What is claimed:

1. A method for culturing and monitoring cells using a microfluidic cell culture system comprising:

introducing one or more groups of cells in to a cell chamber of the microfluidic cell culture system;

culturing the one or more groups of cells in the cell chamber;

creating and controlling a gradient in the cell chamber using one or more microfluidic flow channels;

wherein the cell chamber provides an uninterrupted optical path to the cells during culturing and creation and control of the gradient;

capturing a plurality of images of the cells in the microfluidic culture system during culturing and during control of the gradient;

using an information system to analyze one or more cells in a sequence of the plurality of images to determine migration properties of the one or more cells.

2. The method of claim 1, further comprising:

wherein one or more of the microfluidic flow channels is associated with one or more barriers that restrict cell movement while introducing substances into the cell chamber.

3. The method of any combination of claim 1 to the preceding claim, further comprising:

controlling gradient parameters of one or more substances;

4. The method of any combination of claim 1 to the preceding claim, further comprising:

controlling gradient parameters to provide a steady state or varying gradient or both at different time periods.

5. The method of any combination of claim 1 to the preceding claim, further comprising:

using microfluidic control of cell culture conditions and chemoattractant gradient formation during image capture to determine precise, dynamic quantitation of cell migration in response to stimuli.

6. The method of any combination of claim 1 to the preceding claim, further comprising:

distinguishing chemotaxis towards a substance from nondirectional migration by analyzing movements in an x direction (e.g., perpendicular to gradient) separately from movements in a y direction (e.g., parallel to gradient).

7. The method of any combination of claim 1 to the preceding claim, further comprising:
determining total migration distance and Euclidean distance with respect to gradient conditions.

8. The method of any combination of claim 1 to the preceding claim, further comprising:
determining the effects of signaling molecules, growth factors, or other substances, on migration propensities of cells in one or more of tumors, wounds, developing tissues, immune responses, and other biological systems that can be characterized with respect to active cell migration.

9. The method of any combination of claim 1 to the preceding claim, further comprising:
culturing cells in three distinct phases:
complete substance feeding;
serum starvation; and
exposure to a chemoattractant (e.g., fetal bovine serum (FBS)) gradient.

10. The method of any combination of claim 1 to the preceding claim, further comprising:
reversing a gradient orientation after a selected period of time.

11. The method of claim 10 wherein the selected period of time is a sufficient time to determine cell migration or other properties in one direction of a gradient, e.g., 3, 6, 12, 24 hours or any appropriate time period.

12. The method of any combination of claim 1 to the preceding claim, further comprising:
capturing time-lapse images during a gradient portion or culture portion and storing digital representations of the images for analysis;
wherein images are captured at desired intervals during a gradient exposure time or a culture time or both;
analyzing the images using an information system;
wherein one or more individual cells are tracked for migratory properties across a sequential series or other series of images.

13. The method of claim12, further comprising:

wherein desired intervals are e.g., approximately 20-minute intervals or, any number between 1 minute and 48 hour intervals;

wherein individual cells comprise one or more of about 10, about 20, about 30, about 40, about 50, about 60-100, or about 100-5,000 representative cells;

wherein the series of images are e.g., 10-36 images during 8-16 hours in culture, 36 - 300 images during 24 hours in culture, or any desired regular or irregular capture of images during any desired culture period.

14. The method of any combination of claim 1 to the preceding claim, further wherein one or more individual single cells (e.g., MDA-MB-231) are monitored in response to gradients over the course of a long period (e.g., a period of about 6 hours to about 6 days, or a period of about three days.)

15. The method of any combination of claim 1 to the preceding claim, further determining mechanisms underlying pathological phenomena such as wound healing and tumor metastasis from the migration.

16. The method of any combination of claim 1 to the preceding claim, further comprising:

analyzing cell migration properties by comparing cells in stable medium to equivalent cells exposed to a nutrient or other gradient.

17. The method of any combination of claim 1 to the preceding claim, further comprising:

analyzing cell migration properties by comparing cells migration to determine when cell migration is significantly more directed, thereby indicating a response to a gradient in the culture chamber.

18. The method of any combination of claim 1 to the preceding claim, further comprising:

determining cell migration in response to a gradient by comparing cells in a constant substance and determining whether cells in the gradient are moving substantially farther (about 3-fold or about 1.5-fold to 10-fold) away from their site of origin than cells in a constant substance state.

