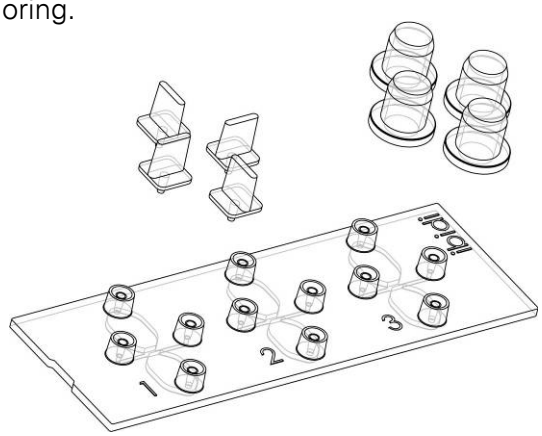
2D Chemotaxis assay using µ-Slide Chemotaxis 2D

0. Important Notes

- Read the related document "Important Notes" first.
- Follow all steps in this protocol carefully.
- Watch the handling movie on www.ibidi.com.
- Start with free samples and the included food coloring.

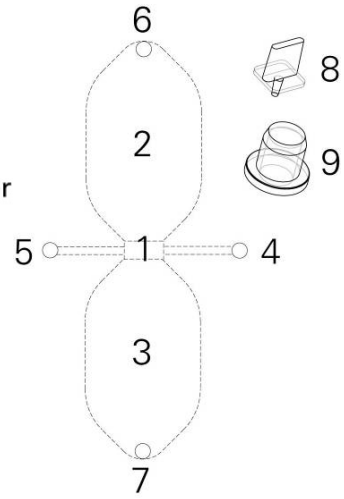
1. General Information

The µ-Slide Chemotaxis 2D is a tool for observing chemotactical responses of adherent migrating cells in 2D over extended periods of time. The linear concentration profile which is required for chemotactical movement is generated by diffusion and stable for at least 48 hours. Three chambers on one slide allow parallel chemotaxis experiments. The following protocol is adapted for adherent cells only.



Color code for liquids in this Application Note:

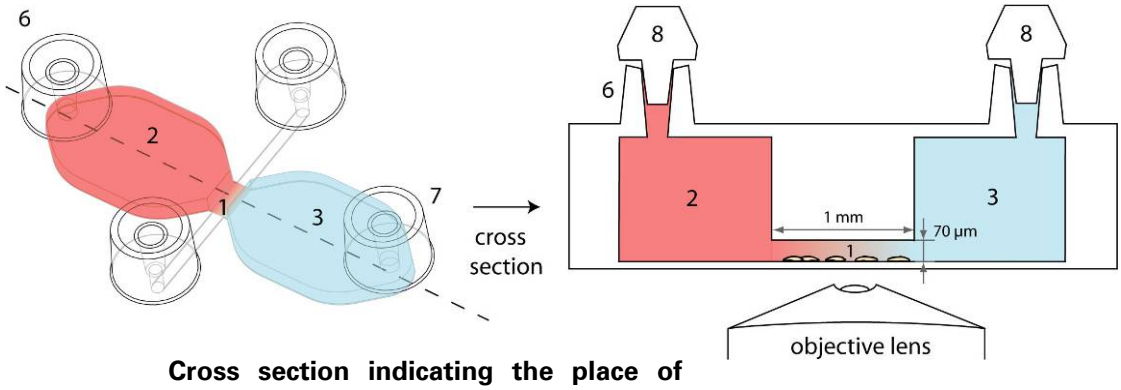
- Coating solution
- Rinsing solution, e.g. water
- Cell suspension
- Cell-free medium
- Chemoattractant



Chamber configuration:
1 Observation area (2000x1000 µm²)
2 Large reservoir (40 µl)
3 Large reservoir (40 µl)
4 Filling port with small side channel
5 Filling port with small side channel
6 Filling port
7 Filling port
8 Plugs
9 Cultivation caps

2. Principle

Two large volume reservoirs are connected by a thin slit. The reservoirs (2, 3) contain different chemoattractant concentrations (indicated below by red and blue color). Inside the connecting slit a linear and stable concentration profile is generated by diffusion.

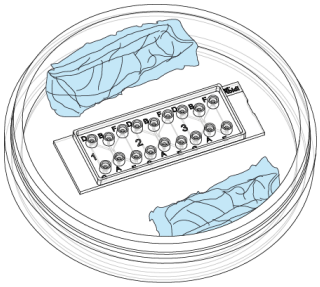


Cross section indicating the place of cells, chemoattractant, and gradient

3. Equipment

It is 100% necessary using the following equipment to conduct chemotaxis experiments with the µ-Slide Chemotaxis 2D:

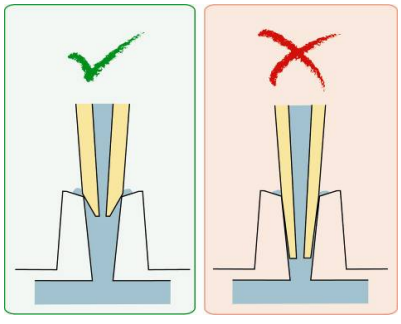
- Adherent cells
- Optimal conditions for cells (heated or incubated stage)
- A humid chamber, such as a 10 cm Petri dish with wet tissue (as shown on the right)
- Inverted microscope (phase contrast, fluorescence, …)
- Time lapse video equipment (CCD camera, video camera, acquisition software)
- Use only correct 10 - 200 µl pipet tips. Others do not work.
  - Greiner Bio-One 739261, 739280, 739290, 772288 or related beveled Greiner tips.
  - Axygen T-200-C, TR-222-C, TR-222-Y or related Axygen beveled tips.
  - STARLAB TipOne RPT S1161-1800 or related beveled TipOne tips.
  - Sorenson BioScience MulTi Fit Tip 10590, 15320, 15330.
- Slant cosmetics tweezers, for plug handling
- Optional: Motorized stage and autofocus (x,y,z) to observe all 3 chambers in parallel.



Humid Chamber
With wet sterile tissue.

