In Vivo Bioluminescent/ Fluorescent Imaging Program

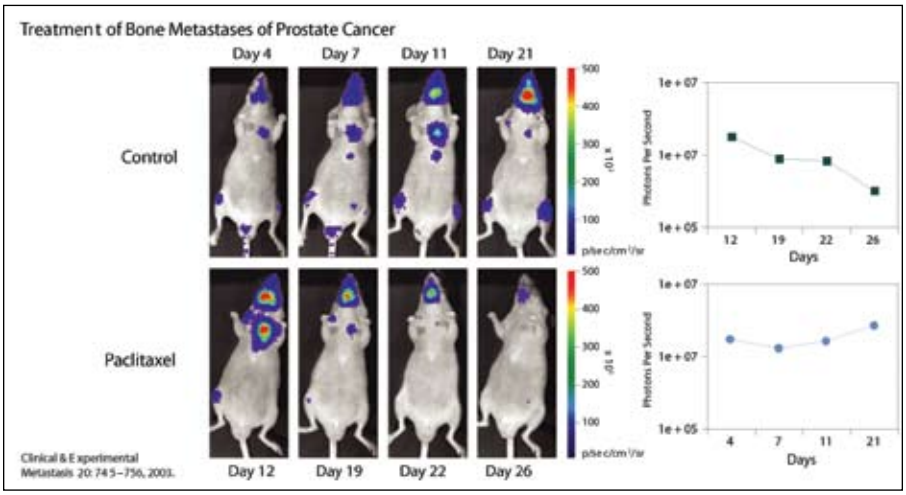
Overview Sheet

CDAS offers extensive *in vivo* expertise, intellectual property, and years of experience with biophotonic *in vivo* imaging.

In recent years, pharmaceutical companies have invested heavily in genomics, proteomics, bioinformatics, combinatorial chemistry, and high throughput *in vitro* screening to identify and develop potential therapeutic agents. Although these technologies identify increasing numbers of compounds as drug development candidates, decades-old animal modeling techniques have shown that only a limited number of these candidates will enter late-stage pre-clinical testing. In addition to being inherently low throughput, traditional animal modeling techniques are marginally predictive of success in human clinical trials, and a high failure rate in drug development persists. New technologies are developed to overcome the bottlenecks in animal testing To that end, CDAS is pleased to offer access to its *in vivo* expertise, intellectual property and know-how with respect to real-time *in vivo* biophotonic imaging. Pharmaceutical and biotechnology companies may now access this proprietary patented technology held by Caliper Life Sciences through research collaborations with its subsidiary CDAS.

Real-time *In Vivo* Imaging

In vivo biophotonic imaging offers increased throughput, allowing in vivo testing on a larger number of drugs than with conventional technologies. Moreover, CDAS' real-time *in vivo* imaging offers a more predictive model, since more and higher quality data can be collected earlier in the development process for those drug candidates that are evaluated *in vivo*. Therefore, the combination of higher throughput and more predictive models is likely to improve the productivity of the drug development process. This real-time *in vivo* imaging utilizes the light emitted by a bioluminescent or fluorescent reporter gene (or fluorescent molecule, such as a dye or quantum dot) expressed in a living organism, and then analyzes the source and strength of that bioluminescent or fluorescent signal non-invasively, allowing extensive longitudinal modeling in the same live animal.



In vivo monitoring of multiple metastatic lesions and their response to paclitaxel treatment over time.

By measuring and analyzing the light emissions, researchers can monitor cellular or genetic activity, and use the results to track gene expression, the spread of disease, or the effect(s) of a new drug candidate *in vivo*.

Capturing, quantifying, and analyzing the light emitted from the animal requires an extremely sensitive camera system capable of detecting exceptionally low light levels.

The IVIS Imaging System and Living Image software controls image acquisition and data analysis for biophotonic imaging

