

Depth-resolved fiber photometry of amyloid plaque signals in freely behaving Alzheimer's disease mice

Nicole Byron,^a Niall McAlinden,^{b,†} Filippo Pisano,^{b,c,d,e,†} Marco Pisanello,^{b,f} Jacques Ferreira,^a Cinzia Montinaro,^c Keith Mathieson,^b Massimo De Vittorio,^{c,g} Ferruccio Pisanello,^{b,c} and Shuzo Sakata^{b,a,*}

^aUniversity of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

^bUniversity of Strathclyde, SUPA, Institute of Photonics, Department of Physics, Glasgow, United Kingdom

^cIstituto Italiano di Tecnologia, Center for Biomolecular Nanotechnologies, Arnesano (Lecce), Italy

^dUniversity of Padua, Department of Physics and Astronomy 'G. Galilei', Padua, Italy

^eUniversity of Padua, Padova Neuroscience Center, Padua, Italy

^fOptogeniX s.r.l., Arnesano (Lecce), Italy

^gTechnical University of Denmark, Department of Health Technology, Kongens Lyngby, Denmark

ABSTRACT. **Significance:** Current preclinical evaluation of Alzheimer's disease pathology in mouse models relies on post-mortem analyses, which hinders the development and optimization of therapeutic approaches. Although *in vivo* methods exist, monitoring amyloid plaque signals across multiple brain regions in freely behaving animals remains a significant challenge.

Aim: We aim to develop an optical approach to address this challenge.

Approach: We used flat and tapered optical fibers in an Alzheimer's mouse model.

Results: We first confirmed that conventional flat fiber-based photometry can detect amyloid plaque signals across multiple brain regions under anesthesia after injecting a blood-brain-barrier-permeable tracer, Methoxy-X04. The depth profile of *in vivo* fluorescent signals is correlated with histological signals. A machine learning approach could distinguish between *in vivo* fluorescent signals of mice with and without amyloid plaques. Next, after validating the feasibility of depth-resolved fiber photometry *ex vivo*, we chronically implanted a tapered fiber to monitor amyloid plaque signals in freely behaving mice. After injecting Methoxy-X04, fluorescent signals increased in a depth-specific manner in Alzheimer's mice, but not in their wild-type littermates.

Conclusions: Our approach expands the capabilities of fiber photometry to monitor molecular pathologies, such as amyloid plaques, even in a freely behaving condition.

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*Address all correspondence to Shuzo Sakata, shuzo.sakata@strath.ac.uk

[†]Equal contributions.

1 Introduction

Alzheimer's disease (AD) is the most common form of dementia. Amyloid plaques have long been recognized as a hallmark of AD.^{1–3} Recent therapeutics targeting amyloid- β protofibrils or deposited amyloid plaques have proven effective in patients^{4,5} and successful in translating early preclinical mouse data⁶ to the clinic. As such, evaluating novel interventions in preclinical mouse models plays a critical role in accelerating further successes.

Although a wide range of pharmacological and non-pharmacological intervention approaches have been examined in mouse models, post-mortem biochemical and histological analysis remains the gold standard in the field. This means that every intervention is a one-time experiment that requires animal sacrifice. To optimize an intervention approach flexibly, it would be ideal to evaluate its efficacy in real-time.

Although non-invasive approaches to assess a pathological state are available for human subjects and plasma-based biomarker detection is an emerging area,^{7–9} options for mouse models remain limited. For example, although microdialysis allows assessment of interstitial fluid contents in the brain,^{10–12} it lacks spatial resolution. Two-photon imaging can detect plaques *in vivo* by combining with a blood-brain barrier permeable tracer, Methoxy-X04.^{13–15} However, depth penetration is limited. It is crucial to assess the plaque distribution at depth, given the fact that amyloid pathology can be seen across multiple deep brain regions. Another approach is opto-acoustic tomography,¹⁶ allowing brain-wide monitoring of plaque signals. However, current technologies do not permit monitoring of amyloid pathology across multiple brain regions in a freely behaving condition.

Fiber photometry is an alternative, versatile optical approach.^{17–20} It allows monitoring of neuronal and non-neuronal population activity in a cell-type-specific manner *in vivo*.^{21–25} Although fiber photometry has also been adopted to monitor intracellular signaling,²⁶ it remains unknown if fiber photometry can monitor extracellular pathological signals such as amyloid plaques. For instance, fiber photometry typically uses fluorescent sensors that require availability and expression of their genetically encoded construct. To overcome this requirement, molecular pathologies could be assessed through a non-genetic strategy by administration of a blood-brain barrier fluorescent plaque dye.

Here, we test the hypothesis that fiber photometry can be adapted to access molecular pathologies, using a non-genetic approach. Specifically, we test if it can be used to monitor amyloid pathology in 5xFAD mice, a widely used AD mouse model carrying five familial AD mutations that develop amyloid pathology from 2 months, which aggressively continues with age.²⁷ To this end, we take two approaches. First, as a proof-of-concept, we utilize conventional flat optical fibers to examine whether amyloid pathology can be monitored across multiple depths in mice. Second, we exploit the photonic properties of tapered optical fibers²⁰ to establish depth-resolved photometry of plaque signals *ex vivo* and *in vivo*. To monitor amyloid pathology, we make use of the blood-brain-barrier-permeable fluorescent compound, Methoxy-x04. Its hydrophobic structure allows entry to the brain, where it specifically binds to beta-sheets found within amyloid fibrils, allowing visualization of amyloid plaques in Alzheimer's disease models.¹³ Our novel photometry approach expands the capabilities of *in vivo* fiber photometry to examine the pathological features of an AD mouse model in a freely behaving condition.

2 Materials and Methods

2.1 Animals

Experiments were performed in accordance with the UK Animals (Scientific Procedures) Act of 1986 Home Office regulations and approved by the Home Office (PP0688944). Mice were housed with sex-matched littermates, if available, on a 12-h/12-h light/dark cycle with access to food and water *ad libitum*. All experiments were performed during the light period. 5xFAD mice²⁷ (JAX006554, The Jackson Laboratory, Bar Harbor, Maine, United States) were obtained and backcrossed onto C57BL/6 background (>F10). Male and female 5xFAD+/-, referred to as 5xFAD, and 5xFAD-/-, referred to as WT, mice aged 3 to 12 months were used (Table S1 in the [Supplementary Material](#)). Fifteen mice (seven 5xFAD, six males and one female; eight WT, five males and three females) were used for *in vivo* flat fiber photometry experiments. Three mice

(two 5xFAD, two males; one male WT) were used for *ex vivo* tapered fiber photometry experiments. Twenty-five mice (14 5xFAD, 6 males and 8 females; 11 WT, 4 males and 7 females) were used for *in vivo* tapered optical fiber (TF)-based photometry experiments. Some mice were excluded from the analysis due to recording or histological issues, including an incomplete recording, inaccurate laser power estimations, or inaccurate registration of histological sections (Table S1 in the [Supplementary Material](#)). No blinding or randomization was adopted, nor was there an analysis of sex differences, as this was a technical development study.

