**FRAP and FLIP**

Fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) exploit the principle that high doses of excitation radiation destroy the fluorophore, resulting in quenching of fluorescence. In FRAP, a brief burst of very intense laser light bleaches a small defined area, whereupon diffusion from surrounding areas will re-establish the signal. FLIP uses continuous irradiation, which is just strong enough to quench fluorescence in certain exposed areas but leaves it at least partly intact in more protected spaces; this allows subcellular compartments to be visualized.

### 1.6. Other Imaging Technologies and Overarching Approaches

**Ultrasound and Photoacoustics**

*Ultrasound* (by definition, sound with a frequency > 20 kHz, although clinical diagnostic equipment operates in the 1- to 20-MHz range) is the most widely used cross-sectional imaging modality worldwide. In its basic mode, ultrasound imaging operates like a sonar: Trains of brief ultrasound pulses are emitted from a transducer, which is placed on the body surface, and the returning echoes are used to reconstruct an image that is initially two-dimensional, containing information on position and ultrasound reflectivity. However, for each point of this image, the signal travel time also contains information on the distance of the target and the type and density of the tissue between the transmitter and the target (which influences speed of sound and attenuation). If the target is moving, the Doppler effect will change the frequency of the echo signal, depending on the target's speed and direction. Modern ultrasound equipment can integrate this additional information into the processed image.

In the context of molecular imaging, ultrasound has received comparatively limited attention. However, recent developments are beginning to change this. Microbubble contrast agents—blood pool agents that spontaneously form gas-filled microscopic bubbles when they are exposed to ultrasound (a phenomenon called cavitation) and emit ultrasound when these microbubbles collapse—can be directed to specific molecular targets, for example, integrins that are expressed on the surface of solid tumors, on inflamed intestinal mucosa, or on growing blood vessels. Contrast-enhanced ultrasound is also able to differentiate vulnerable atherosclerotic plaques from stable ones.
Fluorescence energy transfer (FRET; see Chapter 1) is a well-established embodiment of the proximity assay principle. It requires two independent fluorophores (a primary donor and a secondary acceptor) that have overlapping fluorescence excitation and emission spectra to come within less than 10 nm of one another. If this is the case, FRET will trigger a secondary emission from the acceptor fluorophore as soon as the primary (donor) fluorophore undergoes photoexcitation (which requires a wavelength to which the acceptor fluorophore cannot respond directly). Either fluorophore can be attached to the target or to the probe (Figure 2.1).

**Figure 2.1. Fluorescence Resonance Energy Transfer (FRET) Principle**

- **No FRET signal**
  - CFP is excited by light and emits light
  - CFP is more than 10 nm away from YFP
  - YFP is not excited and emits no light

- **FRET signal**
  - CFP is excited by light but emits only a reduced amount of light
  - CFP in close proximity (1–10 nm) to YFP
  - YFP is not excited by light but emits light

CFP, cyan fluorescent protein; em, emits; ex, excited; YFP, yellow fluorescent protein.

*Source: Insight Pharma Reports*
Diagnostic Imaging at Nuclear Medicine Centers and at the Doctor’s Office

In NSCLC, the relatively modest increase in survival must be balanced against the toxicity of aggressive chemotherapeutic treatment, which makes the case for monitoring therapeutic response especially compelling. A recent small study, conducted at the Universities of Tennessee and Western Ontario, suggests that two sequential FDG-PET scans—1 and 3 weeks after initiation of cancer therapy—can predict success or failure of the therapy.41

All considered, the role of PET and PET/CT in lung cancer can be summarized as follows:

- Improved differentiation between benign and malignant nodules in primary diagnosis
- Staging of lung cancer, especially assessment of hilar/mediastinal nodes and detection of occult distant metastases
- Providing accurate information for choosing between potentially curative and purely palliative treatment modalities
- Assistance in treatment planning (exact tumor delineation and target volume definition, evaluation of treatment response)

Optical Breast Imaging: Beyond Digital Mammography

According to the National Breast Cancer Coalition, approximately 180,000 new cases of breast cancer are diagnosed in the United States every year. Molecular imaging plays an increasing role in confirming these diagnoses and monitoring tumor response to therapy.

It is estimated that, in 2008, close to 40 million x-ray mammograms will be performed at the almost 9,000 sites that have FDA certification to perform this procedure. Mammography, whether conventional or digital (i.e., with image processing and pattern recognition), misses between 25% and 40% of all breast cancers, and its sensitivity is even worse in the group of predominantly young women with dense breasts who have a much higher incidence of breast cancer to begin with. Mammography is also not very specific in differentiating early-stage tumors from cysts or other benign lesions: 60% to 80% of women who undergo follow-up biopsies of conspicuous initial results are confirmed not to have breast cancer.