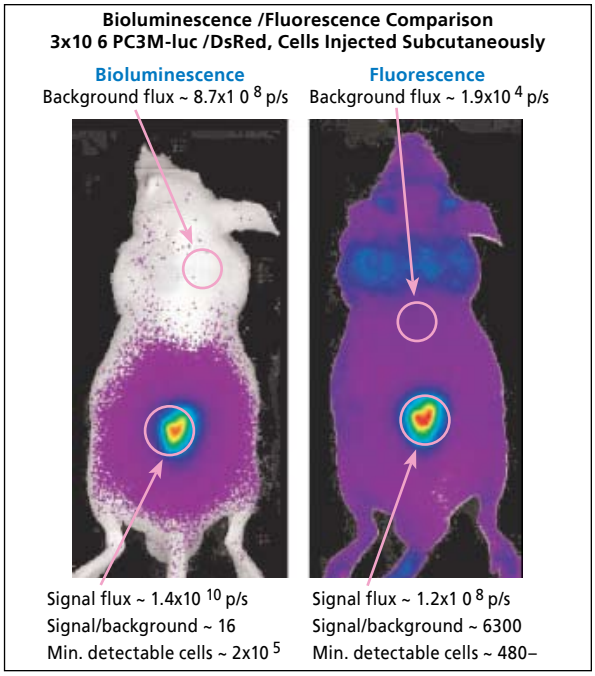
IVIS Imaging Systems from Caliper integrate CCD cameras, optimized imaging chambers, and custom Living Image software to accomplish the task. The IVIS Imaging Systems use back-thinned, thermoelectrically-cooled CCD cameras to provide high-efficiency photon detection, particularly in the red region of the spectrum where tissue transparency is highest, and offering a high signal to noise ratio for maximum sensitivity. Researchers can view an entire animal or focus on one organ or system for added detail and sensitivity. The Living Image software provides an interface for imaging and data capture. Further, the system acquires images per user settings and displays the data as an overlaid color image, recording the emitted and quantified photon data. The colors in the images represent the number of photons emitted per unit area, with an adjustable scale to allow enhancement of detail in any data set if required.

R&D Benefits

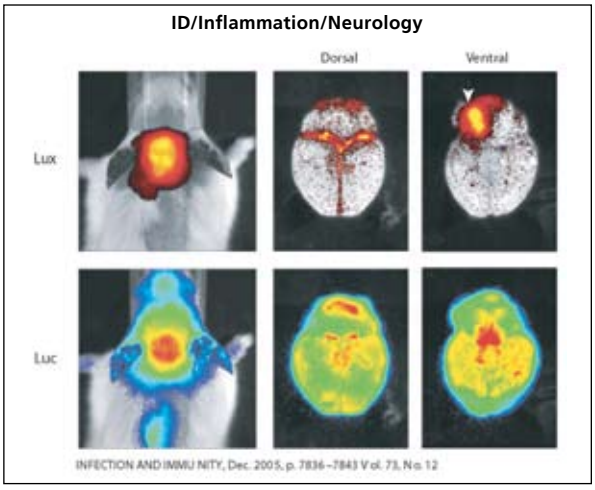
Real-time *in vivo* imaging is designed to provide:

- **Higher Throughput** — Caliper's proprietary imaging technology requires fewer test animals and less time than other animal testing models, allowing more compounds to be tested for efficacy or toxic effects.
- **Higher Data Content and Quality**— temporal and spatial data can be collected from the same animal over multiple time points. Also, the response to treatment can be assessed without the need for measuring circulating markers or terminal histological assessments. This also decreases the statistical error inherent in conventional methodologies, improving data quality.
- **More Predictive Animal Models** — By collecting data from intact, living animals, more accurate predictions can be made earlier in pre-clinical development as to how drug development candidates will perform in the clinic.

- Ideal for small animal imaging
  - Small tissue depths
  - Relatively simple instrumentation
  - Easy to learn
- *In vivo* tracking and monitoring of tumor cells, stem cells, bacteria
- Study of gene function
- Quantitative – light output is proportional to number of labeled cells



Fluorescent signal is limited by tissue autofluorescence. Signal to background is 400x higher for bioluminescent even though the signal level is 120x lower.

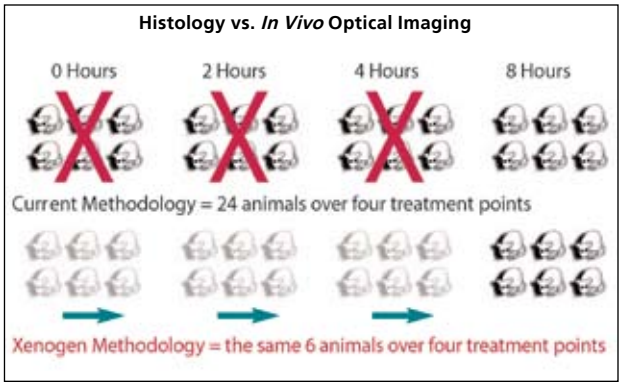


In vivo and *ex vivo* images of brains from mice with meningitis at 19 h post-infection. Dorsal and ventral views of an *ex vivo* brain show the bacterial and GFAP signals individually.