2.2 In Vivo Flat Fiber Photometry

2.2.1 Photometry system

A flat fiber-based photometry system is described elsewhere.²³ To adjust the system for Methoxy-X04 signals, the light from a 405-nm light-emitting diode (LED) (M405L3, Thorlabs, Newton, New Jersey, United States) was collimated by an aspheric lens (AL2520M-A, Thorlabs), passed a bandpass filter (FB405-10, Thorlabs), reflected off two dichroic mirrors (MD498 and DMLP425R, Thorlabs), and focused by an aspheric lens onto the multimode patch cable (400- μm core, 0.5 NA; MAF3L1, Thorlabs) and flat fiber (200- μm core, 0.50 NA; MAF3L1, Thorlabs). Emitted light passed back through the fiber and patch cable and was collimated by an aspheric lens, passed a dichroic mirror (DMLP425R, Thorlabs), and was redirected toward an emission filter by a broadband mirror (BB1-E02, Thorlabs). Filtered light was focused by an aspheric lens onto a photodetector (NewFocus 2151, Newport) for measurement. In total, 440- and 550-nm emission filters (FB440-10 and FB550-10, Thorlabs) were used interchangeably by adding and removing a drop-in filter holder (DCP1, Thorlabs) between each measurement. A NIDAQ device (NI USB-6211, National Instruments, Austin, Texas, United States) and custom LabVIEW software were used to control the LED and photodetector.

2.2.2 Photometry experiments

5xFAD and WT mice were used for *in vivo* flat fiber depth profile photometry experiments. A total of 10 mg/kg of Methoxy-X04 (4920, Tocris, Bristol, United Kingdom) dissolved in 45% propylene glycol/45% 0.1 M phosphate-buffered saline (PBS)/10% DMSO was administered intraperitoneally. The next day, mice were anesthetized with urethane (1.5 g/kg) and placed in a stereotaxic frame (Model 963, KOPF Instruments, Los Angeles, California, United States). Body temperature was maintained at 37°C (ATC 100, World Precision Instruments, Sarasota, Florida, United States), and eye gel (Hylonight or Viscotears) was applied throughout. Craniotomies were made over three sites (site 1: AP +0.49 mm; ML 0.25 mm; site 2: AP −1.79 mm, ML 1.50 mm; site 3: AP −3.30 mm, ML 2.80 mm), which were covered with KWIK-SIL (World Precision Instruments). Throughout these procedures, the anesthetic level was maintained with isoflurane (1% to 1.5% at 0.8 L/min air flow).

Mice were moved to the recording set-up (SR-8N-S, Narishige, Tokyo, Japan) for depth profile measurements, where body temperature was maintained at 37°C (50-7212, Harvard Apparatus, Holliston, Massachusetts, United States). For each craniotomy, KWIK-SIL was removed, and the flat fiber (200- μm core, 0.50 NA; MAF3L1, Thorlabs) was lowered to the brain surface by the substage and micromanipulator (SM-15M and SM-15, Narishige). A measurement was completed with both 440- and 550-nm emission filters, before repeating this process at increasing 100- μm depths until a maximum depth of 4000 μm was reached.

For each depth measurement, the LED went through 10 repetitions of 10 ms on, 5 ms off, separated by a 10-ms baseline for light powers ranging from 0.1 to 1 mW/mm². Data were collected at the photodetector and LED sync channel at 5000 Hz.

2.2.3 Histological analysis

Immediately after photometry experiments, mice were deeply anesthetized with lidocaine (2%) and pentobarbital (200 mg/ml) and transcardially perfused with PBS and 4% paraformaldehyde. Brain tissue was removed and post-fixed in the same fixative at 4°C overnight, and cryoprotected in 30% sucrose/0.1 M PBS for several days. Then, 100- μm coronal sections were prepared using a sliding microtome (SM2010R, Leica, Wetzlar, Germany). As Methoxy-X04 was injected the day before brain retrieval, amyloid pathology was stained accordingly, and no other staining

was necessary. Therefore, sections were washed in PBS (3×5 min), before rinsing in gelatin and mounting onto glass slides. Once dry, slides were sealed with cover slips and fluoromount solution (ThermoFisher Scientific Fluoromount G, Invitrogen, Waltham, Massachusetts, United States). The brain sections anterior and posterior to and containing the flat fiber implant site were imaged. Images were acquired using an upright fluorescence microscope (Eclipse E600, Nikon, Tokyo, Japan) and CMOS camera (C11440-36U, Hamamatsu, Shizuoka, Japan) at $4\times$ magnification.

The post-processing of histological images is summarized in Fig. S1 in the [Supplementary Material](#). Images were stitched automatically, and the stitched images were registered to the Allen Mouse Brain Common Coordinate Framework using a modified version of a freely available software (AMaSiNe, <https://github.com/vsnnlab/AMaSiNe>).²⁸ Plaques were automatically detected as particles between 11 and $20\ \mu\text{m}$ in diameter. Coordinates of the flat fiber track were manually annotated across sections and estimated using a linear regression model.

To estimate the plaque density, as the ipsilateral hemisphere was damaged by the flat fiber, the estimated fiber track was projected onto the contralateral hemisphere, assuming that plaques are distributed homogeneously across hemispheres. To quantify plaques, a cylinder with a radius of $250\ \mu\text{m}$ was generated along the projected fiber track, and plaques within $250\ \mu\text{m}$ from each depth measure were counted every $100\text{-}\mu\text{m}$ step. As virtually no plaque signals were detected in WT mice, randomly generated noise ranging between 0 and 1×10^{-10} was added to the histological quantification of both 5xFAD and WT mice without producing artificial trends. Plaque density was determined by calculating the plaque count per spherical volume at each quantified depth.

2.2.4 Photometry signal processing

Offline analysis was performed using custom MATLAB codes. For all analyses, 440-nm photometry data at $1\ \text{mW}/\text{mm}^2$ were used. To obtain a depth profile, z -scored fluorescence at each depth was calculated by $dF = \frac{F - \text{mean}(F_0)}{\text{std}(F_0)}$, where F is the raw fluorescence and F_0 is the mean fluorescence across 0 to $400\ \mu\text{m}$. A median filter was applied to smooth data (window size: 4). For comparison of photometry to histology signals, Spearman's rho correlation test was completed.

To classify photometry signals, each depth profile was z -scored, and the data matrix containing all samples (animals and recording sites) was constructed. As signals were sampled at 41 depths for each penetration, principal component analysis was applied to reduce the dimension. The first three principal components (PCs) were used to train a support vector machine model to predict genotype. The training was done by taking the leave-one-out procedure, and the genotype of the remaining sample was predicted by the trained model. The same procedures were repeated across all samples. To assess the performance of this classification, a confusion matrix was computed.

2.3 Ex Vivo Tapered Fiber Photometry

2.3.1 Photometry system

A custom-built two-photon laser scanning microscope described in detail in Ref. 29 was used to evaluate the capability of TFs to gather significant data from plaque-originated fluorescence. The microscope is equipped with three different imaging channels: an epi-fluorescence channel provided with a dichroic mirror (FF665-Di02, Semrock, West Henrietta, New York, United States) and bandpass filter (FF01-520/70, Semrock) to acquire the reference image of the brain slices and the TF; a fiber-coupled channel provided with a bandpass filter (FF01-442/42-25, Semrock) and synchronized to the microscope scanner to acquire the TF light collection field; and a wide-field channel provided with a bandpass filter (FBH450-40, Thorlabs) to acquire the TF illumination field on the sensor of a sCMOS camera (Orca Flash lite 4.0, Hamamatsu).

2.3.2 Ex vivo photometry experiments

To label amyloid pathology, Methoxy-X04 (10 mg/kg) (4920, Tocris) was injected intraperitoneally. The following day, brain samples were retrieved as described above. However, for

this procedure, the sample did not undergo cryoprotection in sucrose and was rather stored in PBS. Then, 500- μm coronal sections were prepared using a vibratome (054018, Ted Pella Incorporated, Redding, California, United States) and stored in PBS.

