into a mass spectrometer is characterized by a less than a 0.1% standard error fluctuation in total mass spectrometric signal intensity.

**[0013]** In yet another aspect, disclosed herein is a method for providing stable electrospray ionization performance comprising: a) providing a microfluidic chip comprising a substrate, wherein the substrate comprises: (i) a fluid channel having a distal end that is in fluid communication with an electrospray ionization orifice, and (ii) a gas channel having a distal end that is in fluid communication with a gas outlet orifice; b) flowing a solution through the fluid channel; c) flowing a gas through the gas channel; and d) controlling a flow rate of the gas and a flow rate of the solution such that a ratio of volumetric flow rates for the gas and solution ranges from 1000:1 to 1,000,000:1 or a ratio of flow velocity for the gas at the gas outlet orifice and flow velocity for the solution at the electrospray ionization orifice ranges from 100:1 to 1,000,000:1. In some embodiments, the ratio of volumetric flow rates for the gas and solution ranges from 10,000:1 to 1,000,000:1. In some embodiments, the ratio of volumetric flow rates for the gas and solution ranges from 10,000:1 to 500,000:1, and more particularly from 10,000:1 to 300,000:1.

**[0014]** In some embodiments, the flow of solution is controlled by pressure, gravity, an electrokinetic force, or any combination thereof. In some embodiments, the flow of gas is provided by a compressed gas source. In some embodiments, the volumetric flow rate for the solution is less than 25 μL/min. In some embodiments, the microfluidic chip comprises two or more gas channels, each comprising a distal end that is in fluid communication with a gas outlet orifice, and wherein the two or more gas outlet orifices are disposed symmetrically about and adjacent to the electrospray ionization orifice. In some embodiments, the electrospray ionization orifice is disposed on an edge or corner of the substrate. In some embodiments, the one or more gas outlet orifices are disclosed adjacent to the electrospray ionization orifice on an edge of the substrate. In some embodiments, an electrospray ionization performance achieved when the microfluidic chip is configured to introduce a sample into a mass spectrometer is characterized by a less than a 1.0% standard error fluctuation in total mass spectrometric signal intensity. In some embodiments, an electrospray ionization performance when the microfluidic chip is configured to introduce a sample into a mass spectrometer is characterized by a less than a 0.1% standard error fluctuation in total mass spectrometric signal intensity.

**[0015]** There is disclosed a method for providing stable electrospray ionization performance comprising: a) providing a microfluidic chip comprising a substrate, wherein the substrate comprises: (i) a fluid channel having a distal end that is in fluid communication with an electrospray ionization orifice, and (ii) a gas channel having a distal end that is in fluid communication with a gas outlet orifice; b) flowing a solution through the fluid channel; c) flowing a gas through the gas channel; and d) controlling a flow rate of the gas and a flow rate of the solution

## CLAIMS

WHAT IS CLAIMED IS:

1. A microfluidic chip comprising:

a) a substrate, wherein the substrate comprises:

i) a fluid channel comprising a distal end that is in fluid communication with an electrospray ionization orifice; and

ii) a gas channel comprising a distal end that is in fluid communication with a gas outlet orifice disposed adjacent to the electrospray ionization orifice;

wherein an angle between the distal end of the fluid channel and the distal end of the gas channel ranges from about 0 degrees to about 30 degrees.

2. The microfluidic chip of claim 1, wherein the gas outlet orifice is disposed an edge of the substrate adjacent to the electrospray ionization orifice.

3. The microfluidic chip of claim 2, wherein the substrate comprises two or more gas outlet orifices disposed adjacent to and symmetrically about the electrospray ionization orifice.

4. The microfluidic chip of any one of claims 1 to 3, wherein the angle ranges from about 10 degrees to about 20 degrees.

5. The microfluidic chip of any one of claims 1 to 4, wherein the gas outlet orifice is configured to perform nebulization of a solution expelled from the electrospray ionization orifice.

6. The microfluidic chip of any one of claims 1 to 5, wherein the microfluidic device comprises three or more gas channels each comprising a gas outlet orifice disposed adjacent to the electrospray ionization orifice, and wherein at least one of the three or more gas channels are disposed within the substrate, and at least one of the three or more gas channels are disposed within an auxiliary component of the microfluidic chip that is positioned adjacent to the substrate such that the at least one gas channels are not located within a same plane as the substrate.

7. The microfluidic chip of claim 6, wherein the at least one of the three or more gas channels disposed within the auxiliary component are positioned such that:

(a) their gas outlet orifices lie in a plane that is substantially perpendicular to that of the substrate and are positioned symmetrically about and adjacent to the electrospray ionization orifice, or

(b) their gas outlet orifices lie in one or more planes that are rotated relative to that of the substrate and are positioned in a radially-symmetric pairwise manner about and adjacent to the electrospray ionization orifice.

8. The microfluidic chip of any one of claims 1 to 7, wherein the microfluidic chip is configured to perform an isoelectric focusing or electrophoretic separation of a sample comprising a mixture of analytes in the fluid channel.

