# Whole-body spatial bioimaging by Cryo-Fluorescence Tomography (CFT) imaging and ddPCR of non-human primate to reveal the full spatial expression of AAV after systemic intravenous administration

**Ciara Finucane; Jared B. Smith; Cagri Ansen; Samantha A. Yost; Amy Cao; Janeen Vanhooke; Jacob McDonald; Matthew Silva**

EMIT Imaging, Natick, MA 01760 USA (CF, AC, JV, MS); Research & Early Development, REGENXBIO Inc. Rockville, MD 20850 USA (JBS, SAY); Envol Biomedical, Immokalee, FL, USA (CA, JM)

## Introduction

While rodent models remain the most widely utilized for early adeno-associated virus (AAV) assessment, there are substantial physiological differences between rodents and primates that limit the translatability of the rodent data. However, the large size of primates complicates whole body monitoring and imaging. We reported on the use of cryo-fluorescence tomography (CFT) to compare the whole-body fluorescent expression of AAV7 and AAV9 in mice[1], identifying protein expression differences that were confirmed by droplet digital (ddPCR) analysis of tissue homogenates and microscopy data from tissue processed for immunohistochemistry.

Conventionally, AAV biodistribution in NHP tissues are interrogated similarly, by small biopsies/tissue sampling (punching ~1 $mm^3$/20 mg) each individual tissue of interest at necropsy. Limitations of this method include minimal volume of large targets like skeletal muscle, loss of spatial context in non-homogenous tissues, limited and biased tissue sample selection that miss unexpected tissues. Herein we report the extension of previous methods for whole-body, 3D CFT imaging of the common marmoset *(Callithrix jacchus)* as an unbiased, high-resolution method to evaluate total AAV expression in all tissues via fluorescent reporter proteins.

## Methods

### Methods: Animals and dosing

Marmosets (2–6 years of age, 0.30–0.35 kg) were screened for anti-AAV neutralizing antibodies (NAb), and NAb-negative animals were selected to receive a single intravenous bolus of AAV8 or AAV9 at 5e13 GC/kg via the saphenous vein. One naïve animal served as an autofluorescence control. Animals were monitored for 21 days with serial serum and plasma sampling, then euthanized on Day 22 and flash-frozen whole in a hexane/dry ice bath. All procedures were in strict compliance with animal welfare regulations. Frozen carcasses were submitted for CFT imaging (methods below). During the CFT, the imaging was paused to collect tissue biopsies from liver, heart, skeletal muscle, and brain for ddPCR analysis to enable within-animal comparison of imaging signal against molecular quantification at the same anatomical location.

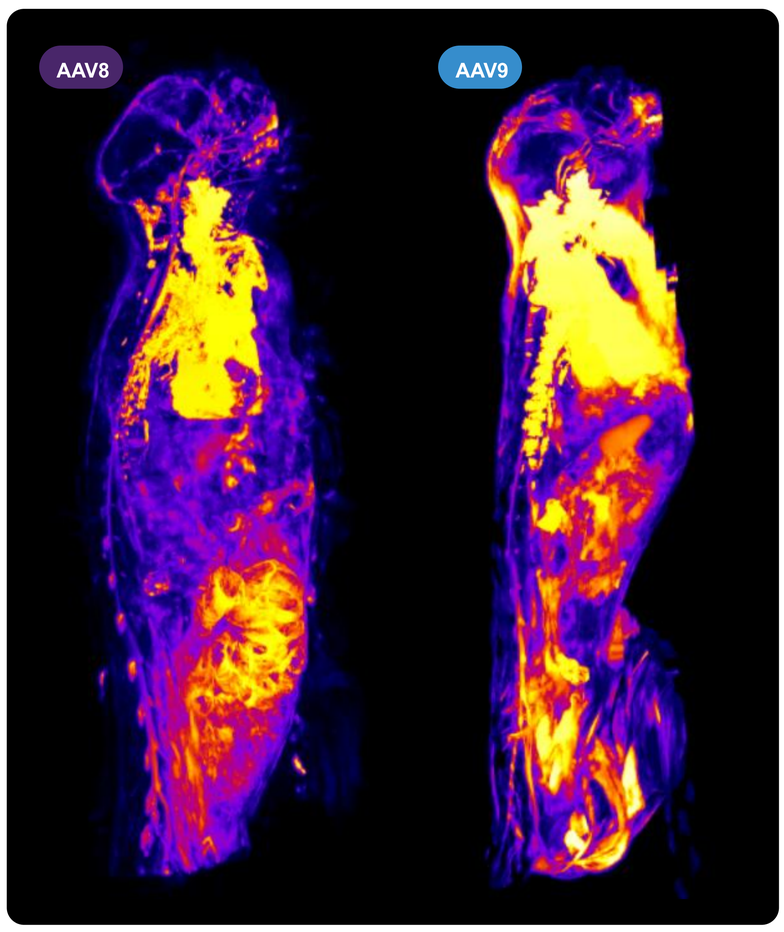


**Figure 2.** Whole-body MIP of tdTomato expression. Left: AAV8, right: AAV9. Note the complex presentation throughput the animal as well as some visible differences such as CNS/PNS and liver.

