Two-photon imaging and 3D neuronal activity recording in behaving animals

Technical Journal Club

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Two-photon microscopy

- Widely used to investigate **brain function across multiple spatial scales**
- Used for investigating circuit function because of its **penetration depth in scattering tissue** and **high spatial resolution**
- Capable of monitoring signal in **3D with high temporal resolution**

Random-access three-dimensional (3D) laser scanning

Acousto-optic lens

Issues with in-vivo recordings of behaving animals

- Brain movements and tissue displacements due to limbs movements, locomotion and licking → they remain even in anaesthetitzed animals (from heart beats and breathing)
- Brain motion-induced artifacts are typically corrected using post hoc processing of two-dimensional images → slow approach and does not correct for axial movements
- Also, effects of brain movement on high-speed imaging of small regions of interest and photostimulation cannot be corrected post hoc

Solving motion artifacts is a main issue to observe neuronal activity in behaving animals

Solving movements artifacts

PAPER 1

nature methods
ARTICLES
https://doi.org/10.1038/s41592-020-0851-7
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Real-time 3D movement correction for two-photon imaging in behaving animals

Victoria A. Griffiths[1,7], Antoine M. Valera[1,7], Joanna YN. Lau[1], Hana Roš[1], Thomas J. Younts[1], Bóris Marin[1,2], Chiara Baragli[1,3], Diccon Coyle[1], Geoffrey J. Evans[1,4], George Konstantinou[1,5], Theo Koimtzis[1,6], K. M. Naga Srinivas Nadella[1], Sameer A. Punde[1], Paul A. Kirkby[1], Isaac H. Bianco[1] and R. Angus Silver[1]✉

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PAPER 2

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Fast optical recording of neuronal activity by three-dimensional custom-access serial holography

Walther Akemann[1], Sébastien Wolf[1,2,3], Vincent Villette[1,3], Benjamin Mathieu[1,3], Astou Tangara[1], Jozsua Fodor[1], Cathie Ventalon[1], Jean-François Léger[1,4], Stéphane Dieudonné[1,4]✉ and Laurent Bourdieu[1,4]✉

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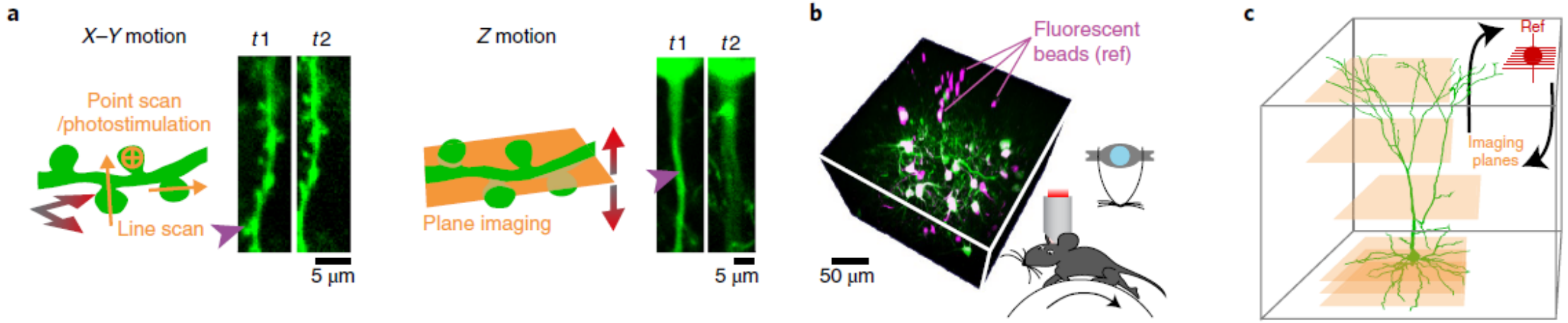
Real-time 3D movement correction for two-photon imaging in behaving animals

- They combined random-access three-dimensional (3D) laser scanning using an acousto-optic lens and rapid closed-loop field programmable gate array processing to track 3D brain movement and correct motion artifacts in real time

- Neuronal recordings in behaving mice and zebrafish demonstrate real-time movement-corrected 3D (RT-3DMC) two-photon imaging with submicrometer precision.

