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## Supplementary information

# Fluorescent labeling of abundant reactive entities (FLARE) for cleared-tissue and super-resolution microscopy

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# Supplementary Information

# Fluorescent Labeling of Abundant Reactive Entities (FLARE) for Cleared-Tissue and Super-Resolution Microscopy

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# Supplementary Method 1: DNA-FISH on Expanded Kidney tissue

### General reagents

- 10 μM Major Satellite (MaSat) DNA oligonucleotide probe **(**5′-GGAATATGGC GAGAAAACTG AAAATCACGG AATGATACGG CGACCACCGA ACTGCTACAG-3′**,** IDT).
- 10 μM Fluorescent oligonucleotide reporter probe (5′-/5ATTO647NN/CTGTAGCAGT TCGGTGGTCG CCGTATCATT-3′, IDT).

### Chemical reagents

- 10× PBS, pH 7.4 (e.g., Fisher Bioreagents # L-5400)
- Sodium azide ($NaN_3$, Fisher Scientific #S227I)
- Deionized water (DI water)
- Triton X-100 (Sigma-Aldrich, #X100)
- Formamide (Fisher Chemical, F84-1) **!Caution**
- 20× SSC (Sigma-Aldrich, S6639)
- 50% OmniPur Dextran Sulfate (EMD Millipore, 3730)
- Tween 20 (Sigma-Aldrich, P9416)

**Caution:** Researchers should consult the safety data sheet (SDS) for these chemicals prior to use. Formamide is toxic. Procedures using formamide should be performed in a fume hood and researchers are advised to be cautious.

### Other materials

- 1.5 mL microcentrifuge tubes (e.g., Fisher Scientific**,** 05-408-129)
- Razor blade (American Line, #66-0089)

### Stock solutions

- 1× PBS (pH 7.4)

- 300 mM sodium azide:
    - Add 1.95 g of sodium azide to 100 mL of $H_2O$.
- 20% Triton X-100 solution (v/v)
    - Add 2 mL of Triton X-100 to 8 mL of $H_2O$.
    - Store at 4 °C until use.
- 20% Tween-20 solution (v/v)
    - Add 2 mL of Tween-20 to 8 mL of $H_2O$.
    - Store at 4 °C until use.
- 20× SSC (pH 7.0)
- 2× SSC (pH 7.0)
- 2× SSCT: 2× SSC containing 0.1% Tween 20 (v/v).
    - Add 5 µL of 20% Tween 20 solution into 995 µL of 2×SSC.
- 20% Triton X-100 solution (v/v)
    - Add 2 mL of Triton X-100 to 8 mL of $H_2O$.
    - Store at 4 °C until use.
- DNA-FISH perm solution: 1× PBS containing 0.5% (v/v) Triton X-100.
    - Add 25 µL of 20% Triton X-100 solution to 975 µL of 1× PBS.
    - Store at 4 °C until use.
- Hybridization buffer solution: 2× SSC with 50% (v/v) Formamide and 0.1% (v/v) Tween-20.
    - Add 10 µL of 20% Tween-20 solution, 1000 µL of Formamide, 200 µL of 20× SSC to 790 µL of $H_2O$.
    - Prepare fresh each time.
- Hybridization mixture solution: 2× SSC with 50% Formamide, 10% Dextran Sulfate, 0.1% (v/v) Tween-20, 3mM sodium azide, 100 nM MaSat probe, 100 nM fluorescent reporter probe.
    - Add 1 µL of 20% Tween-20 solution, 1 µL of 300 mM sodium azide, 100 µL of Formamide, 20 µL of 20× SSC, 40 µL of 50% dextran sulfate, 2 µL of 10 µM of MaSat probe, 2 µL of 10 µM fluorescent reporter probe, 34 µL $H_2O$.
    - Prepare fresh each time.

## Procedure

The following procedure is written as a general procedure for DNA Fluorescence *in situ* hybridization (FISH) labeling of Major Satellite genomic DNA in MAP-expanded mouse Kidney tissue nuclei. This serves as a starting guide and could be further optimized to target other genomic regions.

### Permeabilization and equilibration •Timing ~3 hours

1. Incubate MAP-expanded mouse Kidney sample in 1×PBS **[50 mL per sample]** for 10 min.
2. Remove excess buffer and, using a razor blade, slice a small section of MAP-expanded mouse kidney sample and weigh the sliced sample **[~ 40 mg per gel slice].** ▲**Critical** When removing the excess 1× PBS, avoid aspirating close to the gel, then carefully slice the gelled sample on a clean surface.
3. Prepare a fresh stock of hybridization mixture so that the approximate volume of the mixture is twice that of the sliced sample **[> 0.1 mL per gel slice]** and place the mixture into a 1.5 mL tube.
4. Place weighed sliced sample into a 1.5 mL tube and incubate in DNA Perm solution **[1 mL per tube]** for 120 min. ▲**Critical** Sliced samples must be gently slid into the tube without breaking the gel apart.
5. Carefully remove DNA Perm solution and incubate sample in 1× PBS **[1 mL per tube]** for 10 min. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
6. Carefully remove 1× PBS and incubate sample in 2× SSCT **[1 mL per tube]** for 10 min. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid. ■**Pause Point** Able to store gel sample in 2× SSCT for up to a week.
7. Carefully remove 2× SSCT and incubated in hybridization buffer **[1 mL per tube]** for 10 min at room temperature. ▲**Critical** To avoid puncturing or ripping the gel,

aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.