19. The method of any combination of claim 1 to the preceding claim, further comprising:

determining cell migration in response to the gradient by comparing a velocity of cells in a constant substance and a velocity of cells in the gradient.

20. The method of any combination of claim 1 to the preceding claim, further comprising culturing cells for a substantial period that allows for both cell expansion and movement (e.g. a 4 hour to 6 day period) with images taken some period after gradient induction.

21. The method of any combination of claim 1 to the preceding claim, further comprising:

configuring a microfluidic device comprising: a first channel; a first barrier separating the first channel from a cell chamber; a second barrier separating the cell chamber from a second channel; the first channel configured to contain a first substance; the second channel configured to contain a second substance;

such that cells in the cell chamber are exposed to a gradient of the first substance in the cell chamber attracting the cells towards the first barrier.

22. The method of any combination of claim 1 to the preceding claim, further comprising:

configuring a microfluidic device comprising:

a. a cell chamber;

b. a first flow inlet for flowing a first substance into the cell chamber;

c. a second flow inlet for flowing a second substance into the cell chamber;

d. at least one cell inlet;

such that cells in the cell chamber are exposed to a continuous stable gradient of the first substance and the second substance.

23. The method of any combination of claim 1 to the preceding claim, further comprising:

configuring a microfluidic device according to any combination of claims 26 to 46 such that cells in the cell chamber are exposed to a controlled gradient.

24. The method of any combination of claim 1 to the preceding claim, further comprising:

configuring a microfluidic system according to any combination of claims 47 to 55 such that cells in a plurality of cell chambers in plurality of microfluidic devices are exposed to one or more controlled gradients.

25. A method for culturing and monitoring cells using microfluidic cell culture systems comprising:

monitoring cells (e.g., the metastatic human breast cancer cell line MDA-MB-231) to determine their movement in response to spatial changes in serum concentration;

detecting for cells exposed to a gradient (e.g. FBS), movement of cells along the Y-axis toward the upper barrier of the cell chamber in the positive direction of the gradient;

monitoring one or more control cells cultured in the absence of a spatial serum gradient to detect less movement with far more random directional properties;

wherein cells exposed to gradients are determined to move preferentially toward the source of higher gradient concentration; and wherein both cell expansion and movement occurred during the 4 hour to 6 day intervals, with images taken some period after gradient induction.

26. A microfluidic device comprising:

a. a first channel;

b. a first barrier separating the first channel from a cell chamber;

c. a second barrier separating the cell chamber from a second channel ;

d. the first channel configured to contain a first substance;

d. the second channel configured to contain a second substance ;

e. such that cells in the cell holding region are exposed to a gradient of the first substance in the cell chamber attracting the cells towards the first barrier .

27. A microfluidic device comprising:

a. a cell chamber;

b. a first flow inlet for flowing a first substance into the cell chamber;

c. a second flow inlet for flowing a second substance into the cell chamber;

d. at least one cell inlet;

e. such that cells in the cell chamber are exposed to a continuous stable gradient of the first substance and the second substance.

28. A microfluidic device according to claim 27 further wherein:

the first or the second flow inlets or both comprise a plurality of perfusion passages allowing substance to flow into the cell chamber and inhibiting passage of cells out of the chamber through the first or the second flow inlets.

29. A microfluidic device according to claim 27 further comprising:

f. a third flow inlet for flowing a third substance into the cell chamber.

30. A microfluidic device according to any combination of claim 26 to the preceding claim, further wherein:

the device is configured such that flows in one or more flow channels are driven by capillary force or gravity or an active pneumatic manifold or any combination thereof.

31. A microfluidic device according to any combination of claim 26 to the preceding claim, further the first or second flow channel or both are separated from the cell chamber by a set of cross section microfluidic pores or passages measuring about 8x8 microns.

32. A microfluidic device according to any combination of claim 26 to the preceding claim, further wherein liquids in first and second channel wells and in an outlet well are in contact with the flow channels.

33. A microfluidic device according to any combination of claim 26 to the preceding claim further wherein a well above the culture chamber is left empty for better imaging quality and the bottom surface of the plate is a glass slide.

34. A microfluidic device according to any combination of claim 26 to the preceding claim wherein one or more flow channels are about 550 μm in width and 50 μm in height.

35. A microfluidic device according to any combination of claim 26 to the preceding claim wherein one or more barriers consists of a network of passages of approximately 50x8x8μm (LxWxH).