### Methods: CFT imaging

CFT imaging is performed by serial block-face imaging of frozen samples. The marmosets were embedded in Dark OCT 31 (an EMIT CFT-optimized OCT formulation) in 22×15×10 cm blocks. Due to the length of the animals, they are cut at the hips, such that the upper and lower halves were imaged separately. The CFT imaging system, Xerra, acquires both photographic (white light) anatomical images and fluorescence images. For tdTomato, Xerra excites at 555 nm and collects emission at 586nm (+/-15 nm bandpass). Imaging is performed with a 12 mega-pixel 4,096 × 3,008 camera, resulting in a 55 µm in-plane pixel size. To maintain isotropic voxels, sectioning was performed at 55 µm thickness. To ensure sensitivity, five fluorescence images were acquired from 5 to 2500 ms. Post acquisition, images underwent standard corrections: flat-field, dark-field, chromatic aberration warp. Image corrections, visualization, and analysis are performed using EMIT's XerraRecon and CFT Analyze software platforms.

## Results

CFT resolved AAV-mediated tdTomato expression across the intact animal, with AAV9 and AAV8 demonstrating tropism-specific features. Figure 1 shows the anatomical and fluorescence pattern of AAV8, and Figure 2 shows the comparison of AAV8 and AAV9. As expected, there is extensive expression throughout the body of the marmoset, illustrating the necessity for modified and targeted viral delivery and expression control. In Figure 1, the inset shows the location of the heart biopsy, which was used for ddPCR. The resulting cDNA data was correlated with signal intensity from the CFT image, and Figure 3 shows a strong correlation between RNA and CFT. Figures 4 and 5 show data in spine and heart, respectively, demonstrating the utility of tissue specific investigation in CFT.

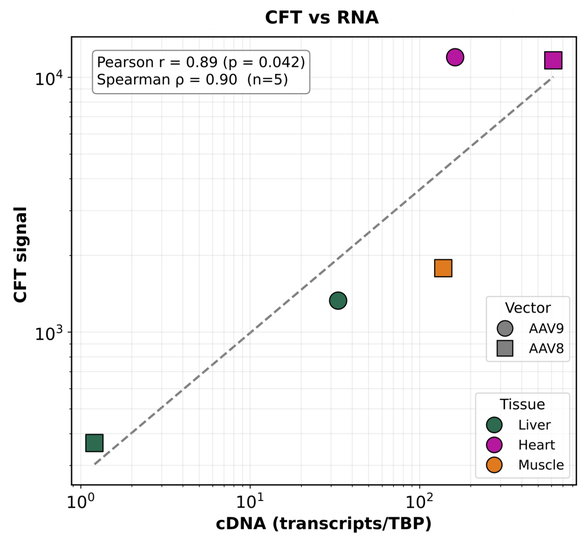


**Figure 3.** CFT signal versus cDNA ddPCR in liver, heart, and muscle.

## Conclusions

These initial data establish the marmoset as a tractable primate model in which whole-body vector biodistribution and expression is visualized in three dimensions and cross-validated against molecular endpoints in the same animal. This spatial bioimaging workflow can improve screening of assets, better predict clinical efficacy and safety. Future work extends into additional capsids, doses, and time points.

