***In vitro* Time-lapse Imaging of Primary Cilium in Migrating Neuroblasts**

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[Abstract] Neuronal migration is a critical step for the development of neuronal circuits in the brain. Immature new neurons (neuroblasts) generated in the postnatal ventricular-subventricular zone (V-SVZ) show a remarkable potential to migrate for a long distance at a high speed in the postnatal mammalian brain, and are thus a powerful model to analyze the molecular and cellular mechanisms of neuronal migration. Here we describe a methodology for *in vitro* time-lapse imaging of the primary cilium and its related structures in migrating V-SVZ-derived neuroblasts using confocal or superresolution laser-scanning microscopy. The V-SVZ tissues are dissected from postnatal day 0-1 (P0-1) mouse brains and dissociated into single cells by trypsinization and gentle pipetting. These cells are then transduced with a plasmid(s) encoding a gene(s) of interest, aggregated by centrifugation, and cultured for 2 days in Matrigel. Time-lapse images of migratory behaviors of cultured neuroblasts and their ciliary structures, including the ciliary membrane and basal body, are acquired by confocal or superresolution laser-scanning microscopy. This method provides information about the spatiotemporal dynamics of neuroblasts' morphology and ciliary structures, and is widely applicable to various types of migrating neuronal and nonneuronal cells in various species.

Keywords: Postnatal neurogenesis, Ventricular-subventricular zone, Neuroblast, Primary cilium, Migration, Time-lapse imaging, Confocal microscopy, Superresolution microscopy

[Background] In the postnatal brain, neural stem cells reside in the ventricular-subventricular zone (V-SVZ) lining the lateral walls of lateral ventricles, and continuously generate immature new neurons (neuroblasts) (Obernier and Alvarez-Buylla, 2019). These neuroblasts form chain-like cell aggregates and migrate along each other through the rostral migratory stream (RMS) toward the olfactory bulb (OB) (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois *et al.*, 1996), where they are integrated into the neuronal circuits and involved in the processing of odor information (Gheusi *et al.*, 2000; Breton-Provencher *et al.*, 2009; Moreno *et al.*, 2009; Sakamoto *et al.*, 2011 and 2014). Following brain injuries such as stroke and trauma, V-SVZ-derived neuroblasts migrate toward the lesion and differentiate into mature neurons, contributing to the recovery of impaired gait behaviors (Arvidsson *et al.*, 2002; Parent

et al., 2002; Yamashita *et al.*, 2006; Yang *et al.*, 2007 and 2008; Kreuzberg *et al.*, 2010; Jinnou *et al.*, 2018; Kaneko *et al.*, 2018). Altering the migration of neuroblasts affects their final positioning and functions in the postnatal brain under both physiological and pathological conditions (Belvindrah *et al.*, 2011; Ota *et al.*, 2014; Petri *et al.*, 2017; Kaneko *et al.*, 2018; Sawada *et al.*, 2018), suggesting that regulation of neuroblast migration is critical for the plasticity of postnatal neuronal circuits.

V-SVZ-derived migrating neuroblasts show typical immature morphology, with a long leading process and a short trailing process (Schaar and McConnell, 2005). These neuroblasts show saltatory migration, which is executed by repeated extension of a leading process and subsequent somal translocation (Schaar and McConnell, 2005). Recently, accumulating evidence suggests that the primary cilium, a tiny cellular protrusion (Gomez-Gamboa *et al.*, 2014; Malicki and Johnson, 2017), and its associated cytoskeletal network play pivotal roles in neuronal migration (Baudoin *et al.*, 2012; Higginbotham *et al.*, 2012; Trivedi *et al.*, 2014; Guo *et al.*, 2017). Therefore, time-lapse imaging of these components is useful for analyzing their subcellular localization and understanding the mechanisms of neuroblast migration (Trivedi *et al.*, 2014 and 2017; Matsumoto *et al.*, 2019).

