Millicell® µ-Migration Assay Kit

Catalogue No. MMA205

FOR RESEARCH USE ONLY
Not for Use in Diagnostic Procedures

USA & Canada Phone: +1(800) 437-7500 Fax: +1 (951) 676-9209
Australia +61 3 9839 2000
www.millipore.com

READ INSTRUCTIONS CAREFULLY BEFORE USE

This is essential for successful assay performance.

Contents

Introduction

Cell migration occurs in a variety of biological processes including wound healing, embryonic development, and immune response. Because of the importance of cell migration in angiogenesis, oncology, neurology, and especially immunology, there is an increased interest in understanding the underlying biological mechanisms at a single cell level.

Traditional migration assays employ a multiwell or Boyden chamber in which cells are seeded on top of the insert in serum-free medium, while serum or similar chemoattractants are placed in the well below. Limitations to multiwell migration assays include the following: (1) the gradients are not linear, well-established, or controlled, (2) the quantification of migratory cells is based on an endpoint measurement rather than a dynamic determination, and (3) imaging of the cells while they are migrating is currently not possible. Millipore's Millicell µ-Migration Assay Kit overcomes the limitations of traditional multiwell migration assays. The innovative design of the µ-Migration Slide promotes a stable diffusion-generated concentration gradient that is consistently linear and lasts for more than 48 hours. Made from a plastic with high optical qualities similar to those of glass, the µ-Migration Slide is specially designed for video microscopy assays. At specific time intervals, images of the observation area can be acquired, allowing real-time monitoring and quantitative measurements of cell migration.

The Millicell µ-Migration Assay Kit includes the following components:

1. **Four µ-Migration Slides, with 12 plugs and 12 cultivation caps per slide.** Each slide has 3 chambers (Figure 1) for three independent experiments, a total of 12 chambers per kit. Based on the ibidi® technology platform, the µ-Migration Slides possess enhanced optical imaging capabilities with low birefringence and autofluorescence properties. The slides are sterile and should not be autoclaved. Specially designed plugs and caps are provided to hinder evaporation and stabilize the diffusion gradient.

   One of the four slides may be used as a practice slide. It is highly recommended that first-time users practice with a slide to minimize errors (e.g., introducing air bubbles). The practice slide should not be re-used later.

2. **One vial of 1 mg/mL Human Collagen IV (100 µg).** A major structural element of basal membranes, human collagen type IV is frequently used to culture a variety of cell types including HUVEC, HT-1080, NIH-3T3, and MDA-MB-231. The Human Collagen IV solution provided is sufficient to coat more than 12 chambers at the recommended 30 µg/mL concentration.

3. **One vial of 0.5 M Acetic Acid Buffer (1.5 mL).** The buffer may be used to dilute the Human Collagen IV to the recommended 30 µg/mL concentration.

4. **One vial of 30% BSA Solution (100 µL).** The BSA solution may be used to make up serum-starvation medium.

5. **Two boxes of 200 µL beveled pipette tips (96 tips per box).** To minimize the risk of introducing air bubbles during assay set-up, these tips should be used for all manipulations involving the slides (e.g., loading of cells and media onto the slides).

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It is not for use in diagnostic procedures.

µ-Migration Slide Specifications

Number of chambers on slide	3
Volume per chamber	80 µL
Observation window dimensions	2 × 1 mm
Total height with plugs	12 mm
Total height without plugs	4.55 mm
Volume of chemoattractant	18 µL
Slide dimensions	25.5 × 76.6 mm

Kit Components and Storage

Kit shelf life: 6 months from date of receipt when reagents are stored and handled appropriately.

1. **Millicell µ-Migration Slides:** Part No. CS204500, four uncoated sterilized slides. Each slide contains 3 chambers. Store at room temperature.
2. **Millicell µ-Migration Caps and Plugs:** Part No. CS204501, twelve caps and twelve plugs for use with the µ-Migration Slides (4 for each of the 3 chambers on a slide). Store at room temperature.
3. **Human Collagen IV:** Part No. CS204498, one 100 µg vial of 1 mg/mL Human Collagen IV. Store at -20 °C.
4. **Acetic Acid Buffer:** Part No. CS204485, one vial containing 1. 5 mL of 0.5 M acetic acid. Store at room temperature.
5. **BSA Solution:** Part No. CS204507, one vial containing 100 µL of 30% BSA Solution. Store at -20 °C.
6. **Beveled Pipette Tips:** USA Scientific® TipOne® 200 µL tips, USA Scientific Cat. No. 1111-1810, two 96-tip boxes. Store at room temperature.

Materials Required but Not Supplied

1. Adherent cells
2. Inverted microscope (phase contrast, fluorescence, etc.)
3. Heating stage (check dimensions to make sure that the slide fits)
4. Time-lapse video equipment (CCD camera, video camera, acquisition software)
5. Motorized stage and autofocus (x,y,z) to observe all 3 chambers in parallel (optional)
6. Precision pipettes and pipette tips (10 µL and 200 µL) for routine cell culture manipulations

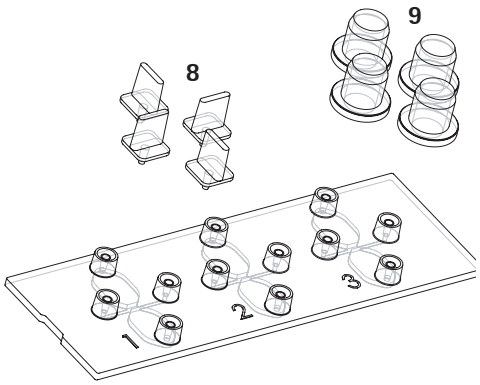
   Note: Pipette procedures that involve loading cells and/or media into the µ-Migration Slide should be performed using only the supplied special beveled pipette tips (USA Scientific TipOne 200 µL tips, Cat. No. 1111-1810)
7. Fisherbrand® Micropipette 0.1–10 µL Tips (Fisher Scientific® Cat. No. 02-707-136)
8. Cell culture growth medium, appropriate for cells under study
9. Accutase™ Cell Dissociation Solution (Cat. No. SCR005)
10. Phosphate-buffered Saline, 1X PBS, (Cat. No. BSS-1005-B)
11. Sterile distilled water (Cat. No. TMS-006-C or TMS-006-B)
12. Low speed centrifuge
13. Sterile microcentrifuge tubes
14. 37 °C incubator with 5% CO_2
15. Scepter™ Handheld Automated Cell Counter (Cat. No. PHCC00000) or Hemacytometer
16. Trypan Blue (optional)

Principle

The µ-Migration Slide has been adapted for adherent cells only. All manipulations should be carried out under sterile conditions. A schematic of the slide and chamber configuration is shown in **Figure 1**. Each slide contains three chambers that may be used in parallel experiments or individually, at different times. Plug all adapters (adapters 1, 5, 6, and 7, **Figure 1B**) of any unused chambers on the slide to ensure sterility for later applications.

