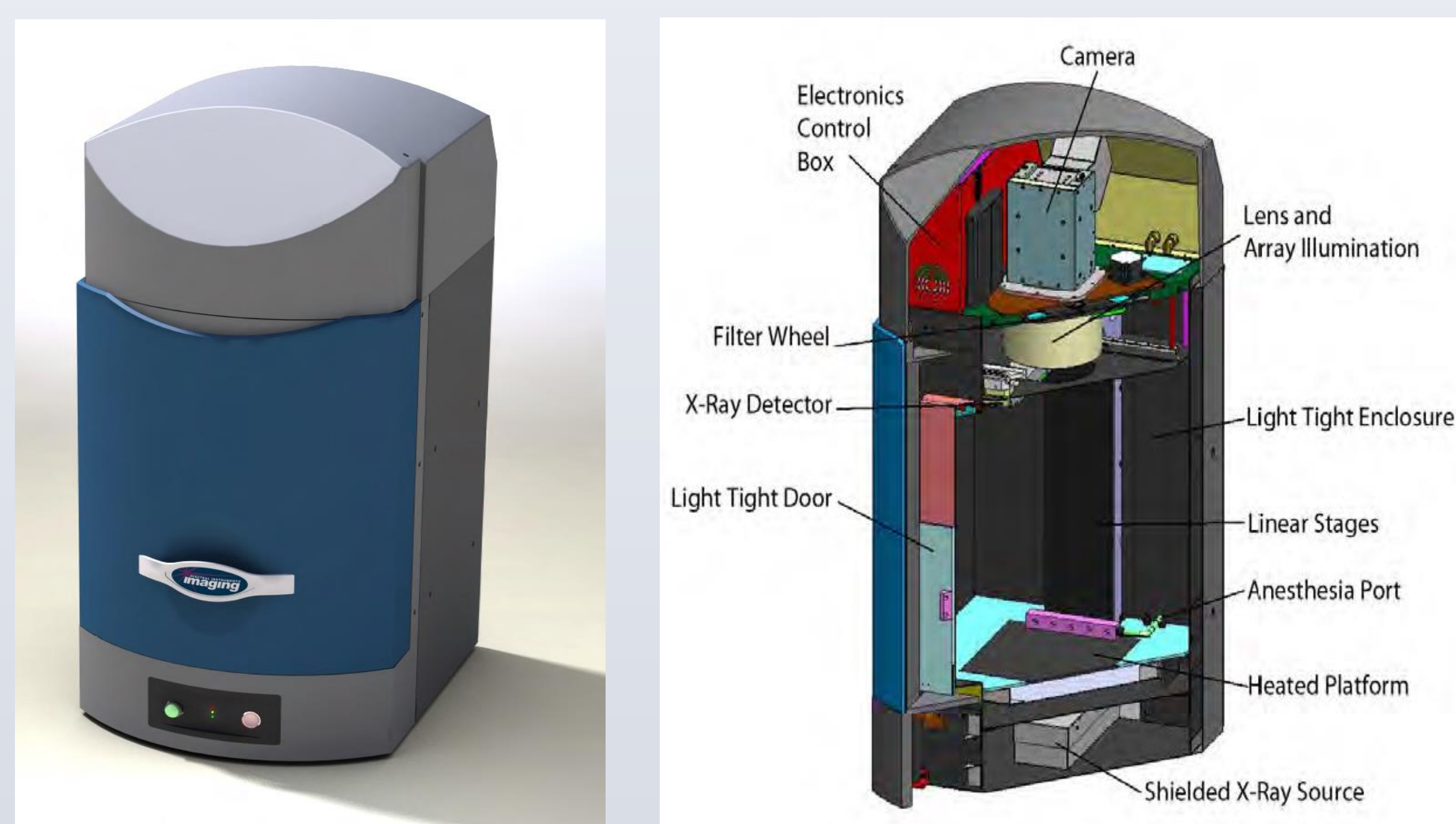


### Introduction

Cerenkov radiation (CR) is visible light that is emitted when a charged particle, such as a positron or electron, exceeds the speed of light in a medium in which it travels. In practice, it can be detected by optical imager with appropriate sensitivity. The goal of this study is to develop standardized protocols to test whether an imager can feasibly perform Cerenkov Luminescence Imaging (CLI). The AMI-1000, a commercially available optical imaging device, was then assessed using these protocols.