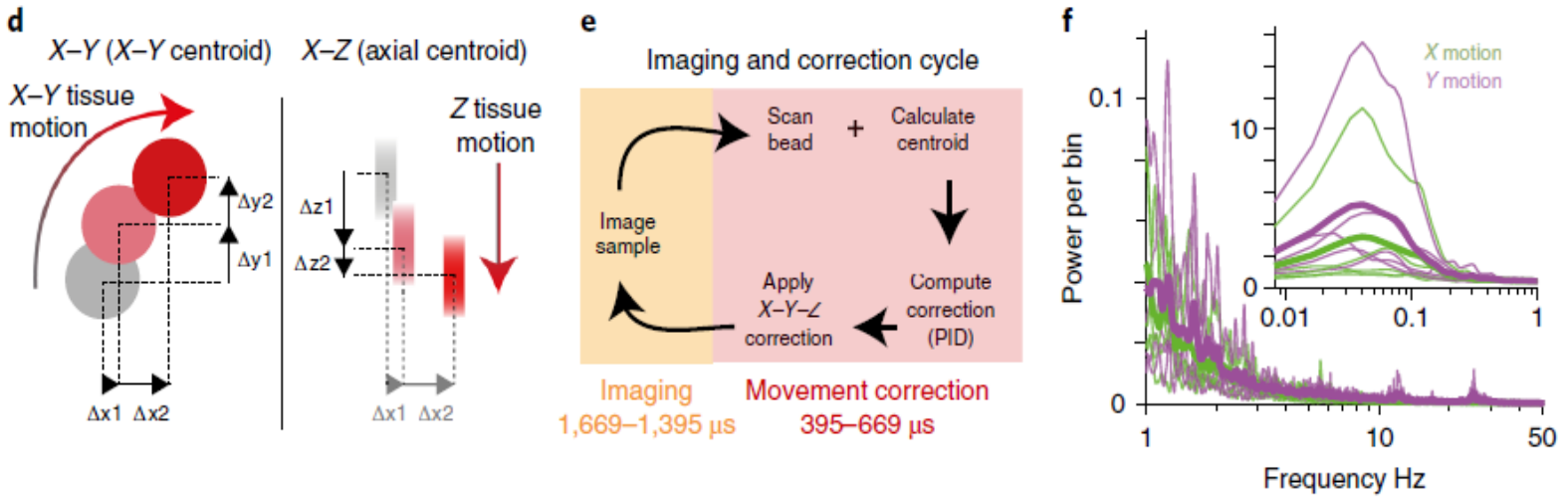
Implementation of real-time 3D movement-corrected imaging



a, Schematic of dendrite and fluorescence images at two time points (*t*1, *t*2) illustrating effect of lateral (left) and axial (right) brain motion. Purple arrows indicate lost features.
b, *Z* stack of L2/3 cortical pyramidal cells (green) and 4-µm fluorescent beads (magenta) used to track brain motion (left) in head-fixed mice running on a treadmill (right). The beads are in this case the reference object
c, Schematic illustration of interleaving functional imaging (orange planes) and monitoring of a reference object (red)

→ They achieved tracking by periodically interrupting functional imaging with an ultra-high-speed scan of the reference object

Quantifying brain imaging in head fixed animals and correcting it



d, The field programmable gate array (FPGA) on the image acquisition board rapidly calculated the 3D position of the reference object (beads) using a weighted centroid algorithm

e, Imaging the reference object, together with tracking and correction of imaging drives, took 395–669 µs

f, Power spectrum of *X* (green) and *Y* (purple) motion of motor cortex from head-fixed mice during locomotion.

