

Claims

1. A method of sequential signal-encoding of analytes in a sample, the method comprising the steps:
 - (1) providing a set of analyte-specific probes, each analyte-specific probe comprising:
 - a binding element (S) that specifically interacts with one of the analytes to be encoded, and
 - an identifier element (T) comprising a nucleotide sequence which is unique to said set of analyte-specific probes (unique identifier sequence);
 - (2) incubating the set of analyte-specific probes with the sample, thereby allowing a specific binding of the analyte-specific probes to the analyte to be encoded;
 - (3) removing non-bound probes from the sample;
 - (4) providing a set of decoding oligonucleotides, each decoding oligonucleotide comprising:
 - a first connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence, and
 - a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;
 - (5) incubating the set of decoding oligonucleotides with the sample, thereby allowing a specific hybridization of the decoding oligonucleotides to the unique identifier sequence;
 - (6) removing non-bound decoding oligonucleotides from the sample;

- (7) providing a set of signal oligonucleotides, each signal oligonucleotide comprising:
 - a second connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and
 - a signal element; and
 - (8) incubating the set of signal oligonucleotides with the sample, thereby allowing a specific hybridization of the signal oligonucleotides to the translator element (c).
2. The method of claim 1, wherein the sample is a biological sample, preferably comprising biological tissue, further preferably comprising biological cells.
3. The method of claim 2, wherein prior to step (2) the biological tissue and/or biological cells are fixed.
4. The method of any of claims 1-3, wherein within the set of analyte-specific probes the individual analyte-specific probes comprise binding elements (S1, S2, S3, S4, S5) which specifically interact with different sub-structures of one of the analytes to be encoded.
5. The method of any of claims 1-4, further comprising:
- (9) removing non-bound signal oligonucleotides from the sample; and
 - (10) detecting the signal, and, optionally,
- further comprising:

- (11) selectively removing the decoding oligonucleotides and signal oligonucleotides from the sample, thereby essentially maintaining the specific binding of the analyte-specific probes to the analyte to be encoded, and, optionally,

further comprising:

- (12) repeating steps (4)-(11) at least once [(4₂)-(11₂)] to generate an encoding scheme consisting of at least two signals.
6. The method of claim 5, wherein said encoding scheme is predetermined and allocated to the analyte to be encoded.
7. The method of claim 5 and 6, wherein decoding oligonucleotides are used in repeated steps (4₂)-(11₂) comprising a translator element (c2) which is identical with or differs from the translator element (c1) of the decoding oligonucleotides used in previous steps (4)-(11).
8. The method of claims 5-7, wherein signal oligonucleotides are used in repeated steps (4₂)-(11₂) comprising a signal element which is identical with or differs from the signal element of the decoding oligonucleotides used in previous steps (4)-(11).
9. The method of any of claims 1-8, wherein the binding element (S) comprise a nucleic acid comprising a nucleotide sequence allowing a specific binding to the analyte to be encoded, preferably a specific hybridization to the analyte to be encoded.
10. The method of any of claims 1-9, wherein the binding element (S) comprise an amino acid sequence allowing a specific binding to the analyte to be encoded, preferably the binding element is an antibody.

11. The method of any of claims 1-10, wherein the analyte to be encoded is a nucleic acid, preferably DNA or RNA, further preferably mRNA.
12. The method of any of claims 1-11, wherein the analyte to be encoded is a peptide or a protein.
13. Use of a set of decoding oligonucleotides to sequentially signal-encode analytes in a sample, each decoding oligonucleotide comprising:
 - a first connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a nucleotide sequence which is unique to a set of analyte-specific probes (unique identifier sequence), and
 - a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.
14. A kit for sequentially signal-encoding of analytes in a sample, comprising
 - a set of analyte-specific probes, each analyte-specific probe comprising:
 - a binding element (S) that specifically interacts with one of the analytes to be encoded, and
 - an identifier element (T) comprising a nucleotide sequence which is unique to said set of analyte-specific probes (unique identifier sequence); and
 - a set of decoding oligonucleotides, each decoding oligonucleotide comprising:
 - a first connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence, and
 - a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

15. The kit of claim 14, further comprising:

- a set of signal oligonucleotides, each signal oligonucleotide comprising:
 - a second connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and
 - a signal element.