Slides preparation guidelines for 10X Genomics Visium polyA-based non-HD (non-Cytassist)

Contents

Submission to GECF

A **submission form**, found on our website, needs to be filled and sent to GECF before the experiment.

Tissue sectioning, fixation, staining, and imaging are typically performed at the EPFL Histology core facility, which you should contact in advance. If you are planning to perform any of these steps yourself, contact us in advance since these guidelines assume you do that with HCF.

The interactions between you/HCF and the GECF will depend very much on which workflow is used. We will give you more details once details of the experiment are discussed.

General information

Please refer to the file named "General information about different Visium methods" on our website, for an overview of the different Visium methods and for help choosing the most appropriate one for your experiment.

Visium polyA non-HD workflow

Visium non-Cytassist is a **polyA-based** method, which can be performed on **all species, but** only on **Fresh Frozen** tissues.

GENERAL INFORMATION, VISIUM CYTASSIST SLIDE AND CAPTURE AREAS

- Visium non-Cytassist is performed on slides with **4 capture areas**. All capture areas must be used.
- Each capture area is **6.5 x 6.5 mm** and contains ca 5'000 spots.
- **Permeabilization optimization is mandatory** prior to the experiment (see Visium Optimization Slide paragraph below).
- You can run 2 tissues with **different permeabilization times on the same gene expression slide**.

- This method was demonstrated with **several tissue types**. Consult compatibility of tissues in 10X Genomics Support website https://support.10xgenomics.com/spatial-gene-expression/tissue-optimization/doc/specifications-visium-spatial-gene-expression-optimized-tissues
- Good quality of starting tissue is critical for optimal results.

VISIUM PERMEABILIZATION TIME OPTIMIZATION SLIDE

- The cost of this optimization is much lower than a true Visium experiment (refer to our price list).
- The optimization must be done in the **exact experimental conditions** that will be used later for the real experiment: exact tissue type, development stage, dissection, freezing, storage method and duration, sectioning, fixation and staining. On user side, preparing the samples for the optimization or the real experiment is identical.
- In case of doubt when assessing optimization results, it is better to opt for the slightly longer time.
- **Thickness of tissues section could also be optimized** if initial results are unsatisfactory.

EMBEDDING AND STORAGE

They must be performed according to the latest version of the 10XG Tissue Preparation Guide (protocol CG000240).In case these guidelines were not followed we would not be able to guarantee the results. Please inform us in advance and we can discuss how to proceed.

ASSESSMENT OF RNA QUALITY

Tissue blocks with **RIN ≥7** are recommended.

- It is **optional** but if the experiment gives poor results and RNA quality hadn't been checked beforehand, the assessment will be done afterwards and, if quality is not sufficient, the GECF will not be held responsible.
- It can be done by GECF if provided with the necessary amount of tissue.

SECTION THICKNESS

- **Between 10-20 um**. Recommended for most tissue types is 10 µm (sections outside these specifications may result in reduced performance).
- **To determine optimal tissue thickness** check whether your tissue of interest is mentioned in this list: https://support.10xgenomics.com/spatial-gene-expression/tissue-optimization/doc/specifications-visium-spatial-gene-expression-optimized-tissues.

If not, 10XG say: "Section thickness for the Visium slides will depend upon tissue type. Customers should try to achieve a good quality section at the minimum section thickness for their tissue type. 5 - 35 µm sections have been tested in-house; however, 10 µm is used for most tissue types. Fatty tissues generally require thicker sections."

SLIDE PREPARATION

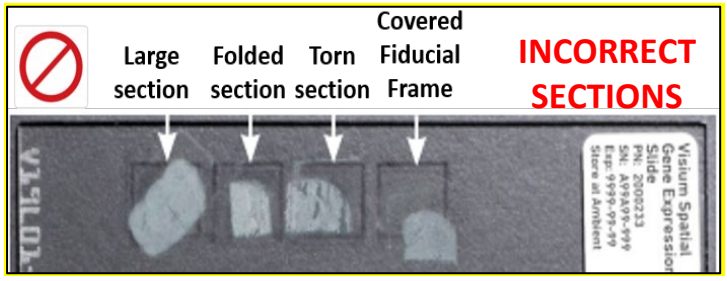
Tissue sectioning, fixation, staining and imaging are typically performed at the **EPFL Histology core facility**. If you are planning to perform any of these steps yourself (not recommended), contact us in advance and we will give you further details.

