



Guidelines for 10X Genomics Visium Cytassist – sectioning and placing tissue on slide

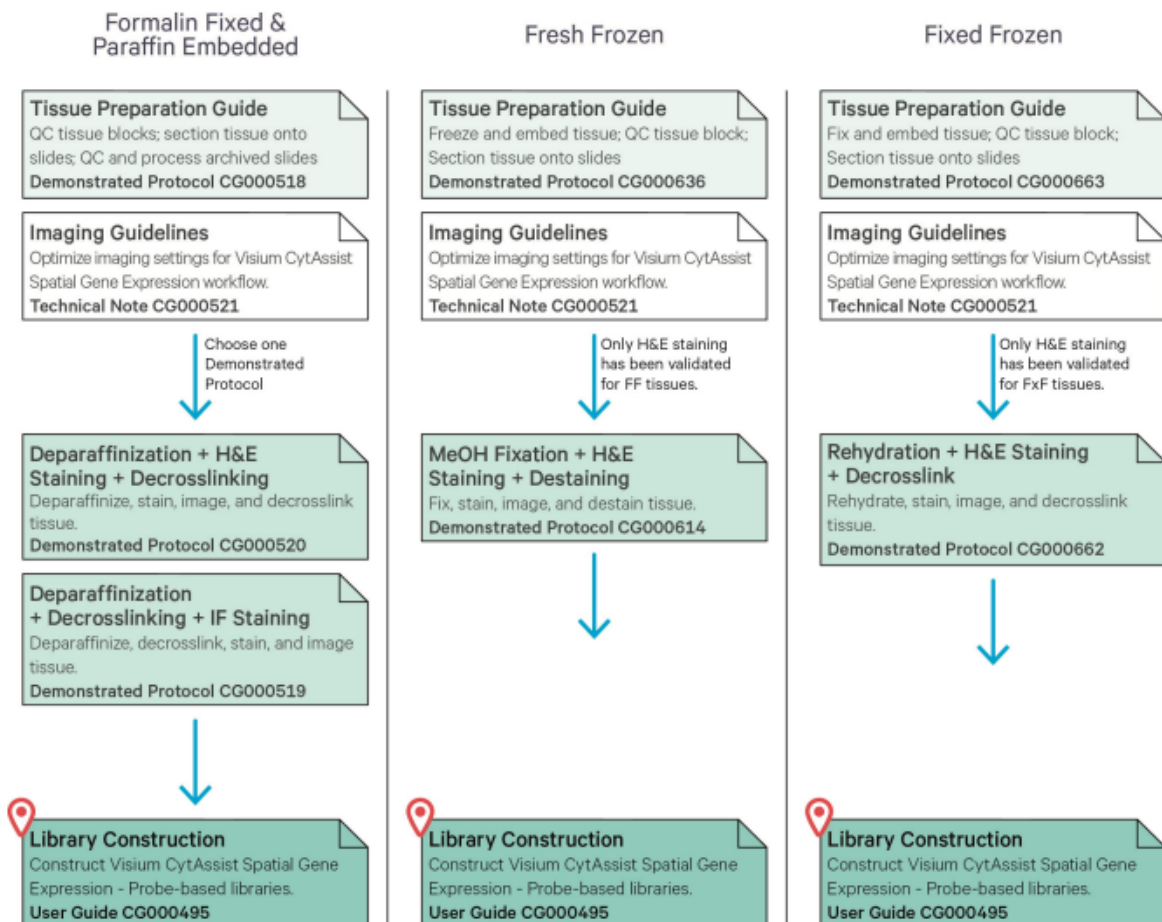
These guidelines relate to the Visium Cytassist protocol for the part of sectioning, fixation, staining and imaging if they are NOT performed at the EPFL Histology core facility.

General information

Visium Cytassist can be performed on **FFPE**, **Fresh Frozen** or **Fixed Frozen** tissues (see Workflow overview below for different protocols to follow).