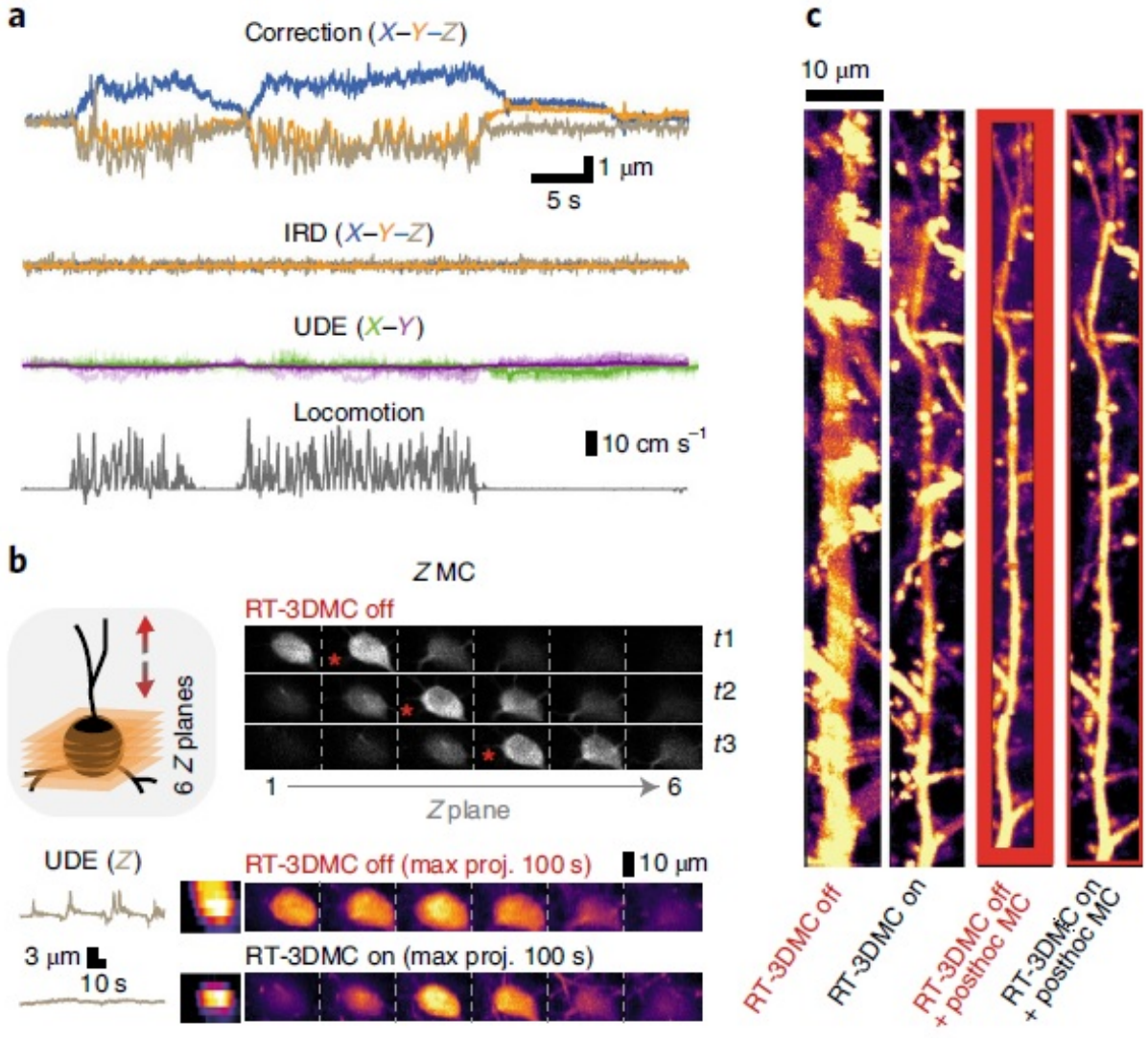
g, they mimicked the head movements using beads embedded in agarose

→ they quantified brain movement in head-fixed mice by imaging neocortex and cerebellum at >100 Hz

→ **RT-3DMC can track a 1-µm bead and keep a laser beam focused on it at speeds and displacements comparable to the maximum observed for brain tissue during locomotion**

Performance of movement-corrected imaging in behaving mice

a, Example of displacement estimated by tracking a bead in motor cortex during locomotion
b, Example of volumetric imaging of a tdTomato-expressing soma during locomotion for six *X*–*Y* planes at three time points (*t*1, *t*2, *t*3). Maximum intensity projections (MIPs) during a 100 s recording.
c, Example of MIPs of a pyramidal cell dendrite over 140 s during locomotion, with RT-3DMC off, RT-3DMC on, after post hoc correction with RT-3DMC off and after post hoc correction with RT-3DMC on. Red region cannot be corrected post hoc due to out-of-frame movement.