Here, we describe a detailed protocol that we have recently used for *in vitro* time-lapse confocal/superresolution imaging of the primary cilium in migrating neuroblasts (Matsumoto *et al.*, 2019). Images of whole-cell morphology and the primary cilium in cultured V-SVZ-derived neuroblasts could be recorded in a four-dimensional (x, y, z, and time) manner. This technique is widely applicable not only to migrating immature neurons but also to migrating nonneuronal cells in various species.

Materials and Reagents

1. 70 μ m Cell Strainer (BD, Falcon, catalog number: 352350)
2. 35 mm multiwell glass bottom dish (Matsunami, catalog number: D141400)
3. 35 mm tissue culture dish (BD, Falcon, catalog number: 353001)
4. 50 ml centrifuge tube (Thermo, catalog number: 339652)
5. 1.5 ml microtube (BIO-BIK, catalog number: CF-0150)
6. 0.6 ml microtube (QSP, catalog number: 502-PLN-Q)
7. Postnatal day 0-1 C57BL/6J mice (Japan SLC)
8. Postnatal day 0-1 *GFP::Cent2* mice (Higginbotham *et al.*, 2004)
9. CSII-CMV-Arl13b::Venus (Matsumoto *et al.*, 2019)
10. pdTomato-C1-Cent2 (dTomato::Cent2) (Matsumoto *et al.*, 2019)
11. pCAGGS-dnKif3A (Matsumoto *et al.*, 2019)
12. pcDNA3.1-EB3-mEGFP (EB3::GFP) (Watanabe *et al.*, 2015) (provided by Dr Kozo Kaibuchi at Fujita Health University)
13. Leibovitz's L-15 medium (GIBCO, catalog number: 11415-064)
14. Neurobasal medium (GIBCO, catalog number: 21103)
15. Penicillin-streptomycin (GIBCO, catalog number: 15140-122)
16. L-glutamine (GIBCO, catalog number: 25030)

17. NeuroBrew-21 (MACS Miltenyi Biotec, catalog number: 130-093-566)
18. DNase I (Roche, catalog number: 10-104-159-001)
19. 0.25% (w/v%) Trypsin-1 mmol/L EDTA·4Na Solution with phenol red 500 ml (WAKO, catalog number: 201-16945)
20. RPMI-1640 medium (WAKO, catalog number: 189-02145)
21. Amaxa Mouse Neural Stem Cell Nucleofector Kit (Lonza, catalog number: VPG-1004)
22. BD Matrigel matrix 10 ml (BD Biosciences, catalog number: 354234)
23. Fetal bovine serum (FBS)
24. KE-106 (Shin-Etsu Silicone)
25. CAT-RG (Shin-Etsu Silicone)
26. India ink
27. Neurobasal final medium (see Recipes)
28. 60% Matrigel/L15 (see Recipes)
29. DNase I solution (see Recipes)
30. 10% FBS/L15 (see Recipes)

Note: Plasmids used in this study (9.-11.) are available on request.

Equipment

1. Stereomicroscope (Olympus, model: SZ61)
2. Light source and fibers (Olympus, model: LG-PS2)
3. Microscopic scissors (Karl Hammacher GmbH, model: HSB014-11)
4. Forceps (Natsume Seisakusyo Co., Ltd., NAPOX A-1)
5. Ultrafine forceps (Dumont, model: DU-5/45)
6. Ophthalmic knife (MANI, model: MST15)
7. P1000 micropipet (Nichiryo, catalog number: NPX-1000)
8. P200 micropipet (Nichiryo, catalog number: NPX-200)
9. P20 micropipet (Nichiryo, catalog number: NPX-20)
10. P2 micropipet (GILSON, catalog number: F144801)
11. Amaxa NucleofectorTM II (Lonza)
12. Centrifuge (TOMY, model: PMC-060)
13. Confocal/superresolution laser-scanning microscope system (Carl Zeiss, model: LSM880 with Airyscan)
14. Laser line 561 nm (Carl Zeiss, catalog number: 000000-1410-117)
15. RE Argon Laser LGK7812 ML5 (Carl Zeiss, catalog number: 000000-2158-591)
16. Stage-top incubation chamber (Tokai Hit Co., Ltd., INUG2-WSKM)
17. CO₂ gas tank
18. CO₂ incubator (SANYO, model: MCO-18AIC)
19. Incubator (EYELA, model: NDS-500)

