



Frequently Asked Questions

Data Processing and Decoding

FAQ Data Processing and Decoding

At CARTANA, we have an ISS Starter Program. Every new user of the technology is enrolled in this program to get started and successful with the technology. This program provides individual technical support based on your specific tissue samples and experimental design.

If you have any question, please contact support@cartana.se

What is the sensitivity of CARTANA ISS?

We estimate that CARTANA ISS can detect about 10% of all expressed transcripts in cells. Therefore, if your transcript is expressed at 100 copies/cell, we expect to detect about 10 signal dot/positive cell.

Is CARTANA ISS a quantitative method?

ISS technology is quantitative as one RNA transcript is detected as one ISS spot. Therefore, it is possible to quantify gene expression levels and compare different experimental conditions. However, absolute quantification is always challenging, due to some bias in detection. For example, factors like RNA structure and sequence GC content do affect it, but changes in expression levels can be observed and measured using CARTANA ISS. Furthermore, we can observe various expression levels between different genes, and we estimate that a 5-fold (or higher) variation in expression levels can be quantified.

With regards to sensitivity, what are CARTANA's typical statistics for biologically interesting genes in terms of dots/cell and how are error bars generated based on those detection counts?

The dots/cell vary depending on the gene of interest and typically, the error bars describe cell-to-cell variability. Depending on the experimental setup, variation between biological replicates can also be considered. The major advantage of the high-throughput feature of ISS is, that it results in a higher statistical power since it can assay many cells at once.

Have ISS and scRNAseq been tested on the same sample?

Yes, ISS and scRNAseq have been performed on the same sample. Please refer to this publication for additional information:

Grundberg, *et al.* *In situ mutation detection and visualization of intratumor heterogeneity for cancer research and diagnostics.* *Oncotarget* (2013). DOI:[10.18632/oncotarget.1527](https://doi.org/10.18632/oncotarget.1527)

What is the dynamic range of transcript detection? For example, how few or how many copies can be detected on the same section?

The number of ISS spots that can be resolved depends directly on the microscope hardware settings. In-house we use a 20x objective with 0.75 numerical aperture. ISS spots are between 0.5-1 μm of size, so the question comes down to how many ISS spots can be resolved with optical settings. An average number of a cell of 20 μm of diameter is in the 10^3 orders of magnitude. When using 20x magnification, we estimate that we can detect 100-200 dots/cell. This number increases to 500-1000 dots/cell when using 40x magnification.

We also have customised solutions in the sequencing pipeline that increase the resolution and that we can discuss with you on case-to-case basis. We also highly recommend informing us when you know that some of your targeted genes have very high expression levels. This allows us to anticipate potential imaging and analysis challenges and use customised solutions. Please contact us at support@cartana.se.

How consistent are the transcript levels from different sections of the same sample?

CARTANA ISS is a reproducible assay. To improve consistency and reproducibility between samples, we highly recommend preparing and processing sections as one batch. Reproducibility is good between CARTANA ISS experiments, but in order to merge data, some batch corrections might still be required.

Does a dot on a tissue represent a single RNA molecule? How do you distinguish signal from noise?

Yes, one signal dot represents one RNA molecule as it is one ISS spot from the RCA reaction. The process of matching colour combinations in a specific order sufficiently removes noise arising from fluorescence imaging like NGS and reads alignment. If imaging rounds are sufficiently long and optimally set-up, it helps to distinguish true signals from noise.

How do you perform segmentation on CARTANA ISS results images?

Traditionally, segmentation has been done based on nuclear counterstain and/or membrane stain. This provides moderately accurate results. For cell typing analysis where the knowledge about cell types exists, the prior information can be used in junction to more accurately define cell boundaries: <http://insitu.cortexlab.net/>

Reference:

Qian, X. *et al.* Probabilistic cell typing enables fine mapping of closely related cell types *in situ*. *Nature Methods* (2020).

Recently, there have been some segmentation-free methods that purely use the relative spatial information of transcripts, with or without cell type information.

References:

Partel, G. & Wählby, C. Spage2vec: Unsupervised detection of spatial gene expression constellations. *bioRxiv* (2020)

Park, J. *et al.* Segmentation-free inference of cell types from *in situ* transcriptomics data. *bioRxiv* (2019)

**For quantity of transcripts/cell, do you only use number of puncta or intensity as well?
For instance, how quantitative is the detection of a 2-fold or 3-fold increase in transcripts?**

For quantity of transcripts/cell we use the number of puncta. CARTANA ISS technology is quantitative as one RNA transcript is detected as one ISS spot. Therefore, it is possible to quantify gene expression levels and compare different experimental conditions.

Brain cells have different shapes and sizes depending on the brain region. How confident are you in assigning genes to individual cells, particularly in dense regions?

This is indeed very hard to achieve. Not only because of the size, shape and density of cells, but also because when assaying a section, we are looking at a plane of a 3D structure. Consequently, we aim for a compromise between efficiency and accuracy. Moreover, single-cell sequencing, also has high noise and suffers from problems like doublets and incomplete RNA content. Biological discovery can be achieved by the statistical power that results from profiling millions of cells, rather than few individual cells with 'perfect' profiles.

What are the specific imaging applications that can be used for the analysis and mapping steps?

For in-house analysis and mapping we use MATLAB, but CARTANA is currently developing a user-friendly platform for data exploration.

Is there another analysis software other than MATLAB that can be used to analyse the results?

We are currently working on the development of a user-friendly software for CARTANA ISS results analysis.

Among the open source packages, are you going to work with CZI starfish?

The CZI starfish platform can be used to analyse CARTANA ISS data analysis. You can find the pipeline and example dataset here:

<https://github.com/spacetx/starfish/blob/master/README.rst>

However, CARTANA is actively integrating starfish into its pipeline. We are currently translating the MATLAB script into Python and also working on the development of a user-friendly platform for data analysis.

For tissues that are very dense, like tumor samples, how reliable is the segmentation to get single-cell resolution?

Segmentation on tumours is difficult to perform since cells are growing in a disordered way and different cell types can be intermixed. One must check the performance of the segmentation to evaluate how well it worked. Many segmentation algorithms exist, and one needs to test which one works best on the tumour sample of interest.