Beveled Pipet Tip
See list for correct models

Slant Tweezers
For convenient plug handling



Correct tip
- Fits on top
- Closes completely
- Does not stuck

Wrong tip
- Fits too tightly
- Gets stuck

4. Coating µ-Slide Chemotaxis

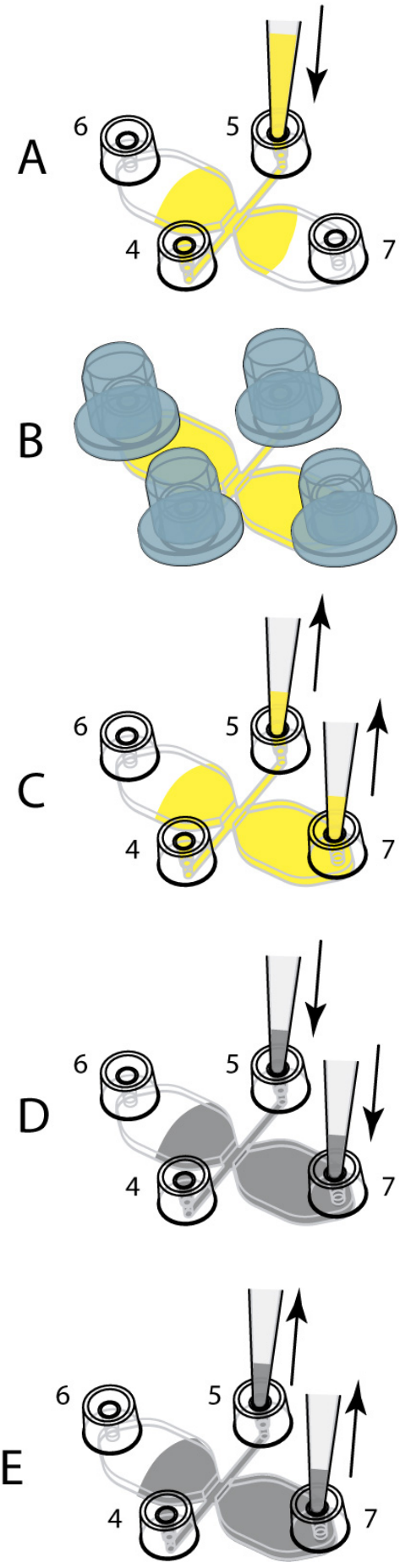
For direct use the tissue culture treated version µ-Slide Chemotaxis 2D, ibiTreat (80306) or the precoated µ-Slide Chemotaxis 2D, Collagen IV, sterile (80302) can be used. Other coatings can be adapted by using the same procedure described below. Work under sterile conditions.

a) Full Coating Protocol with Collagen IV

Please use the following protocol to coat the entire inside of the chamber with Collagen IV.
(µ-Slide Chemotaxis 2D, ibiTreat, 0.8 µg/cm^2, 80 µl, 24 µg/ml, 2.4 cm^2):

- Dilute the Collagen IV (e.g. BD Cat.-Nr. 356233) to a concentration of 24 µg/ml using 0.05 M HCl.
- Inject 80 µl coating solution per chamber using the filling port 5 (step A) until the chamber is completely filled. Take care to fill all channels and filling ports.
- Leave at room temperature for at least 30 minutes. Use the caps to decrease evaporation (step B).
- Completely aspirate the solution using a cell culture aspirator. Take care to aspirate from more than one filling port to remove as much liquid as possible (step C).
- Wash with 80 µl ultra-pure water by injecting into pipet ports 5 and 7 (step D). Please note that filling the chamber with water or washing buffer is difficult as long as it is not completely dried. Don't mind air bubbles at this point.
- Aspirate exhaustively by using a cell culture aspirator. Take care to aspirate from more than one filling port to remove as much liquid as possible. (step E)
- Repeat until the entire inside is washed.
- Let the chamber dry at room temperature overnight under sterile conditions.

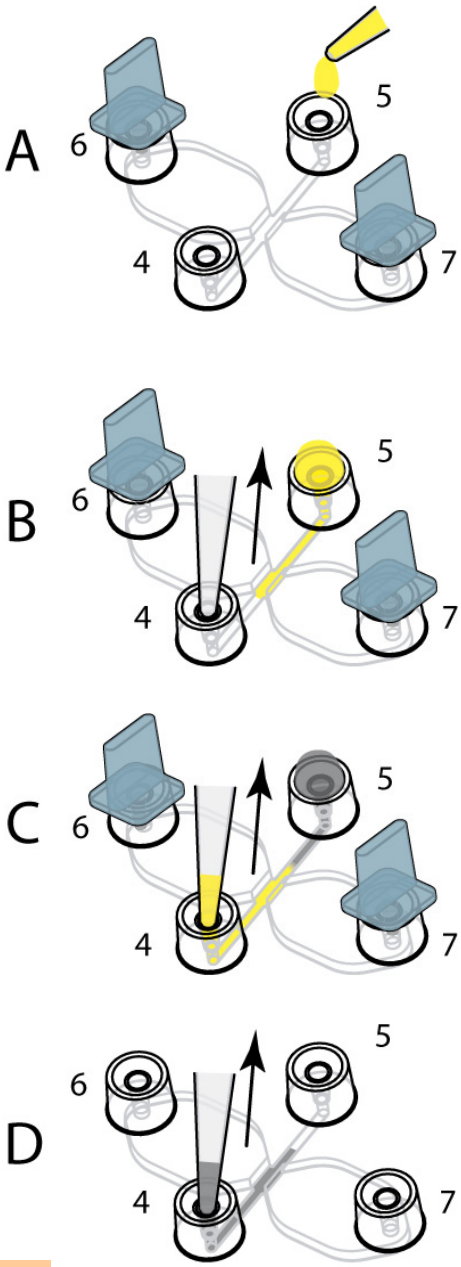
Please note that filling the chamber with cells or washing buffer is difficult as long as it is not completely dried.



b) Observation Area Coating Protocol with Collagen IV (equal to ibidi Cat. No. 80302)

This protocol is used to coat the observation area only. This will create a coating barrier so cells may not be allowed to enter the large reservoirs. Only the observation area and the side channels are coated with Collagen IV.
(µ-Slide Chemotaxis 2D, ibiTreat, 0.8 µg/cm^2, 6 µl, 60 µg/ml, 0.45 cm^2)