**Figure 1.** AMI-1000.

- Developed by Spectral Imaging Instruments, the AMI-1000 is comparable to the IVIS Spectrum, the dominant optical imaging device currently on the market.
- Acquires quantitative images for Fluorescence and Bioluminescence Imaging.
- No studies have yet been performed to investigate the ability of this machine to collect Cerenkov Luminescence Images.

### Methods

#### 1. Spatial Resolution

- A Jaszczak PET/SPECT spatial resolution phantom was injected with 350  $\mu$ Ci of  $^{18}\text{F}$ -FDG and vortexed for homogeneity.

#### 2. Linear Relationship between activity and signal.

- A longitudinal scan was taken of 3 samples with different activities, then a linear regression of all data points was performed using Excel.

#### 3. Cerenkov Radiation Spectrum

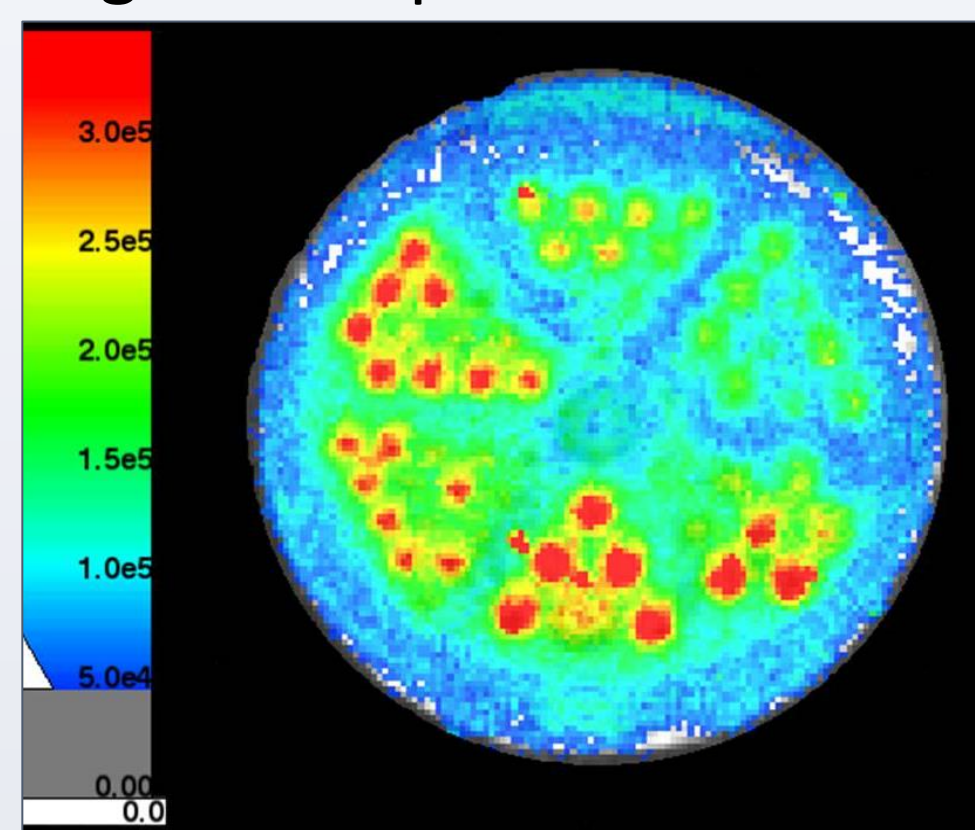
- A well established phenomenon of Cerenkov Radiation is that its spectrum varies with  $\frac{1}{\lambda^2}$  where  $\lambda$ =wavelength. This known relationship was used to assess the quantum efficiency curve of our detector.

