

## Introduction

One of the major hallmarks in Alzheimer's disease (AD) is the increasing levels of amyloid- $\beta$  (A $\beta$ ) deposition within the demented brain. This makes them an attractive target for molecular imaging strategies aimed at early detection and therapy monitoring, especially while currently several AD mouse models are available for the initial preclinical studies. With regard to optical imaging strategies, a number of NIR dyes, like AOI987<sup>1</sup>, have been developed and used in vivo for this purpose. However, the low plaque-to-background contrast, due to non-specific binding and slow washout, has meant that planar optical imaging might not give a complete window into the disease. With new whole animal NIR imaging systems now available that include 3D volume and lifetime measurements, we investigated whether the use of such systems could yield additional data on the in vivo properties of NIR probes (AOI987) within an AD mouse model.

## Results

- Consistent with previous reports<sup>1</sup>, signal intensity due to AOI987 injection was higher in Tg mice compared to Wt with decreasing intensity within 24hrs. (Fig.A & Table A)
- Analysis of the lifetime images showed similar effects (Fig.B & Table A).
- Upon ROI analysis, lifetime differences resulted in lower  $p$  values when comparing similar ROIs within Tg and Wt at both time points following AOI987 injection, respectively  $p = 0.00$  vs.  $p = 0.05$  and  $p = 0.02$  vs.  $p = 0.06$ .
- 3D reconstructed images (Fig.C) with their corresponding volume measurements (Table A) confirmed the temporal behavior of AOI987 spatially, where significantly more volume was detected in the Tg mice.

## Conclusions & Discussion

Besides known signal intensity differences, this study shows that time domain lifetime and volume measurements not only confirm these findings, but possibly lead to a more specific follow-up of the spatial and temporal behavior of dyes like AOI987.

Even though, from literature it is not known if AOI987's lifetime changes upon binding, significant differences between Wt and Tg were found. The higher lifetime in Tg mice may reflect binding specificity of the dye to A $\beta$  plaques, especially while the unspecific binding of the background has been removed for each animal. Thus in vivo lifetime measurements may provide a more sensitive parameter in distinguishing bound from unbound probe and therefore a better indicator of A $\beta$  load.

It is however important to be well aware of what ROI has to be used for which background correction, while this has a major influence on the final results. For example, intensity data corrected in a similar fashion to the shown lifetime data resulted in no significant differences at all for AOI987 injected Tg versus Wt.

Interestingly, intensity data in negative Tg and Wt also tend to be different, although statistically sufficient power could not be reached partly due to errors by the darkened skin found within the several animals.