36. A microfluidic device according to any combination of claim 26 to the preceding claim wherein one or more barriers consists of a network of passages of approximately 100x4x2 μm (LxWxH) and further wherein a narrow cross section of the passages prevents cells from passing through the barrier and further wherein medium and/or any substance such as chemoattractants, dyes, or other materials diffuse across the barrier and form a gradient to the cells.

37. A microfluidic device according to any combination of claim 26 to the preceding claim wherein the chamber is about 4.8 x 0.5 x .05 mm in dimension (LxWxH) and is used to count the number of cells that migrate.

38. A microfluidic device according to any combination of claim 26 to the preceding claim wherein cells in the chamber are counted by a manual or an automated microscope and quantified.

39. A microfluidic device according to any combination of claim 26 to the preceding claim wherein one or more flow channels is about 100x50 μm (WxH) and at least one flow channel carries fluid from a flow inlet well along a barrier and empties the fluid to a flow outlet well allowing diffusion of materials from the flow channel through the barrier.

40. A microfluidic device according to any combination of claim 26 to the preceding claim wherein the device is configured into a well plate to allow for simultaneous running of one or more assay experiments, including assays that expose cells to different substance, hormonal or other stimuli, drugs, chemoattractants, etc.

41. A microfluidic device according to any combination of claim 26 to the preceding claim further containing structures providing a culture environment in the chamber that sustains the cells and air diffusion through the material that defines the channels facilitated by air passages and air holes.

42. A microfluidic device according to any combination of claim 26 to the preceding claim further wherein flow of substance or liquid in one or more flow channels diffuses into the cell culture chamber and nourishes the cells for culture while providing an attractant for cells.

43. A microfluidic device according to any combination of claim 26 to the preceding claim further wherein each chamber is about 1.5 x 0.5 mm in size, with a height of about 200 μm.

44. A microfluidic device according to any combination of claim 26 to the preceding claim wherein an expected number of cells per unit is about 500 cells.

45. A microfluidic device according to any combination of claim 26 to the preceding claim wherein an example perfusion rate is about 40 ul/day.

46. A microfluidic device according to any combination of claim 26 to the preceding claim wherein an example cell chamber volume is about 150 nL, and the chamber dimensions are about 1.5x0.5x0.2 mm.

47. A microfluidic system comprising:

a. one or more devices according to any combination of claim 26 to the preceding claim;

b. a microfluidic plate with at least three wells associated with each of the one or more devices;

c. wherein at least one well is configured so that it introduces a chemoattractant agent into the first channel or the second channel.

48. A microfluidic system comprising:

a. one or more devices according to any combination of claim 26 to claim 46;

b. an active control microfluidic plate with at least three wells connected to each of the devices;

c. wherein at least one well is configured so that it introduces a chemoattractant agent into the first channel or the second channel; and

d. a manifold for controlling pressure and or contents in at least one well connected to at least one device during cell culture.

49. The system according to claim 48 further comprising:

in addition to said at least one well where pressure is controlled by said manifold, at least one well configured to receive fluid and perfuse fluid into the device via passive gravity driven flow.

50. The system according to claim any combination of claims 48 to the preceding claim further comprising:

at least three inlet wells and one outlet well associated with each of the one or more devices.

51. The system according to any combination of claims 48 to the preceding claim further comprising:

at least three flow inlet wells, at least one cell inlet well, at least one cell outlet well, and at least one imaging/viewing window or well associated with each of the one or more devices.

52. A microfluidic system comprising:

a. one or more devices according to any combination of claim 26 to claim 47;

b. an active control microfluidic plate with at least three inlet wells connected to each of the devices;

c. wherein at least one well is configured so that it introduces a chemoattractant agent into the first channel or the second channel; and

d. a manifold for controlling pressure and or contents in at least one well connected to at least one device during cell culture.

53. The system according to claim 52 further comprising:

at least three inlet wells and one outlet well associated with each of the one or more devices.

54. The system according to any combination of claim 52 to the preceding claim further comprising:

at least four inlet wells and one outlet well associated with each of the one or more devices.

55. The system according to any combination of claim 52 to the preceding claim further comprising:

at least four of the devices, each associated with at least two inlet wells, at least one outlet well, and an imaging well or region.

56. The system according to any combination of claims 48 to the preceding claim further comprising:

a well plate containing one or more microfluidic devices, each device associated with a plurality of inlet wells; and

a pneumatic manifold for mating to the plate and allowing pressure driven control of solutions in the inlet wells.

57. The system according to claim 56 further comprising:

an automated digital controller with a logic processor for controlling pressure in the wells via the manifold.