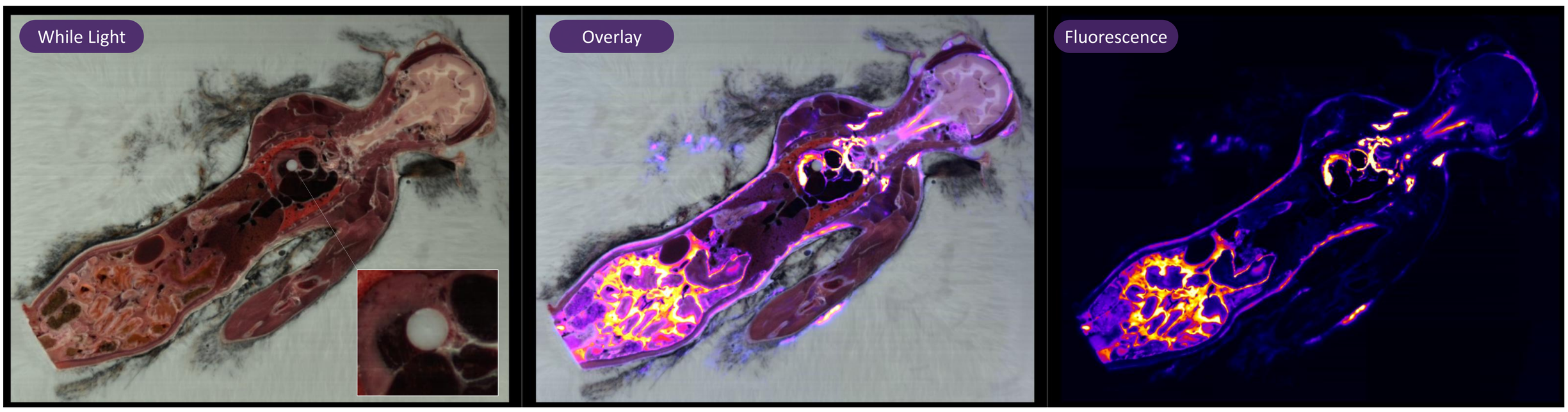


**Figure 1.** AAV8 upper body: white light anatomical image, overlay of the fluorescence and anatomical images, and fluorescence image. The inset shows the heart biopsy location.

### Spinal cord

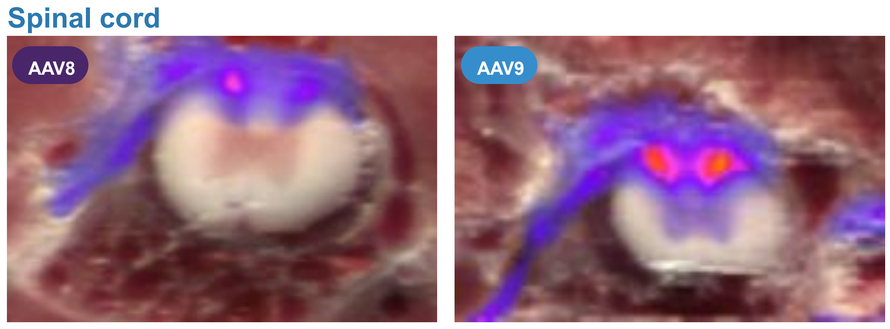


**Figure 4.** Co-registered white light and fluorescence image of spinal cord. Note dorsal cord and nerve signal

### Heart

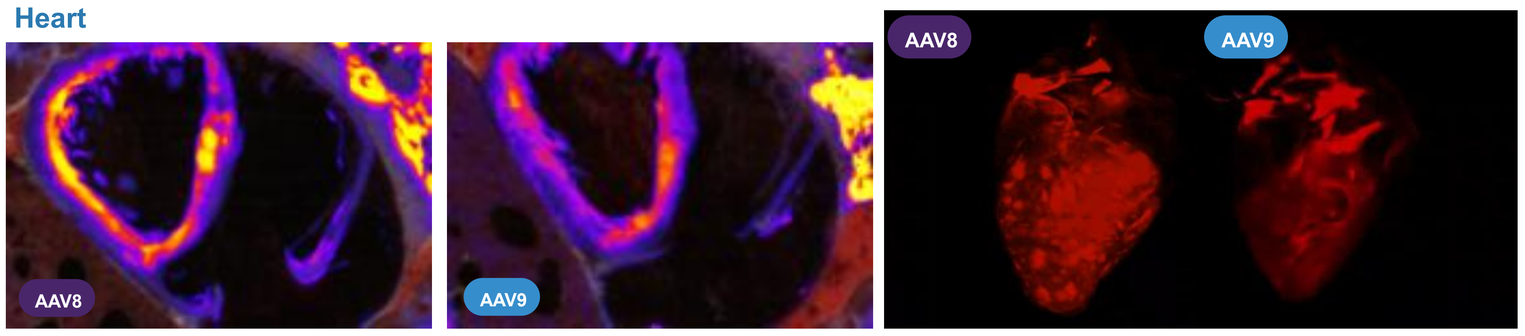


**Figure 5.** Heart, AAV8 and AAV9. For each vector, co-registered white light and fluorescence (left) and MIPs of both hearts (right).

### Acknowledgments

We acknowledge the efforts of EMIT, REGENXBIO and Envol colleagues, notably Yelena Engelson, Aaron Hogan, and Eli White.

### Reference

1. Yost et al., J. Virol. 2023

DISCOVER MORE

emitimaging.com