- Dilute the Collagen IV (e.g. BD Cat.-Nr. 356233) to a concentration of 60 µg/ml using 0.05 M HCl.
- Close filling ports 6 and 7 by plugs (not shown). Handle plugs with appropriate blunt tweezers.
- Use a 20 µl pipet (e.g. Gilson P-20) and apply 6 µl coating solution onto filling port 5 (step A).
- Use the same pipet settings and aspirate air from the opposite filling port 4. (step B) Press the pipet tip directly into filling port 4. The coating solution on port 5 will be flushed inside filling the entire channel homogeneously. Gently aspirate until the solution reaches the pipet tip.
- Remove the plugs and cover with lids. Leave at room temperature for 60 minutes (not shown).
- Gently close filling ports 6 and 7 by plugs (not shown).
- Put 10 µl ultra-pure water on top filling port 5 (step C). Don't trap air bubbles.
- Aspirate the same amount of liquid from cell inlet adapter 5 as shown (step C). Repeat this step twice.
- Aspirate intensively all liquid from filling port 4 and 5 (step D).
- Let the chamber dry at room temperature overnight under sterile conditions.



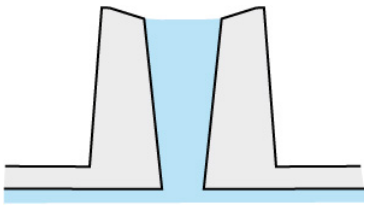
After coating, the chamber needs to be completely dry. Otherwise, the cell seeding protocol does not work correctly.

5. Experiment

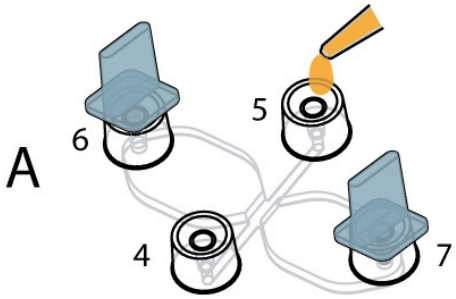
a) Seeding cells

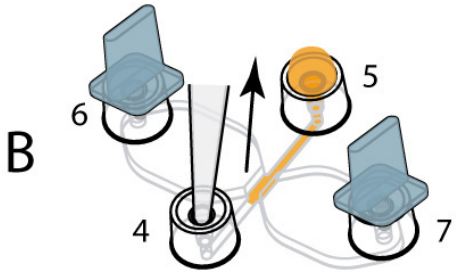
The day before seeding cells and conducting the experiment, it is necessary to place all cell media, the µ-Slide, and plugs into the incubator for gas equilibration. Media is put in a slightly opened vial. This will prevent the medium inside the slide and the slide itself from emerging air bubbles over the incubation time.

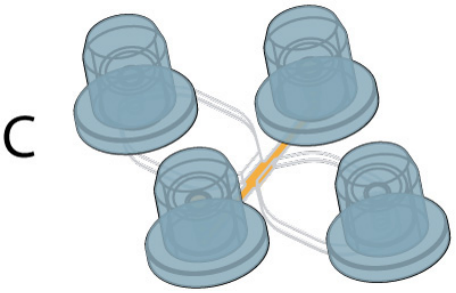
- Unpack the µ-Slide Chemotaxis and put into a 10 cm Petri dish with sterile, wet tissue around the slide decreasing evaporation.
- Prepare your cell suspension as usual. Use cell concentration of ca. 3 x 10^6 cells/ml. High cell concentrations are needed due to the small height of the observation area.
- Close filling ports 6 and 7 by plugs (not shown). Handle plugs with appropriate blunt tweezers.
- Use a 20 µl pipet (e.g. Gilson P-20) and apply 6 µl cell suspension on top of filling port 5 (step A). Do not inject directly.
- Use the same pipet settings and aspirate air from the opposite filling port 4. Press the pipet tip directly into filling port 4. The cell suspension on top of port 5 will be flushed inside filling the entire channel homogeneously. Gently aspirate until the cell suspension reaches the pipet tip. The hydrophilic ibiTreat surface (TC-treated) supports this step so different surface coatings can behave differently.
- Leave both filling ports (4 and 5) filled with cell suspension. Level out the liquid heights in both filling ports like this:



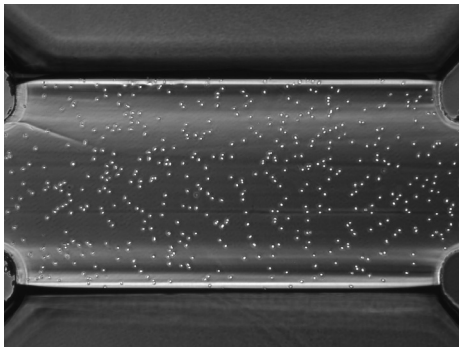
Do not overfill or underfill both filling ports to avoid leakage. To achive this, the 6 µl seeding volume may have to be adjusted.







- Directly after cell seeding, gently remove the plugs from filling port 6 and 7 by tweezers (not shown).
- Cover all filling ports with cultivation caps (step C). Do not use the plugs during cell attachment.
- Control cell density and cell distribution by phase contrast microscopy.
- Incubate the slide inside a sterile and humid atmosphere to minimize evaporation until cells have attached. This should occur within 1-5 hours depending on your cell type and surface. Make sure evaporation is low by using a sterile 10 cm Petri dish with extra wet tissue around the slide.



Observation area with recommended cell density right after seeding.

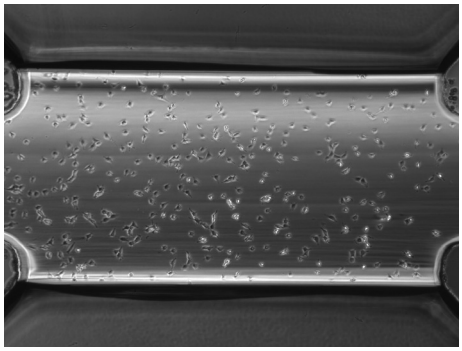
b) Cell Attachment

- Control cell attachment and morphology by phase contrast microscopy. Control evaporation by checking the amount of medium in filling port 4 and 5.