A brain slice containing the septal area was placed in the imaging plane of the custom-built two-photon laser scanning microscope, and a TF (0.39 NA, 200- μm core, 225- μm cladding, 1.8-mm emission length, OptogeniX, Arnesano, Italy) was inserted into the slice so that the 1.8-mm optically active region of the taper was fully within the septal area.

Femtosecond pulsed laser light at 740 nm was used to elicit Methoxy-X04 fluorescence in brain slices through the microscope to measure the reference image and TF collection. Continuous wave 405-nm laser light (MDL-III-405-50-mW, CNI laser) was used to elicit Methoxy-X04 fluorescence in brain slices through the TF to measure the TF illumination field. Control on the angle at which light couples to the fiber was achieved using a galvo mirror-based scanning system identical to the one employed for the *in vivo* experiments (described later in the text).

2.3.3 Ex vivo data analysis

Images were processed with the FIJI software³⁰ and custom Python scripts. Images acquired in the reference and fiber channels were intrinsically registered by syncing both photodetectors (PMTs) with the laser scanning. Illumination fields acquired on a charge-coupled device (CCD) camera were rescaled and manually registered on the reference and fiber image. All images were preprocessed with background subtraction and thresholding before performing automated amyloid plaque localization and counting (analyze particles). The distribution of the plaques along the fiber axis was calculated on the images from the reference and fiber channels by computing the number of plaque centers falling within a circular region of interest (ROI) of radius 250 μm and centered on the fiber axis that was moved along the fiber at steps of 40 μm . The correlation between the quantified plaques in the reference image and fiber collection image was calculated using Spearman's rho correlation test.

The image stack of the illuminated areas of tissue against the input angles (galvo voltages) was pre-processed by background subtraction and normalized to the maximum value of the stack, to account for the dynamic in illumination density. For each frame—corresponding to an input angle—a centroid of the illumination peak was calculated after subtracting an intensity baseline corresponding to the intensity of the terminal input angles, where a negligible amount of light is delivered in the tissue.

The photometry stack was then calculated by multiplying the illumination stack against the fiber channel image pixel by pixel, as described in Ref. 29. The simulated photometry intensities were calculated from the integral intensity in the photometry stack. Each value of photometry intensity was then attributed to a spatial location along the fiber corresponding to the centroid of the peak of the illumination profile for the corresponding input angle. This allowed us to compare the photometry intensity profiles against the plaque density profile in the reference image (intensity scaled by dividing by the maximum value). The correlation between the photometry intensity profiles and the intensity-scaled plaque density in the reference image was calculated using Spearman's rho correlation test.

2.4 In Vivo Tapered Fiber Photometry

2.4.1 Photometry system

A 405-nm laser (MDL-III-405-50-mW, CI90055, CNI lasers or Cobolt 06-01 Series, Hubner Photonics, Solna, Sweden) delivered excitation light. A portion of this light beam was reflected toward a photodetector (PDA25K-EC, Thorlabs) by a glass slide to monitor laser stability. The rest passed broadband mirrors before being focused onto a galvo mirror (GVS001, Thorlabs) by an aspheric lens (AC254-050-A-ML, Thorlabs). The galvo mirror controlled the angle of reflected light, and therefore, the angle of light coupled into the tapered fiber (0.39 NA, 200- μm core, 225- μm cladding, 1.8-mm emission length, OptogeniX). Reflected light was collimated by an aspheric lens (AC254-050-A-ML, Thorlabs), passed through a dichroic mirror (DMSP425T, Thorlabs), and then focused by an aspheric lens (AC254-030-A-ML, Thorlabs) onto a multimode patch cable (200- μm core, 0.39° NA) and an implanted tapered fiber. Emitted light passed back

through the tapered fiber and patch cable and was collimated by an aspheric lens and reflected off a dichroic mirror (DMSP425T, Thorlabs) toward a dichroic mirror (DMSP490R, Thorlabs), which separated 440- and 550-nm light beams, with the use of emission filters (FB440-10 and FB550-10, Thorlabs). Each beam was then focused onto the photodetector by an aspheric lens (AC254-030-A-ML, Thorlabs) for measurement. A NIDAQ device (NI USB-6343, National Instruments) and custom LabVIEW software were used to control the laser, galvo mirror, and photodetectors.

2.4.2 Calibration and photometry protocols

For equalizing the light power across the tapered fiber, the fiber was placed in a uniform Methoxy-X04 solution (0.1 mM) and underwent an illumination protocol. The laser was activated in cycles of 10 ms on and 5 ms off at 60 and 80 μ W for five repetitions. In parallel with laser on periods, the galvo mirror was driven from -1 to 4.5 V, remaining at 0 V during the off period. Data were collected at 5000 Hz. The fluorescence at each galvo measure was used to calculate the required power for uniform fluorescence across the tapered fiber. For each galvo measure, the mean fluorescence was transformed to a relative required power by $P_R = \frac{1}{F} \div \min(\frac{1}{F})$, where P_R is the required power and F is the fluorescence.

2.4.3 Photometry experiments

5xFAD and WT mice were used. Mice were anaesthetized with isoflurane (5% for induction and 1% to 1.5% for maintenance with 0.8 L/min airflow). Mice were placed in a stereotaxic frame (Model 963, KOPF Instruments), reflexes were monitored, body temperature was maintained at 37°C (ATC 100, World Precision Instruments), and eye gel (Hylonight or Viscotears) was applied throughout. Naropin (8 mg/kg) was administered to the surgical area subcutaneously, while Vetergesic (0.1 mg/kg), Rimadyl (20 mg/kg), and saline (0.3 ml, FKE1323, Baxter, Deerfield, Illinois, United States) were administered subcutaneously to the rump. Skull screws (418 to 7123, RS Components, Corby, United Kingdom) were implanted in the skull: two in the front (AP +1.50 mm, ML $\pm$ 1.00 mm), one over the right hemisphere (AP -1.00 mm, ML +4 mm), one over the left hemisphere (AP -3.00 mm, ML -4.00 mm), and two in the back (AP -2.00 mm from lambda, ML $\pm$ 2.00 mm) as anchors. The tapered fiber was implanted at the target site (AP -3.50 or -3.60 mm, ML 2.25 or 2.80 mm, DV 2.50 or 2.00 mm) before being secured with KWIK-KAST (World Precision Instruments) and layers of superglue (918-6872, RS-Pro) and dental cement (Kemdent). Dental cement was applied across the skull and skin edges. After 5 days of recovery, mice were habituated to handling and being tethered to the photometry system. Although scruffed, they were attached to the patch cable via a mating sleeve (ADAL1-5, Thorlabs) and placed into a recording chamber (46.5 $\times$ 21.5 $\times$ 20.5 cm). Recordings included a baseline measurement with no Methoxy-X04 for 5 h. Twenty-four hours later, a 5-h measurement was completed, with Methoxy-X04 (10 mg/kg) (4920, Tocris) being injected intraperitoneally at 30 min. Twenty-four hours later, the fluorescence was monitored for a further 2 h. The laser was activated in cycles of 10 ms on, 5 ms off across four powers ranging from 60 to 140 μ W. In parallel with laser on periods, the galvo mirror was driven from -1 to 4.5 V, remaining at 0 V during the OFF period. This protocol was repeated three to five times at a sampling interval of 60 or 300 s. Data were collected at 5000 Hz. After several recordings, mice were culled for histological analysis.