20. Water bath

Software

1. ZEN (Carl Zeiss)
2. FIJI (<https://fiji.sc>) (Schindelin *et al.*, 2012)
3. IMARIS (<https://imaris.oxinst.com>) (Bitplane, version 7.5.2)
4. EZR (<https://www.softpedia.com/get/Science-CAD/EZR.shtml>) (Kanda, 2013)

Procedure

A. Preparation of 35 mm dissection dish

1. Add KE-106 23 ml, CAT-RG 2.3 ml, and India ink 0.6 ml into a 50 ml tube, and mix them using a stirring rod until the silicone solution becomes homogeneous.
2. Centrifuge the tube at 3,300 x *g* for 1 min to remove bubbles in the silicone solution.
3. Add an appropriate amount of the silicone solution to a 35 mm dish.
4. Polymerize the silicone solution by incubation at 60 °C for 3 h in an incubator.

B. Dissection of V-SVZ tissues from postnatal day 0-1 (P0-1) mouse brains

A video and detailed procedure for dissection of V-SVZ tissue in the adult brain have been published previously (Mirzadeh *et al.*, 2010). Our method is modified from that of (Mirzadeh *et al.*, 2010) to increase the relative proportion of neuroblasts in the neonatal V-SVZ- tissues. For *in vitro* time-lapse imaging of migrating V-SVZ-derived neuroblasts, use P0-1 mouse brains. Older brains contain fewer migrating neuroblasts in the V-SVZ.

1. Anesthetize a P0-1 pup by hypothermia (1-2 min) and sacrifice it by cervical dislocation. Use 3-5 pups per experimental group. Tissue dissection (3.-10.) should be performed at 4 °C and within 10 min per pup.
2. Make an incision in both the scalp and skull along the midline of the head using scissors, and remove the dorsal part of the scalp and skull using forceps.
3. Extract the brain into L15 medium (2-3 ml) in a 35 mm dissection dish.
4. Divide the brain into two hemispheres by cutting along the midline under a stereomicroscope with a light source (Figure 1, Step 4).
5. View the brain hemisphere from the dorsal view, and remove the septum and the medial part of the OB (Figure 1, Step 5). This cutting enables one to visualize the anterior lateral wall of the lateral ventricle.
6. View the lateral wall from the en face view, and remove the cerebral cortex by cutting along the corpus callosum (Figure 1, Step 6).
7. View the brain from the dorsal view, and remove the hippocampus by cutting along the posterior part of the lateral ventricle, which is visualized as a cleft (Figure 1, Step 7).

8. View the lateral wall from the en face view, and remove the thalamus and olfactory bulb (Figure 1, Step 8). As a result, the whole lateral wall of the lateral ventricle can now be visualized.
9. View the brain from the ventral or dorsal view, and collect the V-SVZ tissue by cutting along the border between the V-SVZ and striatum (Figure 1, Step 9). Collected V-SVZ tissue should be semi-transparent. Contamination by a large amount of striatal tissue will decrease the number of neuroblasts migrating out from the cell aggregates embedded in Matrigel.
10. Trim the collected V-SVZ tissue by removing the ventral and posterior V-SVZ (Figure 1, Step 10), to increase the relative proportion of neuroblasts in the V-SVZ tissue (Doetsch and Alvarez-Buylla, 1996).

Figure 1. Dissection of V-SVZ tissues from postnatal mouse brains. Illustrations for steps 1-10 correspond to the descriptions in Procedure B Steps 1-10, respectively. Red arrows indicate lines of cutting. A video of the original procedure for dissection of V-SVZ tissues is shown in Mirzadeh *et al.* (2010).