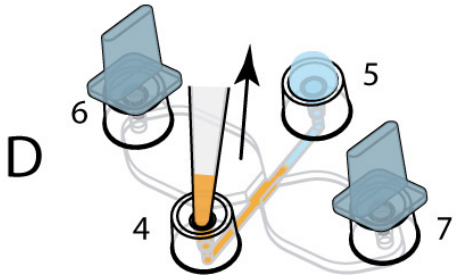
After cell attachment (1 – 5 h) remove non-adherent cells and old medium:

- Remove all cultivation caps from the filling ports (not shown).
- Gently close filling ports 6 and 7 by plugs (not shown).
- Fill 10 µl cell free medium onto filling port 5. Don't trap air bubbles (step D).
- Aspirate the same amount of liquid from cell inlet adapter 4 as shown (step D).
- Repeat this step twice.

This washing protocol can also be used to pretreat the cells before the chemotaxis experiment is started.

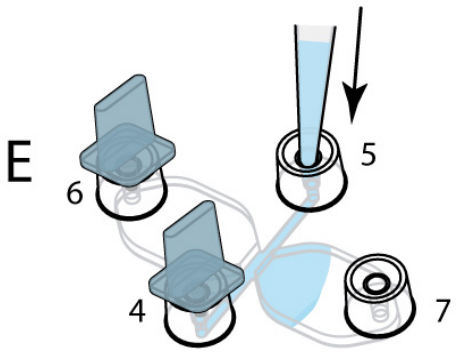


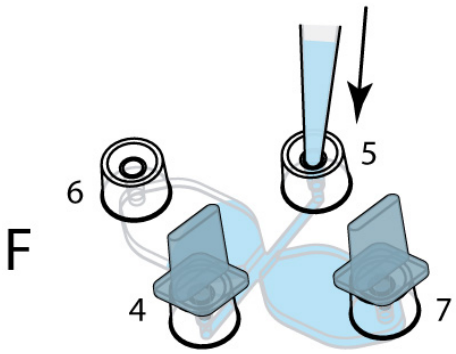
Cells in observation area after attachment.



c) Filling the Reservoirs

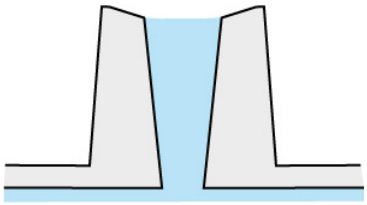
- Transfer the plug from filling port 7 to filling port 4. (not shown)
- Fill one reservoir by gently injecting 45 µl cell-free medium into filling port 5 (step E). In this step it is crucial not to detach cells. This can be achieved by very gently pipetting. Slowly inject media until filling port 7 is completely filled. (This is a very critical step because cells might be flushed away due to high flow rates. The longer the filling takes the fewer your cells are stressed. Approx. 30 seconds per reservoir are fine.)
- Transfer the plug from filling port 6 to filling port 7. (not shown)
- Fill the other reservoir by gently injecting 45 µl cell-free medium into filling port 5 (step F). (Keep in mind that all filling ports must be completely filled but not overfilled, as shown.)
- Now, the chamber is completely filled and cells grow in the observation area only. Control your cells under the phase contrast microscope.

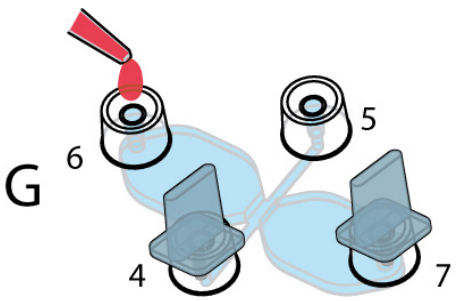




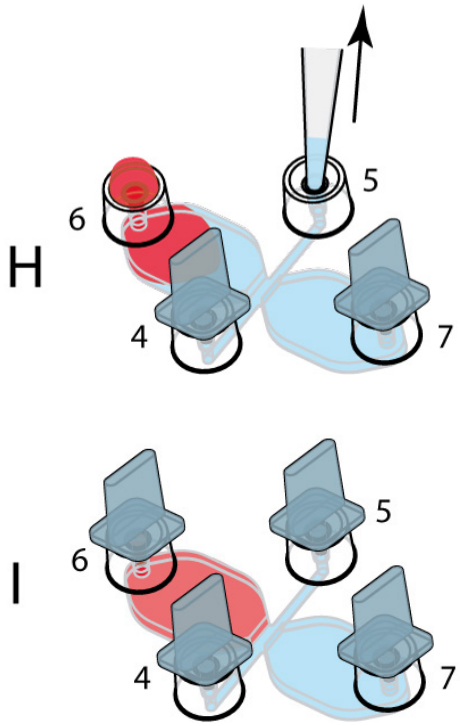
d) Apply a Chemoattractant in one Reservoir

Make sure the two open filling ports 5 and 6 are completely filled like this:





- Apply 18 µl of chemoattractant onto filling port 6 as shown. Don't trap air bubbles. Don't inject the chemoattractant directly (step G).
- Aspirate the same amount of liquid (18 µl) from filling port 5 (step H). This will flush the chemoattractant into the large reservoir. Don't aspirate too fast as this might detach the cells.
- Close both filling ports 5 and 6 with plugs. Start with the non-attractant containing filling port 5 (step I). Don't trap air bubbles.
- After a short time the chemoattractant will diffuse through the observation area and establish a linear concentration profile over the cells. See § 10. (fluorescence measurements) for details.



Please visit www.ibidi.com for movies on handling and applications with µ-Slide Chemotaxis!

6. Chemoattractant Concentrations and Recommended Experimental Setups

a) Definition

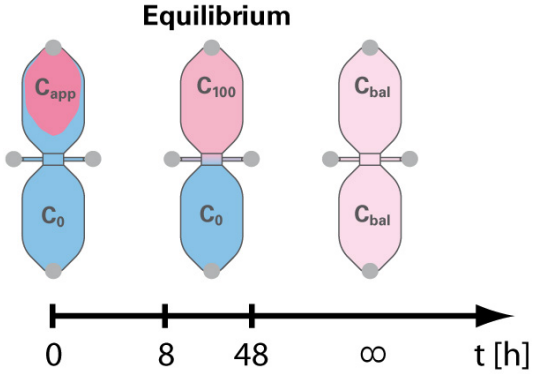
For optimal results we recommend performing two control experiments by filling the chamber completely with/without chemoattractant solution. That gives access to the question whether a compound is influencing directed movement of cells (chemotaxis) and/or influencing random migration (chemokinesis).

