	Hours	Hours to Months
Source of Radioactivity	Near-by Cyclotron	Vender	Vender
Tagging Chemistry	Easy, but requires very fast (expensive) hot lab processing.	Complicated, but more time is available.	Many long half-life molecules can be purchased already tagged.
Volume Resolution (nanoliter)	Limited to 7000 nL by position travel	Depends on aperture, ~3500 nL for rats	125 nL, constant over full 50mm aperture.
Sensitivity	Intrinsically high, no collimation to absorb radiation	Very low, gamma rays must get through pinhole(s)	High, eight very large solid angle (f/0.9) collimators
Radioactivity required	Large because of short life-time	Large because of low sensitivity	Extremely low, 100 nCi in target can be imaged
Ease of Use	Requires large scientific staff to operate cyclotron, hot lab, and scanner	Requires physicists to keep gamma cameras running properly	Any laboratory technician can operate scanner
Coregistered Physical Imaging	CT	CT	MRI
Acronym	PET	SPECT	MollyQ™

The Decision Tree to Optimal Molecular Imaging

Starting at the bottom of the figure on the right, the first decision is whether or not to use positron isotopes and PET scanners. As has been pointed out, the chemistry of tagging organic molecules is easy. The disadvantage is the huge commitment of capital, staff and operating funds. For these reasons, positron systems are being less used and few new ones are being installed.

Moving up the tree, molecular imaging systems that do not require positron-emitting isotopes are more convenient and less expensive than PET. Here we must choose between rotating gamma cameras with multiple pinholes (SPECT) and the new MollyQ Scanning Focal-Point Microscope. While the rotating gamma camera has been available for some time and is more widely used than positron systems, the MollyQ provides molecular imaging with the highest sensitivity and resolution at the lowest cost. Unlike rotating gamma cameras, it can sit on a desk and be operated by a laboratory technician.



Many tagged biologically important molecules are readily available from suppliers.¹⁰

The MollyQ is available with holders and coils for a variety of laboratory animals. These make possible the coregistration of MRI soft tissue anatomy with the distribution of tagged molecules.

A comparison of various molecular and anatomical imaging modalities is shown at the left.

¹⁰ The wide choice of "off-the-shelf" tagged molecules uniquely available to the MollyQ is the result of their applications in autoradiography!