# Samples preparation guidelines for 10X Genomics Visium

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## Submission to GECF

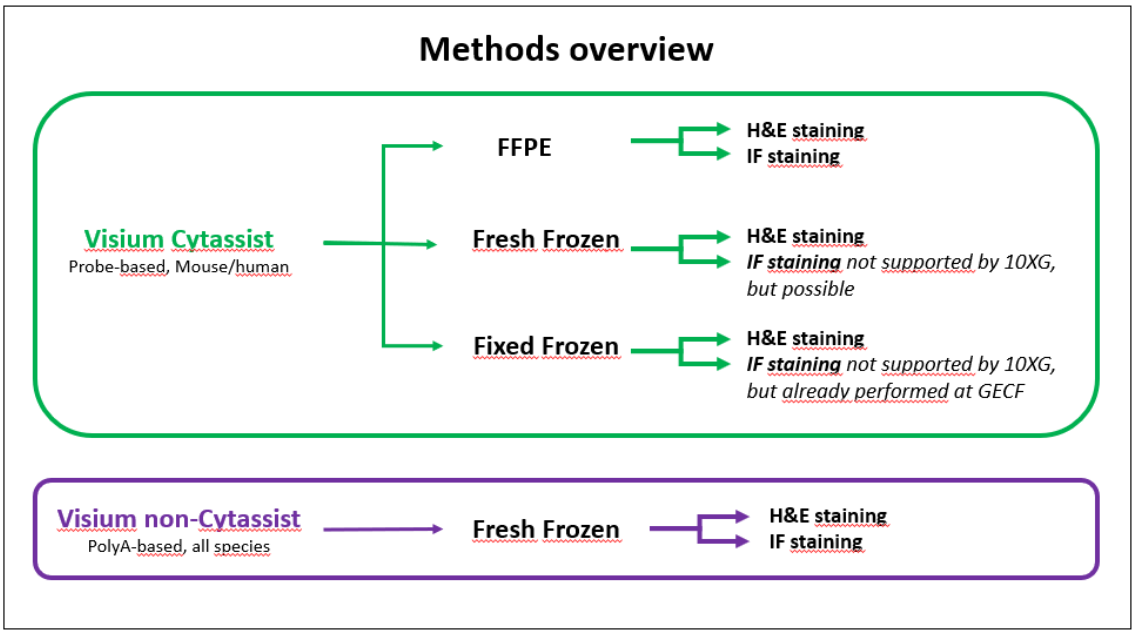
A **submission form** needs to be filled and sent to GECF before the experiment.

**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 – Cytassist or non-Cytassist?

Both methods ultimately employ a **Visium slide** containing **Capture Areas** (one area per tissue section) composed of **spots of spatially barcoded oligonucleotides** that capture either gene expression probes (Cytassist) or polyA mRNAs (non-Cytassist). Each spot is 55 µm in diameter, with a 100 µm centre to centre distance between spots. The resolution is therefore **NOT SINGLE CELL (Visium HD coming in April 2024 with increased resolution)**.



**Visium Cytassist**

**- Visium Cytassist is our method of choice**. Advantages are higher sensitivity, more robustness, and the usage of standard histology slides for the tissue sections positioning.

- It is a **probe-based** method, which improves sensitivity, but comes with a few caveats:
    - Detection of exogenous genes (GFP, reporters, viral genes....) requires designing custom probes before starting the experiment.
    - It gives no information on SNPs or isoforms (anyway very scarce with non-Cytassist method as well).
    - It can be performed only on **human and mouse** tissues
- It can be performed on **FFPE**, **Fixed Frozen** or **Fresh Frozen** tissues.

**Visium non-Cytassist**

- Visium non-Cytassist, which is less robust since the slides used are not standard histology slides, should be employed only if Visium Cytassist cannot be used (e.g. if species is not human or mouse).

- It is a **polyA-based** method, which can be performed on **any species,** but only on **Fresh Frozen** tissues.

# Visium Cytassist

## VISIUM CYTASSIST SLIDE AND CAPTURE AREAS

- Each Visium Cytassist Slide can hold **2 tissue sections**.
- The **capture area** in a Visium Cytssist Slide can be either **6.5x6.5 mm** (around 5'000 barcoded spots) or **11x11 mm** (around 14'000 barcoded spots).
- In case of **tissues larger than the selected Capture Area**, a microscope image of the full tissue, with a square delimiting the region of interest, needs to be provided.

## PROBE-BASED METHOD

- Visium Cytassist is a **probe-based** method, with probes targeting the **whole transcriptome**. The human or mouse whole transcriptome probe panels consist of ~3 or ~1 specific probes for each targeted gene respectively.
- Because of this probe-based nature:
    - detection of exogenous genes (GFP, reporters, viral genes....) requires designing **custom probes** in advance. See technical note CG000621 for guidance.
    - no information regarding SNPs or isoforms (anyway very scarce with non-Cytassist method as well)

## TISSUE TYPES AND SPECIES

- Visium Cytassist is available only for **human** and **mouse** (for samples other than human and mouse, Visium-non Cytassist protocol (CG000239) can be used instead).
- It was demonstrated with **several tissue types**. **Additional optimization** may be required for the preparation of specialized tissues, such as tissue with high fat content.