2.4.4 Histological analysis

The brain sections (50 μ m) were prepared as described above. As Methoxy-X04 was injected several days before brain retrieval, amyloid pathology will be stained accordingly, but sections were co-stained with another plaque marker as a backup. To identify the TF track, sections were co-stained with a microglial marker. After washing in PBS (3 $\times$ 5 min), the sections were incubated in 10% normal goat serum in 0.3% Triton X in PBS (PBST) for 1 h. Then, the sections underwent primary antibody incubation with anti-Iba1 antibody (1:1000 in 3% normal goat serum in PBST; Abcam, cat. no. ab178846) overnight at 4°C. After washes in PBS (3 $\times$ 5 mins), a 2-h secondary antibody incubation with Alexa Fluor 594 (1:1000 in 3% normal goat serum in PBST; ThermoFisher, cat. no. A-11005) was completed. Sections were washed in PBS

(3 × 5 mins) before co-staining with Thioflavin-S (0.01% in PBS; Sigma-Aldrich, cat. no. T1892, St. Louis, Missouri, United States) for 15 min. The sections were washed in PBS (3 × 5 min) before rinsing in gelatin and mounting onto glass slides. Once dry, slides were sealed with cover slips and fluoromount solution (ThermoFisher Scientific Fluoromount G, Invitrogen). The brain sections were imaged as described above.

Image processing was the same as described above. Plaque quantification was the same as described above but was completed at 41 steps every 39.5 μm (galvo measure resolution). Also, to account for overestimations occurring during the estimation of the TF penetration track, we shifted the penetration track up 600 μm [Fig. S2(a) in the [Supplementary Material](#)].

2.4.5 Photometry signal processing

Offline analysis was performed using custom MATLAB codes. To estimate Methoxy-X04-related fluorescent signals, the dynamics of the autofluorescence were predicted based on the signals without Methoxy-X04 administration (day 0). To this end, a linear model was established at each galvo scanning level as $\text{AF} = a(t) + b$, where AF was predicted autofluorescence, t was the time samples, and a and b are the model coefficients, which were estimated by twofold cross-validation. Methoxy-X04 signals were estimated at each galvo scanning level as $F_m = F - \text{AF}$, where F_m was the estimated Methoxy-X04 signals and F was raw fluorescent signals. Measurements from 0- to 4.5-V galvo scanning levels were taken in 5-min time bins across recordings. The depths containing the minimum and maximum median signal from 30 to 240 min were z-scored by $dF = \frac{F_m - \text{mean}(F_0)}{\text{std}(F_0)}$, where dF was the z-scored fluorescence, F_m was estimated from the Methoxy-X04 signals, and F_0 was the mean fluorescence in the first 25 min of recording. Data were smoothed using a moving median filter. For all analyses, 440-nm photometry data at 120 μW were used.

2.5 Statistical Analysis

All statistical analyses were performed using MATLAB unless otherwise stated. A Kolmogorov–Smirnov test was completed to determine the normality of the data. Accordingly, in Figs. 1–3, correlation analysis was completed using Spearman’s rho test. A two-sample t -test was performed in Figs. 1 and 3 to compare the correlation coefficients. In Fig. 3, the maximum and minimum fluorescent profiles for 5xFAD and WT were tested with a Wilcoxon signed-rank test. All data were shown as mean $\pm$ standard error of mean unless otherwise stated.

3 Results

3.1 Flat Fiber-Based Photometry to Monitor Amyloid Pathology in Anesthetized 5xFAD Mice

As a proof-of-concept experiment, we first examined whether conventional flat fiber-based photometry [Fig. 1(a)] allows monitoring of plaque signals across multiple depths *in vivo*. To this end, Methoxy-X04 was injected a day before experiments in either 5xFAD mice ($n = 6$) or their littermate wild-type (WT) controls ($n = 7$). Under urethane anesthesia, we performed fiber photometry at increasing depths by gradually lowering a flat fiber into the brain [Fig. 1(b)]. As we lowered the flat fiber, we observed variable fluorescent signals along the penetration track in a 5xFAD mouse [Fig. 1(c)]. After photometry experiments, we performed histological analysis to reconstruct the depth profile of plaque density along the penetration track [Fig. 1(c)]. We took the depth profile from the contralateral hemisphere to estimate signals in the intact brain based on previous literature that demonstrates homogenous labeling of amyloid pathology across brain regions and hemispheres.³¹ We confirmed a significant positive correlation between *in vivo* fluorescent and post-mortem histological depth profiles ($r = 0.855$, $p < 0.0001$, Spearman’s rho) [Fig. 1(c)]. The depth-dependent trend of lower correlation is likely attributable to anatomical factors that vary across regions and mice. In contrast, signals in a control animal showed lower variability. As the control animal did not form plaques, no correlation was found [Fig. S3(a) in the [Supplementary Material](#)]. We repeated the same experiments across three different sites across animals to find statistically significant positive correlations in 5xFAD mice (0.499 ± 0.081) compared with control mice (-0.101 ± 0.061) ($t(22) = 5.70$, $p < 0.0001$,

