

CLAIMS

WHAT IS CLAIMED IS:

1. A computer-implemented method, comprising:
 - (a) receiving, using a processor, a time-series imaging data set comprising a plurality of images of an isoelectric focusing separation performed in a separation channel, wherein each image of the plurality of images corresponds to a different time point;
 - (b) converting, using the processor, each image of the plurality of images to an intensity or absorbance measurement as a function of position along the length of the separation channel for the corresponding time point; and
 - (c) generating, using the processor, a heat map or a 3-dimensional plot of the intensity or absorbance measurements as a function of position along the length of the separation channel and as a function of time.
2. The computer-implemented method of claim 1, wherein the time-series imaging data set further comprises a plurality of images of the mobilization of separated analyte peaks in the separation channel.
3. The computer-implemented method of claim 1 or claim 2, wherein the time-series imaging data set comprises a plurality of ultraviolet (UV) absorbance images.
4. The computer-implemented method of any one of claims 1-3, wherein the time-series imaging data set comprises a plurality of fluorescence images.
5. The computer-implemented method of claim 4, wherein the fluorescence images comprise images of native fluorescence.
6. The computer-implemented method of any one of claims 1-5, wherein the time-series imaging data set comprises a plurality of images acquired at a frame rate of at least one image per minute.
7. The computer-implemented method of claim 6, wherein the time-series imaging data set comprises a plurality of images acquired at a frame rate of at least one image per 30 seconds.
8. The computer-implemented method of claim 7, wherein the time-series imaging data set comprises a plurality of images acquired at a frame rate of at least one image per 10 seconds.
9. The computer-implemented method of any one of claims 1-8, further comprising, imaging the separation channel while the isoelectric focusing separation is performed, wherein (a)-(c) are performed iteratively as the images are acquired.
10. The computer-implemented method of any one of claims 1-9, wherein the heat map or the 3-dimensional plot is used to perform one or more tasks selected from the group consisting of: comparing the isoelectric focusing separation with an additional isoelectric focusing separation, comparing a mobilization reaction with the isoelectric focusing separation, comparing the mobilization reaction with an additional mobilization reaction,

determining a completion of the isoelectric focusing separation, monitoring a progress of the mobilization reaction, determining a presence of electroosmotic flow, determining a separation performance parameter.

11. The computer-implemented method of claim 10, wherein the separation performance parameter is separation resolution.
12. A computer-implemented method, comprising:
 - (a) receiving, using a processor:
 - (i) a first data set comprising a plurality of intensity or absorbance measurements as a function of length along a separation channel from an isoelectric focusing separation performed in the separation channel; and
 - (ii) a second data set comprising a plurality of mass spectrometer total ion measurements as a function of time;
 - (b) converting the second data set, using the processor, into a third data set comprising ion count measurements as a function of mass; and
 - (c) overlaying, using the processor, plots of the first data set and the third data set.
13. The computer-implemented method of claim 12, wherein (c) further comprises overlaying, using the processor, a plot of the second data set with the plots of the first data set and the third data set.
14. The computer-implemented method of claim 12 or 13, wherein (c) comprises deconvolving the second data set to generate the third data set.
15. The computer-implemented method of any one of claims 12-14, wherein, in (c), a first peak in intensity or absorbance of the first data set is mapped to a set of peaks of the third data set.
16. The computer-implemented method of claim 15, wherein the second data set is used to map the first data set to the set of peaks of the third data set.
17. The computer-implemented method of claim 15, further comprising, using the processor, correlating the first peak to the set of peaks to determine a mass distribution and an isoelectric point of at least one analyte species of said first peak.
18. The computer-implemented method of claim 15, wherein the first peak corresponds to an analyte peak and yields information on an isoelectric point of one or more analyte species in the analyte peak.
19. The computer-implemented method of claim 18, wherein the set of peaks corresponds to a mass distribution of the one or more analyte species in the analyte peak.
20. The computer-implemented method of claim 19, further comprising, using the processor to determine, for a given isoelectric point, the identities of the one or more analyte species in the analyte peak.

