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## Introduction

In a recent first-in-human study a novel approach was introduced utilizing a FDA approved therapeutic antibody for diagnostic application using an IRDye800 label (Rosenthal et al. 2015). Resected tumors were processed for standard pathological evaluation to determine the distribution of Cetuximab in the tumor and its colocalization with EGFR (De Boer et al. 2015). This conventional pathological examination, while providing high-resolution imaging of Cetuximab in the tissue, is highly limited by its spatial resolution and is randomized in section collection.

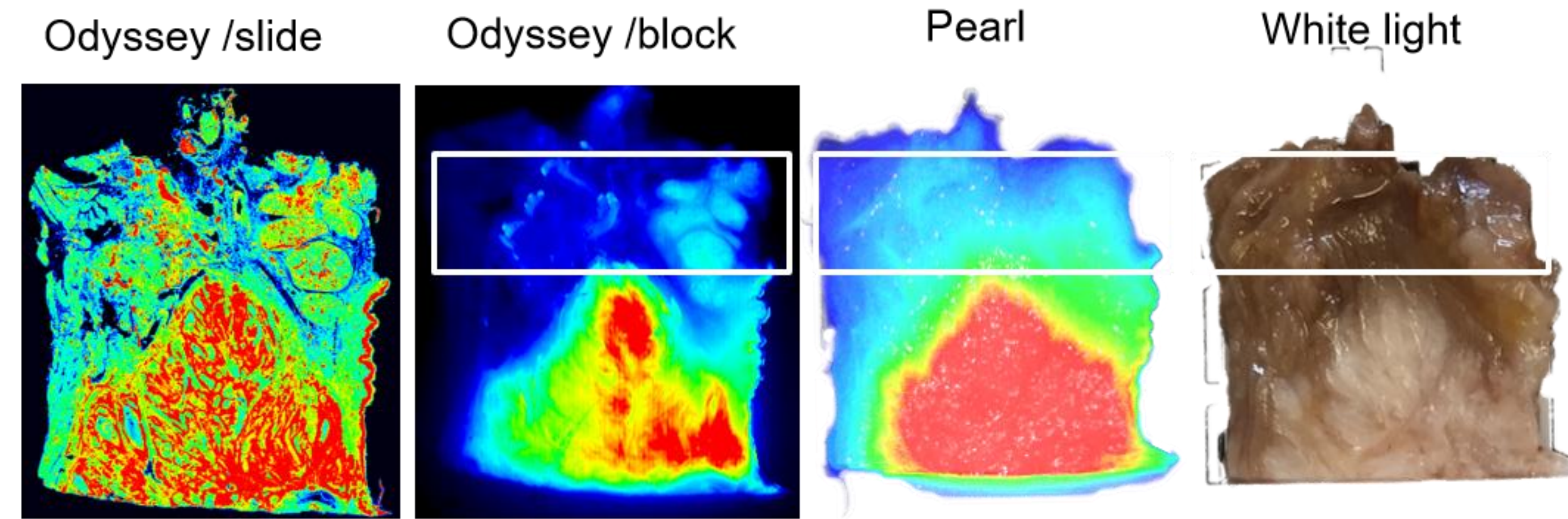
Cryofluorescence tomography (CFT) is a unique approach for high-resolution 3D imaging of large samples and can be utilized to visualize antibody bio-distribution across an entire tumor sample.