C. Dissociation of V-SVZ cells

1. Incubate the V-SVZ tissues with 200 μ l of pre-warmed trypsin-EDTA (37 $^{\circ}$ C) at room temperature (RT) for 3-4 min without pipetting in a 1.5 ml microtube.
2. Briefly centrifuge the tube and remove the trypsin-EDTA.
3. Add 200 μ l of 10% FBS/L15 medium (for trypsin inactivation) and 8 μ l of 2 mg/ml DNase, and dissociate the V-SVZ tissues into single cells by pipetting using a P200 micropipet.
4. Pass the cell suspension through a 70- μ m cell strainer to remove tissue debris.
5. Add 800 μ l of DNase I solution with gentle pipetting.
6. Centrifuge the tube for 1 min at RT, and remove the supernatant.
7. Wash the cells with 800 μ l of DNase I solution with gentle pipetting.
8. Centrifuge the tube for 1 min at RT, and remove the supernatant.
9. Proceed to Procedure D (Nucleofection and Matrigel embedding). When using a transgenic mouse line that labels specific cells and/or subcellular organs by fluorescent proteins (e.g., *GFP::Cent2* mice), skip the steps for plasmid transduction (Steps D1-D8).

D. Nucleofection and Matrigel embedding

1. Prepare Mouse Neural Stem Cell Solution (Lonza, see manufacturer's instructions).
2. Resuspend the cells with 100 μ l of Mouse Neural Stem Cell Solution with gentle pipetting.
3. Add ~2.0 μ g of plasmid(s) to the cell suspension. If two or more plasmids are introduced, the total amount of those plasmids should be ~2.0 μ g.

For labeling of the primary cilium, we use a plasmid encoding Arl13b::Venus (Matsumoto *et al.*, 2019). For labeling of the basal body, we use a plasmid encoding dTomato::Cent2 (Matsumoto *et al.*, 2019). A GFP::Cent2 transgenic mouse line (Higginbotham *et al.*, 2004) is also useful for time-lapse imaging because all neuroblasts express GFP::Cent2. For labeling of the microtubule plus-end, we use a plasmid encoding EB3::GFP (Watanabe *et al.*, 2015).

4. Transfer the cell suspension to a cuvet using a dropper (included in the Amaxa Nucleofector Kit).
5. Transduce the plasmid(s) into the cells using Amaxa Nucleofector™ II (program: G-013).
6. Add 400 μ l of pre-warmed RPMI-1640 medium (37 °C) to the cuvet.
7. Transfer the cell suspension (a total of 500 μ l) to a 1.5 ml microtube and incubate it at 37 °C (water bath) for 15 min to allow the cells to recover.
8. Centrifuge the tube for 1 min at RT, and remove the supernatant.
9. Wash the cells with 200 μ l of DNase I solution with gentle pipetting, and transfer the cell suspension to a 0.5 ml PCR tube.
10. Centrifuge the tube for 2 min at RT.
11. Carefully transfer the cell pellet (while maintaining its shape) from the PCR tube to L-15 medium in a 35 mm dissection dish by gentle pipetting (Figure 2, Step 1).
12. Prepare 500 μ m x 500 μ m x 500 μ m cell aggregates from the cell pellet using an ophthalmic knife and ultrafine forceps (Figure 2, Step 2).
13. Carefully transfer 1-5 cell aggregates into a 1st drop (8 μ l) of 60% Matrigel/L15 on a 35 mm dish (4 °C), to replace L-15 medium surrounding the cell aggregates with 60% Matrigel/L15 (Figure 2, Step 3). The percentage of Matrigel will affect the migration behaviors of cultured neuroblasts: individual and chain migration will be observed in 50% and 70-100% Matrigel, respectively. Matrigel concentration less than 50% will result in the failure of gelation (Step D16).
14. Carefully transfer the cell aggregates from the 1st drop into a 2nd drop (8 μ l) of 60% Matrigel/L15 on a 35 mm dish (4 °C) (Figure 2, Step 4). Carrying in an excess volume of the 1st drop with the cell aggregates will result in a decrease of the Matrigel concentration in the 2nd drop and the failure of gelation (Step D16).
15. Add 60% Matrigel/L15 containing 2-5 cell aggregates to a 35 mm multiwell glass-bottom dish (Figure 2, Step 5).
16. Incubate the dish for 3 min at 4 °C for sedimentation of cell aggregates within 60% Matrigel/L15, and subsequently for 15 min at 37 °C in the presence of 5% CO₂ for Matrigel gelation.
17. Carefully add 200 μ l of Neurobasal final medium and culture the cell aggregates for 2 days at 37 °C in a CO₂ incubator in the presence of 5% CO₂.