21. The computer-implemented method of claim 20, wherein the one or more analyte species comprises different protein isoforms.
22. The computer-implemented method of claim 21, wherein the protein isoforms comprise different post-translational modifications of a protein.
23. The computer-implemented method of any one of claims 12-22, wherein the overlay plot shows (i) an intensity or absorbance measurement of the plurality of intensity or absorbance measurements as a function of the length along the separation channel and (ii) a time series of ion count measurements as a function of mass.
24. The computer-implemented method of any one of claims 12-23, further comprising, performing the isoelectric focusing separation, mobilization, and electrospray ionization using a single, integrated microfluidic device coupled to a mass spectrometer to obtain the first data set and the second data set.
25. The computer-implemented method of claim 24, wherein (b) and (c) are performed within 1 minute of or concurrently with the ESI-MS.
26. The computer-implemented method of any one of claims 12-25, wherein (b) or (c) is performed automatically as part of a software package for acquiring or processing electrospray ionization-mass spectrometry (ESI-MS) data.
27. The computer-implemented method of any one of claims 12-26, wherein (b) and (c) are performed automatically as part of a software package for acquiring or processing electrospray ionization-mass spectrometry (ESI-MS) data.
28. A method, comprising:
 - using (i) mass spectrometry data for one or more analyte species and (ii) isoelectric focusing data of the one or more analyte species to assign a post-translational modification to the one or more analyte species.
29. The method of claim 28, wherein the post-translational modification is selected from the group consisting of: a hydroxylation, a methylation, a lipidation, an acetylation, a disulfide bond, a sumoylation, a ubiquitination, a glycosylation, a glycation, an amino acid addition or removal, an amidation, a deamidation, an isomerization, an oxidation, a fucosylation, a sialylation, and a phosphorylation.
30. The method of claim 28 or 29, further comprising, performing an isoelectric focusing separation on a mixture of analytes comprising the one or more analyte species to generate the isoelectric focusing data, mobilizing the one or more analyte species, and performing electrospray-ionization mass spectrometry (ESI-MS) to generate the mass spectrometry data.
31. The method of claim 30, wherein the isoelectric focusing separation and mobilization are performed using a single microfluidic device comprising a separation channel and an integrated electrospray tip.
32. The method of any one of claims 28-31, wherein the isoelectric focusing data comprises one or more intensity or absorbance measurements as a function of distance along a separation channel, and wherein a peak in the intensity or absorbance measurements

corresponds to an analyte peak comprising the one or more analyte species having a same given isoelectric point.

33. The method of claim 32, further comprising, for the given isoelectric point, using the mass spectrometry data to distinguish at least one post-translational modification of the one or more analyte species.
34. The method of any one of claims 28-33, wherein the isoelectric focusing data comprise information on an isoelectric point of the one or more analyte species and the mass spectrometry data comprise information on the mass of the one or more analyte species.
35. The method of any one of claims 28-34, further comprising, using known values of isoelectric point shifts and mass shifts of a plurality of post-translational modifications to assign the post-translational modification to at least one of the one or more analyte species.
36. The method of any one of claims 28-35, wherein the assigning of the post-translational modification occurs within 1 minute of acquiring the ESI-MS data.
37. The method of any one of claims 28-36, wherein the isoelectric focusing data comprises information on one or more isoelectric points of the one or more analyte species, wherein the mass spectrometry data comprises information on one or more masses of the one or more analyte species, and wherein the post-translational modification is assigned by matching the one or more isoelectric points and the one or more masses to a reference comprising a plurality of known isoelectric points and mass values of a plurality of post-translational modifications.
38. The method of claim 37, wherein the reference comprises publicly available data.
39. A method for maintaining a constant voltage difference between an electrospray ionization (ESI) tip and a mass spectrometer inlet, the method comprising:
 - (a) applying a first voltage to a proximal end of a separation channel, wherein a distal end of the separation channel is in fluid and electrical communication with the ESI tip;
 - (b) applying a second voltage to a proximal end of an auxiliary fluid channel, wherein a distal end of the auxiliary fluid channel is in fluid and electrical communication with the distal end of the separation channel;
 - (c) performing a separation reaction to separate a mixture of analytes, wherein the separation reaction takes place within the separation channel; and
 - (d) monitoring a change in resistance of the separation channel or a change in voltage at the ESI tip in a feedback loop that adjusts a third voltage applied to the mass spectrometer inlet to maintain the constant voltage difference between the ESI tip and the mass spectrometer inlet.
40. The method of claim 39, wherein the separation channel is a lumen of a capillary.
41. The method of claim 40, wherein the capillary comprises a microvial spray tip.