- Briefly, the tissues must be prepared, sectioned and positioned directly on Visium slides according to the latest version of the 10XG Tissue Preparation Guide (Visium non-Cytassist: **protocol CG000240**).
- The fixation and imaging must be done according to either one of these protocols:

  - Methanol Fixation, H&E Staining Protocol (CG000160)
  - Methanol Fixation, Immunofluorescence Staining Protocol (CG000312)

CAUTION: obtaining good data requires that the tissue is properly sectioned, without cracks, or other freezing/conservation/cutting/staining artefact. This is likely the most important and often most tricky step of the whole 10XG Visium procedure. Therefore, we strongly recommend that you test and optimize the histology procedure beforehand on similar but dummy samples and slides.

- If **sections are incorrectly placed** on Visium slide or quality is poor (folds, breaks, see image below), it is possible to do a **slide reset** prior to tissue permeabilization (see guide CG000332).



DIVERSE NOTES FOR VISIUM NON_CYTASSIST

- Some **tips for samples preparation** for Visium non-Cytassist can be found here: https://kb.10xgenomics.com/hc/en-us/sections/360007223212-Sample-Preparation
- If you want to **monitor a GFP expression**, here are comments by 10XG: "Based upon results from limited in-house testing, fluorescent GFP reporters show compatibility with the Visium assay, but we have not yet fully validated this technique.
  - we recommend performing the fluorescent imaging immediately after the isopropanol drying step and before the H&E staining to avoid background autofluorescence from the H&E stain. Keep the imaging time to a minimum.
  - An alternative solution is to perform the fluorescent imaging on a subsequent section on a plain glass slide and overlay this with the H&E image generated from the Visium slide. Unfortunately, with this method you will only get a general GFP+ cell distribution, as opposed to a direct 1:1 relationship."

Sequencing and analysis

- **Sequencing depth**: **Visium non-Cytassist**: a minimum of **50'000 reads per tissue covered spot** are required. More reads will give better sensitivity.
  So, for example 200 mio reads for a sample covering around 80% of the Capture Area of a 6.5 mm slide (0.8 x 5'000 x 50'000).

- SpaceRanger results of single samples coming from different biological conditions of the same sample or from consecutive sections of the same tissue block can be aggregated into a single feature-barcode matrix by running SpaceRanger aggr.
- SpaceRanger v2.1 has introduced a reference-free spot deconvolution function. This allows the deconvolution for cell-typing, as described here: https://www.10xgenomics.com/support/software/space-ranger/algorithms-overview/gene-expression#lda-based-spot-deconvolution-7e5466 . The results can be explored through the Spot Deconvolution feature of Loupe Browser. 10XG provide a tutorial for this method: https://www.10xgenomics.com/analysis-guides/exploring-your-visium-data-a-spot-deconvolution-story

Results

Once the experiment is finished, user will get a folder of the sequencing data and a folder of the Spaceranger analysis.

For the Spaceranger results, we recommend to inspect in particular the **websummary.html** file and the **cloupe.cloupe** file (for the latter, Loupe Browser software is needed). These files are located in the "out" folder inside each sample's results

Visium integration with single cell data

Visium data can be combined with single cell data in order to get additional information on the sample (https://www.10xgenomics.com/analysis-guides/integrating-single-cell-and-visium-spatial-gene-expression-data).

Starting from the same blocks used for the Visium experiment it is possible to isolate nuclei/cells for Flex single cell gene expression (protocol for Fresh frozen OCT embedded samples can be found here).

Versions log

- vA.01 (22.04.2025): 1st version.