quantification further increases the value of dPCR technology. Existing dPCR solutions have used elastomeric valve arrays, silicon through-hole approaches, and microfluidic encapsulation of droplets in oil. Despite the growing number of available dPCR platforms, dPCR has been at a disadvantage when compared to the older qPCR technology which relies on counting the number of PCR amplification cycles. The combination of throughput, ease of use, performance and cost are the major barriers for gaining adoption in the market for dPCR.

SUMMARY

[0007] Provided herein are methods and devices that may be useful for amplifying and quantifying nucleic acids. The present disclosure provides methods, systems, and devices that may enable sample preparation, sample amplification, and sample analysis through the use of dPCR. This may enable a nucleic acid to be amplified and quantified at a reduced cost and complexity as compared to other systems and methods.

[0008] In a first aspect of the present disclosure, there is provided a method for forming a microfluidic device, comprising: providing a microfluidic structure and a film; (b) treating a surface of the microfluidic structure, a surface of the film, or both with a solvent; (c) subsequent to (b), pressing the microfluidic structure together with the film under a first heating condition to form the microfluidic device comprising the solvent; and (d) applying a negative pressure to the microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes and at a pressure less than 20 kilopascals (kPa) to remove at least a portion of the solvent from (b).

[0008a] In a second aspect of the present disclosure, there is provided a method for forming a microfluidic device, including: a) providing a microfluidic structure and a film; b) treating a surface of said microfluidic structure, a surface of said film, or both with a solvent; c) subsequent to (b), pressing said microfluidic structure together with said film under a first heating condition to form said microfluidic device comprising said solvent, wherein said first heating condition comprises heating to a temperature of at least about 60 °C; and d) applying a negative pressure to said microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes or at a pressure less than 20 kilopascals (kPa) to remove at least a portion of said solvent from (b), and said second heating condition comprising heating said microfluidic device to a temperature of at least about 70 °C.

[0008b] In a third aspect of the present disclosure, there is provided a method for forming a microfluidic device, including: a) providing a microfluidic structure and a film; b) treating a surface of said microfluidic structure, a surface of said film, or both with a solvent; c) subsequent to (b), pressing said microfluidic structure together with said film under a first heating condition to form said microfluidic device comprising said solvent; and d) applying a negative pressure to said microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes or at a pressure less than 20 kilopascals (kPa) to remove at least a portion of said solvent from (b), and said applying said negative pressure comprising applying said negative pressure for at least about 2 hours.**[0009]** In some embodiments, the microfluidic structure comprises a microchannel, a plurality of microchambers, a plurality of siphon apertures, or any combination thereof. In some embodiments, the treating comprises application of one or more solvents. In some embodiments, the one or more solvents include a solvent selected from the group consisting of isopropyl alcohol, acetone, ethyl alcohol, hexanes, cyclohexane, toluene, and benzene. In some embodiments, the pressing comprises applying a force of at least about 0.5 kilo-Newtons (kN). In some embodiments, the first heating condition comprises heating to a temperature of at least about 60 °C. In some embodiments, the applying the negative pressure comprises applying a pressure less than about 7 kPa. In some embodiments, the second heating condition comprises heating the microfluidic device to a temperature of at least about 70 °C. In some embodiments, the heating the microfluidic device to a temperature of at least about 70°C removes at least about 75% of the solvent from the microfluidic device. In some embodiments, the applying the negative pressure comprises applying the negative pressure for at least about 2 hours. In some

CLAIMS

1. A method for forming a microfluidic device, comprising:

a) providing a microfluidic structure and a film;

b) treating a surface of said microfluidic structure, a surface of said film, or both with a solvent;

c) subsequent to (b), pressing said microfluidic structure together with said film under a first heating condition to form said microfluidic device comprising said solvent; and

d) applying a negative pressure to said microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes and at a pressure less than 20 kilopascals (kPa) to remove at least a portion of said solvent from (b).

2. The method of claim 1, wherein said microfluidic structure comprises a microchannel, a plurality of microchambers, a plurality of siphon apertures, or any combination thereof.

3. The method of claim 1, wherein said treating comprises application of one or more solvents.

4. The method of claim 3, wherein said one or more solvents include a solvent selected from the group consisting of isopropyl alcohol, acetone, ethyl alcohol, hexanes, cyclohexane, toluene, and benzene.

5. The method of claim 1, wherein said pressing comprises applying a force of at least about 0.5 kilo-Newtons (kN).

6. The method of claim 1, wherein said first heating condition comprises heating to a temperature of at least about 60 °C.

7. The method of claim 1, wherein said applying said negative pressure comprises applying a pressure less than about 7 kPa.

8. The method of claim 1, wherein said second heating condition comprises heating said microfluidic device to a temperature of at least about 70 °C.

9. The method of claim 8, wherein said heating said microfluidic device to a temperature of at least about 70°C removes at least about 75% of said solvent from said microfluidic device.

10. The method of claim 1, wherein said applying said negative pressure comprises applying said negative pressure for at least about 2 hours.

11. The method of claim 1, wherein said applying said negative pressure removes at least about 50 % of said solvent from said microfluidic device.

12. The method of claim 1, wherein said applying said negative pressure under said second heating condition reduces separation between said microfluidic structure and said film.

13. The method of claim 1, wherein said microfluidic structure comprises a channel or chamber with a feature size of at most about 500 micrometers.

14. The method of claim 1, wherein said removing said solvent increases a yield of a microfluidic device generation process by at least about 25%.

15. The method of claim 1, wherein said microfluidic device has a usable feature fraction of at least about 0.5.

16. The method of claim 1, further comprising applying an increased pressure to said microfluidic device, wherein said increased pressure is sufficient to expel at least a portion of said solvent.

17. A method for forming a microfluidic device, comprising:

a) providing a microfluidic structure and a film;

b) treating a surface of said microfluidic structure, a surface of said film, or both with a solvent;

c) subsequent to (b), pressing said microfluidic structure together with said film under a first heating condition to form said microfluidic device comprising said solvent, wherein said first heating condition comprises heating to a temperature of at least about 60 °C; and

d) applying a negative pressure to said microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes or at a pressure less than 20 kilopascals (kPa) to remove at least a portion of said solvent from (b), and said second heating condition comprising heating said microfluidic device to a temperature of at least about 70 °C.

18. A method for forming a microfluidic device, comprising:

a) providing a microfluidic structure and a film;

b) treating a surface of said microfluidic structure, a surface of said film, or both with a solvent;

c) subsequent to (b), pressing said microfluidic structure together with said film under a first heating condition to form said microfluidic device comprising said solvent; and

d) applying a negative pressure to said microfluidic device under a second heating condition, said negative pressure being applied for a time period greater than 30 minutes or at a pressure less than 20 kilopascals (kPa) to remove at least a portion of said solvent from (b), and said applying said negative pressure comprising applying said negative pressure for at least about 2 hours.