42. The method of claim 39, wherein the separation channel is a fluid channel within a microfluidic device.
43. The method of any one of claims 39 to 42, wherein the separation reaction comprises an isoelectric focusing reaction.
44. The method of any one of claims 39 to 42, wherein the separation reaction comprises an electrophoretic separation reaction.
45. The method of any one of claims 39 to 44, wherein the first voltage is applied at a cathode coupled to the separation channel and the second voltage is applied at an anode coupled to the separation channel.
46. The method of any one of claims 39 to 45, wherein the voltage at the ESI tip or the mass spectrometer inlet is held at ground.
47. The method of any one of claims 39 to 46, wherein the voltage at the mass spectrometer inlet is held at the third voltage.
48. The method of any one of claims 39 to 47, wherein the third voltage is adjusted by adding a transient voltage change measured at the ESI tip to the third voltage.
49. The method of any one of claims 39 to 48, wherein the voltage at the ESI tip is measured using a power supply.
50. The method of claim 49, wherein the power supply is coupled to the separation channel.
51. The method of claim 50, wherein the power supply is coupled to another channel that is coupled to the separation channel.
52. The method of claim 11, wherein the power supply is set to 0 microamps.
53. The method of any one of claims 39 to 48, wherein the voltage at the ESI tip is measured using an electrode disposed at the ESI tip, and wherein the electrode is configured to output 0 microamps of current.
54. The method of any one of claims 39 to 53, wherein the feedback loop operates at a frequency of at least 0.1 Hz.
55. The method of any one of claims 39 to 53, wherein the feedback loop operates at a frequency of at least 10 Hz.
56. The method of any one of claims 39 to 55, wherein the feedback loop maintains the voltage at the ESI tip to within $\pm 10\%$ of a pre-set value.
57. The method of any one of claims 39 to 55, wherein the feedback loop maintains the voltage at the ESI tip to within $\pm 1\%$ of a pre-set value.
58. The method of any one of claims 39 to 55, wherein the feedback loop maintains the constant voltage difference between the ESI tip and the mass spectrometer inlet to within $\pm 10\%$ of a pre-set value.
59. The method of any one of claims 39 to 55, wherein the feedback loop maintains the constant voltage difference between the ESI tip and the mass spectrometer inlet to within $\pm 1\%$ of a pre-set value.
60. A method for maintaining a constant voltage difference ($\Delta V_{\text{TIP-MS}}$) between an electrospray ionization (ESI) tip and a mass spectrometer inlet, the method comprising:
 - (a) setting a target value (ΔV_{TARGET}) for $\Delta V_{\text{TIP-MS}}$;
 - (b) periodically or continuously monitoring a first voltage at the ESI tip, wherein the ESI tip is in fluid and electrical communication with a separation channel;

