**Introduction**

Despite recent technological advances in cancer diagnosis including CT, MRI, and PET imaging, the main treatment modality for solid tumors is surgery. However, surgical excision of tumors is dependent on the surgeon’s ability to differentiate tumor from normal tissue using non-quantifiable criteria such as tumor location, texture, color, relationship to surrounding structures etc. Under these circumstances, the ability to detect and treat micrometastases and residual tumor in real-time is limited.

**Methods**

**Animal model**: Spontaneous tumors in transgenic mice (MMTV-PyMT), and human cancer cell lines derived from a variety of anatomic sites (breast, prostate, colon, larynx) xenografted into nude mice, were imaged after intravenous injection of Cy5-labeled ACPPs and waiting sufficient time for elimination of uncleaved ACPPs.

**Surgical Set-up**

Tumors are resected using a Zeiss Lumar fluorescence dissecting microscope equipped with a rotating filter set capable of exciting and detecting the emission from GFP, RFP and Cy-5.

Using the commercially available Metamorph software and a CoolSnap Zeiss CCD camera, a live video of the surgical field can be displayed on the monitor adjacent to the dissecting microscope. Tumor resection is done either brightfield illumination or using tumor specific labeling with the Cy-5 labeled ACPP.

**Results**

Preoperative MRI (A), fluorescence (B) and brightfield (C) images from a transgenic PyMT animal with spontaneous breast tumors showing the tumor enhancement in both MRI images and fluorescence images after IV injection of our dual probe (ACPP-Gadolinium-Cy5). Following preoperative imaging, the animal underwent surgical excision with fluorescence guidance to ensure complete tumor removal. In the postoperative images (D-F), axial MRI section (D) at the same level as the preoperative scan revealed complete tumor removal as confirmed by fluorescence imaging (E) and by brightfield imaging (F).

**Conclusion**

We describe a novel method for visualizing the margin between tumor and normal tissue in vivo during surgery using ACPP-guided molecular fluorescence imaging (MFI). We showed that ACPP guidance resulted in fewer residual tumor cells at the surgical site and tumor-free survival is superior compared to unguided surgery using standard technique. The clinical implication of these results is that tumor burden may be similarly reduced using ACPP guided surgery in human patients compared to standard unguided surgery.