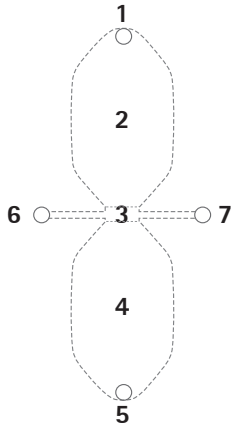
Figure 1. Slide and Chamber Configuration

A. Slide Configuration



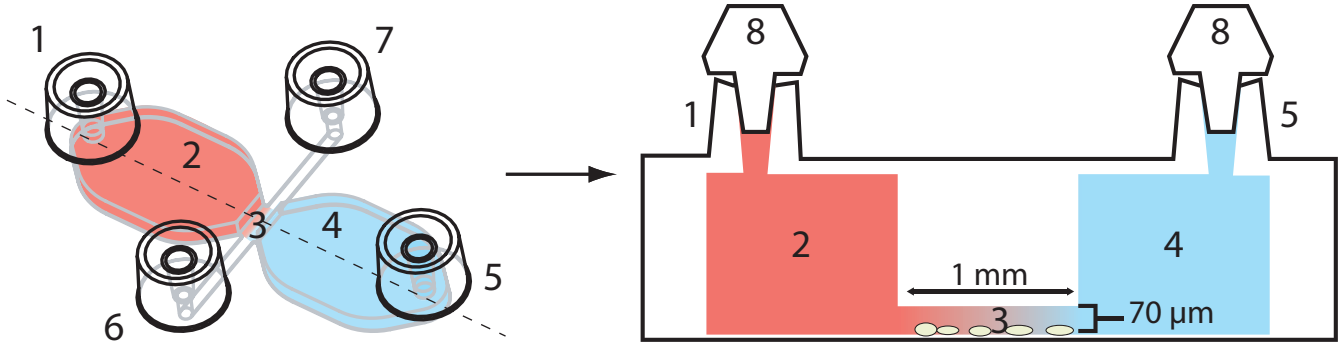
1 - Pipette adapter
2 - Reservoir (40 µL)
3 - Observation window
4 - Reservoir (40 µL)
5 - Pipette adapter
6 - Cell inlet adapter
7 - Cell inlet adapter
8 - Plugs
9 - Cultivation caps

B. Chamber Configuration



Two large volume reservoirs (2 and 4) are connected by a thin slit called the observation window (**Figure 2**). When reservoirs 2 and 4 are filled with different chemoattractant concentrations (as indicated below in red and blue), a linear and stable diffusion-generated concentration gradient is set up in the observation window. The migration of adherent cells plated in the observation window can be monitored in real time through the acquisition of images obtained using a time-lapse video camera.

Figure 2. Reservoir Configuration



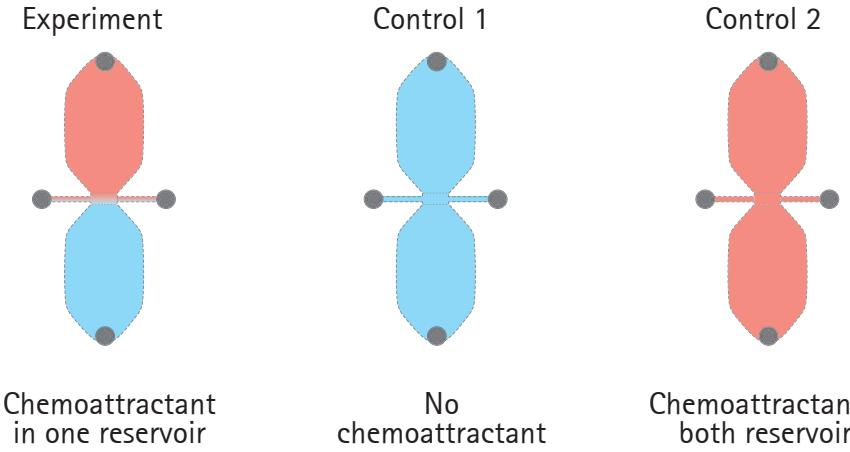
Cross section showing location of cells, chemoattractant, and gradient

Principle, continued

For optimal results, we recommend performing two control experiments by filling two additional chambers on a slide (both reservoirs) with or without chemoattractant solution. This helps determine whether a compound is influencing directed movement of the cells (chemotaxis) and/or influencing random migration (chemokinesis).

Assay Instructions

Figure 3. Recommended Experimental Setup



-- Critical Tip -- Recommended Tip -- Optional Tip

1. Day Minus 1 (two days before cell migration experiment) Preparation of Coated Slides

The observation window in the µ-Migration Slide must be coated with ECM to promote cell adhesion. Uncoated slides will not permit cell adhesion and growth.

The following protocol for coating the µ-Migration Slide with 30 µg/mL Human Collagen IV has been determined empirically to work for HT-1080, HUVEC, MDA-MB-231, and NIH-3T3 cell types. ECM requirements may differ with different cell types and should be optimized by the user. Because adhesion proteins are biological reagents, there may be quality differences between manufacturer lots. It is recommended that tests be performed with each new lot of ECM. Prepare coating substrates according to the manufacturer's specifications. Coating with other ECMs (not included) can be adapted using similar procedures.

Human Collagen IV coating: Coating of the µ-Migration Slides should be performed at least 2 or more days before initiating the migration experiments. The µ-Migration Slides may be coated beforehand and stored at room temperature for up to one month. Perform the following steps in a sterile tissue culture hood.

1.1 Bring the 1 mg/mL Human Collagen IV Solution (supplied) to room temperature.

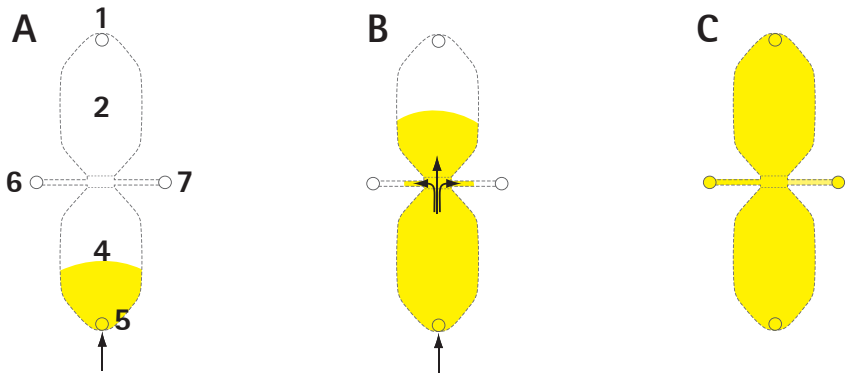
1.2 Dilute the 1 mg/mL Human Collagen IV Solution to the desired stock concentration of 30 µg/mL with 0.5 M Acetic Acid Buffer (supplied).

1.3 Using a beveled pipette tip (supplied), pipette up 82 µL of the 30 µg/mL Human Collagen IV solution.

Note: It is critical that the supplied beveled pipette tips be used rather than normal pipette tips.

