

## Detailed description for GeoMx Digital Spatial Profiling (DSP) Workflow

The figure is a flowchart showing the five phases of the GeoMx Digital Spatial Profiling (DSP) workflow. Each phase has 1-2 icons with two gray parallel lines (one labeled protein and one labeled RNA) going through phases 1-4 and becoming a single line between phases 4 and 5.

Below, the workflow is described as lists in which each subsequent step is listed beneath each phase.

### Phase 1: Stain

- a. Protein line: Gray circle denoting detection of protein using DSP barcoded antibodies and one fluorescent antibody.
- b. RNA Line: Gray circle denoting detection of RNA using two DSP barcoded RNA probes and one RNAscope probe or fluorescent antibody.

### Phase 2: Select Regions of Interest (ROI)

- a. Both lines: Icon of DSP instrument and computer with input of two gray lines from each of the protein and RNA circles.
- b. Both lines: Close up of computer screen icon with ROI circled.

### Phase 3: UV-Cleave and Collect

- a. Protein line: Circle on the gray protein line with two DSP barcoded antibodies and one fluorescent antibodies being aspirated by a micropipette.
- b. RNA Line: Circle on the gray RNA line with two DSP barcoded RNA probes and one RNAscope probe or fluorescent antibody being aspirated by a micropipette.

### Phase 4: Dispense

- a. RNA and Protein lines are now merged. Circle with two DSP barcodes being dispensed into a collection tube.

### Phase 5. Barcode Count

- a. Icons of an nCounter and of an Illumina sequencer used to count the collected barcodes.