CLAIMS

1. A method comprising:

loading an analyte of interest into a microfluidic device, wherein the microfluidic device comprises:

a flow cell including an inlet port, an outlet port, and at least one flow channel extending between the inlet port and the outlet port, the flow channel to receive the analyte of interest and one or more reagents; and

at least one electrothermal mixing device coupled to the flow cell and positioned proximate to the at least one flow channel, the electrothermal mixing device comprising a passivation layer positioned between the flow channel and an electrode array of the electrothermal mixing device;

loading a reagent into the flow cell; and

actively mixing the reagent with the analyte of interest in said flow channel via electrothermal mixing by the electrothermal mixing device.

2. The method of claim 1, wherein the flow channel has a height of 20 μm, inclusive, to 400 μm, inclusive.

3. The method of claim 1 or 2, wherein the electrode array is electrically connected to an AC electric field.

4. The method of claim 1, 2 or 3, wherein the electrode array comprises a plurality of electrodes, spaced apart to create gradients in conductivity and permittivity in liquid in the flow channel.

5. The method of claim 4, wherein the plurality of electrodes are arranged in a coplanar arrangement.

6. The method of claim 4, wherein the plurality of electrodes are arranged facing each other.

7. The method of any of claims 4-6, wherein at least some of the plurality of electrodes have a thickness of 20 nm, inclusive, to 2 μm, inclusive, and a width of 200 μm, inclusive, to 800 μm, inclusive.

8. The method of any of claims 4-7, wherein a first electrode of the plurality of electrodes has a first planar area dimension and a second electrode of the plurality of electrodes has a second planar area dimension, wherein the first planar area dimension and the second planar area dimension are different.

9. The method of any of claims 4-8, wherein a first electrode of the plurality of electrodes is located at a first distance from a second electrode of the plurality of electrodes, wherein the second electrode of the plurality of electrodes is located at a second distance from a third electrode of the plurality of electrodes, wherein the first distance and the second distance are different.

10. The method of any of claims 4-9, wherein a first electrode and a second electrode of the plurality of electrodes are separated by a gap, the gap comprising a first distance between the first and second electrodes and a second distance between the first and second electrodes, wherein the first distance and the second distance are different, such that the gap is non-uniform.

11. The method of any of claims 4-10, wherein the plurality of electrodes comprises a first crescent-shaped electrode, a second crescent-shaped electrode, and a third central electrode,

wherein the first and second crescent-shaped electrodes each comprise a center portion, a first distal end, and a second distal end,

wherein the first and second crescent-shaped electrodes are arranged concentrically such that the first distal ends are facing one another and are separated by a first opening and the second distal ends are facing one another and are separated by a second opening, and

wherein the third central electrode comprises a substantially circular shape having a first wing portion and a second wing portion extending from opposite sides of the substantially circular shape, the third central electrode positioned between the first and second crescent-shaped electrodes such that the first wing portion extends through the first opening and the second wing portion extends through the second opening.

12. The method of claim 11, wherein the first wing portion is distanced from the first distal end of the first crescent-shaped electrode by a first gap.

13. The method of claim 12, wherein the first wing portion comprises a proximal end connected to the substantially circular shape and a distant end extending away from the substantially circular shape, and wherein the width of the first wing portion increases gradually from the proximal end to the distant end, such that the first gap is non-uniform.

14. The method of any of the preceding claims, wherein the electrode array is embedded within at least one of an inner surface of a base of the flow cell, an inner surface of a lid of the flow cell, or at least a portion of the passivation layer.

15. The method of any of the preceding claims, wherein the passivation layer comprises a dielectric layer, wherein the dielectric layer comprises one of SiO_2, Si_3N_4, Ta_2O_5, or a polymer.

16. The method of any of the preceding claims, wherein the passivation layer has a thickness of 20 nm, inclusive, to 2 μm, inclusive.

17. The method of any of the preceding claims, wherein the flow cell comprises a glass layer having a thickness in the range of 80 μm, inclusive, to 1 mm, inclusive, and wherein the electrode array is arranged on a printed circuit board positioned on an outer surface of the flow cell.

18. The method of any of the preceding claims, wherein the flow cell further comprises a nanowell layer comprising a plurality of nanowells, the nanowells adapted for the analyte of interest to bind to an interior surface thereof.

19. The method of any of the preceding claims, wherein the analyte of interest comprises a nucleic acid.

20. The method of claim 18, wherein the analyte of interest is bound to an interior surface of the nanowells via a hydrogel layer.

21. The method of claim 18 or 19, wherein the electrode array comprises a plurality of transparent electrodes, and wherein a highly conductive material is deposited onto a portion of the plurality of transparent electrodes, outside of an imaging area of the flow cell.

22. The method of claim 21, wherein the highly conductive material comprises a metal or conductive ink with a resistivity of 2 $x10^{-8}$ Ω·m, inclusive, to 5 $x10^{-6}$ Ω·m, inclusive.

23. The method of claim 21 or 22, wherein the flow cell further comprises a planarization layer deposited onto a portion of the plurality of transparent electrodes, within an imaging area of the flow cell.

24. The method of claim 23, wherein the planarization layer is an index matching layer.

25. The method of claim 24, wherein the index matching layer comprises an insulating material.

26. The method of any of claims 21-25, wherein a first transparent electrode of the plurality of transparent electrodes has a thickness of 20 nm, inclusive, to 1μm, inclusive, and a width of 200 μm, inclusive, to 800 μm, inclusive.

27. The method of claim 26, wherein the first transparent electrode of the plurality of transparent electrodes is separated from a second transparent electrode of the plurality of transparent electrodes by a gap in the range of 50 μm, inclusive, to 750 μm, inclusive.

28. The method of any of the preceding claims, wherein the microfluidic device further comprises an external loop connecting the outlet port back to the inlet port such that fluid within the flow cell is recirculated through the flow cell.

29. The method of claim 28, wherein the external loop further comprises a recirculation pump.

30. The method of any of the preceding claims, wherein the flow cell further comprises a nanowell layer comprising a plurality of nanowells, the nanowells adapted for the analyte of interest to bind to an interior surface thereof, wherein the analyte of interest comprises a nucleic acid, and wherein the analyte of interest is bound to an interior surface of the nanowells via a hydrogel layer.