# Microtubule Dynamics in Primary Hippocampal Neurons

*Day 1*:

*Timing: 10 min.*

1. Prepare MatTek live imaging dishes (35 mm petri dish, 14 mm microwell) with 0.1 mg/mL PDL in 1x sterile borate buffer for 24 hours at 37º C. The microwell holds ~300 µL volume. PDL only needs to cover the microwell.

*Day 2*:

*Timing: 2-3 hrs.*

2. Culture primary hippocampal neurons from E18 rat. Seed 50,000-70,000 cells into microwells of coated MatTek dishes. Spread the cells evenly in the center. After two hours, add 2 ml of culturing medium.

*Day 3-16*:

*Timing: 30 minute.*

3. To maintain the cultures, change medium every 3-4 days. If starting volume is ~2 mL, take 300-400 µL out and add 500-600 µL of conditioned medium.

4. Healthy neurons will be evenly spread in the imaging chamber with multiple extended and branched dendrites and high spine density. This is critical for transfection efficiency and expected EB3 puncta motility.

*Day 16:*

*Timing: 3 hrs.*

5. Hippocampal neurons are transfected at 16 DIV with Lipofectamine 2000 reagent. For one dish, mix 1-1.5 µg of plasmid DNA (GFP-EB3) with 300 µL of incomplete neurobasal medium (no B27, no glutamax, no antibiotics).

6. Add 3 µL of Lipofectamine 2000 to the diluted plasmid DNA-media mix followed by vortexing. Incubate Lipofectamine-DNA mix at room temperature for 30 min.

7. Pipette original conditioned medium out from the dishes and save in conical tubes. Add lipofectamine-plasmid DNA mix dropwise to the microwells and make sure the

microwells are well covered. Put dishes back into the incubator and the conditioned medium in conical tube in a 37ºC water bath.

8. Incubate for 2 hours.

9. Take dishes out of the incubator and aspirate out transfecting reagents. Tilt the dish so that the liquid is aspirated out completely and the pipette tip doesn't touch the cells. Add back 2 mL of conditioned medium immediately. Do not let the cells dry.

**Live cell imaging in primary hippocampal neurons.**

10. Live cell imaging of EB3 comets in hippocampal neuronal culture is performed 24-48 hours after transfection.

11. Prepare complete HBSS as recording medium and warm it up in a 37 ºC water bath.

| | Stock Solution | Volume | Final concentration |
|---|---|---|---|
| **Complete HBSS (cHBSS) 500 mL** | 10x HBSS | 50 mL | 1x |
| | 1 M Hepes (pH7.4) | 1.25 mL | 2.5 mM |
| | 1 M D-glucose | 15 mL | 30 mM |
| | 100 mM CaCl$_2$ | 5 mL | 1 mM |
| | 100 mM MgSO$_4$ | 5 mL | 1 mM |
| | 1 M NaHCO$_3$ | 2 mL | 4 mM |
| Add ddH$_2$O to a total volume of 500 mL. Sterile filter with a 0.2 uM filter. Store at 4°C. | | | |

12. Movies are acquired using an epifluorescence microscope (Olympus IX81) equipped with a controlled temperature and CO$_2$ incubator. Stabilize the temperature and humidity of incubator before imaging. The microscope was equipped with a 60×/0.7 Plan Apochromat objective, perfect focus controller, encoded XY stage, filter wheels, and epifluorescence illumination. Videos were acquired with a monochrome charge-coupled device camera (Sensicam QE; Cooke Corporation) and integrated Slidebook software at 2s/frame for 3 min.

13. Select dendrites and axons (smaller diameter than dendrites and no spine structures along the neurite) based on morphology. Proximal axonal and dendritic segments (within 100 μm from the cell body according to our definition) are determined by first locating the cell body and then tracing the distance from cell body based on the real distance in μm in a still image taken with camera.

**Quantification:**

14. Tiff files can be opened by ImageJ software. To better visualize the comets and reduce background, the average intensity projection image of a time-lapse can be subtracted from each frame. Click Image → stacks → z projects, then choose average intensity. The average intensity projection image of the movie will show up. Click process → image calculator, then subtract the average intensity projection image from every frame of the movie. Save as tiff file.

15. Kymographs are generated by drawing a region on the axons/dendrites from the cell body to the most distal visible end of the neurite. Axons are identified based on morphology and uniform anterograde movement of EB3-labeled MT plus ends. Open the tiff file movie in MetaMorph software to generate kymographs. Draw a line along the neurite from the cell body to the most distal visible end of the the axon. Click stack → kymograph. Click measure → calibrate distance to set pixel to μm conversion. Measure only comet tracks moving within 100 μm from the cell body. If doing the analysis in ImageJ, install KymoResliceWide plugin. Click Analyze → set scale to convert px to μm. Draw the line along axons from cell the body to the end of the neurite. Normally axons need 10-15 px wide lines. Click plugin → KymoRescliceWide to generate a kymograph. Length of growth can be measured directly on the comet track. Comet lifetime has to be converted by the Y-axis length in the ratio of kymograph width which is the total length of movie time. Then growth rate can be calculated.

Parameters describing MT dynamics are defined as follows:

| MT dynamics parameter | definition |
| --- | --- |
| rescue/nucleation frequency | number of rescue or nucleation events per $\mu m^2$ per min |
| catastrophe frequency | number of full tracks/total duration of growth |
| Comet density | number of comets per $\mu m^2$ per min |
| growth length | comet movement length in $\mu m$ |
| comet lifetime | duration of growth in sec |
| growth rate | growth length/comet lifetime |