#### 4. Correlational PET Studies

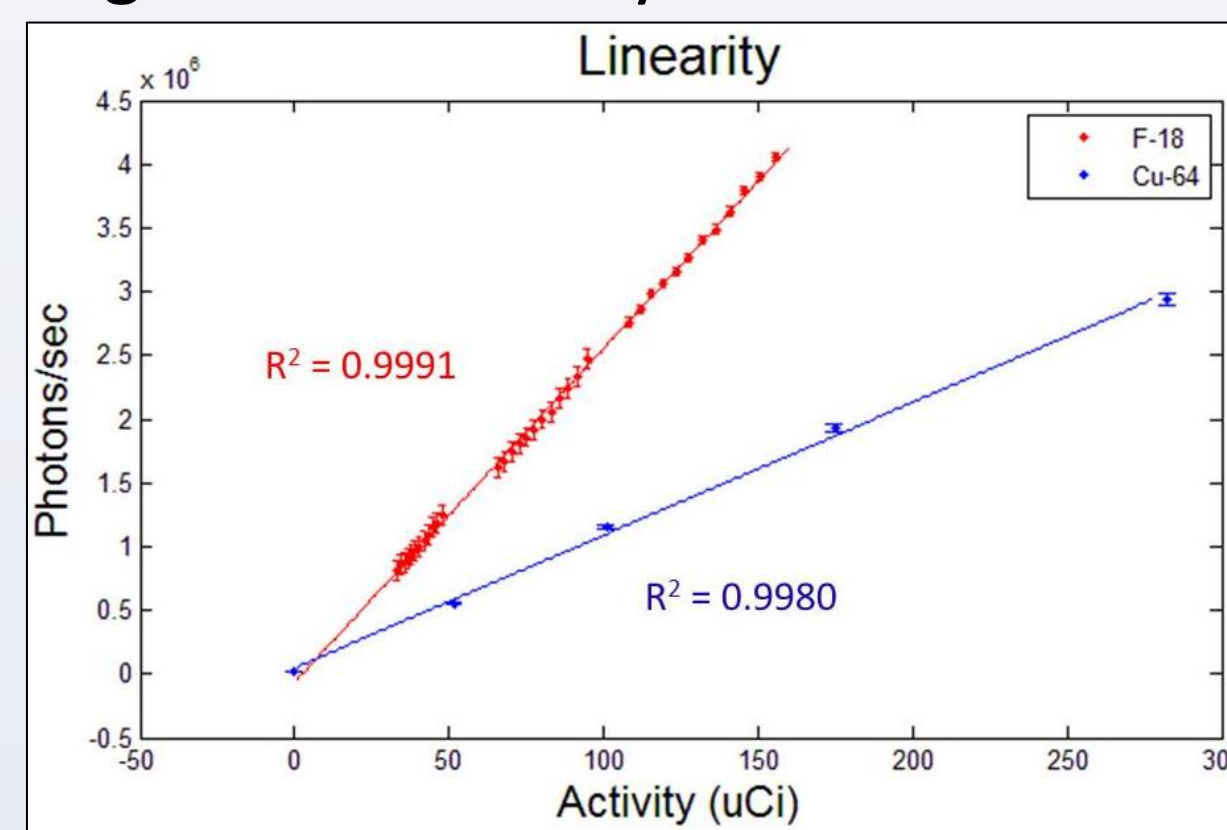
- The well-plate containing these samples was placed in the Genysis4 micro-PET scanner to investigate the correlation with the previously obtained CLI images.
- Lastly, mice injected with the two isotopes listed above were imaged in both the micro-PET and the AMI-1000 to prove that Cerenkov Luminescence Imaging is correlated with PET.

