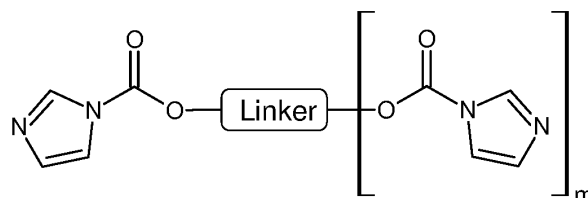


CLAIMS

What is claimed is:

1. A method for preparing a biological sample comprising contacting the sample with a first fixing reagent composition comprising a compound of formula (I)

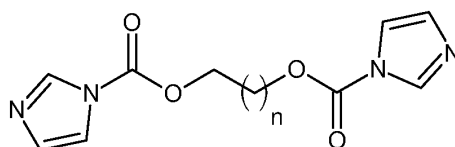


(I)

wherein, “Linker” comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

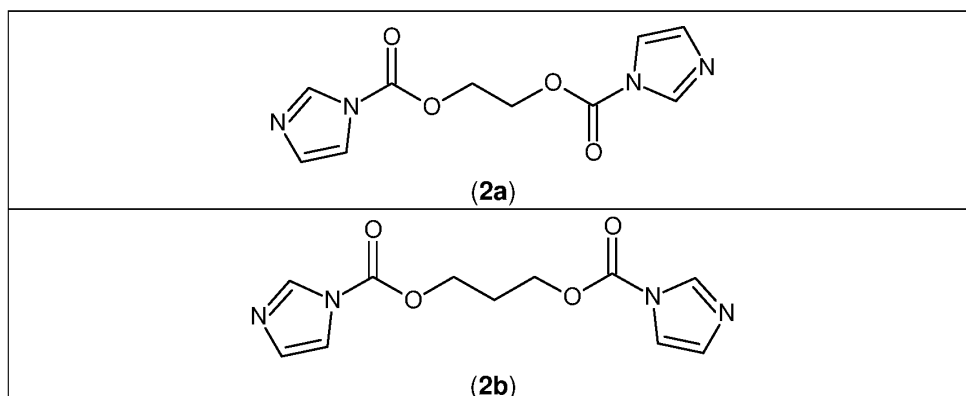
optionally, wherein:

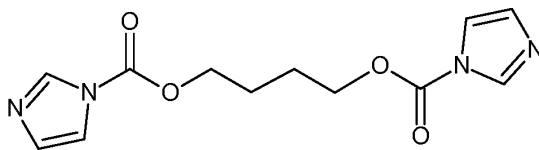
- i) $m = 1$;
 - ii) the “Linker” comprises a linear alkane moiety of 2-24 carbons;
 - iii) the “Linker” comprises an ethylene glycol moiety;
 - iv) the “Linker” comprises a disulfide bond; and/or
 - v) the “Linker” comprises a branched alkane moiety.
2. The method of claim 1, wherein the compound of formula (I) is a compound of formula (II)



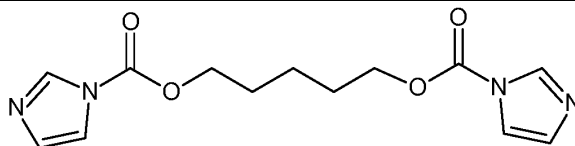
(II)

wherein n is 1 to 13; optionally, wherein the compound of formula (II) is selected from compounds (2a) – (2k)

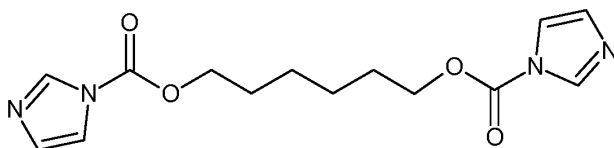




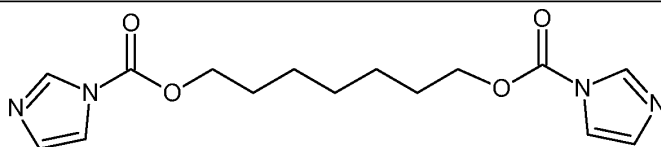
(2c)