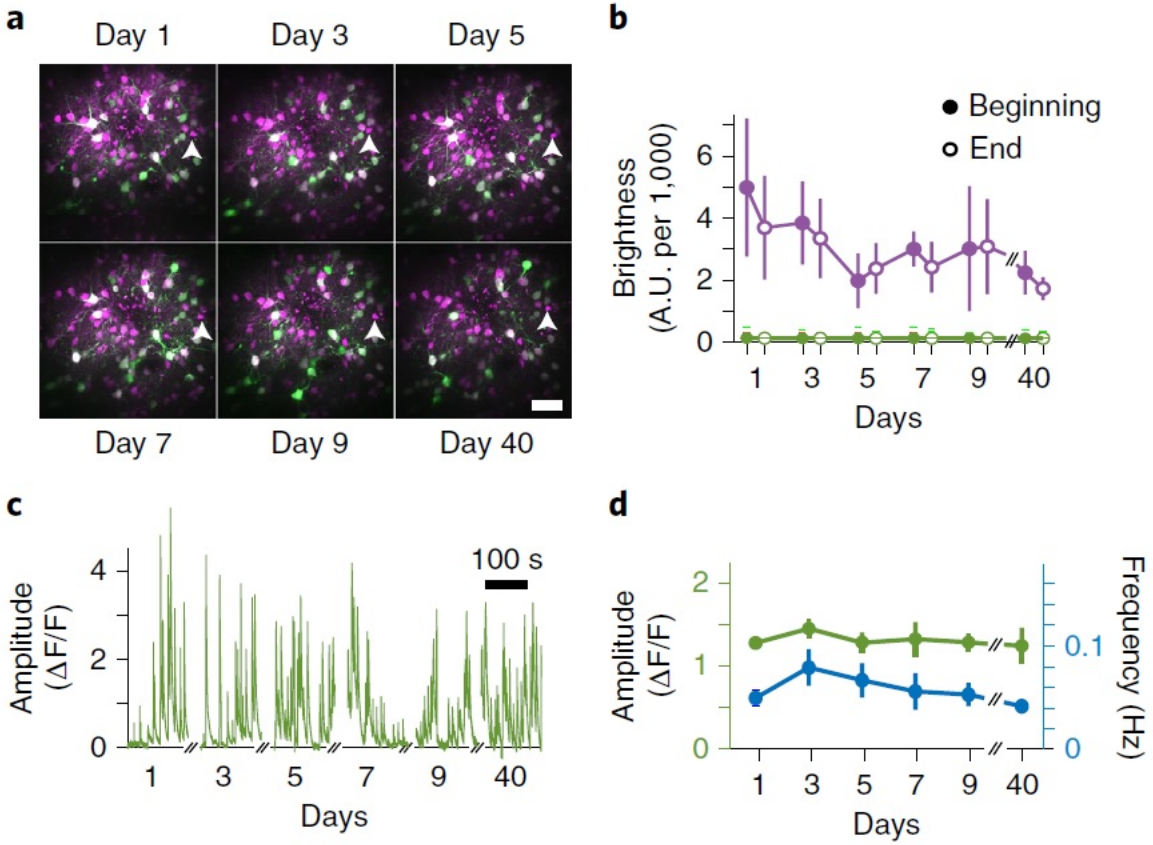


→ Imaging of layer 2/3 pyramidal cells expressing GCaMP6f and tdTomato in the motor cortex, with mouse on a treadmill

→ Without RT-3DMC, the midpoint of the soma moved between planes over time

→ **RT-3DMC is therefore key for high-speed selective imaging of small structures in head-fixed mice.**

