CLAIMS

1. A method for preparing a three-dimensional polymerized matrix, comprising:

a) delivering a matrix-forming material in a space between a surface of a first planar substrate and a surface of a second planar substrate, wherein:

a biological sample is immobilized on the surface of the first planar substrate,

the space is at least partially enclosed by a spacer between the first and second planar substrates, and

the spacer is non-integral to the first or second planar substrate; and

b) forming the three-dimensional polymerized matrix from the matrix-forming material in the space, wherein the three-dimensional polymerized matrix comprises the biological sample embedded therein and has an average thickness between about 5 μm and about 200 μm.

2. The method of claim 1, further comprising c) removing the spacer from the first and/or the second planar substrates.

3. The method of claim 1 or claim 2, wherein the spacer is an adhesive tape having a thickness of about 100 μm.

4. The method of claim 1 or claim 2, wherein the spacer is an adhesive tape having a thickness between about 10 μm and 20 μm.

5. The method of claim 1 or claim 2, wherein the spacer comprises a plurality of microparticles.

6. The method of claim 5, wherein the average diameter of the rigid microparticles is between about 10 μm and 50 μm.

7. The method of claim 5 or claim 6, wherein the plurality of microparticles is monodisperse.

8. The method of any one of claims 5 to 7, wherein the plurality of microparticles comprises a plurality of silica monospheres.

9. The method of any one of claims 1 to 8, further comprising:

applying the spacer to the surface of the first planar substrate and/or the surface of the second planar substrate, and

bringing the first planar substrate and second planar substrate together such that the spacer separates the first and second planar substrates to provide the space.

10. The method of claim 9, wherein the spacer comprises a plurality of microparticles, and the applying step comprises adding a suspension to the surface of either the first planar substrate or second planar substrate, wherein the suspension comprises the plurality of microparticles and a carrier.

11. The method of claim 10, wherein the carrier comprises mineral oil or an organic solvent, optionally wherein the organic solvent is ethanol.

12. The method of claim 10 or claim 11, wherein the plurality of microparticles are not mixed with the matrix-forming material and do not contact the three-dimensional polymerized matrix.

13. The method of claim 1, further comprising fixing the biological sample prior to the delivering step.

14. The method of any one of claims 1 to 13, wherein the first planar substrate comprises a coating with or is functionalized with one or more substances to facilitate attachment of the biological sample.

15. The method of claim 14, wherein the one or more substances comprise lectins, poly-lysine, antibodies, polysaccharides, or covalently binding moieties.

16. The method of claim 14 or 15, wherein the one or more substances comprise acryloyls.

17. The method of any one of claims 1 to 16, further comprising permeabilizing the biological sample after the fixing step and prior to the delivering step.

18. The method of any one of claims 1 to 17, further comprising cross-linking the biological sample embedded within the three-dimensional polymerized matrix.

19. The method of any one of claims 1 to 18, further comprising clearing the biological sample embedded within the three-dimensional polymerized matrix.

20. The method of any one of claims 1 to 19, wherein the biological sample is a tissue slice between about 1 µm and about 50 µm in thickness.

21. The method of claim 20, wherein the tissue slice is between about 5 µm and about 35 µm in thickness.

22. The method of any one of claims 1 to 21, wherein the matrix-forming material comprises a plurality of fluorescent beads, and wherein the thickness of the three-dimensional polymerized matrix is measured by imaging the fluorescent beads, optionally wherein the fluorescent beads have an average diameter of about 0.2 µm.

23. The method of any one of claims 1 to 22, further comprising applying a force to the first and/or second planar substrates prior to forming the three-dimensional polymerized matrix.

24. The method of any one of claims 1 to 23, wherein the three-dimensional polymerized matrix is formed by subjecting the matrix-forming material to polymerization.

25. The method of claim 24, wherein the polymerization is initiated by adding a polymerization-inducing catalyst, UV light or functional cross-linkers.

26. The method of any one of claims 1 to 25, wherein the matrix-forming material comprises polyacrylamide, cellulose, alginate, polyamide, cross-linked agarose, cross-linked dextran or cross-linked polyethylene glycol.

27. The method of any one of claims 1 to 26, wherein the surface of the first or second planar substrate has a recessed cavity.

28. The method of any one of claims 1 to 27, wherein the surface of the first or second planar substrate comprises one or more positional markers and/or fiducial markers.

29. A method for embedding a biological sample in a three-dimensional polymerized matrix, comprising:

a) applying a spacer to a surface of a first planar substrate or a surface of a second planar substrate, wherein the spacer is non-integral to the first or second planar substrate, and wherein a biological sample is immobilized on the surface of a first planar substrate;

b) bringing the first and second planar substrates together to form a space between the first and second substrates such that the biological sample is in the space at least partially enclosed by the spacer;

c) delivering a matrix-forming material in the space; and

d) forming a three-dimensional polymerized matrix from the matrix-forming material in the space, wherein the three-dimensional polymerized matrix has an average thickness between about10 μm and about 100 μm,

thereby embedding the biological sample in the three-dimensional polymerized matrix.

30. The method of claim 29, wherein the spacer is applied to the second planar substrate which is downward facing, and the method further comprises e) removing the second planar substrate from the first planar substrate, thereby removing the spacer at least partially enclosing the biological sample.

31. The method of claim 29, wherein the spacer is applied to the first planar substrate which is upward facing, and the method further comprises e) removing the second planar substrate from the first planar substrate, and optionally further removing the spacer from the first planar substrate.

32. The method of any of claims 29-31, further comprising crosslinking the biological sample embedded in the three-dimensional polymerized matrix.

33. The method of any of claims 29-32, further comprising clearing the biological sample embedded within the three-dimensional polymerized matrix.

34. The method of claim 33, wherein the clearing is performed for less than about 60 minutes.

35. The method of claim 33, wherein the clearing is performed for less than about 30 minutes.