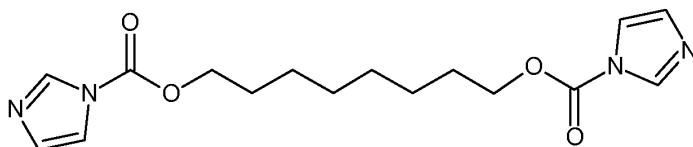
(2d)



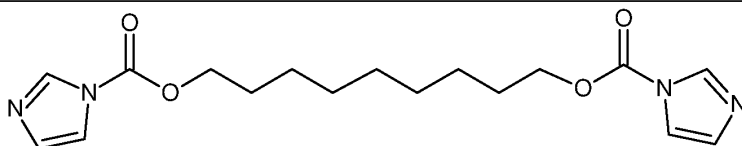
(2e)



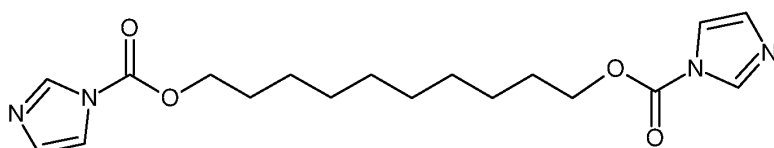
(2f)



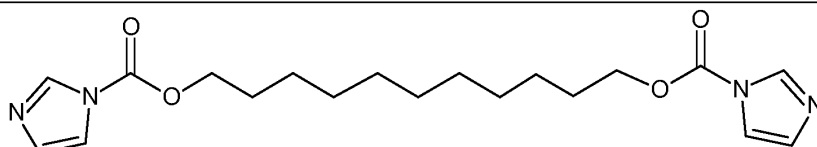
(2g)



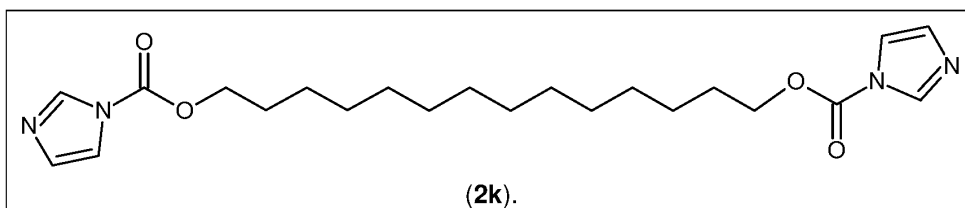
(2h)



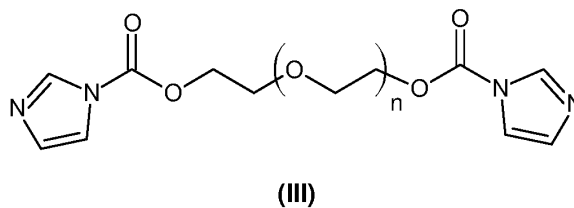
(2i)



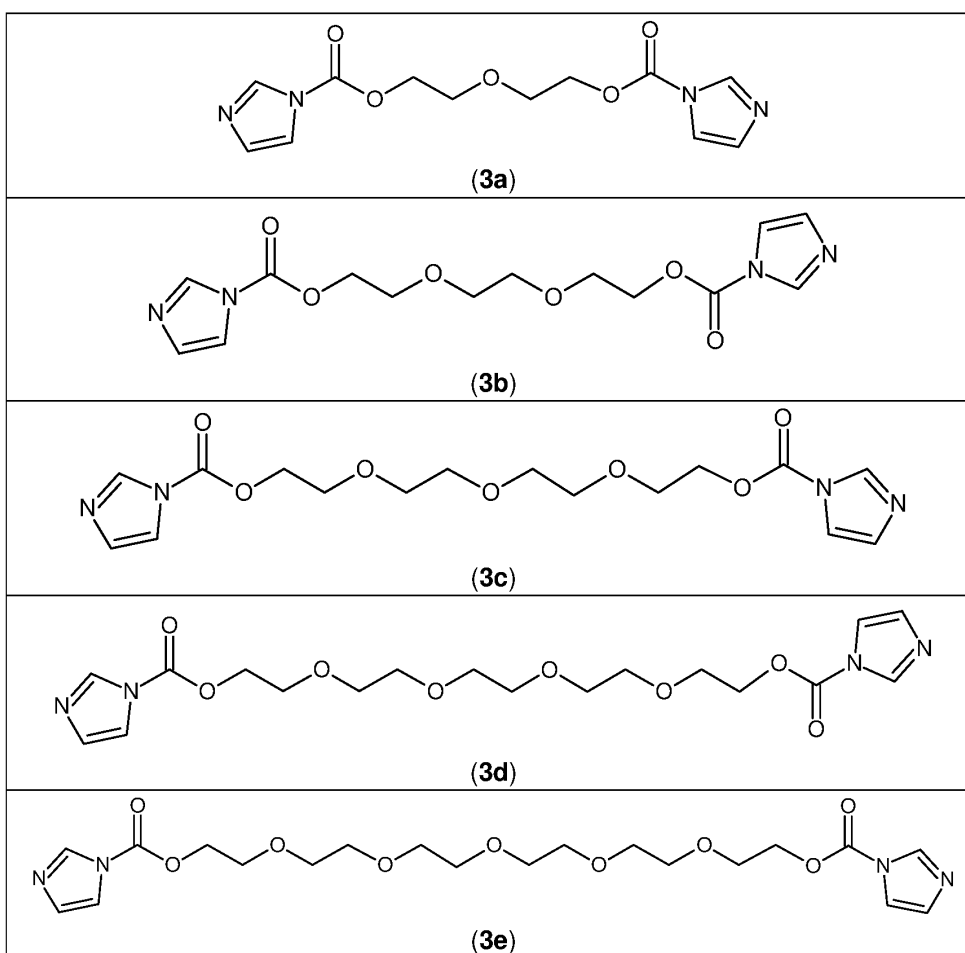
(2j)