## Materials & Methods

### Subjects and Samples

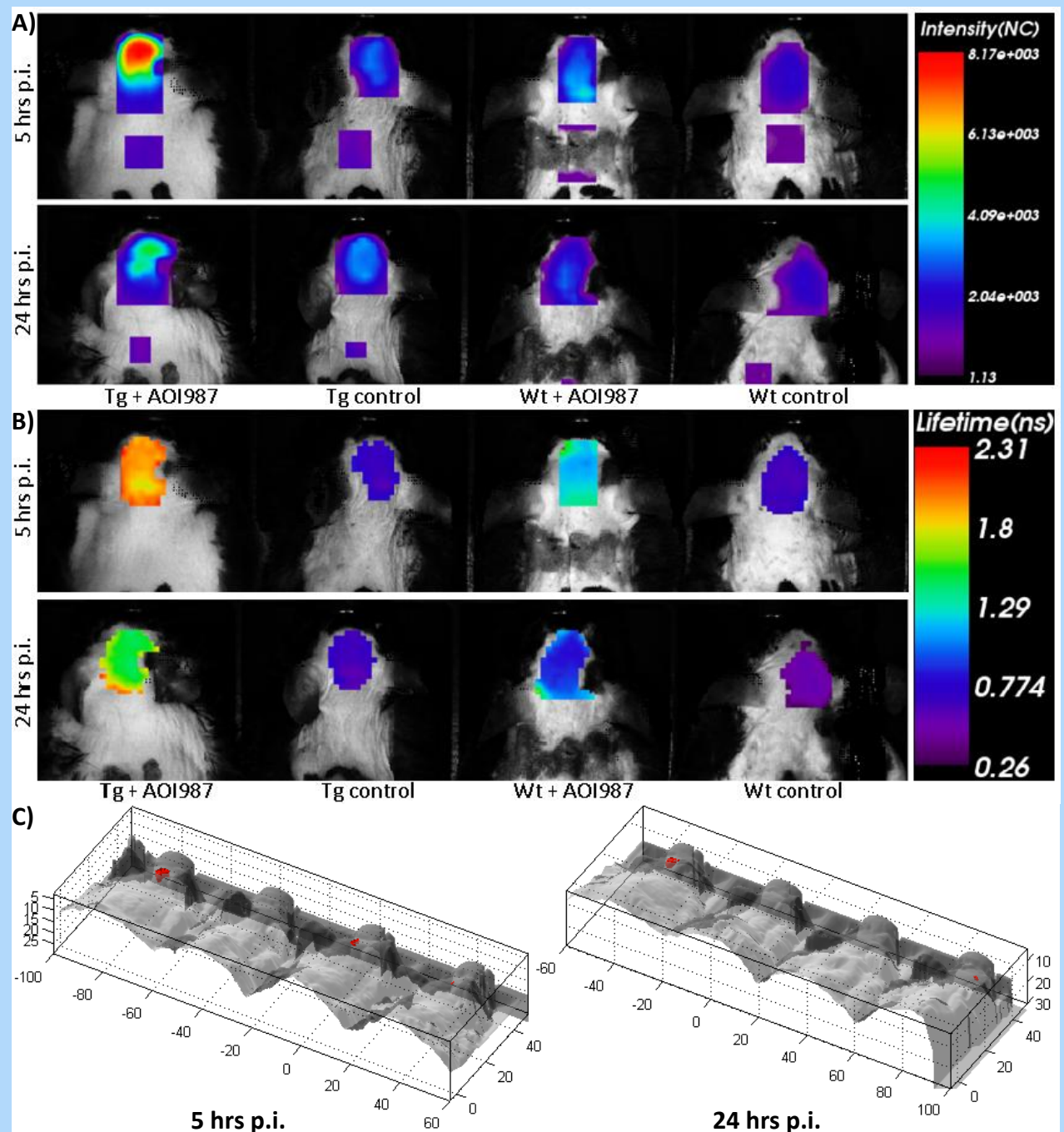
Six 12-14 month old APP-PS1 mice (Tg) and six wildtype (Wt) littermates were injected i.v. with 150  $\mu$ l phosphate buffered saline with or without 0.1mg/kg AOI987. Animals were divided into three groups of four animals per group and hair was removed before the experiment.

### Image protocol

All imaging was performed using the Optix MX3 (ART, Canada) at 670nm laser excitation, while animals were anesthetized with 1-2% isoflurane. A pre-injection scan was obtained for one animal. Each group was imaged at  $t = 5 - 24$  hrs post injection. For efficiency, only the head and a small part of the thorax of each animal was selected for imaging. Following the last session animals were perfused/fixated and 30  $\mu$ m brains sections were stained with Thioflavin T to confirm the presence of A $\beta$  plaques.

### Image processing and Analysis

Using OptiView v3.00.01 (ART, Canada), cleaned fluorescence intensity were computed without background removal. Corresponding lifetime images were calculated by removal of the background specific for each mouse. For analysis, regions of interest (ROI) were determined based upon the intensity image to be overlaid onto the lifetime data. 3D volumes were reconstructed using MatLab. Statistical analysis was performed using a (un)paired student t-tests.



**Figure A**) Clean signal intensities of APP-PS1 and wildtype littermate 5hrs following i.v. injection of 0.1 mg/kg AOI987 with their associative lifetime images shown in B). Corresponding 3D reconstruction images of this group including the transgenic and wildtype controls are shown in C).

Animal	time point	Intensity (nc)	s.d.	Lifetime (ns)	s.d.	Volume (ml)	s.d.
Tg+	t = 5	6163	1603	1.81	0.14	100.5	20.9
	t = 24	3867	604	1.39	0.10	31.1	2.8
Tg-	t = 5	2287	391	0.67	0.05	0.3	0.6
	t = 24	2537	527	0.64	0.13	3.7	6.0
Wt+	t = 5	3480	296	1.20	0.10	0.3	0.4
	t = 24	2893	257	0.82	0.26	12.1	6.2
Wt-	t = 5	1840	113	0.60	0.04	0.0	0.0
	t = 24	1995	163	0.53	N/A	0.0	0.0

**Table A.** Summary of both intensity and lifetime ROI analysis of all three groups. Due to darkening of the skin some values could not be calculated.

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## References

<sup>1</sup>Hintersteiner *et al.* Nature Biotech 2005;23(5):577-83