1.4 In one hand, hold and tilt the µ-Migration Slide at a 30° angle from the horizontal. With the other hand, place the beveled pipette tip containing the Human Collagen IV Solution at a 90° angle into pipette adapter 5 and **very** slowly pipette in the 82 µL of 30 µg/mL Human Collagen IV Solution (**Figure 4A**) until reservoirs 2 and 4 are completely filled (**Figures 4B, 4C**). It should take approximately 30 seconds to fill both reservoirs. Do not push forcibly on the pipette plunger to eject any remaining liquid as this may force air into the µ-Migration Slide and generate unwanted bubbles. Instead, keep the pipette plunger pressed down as you remove the empty pipette tip from the adapter. This ensures that liquid is not drawn back into the pipette tip due to negative back-pressure. When applied properly, the coating volume should be sufficient to fill both reservoirs as well as most of pipette adapter 1 completely. Some liquid spill-over is acceptable.

Figure 4. Filling the Chambers with Coating Solution



Air bubbles that form due to improper pipetting techniques may affect the quality of the results. Below are tips to avoid air bubbles during pipetting:

- Use the beveled pipette tips (supplied) when performing cell and/or media manipulations involving the µ-Migration Slide. The beveled pipette tips are critical in helping to prevent air bubbles.
- Tilt the µ-Migration Slide while filling the chambers to allow the air that is being displaced by the coating solution to escape through pipette adapter 1.
- Always place the pipette tip into the desired pipette adapter at a 90° angle. Do not move the tip around.
- Inject solutions **very slowly** into the µ-Migration Slide. This will help to ensure an even coating. It should take at least 30 seconds or more to fill reservoirs 2 and 4.
- Once most of the liquid solution has been injected, do not try to eject the last bit of liquid by exerting additional pressure on the pipette plunger. This may introduce air from the emptied tip and create bubbles in the chamber.
- Do not release the pipette plunger before removing the pipette tip from the adapter, as some liquid may be drawn back into the pipette tip by negative back-pressure. Instead, maintain constant downward pressure on the pipette plunger as you remove the empty pipette tip from the adapter.

1.5 If reservoir 2 is not completely filled, more solution should be applied through pipette adapter 5 until reservoir 2 is filled (**Figure 4B, 4C**). Keep the µ-Migration Slide tilted (see tips above), to allow air to escape through pipette adapter 1.

1.6 Leave the slide at room temperature in a dry environment (e.g., the tissue culture hood) for one hour. One hour is sufficient time to achieve coating without drying out the slide.

1.7 After one hour, completely remove the coating solution using the provided beveled pipette tip as follows: Set the pipette to 82 µL, depress the pipette plunger as if to withdraw liquid and then insert the beveled tip into pipette adapter 1. Slowly pipette up the coating solution (approximately 82 µL in volume). Do not use a vacuum aspirator to remove the coating solution.

1.8 Using a new beveled pipette tip, **very slowly** add 82 µL of sterile water to the slide chambers through pipette adapter 5. Ensure that both reservoirs 2 and 4 are filled. Remove the water using a new beveled tip applied at pipette adapter 1 (see step 1.7 above for detailed instructions). Repeat the wash steps at least 3 times using a new beveled tip for each wash step. It is important to make sure that both reservoirs 2 and 4 are completely filled after each introduction of sterile water.

1.9 After the last aspiration, let the chambers dry at room temperature or in a 37 °C incubator overnight. Coated slides can be stored at room temperature for up to one month.

The chambers must be completely dry, otherwise cell attachment will be adversely affected.

2. Day 0 (one day before the experiment)
Gas and Temperature Equilibration of the µ-Migration Slides and Medium

Equilibrate the µ-Migration Slides, plugs, cultivation caps, and media in an incubator one day **before** seeding the cells and conducting the experiment. This will prevent air bubbles from emerging within the medium or the slide over the incubation period.

2.1 Place the coated µ-Migration Slide, plugs, cultivation caps, and cell culture media to be used in the experiment (i.e., media used for attaching the cells and/or for serum-starvation) inside a 37 °C, 5% CO_2 tissue culture incubator overnight to allow for gas and temperature equilibration. The cell culture media (10 mL) can be stored in T25 flasks with ventilated caps. Plugs and cultivation caps come in a sterile package, which can be put directly into the incubator without opening, to maintain sterility.

3. Day 1 – Seeding the Cells

3.1 Prepare your cell suspension of adherent cells as usual to obtain **3×10^6 cells/mL** (i.e., harvest using Accutase solution or trypsin/EDTA, centrifuge gently, resuspend the cell pellet in **gas-equilibrated** medium, count, and adjust the volume to achieve the desired cell concentration). A cell suspension of **3×10^6 cells/mL** is recommended to start (see notes below). In subsequent experiments, the cell density can be adjusted if necessary to obtain the desired number of cells in the observation window.

3.2 Transfer the gas-equilibrated, coated slide to a sterile tissue culture hood. Using sterile forceps, firmly insert the plugs (provided) into pipette adapters 1 and 5 of the slide (**Figure 6**).

Cell Loading Tips

- Applying a cell suspension of the recommended **3×10^6 cells/mL** concentration will yield approximately 100–200 cells in the observation window, optimal for viewing with a 5X objective. If fewer or more cells are preferred, the starting cell concentration may be reduced or increased.
- The recommended volume for the cell suspension is 6–8 µL. A larger volume may cause the cell suspension to wash out of the observation window and into reservoirs 2 and/or 4, whereas too small a volume may result in the cells drying out.
- To ensure even cell loading, it is important to resuspend the cells thoroughly between loading each chamber.
- Care must be taken to avoid the formation of small bubbles during pipetting. If air bubbles are present, allow them to rise to the liquid surface in the tip before transferring cells onto the pipette adapter.
- Tilt the pipette tip and place the cell suspension as a drop on top of the pipette adapter (**Figure 5**). Do not apply pressure or otherwise force the suspension into the chamber. The drop will be kept at the top of the adapter due to the hydrophobic nature of the surface.
- Avoid generating and trapping bubbles when loading the cells onto the adapter.

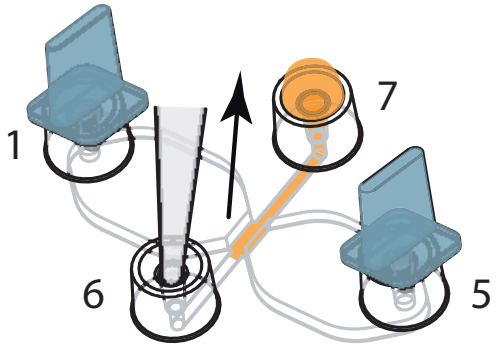
3.3 Using a new beveled tip, pipette up 6 µL (no more than 8 µL) of the cell suspension and with the tip tilted slightly to the side, lightly place the cell suspension as a drop on top of cell inlet adapter 7 (orange drop as shown in **Figure 5**). **Do not apply pressure or otherwise force the cell suspension into the chamber.**

Figure 5. Loading Cells onto the Inlet Adapter

3.4 Using a new beveled pipette tip and the same pipette setting, depress the plunger as if to withdraw medium. Maintaining this pressure on the plunger, insert the tip into adapter 6 and **very slowly** aspirate the same volume of air (6–8 µL) until the cell suspension on top of adapter 7 reaches the pipette tip (**Figure 6**). Do not draw all of the cell suspension into the pipette tip. Both cell inlet adapters 6 and 7 must be filled with the cell suspension, otherwise air bubbles may form in the chamber. Overflow in either of these adapters is acceptable and is preferred to underfilled adapters. Done correctly, this step should take approximately 30 seconds.