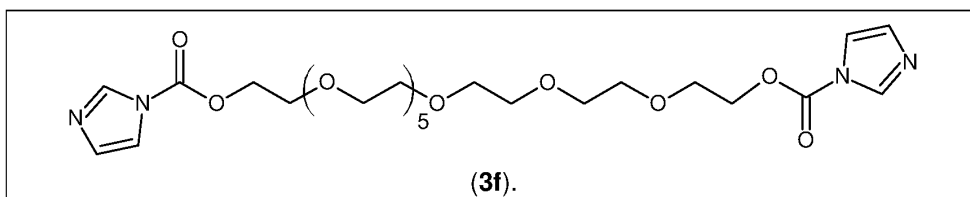


3. The method of claim 1, wherein the compound of formula (I) is a compound of formula (III)

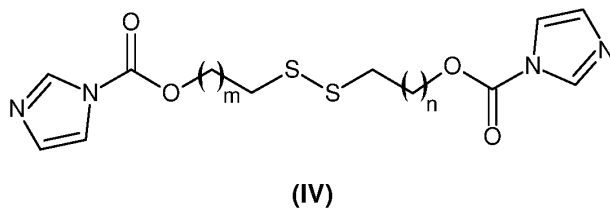


wherein n is 1 to 12; optionally, wherein the compound of formula (III) is selected from compounds (3a) – (3f)