### Results (figures)

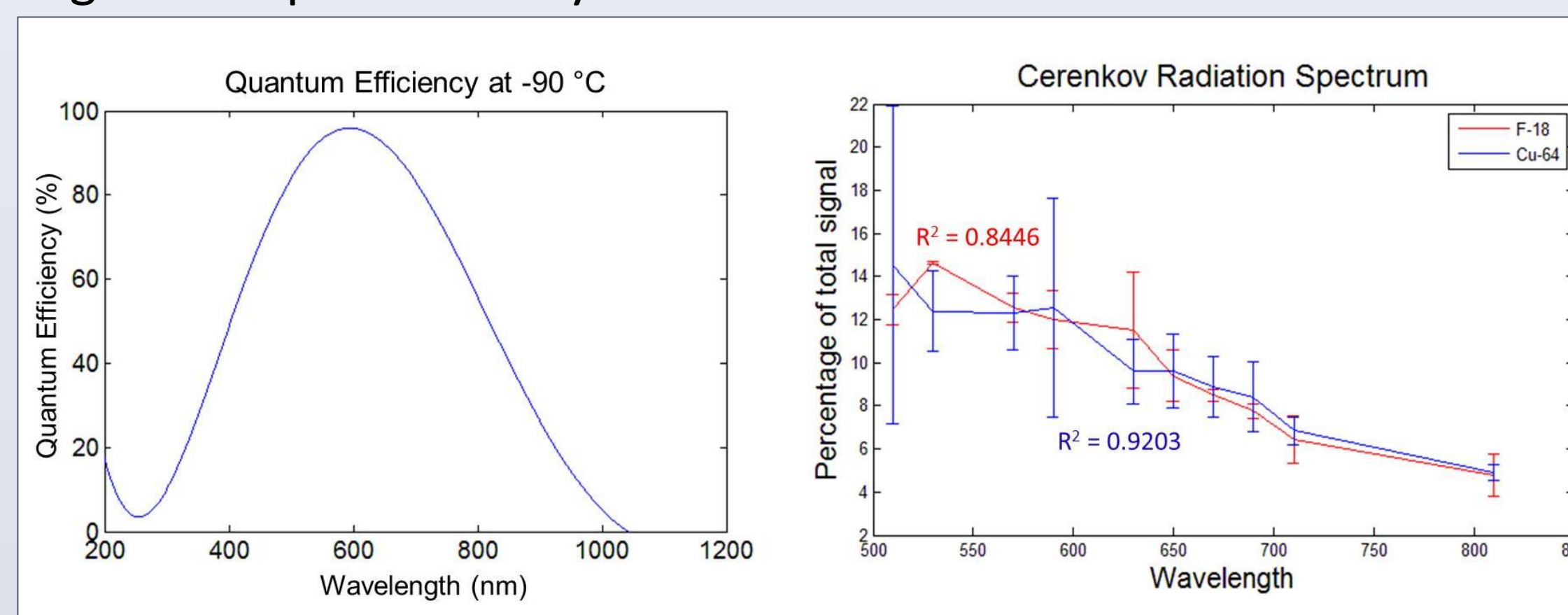
**Figure 1: Spatial Resolution**