58. The system according to any combination of claims 56 to the preceding claim further comprising:

wherein the multiple liquid or reagent inlet wells enable controlled experiments according to any combination of the methods of claims 1 to 25.

59. The system according to any combination of claims 56 to the preceding claim further comprising:

wherein the multiple liquid or reagent inlet wells enable controlled experiments regarding how cells respond to different drugs or other stimulus introduced via the inlets.

60. The system according to any combination of claims 56 to the preceding claim further comprising:

a gravity well associated with one or more devices to facilitate maintaining cells before the plate is attached to the manifold and experiments are performed.

61. The system according to any combination of claims 56 to the preceding claim wherein the multiple liquid or reagent inlet wells enable controlled experiments regarding how cells respond to different drugs or other stimulus introduced via the inlets where solutions are quickly changed over the cells.

62. The system according to any combination of claims 56 to the preceding claim further comprising:

a digital image capture device for capturing images of cells in one or more cell chambers;

an information processing system for reading captured images to count cells or determine migration of individual cells or groups of cells or both.

63. The system according to claim 62 wherein the information processing system controls pressure driven flow of the manifold.

What is claimed:

1. A method for culturing and monitoring cells using a microfluidic cell culture system, wherein the microfluidic cell culture system comprises a cell chamber, separated from a first microfluidic flow channel by a perfusion barrier and from a second microfluidic flow channel by a second perfusion barrier on an opposite side of the cell chamber, a gravity flow inlet well, and an outlet well, the method comprising:

introducing one or more groups of cells into the cell chamber of the microfluidic cell culture system;

culturing the one or more groups of cells in the cell chamber;

continuously perfusing the cultured one or more groups of cells by supplying a media solution to the gravity flow inlet well, wherein a medium level difference between the gravity flow inlet well and outlet well creates a perfusion flow rate;

creating and controlling a first gradient in the cell chamber by supplying a first substance to the first microfluidic flow channel and a second substance to the second microfluidic flow channel, the first gradient forming within the cell chamber perpendicular to the first perfusion barrier and the second perfusion barrier;

creating and controlling a second gradient in the cell chamber, after a selected period of time, by supplying a third substance to the first microfluidic flow channel and a fourth substance to the second microfluidic flow channel, the second gradient forming within the cell chamber perpendicular to the first perfusion barrier and the second perfusion barrier;

wherein the cell chamber provides an uninterrupted optical path to the cultured one or more cells during culturing and creation and control of the gradients;

capturing a plurality of images of the cultured one or more cells in the microfluidic culture system during culturing and during control of the gradients;

using an information system to analyze the cultured one or more cells in a sequence of the plurality of images to determine migration properties of the cultured one or more cells in response to the first and second gradients.

2. The method of claim 1, wherein one or more of the microfluidic flow channels is associated with one or more barriers that restrict cell movement while introducing substances into the cell chamber.

3. The method of claim 1 further comprising: controlling gradient parameters to provide a steady state or varying gradient or both at different time periods.

4. The method of claim 1 further comprising:
using microfluidic control of cell culture conditions and chemoattractant gradient formation during image capture to determine precise, dynamic quantitation of cell migration in response to stimuli.

5. The method of claim 1 further comprising:
distinguishing chemotaxis towards a substance from nondirectional migration by analyzing movements in a direction perpendicular to a gradient separately from movements in a direction parallel to the gradient.

6. The method of claim 1 further comprising:
determining total migration distance and Euclidean distance with respect to gradient conditions.

7. The method of claim 1 further comprising:
determining the effects of signaling molecules, growth factors, or other substances, on migration propensities of cells in one or more of tumors, wounds, developing tissues, immune responses, and other biological systems that can be characterized with respect to active cell migration.

8. The method of claim 1 further comprising:
culturing cells in three distinct phases: complete substance feeding;
serum starvation; and
exposure to a chemoattractant.

9. The method of claim 1 wherein
the first microfluidic channel is in communication with a first and second solution inlet well;

the second microfluidic channel is in communication with a third and fourth solution inlet well; and

the first gradient is created by supplying solutions from the first and third solution inlet wells and the second gradient is created by supplying solutions from the second and fourth solution inlet wells.

10. The method of claim 9, wherein complete medium is provided in the first and fourth solution inlet wells, and complete medium without chemoattractant is provided in the second and third solution inlet wells.

11. The method of claim 1 wherein the selected period of time is a sufficient time to determine cell migration or other properties in one direction of a gradient.

