CLAIMS

What is claimed is:

1. A method of characterizing cells in a biological sample *in situ*, comprising:
   a. contacting the biological sample with a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein the plurality of pre-determined genes comprises at least one gene and at least one other gene that show coordinated changes in their expression levels, and

      wherein each probe comprises:

         i) an identical detectable label, and

         ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes;

      wherein a signal is emitted when the probe binds to the ribonucleic acid transcript;
   b. detecting a combination or plurality of emitted signals from the plurality of probes; and
   c. characterizing the cells based on the combination or plurality of emitted signals.

2. The method of claim 1, wherein steps a and b are repeated one or more times using a plurality of probes that bind to RNA transcripts of a plurality of different pre-determined genes.

3. The method according to claim 1 or 2, further comprising a step of quantifying the level of the emitted signal detected in step b, processing the signal, or both, prior to characterizing the cell.

4. The method according to claim 1, wherein the at least one gene and at least one other gene that show coordinated changes in their expression levels both are:
   a) markers of a specific cell type;
   b) differentially expressed genes of a specific cell type;
   c) markers of a gene expression program or gene regulatory module;
   d) markers of a biological pathway;

   or combinations thereof; wherein the at least one other gene is selected from one or more input datasets.

5. The method according to claim 4, wherein the input dataset is a bulk RNA sequencing or single-cell RNA sequencing or microarray dataset or chromatin accessibility sequencing or methylation sequencing or DNA-associated proteins sequencing or spatial transcriptomics sequencing or

multiplexed RNA fluorescence *in situ* hybridisation or multiplexed immunohistochemistry or bioinformatics database or any user-defined dataset or combinations thereof.

6. The method according to any one of claims 1 to 5, wherein selection of the plurality of predetermined genes is an unsupervised selection, a supervised selection, or a combination thereof.

7. The method according to any one of claims 4 to 6, wherein the coordinated changes in their expression levels of the at least one gene and at least one other gene is determined by correlation analysis or clustering analysis or dimensionality reduction analysis or differential expression gene analysis or combinations thereof of the input dataset.

8. The method according to any one of claims 4 to 7, wherein the genes showing coordinated changes in their expression levels are further analyzed using signal gain (SG) or signal specificity ratio (SSR) to identify the plurality of pre-determined genes.

9. The method according to any one of claims 1 to 8, wherein the domain of the probe is a ribonucleic acid (RNA) oligonucleotide that binds specifically to RNA.

10. The method according to any one of claims 1 to 9, wherein the biological sample comprises a homogenous or heterogenous population of cells.

11. The method according to any one of claims 1 to 10, wherein characterisation of the cell includes one or more of mapping the location of the cell in the biological sample, identifying an interaction between the cell and one or more other cells, identifying gene expression patterns of the cell or biological sample and visualizing the spatial transcriptome of the cell or biological sample, stratifying cancer subtypes, determine severity of cancer.

12. The method according to any one of claims 4 to 11, further comprising pre-processing of the input dataset prior to performing correlation analysis or clustering analysis or dimensionality reduction analysis or differential expression gene analysis.

13. The method according to any one of claims 1 to 12, wherein the plurality of pre-determined genes are expressed in cells associated with cancer.

14. A method to determine the prognosis of a subject suffering from cancer, comprising:
    a. obtaining a sample of the subject;
    b. characterizing one or more cancer cells in the sample using the method of any one of claims 1 to 13 to determine the stage of the cancer; and
    c. determining the prognosis based on the stage of the cancer.

15. A kit for characterising cells in a biological sample *in situ* comprising:
    a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes; wherein the plurality of pre-determined genes comprises at least one gene and at least one other gene that show coordinated changes in their expression levels, and wherein each probe comprises
        i) an identical detectable label, and
        ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes, and instructions for use.

16. The kit according to claim 15, wherein the at least one gene and at least one other gene that show coordinated changes in their expression levels both are:
    e) markers of a specific cell type;
    f) differentially expressed genes of a specific cell type;
    g) markers of a gene expression program or gene regulatory module;
    h) markers of a biological pathway;
    or combinations thereof; wherein the at least one other gene is selected from one or more input datasets.

17. The kit according to claim 15 or 16, wherein the plurality of pre-determined genes are expressed in kidney, brain, cancer or combinations thereof.

18. A kit for characterizing a colorectal cancer in a biological sample *in situ* comprising:
    a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein the plurality of pre-determined genes is selected from the genes listed in Table 6 (6a) – (6d);
    wherein each probe comprises:
        i) an identical detectable label, and
        ii) a domain that binds specifically to a ribonucleic acid transcript of the plurality of pre-determined genes, and instructions for use.