C_{100} Concentration in stable gradient equilibrium (= max. concentration reaching the cells on one edge of the observation area).

$C_{applied}$ Concentration applied into the chamber with a volume of 18 µl. **$C_{applied} = C_{100} \times 2.5$**[1)]

$C_{balanced}$ Balanced chemoattracntant concentration after infinite time calculated with the total chamber volume of 80 µl. **$C_{balanced} = C_{applied} \times 18\ µl / 80\ µl$.**

C_0 Solution without (or low concentration) of chemoattractant.



[1)] In theory this value should be 40 µl / 18 µl = 2.22. Fluorescence measurements revealed that a slightly higher concentration is needed due to the fact that in equilibrium the chemotattractant containing reservoir already lost molecules for gradient establishment.

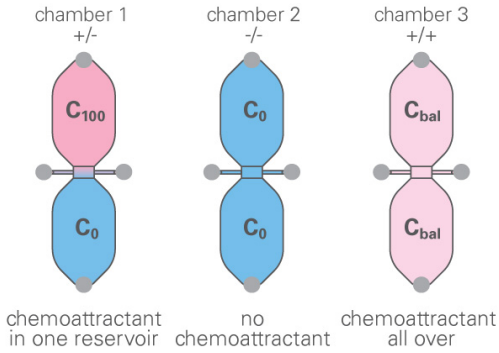
b) Calculation Example

Chemoattractant concentration profile over cells between 100 µg/ml (=C_{100}) and 0 µg/ml (=C_0) in equilibrium.

C_{100} = 100 µg/ml
$C_{applied}$ = 100 µg/ml x 2.5 = 250 µg/ml
$C_{balanced}$ = 250 µg/ml x 18 µl / 80 µl = 56.25 µg/ml
C_0 = 0 µg/ml

The used solutions are: $C_{applied}$ for +/- experiment and C_0 and $C_{balanced}$ for the two control experiments (-/- and +/+). Preparation of C_{100} is not necessary.

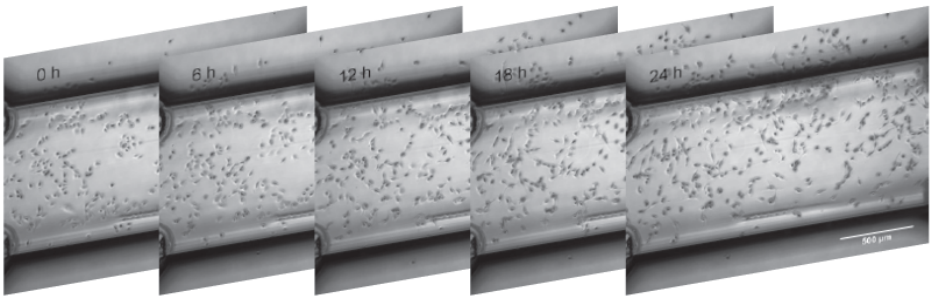
Recommended setup for one slide:



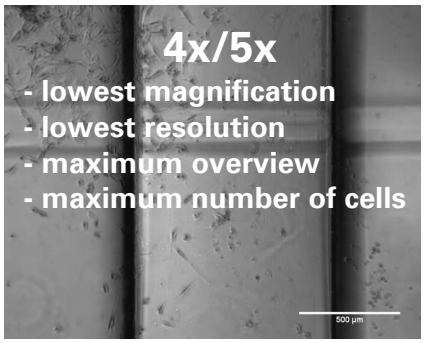
7. Video Microscopy

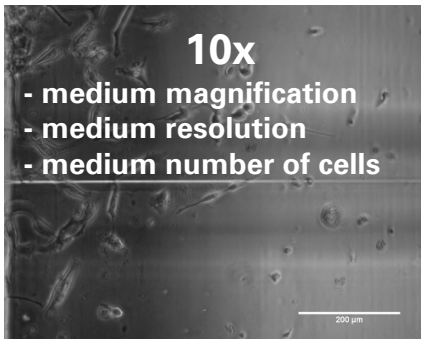
Video microscopy is an absolutely necessary tool for ibidi's µ-Slide Chemotaxis. Without, there is no access and analysis of chemotaxis and migration effects.

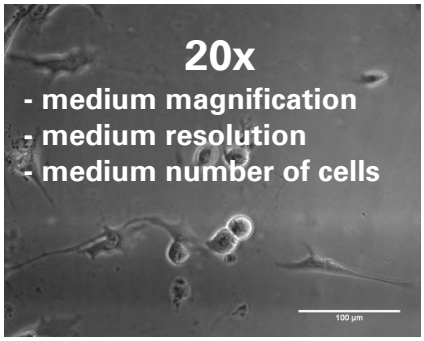
- Mount the µ-Slide Chemotaxis on the stage of your inverted microscope and observe cell movement within the observation areas. Depending on the cells´ velocity one frame per 1-10 minutes should be sufficient. For optimal migration data of slow migrating cells we recommend starting the experiment immediately after filling in the attractant and observe cell movement for 24 hours. After initial experiments this time can be varied for efficient use of experimental time.
- Depending on your cells´ requirements heating and incubation devices are necessary. Please contact us for more details on incubation systems right on the microscope.
- After mounting the slide on the microscope stage, wait 20 min for temperature equilibration.
- Start a time-lapse experiment with a 5x or 10x objective. Higher magnifications are possible but for optimal migration data it is necessary to track at least 20 – 40 cells.
- After the time-lapse export your images as uncompressed single page .tif files.

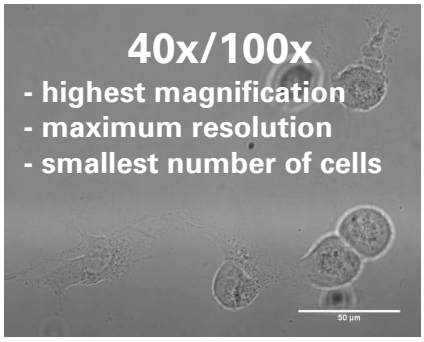


Depending on your focus of interest, choose a magnification:









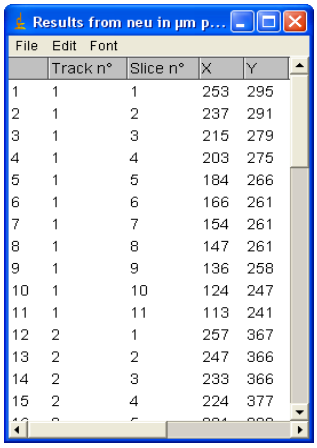
8. Tracking Cells

After the experiment, it is necessary to track the cells with the appropriate software. We recommend, for example, the ImageJ plugin, Manual Tracking. This plugin is able to quantify the movement of objects between frames of a temporal stack.

