



Apr 14, 2020

Quasi-periodic migration of single cells on short microlanes [↗](#)

PLOS One

Fang Zhou¹¹Faculty of Physics and Center for NanoScience, Ludwig-Maximilians-Universität München, Munich, Germany**1** Works for me dx.doi.org/10.17504/protocols.io.bcdiis4e

ABSTRACT

Cell migration on microlanes represents a suitable and simple platform for the exploration of the molecular mechanisms underlying cell cytoskeleton dynamics. Here, we report on the quasi-periodic movement of cells confined in stripe-shaped microlanes. We observe persistent polarized cell shapes and directed pole-to-pole motion within the microlanes. Cells depolarize at one end of a given microlane, followed by delayed repolarization towards the opposite end. We analyze cell motility via the spatial velocity distribution, the velocity frequency spectrum, and the reversal time as a measure for depolarization and spontaneous repolarization of cells at the microlane ends. The frequent encounters of a boundary in the stripe geometry provides a robust framework for quantitative investigations of the cytoskeleton advancement and repolarization dynamics. In a first advance to rigorously test physical models of cell migration, we find that the statistics of the cell migration is recapitulated by a Cellular Potts model with a minimal description of cytoskeleton dynamics. Furthermore, we investigate whether the microlane tip shape has an effect on cell dynamics. Using LifeAct-GFP transfected

cells and microlanes with differently shaped ends, we show that the local deformation of the leading cell edge in response to the tip geometry can locally either amplify or quench actin polymerization.

EXTERNAL LINK

<https://doi.org/10.1371/journal.pone.0230679>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Zhou F, Schaffer SA, Schreiber C, Segerer FJ, Goychuk A, Frey E, Rädler JO (2020) Quasi-periodic migration of single cells on short microlanes. PLoS ONE 15(4): e0230679. doi: [10.1371/journal.pone.0230679](https://doi.org/10.1371/journal.pone.0230679)

1 Preparation of micropatterns.

1.1 Production of stamp masters by photolithography

- 1). A silicon wafer was coated with TI Prime adhesion promoter and then baked on a heating plate.
Settings of spincoating: firstly 500 rpm for 5s, then 5000 rpm for 30s
Settings of baking: 120 °C for 2 mins
- 2). The wafer was further spincoated by AZ40XT (MicroChemicals) photoresist and baked again on the heating plate.
Settings of spincoating: 800 rpm for 3s, then 3000 rpm for 30s.
Settings of baking: 80°C for 1 min, 100 °C for 1 min and 125°C for 1 min.
- 3). The wafer was further exposed to UV light using laser direct imaging (Protolaser LDI, LPKF) to write the designed patterns and was baked again.
Settings of laser: laser spot 1 µm, spot spacing 0.2 µm, illumination dose 450 mJ/cm²
Setting of baking: 105°C for 1.5 min
- 4). Afterwards, the photoresist was developed (AZ 826 MIF, MicroChemicals) for about 2 min to remove the photo resist,
- 5). Then, it was silanized with Trichloro(1H,1H,2H,2H-perfluorooctyl)silane, (Sigma-Aldrich).

1.2 Production of PDMS stamps

- 1). To fabricate the stamp, polydimethylsiloxane (PDMS) monomer and crosslinker (DC 184 elastomer Kit, Dow Corning) were mixed in a 10:1 ratio (w/w), and degassed in a vacuum oven for 30 min.
- 2). The mixer was poured onto the master mold, and cured 3 h or overnight at 50°C.
- 3). Finally, the crosslinked PDMS layer was peeled off and manually cut into stamps.

1.3 Microcontact printing

- 1). PDMS stamps were exposed with UV-light (PSD-UV, Novascan Technologies) for 5 min.
- 2). The stamps were then immersed in an aqueous solution of 40 µg/ml fibronectin (Yo Proteins) containing 10 µg/ml Alexa Fluor 488 dye (Life Technologies) labeled fibronectin for 45 min.
- 3). The stamps were subsequently washed with ultrapure water.
- 4). The stamps were dried under filtered airflow and then stamped onto a hydrophobic uncoated µ-Dish (Ibidi GmbH) bottom that underwent UV exposure for 15 min beforehand.
- 5). The stamps were gently pressed with tweezers for a few seconds to ensure contact with the bottom.
- 6). To fabricate the cell-repelling areas, 30 µL of 2 mg/ml poly-L-lysine-grafted polyethylene glycol (PLL-g-PEG) (2 kDa PEG chains, SuSoS) dissolved in 10 mM Hepes and 150 mM NaCl solution was added.
- 7). The stamps were removed. A glass cover slip was placed on the printed bottom to assure complete coverage with the PEG solution and then incubated for 30 min at room temperature.
- 8). Finally, the printed bottom was washed with phosphate buffered saline (1xPBS) three times and stored in 1x PBS for further cell seeding.

2 Cell culture

2.1 Cell seeding

- 1). MDA-MB-231 breast cancer cells were cultured in modified Eagle's medium (MEM-F10, c.c.pro) supplemented with 10% fetal calf serum (FCS, Invitrogen) and 2.5 mM L-glutamin (c.c.pro) at 37°C in 5% CO₂ atmosphere.
- 2). For time-lapse phase-contrast images, cells were seeded at a density of 1×10^4 cells per dish (µ-Dish, IBIDI).
- 3). After 2 h, cell medium was replaced by 1 ml Leibovitz's L-15 Medium (c.c.pro) containing 10% FCS and 25 nM Hoechst 33342 nucleic acid stain (Invitrogen) and incubated for 1 h at 37°C before imaging.

2.2 Cell transfection.

- 1). For actin dynamics studies, seeded cells were further transfected with LifeAct-GFP mRNA.
~ 1×10^4 cells were seeded into a 35mm µ-Dish and incubated 2 h at 37°C in 5% CO₂ for cell adhesion.
- 2). 1.25 µl Lipofectamine MessengerMax Reagent (Invitrogen) was diluted in 123.75µl OptiMEM (Life Technologies) transfection medium and incubated 10 min at room temperature.
- 3). 500 ng mRNA (0.5 µl x 1000 ng/µl) was diluted in 124.5 µl OptiMEM.
- 4). Both solutions were mixed and incubated for 5 min at room temperature for lipoplex formation.
- 5). Adhered cells were washed with 1xPBS, and carefully added to the 250 µl transfection mix.
- 6). After a 1 h incubation at 37°C in 5% CO₂, the cell transfection mix was replaced by 1 ml Leibovitz's L-15 Medium (c.c.pro) containing 10% FCS before proceeding to time lapse imaging.



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