[0278] CLAIMS

What is claimed:

1. A multiplex method for co-detecting a plurality of different types of biomarkers in a tissue sample on a single slide, said method comprising:

a) providing a tissue sample on a single slide;

b) contacting said tissue sample with a plurality of different antibodies to effect binding of the antibodies to the tissue sample, each of said antibodies reactive with a different biomarker, and each of said antibodies conjugated to a unique photocleavable mass-tag;

c) illuminating said photocleavable mass-tags with light so as to photocleave at least a portion of said mass-tags prior to step d); and

d) detecting, using mass spectrometric imaging, said mass-tags, or fragments thereof, as molecular ions.

2. The method of claim 1, further comprising performing, after step a) but before step b), direct mass spectrometric imaging on said tissue sample.

3. The method of claim 1, wherein said a plurality of antibodies are in a mixture and said tissue sample in step b) is contacted with said mixture.

4. The method of claim 1, wherein said mass-tags are non-rare-earth-metal mass-tags.

5. The method of claim 1, wherein said mass-tags comprise a plurality of amino acids.

6. The method of claim 1, wherein said tissue sample was fresh frozen and thin-sectioned prior to being mounted on said single slide.

7. The method of claim 1, wherein said slide comprises gold.

8. The method of claim 7, wherein said slide is a glass slide with a gold layer.

9. The method of claim 1, wherein said tissue sample was formalin-fixed and paraffin embedded and thin-sectioned prior to being mounted on said single slide.

10. The method of claim 9, wherein the tissue sample is subjected to a treatment prior to the steps of contacting the sample with antibody, said treatment comprising deparaffinization.

11. The method of claim 10, wherein said deparaffinization is performed with xylene.

12. The method of claim 10, wherein the tissue sample is further subjected to a treatment, said treatment comprising rehydration.

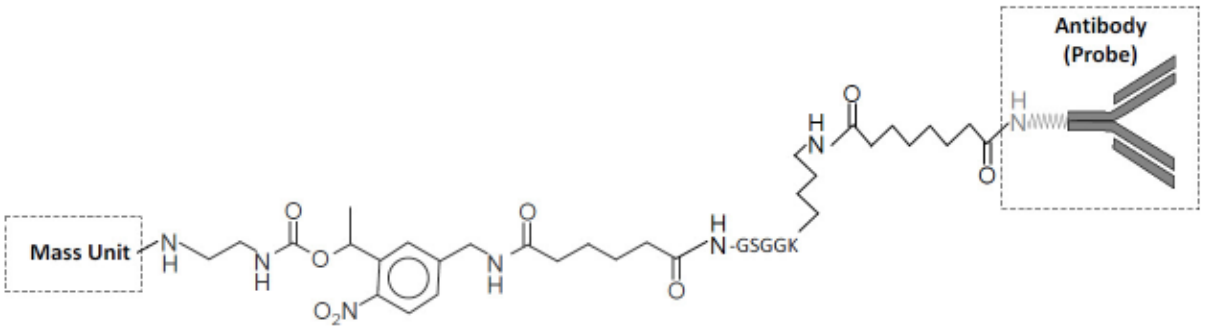
13. The method of claim 12, wherein said rehydration is performed with a series of ethanol/water mixtures and aqueous saline buffers.

14. The method of claim 12, wherein the tissue sample is further subjected to a treatment, said treatment comprising antigen retrieval.

15. The method of claim 14, wherein said antigen retrieval is performed by heating in citrate buffer, pH 6.

16. The method of claim 14, wherein said antigen retrieval is performed with the use of formic acid.

17. The method of claim 1, wherein said antibodies conjugated to a photocleavable mass-tag have the following general structure:



18. The method of claim 1, wherein a matrix compound is applied to said mass-tags before step d).

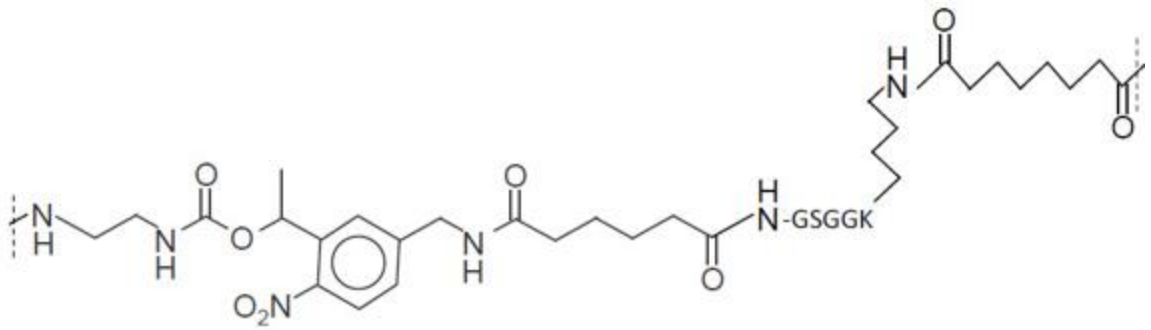
19. The method of claim 18, wherein said matrix compound is selected from the group consisting of alpha-cyano-4-hydroxycinnamic acid (CHCA), 2,5-Dihydroxybenzoic acid (DHB) and 3,5-Dimethoxy-4-hydroxycinnamic acid (sinapinic acid).

20. The method of claim 18, wherein said matrix compound is applied by sublimation.

21. The method of claim 18, wherein the tissue sample is subjected to a treatment after the steps of contacting the sample with a matrix compound, said treatment comprising matrix recrystallization.

22. The method of claim 1, wherein said photocleavable mass-tags conjugated to said antibodies comprise a core structure which is conjugated to a mass unit, said antibodies having the following general structure: Mass Unit – Core Structure – Antibody.

23. The method of claim 22, wherein said core structure has the structure:



24. The method of claim 1, wherein said tissue is from a tumor.

25. The method of claim 24, wherein said tumor is a breast tumor.

26. The method of claim 1, wherein at least one of said plurality of different antibodies comprises a fluorescent moiety in addition to said mass-tag.

27. The method of claim 1, wherein the number of said different antibodies is 8 or more.

28. The method of claim 27, wherein a subset of said different antibodies reacts with i) the estrogen receptor (ER), ii) the progesterone receptor (PR), iii) human epidermal growth factor receptor 2 (HER2), and iv) Ki67.

29. The method of claim 27, wherein a subset of said different antibodies reacts with T-cell biomarkers i) CD3 (T-cells), ii) CD4 (T-helper), iii) CD8 (cytotoxic T-cells), and iv) CD45RO (memory T-cells).