12. The method of claim 1 further comprising:

capturing time-lapse images during a gradient portion or culture portion and storing digital representations of the images for analysis;

wherein images are captured at desired intervals during a gradient exposure time or a culture time or both;

analyzing the images using an information system;

wherein one or more individual cells are tracked for migratory properties across a sequential series or other series of images.

13. The method of claim 12, further comprising:

wherein desired intervals are any number between 1 minute and 48 hour intervals;

wherein individual cells comprise one or more of about 10, about 20, about 30, about 40, about 50, about 60-100, or about 100-5,000 representative cells;

wherein the series of images are any desired regular or irregular capture of images during any desired culture period.

14. The method of claim 1 further wherein one or more individual cells are monitored in response to gradients over the course of a period of between about 6 hours to about 6 days.

15. The method of claim 1 further comprising: analyzing cell migration properties by comparing cells in stable medium to equivalent cells exposed to a nutrient or other gradient.

16. The method of claim 1 further comprising: analyzing cell migration properties to determine when cell migration is significantly more directed, thereby indicating a response to a gradient in the culture chamber.

17. The method of claim 1 further comprising: determining cell migration in response to a gradient by comparing cells in a constant substance and determining whether cells in the gradient are moving substantially farther away from their site of origin than cells in a constant substance state.

18. The method of claim 1 further comprising: determining cell migration in response to a gradient by comparing a velocity of cells in a constant substance and a velocity of cells in the gradient.

19. The method of claim 1 further comprising culturing cells for a period of between 4 hours and 6 days that allows for both cell expansion and movement with images taken some period after gradient induction.

20. A method for culturing and monitoring cells using microfluidic cell culture systems comprising:

culturing cells within a cell chamber of a microfluidic cell culture system, the cell chamber comprising a first perfusion barrier in communication with a first microfluidic flow channel and a second perfusion barrier positioned opposite from the first perfusion barrier and in communication with a second microfluidic flow channel;

continuously perfusing cells with media using gravity-driven perfusion;

creating and controlling a gradient in the cell chamber by supplying a first substance to the first microfluidic flow channel and a second substance to the second microfluidic flow channel, the gradient forming within the cell chamber along a Y-axis towards the first perfusion barrier;

monitoring cells to determine their movement in response to the gradient;

detecting, for cells exposed to the gradient, movement of cells along the Y-axis toward the first barrier of the cell chamber in the positive direction of the gradient;

monitoring one or more control cells cultured in the absence of the gradient to detect less movement with far more random directional properties;

wherein cells exposed to the gradient are determined to move preferentially toward the source of higher gradient concentration;

and wherein both cell expansion and movement occurred during the 4 hour to 6 day intervals, with images taken some period after gradient creation.

21. The method of claim 10, where the chemoattractant is fetal bovine serum (FBS).

22. A method of analyzing cells exposed to a gradient in a microfluidic device, comprising:

culturing cells within a cell chamber of a microfluidic device;

supplying a first substance to a first microfluidic flow channel in communication with a first perfusion barrier, the first perfusion barrier separating the first microfluidic flow channel from a cell chamber;

supplying a second substance to a second microfluidic flow channel in communication with a second perfusion barrier, the second perfusion barrier spaced opposite from the first perfusion barrier and separating the second microfluidic flow channel from the cell chamber;

wherein the first substance passes at least in part through the first perfusion barrier into the cell chamber, and the second substance passes at least in part through the second perfusion barrier into the cell chamber, the first and second substances forming a first gradient within the cell chamber, the first gradient established perpendicular to the first perfusion barrier and second perfusion barrier;

and analyzing a response of the cultured cells within the cell chamber in response to the first gradient.

23. The method of claim 22, wherein the response of the cultured cells is at least one of cell movement and cell expansion.

24. The method of claim 22, further comprising:

supplying a third substance to the first microfluidic flow channel;

supplying a fourth substance to the second microfluidic channel;

such that the third substance passes at least in part through the first perfusion barrier into the cell chamber, and the fourth substance passes at least in part through the second perfusion barrier into the cell chamber, the third and fourth substances forming a second gradient within the cell chamber;

and analyzing a response of the cultured cells within the cell chamber in response to the second gradient.

25. The method of claim 24, wherein the second gradient is created concurrently with the first gradient.

26. The method of claim 22, wherein the cultured cells migrate in a profile that is substantially linear in an axis perpendicular to the first perfusion barrier and second perfusion barrier.