3.5 To avoid introducing air bubbles into the adapter, maintain steady downward pressure on the pipette plunger as you remove the beveled pipette tip from the adapter.

Figure 6. Drawing Cells into the Observation Chamber

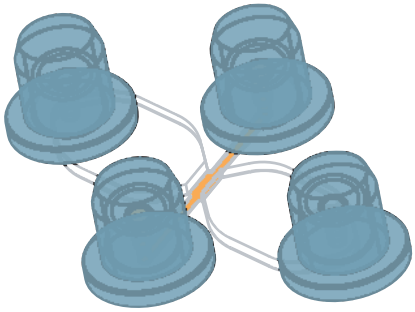


	Draw the cells into the observation chamber **very slowly** so that the cell suspension reaches the pipette tip inserted in pipette adapter 6 in approximately 30 seconds. Drawing the cell suspension through too fast may force the cells out of the targeted observation window and into reservoirs 2 and 4 instead.

3.6 Gently remove the plugs from adapters 1 and 5. Use sterile forceps if necessary.

3.7 Cover all adapters with cultivation caps (**Figure 7**) to prevent evaporation. Do not use the plugs.

Figure 7. Cultivation Caps



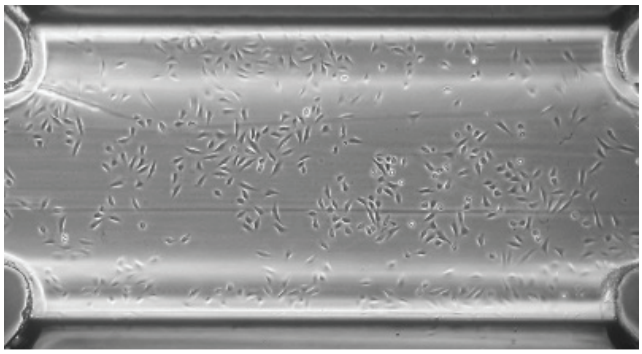
3.8 Carefully place the µ-Migration Slide inside a sterile 10 cm Petri dish containing moistened Kimwipes® wipers. This acts as a make-shift humidified chamber to minimize evaporation.

	Evaporation may occur due to the small volume of the µ-Migration Slide. It is thus critical to maintain high humidity during cell attachment to avoid evaporation. Create a make-shift humidified chamber for the µ-Migration Slide by placing it in a sterile Petri dish with sterile, moistened Kimwipes wipers. To maintain stable conditions, minimize the opening and closing of the incubator door.

3.9 Place the make-shift humidified chamber containing the µ-Migration Slide inside a 37 °C, 5% CO_2 incubator to allow the cells to attach. Cell attachment typically occurs within 3–6 hours depending upon the cell type and ECM substrate (**Figure 8**).

3.10 After 3–6 hours, observe the slide under a bright-field microscope to ensure that the cells are homogeneously attached.

Figure 8. Even Monolayer of Adherent Cells Viewed Through the Slide Observation Window



4. Removing Non-adherent Cells and Serum Starving the Cells

After 3—6 hours of attachment, the µ-Migration Slide is washed with gas and temperature equilibrated serum-starvation medium to remove non-adherent cells and replace the cell attachment medium with gas and temperature equilibrated serum-free medium.

4.1 Make serum-starvation medium (0.5% BSA in serum-free medium) using the supplied 30% BSA solution and gas and temperature equilibrated serum-free medium (from step 2).

4.2 Transfer the µ-Migration Slide with the fully adherent cells to a tissue culture hood and remove all the cultivation caps. Keep the caps sterile in a separate Petri dish for later use.

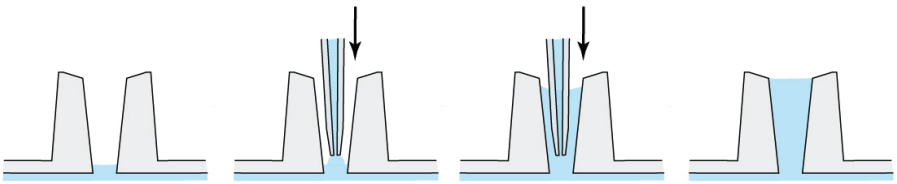
4.3 Check pipette adapters 1 and 5 for the presence of air bubbles. Remove any air bubbles using a 10 µL pipette with a micropipette tip attached (see step 4.5).

4.4 Insert plugs into pipette adapters 1 and 5 using sterile forceps.

4.5 Check cell inlet adapters 6 and 7 to make sure that both adapters are completely filled with medium and no air bubbles are present. After overnight incubation, some of the medium in adapters 6 and 7 may have evaporated.

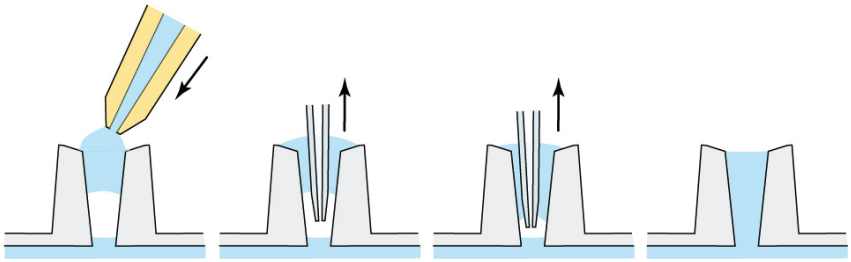
If no bubbles are present, use a 10 µL pipette with a micropipette tip attached to slowly top off both adapters 6 and 7 with equilibrated serum-free medium (**Figure 9A**).

Figure 9A. Topping Off Cell Inlet Adapters 6 and 7 with Equilibrated Serum-Free Medium



If an air bubble is present, use a new beveled tip to add 3 µL of equilibrated serum-free medium on top of the pipette adapter. Then, using a 10 µL pipette with a new 10 µL micropipette tip attached, remove the air bubble (**Figure 9B**).

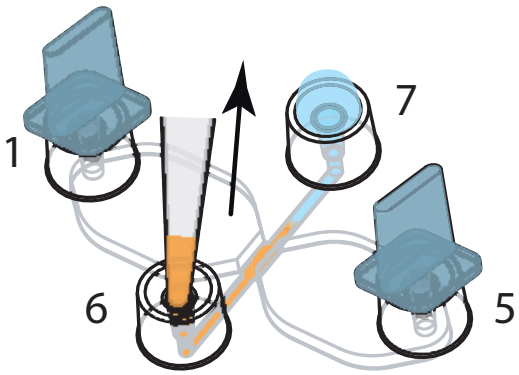
Figure 9B. Adding Medium and Removing Bubbles from Cell Inlet Adapters 6 and 7



4.6 Using a new beveled tip, pipette up 10 µL of the gas-equilibrated serum-starvation medium (from step 4.1) and with the tip slightly tilted, lightly place the liquid as a drop on top of cell inlet adapter 7. **Do not apply pressure or otherwise force the suspension into the chamber.** The drop will be suspended on top of the adapter due to the hydrophobic nature of the surface.