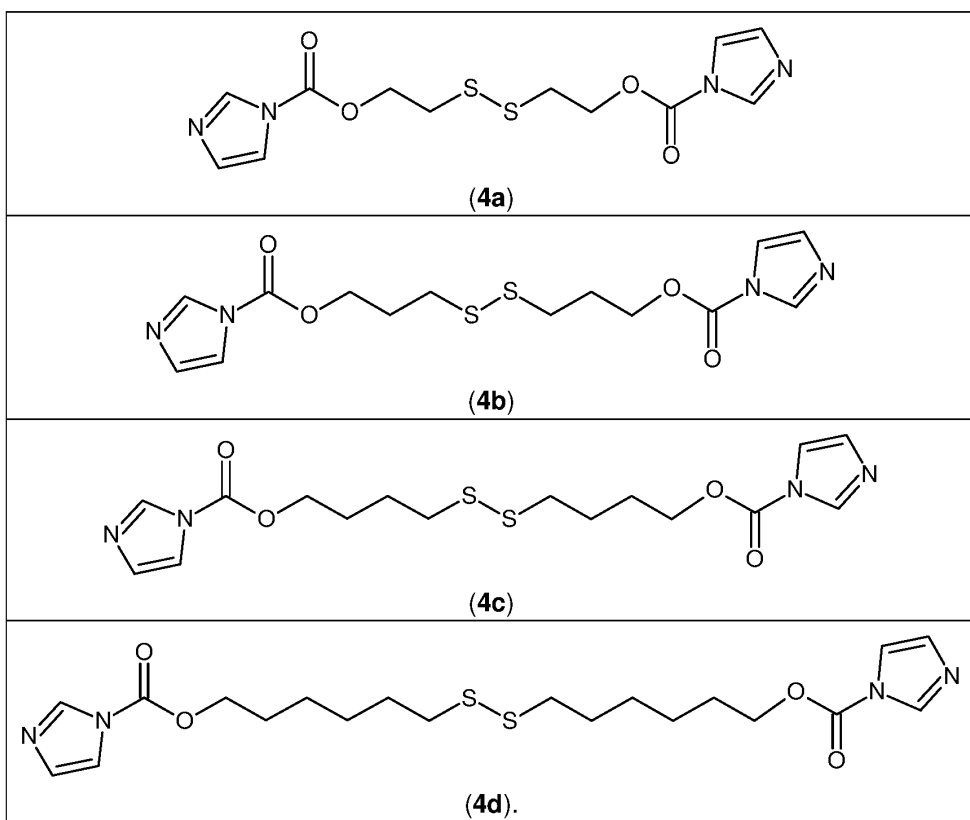




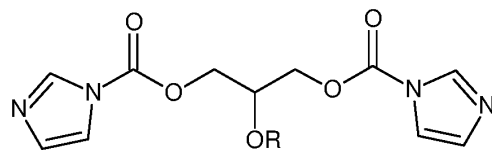
4. The method of claim 1, wherein the compound of formula (I) is a compound of formula **(IV)**



wherein m is 1 to 13, and n is 1 to 13; optionally, wherein the compound of formula **(IV)** is selected from compounds **(4a)** – **(4d)**

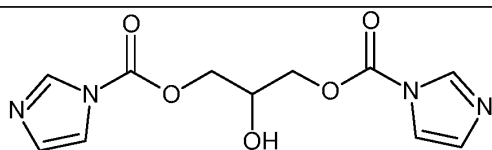


5. The method of claim 1, wherein the compound of formula (I) is compound (V)

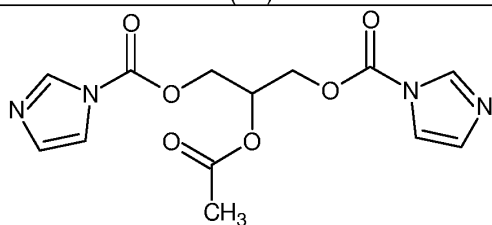


(V)

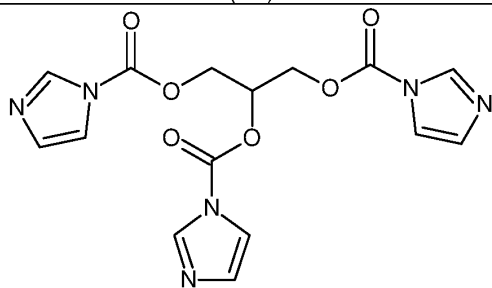
wherein R is selected from -H, -O(CO)-CH₃, and -O(CO)-imidazole; optionally, wherein the compound of formula (V) is selected from compounds (5a) – (5c)



(5a)

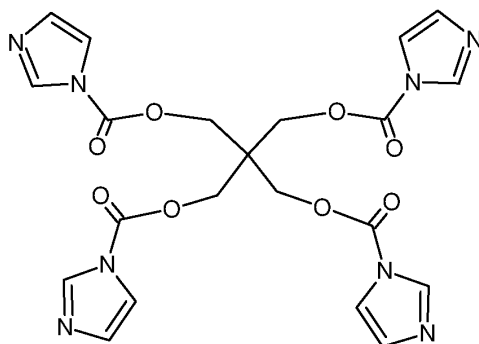


(5b)



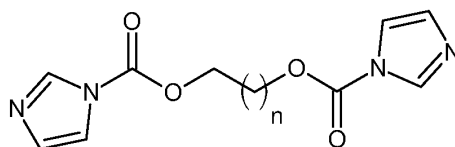
(5c)

6. The method of claim 1, wherein the compound of formula (I) is compound (6)



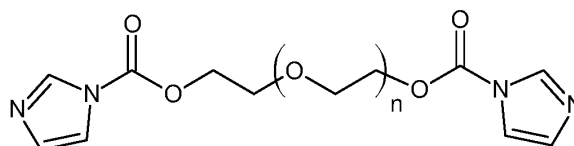
(6).