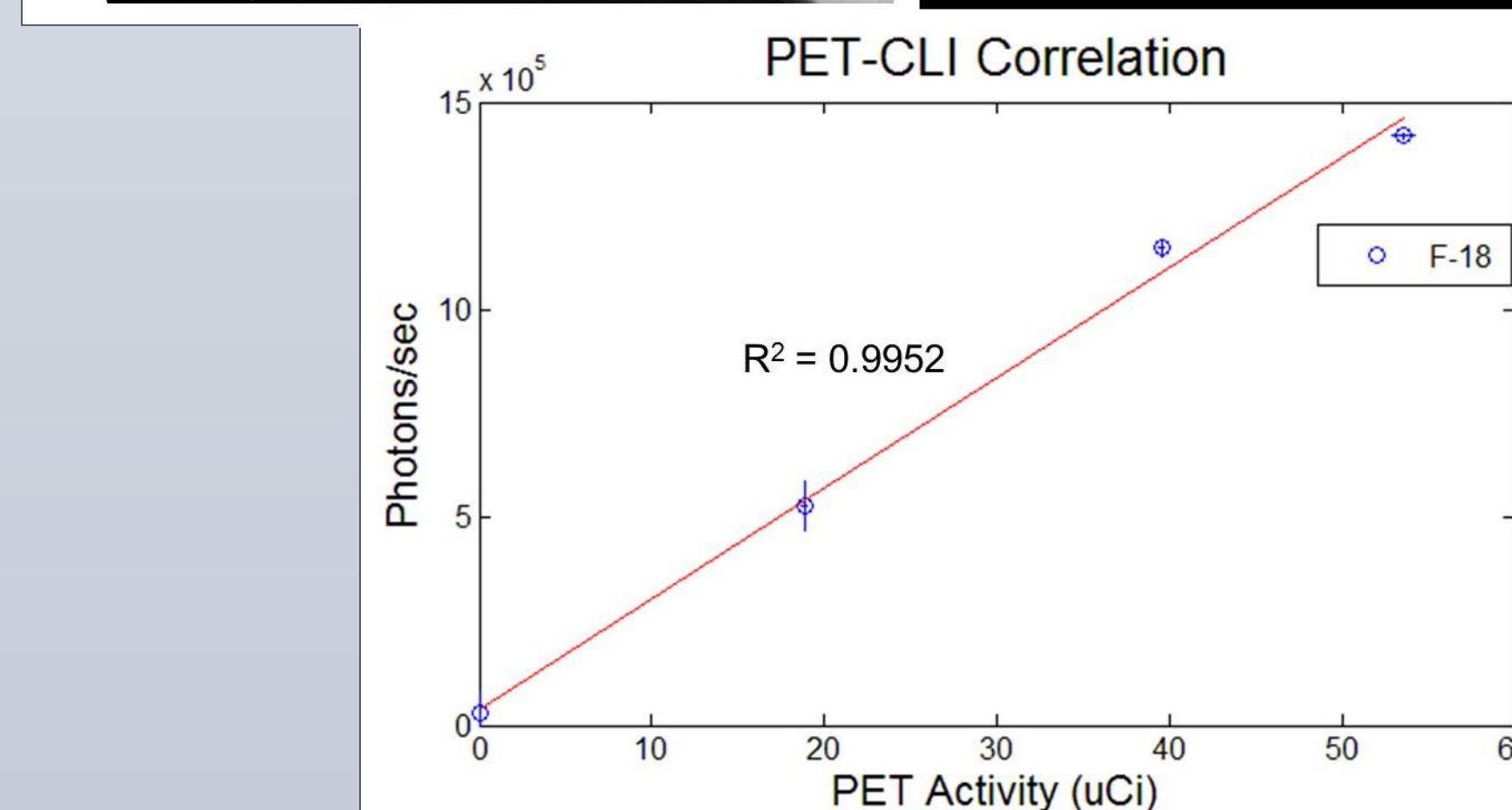
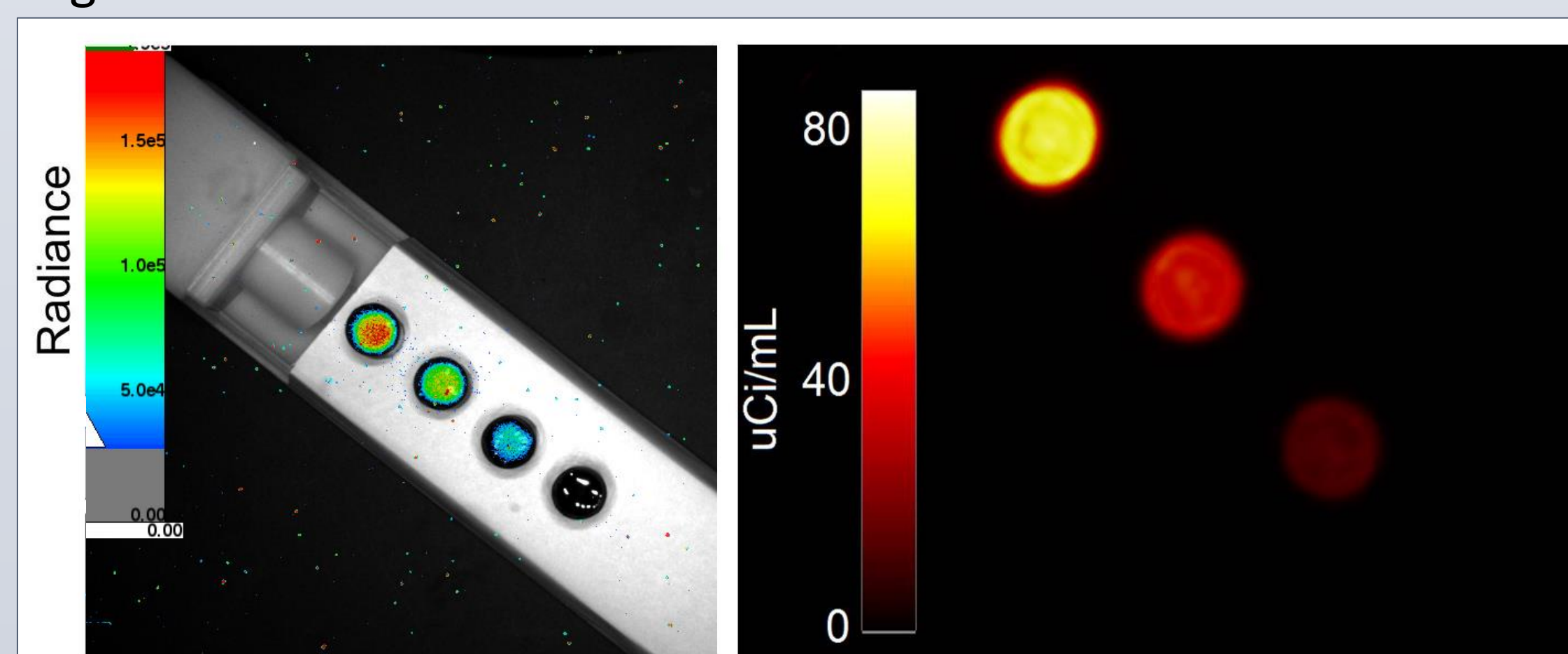
**Figure 2: Linearity**



