



LI-COR<sup>®</sup> Biosciences  
November 30, 2010

Upcoming webinars: [request registration reminder](#)

Part 5. ***In Vivo* Cancer Imaging: Targeted Fluorescent Contrast Agents for Image-Guided Cancer Surgery**, February 1, 2011 (*note: date has changed from announcement at webinar*)

On-demand video webinars:

Part 1. **Introduction to Near-Infrared (NIR) Fluorescent Methods:** [Watch now!](#)

Part 2. **Quantitative Protein Analysis in Cancer Research:  
NIR Fluorescent Western Blots and Cell-Based Assays:** [Watch now!](#)

Part 3. ***In Vivo* Cancer Imaging with IRDye<sup>®</sup> Optical Probes:** [Watch now!](#)

Part 4: **Development of Custom NIR Probes:** [Watch Now!](#)

1. Targeted Optical Agent Development Workflow
  - a. Identify Agent
  - b. *In vitro* testing: Immunocytochemical assays and microscopy
  - c. *In vivo* Clearance
  - d. Establish tumor model and dosing
  - e. Tumor studies
  - f. Whole organ
  - g. Histology
2. IRDye 800CW Labeling Chemistries
  - a. NHS-ester
  - b. Maleimide
  - c. Carboxylate
  - d. Optimize Dye:Protein (D/P) Ratio
  - e. Purification
3. Begin Characterization: *In vitro*
  - a. Desired outcome
  - b. Undesired outcome
  - c. Carboxylate controls
  - d. Assessing signaling pathway involvement

[Gong, H, J Kovar, G Little, H Chen, and DM Olive. \(2010\) Neoplasia 12\(2\):139-149](#)

e. Immunocytochemical plate-based assays

- i. Binding
- ii. Competition

[Kovar JL, W Volcheck, E Sevick-Muraca, MA Simpson, and DM Olive \(2009\) Anal Biochem 384\(2\): 254-262](#)

f. Data examples:

- i. Binding
- ii. Competition
- iii. Stimulation

[Kovar JL, W Volcheck, E Sevick-Muraca, MA Simpson, and DM Olive \(2009\) Anal Biochem 384\(2\): 254-262](#)

[Gong, H, J Kovar, G Little, H Chen, and DM Olive. \(2010\) Neoplasia 12\(2\):139-149](#)

4. [Microscopy](#)

- a. Cytoplasmic location
- b. Cell-surface binding
- c. Endocytotic uptake

5. Clearance, Dosing, and Tumor studies

- a. Clearance Profiles
- b. Route of Administration
- c. Dosing

[Kovar JL, MA Simpson, A Schutz-Geschwender, and DM Olive \(2007\) Anal Biochem 367\(1\): 1-12.](#)

6. Organ Analysis and Histology

[Kovar JL, W Volcheck, E Sevick-Muraca, MA Simpson, and DM Olive \(2009\) Anal Biochem 384\(2\): 254-262](#)

[Gong, H, J Kovar, G Little, H Chen, and DM Olive. \(2010\) Neoplasia 12\(2\):139-149](#)

7. Summary

- a. IRDye 800CW is ideally situated in the spectral region to provide high signal in an environment of low autofluorescence and background. IRDye 800CW-labeled agents are valuable tools in the study of tumorigenesis and metastasis. As well as, preclinical studies evaluating the effects of therapeutic agents and studies looking at drug-to-target interactions.

Fully characterizing and validating your labeled optical imaging agent will allow you to visualize molecular events as they impact your cancer research in vivo. We hopefully have shown you a systematic workflow to achieve successful results as you develop labeled agents and implement their use in your cancer studies.

## **Reagents**

[Protein Labeling Kits](#)

[NHS Ester Dyes](#)

[Maleimide Dyes](#)

[BrightSite™ Small Animal Imaging Agents](#)

## **Instruments and Accessories**

[Pearl Impulse Small Animal Imaging System](#)

[SmartFlow Anesthesia System](#)

## **References:**

[\*Gong, H, J Kovar, G Little, H Chen, and DM Olive. \(2010\) Neoplasia 12\(2\):139-149.\*](#)

[\*Kovar JL, W Volcheck, E Sevick-Muraca, MA Simpson, and DM Olive \(2009\) Anal Biochem 384\(2\): 254-262\*](#)

[\*Kovar JL, MA Simpson, A Schutz-Geschwender, and DM Olive \(2007\) Anal Biochem 367\(1\): 1-12.\*](#)

[\*Kovar JL, MA Johnson, MW Volcheck, J Chen, and MA Simpson \(2006\) Am J Pathol 169:1415-1426\*](#)