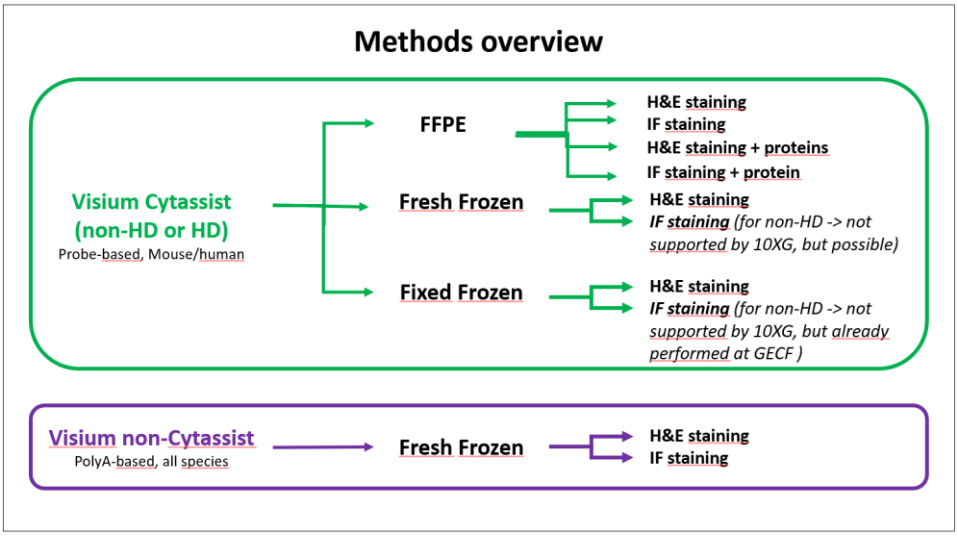
General information about different Visium methods

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

We recommend **VisiumHD** for all projects, apart when it cannot be used (non-human/mouse tissue). *Visium HD Poly-A is arriving in mid-2025. With this method all species can be used. It can be performed on Fresh frozen, OCT-embedded tissue blocks. Staining supported is H&E.*

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.

Additional info on how to choose can be found at https://kb.10xgenomics.com/hc/en-us/articles/29981279172237 .

Visium non-Cytassist

- Visium non-Cytassist, 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
- vA.03 (19.05.2025): mention to Visium HD Poly-A. Minor edits.
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