Figure 2. Preparation of V-SVZ cell aggregates. Illustrations for steps 1-5 correspond to the descriptions in Procedure D Steps 11-15, respectively. Blue and red arrows in steps 1 and 3-5 indicate the direction of blowing and aspiration, respectively. Red arrows in step 2 indicate lines of cutting. Cell aggregates indicated by asterisks (step 2) should be discarded. These procedures have been used previously (Hirota *et al.*, 2007).

E. *In vitro* time-lapse imaging of migrating neuroblasts

1. Start up the confocal laser-scanning microscope system and stage-top incubation chamber set at 37 °C. To stabilize the culture environment within the stage-top incubation chamber, incubate the chamber without cells for 1 h at 37 °C in the presence of 5% CO₂.
2. Set a 35 mm glass-bottom dish containing cultured neuroblasts in the stage-top incubation chamber, and incubate it for 2 h at 37 °C in the presence of 5% CO₂.
3. Choose the appropriate objective lens. To perform time-lapse imaging of the basal body and primary cilium, a 63x oil-immersion objective lens is preferable. Lengths of a neuroblast and its primary cilium are typically 0.5-3.0 μm and 60-80 μm, respectively. Since the primary cilium and its associated structures are tiny subcellular components, confocal or superresolution laser-scanning microscopy is highly recommended for time-lapse imaging to understand their spatiotemporal dynamics in migrating neuroblasts. The optical zoom and resolution setting in the confocal laser-scanning microscope system are also useful to increase the imaging resolution.
4. Set the laser configuration for image acquisition in the confocal laser-scanning microscope system by adjusting the pinhole size, laser intensity, detector gain and offset in all channels. Set the pinhole to 1 Arbitrary Unit for clear image acquisition of the basal body and primary cilium. Excessive laser intensity will induce phototoxicity or photobleaching in targeted neuroblasts and

their primary cilium. High-sensitivity GaAsP detectors can be used for brighter time-lapse imaging. Excitation wavelengths used in this study were as follows: 488 nm for GFP/Venus, 561 nm for dTomato.

5. (Optional) Improve the resolution of time-lapse images by using superresolution methodology. For settings used for superresolution imaging, follow the instructions of the microscope's supplier. In our EB3::GFP and dTomato::Cent2 imaging, which visualizes microtubule polymerization and centrioles, respectively, we use an LSM880 with Airyscan imaging system (Carl Zeiss), and its image acquisition settings are as follows: 63x (NA 1.4) oil-immersion objective lens, S-R mode, Optical zoom 1.8x, 0.043 $\mu\text{m}/\text{pixel}$, and pixel dwell 2.38 μsec (Matsumoto *et al.*, 2019).
6. Set time- and z-intervals, which are determined depending on the experimental purpose. In our experiments (Matsumoto *et al.*, 2019), time-lapse images of primary cilium and microtubule polymerization dynamics in neuroblasts are acquired at 1-2 min and 4-sec intervals, respectively. To monitor the dynamics of the basal body and primary cilium in migrating neuroblasts, z-stack images are acquired at 0.6 μm intervals.
7. Set the imaging period, which is determined depending on the experimental purpose. If photobleaching or phototoxicity is observed, decrease the laser intensity, and/or increase the z-/time-intervals.
8. Start the image acquisition. After finishing image acquisition, save the imaging file in a format specified by the confocal microscope supplier (e.g., .ism or .czi format for Carl Zeiss). If necessary, save z-stack projection images as well.

