21;7(17):eabg4755) measure genome-wide single-cell expression but at low spatial resolution (500 µm resolution) with less mRNA reading and less detected genes than standard scRNA-seq. Fluorescence *in situ* sequencing (FISSEQ) uses *in situ* sequencing to spatially localise the expression of multiple genes in fixed tissues, with a short reading (30 bases) and with only about 200 mRNA readings per cell (compared to about 40,000 in scRNA-seq). Digital Spatial Profiler (DSP) is a platform developed by Nanostring based on the sequencing of photocleavable oligonucleotide markers released from a targeted tissue area by UV exposure. Data on the localisation of cells in the tissue provide a numerical and spatial profile of RNA or target abundance. However, this technique does not provide access to the complete transcriptome, does not have unicellular resolution, and allows analysis of only a small number of areas.

In summary, the current technological tools to study cellular interactions in tissues, interactions at the single-cell level, are still limited because they only allow spatial localisation of signals from a limited number of molecular targets for a large number of cells, or measurement of a large number of molecular targets for a limited number of localised (microdissected) cells, or measurement of a large number of molecular targets on thousands of non-localised cells.

Currently, the coupling, for thousands of individual cells, of (i) intra-tissue spatial localisation information of each cell to (ii) the measurement of molecular signals from each of these same cells is clearly identified as a major technological challenge and as a clinical need in order to better understand, for example, the adaptation mechanisms occurring in tumours and to adjust patient treatment accordingly.

The methods and kits provided by the invention fulfil this need for a system allowing single-cell "omics" analysis of thousands of cells with a spatial resolution of a few dozen cells, or less, from a tissue.

SUMMARY

The invention relates to a method for labelling individual cells or organelles within a biological sample with an identification nucleic acid sequence, the method comprising:

a) providing a first set of nucleic acids (“Emitter nucleic acids”) wherein each nucleic acid molecule comprises an amplification sequence, an identification sequence, and a capture sequence;

b) providing a second set of nucleic acids (“Receptor nucleic acids”), wherein each Receptor nucleic acid is coupled, covalently or non-covalently, to a ligand of a cell target or organelle target, wherein the Receptor nucleic acid comprises i) an amplification sequence matching all or part of the amplification sequence of the set of Emitter nucleic acids, or a

sequence thereof, contained in the nucleic acids produced at step e), thereby combining mapping and sequencing information of the individual cells of the biological sample;

h) Mapping of the position of the single cell originally on the biological sample by determining the relative proportions of identical identification sequence(s), or reverse complementary sequence thereof, contained in the nucleic acids produced at step e) to estimate the distance between cells, on the principle of triangulation; and

i) Optionally, mapping the sequencing information back onto an image from microscopy of the biological sample, taken before dissociation.

In a third aspect, the invention relates to a kit comprising:

a) Emitter nucleic acids wherein each emitter nucleic acid comprises an amplification sequence, an identification sequence, and a capture sequence;

b) Receptor nucleic acids wherein each receptor nucleic acid comprises i) an amplification sequence matching all or part of the amplification sequence of the set of Emitter nucleic acids, or a complement thereof, or ii) a capture sequence matching all or part of the capture sequence of the set of Emitter nucleic acids, or a complement thereof;

c) Ligands of a cell target or organelle target;

d) Optionally a nicking endonuclease and a polymerase with strand displacement activity.

In another aspect, provided herein is a method of mapping and sequencing individual cells or organelles of a biological sample, the method comprising:

a) Providing individualized cells labelled with (i) a nucleic acid that comprises an amplification sequence, an identification sequence, and a capture sequence, or (ii) the reverse complement thereof (i), as obtainable by the method as described herein;

b) Trapping the individualized cells or organelles labelled with said nucleic acid or the reverse complement thereof in a compartment, wherein the compartment comprises a compartment-specific nucleic acid and at least one of the following sequences for nucleic acid labeling and further sequencing: hybridization site, ligation site or recombination site;

c) Optionally analysing captured cells, organelles and/or molecules they secrete, using optical detection;

d) Lysing trapped cells, or cells and organelles, thereby releasing nucleic acids from the cells or organelles in the compartments;

e) Associating i) the compartment-specific sequence with ii) the nucleic acids released from the cells or organelles in the compartments and iii) the nucleic acids comprising the

amplification sequence, the identification sequence, and the capture sequence, or the reverse complement thereof;

f) Recovering the nucleic acids produced at step e) from the compartments and sequencing recovered nucleic acids; and

g) Defining nucleic acids comprising the same compartment-specific sequence as originating from the same single cell, and mapping the position of the single cell originally on the biological sample based on the identification sequence(s), or reverse complementary sequence thereof, contained in the nucleic acids produced at step e), thereby combining mapping and sequencing information of the individual cells of the biological sample;

h) Mapping of the position of the single cell originally on the biological sample is performed by determining the relative proportions of identical identification sequence(s), or reverse complementary sequence thereof, contained the nucleic acids produced at step e) to estimate the distance between cells, on the principle of triangulation; and

i) Optionally, mapping the sequencing information back onto an image from microscopy of the biological sample, taken before dissociation.

DETAILED DESCRIPTION

The present invention concerns a solution to assign identification nucleic acids by diffusion to areas of a biological sample, such as a tissue section, comprising a plurality of cells or organelles through cells (optionally permeabilized), e.g. about two to ten cells. As cells carrying the same identification nucleic acid are in the same area, it allows the reconstruction of the network of neighbouring cells after dissociation of the biological sample, and single-cell analysis of the cellular nucleic acids, and therefore reconstruct the spatial organisation of cells prior to dissociation. Different proportions of specific identification nucleic acids allow to estimate the original distance between cells, based on the principle of triangulation. The present invention relies on local isothermal amplification or pre-amplification of identification nucleic acid sequences followed by their diffusion. The identification nucleic acid sequences can be associated to the surrounding cells via a ligand modified to conjugate with the produced nucleic acids.

The invention thus makes it possible for cells of a biological sample to be dissociated, analysed and sequenced individually (single cell) using current techniques (droplet