Longitudinal imaging using RT-3DMC

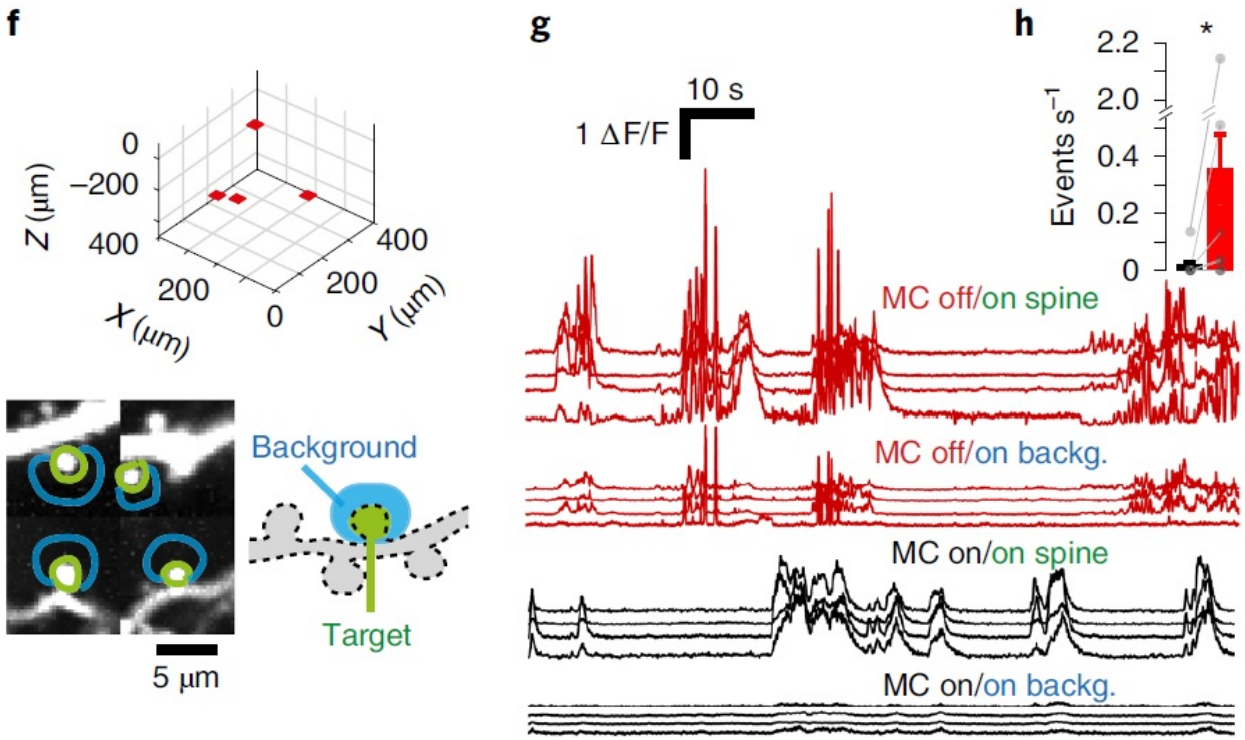


a, functional Ca2+ recordings from L2/3 pyramidal cells by imaging multiple times from the same volume, using the same bead as reference
b, the fluorescence of the bead remained more than 20-fold brighter than the background fluorescence
c and **d**, baseline GCaMP6f fluorescence of identified pyramidal cells and the amplitude and frequency of their somatic activity remained stable suggesting that the cells remained healthy

→ **Cells remain healthy during multiple recordings**

→ **RT-3DMC is compatible with repeated functional imaging of the same neuronal structures**