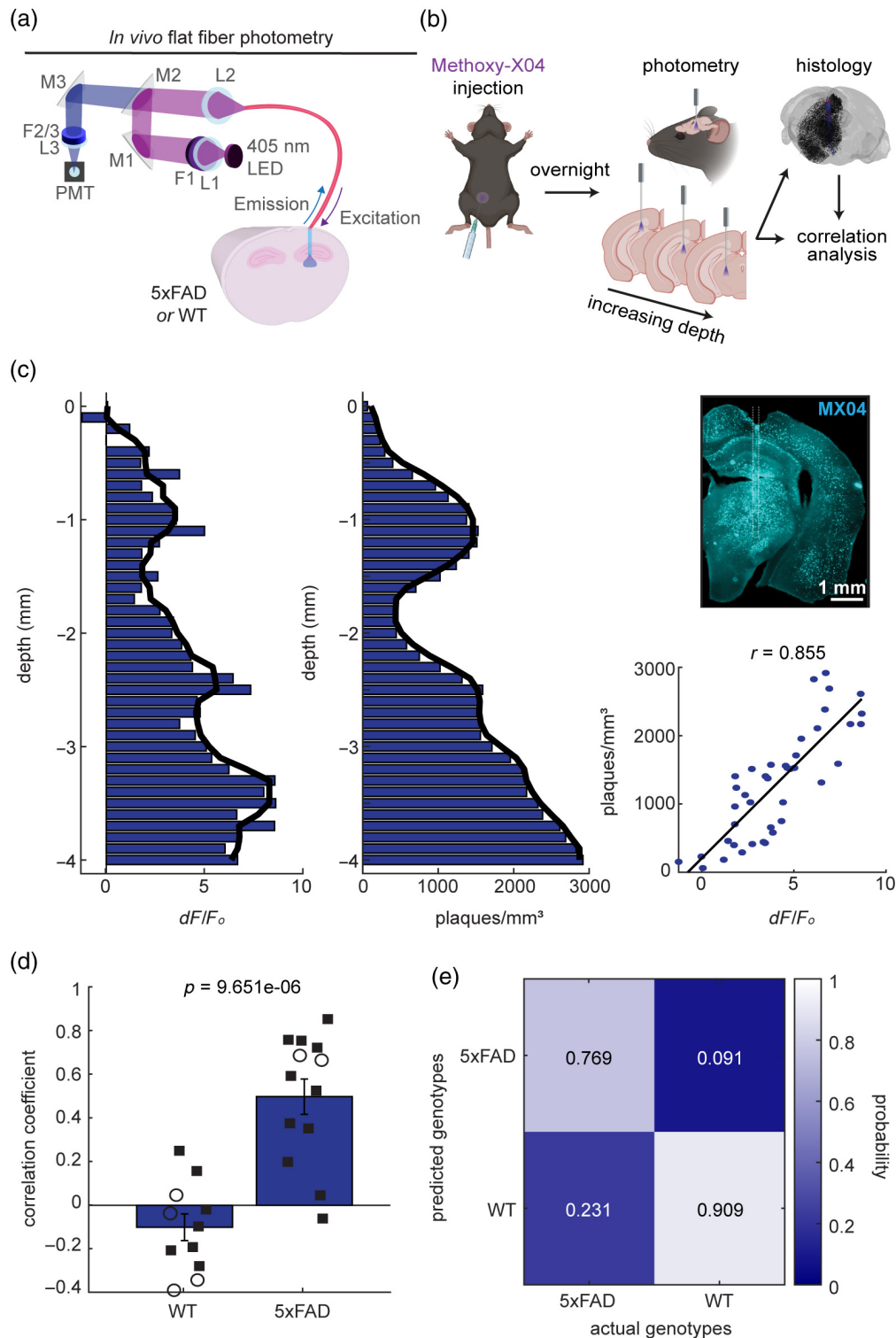


Fig. 1 Flat fiber-based photometry realizes fluorescent signals reflective of amyloid pathology in 5xFAD mice. (a) Flat fiber-based photometry system allows 405-nm LED light to pass through the flat fiber implanted in either 5xFAD or WT mouse brain. Emitted fluorescence light passes back through the flat fiber before being filtered to cut out excitation light and being focused onto a PMT. The light path was controlled by a series of mirrors (M), filters (F), and lenses (L). (b) Schematic of the experimental design. Mice were injected with Methoxy-X04 the day before being terminally anesthetized for photometry recordings. After, brain tissue was retrieved for histology, where photometry and histology signals underwent correlation analysis. Part of the diagram was created in BioRender. (c) Left: example *in vivo* fluorescent and post-mortem histology depth profiles. Solid

Fig. 1 (Continued) line shows the median smoothed signal (window size: 4). Top right: coronal brain slice showing the flat fiber penetration track surrounded by Methoxy-X04-stained amyloid pathology. The white dashed line shows the penetration track. Scale: 1 mm. Bottom right: correlation analysis comparing photometry and histology depth profiles (r , Spearman's rho). The black line shows a fitted linear regression. (d) Summary correlation coefficients across three different implant sites (two-sample t -test). 5xFAD, $n = 13$ recordings from six mice. WT, $n = 11$ recordings from seven mice. Squares, males; circles, females. (e) Classification performance based on *in vivo* photometry-based depth profiles.

two-sample t -test) [Fig. 1(d)]. Our findings also confirm positive correlation in the implanted (ipsilateral) hemisphere, with comparable plaque densities across hemispheres (Fig. S4 in the [Supplementary Material](#)). These results indicate that *in vivo* photometry signals reflect plaque signals at depth.

We also examined whether photometry-based depth profiles alone can discriminate genotypes (i.e., 5xFAD or WT). To this end, we took a machine learning approach. After reducing the dimension to 3 from 41 data points across the penetration by performing principal component analysis, we trained a support vector machine model by leaving one sample out. Then, we tested the model to assess if the remaining sample was accurately classified [Fig. 1(e)]. The overall performance was 83.3%: out of 13 5xFAD recordings, 10 were properly classified (76.9% hit), whereas out of 11, 10 WT recordings were correctly rejected (90.9%). These results indicate that *in vivo* photometry signals alone are capable of classifying genotypes.

3.2 Ex Vivo Evaluation of Tapered Fiber-Based Photometry

Given the success of the flat fiber-based approach, we sought to develop a method to monitor amyloid pathology across brain regions in freely behaving animals. To this end, we adopted TF technology.^{20,32} Before using TFs *in vivo*, we examined if a TF allowed depth-resolved photometry of amyloid plaques *ex vivo* in a controlled, relevant environment (Fig. 2). To this end, we injected Methoxy-X04 into a 5xFAD mouse and extracted the brain tissue on the following day. We then inserted a TF (0.39NA, 200- μm core, 225- μm cladding, 1.8-mm emission length) in a 500- μm -thick coronal brain slice containing the septal area and used a two-photon scanning microscope equipped with an epi-fluorescence channel and a fiber-coupled channel [Fig. 2(a)]^{29,33} to simultaneously collect a reference image of the brain slice [Fig. 2(c)] and a measurement of the TF collection field $C(x, y)$, where (x, y) denotes a location in the slice [Fig. 2(d)]. A similar approach was described in previous works.^{20,29} To determine if the fiber collection field was large enough to gather a significant amount of plaque-originated fluorescence, we compared the number of plaques enclosed inside a 250- μm radius sphere with the center moving along the TF axis on these two images [Fig. 2(g)]; despite the fiber collecting a lower number of plaques than the epi-fluorescence channel, mainly due to the decay in collection efficiency while moving radially farther from the fiber axis, the two curves are consistent ($r = 0.884$, $p < 0.0001$, Spearman's rho).

With the TF in the same position, we then delivered 405-nm light across different positions along the TF to the brain slice while changing the laser input angle, acting on the controlling voltage V of a galvanometer mirror-based scanning system.²⁰ For each input angle and resulting emission pattern, we collected an image of the emission of light in tissue using a sCMOS camera on a wide-field path of the two-photon microscope [Fig. 2(b)]. In this way, we measured the light illumination fields $I(x, y, V)$ produced by the TF at every input voltage V of the galvo [Fig. 2(e)], where (x, y) denotes a position in tissue. Then, we used the $I(x, y, V)$ fields as a pixel-by-pixel multiplicative mask to estimate the portion of the fiber collection field $C(x, y)$ involved in fluorescence excitation for different input angles [Fig. 2(f)]. This led us to calculate a photometry stack of images $P(x, y, V)$ representing the photometry collection fields according to

$$P(x, y, V) = C(x, y) \times I(x, y, V),$$

where $\times$ indicated a pixel-by-pixel multiplication.

The images in the illumination fields stack $I(x, y, V)$ measured on the sCMOS camera were also used to locate the centroid of the area of tissue recruited at every input angle using