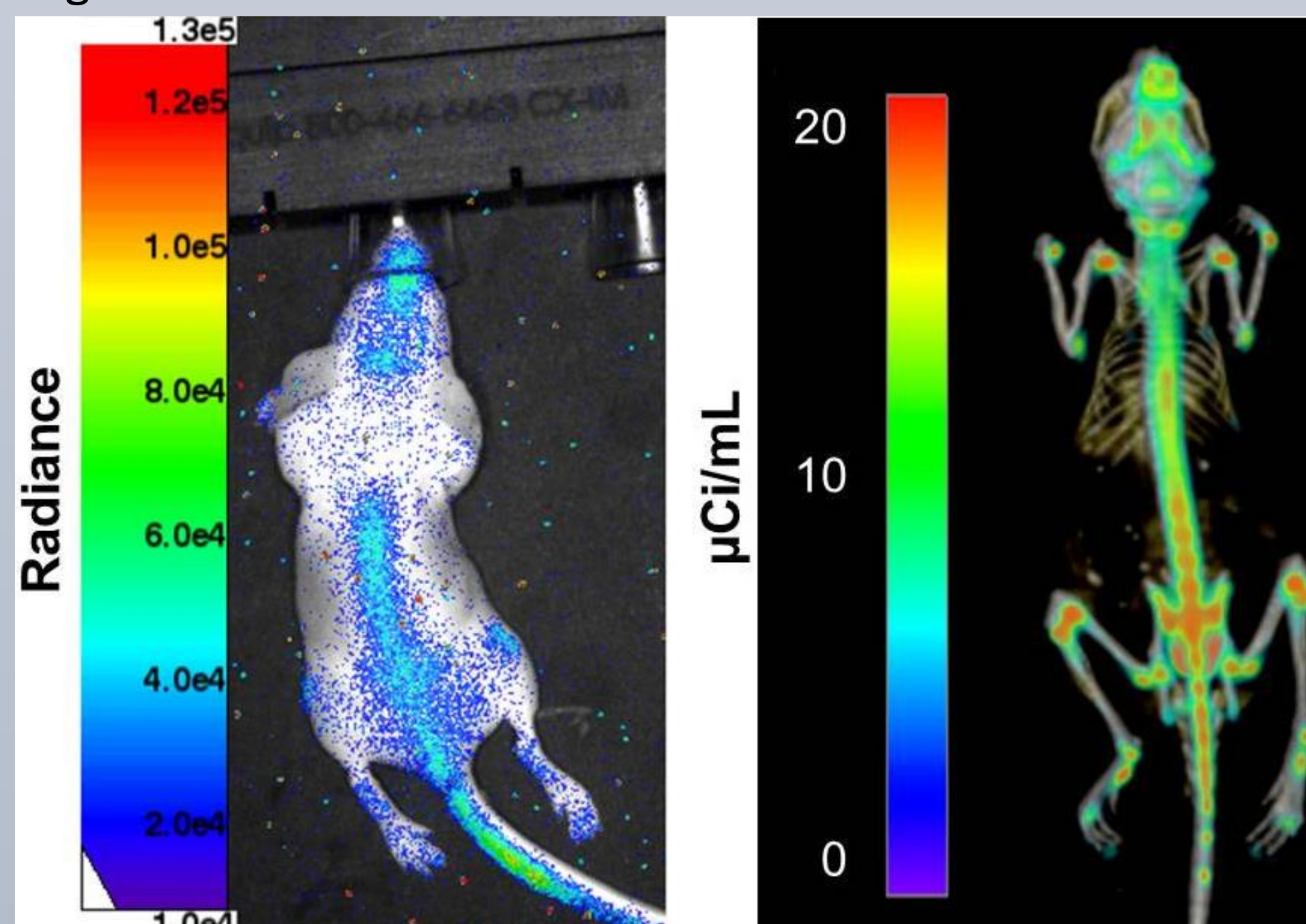
**Figure 3: Spectral Analysis**



**Figure 4: In Vitro PET-CLI Correlation**



**Figure 5: In Vivo PET-CLI Correlation**



### Results (description)

1. Spatial Resolution: A column PET/SPECT Spatial Resolution phantom was injected with 350  $\mu$ Ci  $^{18}\text{F}$ -FDG, then analyzed visually.

#### **Figure 1. Spatial Resolution.**

Visual analysis yielded a spatial resolution of 1.2 mm.

2. Linearity: It is expected that the relationship between visible light signal and activity of the source will be linear, since this should be the case if signal is coming directly from each radioactive decay.

**Figure 2.** Linearity was assessed by placing samples of varying activities in the AMI-1000 and performing a longitudinal scan. The  $R^2$  of the linear regression was determined to have a value of 0.9988.

3. Cerenkov Radiation Spectrum: We validated a quantum efficiency curve of our detector using filters of different wavelengths.

**Figure 3.** Quantum Efficiency of detector at wavelengths from UV to NIR (Left). At right, experimental data was normalized by the quantum efficiency curve by integrating over the filter wavelength  $\pm 10$  nm, then acquiring the mean value of that data. When this was compared to the theoretical Cerenkov Radiation curve, a very strong correlation was achieved.

4. Studies of *In Vitro* PET Correlation: Since CLI is quantitative in principle, we can draw quantitative conclusions about our correlation with PET. Here we compare radiance detected by the AMI-1000 to the activity measured in the PET scan.