4.7 Using a new beveled pipette tip and the same pipette setting, depress the plunger as if to withdraw medium. Maintaining this pressure, insert the tip into cell inlet adapter 6 and **very slowly** aspirate the same volume of air (10 µL) until the liquid on top of adapter 7 is aspirated from cell inlet adapter 6 (**Figure 10**). Aspiration should be done **very slowly** (approximately 30 seconds) to avoid detaching the cells. Avoid trapping any air bubbles. Repeat wash steps 4.6 through 4.7 two more times. If an air bubble does get trapped, it can be removed by filling the cell inlet adapter that is furthest from the trapped air bubble with equilibrated serum-free medium and slowly aspirating from the cell inlet adapter that is closest to the air bubble. This prevents the bubble from being drawn into the observation window.

Figure 10. Washing with Serum-free Starvation Medium



4.8 Remove the plugs from adapters 1 and 5 and check the adapters for air bubbles. Remove any air bubbles with a 10 µL pipette (micropipette tip attached).

4.9 Cover all the adapters with cultivation caps and place the µ-Migration Slide in an incubator for 12–18 hours. At this point, the cells are no longer in the presence of serum.

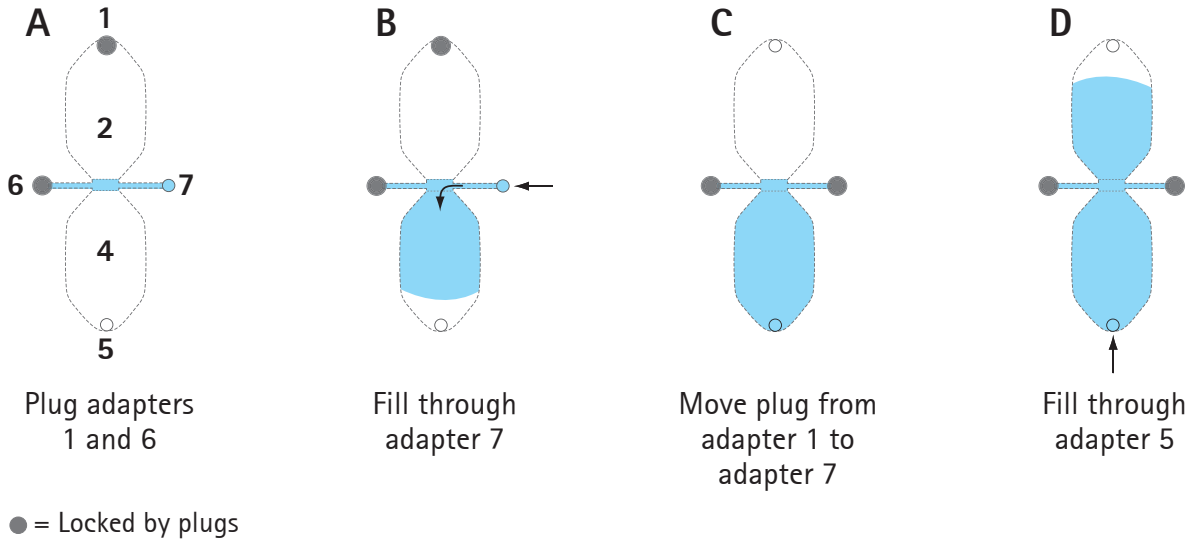
5. Day 2 – Filling the Reservoirs with Medium

5.1 After 12—18 hours of serum-starvation, transfer the µ-Migration Slide to a sterile tissue culture hood and remove all the cultivation caps.

5.2 Insert plugs into pipette adapters 1 and 6 (**Figure 11A**).

5.3 In one hand, hold and tilt the µ-Migration Slide at a 30° angle from the horizontal. With the other hand, place the beveled pipette tip containing 42 µL of gas and temperature equilibrated serum-free medium at a 90° angle into pipette adapter 7 and **very slowly** pipette in the 42 µL of liquid (**Figure 11A**). The liquid will move into reservoir 4 until pipette adapter 5 is also filled (i.e., the liquid surface reaches the top of adapter 5 (**Figure 11B**)). Adapter 5 must be completely filled (overflow is acceptable), otherwise air bubbles may form inside the chambers at later steps. Filling reservoir 4 should take approximately 30 seconds. Do not fill the reservoirs too quickly as it may cause cell detachment and/or create air bubbles.

Figure 11. Filling the Reservoirs with Serum-Free Medium



5.4 Once reservoir 4 is completely filled, maintain constant downward pressure on the pipette plunger as you remove the pipette tip from the adapter. This is to ensure that liquid is not drawn back into the pipette tip due to negative back-pressure.

	The beveled pipette tip (provided) should be inserted into the adapter at a 90° angle to the slide. Pipette **very slowly** and carefully, allowing approximately a 30 second injection time to fill up each reservoir. This slow, careful filling minimizes the stress on the cells and prevents the cells from detaching and being flushed away. After pipetting, be sure to maintain downward pressure on the pipette plunger as you remove the pipette tip from the adapter. This prevents the backwash of medium up the tip which can result in air bubbles forming in the chamber.

5.5. Unplug pipette adapter 1 and insert the plug into pipette adapter 7 (**Figure 11C**).

5.6 In one hand, hold and tilt the µ-Migration Slide at a 30° angle from the horizontal. With the other hand, place the beveled pipette tip containing 42 µL of equilibrated serum-free medium at a 90° angle into pipette adapter 5 and **very slowly** pipette in the 42 µL of liquid (**Figure 11D**). Once again, the optimal time to fill reservoir 2 is about 30 seconds. Maintain constant downward pressure on the pipette plunger as you remove the empty pipette tip from the adapter.

5.7 If there is any excess medium remaining on the adapters, leave it as is. Do not remove the excess liquid from the adapters.

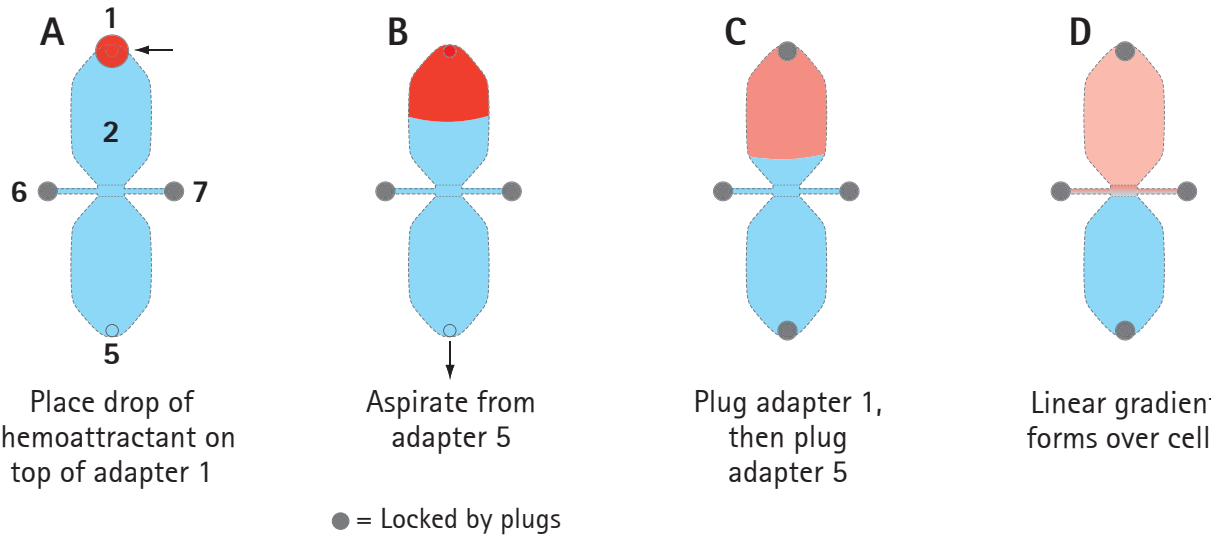
5.8 The chamber should now be completely filled and the cells should grow in the observation window only. Monitor the cells with a phase contrast microscope to ensure that cells are still attached and visible in the observation window.