9. The microfluidic chip of any one of claims 1 to 8, wherein the fluid channel has one or more of the following dimensions:

(a) a width ranging from about 20 µm to about 600 µm,

(b) a depth ranging from about 10 µm to about 100 µm, or

(c) a length ranging from about 0.25 cm to about 30 cm and/or

the gas channel has one or more of the following dimensions:

(a) width ranging from about 20 µm to about 200 µm,

(b) a depth ranging from about 10 µm to about 100 µm, or

(c) a length ranging from about 0.2 cm to about 20 cm.

10. The microfluidic chip of any one of claims 1 to 9, wherein the electrospray ionization orifice has a maximum cross-sectional dimension ranging from about 10 µm to about 100 µm.

11. The microfluidic chip of any one of claims 1 to 10, wherein the gas outlet orifice has a maximum cross-sectional dimension ranging from about 10 µm to about 50 µm.

12. The microfluidic chip of any one of claims 1 to 11, wherein the gas outlet orifice is disposed within 100 µm, 50 µm, or within 15 µm of the electrospray ionization orifice.

13. A microfluidic chip comprising:

a) a substrate, wherein the substrate comprises:

i) two or more gas channels of different lengths, each configured to deliver a gas to a gas outlet orifice;

wherein a dimension of at least one of the two or more gas channels is adjusted along a portion of its length so that each of the two or more gas channels has about the same hydrodynamic flow resistance.

14. The microfluidic chip of claim 13, wherein a cross-sectional area of at least one of the two or more gas channels is adjusted along a portion of its length.

15. The microfluidic chip of claim 13 or claim 14, wherein a difference in length of the two or more gas channels ranges from about 1 cm to about 10 cm.

16. The microfluidic chip of any one of claims 13 to 15, wherein the substrate further comprises a fluid channel comprising a distal end that is in fluid communication with an electrospray ionization orifice.

17. The microfluidic chip of claim 16, wherein the two or more gas outlet orifices are disposed symmetrically about and adjacent to the electrospray ionization orifice and are configured to perform nebulization of a solution expelled from the electrospray ionization orifice.

18. The microfluidic chip of claim 16 or claim 17, wherein the electrospray ionization orifice is disposed on an edge or corner of the substrate and the two or more gas outlet orifices are disposed on an edge of the substrate adjacent to the electrospray ionization orifice.

19. The microfluidic chip of any one of claims 13 to 18, wherein the microfluidic chip is configured to perform isoelectric focusing or electrophoretic separations.

20. The microfluidic chip of any one of claims 13 to 19, wherein the gas is a nebulizer gas comprising air, nitrogen, oxygen, nitrous oxide, fluorourethane, helium, argon, methanol, or any combination thereof.

21. The microfluidic chip of any one of claims 13 to 20, further comprising a hydrophobic coating on at least a portion of an edge of the substrate or corner of the substrate on which the electrospray ionization orifice is disposed.

22. A microfluidic chip comprising:

a) a substrate, wherein the substrate comprises:

i) a fluid channel comprising a proximal end that is in fluid communication with a fluid inlet port and a distal end that is in fluid communication with an electrospray ionization orifice; and

ii) at least two gas channels, each comprising a proximal end that is in fluid communication with a gas inlet port and a distal end in fluid communication with a gas outlet orifice;

wherein the at least one fluid inlet port and the at least two gas inlet ports are disposed along a first edge of the substrate.

23. The microfluidic chip of claim 22, wherein the electrospray ionization orifice is positioned on a second edge of the substrate or on a corner of the substrate that does not comprise the first edge.

24. The microfluidic chip claim 22 or claim 23, wherein the substrate is less than about 2.0 mm thick.

25. The microfluidic chip of any one of claims 22 to 24, wherein the fluid channel comprises a separation channel configured to perform an electrophoretic separation or an isoelectric focusing separation.

26. The microfluidic chip of any one of claims 22 to 25, wherein the substrate comprises a first separation channel and a second separation channel, wherein a distal end of the first separation channel is in fluid communication with a proximal end of the second separation channel, wherein a distal end of the second separation channel is in fluid communication with the electrospray ionization orifice, and wherein the first separation channel is configured to perform a chromatographic separation, and wherein the second separation channel is configured to perform an electrophoretic separation or an isoelectric focusing separation.

27. The microfluidic chip of any one of claims 22 to 26, wherein the fluid channel comprises a separation channel configured to perform isoelectric focusing separation of a sample comprising a mixture of analytes, and the substrate further comprises a mobilization electrolyte channel that is in fluid communication with a distal end of the separation channel and is configured to provide electrophoretic introduction of a mobilization electrolyte at the distal end of the separation channel.

28. A method for performing electrospray ionization from a microfluidic chip comprising:

a) providing a microfluidic chip comprising a substrate, wherein the substrate comprises:

i) at least one fluid channel comprising a distal end that is in fluid communication with an electrospray ionization orifice; and

ii) at least one gas channel configured to deliver a gas to a gas outlet orifice that is adjacent to the electrospray ionization orifice;

b) flowing a solution through the at least one fluid channel such that the solution is expelled from the electrospray ionization orifice; and

c) flowing a gas through the at least one gas channel such that the gas is expelled from the gas outlet orifice;

wherein a temperature of the substrate is controlled by a temperature of the gas flowing through the at least one gas channel.