- (c) calculating an instantaneous value for $\Delta V_{\text{TIP-MS}}$; and
 - (d) using a feedback loop, periodically or continuously adjusting a second voltage at the mass spectrometer inlet so that $\Delta V_{\text{TIP-MS}} = \Delta V_{\text{TARGET}}$.
61. The method of claim 60, wherein the separation channel is a lumen of a capillary.
 62. The method of claim 61, wherein the capillary comprises a microvial spray tip.
 63. The method of claim 60, wherein the separation channel is a fluid channel within a microfluidic device.
 64. The method of any one of claims 60 to 63, wherein a separation reaction performed in the separation channel comprises an isoelectric focusing reaction.
 65. The method of any one of claims 60 to 63, wherein a separation reaction performed in the separation channel comprises an electrophoretic separation reaction.
 66. The method of any one of claims 60 to 65, wherein the first voltage at the ESI tip or the second voltage at the mass spectrometer inlet is held at ground.
 67. The method of any one of claims 60 to 66, wherein the first voltage at the ESI tip is monitored using an electrode disposed at the ESI tip.
 68. The method of claim 67, wherein the electrode disposed at the ESI tip is configured to output 0 microamps of current.
 69. The method of any one of claims 60 to 30, wherein the first voltage at the ESI tip is monitored using a power supply that is in electrical communication with a fluid channel that intersects the separation channel at a position near the ESI tip, and that is configured to output 0 microamperes of current.
 70. The method of any one of claims 60 to 69, wherein the feedback loop operates at a frequency of at least 0.1 Hz.
 71. The method of any one of claims 60 to 70, wherein the feedback loop operates at a frequency of at least 10 Hz.
 72. The method of any one of claims 60 to 71, wherein the feedback loop operates at a frequency of at least 100 Hz.
 73. The method of any one of claims 60 to 72, wherein the feedback loop maintains $\Delta V_{\text{TIP-MS}}$ to within $\pm 10\%$ of ΔV_{TARGET} .
 74. The method of any one of claims 60 to 73, wherein the feedback loop maintains $\Delta V_{\text{TIP-MS}}$ to within $\pm 1\%$ of 61.
 75. A computer-implemented method for maintaining a constant voltage difference between an electrospray ionization (ESI) tip and an inlet of a mass spectrometer, the method comprising:
 - (a) receiving, using a processor, a measurement of a first voltage at the ESI tip, wherein the ESI tip is in fluid and electrical communication with a separation channel;
 - (b) receiving, using the processor, a measurement of a second voltage at the inlet of the mass spectrometer; and
 - (c) comparing the first voltage to the second voltage using the processor, wherein if the second voltage differs from the first voltage, the processor causes the voltage at the inlet of the mass spectrometer or the ESI tip to be adjusted, such that a

difference between the voltage at the ESI tip and the voltage at the inlet of the mass spectrometer remains constant.

76. The computer-implemented method of claim 75, further comprising:
 - (d) repeating steps (a) through (c) at a specified frequency using a feedback loop.
77. The computer-implemented method of claim 75 or claim 76, wherein the separation channel comprises (i) a lumen of a capillary or (ii) a fluid channel within a microfluidic device.
78. The computer-implemented method of claim 77, wherein the capillary comprises a microvial spray tip.
79. The computer-implemented method of any one of claims 75 to 78, wherein a separation reaction is performed in the separation channel, and wherein the separation reaction comprises an isoelectric focusing reaction.
80. The computer-implemented method of any one of claims 75 -79, wherein the voltage at the ESI tip or the voltage at the inlet of the mass spectrometer is held at ground.
81. The computer-implemented method of any one of claims 75 -80, wherein the voltage at the ESI tip is measured using an electrode placed at the ESI tip.
82. The computer-implemented method of any one of claims 75 -80, wherein the voltage at the ESI tip is monitored using a power supply that is in electrical communication with a fluid channel that intersects the separation channel near the ESI tip, and that is configured to output 0 microamperes of current.
83. The computer-implemented method of claim 76, wherein the feedback loop operates at a frequency of at least 0.1 Hz.
84. The computer-implemented method of claim 76 or claim 83, wherein the feedback loop operates at a frequency of at least 10 Hz.