## Summary

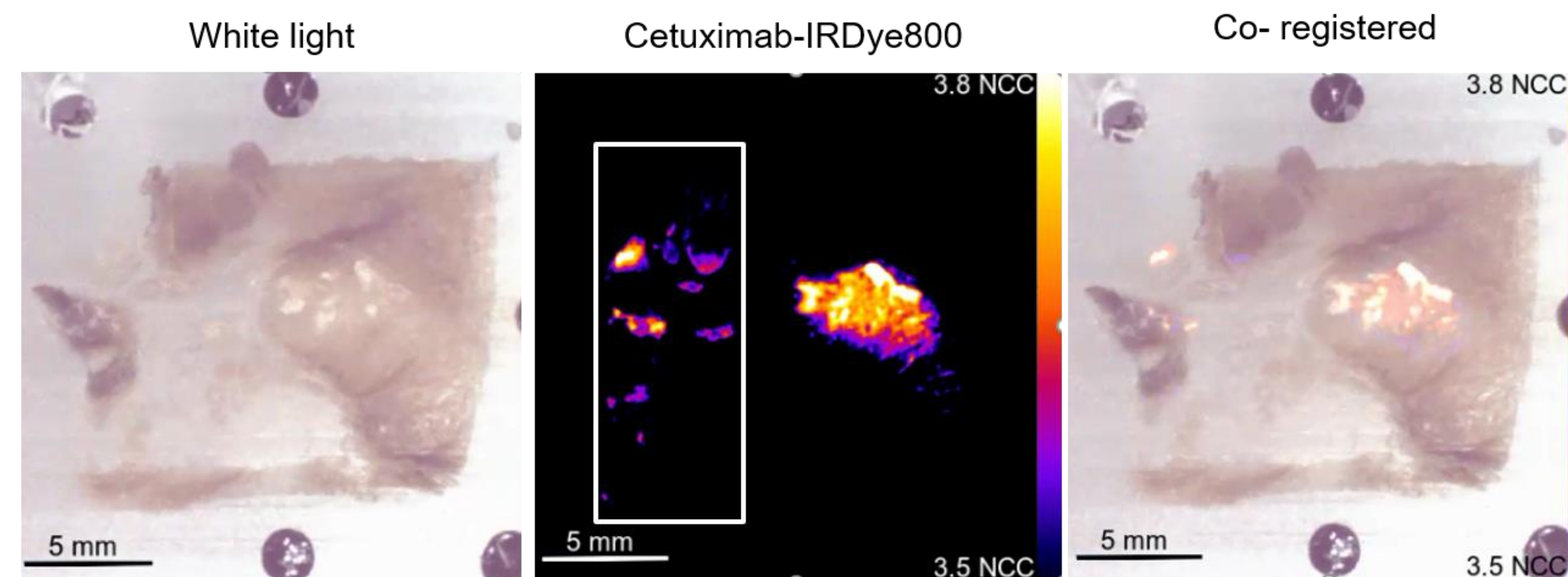
We demonstrate 3D imaging of Cetuximab-IRDye800, acquired using high-resolution fluorescence tomography across an entire formalin fixed paraffin embedded (FFPE) clinical squamous cell carcinoma sample. Maximum intensity projections (MIP) demonstrate the heterogenous distribution of Cetuximab in the sample and white light images provide anatomical information to the localization of Cetuximab in the tissue. The added information of obtained images is further emphasized when compared to tumor images acquired in the operating room, pre and post resection (figures 1 and 2, white rectangle).

Fluorescence tomography for 3D imaging of large tissue samples that is supportive of standard histology, provides an opportunity to create a digital image bank of entire clinical tumor samples. Together with a software solution, this technology can be extremely valuable to better characterize the 3D bio-distribution and localization of antibody drug conjugates, or diagnostic antibodies, across an entire sample. Opportunities to better localize drug delivery to specific compartments of the tumor or differentiate antibodies that attach to cancer cells, endothelium or immune cells will be valuable during the process of drug discovery and interrogating drug-host interactions.