- ImageJ is available here: http://rsb.info.nih.gov/ij/
- The Manual Tracking plug-in (including a PDF documentation) by Fabrice Cordelières, Institut Curie, Orsay, France is available here: http://rsbweb.nih.gov/ij/plugins/track/track.html
- Download Manual_Tracking.class into the plugins folder of ImageJ on your computer and restart ImageJ. Make sure you have administrator rights.

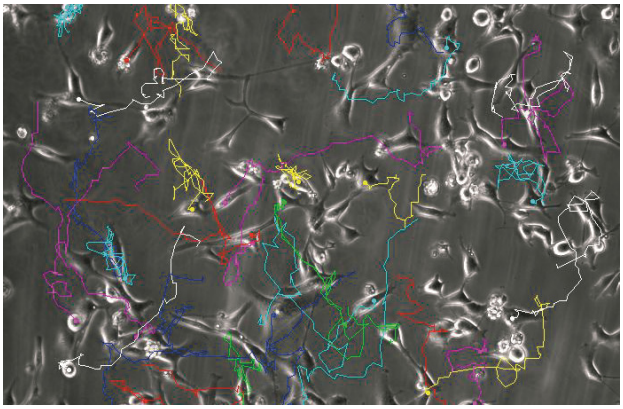
Quick guide for tracking with Manual Tracking:

1) Import a movie as single page .tif files, by using „File/Import/Image_Sequence".
2) Open plugin "Manual Tracking".
3) Select "Add track".
4) Follow the first cell, through all of the time points, by clicking on the cell's midpoint. After the first click, the software creates the results table in an extra window. This table is filled with the x/y data of each cell, at each time point.
5) Save the data table after tracking is completed.
6) The data table contains all tracked cells (=tracks) and time points (=slices) with (x,y) positions.



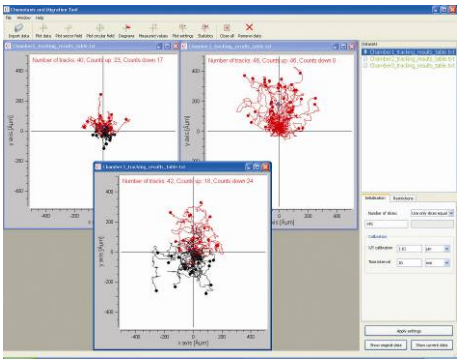
	Track n°	Slice n°	X	Y
1	1	1	253	295
2	1	2	237	291
3	1	3	215	279
4	1	4	203	275
5	1	5	184	266
6	1	6	166	261
7	1	7	154	261
8	1	8	147	261
9	1	9	136	258
10	1	10	124	247
11	1	11	113	241
12	2	1	257	367
13	2	2	247	366
14	2	3	233	366
15	2	4	224	377

- For tracking, we recommend printing out the first image of each image stack before the movie is watched for the first time. At least 40 cells are uniformly marked in order to 1) ensure homogeneity and 2) avoid tracking the same cell twice. At least 30 cells in the observation field need to be tracked during the entire experiment. Some cells will be lost through cell death, cell division, and cells leaving the observation field. We do not recommend using such cells for data analysis.
- You can also export "Overlay Dots & Lines" as a movie file (.avi).



9. Analyzing Chemotaxis

ibidi provides a free software tool for plotting and analyzing the tracked data. For a copy, go to: http://www.ibidi.de/applications/ap_chemo.html



Here is a quick guide for data analysis:

1) Import the data table from "Manual Tracking" (tab-separated .xls file)
2) Select the number of slices (=number of pictures used for tracking). The number of slices can be found in your original data table ("Show original data").
3) Calibrate the software by setting the x/y calibration and the time interval. x/y calibration is the length of one pixel in µm.
4) Press "Apply settings", after changing values and parameters.
5) Create trajectory plot, and then export as an image.
6) Export values of center of mass, FMI, and Rayleigh test.
7) Calculate a two-tailed unpaired Student t-test with independent variances for the single values from 6). Compare the chemotaxis experiment +/- and the two control experiments -/- and +/+ with each other to show statistical significance.

Example: Use the following command in Microsoft Excel.

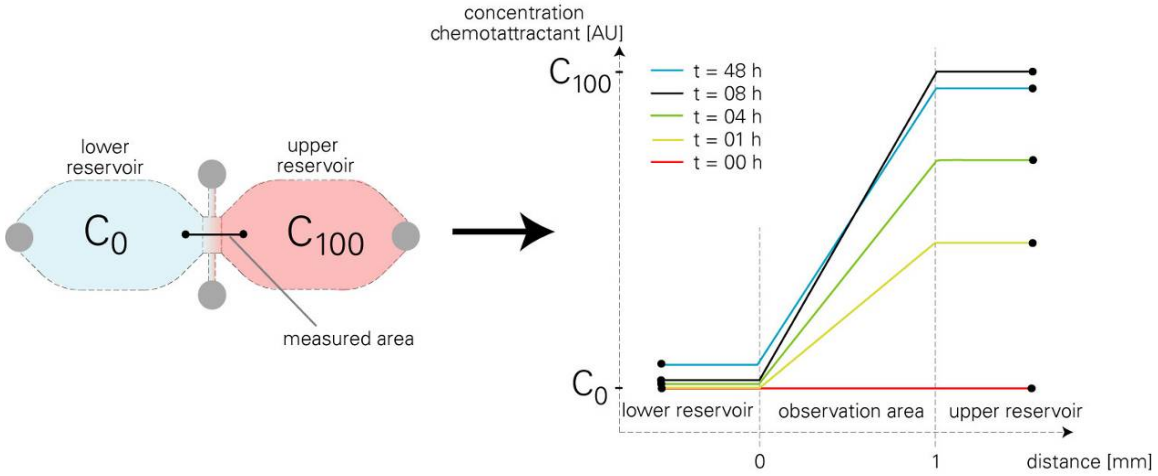
=TTEST(A2:A4;B2:B4;2;3)

Here are some recommendations for presenting the results (in talks or publications):

- Show the original movie (time lapse film of your cells).
- Play the trajectory movie (time lapse film + overlaid cell trajectories).
- Display the trajectory plot (graph).
- Show the table or bar graph of center of mass, FMI, and Rayleigh test.
- Include the results of the Student's t-test.