Applications

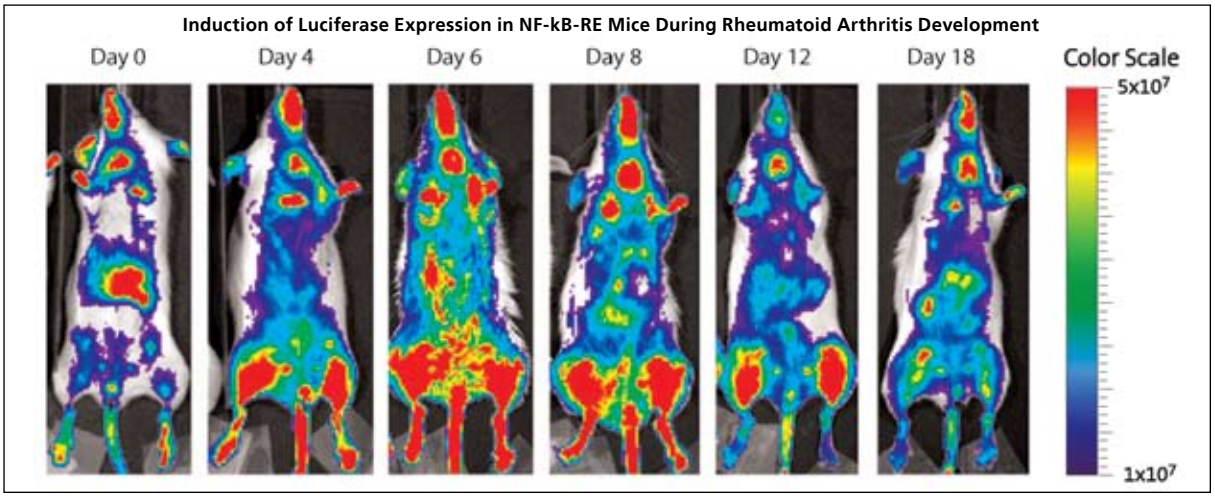
Real-time *in vivo* imaging offers the advantage of noninvasively tracking the progression of cellular growth and/or monitoring the expression of a reporter gene when challenged in a disease model or with a compound. Specifically, biophotonic imaging has been successfully applied to a broad range of critical drug discovery/development challenges that span many therapeutic areas, including:

- Oncology (monitoring tumor growth and metastasis, and the expression of key genes involved in oncology and related processes, such as angiogenesis or apoptosis)
- Infectious diseases (tracking and monitoring bacterial, fungal, protozoal, and viral infections)
- Immunology, Inflammation and Autoimmune Diseases (monitoring the expression of key genes involved in inflammatory pathways and/or tracking and monitoring T- or B- lymphocytes).



Same group of anesthetized test animals at each time point of an experiment uses far fewer animals than current methodology. By using the same set of animals at each time point yields improved statistical relevance.

- Cardiovascular (monitoring the expression of key genes involved in the cardiovascular and lipid metabolism pathways)
- Diabetes and Obesity (monitoring endogenous or transplanted pancreatic beta cells; monitoring the expression of key genes involved in the diabetes and obesity pathways)
- Neurology (monitoring the expression of key genes involved in neurological processes such as neuroinflammation (GFAP), neuroprogenitor cell lines and neurodegeneration (fluorescent probes are currently available to image plaques in the brain).



Female NF-kB-luc mice were i.v. injected with arthrogen-CIA monoclonal antibody cocktail to induce rheumatoid arthritis development.

In addition biophotonic imaging has been used to assess:

- Drug Metabolism (monitoring the expression of key genes involved in drug metabolism)
- Gene Therapy Vectors (Biodistribution of potential vectors)
- Formulations (Biodistribution of proprietary formulations and related drug delivery systems)
- Stem Cell Research (tracking and monitoring stem cell populations)

In addition, CDAS offers a comprehensive program for commercial-scale transgenic and gene targeted animal creation and characterization. This includes the creation of Light-Producing Animal Models for use with Caliper's *in vivo* biophotonic imaging technology.

Please contact us to discuss how we can develop a program using this technology to make your research faster and more efficient. Caliper Life Sciences holds the exclusive license under U.S. patent numbers 6,649,143, 5,650,135, 6,217,847, 6,916,462, 6,890,515 and 6,908,605 relating to methods of non-invasive, biophotonic imaging across a wide range of wavelengths and specifically including light generated by bioluminescence and fluorescence.

Contact Information

For all *In vivo* Biophotonic Imaging and other Animal Model Creation and Characterization Programs, please call: 1.609.860.0806 or e-mail: transgenics@caliperls.com

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