6. Adding Chemoattractant

6.1 Prepare the chemoattractant (e.g., FBS or other compounds) to the desired concentration. Because of a three-fold dilution occurring inside the slide (shown in the calibration in **Figure 15**), the starting concentration should be three times the desired final concentration. For example, to achieve 10% FBS in the µ-Migration Slide, prepare medium containing 30% FBS.

6.2 Using a new beveled tip, pipette up 18 µL of chemoattractant solution and with the tip slightly tilted, lightly place the liquid as a drop on top of pipette adapter 1 (**Figure 12A**). **Do not apply pressure or otherwise force the suspension into the chamber.**

Figure 12. Adding Chemoattractant



6.3 Using a new sterile beveled pipette tip and the same pipette setting, depress the plunger as if to withdraw medium. Maintaining this pressure, insert the tip into pipette adapter 5 and **very slowly** aspirate the same volume of air (i.e., 18 µL) until the chemoattractant on top of adapter 1 is drawn into reservoir 2 through negative pressure (**Figure 12B**). To avoid detaching cells inside the observation window, this step should be done **very slowly** with reservoir 2 filling in approximately 30 seconds. Avoid trapping any air bubbles.

6.4 Insert a plug into pipette adapter 1, then insert a plug into pipette adapter 5 (**Figure 12C**).

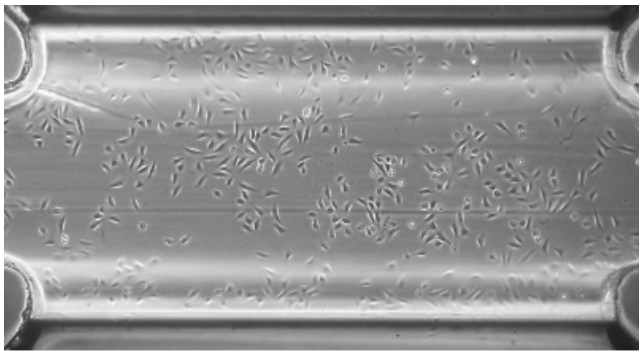
6.5 After a short period of time, the chemoattractant will diffuse through the observation window and establish a linear concentration gradient profile over the cells (**Figure 12D**).

7. Monitoring Cell Migration with Video Microscopy

Video microscopy is an absolutely vital tool for analysis and quantification of chemotaxis and migration effects in the µ-Migration Slide.

7.1 Mount the µ-Migration slide on the stage of an inverted microscope. Confirm that the cells are evenly attached in the observation window (**Figure 13**).

Figure 13. Even Monolayer of Adherent Cells Viewed Through the Slide Observation Window



7.2 After mounting the slide on the microscope stage, wait approximately 20 minutes for the temperature of the slide to equilibrate.

7.3 Depending on the cells' requirements, heating and incubation devices may be necessary.

7.4 Start the time-lapse experiment.

For optimal results with slowly migrating cells, record one frame every 10–15 minutes for a minimum of 12 hours.

7.5 After the time-lapse, export your images as uncompressed single page .tif files.

9. Troubleshooting Tips

9.1 Poor cell attachment

Poor cell attachment may cause cells in the observation window to be flushed out during media exchange steps, resulting in an insufficient number of cells in the observation window for the cell migration experiment. Factors contributing to poor cell attachment include poor or incomplete ECM coatings and improper pipetting of cells and/or media into the adapters.

9.1.1 Poor ECM Coatings

- Make sure that the whole observation window is evenly coated. This can be monitored indirectly by confirming that the coating solution fills up reservoir 4 and then spreads outwardly to completely fill adapters 6 and 7 before filling reservoir 2 (refer to direction of arrows in **Figure 4**).
- ECM adhesion proteins may have lot-to-lot variation and may vary between manufacturers. Each new lot of ECM should be tested before being used in a migration assay.
- Prepare and use coating substrates according to the supplier's specifications or trusted references.
- Optimize the incubation time for cell attachment for each cell line.

9.1.2 Improper pipetting of cells and media through the pipette adapters

- In general, addition of cells and media through pipette adapters should take approximately 30 seconds. Too fast a flow rate may dislodge attached cells.

9.2 Air bubbles

Air bubbles may be generated in several phases of the assay. Bubbles that appear in the observation window after filling with media will compromise the assay because they disturb the diffusion-driven concentration gradient (**Figure 14**). Bubbles trapped in the observation area are difficult to remove, but bubbles formed in preceding steps can be washed out (refer to step 4.7).

Figure 14. Air Bubble in Observation Window

- Air bubbles may emerge from non-equilibrated media and/or slides. This can be avoided by pre-incubating the µ-Migration Slides, plugs, cultivation caps, and the media in a 37 °C 5% CO_2 incubator one day prior to the start of the experiment.
- Air bubbles may emerge from pipette adapters that are not completely filled. Always top off pipette adapters with equilibrated media.
- Always use the supplied beveled pipette tips and follow detailed instructions for pipetting.
- Slides may dry out if placed directly in a 37 °C incubator without properly humidified surroundings. For long-term incubations always place the µ-Migration Slide inside a humidified chamber such as a Petri dish with moistened Kimwipes wipers or cotton balls.

Troubleshooting Tips, continued

9.3 Uneven cell distribution inside the observation window

Uneven cell distribution negatively impacts analysis of the cell migration and should be avoided.

- Resuspend the cell suspension thoroughly just before each loading.
- Never inject the cell suspension directly into a chamber. Instead, add the cell suspension as a drop on top of the adapter.
- Do not load excess cell suspension into the chamber; pressure from the excess solution will push the cells out of the observation window and into the reservoirs.
- Carefully follow all steps in the protocol to avoid flushing the cells into the reservoirs.

9.4 Temperature instability

Temperature changes during an experiment can cause convection, which can disturb the diffusion-driven gradient.

- Always use a heated stage.
- Keep the temperature as stable as possible.
- Do not change the temperature during experiments. Avoid opening doors and windows as it may cause the temperature to fluctuate.
- Minimize the number of times that an incubator is opened and closed.

9.5 Focus instability

Focus stability is mainly influenced by mechanical changes and temperature fluctuations.