RT-3DMC for imaging at optical resolution limit



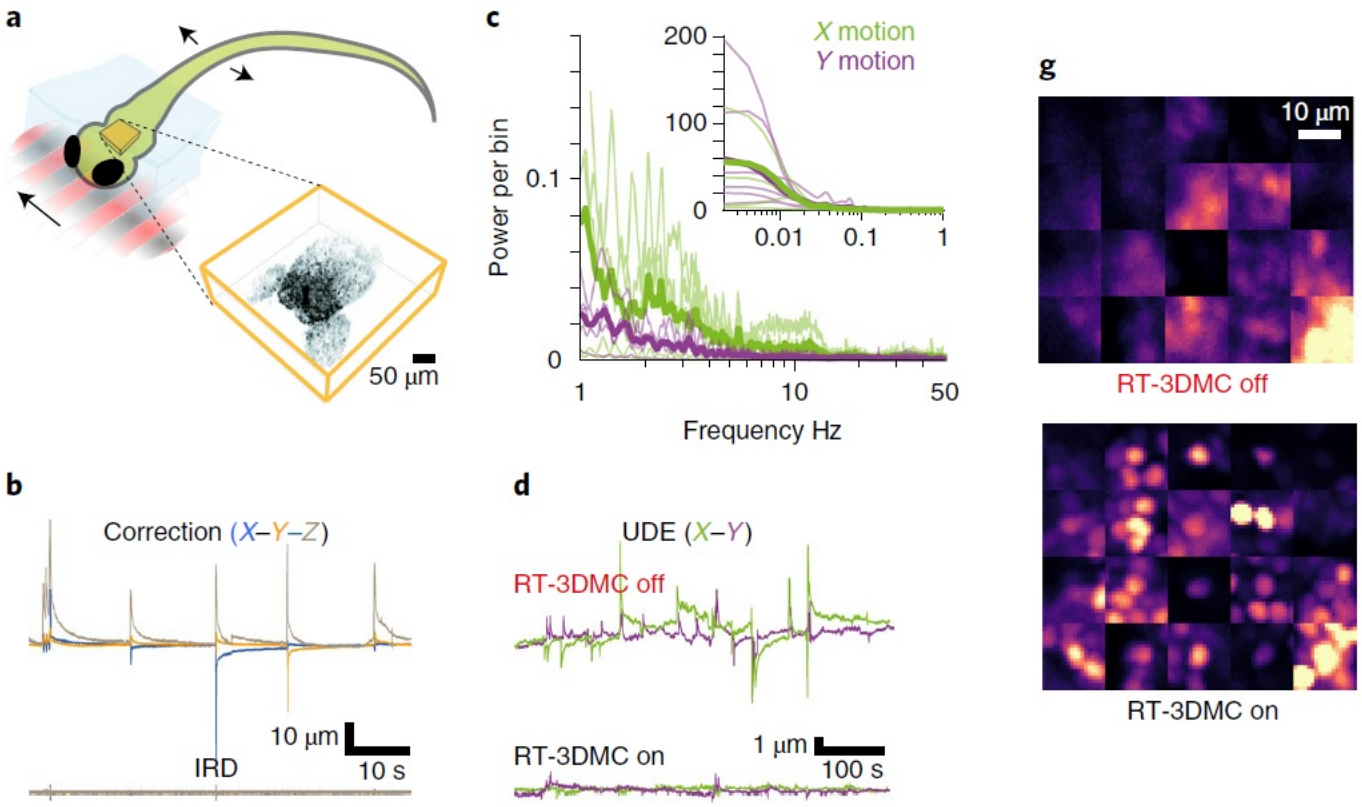
f, Imaging volume with 3D location (top) of four imaged ROIs with dendritic spines (red patches) on a layer 2/3 pyramidal cell expressing GCaMP6f in motor cortex. Imaged patches with regions on (green) and adjacent to (blue) the spines from which functional signals were extracted (bottom).
g, Example of ΔF/F traces from green and blue regions in **f** when RT-3DMC was off (top, red) or on (below, black)
h, average rate of false positive events (peak >2× background in the dark background area) during locomotion with RT-3DMC on or off

→ Images obtained with RT-3DMC exhibited activity localized to spine heads with almost no activity spilling into adjacent pixels

→ **RT-3DMC can track and compensate for brain movements with high precision**

Performance of movement-corrected imaging in zebrafish

a, zebrafish larva with the rostral body embedded in agarose. Inset shows a RT-3DMC *Z*-stack image of the forebrain expressing GCaMP6s within the imaging volume.
b, Example of displacement of brain during swimming bouts and how it is corrected with RT-3DMC on.
c, Power spectrum analysis of *X* and *Y* brain motion during swimming
d, Mean UDE from 20 patches in *X* and *Y* directions with RT-3DMC off and on.
g, Time-averaged images of neurons distributed throughout the forebrain in 20 imaging patches during swimming bouts without and with RT-3DMC



→ Zebrafish larvae expressing nuclear GCaMP6s partially embedded in agarose and presented with visual stimuli

→ **RT-3DMC can image even with more abrupt movements of the sample**