7. The method of any one of claims 1-6, wherein the first fixing reagent composition comprises:
- (a) the compound of formula (I) at a concentration of 50 mM or less, 25 mM or less, 15 mM or less, 10 mM or less, or 5 mM or less; and/or
 - (b) DMSO at a concentration of 5% or less, 2.5% or less, or 1.5% or less.
8. The method of any one of claims 1-7, wherein contacting with the first fixing reagent composition at room temperature (RT) for 3 h or less, 60 min or less, 30 min or less, 15 min or less, or 5 min or less.
9. The method of any one of claims 1-8, wherein the method further comprises contacting the sample with a second fixing reagent composition; optionally, wherein:
- i) the second fixing reagent composition comprises paraformaldehyde; optionally, wherein the paraformaldehyde concentration is 1% or less;
 - ii) the first fixing reagent composition comprises a compound of formula (II)



(II)

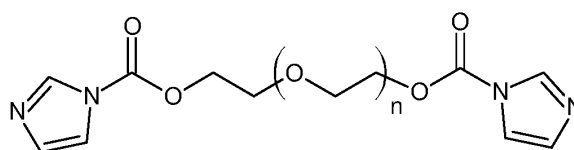
wherein n is 1 to 13; and the second fixing reagent composition comprises a compound of formula (III)



(III)

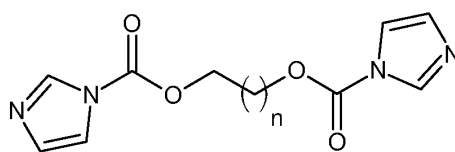
wherein n is 1 to 12;

- iii) the first fixing reagent composition comprises a compound of formula (III)



(III)

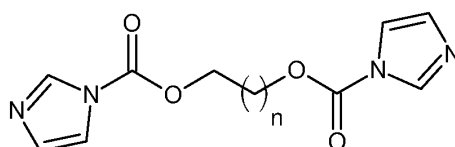
wherein n is 1 to 12; and the second fixing reagent composition comprises a compound of formula (II)



(II)

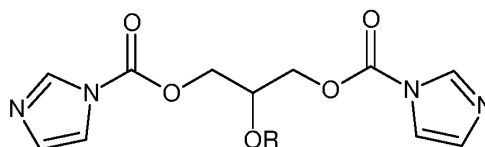
wherein n is 1 to 13; or

iv) the first fixing reagent composition comprises a compound of formula (II)



(II)

wherein n is 1 to 13; and the second fixing reagent composition comprises a compound of formula (V):