Data analysis

- A. Three-dimensional reconstructions of migrating neuroblasts and their primary cilium
 1. Open the original imaging file (e.g., .ism or .czi format for Carl Zeiss) to visualize fluorescent objects using IMARIS software.
 2. Select "Surfaces" from the "Surpass" tab to add a new surface (named "Surfaces 1") in the "Surpass Scene" folder shown in the left upper box of the IMARIS interface (Figure 3, No.1-No.3).

Figure 3. IMARIS interfaces in step A-2. Numbers indicate the sequence of procedures.

3. [Step 1/4 Algorithm] Select “Surfaces 1” in the “Surpass Scene” folder (Figure 3, No.3), and select “Default” in “Load Parameters” in the “Create” tab shown in the bottom box of the IMARIS interface (Figure 4A, No.1 and No.2). Then press the “Next” button (Figure 4A, No.3).

Figure 4. IMARIS interfaces in steps A3-A6. Panels (A-D) indicate the sequence of procedures in steps A3-A6.

4. [Step 2/4 Source Channel] Select the channel of interest from “Source Channel”, and check “Smooth” (Figure 4B, No.1 and No.2) “Surfaces Area Detail Level” is originally set to 0.2 μm , which affects the smooth level of 3D reconstruction. Then select “Absolute Intensity” in “Thresholding”, and press the “Next” button, which will result in generating a 3D reconstruction (Figure 4B, No.3 and No.4).
5. [Step 3/4 Threshold] Quality of generated 3D reconstruction will be revised by adjusting the upper/lower limit values (active zone is indicated by yellow) of “Threshold”. Then press the “Next” button (Figure 4C, No.1 and No.2).
6. [Step 4/4 Classify Surfaces] Select the “Filter Type,” and/or adjust the upper/lower limit values of the histogram (active zone is indicated by yellow), to optimize the morphology of the generated 3D reconstruction (Figure 4D, No.1). If the 3D reconstruction is not visible, select

“Surface” in “Surfaces Style/Quality” of the “Settings” tab (Figure 4D, No.2). Then press the “Finish” button (Figure 4D, No.3). Colors and transparency can be changed using the “Color” tab shown in the left bottom of the IMARIS interface (Figure 4D, No.4).

7. Repeat steps A2-A6 until the complete structure of interest is reconstructed.
8. Select “Show Display Adjustment” from the “Edit” tab, and the uncheck mark in all channels to hide the original fluorescent objects (Figure 5, No.1-No.3).

Figure 5. IMARIS interfaces in step A8. Numbers indicate the sequence of procedures.

9. Save the file using bitplane format.
10. A time-lapse movie file is generated by selecting “recording (red circle)” shown in the left bottom of the Imaris interface.

Examples of time-lapse images and 3D reconstructions of a migrating neuroblast and its primary cilium are shown in Figure 6, and in Matsumoto *et al.* (2019).

Figure 6. Examples of a time-lapse image and 3D reconstruction of a cultured migrating

neuroblast and its primary cilium. Representative time-lapse image of a cultured migrating neuroblast expressing GFP::Cent2 and Arl13b::Venus (A) and its 3D reconstruction (B). Arl13b::Venus+ primary cilium and GFP::Cent2+ basal body are shown in white (A). Green and red colors in (B) indicate cell body and primary cilium of a neuroblast, respectively. Scale bar, 2 μ m (A). Images are modified from Matsumoto *et al.* (2019) with permission.