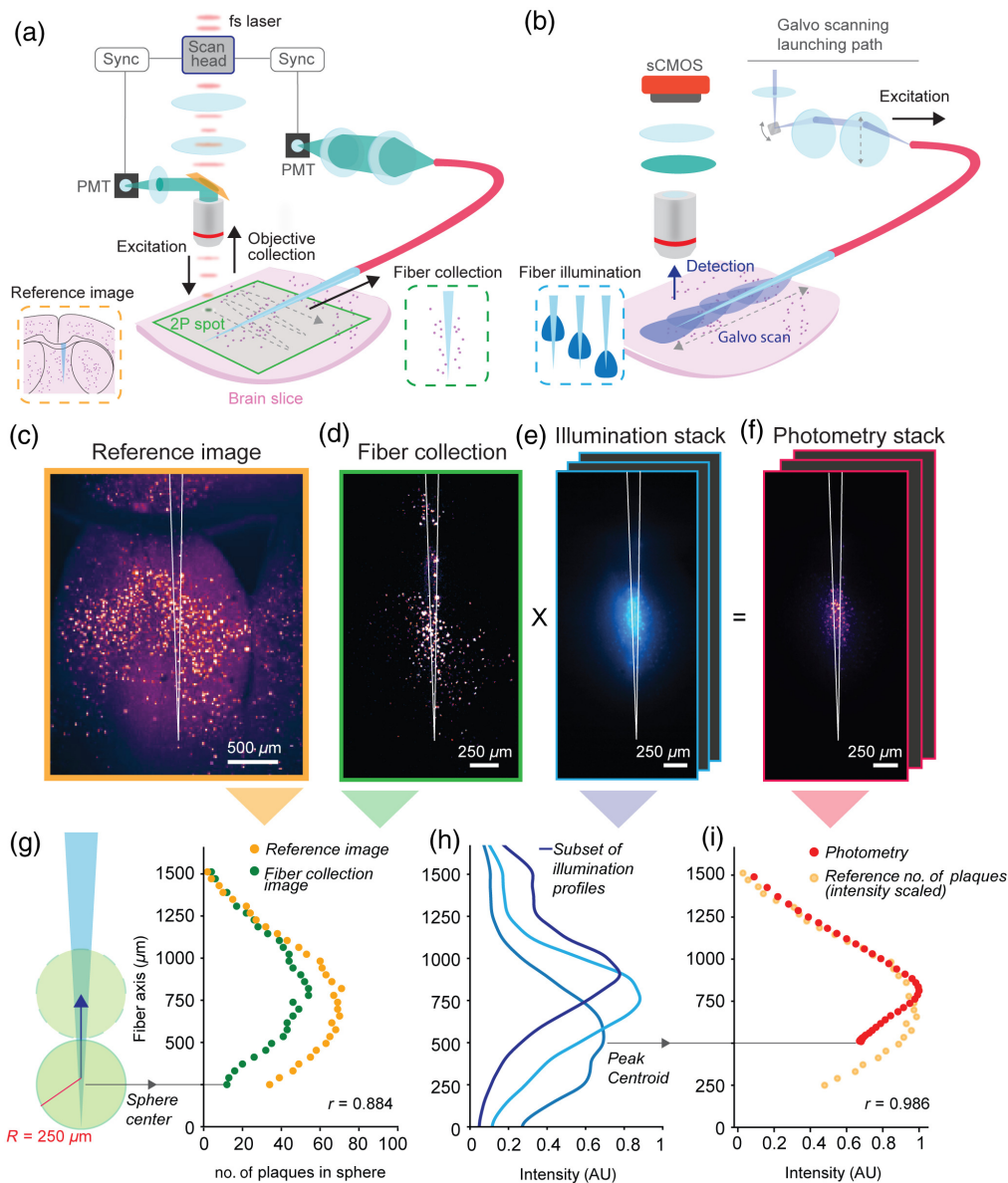


Fig. 2 Ex vivo TF-based photometry of amyloid plaques. (a) Schematic of the two-photon imaging system used to acquire co-registered reference images and fiber collection images. (b) Schematic of the angle-selective light injection system and imaging system to acquire images of the illumination profiles obtained by the TF in brain tissue. (c) Reference image of the amyloid plaques around the implanted TF outlined in white. (d) Fiber collection image of the amyloid plaques, with the fiber profile outlined in white. (e) Example of an illumination image collected by varying the injection angle in the TF. (f) Calculated photometry stack, obtained as a pixel-by-pixel multiplication of the illumination stack versus the collection image. (g) Number of plaques in a moving region of interest modelled as a circle with 250- μm radius for the reference (orange dots) and collection (green dots) image (r , Spearman's rho). (h) Example of a subset of the illumination profiles measured along the TF for each frame in the illumination stack corresponding to an input galvo voltage (e). The centroid of the peak illumination intensity for each input voltage is used as a reference position of the photometry intensity. (i) Simulated photometry intensity profile extracted from the photometry stack; the photometry intensity profile (red dots) is consistent with the distribution of plaques extracted from the reference image (orange dots, rescaled for comparison) (Spearman's rho correlation test).

the position of the centroid of the intensity profile taken along the fiber [Fig. 2(h)]. An *ex vivo* simulated photometry signal was then calculated as the sum of all the pixel intensities in the photometry stack, and each data point was positioned according to the corresponding illumination profile. We compared the curve obtained in this way with a rescaled version of the plaque count in the reference image and found a significant correlation between the two measurements ($r = 0.986$, $p < 0.0001$, Spearman's rho) [Fig. 2(i)]. These results suggest that TF-based photometry can be used to monitor amyloid pathology across brain regions *in vivo*.

3.3 Depth-Resolved Photometry to Monitor Amyloid Pathology in Freely Behaving 5xFAD Mice

To monitor plaque signals in a freely behaving condition, we constructed a photometry system with a 405-nm laser and galvo mirror [Fig. 3(a)]. We configured the galvo mirror to scan signals along the TF (~ 1.6 mm) three or five times at 66 Hz (67% duty cycle). We repeated this scanning every 1 or 5 min to monitor fluorescence signals along the TF [Fig. 3(b)]. As amyloid plaques densely appear in the subiculum in this mouse model,^{27,31} we chronically implanted a TF into the subiculum and surrounding regions (5xFAD, $n = 18$ recordings from 12 mice; WT, $n = 8$ recordings from seven mice).

Our typical recording schedule consisted of a baseline recording without Methoxy-X04 (day 0) and a recording with a Methoxy-X04 injection (day 1) [Fig. 3(c)]. On day 1, after Methoxy-X04 injection, the signals gradually increased in 5xFAD mice with variable signal intensity across depth, but not in WT mice [Fig. 3(d)].

To assess if the increased fluorescent signals reflect depth-resolved plaque distribution, we conducted two analyses: first, we compared the fluorescent dynamics on day 1 at the galvo levels, which provided the maximum and minimum signal intensities [Fig. 3(e)]. We clearly observed differential intensity changes ~ 1.5 h after Methoxy-X04 injections in 5xFAD mice [Fig. 3(e)]. On the other hand, we did not see such differential changes in WT. Specifically, at 1.5 h post-injection, we see a depth-resolved change in intensity in 5xFAD mice ($p = 0.004$, Wilcoxon signed-rank test) but not in WT mice ($p = 0.742$, Wilcoxon signed-rank test) [Fig. 3(f)]. In addition, the depth-resolved fluorescence increases in an age-dependent manner in 5xFAD mice (Fig. S5 in the [Supplementary Material](#)).

Second, to correlate the depth profiles between *in vivo* photometry and post-mortem histology, we compared the photometry depth profile from 210 to 240 min to the reconstructed histology plaque density along the penetration track [Fig. 3(g)]. Similar to the acute experiments (Fig. 2), we took the depth profile from the contralateral intact hemisphere to estimate histological signals. We confirmed a significant positive correlation between *in vivo* fluorescent and post-mortem histological depth profiles ($r = 0.709$, $p < 0.0001$, Spearman's rho) [Fig. 3(h)]. In contrast, as the WT animal did not form plaques, no correlation was found [Fig. S3(b) in the [Supplementary Material](#)]. Overall, we found significant positive correlations in 5xFAD mice (0.750 ± 0.042) compared with WT mice (0.015 ± 0.066) ($t(17) = 9.911$, $p < 0.0001$, two-sample *t*-test). We also assessed how robust the correlation between *in vivo* and histological signals was against a range of parameters (i.e., the TF tip estimation and light dispersion radius, see also Sec. 2). We found that the high correlation in 5xFAD mice was generally preserved across parameter values (Fig. S2 in the [Supplementary Material](#)). Finally, we observed less tissue damage with a TF compared with a flat fiber (Fig. S6 in the [Supplementary Material](#)), consistent with a previous report.³⁴ Thus, we established a TF-based photometry approach to monitor amyloid plaque signals across brain regions in a freely behaving condition.