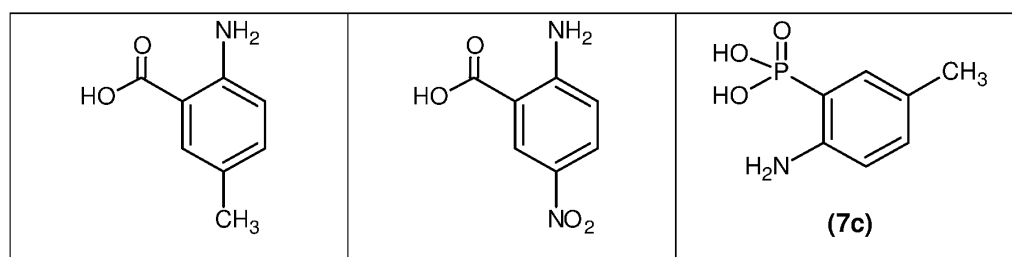


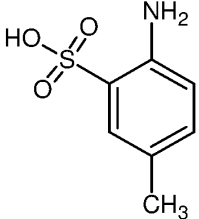
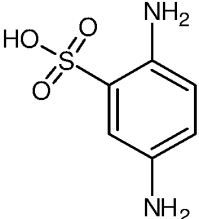
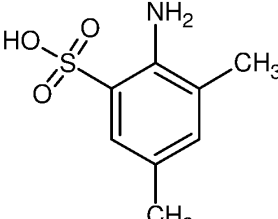
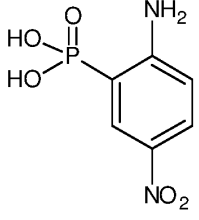
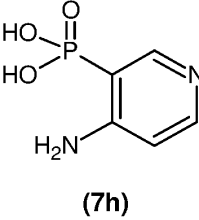
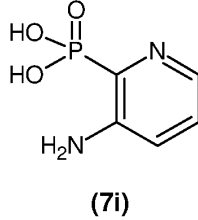
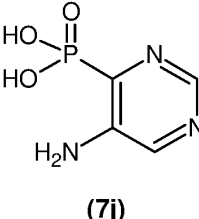
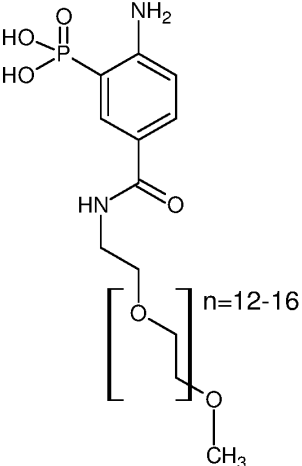
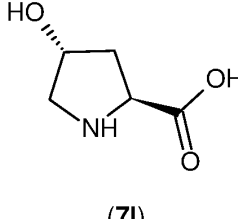
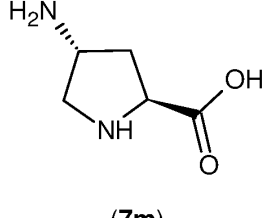
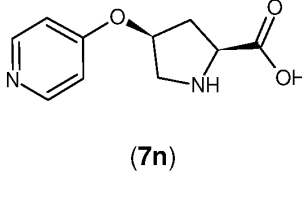
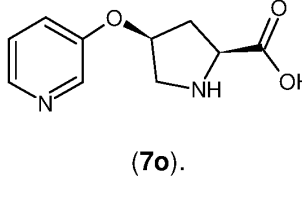
(V)

wherein R is selected from -H, -O(CO)-CH₃, and -O(CO)-imidazole.

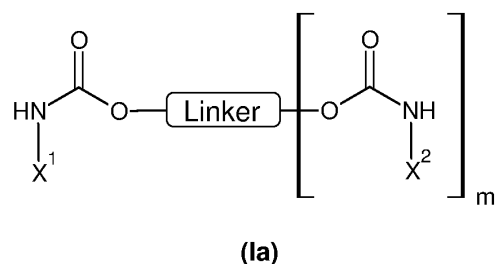
10. The method of any one of claims 1-9, wherein the method further comprises contacting the sample with a lysis and/or an unfixing agent; optionally, wherein the unfixing agent comprises:

- i) a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof;
- ii) a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT; and/or
- iii) a compound capable of reversing paraformaldehyde fixation; optionally, wherein the compound capable of reversing paraformaldehyde fixation is selected from any one of compounds (7a) – (7o)



(7a)	(7b)	
 (7d)	 (7e)	 (7f)
 (7g)	 (7h)	 (7i)
 (7j)	 (7k)	 (7l)
 (7m)	 (7n)	 (7o).

11. A composition comprising a fixed biological sample, wherein the sample comprises crosslinked biomolecules of formula (Ia)



wherein,

X¹ and X² are amine-bearing moieties of the same or different biomolecules of the sample; and

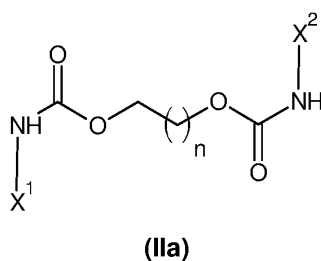
“Linker” comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

optionally, wherein:

- i) m = 1;
- ii) the “Linker” comprises a linear alkane moiety of 2-24 carbons;
- iii) the “Linker” comprises an ethylene glycol moiety;
- iv) the “Linker” comprises a disulfide bond; and/or
- v) the “Linker” comprises a branched alkane moiety.