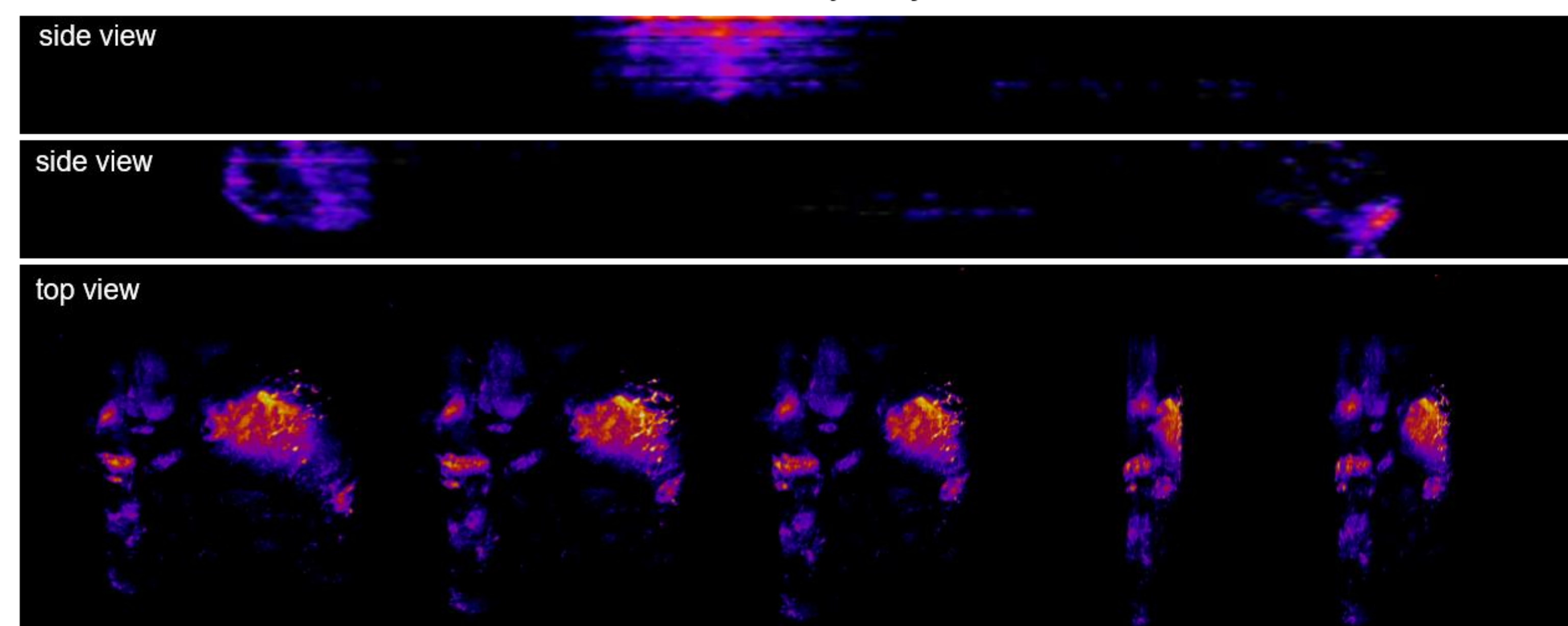
## Results



**Figure 1:** Following tumor resection 2D images of the whole tumor, or a sections of it, were acquired. Difference in Cetuximab signal across imaging modalities demonstrates the tradeoff in resolution and sensitivity when imaging the surface/subsurface fluorescence of the whole tumor compared to imaging a single section



**Figure 2:** Following the standard imaging work-flow, remaining FFPE tumor sample was imaged using CFT. Fluorescence and white light planar images were sequentially acquired off the block-face throughout the entire sample as it was sectioned. Collected 2D white light (left) and fluorescent images (middle) were co-registered (right) to provide anatomical reference to Cetuximab-IR800Dye. Collected voxel size: 31.5µm x 31.5µm x 10µm