Advanced Research Technologies (Montreal, Quebec, Canada) has developed SoftScan, a transillumination optical breast imaging device that is essentially a much-enhanced version of a decades-old visible-
With PET and SPECT being the imaging modalities with the highest reimbursement rates, it came as no surprise that these were among the hardest hit imaging procedures. By mid-2007, actual imaging center experience showed that the new regulations had caused revenues from the technical component of imaging to fall 35% across the board. PET/CT centers, which are particularly dependent on the federal insurance program, saw their technical rates fall by 63%. Centers closed, and center valuations plummeted. The medical imaging device industry saw sales of scanners fall by $125 million.

On March 1, 2007, a new version of a bipartisan bill, entitled the “Access to Medicare Imaging Act of 2007,” was introduced into the House of Representatives, seeking a 2-year moratorium on the DRA’s reimbursement cuts. By September 2007, the bill had 155 cosponsors, including members of the Energy and Commerce Committee from both political parties. A similar measure in the Senate had 27 cosponsors by this time. These motions were still in progress in late 2008, without substantial new developments.

4.4. Regulation of Tomographic Scanners and Picture Archiving Systems

Tomographic Scanners

All clinical scanners for human diagnostic use are rated as medical devices by the US Food and Drug Administration, which means that they need a 510(k) premarket clearance under the Medical Device Amendments to the Federal Food, Drug and Cosmetic Act of 1976. Data must be submitted to demonstrate their “substantial equivalence to a legally marketed predicate product.” All nuclear tomography systems are currently classified as Class II devices.

On December 3, 1998, the FDA issued a “Guidance for Industry” document entitled “Guidance for the Submission of Premarket Notifications for Emission Computed Tomography Devices and Accessories (SPECT and PET) and Nuclear Tomography Systems.” Although it is already 10 years old, it is without a successor, and therefore it is still supposed to represent current thinking at the FDA. The guidance is available at www.fda.gov/cdrh/ode/2240.pdf.
A small opening on the internal door of the incubator allows the operator to add liquids to the culture using motorized opening and closure of the respective dish’s lid. The Olympus MetaMorph software is used for image processing.

Olympus has set up a dedicated Web site (http://fsx.olympus-global.com/en/) for its FX100 fluorescence microscopy imaging station that was launched in 2008. This machine, which looks more like a plate reader than the computerized microscope that it actually is, focuses on easy image acquisition in three microscopy modes (fluorescence, phase contrast, and bright field) and four acquisition modes. Once these modes are chosen and the specimen is inserted, a section of the field of view can be framed, and fluorescence channels can be changed simply by using the mouse. Up to 30 bookmarks can be attached to an image. Target search and autofocus functions are available and can be configured.

Nikon

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As with Olympus, Leitz, and Zeiss, Nikon is a leading camera manufacturer that has a traditional standing in microscopy; the company says it had a 25% share in the global microscopy market in 2007. Its involvement in live cell imaging has been particularly strong. By the end of the year 2007, Nikon had established seven collaborative cellular imaging centers, including the Nikon Oxford Molecular Imaging Centre (NOMIC) at the University of Oxford, United Kingdom (opened in March 2006; www.nikonomic.co.uk), and the Nikon Imaging Centre (NIMCE; http://nimce.curie.fr) at the Curie Institute, which is part of France’s National Center for Scientific Research (CNRS).

In January 2008, Nikon launched the fully automated A1 series of confocal laser scanning systems that fully integrates with the company’s Ti-E research inverted microscope. The A1 series offers many new features, including Nikon’s exclusive DISP (Dual Integration Signal Processing) and a high-speed fiber-optic communication data transfer.
the brakes on commercial molecular imaging (Figures 12A–14A). The latter belief is in line with our own assessment of the perspectives for clinical applications.

The fact that no less than 82% of respondents from academia performed their imaging studies for drug or label development, and that 63% had been involved in preparing their data for regulatory submissions, illustrates how much transitional research in the molecular imaging sector is going on at universities. Virtually all of our respondents who conducted optical imaging in live animals came from academia, and performed their experiments in the framework of a drug or label development project. Much of this certainly reflects collaboration with pharmaceutical companies, which have implemented significant intramural molecular imaging efforts in high-content screening but prefer to rely on academia in other matters. Most certainly, universities are the leading force in developing new tracers and probes.

**Survey Questions**

**Figure 1A. Definition of Molecular Imaging**

What definition of molecular imaging do you personally prefer?

- “Techniques to directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications” (RSNA definition) 7
- “The visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems” (SNM/MICoE definition) 22
- Other 1

n = 30

*Source: Insight Pharma Reports Molecular Imaging Survey—November 2008*