10XG provide guidance on H&E or IF stained FFPE sections or H&E Fresh Frozen or Fixed Frozen sections. GECF have performed experiments on IF Fixed Frozen sections with good results, and possibly IF Fresh Frozen slides and be used also, but 10XG does not support this application.

Workflow Overview





Slide submission to GECF

User needs to hand-in **2 tissue slides, each containing 1 tissue section**. These slides need to be prepared, stained and imaged according to 10x Genomics protocols.

In case of tissues larger than the selected Capture Area, a microscope image of the full tissue, with a **square delimiting the area of interest**.

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

Requirements

- Tissues and sections need to be prepared according to the latest versions of 10X Genomics protocols (see Workflow overview in the previous page).
- **Good quality of starting tissue is critical** for optimal results.
- Ensure that tissue sections are on **Superfrost or positively charged slides**. To ensure compatibility with the Visium CytAssist, tissue sections must be placed in specific areas on a blank slide. **Validated slides**, as well as appropriate tissue placement areas, are listed in the Tissue Preparation guides (CG000518, CG000636 or CG000663 depending on method chosen).
- Each tissue section needs to be placed on a different tissue slide (only 1 section per slide).
- **Tissue size and placement**
 - o Areas of interest should be **maximum 6x6 mm** (for 6.5 mm Visium Cytassist Slides) or **maximum 10.5x10.5 mm** (for 11 mm Visium Cytassist Slides).
 - o **Larger tissue sections** may be placed on the slide, but an **area of interest** needs to be defined (only the tissue within the Capture Area will be processed by the CytAssist instrument). When handing in the slides, provide GECF with the microscope image of the full tissue and a square (6x6 mm or 10.5x10.5 mm depending on the Capture Area chosen) delimiting the area of interest.
 - o Tissue slide dimensions must be within
 - 24.8 mm - 25.3 mm in width
 - 74.4 mm - 76.2 mm in length

While slides are specified as being 25 mm x 75 mm, manufacturing tolerances may lead to dimensions that are too small or large to be compatible with Visium Cytassist.

- o Refer to image at the end of these guidelines “Determining Allowable Area” and to “Visium CytAssist for Accessory Kit Instruction Quick Reference Card (CG000548)” for more information regarding tissue size and placement on slide.

- **Section thickness** (refer to tissue preparation guide for more information):

- o FFPE -> 3-10 um.
- o Fresh Frozen-> 10-20 um.
- o Fixed Frozen -> 10-20 um.

Sections outside these specifications may result in reduced performance.



IMPORTANT: in case of samples prepared with any significant deviations from the 10x Genomics protocols, the user needs to inform GECF and a discussion will be needed to determine whether proceeding is possible.

Determining Allowable Area

Use the following diagrams to verify that freshly placed tissue sections are compatible. Reference the images below to draw the allowable area on the back of blank slides (remove after tissue placement). Images are to scale if scaling settings are not modified. To verify, ensure that the first block in section A measures 6.5 x 6.5 mm.

A Ensure that the area of interest will fit within the Capture Area of a Visium CytAssist Spatial Gene Expression Slide.
 Tissue sections larger than these Capture Areas may be placed on the slide, but only the tissue within the Capture Area will be processed by the CytAssist instrument.
 Overlay the slide, centering the tissue on either square.
 6.5 mm Capture Area 11 mm Capture Area

B If slide has no frosted areas, overlay on this diagram.
 Tissue should lie within the green allowable area:
15 mm from top and bottom edges
5 mm from the sides

If slide has a frosted end and/or marks, overlay on diagram 1 and then on diagram 2.
 Check the allowable area from both the top (1) and bottom (2) to ensure the tissue lies within the green allowable area (area is variable due to variability in the dimensions of frosted areas across slide brands):
15 mm from edge of frosted area/marks
5 mm from the sides

**If text is present below frosted area of slide, align bottom of the text to the line*
***If markings are present at bottom edge of slide, align markings to the + signs*

C Examples of Good Tissue Placement **Examples of Bad Tissue Placement**

Unfrosted Slide Frosted Slide Unfrosted Slide Frosted Slide

Versions log

- vA.01: initial release