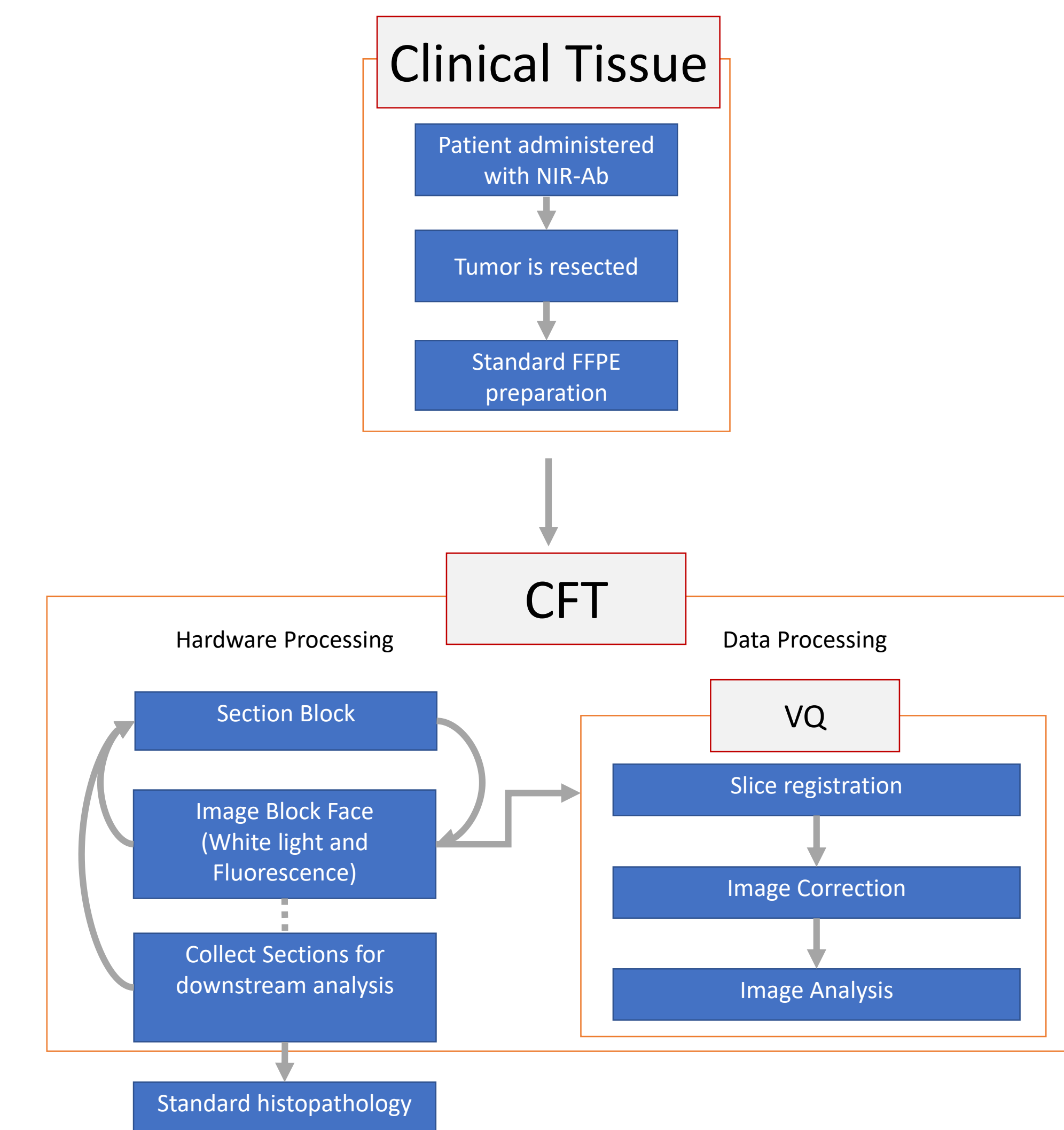


**Figure 3:** Planar fluorescent images were 3D reconstructed using fiducials markers and are visualizing the bio-distribution of Cetuximab-IR800Dye as a MIP. The collected non-isotropic voxels (31.5µm x 31.5µm x 10µm) were interpolated to isotropic voxels to allow multi-planar view.

## Methods

1. Enrolled patients were administered with 2.5mg/m<sup>2</sup>, 25mg/m<sup>2</sup> or 62.5mg/m<sup>2</sup> Cetuximab-IR800Dye
2. Whole resected tumors were imaged using several NIR imaging systems (figure 1)
3. Tumor samples were embedded in paraffin for histopathological evaluation
4. Remaining FFPE block was sectioned in the microtome with a dedicated imaging system placed in front of the block (FLARE system)
5. Fluorescent and bright field images were captured off the block face using the FLARE system
6. The acquired 2D images (white light and fluorescent) were co-registered (figure 2)
7. Planar fluorescent images were 3D reconstructed using VivoQuant (figure 3)

## CFT workflow



### References:

1. Rosenthal et al. Safety and Tumor-specificity of Cetuximab-IR800Dye for Surgical Navigation in Head and Neck Cancer. *Clin Cancer Res.* 2015 August 15; 21(16): 3658–3666
2. De Boer et al. *In Vivo* Fluorescence Immunohistochemistry: Localization of Fluorescently Labeled Cetuximab in Squamous Cell Carcinomas. *Scientific Reports.* 2015 June 29; 5:10169