B. Analyses of dynamics of migrating neuroblasts and their ciliary structures

1. Convert the imaging file to an image sequence (e.g., .tif format). In the case of superresolution imaging, first make super-resolution images from the raw data by using the microscope supplier's software (e.g., ZEN for Carl Zeiss), and then convert it to an image sequence.
2. Open the image sequence file in FIJI.
3. Select the "Manual Tracking" plugin from "Plugins" -> "Tracking".
4. Enter the parameters for "Time-interval" and "x/y calibration" (resolution) (Figure 7, No.1), which are available in the original imaging files (format for confocal microscope supplier).
5. Press "Add track" in the *Tracking* section to start tracking of the structure of interest (cell, basal body, microtubule plus-end, etc.) (Figure 7, No.2). Since the somata change their morphology during saltatory neuronal movement, keep clicking at the center of the soma (center of longitudinal and transverse axes) for analysis of somal movement in migrating neuroblasts. For smaller cellular structures, including basal body and microtubule plus-end proteins, we trace the center of dot-like fluorescent signals.
6. After tracking several sequential images, press "End track" in the *Tracking* section to finish tracking (Figure 7, No.3).
7. Repeat steps B5 and B6 until all of the structure of interest are traced.
8. After finishing tracking, put a check mark in the box "Show text?" in the *Drawing* section (Figure 7, No.4), and generate a new file containing all the traces by pressing "Overlay Dots and Lines" in the *Drawing* section (Figure 7, No.5). Save the generated file as a tif file (Figure 8, Video 1). This file is useful for referring to tracking numbers in step B11.

Video 1. Manual tracking of neuroblast's soma (No.2, green) and basal body (No.1, blue)

Figure 7. Analysis of dynamics of migrating neuroblasts and their ciliary structures using FIJI. Manual Tracking plugin interface in FIJI is shown. Numbers indicate the sequence of procedures.

Figure 8. Representative images of manual tracking of neuroblast's soma (No.2, green) and basal body (No.1, blue). Scale bars, 5 μm. Images are modified from Matsumoto *et al.* (2019) with permission. Also see Video 1.

9. Save the generated table recording the tracking results as a .csv file.
10. Open the table file (.csv or .xls) using Microsoft Excel.
11. Relative coordinates, migration distance, and migration speed in every session of all the tracked structures are shown. Tracking number in the table corresponds to the number in the tif file generated in step B8. Calculate net migration speed by averaging migration speed in every

session.

12. For saltatory migration analysis, migratory behavior of neuroblasts is divided into two phases, resting and migratory phases, based on the speed criterion (12 $\mu\text{m}/\text{h}$) (Matsumoto *et al.*, 2019), which depends on the cell types and experimental conditions. Measure the exact time of resting/migratory phases in one saltatory migration cycle.
13. For analyses of subcellular structure dynamics (leading process, basal body, microtubule plus-end, *etc.*), analyze their direction, trajectory, migration distance (stride), and speed based on the numerical data obtained in step B9. Direction, trajectory, and stride of subcellular structures are visualized by making a graph using the relative coordination data. Speed of subcellular structures is analyzed as described in step B11. By combining the information for resting and migratory phases (step B12), dynamics of subcellular structures in those migration phases will also be analyzed. Perform statistical analyses using EZR (Kanda, 2013).
14. Examples of quantitative analyses of the dynamics of the leading process, basal body, soma, and microtubule polymerization during saltatory movement in cultured migrating neuroblasts are shown in (Matsumoto *et al.*, 2019).

Recipes

1. Neurobasal final medium (can be stored at 4 °C for up to one week)
Neurobasal medium 10 ml
2% NeuroBrew-21
2 mM L-glutamine
50 U/ml penicillin-streptomycin
2. 60% Matrigel/L15 (store at 4 °C, freshly prepared on the day of each experiment)
Matrigel 60 μl
Leibovitz's L-15 medium 40 μl
3. DNase I solution (can be stored at 4 °C for up to one week)
1 mg/ml DNase 400 μl
Leibovitz's L-15 medium 10 ml
4. 10% FBS/L15 (can be stored at 4 °C for up to one month)
FBS 1 ml
Leibovitz's L-15 medium 9 ml

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Competing interests

The authors declare no competing interests.

Ethics

All experiments involving live animals were performed in accordance with the guidelines and regulations of Nagoya City University (Approval ID, H27M-10; Validity period, April 2015 – March 2020).

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