Consult **compatibility of tissues** in 10X Genomics Support website (https://kb.10xgenomics.com/hc/en-us/articles/7776229391373-What-tissue-types-are-compatible-with-the-Visium-CytAssist-Spatial-Gene-Expression-solution-).

## EMBEDDING AND STORAGE

Embedding and storage must be performed according to the latest version of the 10XG Tissue Preparation Guide:
- Cytassist FFPE: protocol CG000518.
- Cytassist Fresh Frozen: protocol CG000636.
- Cytassist Fixed Frozen: protocol CG000663.

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

- It can be done by GECF if providing the necessary amount of tissue.
- It is **mandatory for FFPE** tissues (DV200), strongly recommended for **Fixed Frozen** tissues (DV200), and is **optional** for **Fresh Frozen** (RIN). If the experiment gives poor results and RNA quality hadn't been assessed beforehand, the assessment will be done afterwards and, if quality is not sufficient, the GECF will not be held responsible.
- RNA quality recommendations:
    - **Cytassist FFPE** -> tissue blocks with a **DV200 ≥30%** are recommended.
    - **Cytassist Fixed Frozen** -> tissue blocks with a **DV200 ≥50%** are recommended.
    - **Cytassist Fresh Frozen**-> tissue blocks with **RIN ≥4** are recommended.

## SECTION THICKNESS

Section thickness for:
- **Cytassist FFPE** -> 3-10 um. Most validation was performed by 10XG with sections of 5 um.
- **Cytassist Fresh Frozen**-> 10-20 um. Recommended for most tissue types is 10 µm. Tissues with higher fat content (e.g., breast tissue) may require sections closer to 20 µm.
- **Cytassist Fixed Frozen** -> 10-20 um. Recommended for most tissue types is 10 µm.

Sections outside these specifications may result in reduced performance.

## 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, please contact us in advance
- The tissues must be prepared, sectioned and positioned on slides according to the latest version of the 10XG Tissue Preparation Guide:
    - Visium Cytassist FFPE: protocol CG000518.
    - Visium Cytassist Fresh Frozen: protocol CG000636.
    - Visium Cytassist Fixed Frozen: protocol CG000663.

## TIPS FOR SAMPLE PREPARATION

Some tips for samples preparation for:

- Visium Cytassist FFPE https://kb.10xgenomics.com/hc/en-us/sections/7622802025869-Tissue-Preparation-FFPE
- Visium Cytassist Fresh Frozen and Fixed Frozen https://kb.10xgenomics.com/hc/en-us/sections/12233648909325-Tissue-Preparation-Fresh-Frozen-Fixed-Frozen

### DIVERSE NOTES

Labelled antibodies panels for concomitant protein detection are available from 10XG. These are only available for human tissues, and only one panel exist for now, specific for immune markers. This technology is only validated for Cytassist for FFPE.

## Visium non-Cytassist

**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 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 assessed 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.

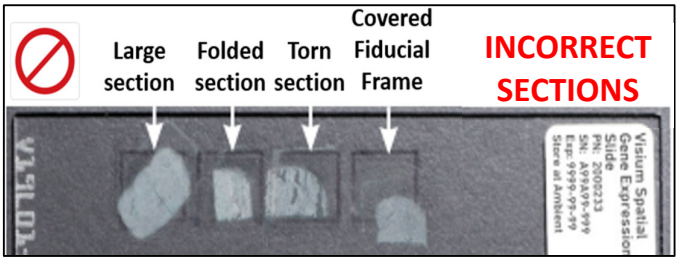
If not, 10XG say: "Section thickness for the Visium slides will depend upon tissue type. Customers should try to achieve a good quality section at the minimum section thickness for their tissue type. 5 - 35 µm sections have been tested in-house; however, 10 µm is used for most tissue types. Fatty tissues 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**).
*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: "We have performed limited in-house testing on imaging tissues that express a GFP reporter. Based

upon results from these experiments, fluorescent GFP reporters show compatibility with the Visium assay, but we have not yet fully validated this technique.

- If you are interested in testing, 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. We also recommend keeping the imaging time to a minimum.
- An alternative solution to detecting the GFP signal directly on a Visium slide 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 Cytassist**: a minimum of **25,000 reads per tissue covered spot** are required. More reads will give better sensitivity.
  - **Visium non-Cytassist**: a minimum of **50,000 reads per tissue covered spot** are required. More reads will give better sensitivity.

  So, for example, for Visium Cytassist
  - at least 100 million reads will be necessary for a sample covering around 80% of the Capture Area of a 6.5 mm slide (0.8 x 5'000 x 25'000),
  - at least 25 million reads will be required for a sample covering 20% of the Capture Area of a 6.5 mm slide
- 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/resources/analysis-guides/exploring-your-visium-data-a-spot-deconvolution-story?mkt_tok=NDQ2LVBCTy03MDQAAAGLZu1r19FfZio2idtnT9h1d5Vj8z0TTTVmavJx7pTxSWkWfu4D3JeR0wDgfYH198CKGDp3aCctAkqrnvMwhfpSDX7t-Av3Etkz0wRCoqc_xWyuwA

**Versions log**

- vA.01: initial release