#### **Figure 4. Correlational in vitro PET Studies.**

- As seen visually, the signals of the CLI image (Top) has a very strong correlation with the PET image (Bottom Right). The graph (Bottom Left) shows that this holds true quantitatively as well.
- The significance of this correlation is instrumental in CLI, since it conveys that CLI is measuring the same mechanism as PET. In this way, CLI can come to be accepted as a legitimate tool in pre-clinical nuclear imaging.

5. Investigation of *In Vivo* PET Correlation: Mice were injected with  $\text{Na}^{18}\text{F}$ , a tracer clinically used to assess bone activity. PET, CT, and CLI scans were obtained and compared visually.

#### **Figure 5. Correlational PET-CLI studies in mice.**

- The CLI image (Top) and the PET image (Bottom) both show uptake in the spine and growth plates in the knees.
- Note that the signal in the CLI image is blurred. The cause of this decreased spatial resolution is the high attenuation (scattering or absorption) of visible light compared to the low level of interaction of imaged  $\gamma$ -rays in PET.

### Conclusion

- We were the first group to perform CLI on the AMI-1000, and have concluded that the device is indeed capable of obtaining images for this modality.
- We also have developed several new phantoms and incorporated them into the standard protocols for Cerenkov Luminescence Imaging on optical imagers.

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