10. Fluorescence Measurements

Fluorescence measurements with Alexa488 (diffusion coefficient D=316 µm²/s) at room temperature (22 °C) revealed the concentration profile as linear over the 1 mm observation area. The profile is established after 1-8 h. After that it reaches steady state (equilibrium). Please note that there is no gradient inside the reservoirs. The profile is maintained linear for over 48 h. After that it slowly flattens due to the concentration equilibration between the reservoirs.



11. Different Chemoattractants and Time Dependencies

If you use a chemoattractant with different molecular weight $M_{molecule}$ (and diffusion constant $D_{molecule}$), different temperatures T, and solutions with different dynamic viscosities $\eta_{solution}$, diffusion will be faster or slower. The following example (Alexa488 in water vs. VEGF in endothelial cell growth medium) shows how to compare our fluorescence measurements with different chemoattractants in different solutions.

Parameters:

$\eta_{Alexa488\ (22\,°C)} = 1 \cdot 10^{-3}\ kg/s \cdot m$

$\eta_{EC\ growth\ medium\ (37\,°C)} = 0.75 \cdot 10^{-3}\ kg/s \cdot m$

$T_1 = 37\,°C = 310\,K$

$T_2 = 22\,°C = 295\,K$

To simplify matters the diffusing molecules are assumed to be globated. The hydrodynamic radius is therefore dependent on the molecular weight only:

$$R_h \approx \sqrt[3]{M_{molecule}}$$

with:

$M_{VEGF} = 45{,}000\,Da$

$M_{Alexa488} = 570\,Da$

Comparison:

$$\frac{D_{VEGF}}{D_{Alexa488}} = \frac{\frac{k_B T_1}{6\pi\eta_1 R_{h1}}}{\frac{k_B T_2}{6\pi\eta_2 R_{h2}}} = \frac{\frac{T_1}{\eta_1 R_{h1}}}{\frac{T_2}{\eta_2 R_{h2}}} = \frac{\frac{T_1}{\eta_{EC\,growth\,medium(37°C)} \sqrt[3]{M_{VEGF}}}}{\frac{T_2}{\eta_{Alexa\,488\,solution\,(22°C)} \sqrt[3]{M_{Alexa488}}}} = \frac{\frac{310\,K}{0.75\cdot 10^{-3}\ kg/s\cdot m\sqrt[3]{45{,}000\,Da}}}{\frac{295\,K}{1\cdot 10^{-3}\ kg/s\cdot m\sqrt[3]{570\,Da}}}$$

$$\frac{D_{VEGF}}{D_{Alexa488}} = 0.33$$

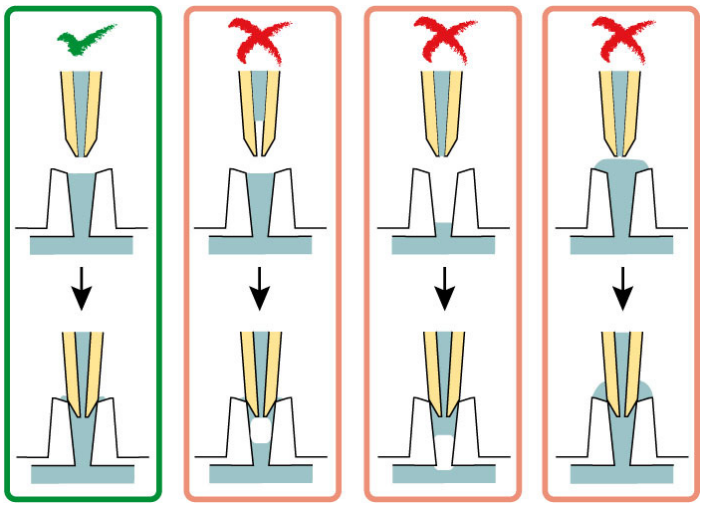
By using VEGF in endothelial growth medium as chemoattractant at 37°C the diffusion is slower with factor 3. The concentration profile is build up slower with that factor. The diffusion of different chemoattractants can be calculated identical.

12. Tips, Tricks and Troubleshooting

a) Avoiding Air Bubbles

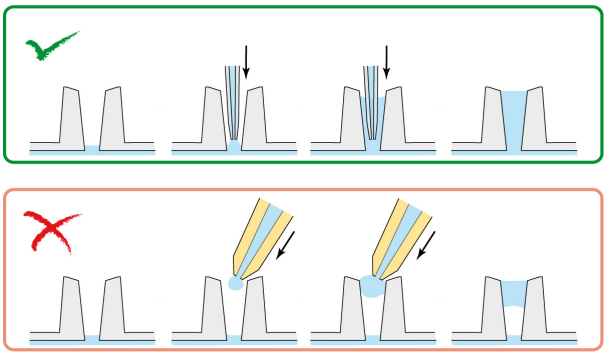
Air bubbles are 1) emerging over time due to not equilibrated media and slides or 2) injected or brought into the chamber by the user. Both types will disturb the diffusion driven concentration gradient by convection and must be avoided.

1) Emerging air bubbles over time can be avoided by equilibrating all media and equipment inside the incubator overnight. Media are put into a slightly opened vial. Slides and plugs can be left in the sterile packaging.
2) The second type of bubbles can be avoided by correct handling. Please avoid unfilled filling ports and trapped air bubbles inside the pipet tip! Keep in mind that an air bubble might be trapped when closing an empty adapter by a plug. Always make sure the filling ports are completely filled but not overfilled.