4 Discussion

In the present study, we reported a novel fiber photometry-based approach to monitor amyloid plaque signals *in vivo*. First, using a conventional flat fiber-based approach, we confirmed that amyloid plaque signals can be monitored across depth, under anesthesia, *in vivo* (Fig. 1). Second, we assessed if TFs allow depth-resolved photometry for plaque signals *ex vivo* (Fig. 2). After confirming the feasibility *ex vivo*, we validated our TF-based approach in a freely behaving condition (Fig. 3).

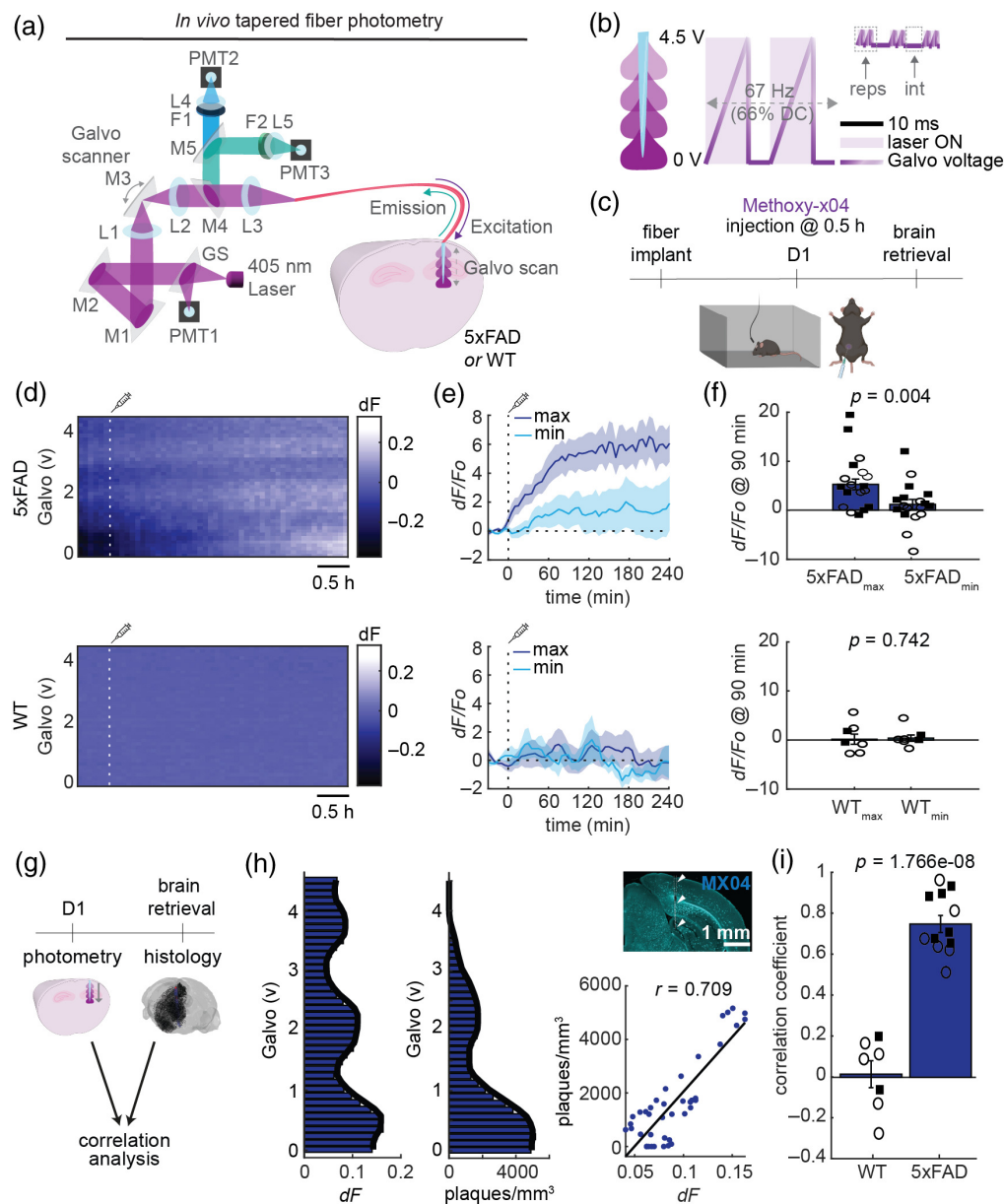


Fig. 3 TF-based photometry of amyloid plaques in freely behaving 5xFAD mice. (a) TF-based photometry system allows 405-nm laser light to pass through the tapered fiber implanted in either 5xFAD or WT mouse brain. Emitted light passes back through the tapered fiber before being focused onto a PMT for each emission wavelength. Light propagation along the tapered fiber was controlled by a galvo mirror, allowing increasing depths to be achieved in one complete galvo scan. The light path was controlled by a series of glass slides (GS), mirrors (M), filters (F), and lenses (L). (b) Illumination protocol. The 67-Hz cycle was repeated (reps) three or five times, at a 1- or 5-min interval (int). (c) Schematic of the experimental design. Methoxy-X04 was injected (i.p.) after 0.5 h on day 1 (D1) of the recording session. Part of the diagram was created in BioRender. (d) Example heatmaps of *in vivo* fluorescence depth profiles, shown over galvo voltage, for the D1 recording session. The white line shows the Methoxy-X04 injection. (e) Depths (galvo voltages) providing the maximum and minimum fluorescence changes for 5xFAD and WT mice. 5xFAD, $n = 18$ recordings from 12 mice; WT, $n = 8$ recordings from 7 mice. Some mice were recorded more than once. (f) Depth-specific fluorescence change at 90 min (Wilcoxon signed-rank test). Squares, males; circles, females. (g) Comparisons between photometry and histology signals. (h) Left: example *in vivo* fluorescent and post-mortem histology depth profiles. Solid line shows the median smoothed signal (window size: 4). Top right: Methoxy-X04-stained coronal section showing the tapered fiber penetration track (white dashed line and arrow heads). Bottom right: correlation between photometry and histology depth profiles (r , Spearman's rho). (i) Summary correlation coefficients (two-sample t -test). 5xFAD, $n = 12$ mice; WT, $n = 7$ mice. Squares, males; circles, females.

4.1 Comparisons to Other Methods

Amyloid pathology in mouse models has been assessed by various methods, yet post-mortem histological analysis remains the gold standard. Although positron emission tomography scan or microdialysis has been used to detect amyloid plaques,^{10,35} the former is not readily available for many researchers, and the latter cannot provide spatially resolved signals. Optical approaches have been sought before: although two-photon microscopy has been used,^{13–15} it is challenging to obtain signals in deep brain structures. Although optoacoustic tomography allows monitoring of brain-wide plaque signals,¹⁶ the imaging needs to be done under anesthesia. Our approach offers a unique, novel solution to monitor depth-resolved plaque signals in a freely behaving condition.

