**Supplementary Files**:

These files are available through Figshare at <https://doi.org/10.6084/m9.figshare.19729567.v1> [(48)](https://sciwheel.com/work/citation?ids=12945427&pre=&suf=&sa=0).

**Supplementary File 1: Human\_DLPFC\_IF.zip**. This folder contains files to run *spotspotcheck* on the human DLPFC IF sample. These files are:

* **scalefactors\_json.json.** JSON file from Space Ranger providing the spot diameter estimation in pixels for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **tissue\_positions\_list.csv.** CSV file containing spot barcode, if the spot was called under (1) or out (0) of tissue, the array position, image pixel position X, and image pixel position Y for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **tissue\_spot\_counts.csv.** CSV file containing all the information in tissue\_positions\_list.csv and the signal count and percentage area of signal per spot.
* **V10B01-087\_A1\_segmentation.mat.** MATLAB mat file consisting segmentations of all channels (AF-Autofluorescence, DAPI, GFAP, NeuN, OLIG2, TMEM119) from the raw TIFF image of human DLPFC stored as V10B01-087\_A1.tif.
* **V10B01-087\_A1.tif.** Raw multichannel (AF-Autofluorescence, DAPI, GFAP, NeuN, OLIG2, TMEM119) TIFF image of human DLPFC sample used in Figure 4.

**Supplementary File 2: Mouse\_brain.zip**. This folder contains files to run *spotspotcheck* on the mouse brain H&E sample. These files are:

* **scalefactors\_json.json.** JSON file from Space Ranger providing the spot diameter estimation in pixels for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **tissue\_positions\_list.csv.** CSV file containing spot barcode, if the spot was called under (1) or out (0) of tissue, the array position, image pixel position x, and image pixel position y for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **V1\_Adult\_Mouse\_Brain\_image.tif.** Raw H&E histology image of mouse brain tissue section.
* **V1\_Adult\_Mouse\_Brain\_image\_cluster.mat.** MATLAB mat file with different color gradient clusters obtained from V1\_Adult\_Mouse\_Brain\_image.tif image.
* **V1\_Adult\_Mouse\_Brain\_image\_nuclei.mat.** MATLAB mat file with nuclei segmentation from V1\_Adult\_Mouse\_Brain\_image.tif.

**Supplementary File 3: Human\_heart.zip**. This folder contains files to run *spotspotcheck* on the human heart H&E sample. These files are:

* **scalefactors\_json.json.** JSON file from Space Ranger providing the spot diameter estimation in pixels for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **tissue\_positions\_list.csv.** CSV file containing spot barcode, if the spot was called under (1) or out (0) of tissue, the array position, image pixel position x, and image pixel position y for the high-resolution raw histology image. See <https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/using/count> for more information about this file.
* **tissue\_spot\_counts.csv.** CSV file containing all the information in tissue\_positions\_list.csv and the signal count and percentage area of signal per spot.
* **V1\_Human\_Heart\_image.tif.** Raw H&E histology image of human heart tissue section.
* **V1\_Human\_Heart\_image\_cluster.mat.** MATLAB mat file with different color gradient clusters obtained from V1\_Human\_Heart\_image.tif.
* **V1\_Human\_Heart\_image\_nuclei.mat.** MATLAB mat file with nuclei segmentation from V1\_Human\_Heart\_image.tif