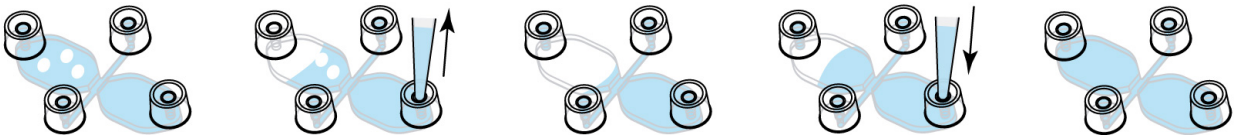
Avoid unfilled filling ports and air bubbles inside the pipet tip!

Accidentally empty filling ports can be refilled by a 10 µl pipet with a very thin pipet tip. Never use the recommended yellow pipet tips (see § 3) for this. An air bubble might be trapped.

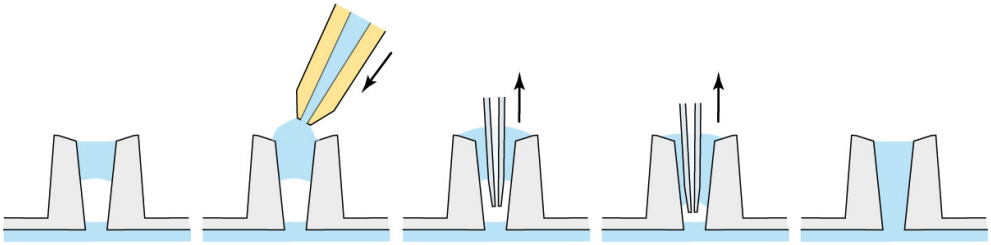


b) Removing Air Bubbles

Air bubbles in channels and reservoirs can often be removed by the following procedure. Gently empty the reservoir containing the air bubble from the opposite side. Aspirate until the atmospheric air reaches the air bubble. After the air bubble bursts re-inject the aspirated liquid. Always incline the slide so liquid is beneath the air phase.

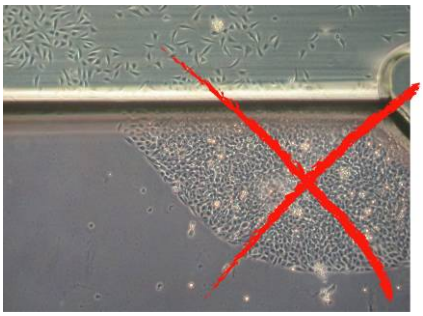


Trapped air bubbles in filling ports can be removed by aspiration. Puncture them and aspirate with a 10 µl pipet with a very thin pipet tip. To make sure there is enough liquid for a correctly filled filling port, add a drop of liquid onto the trapped air bubble before the procedure.



c) Inhomogeneous Cell Distribution

Inhomogeneous cell distribution, especially high cell densities close to the observation area, negatively overlays directed migration and must be avoided. Carefully conduct all steps following the protocol at § 5a to avoid spilling cell suspension into the reservoirs. Never inject cell suspension directly into filling ports.



d) Chamber Fills not Properly

There are some general tips and tricks for filling the chamber:

- During filling by injection of liquid incline the slide in a way that liquid is beneath air. Doing this air can escape trough the filling ports.
- Always use chambers that are completely dry.
  Especially after coating the chambers need to be completely dry. Small liquid remains might hinder proper handling.
- Use a pipet that is serviced routinely.
  After some years of use pipets can loose pressure during pipetting. In normal work they might perform great but there can be problems when used with µ-Slide Chemotaxis.

e) Cells do not Attach Properly

The chamber's geometry is very special. Especially the cell seeding step is critical because the volume is very low. Most problems with cell attachment can be avoided by checking the following questions:

- Are the cells seeded in optimal growth medium?
  Adherent cells will not attach properly when they are seeded in starvation medium. Use normal growth medium with all necessary supplements like serum.
- Is the slide put into an extra humid chamber?
  Evaporation is one of the most crucial issues during cell attachment. Make sure to provide a maximum of humidity during cell attachment.
- Is the incubator frequently opened during cell attachment?
  Frequent door openings must be avoided because this lowers humidity inside the incubator drastically for a short time. A cell culture incubator that is not used by others during cell attachment is perfect.
- Is the surface/coating suitable for the cell type you use?
  Use a surface coating which is known to provide cell attachment for your cell type.

f) Cells Are Flushed Away When Filling the Chamber

Most problems can be solved by checking the following points:

- Use adherent cells only.
- Fill the chamber with cell-free medium very carefully.
  Depending on the filling speed there can be enormous shear forces during this step. Control your cells under the phase contrast microscope after each pipetting step to check during which step the problem occurs.
- Change the surface.
  Since evaporation is critical during cell attachment it is favorable using a precoated surface. Coatings decrease attachment time because cells do not have to produce their own extracellular matrix (ECM).
- Use µ-Slide Chemotaxis 3D
  Our µ-Slide Chemotaxis 3D can also be used to conduct 2D experiments without the use of gel. For this method, cells need to be slightly adherent to the surface of the observation area only. More details can be found in our Application Note 17 in the section 2D assay with slightly adherent cells.

g) Scratches Inside the Observation Area

Scratches on the bottom of the slide can be due to putting the slide directly on a flat metal surface as the work bench. Avoid draggling the slide on the bench. Always use the slide directly inside a 10 cm Petri dish.

Some scratches are part of the chamber itself. Unfortunately, those are normal in this slide and unavoidable. These scratches are on the ceiling of the channel, not on the bottom, where the cells are. Depending on the objective lens used (depth of focus) they are more or less visible.

h) Temperature Instabilities

Major changes in temperature during the experiment may cause convection thus disturbing the diffusion driven gradient. Keep the temperature as stable as possible.

i) Focus not Stable

Especially during time lapse experiments focus drift is an annoying effect. Focus stability is mainly influenced by mechanical changes and temperature instabilities. Follow these recommendations to keep your cells in focus:

- Switch on all components (heating, gas incubation, computer, other equipment) at least 60 min before starting the time-lapse recording.
- After you put the µ-Slide onto the microscope wait 20 min before starting a time lapse experiment to achieve temperature (and immersion oil) equilibration.
- Keep the room temperature as stable as possible. Air conditioning should either be working continuously or should be switched off.
- Do not change temperature during experiments. Avoid door/window openings as this could change temperature rapidly.
- Eliminate all sources of mechanical vibrations. Use a damped table for your microscope.
- Use an autofocus system.

Please contact info@ibidi.com for more information.