29. The method of claim 28, wherein the temperature of the gas ranges from about 4 $^{o}$C to about 100$^{o}$C, and the temperature of the substrate ranges from about 10 $^{o}$C to about 50 $^{o}$C.

30. The method of claim 28 or claim 29, wherein the separation channel is configured to perform an isoelectric focusing separation or an electrophoretic separation of a sample comprising a mixture of analytes.

31. The method of any one of claims 28 to 30, wherein an electrospray ionization performance, when the microfluidic chip is configured to introduce a sample into a mass spectrometer, is characterized by a less than a 1.0% or by a less than a 0.1% standard error fluctuation in total mass spectrometric signal intensity.

32. A method for providing stable electrospray ionization performance comprising:

a) providing a microfluidic chip comprising a substrate, wherein the substrate comprises: (i) a fluid channel having a distal end that is in fluid communication with an electrospray ionization orifice, and (ii) a gas channel having a distal end that is in fluid communication with a gas outlet orifice;

b) flowing a solution through the fluid channel;

c) flowing a gas through the gas channel; and

d) controlling a flow rate of the gas and a flow rate of the solution such that a ratio of volumetric flow rates for the gas and solution ranges from 1000:1 to 1,000,000:1 or a ratio of flow velocity for the gas at the gas outlet orifice and flow velocity for the solution at the electrospray ionization orifice ranges from 100:1 to 1,000,000:1.

33. The method of claim 32, wherein the flow of solution is controlled by pressure, gravity, an electrokinetic force, or any combination thereof and wherein the flow of gas is provided by a compressed gas source.

34. The method of claim 32 or 33, wherein the volumetric flow rate for the solution is less than 25 µL/min.

35. The method of any one of claims 32 to 34, wherein the microfluidic chip comprises two or more gas channels, each comprising a distal end that is in fluid communication with a gas outlet orifice, and wherein the two or more gas outlet orifices are disposed symmetrically about and adjacent to the electrospray ionization orifice.

36. The method of any one of claims 32 to 35, wherein the electrospray ionization orifice is disposed on an edge or corner of the substrate and wherein the one or more gas outlet orifices are disclosed adjacent to the electrospray ionization orifice on an edge of the substrate.

37. The method of any one of claims 32 to 36, wherein an electrospray ionization performance, when the microfluidic chip is configured to introduce a sample into a mass spectrometer, is characterized by a less than a 1.0% or by a less than a 0.1% standard error fluctuation in total mass spectrometric signal intensity.

38. The method of any one of claims 32 to 37, wherein the ratio of flow velocity for the gas at the gas outlet orifice and flow velocity for the solution at the electrospray ionization orifice ranges from 500:1 to 5,000:1 or from 1,000:1 to 3,000:1.

39. A microfluidic cartridge comprising:

a) a microfluidic chip comprising at least one fluid port and at least two gas ports disposed on an edge of the microfluidic chip; and

b) a microfluidic cartridge component that is in fluid communication with the microfluidic chip and is configured to encompass at least a portion of the microfluidic chip, the microfluidic cartridge component comprising at least one fluid port and at least two gas ports that align with the at least one fluid port and at least two gas ports of the microfluidic chip.

40. The microfluidic cartridge of claim 39, further comprising one or more elastomeric components disposed between the edge of the microfluidic chip and a surface of the cartridge; and wherein the one or more elastomeric components form a substantially leak-proof seal between the at least one fluid port and at least two gas ports of the microfluidic chip and the at least one fluid port and at least two gas ports of the microfluidic cartridge component upon application of force.

41. The microfluidic cartridge of claim 39 or claim 40, wherein the edge of the microfluidic chip is less than about 2.0 mm thick.

42. A system comprising:

a) a microfluidic cartridge comprising two or more fluid ports and configured to be removeable from the system; and

b) an instrument comprising two or more fluid interconnects;

wherein each of the two or more fluid interconnects is configured to provide a substantially leak-proof fluid coupling between a fluid line of the instrument and a fluid port of the microfluidic cartridge upon application of force to an assembly comprising the two or more fluid interconnects and the two or more fluid ports of the microfluidic cartridge, and

wherein the substantially leak-proof fluid couplings are maintained when a relative fluid pressure within two of the two or more fluid lines varies by a factor of at least 10-fold.

43. The system of claim 42, wherein the substantially leak-proof fluid couplings are maintained when the relative fluid pressure within two of the two or more fluid lines varies by a factor of at least 100-fold.

44. The system of claim 42 or claim 43, wherein each of the two or more fluid interconnects comprises an independently spring-loaded fitting.

45. The system of claim 44, wherein the independently spring-loaded fittings comprise a flat face-sealing fitting that mates with a fluid port comprising a hole in the microfluidic cartridge.

46. The microfluidic chip of claim 25 or 26, wherein isoelectric focusing is performed while flowing a fluid from the fluid inlet port through the separation channel.