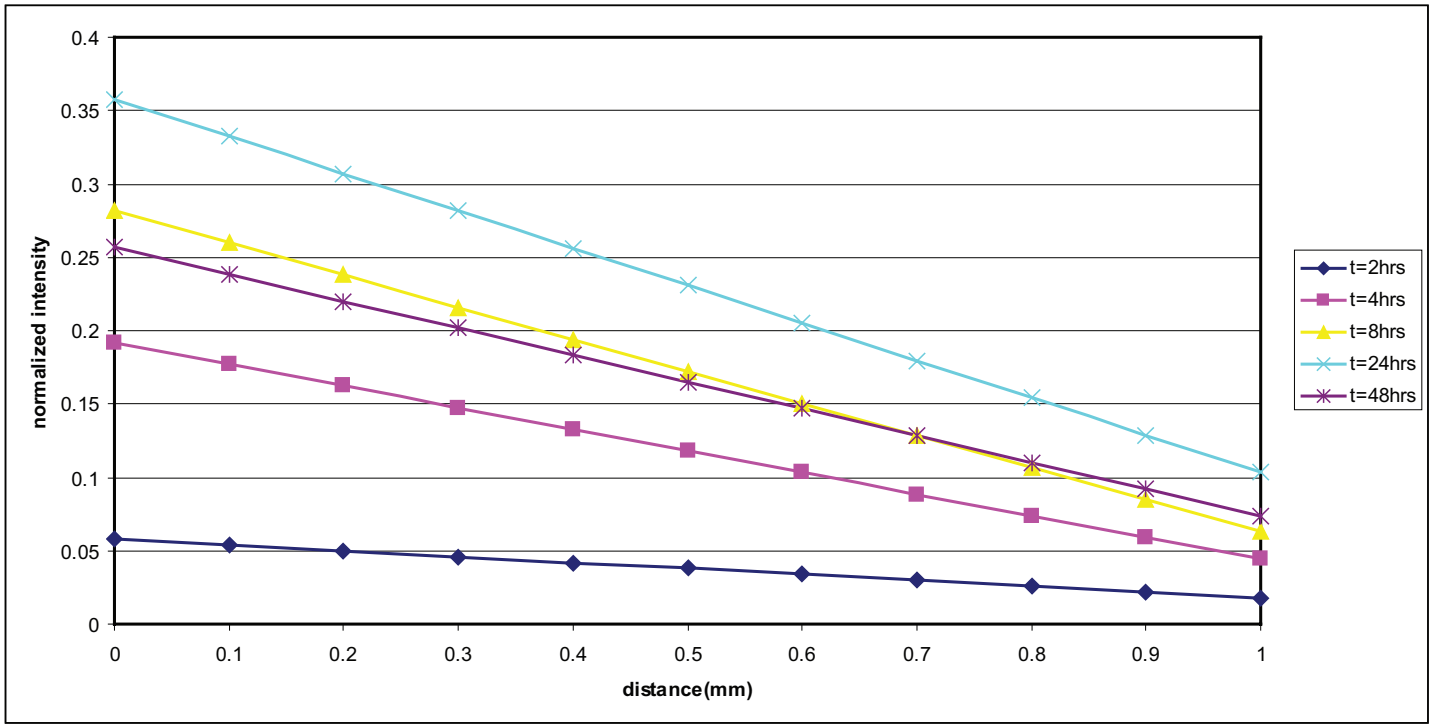
- Turn on all instruments and equipment (heating, incubator, computer, other) at least 60 minutes before starting an experiment.
- After placing the µ-Migration Slide onto the microscope, wait 20 minutes before starting a time-lapse experiment.
- Eliminate all sources of mechanical vibration on the microscope.

Quantitation

For information on tracking cells and data analysis, go to www.millipore.com and use search term "**umigration"**.

Figure 15. Calibration of gradient

Fluorescence measurements show that the maximum working concentration (C_{100}) reaching the cells is only 33% of the applied concentration ($C_{applied}$), as a result of the chemoattractant dilution and diffusion from one reservoir into the other.



Results

The results obtained using the Millicell µ-Migration Assay may be expressed and graphically illustrated as migration indices, directed velocities, percentages of migrated cells, etc.

Figure 16. A typical example of cells with tracked lines

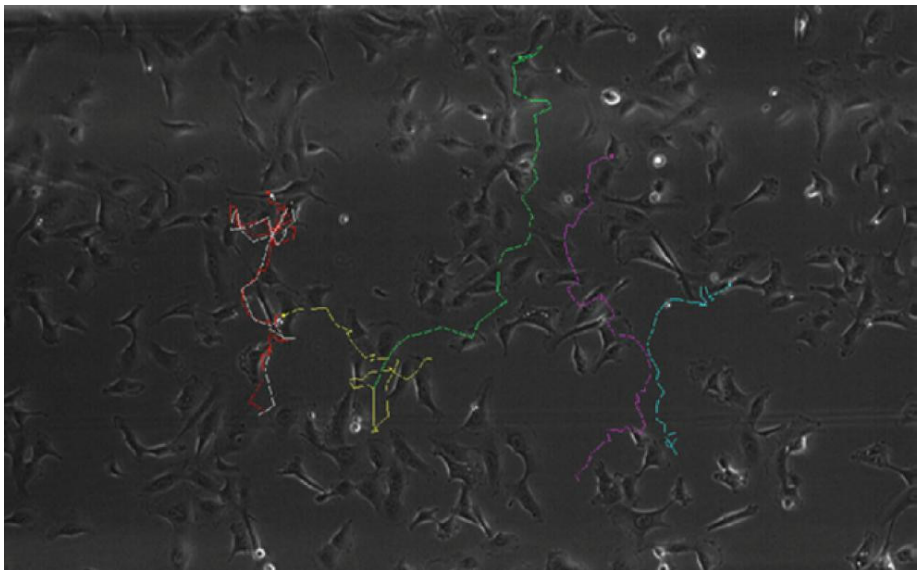


Figure 17. A typical data set to assess the effect of serum at various concentrations (0, 5, 10, and 15%) on the migration propensity of HT1080 cells on collagen-coated surface. **Figure 17A**: Plot graphs of tracked cells, **Figure 17B**: Directionality of tracked cells, **Figure 17C:** Migration index of tracked cells, **Figure 17D:** Migration velocity of tracked cells. The results show that the cells have directed migration towards 10% and 15% FCS.