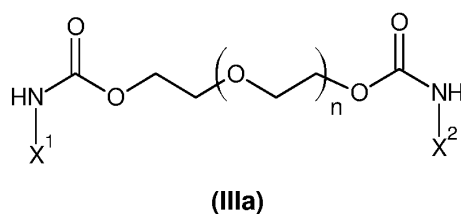
12. The composition of claim 11, wherein the sample comprises:

- (a) crosslinked biomolecules of formula (IIa)



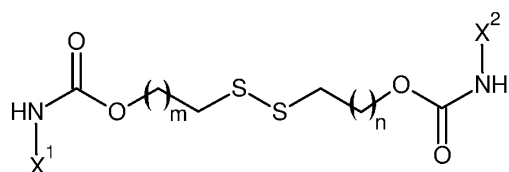
wherein, n is 1 to 13;

- (b) crosslinked biomolecules of formula (IIIa)



wherein, n is 1 to 12;

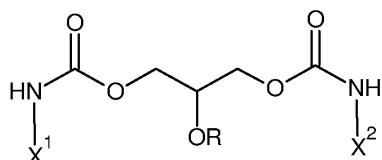
- (c) crosslinked biomolecules of formula (IVa)



(IVa)

wherein m is 1 to 13, and n is 1 to 13;

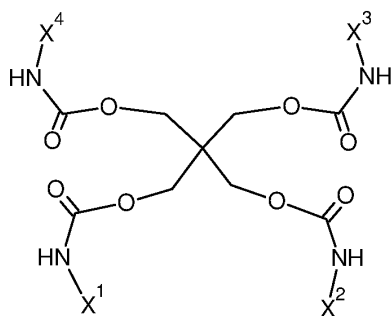
(d) crosslinked biomolecules of formula (Va)



(Va)

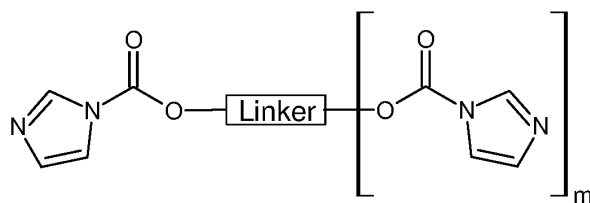
wherein R is selected from -H, -O(CO)-CH₃, and -O(CO)-imidazole; or

(e) crosslinked biomolecules of formula (Via)



(Via).

13. The composition of any one of claims 11-12, wherein the fixed biological sample is derived from a tissue sample, a biopsy sample, or a blood sample; optionally, wherein the fixed biological sample is a single cell;
14. The composition of any one of claims 11-13, wherein the fixed biological sample has been fixed with a fixing reagent composition comprising a compound of formula (I)



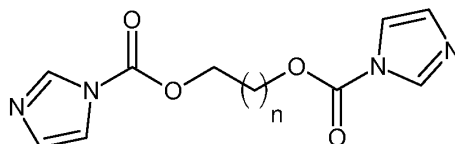
(I)

wherein,

“Linker” comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

optionally, wherein the compound of formula (I) is selected from:

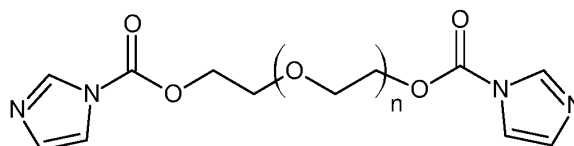
i) a compound of formula (II)



(II)

wherein n is 1 to 13; optionally, wherein the compound of formula (II) is selected from any one of compound (2a) – compound (2k);

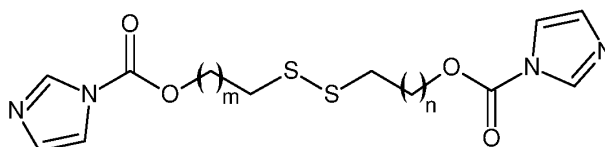
ii) a compound of formula (III)



(III)

wherein n is 1 to 12; optionally, wherein the compound of formula (III) is selected from any one of compound (3a) – compound (3f);

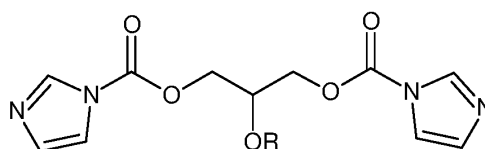
iii) a compound of formula (IV)



(IV)

wherein m is 1 to 13, and n is 1 to 13; optionally, wherein the compound of formula (IV) is selected from any one of compound (4a) – compound (4d); and/or

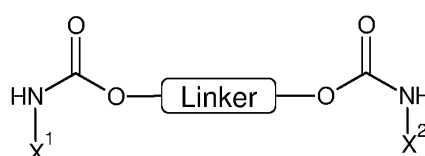
iv) a compound of formula (V)



(V)

wherein R is selected from -H, -O(CO)-CH₃, and -O(CO)-imidazole.