Although fiber photometry has been adopted for a wide range of experiments in neuroscience,^{17,18} our approach has expanded its potential to assess molecular pathology in an AD mouse model *in vivo*. We demonstrated that fiber photometry can be used without a genetic strategy, rather with the injection of a blood-brain-barrier-permeable fluorescent dye. This enhances the adaptability of this technology for various cellular and molecular processes, without the requirement of a genetically encoded construct. Also, compared with flat fiber-based photometry (Fig. 1), our TF-based photometry offers unique advantages. In addition to less invasiveness, we demonstrated that TF-based technology enables us to obtain depth-resolved plaque signals in a freely behaving condition for the first time. Although TF technology has been applied in various ways,^{20,26,36,37} the present study opens a new avenue for preclinical applications of TF-based technology in neurodegenerative disease mouse models.

4.2 Limitations of the Study

Despite the success of this novel approach, we are aware of several limitations. First, although Methoxy-X04 has been widely used, the excitation spectrum is blue-shifted (i.e., ~405 nm). This requires a dedicated laser to excite Methoxy-X04. As a ~470-nm light source is commonly used in the field, the adoption of the reported methodology requires extra investment in an optical setup. On the other hand, combining another wavelength of light will be straightforward. For example, it may be interesting to apply an optogenetic approach by expressing red-shifted opsins to modulate neural activity.³⁸

Second, although a TF allows depth-resolved photometry, the depth resolution can vary depending on taper type and implant location. For example, here, we use a fiber with a taper length of ~1.8 mm, but modification of taper geometry, such as numerical aperture and taper angle, can extend the taper length and depth-resolved properties.³² Although the length of the taper can be up to 3 mm,³² this is not practical to monitor brain-wide plaque distribution. Multi-fiber arrays^{24,39} may be an alternative approach to monitor plaque signals across brain regions in a freely behaving condition. Another important consideration is the influence of anatomical features when using fiber photometry, as the cytoarchitecture of brain regions, as well as amyloid pathology, can modify the collection fields due to scattering contributions and refractive index inhomogeneity.³³ Consequently, recent advances in deep brain spectroscopy using tapered fibers also open an interesting perspective to complement our method with reporter-free detection.⁴⁰

Third, like all other photometry-based approaches, our technique cannot resolve individual plaque signals. Also, we note that our *ex vivo* approach for measuring the photometry intensity collected from different regions of tissue while sweeping the input angle of light injection is an indirect approximation of the signal collected *in vivo*; for example, it does not account for the presence of blood in the brain vasculature. Nonetheless, we confirmed that the depth profile of photometry signals is correlated with histological signals across experimental conditions. It may be interesting to explore advanced optical approaches, such as an endo-microscope,⁴¹ to monitor individual plaques, although the issue with the absorption spectrum of Methoxy-X04 needs to be addressed.

5 Conclusion

Our fiber photometry approach allows us to monitor amyloid plaque signals at depth in a freely behaving condition. Our results demonstrate the potential of *in vivo* fiber photometry, which has been widely used in the neuroscience community. Our TF-based photometry approach also opens new avenues for monitoring plaque signals to optimize therapeutic approaches and to develop intervention strategies for AD in a preclinical setting.

Disclosures

The authors declare no competing financial interests.

Code and Data Availability

The datasets generated and/or analyzed during the current study are available at <https://doi.org/10.15129/4e2b6d72-4f58-4c61-a796-9aa212a478b2>.

Author Contributions

N.B. and S.S. conceived the *in vivo* experiments. N.B. and N.M. developed the optical systems for *in vivo* experiments. N.B. performed all *in vivo* experiments and data analysis. N.B. and J.F. performed the histological experiments. N.B., Fi.P., M.P., and S.S. conceived the *ex vivo* experiments. Fi.P., M.P., and C.M. performed the *ex vivo* experiments and data analysis. K.M., M.D.V., Fe.P., and S.S. supervised the work. N.B., Fi.P., M.P., and S.S. wrote the original paper. N.B., Fi.P., J.F., C.M., K.M., M.D.V., Fe.P., and S.S. reviewed and edited the paper.

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Nicole Byron is a research associate in Professor Shuzo Sakata’s laboratory at the University of Strathclyde, United Kingdom. She earned her PhD in neuroscience from the University of Strathclyde in 2024. Her current research focuses on optical interrogation of

Alzheimer's disease pathology using state-of-the-art tapered fiber-based photometry in freely behaving mice.

Niall McAlinden was awarded his PhD in surface and interface optics from Trinity College Dublin in 2010. Since then, he has served at the Institute of Photonics at the University of Strathclyde. He investigates optical and electronic systems for interfacing with the brain, primarily focusing on the development of microLED devices for optogenetics and photometry.

Filippo Pisano is an associate professor at the University of Padua, Department of Physics and Astronomy "G. Galilei" and an affiliated researcher at the Padova Neuroscience Center. He received his PhD from the Institute of Photonics, University of Strathclyde, in 2017. His research group focuses on the development of label-free optical tools and methods to capture multiple types of signals from the central nervous system.

Marco Pisanello is CTO at OptogeniX s.r.l., a company that offers tapered fiber probes and equipment for optogenetics. He received his PhD in materials and structures engineering and nanotechnologies from Università del Salento and Istituto Italiano di Tecnologia in 2017. He currently develops the product lineup of OptogeniX, with particular emphasis on the optoelectronic hardware and software components.

Jacques Ferreira is a PhD student at the University of East Anglia, United Kingdom. Previously, he was a technician in the Sakata lab at the University of Strathclyde, United Kingdom, where he provided technical support and insight to lab members. His current research investigates the role of a novel *C. elegans* protein in the unfolded protein response pathway of the endoplasmic reticulum.

Cinzia Montinaro graduated in biological sciences and obtained a professional biology qualification. In October 2023, she received her PhD in biological and environmental sciences and technologies from the University of Salento. She is currently a postdoctoral researcher at Istituto Italiano di Tecnologia, Center for Biomolecular Nanotechnologies (IIT@CBN). She focuses on multifunctional neural interfaces targeting deep brain regions with research interests in fiber photometry, two-photon microscopy, and Raman spectroscopy.

Keith Mathieson is a professor at the University of Strathclyde, United Kingdom. He holds a 10-year award from the Royal Academy of Engineering as part of their Chair in Emerging Technologies scheme, with a focus on neurotechnology. His research is focused on the development of devices to interface with neural systems. These are microfabricated, optoelectronic, implantable devices to record and activate neural activity within the brain.

Massimo De Vittorio is a full professor at the Technical University of Denmark (DTU) and principal investigator of the Center for Biomolecular Nanotechnologies of the Istituto Italiano di Tecnologia in Lecce, Italy, where he has served as a director for the past 10 years. His research activity deals with the development of science and technology applied to nanophotonics, nanoelectronics, and nano and micro electromechanical systems (NEMS/MEMS) for sensors and actuators for the human body in healthcare applications.

Ferruccio Pisanello is the coordinator of the Center for Biomolecular Nanotechnologies at the Italian Institute of Technology, where he leads the research unit on multifunctional neural interfaces for deep brain regions. His study focuses on developing advanced neurotechnologies for multimodal sensing and actuation of neural activity. He has coordinated national and international research initiatives and authored over 70 peer-reviewed publications in peer-reviewed journals.

Shuzo Sakata is a professor at the University of Strathclyde, United Kingdom. He earned his PhD in biophysics from Kyoto University in 2002. His research group applies a wide range of *in vivo* approaches to investigate state-dependent and cell-type-specific information processing in the brain. He is also a scientific advisory board member of NeuroVLC. The topic from this perspective is not related to this company.