Figure 17A. Plot Graphs of Tracked Cells

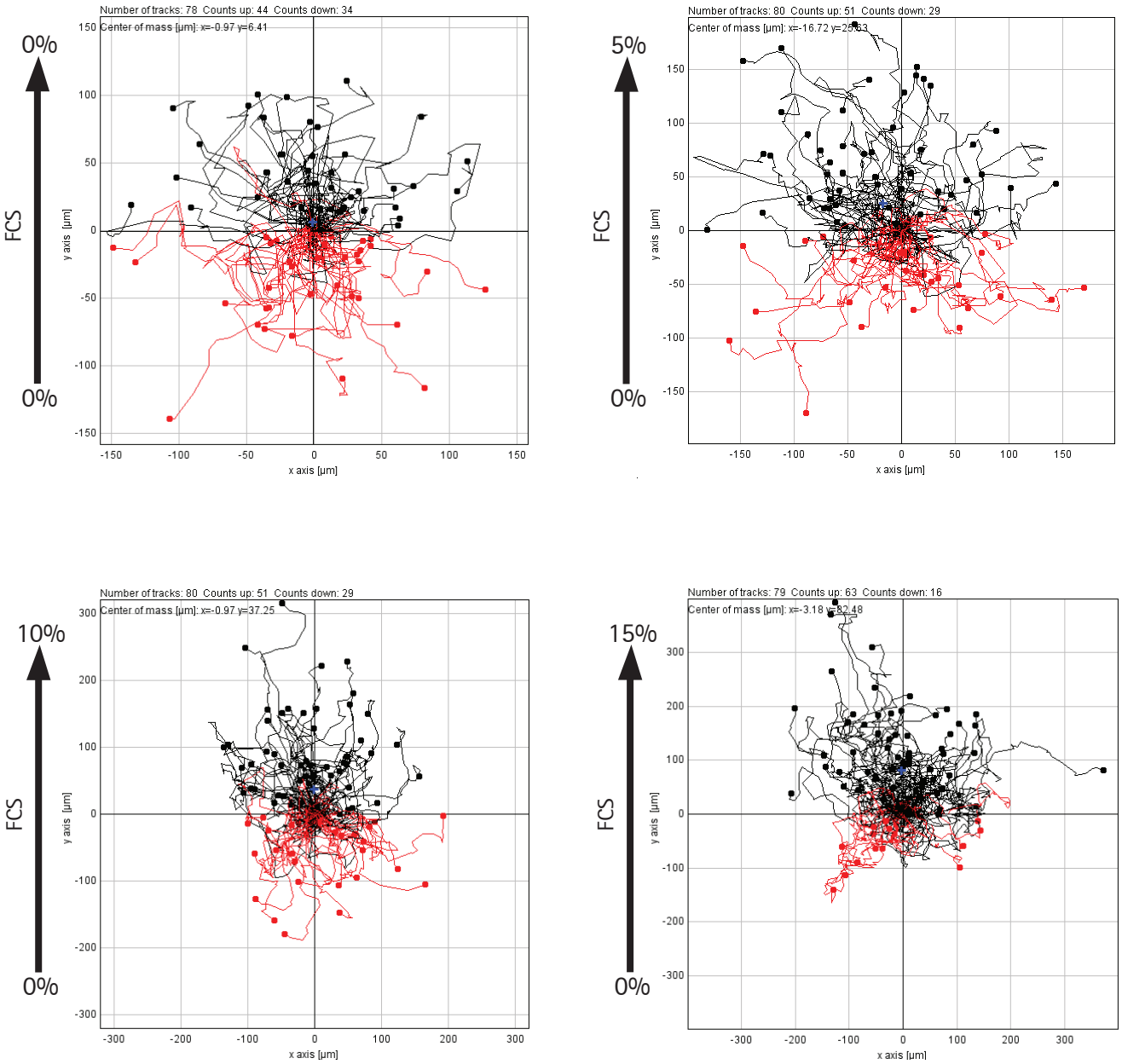


Figure 17B. Directionality of tracked cells

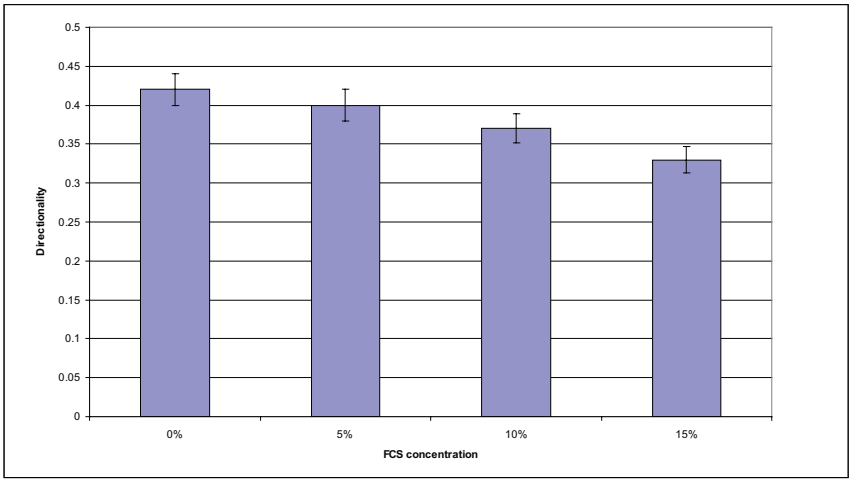


Figure 17C. Migration Index of Tracked Cells

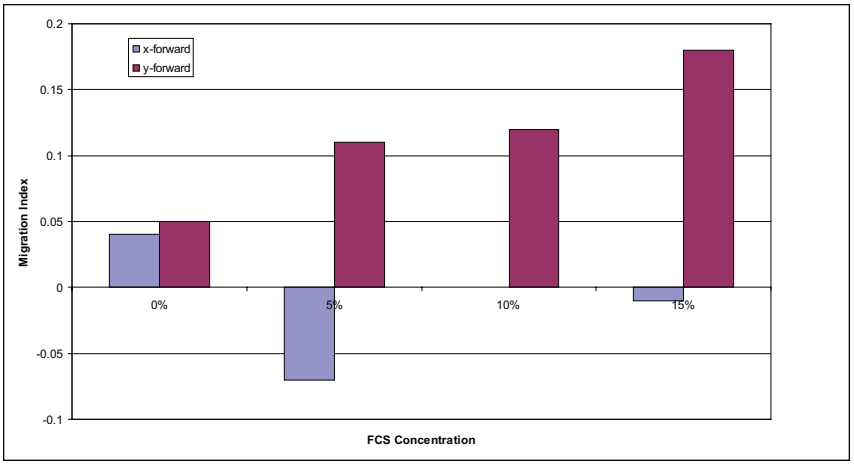
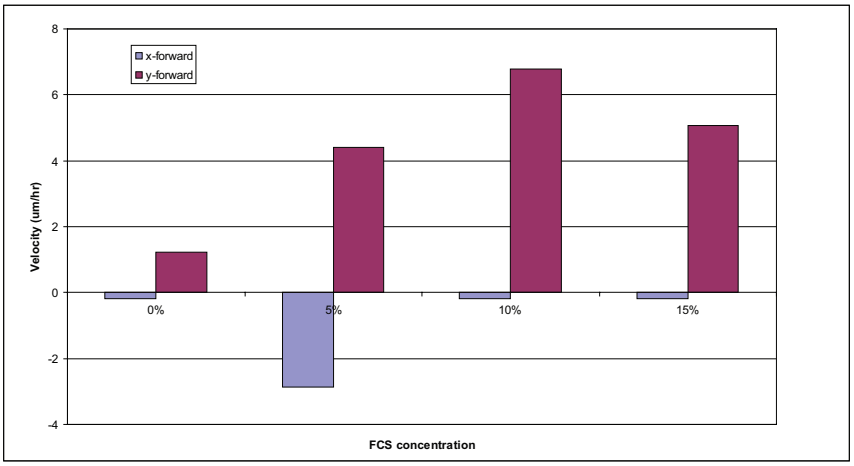


Figure 17D. Migration Velocity of Tracked Cells



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