15. The composition of any one of claims 11-14, wherein the fixed biological sample is provided in a partition; optionally, wherein the partition is a discrete droplet.
16. The composition of claim 15, wherein the partition further comprises:
- (a) a lysis agent;
 - (b) an unfixing agent; optionally, wherein the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof;
 - (c) a bead; optionally, wherein the bead comprises an unfixing agent;
 - (d) assay reagents; optionally, wherein the assay reagents are contained in a bead; and/or
 - (e) a barcode optionally, wherein the barcode is contained in a bead.
17. An assay method comprising:
- (a) generating a partition comprising a fixed biological sample, and assay reagents, wherein the fixed sample comprises crosslinks of formula (Ia)



(Ia)

wherein,

X¹ and X² are amine-bearing moieties of the same or different biomolecules of the sample;

"Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

and

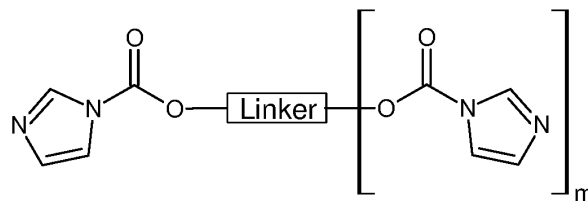
(b) detecting analytes from the reaction of the assay reagents and the unfixed biological sample.

18. The method of claim 17, wherein the partition further comprises a lysis and/or an unfixing agent.
19. The method of claim 18, wherein the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof.
20. The method of claim 18, wherein the partition further comprises a lysis agent in the absence of an unfixing agent.

21. A kit comprising:

assay reagents; and

a fixing reagent composition comprising a compound of formula (I)



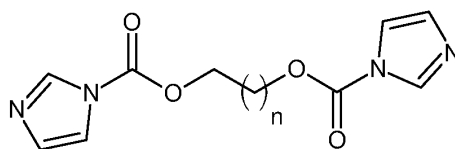
(I)

wherein,

“Linker” comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12.

22. The kit of claim 21, wherein the composition comprises a compound selected from:

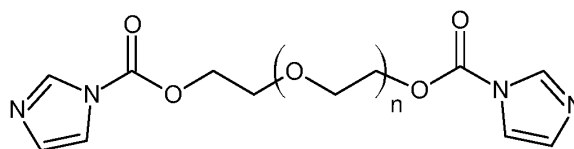
(a) a compound of formula (II)



(II)

wherein n is 1 to 13; optionally, wherein the compound of formula (II) is selected from any one of compound (2a) – compound (2k);

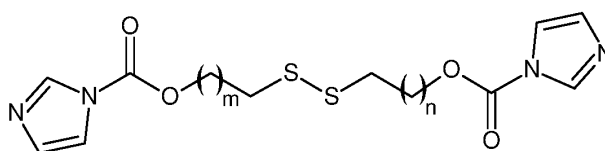
(b) a compound of formula (III)



(III)

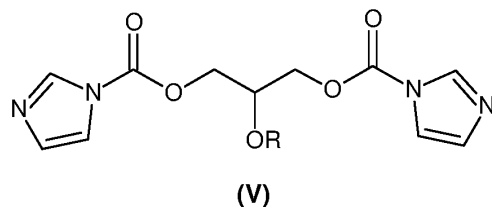
wherein n is 1 to 12; optionally, wherein the compound of formula (III) is selected from any one of compound (3a) – compound (3f);

(c) a compound of formula (IV)



(IV)

wherein m is 1 to 13, and n is 1 to 13; optionally, wherein the compound of formula (IV) is selected from any one of compound (4a) – compound (4d); and/or
(d) a compound of formula (V)



wherein R is selected from -H, -O(CO)-CH₃, and -O(CO)-imidazole.

23. The kit of claim 21, wherein the kit further comprises:

- (a) a lysis agent; and/or
- (b) an unfixing agent; optionally, wherein the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof.