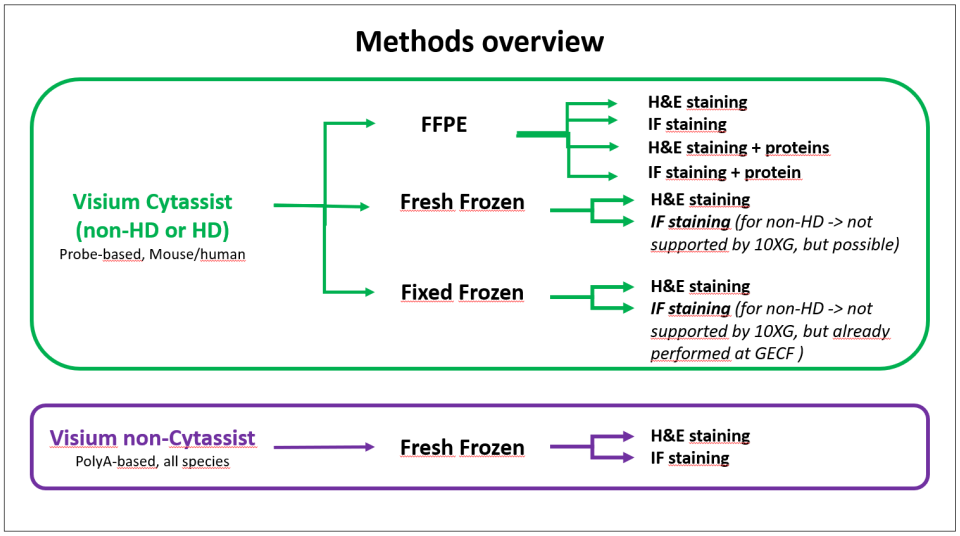
General information about different Visium methods

Cytassist or non-Cytassist? HD or non-HD?

We recommend **VisiumHD** for all projects, apart when it cannot be used (non-human/mouse tissue).

All 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, both non-HD and HD) or polyA mRNAs (non-Cytassist). The original Visium slide contains spots of 55 µm in diameter, with a 100 µm centre-to-centre distance between spots. The newer VisiumHD contains spots of 8um with no space in between (these spots are actually composed of 2um sub-spots that can be analysed individually, but with lower sensitivity).



Visium Cytassist (non-HD or HD)

- 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 available both in HD version (recommended) and in non-HD version (cheaper).

- 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 too).
  - It can be performed only on **human and mouse** tissues

- It can be performed on **FFPE**, **Fixed Frozen** or **Fresh Frozen** tissues. How to choose if you have the choice?:

  - FFPE is the best for preserving morphology and is the most robust.

- Fresh frozen is the best for getting highest UMIs (but its RNA is also the most fragile).
- Fixed frozen is typically chosen only when it is the only available option, as it can be more challenging. See https://kb.10xgenomics.com/hc/en-us/articles/29981279172237 .

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 (typically if tissue is not human or mouse).

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

Versions log

- vA.01 (22.04.2025): First version.
- vA.02 (22.04.2025): minor edits