**DNA denaturation and *in situ* hybridization** •Timing **~20 hr**

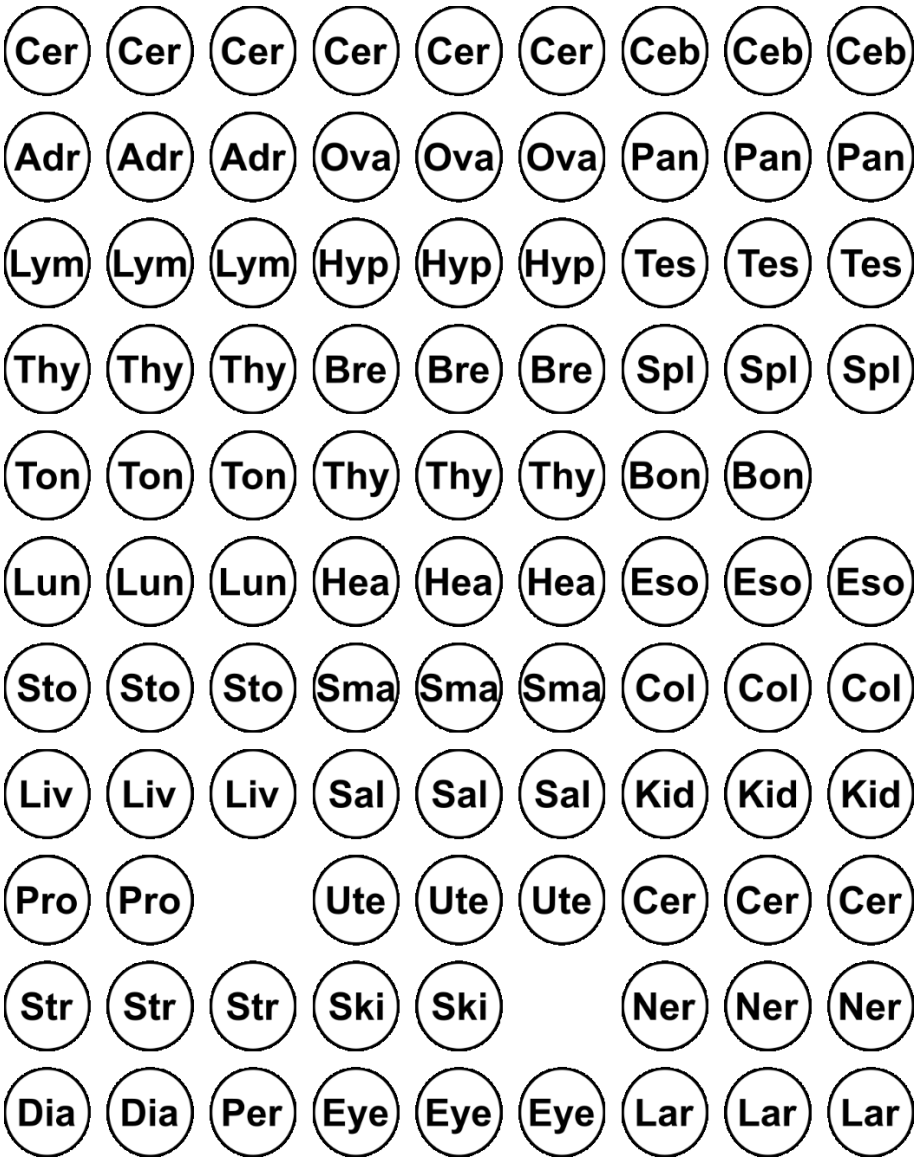
8. Carefully remove hybridization buffer and add pre-heated hybridization buffer **[1 mL per tube],** and incubate for 25 min at 60 °C. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
9. Incubate hybridization mixture made in **Step 1** for 5 min at 92.5 °C as the sliced sample remains in hybridization buffer for another 5 min at 60 °C.
10. Carefully remove hybridization buffer from the sliced sample, then slide sample with a disposable plastic tip into the preheated tube with hybridization mixture. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
11. Denature sample in hybridization mixture for 10 min at 92.5 °C.
12. Incubate tube overnight (~18 hr) at 37 °C.

**Wash and expansion** •Timing **~1 hr**

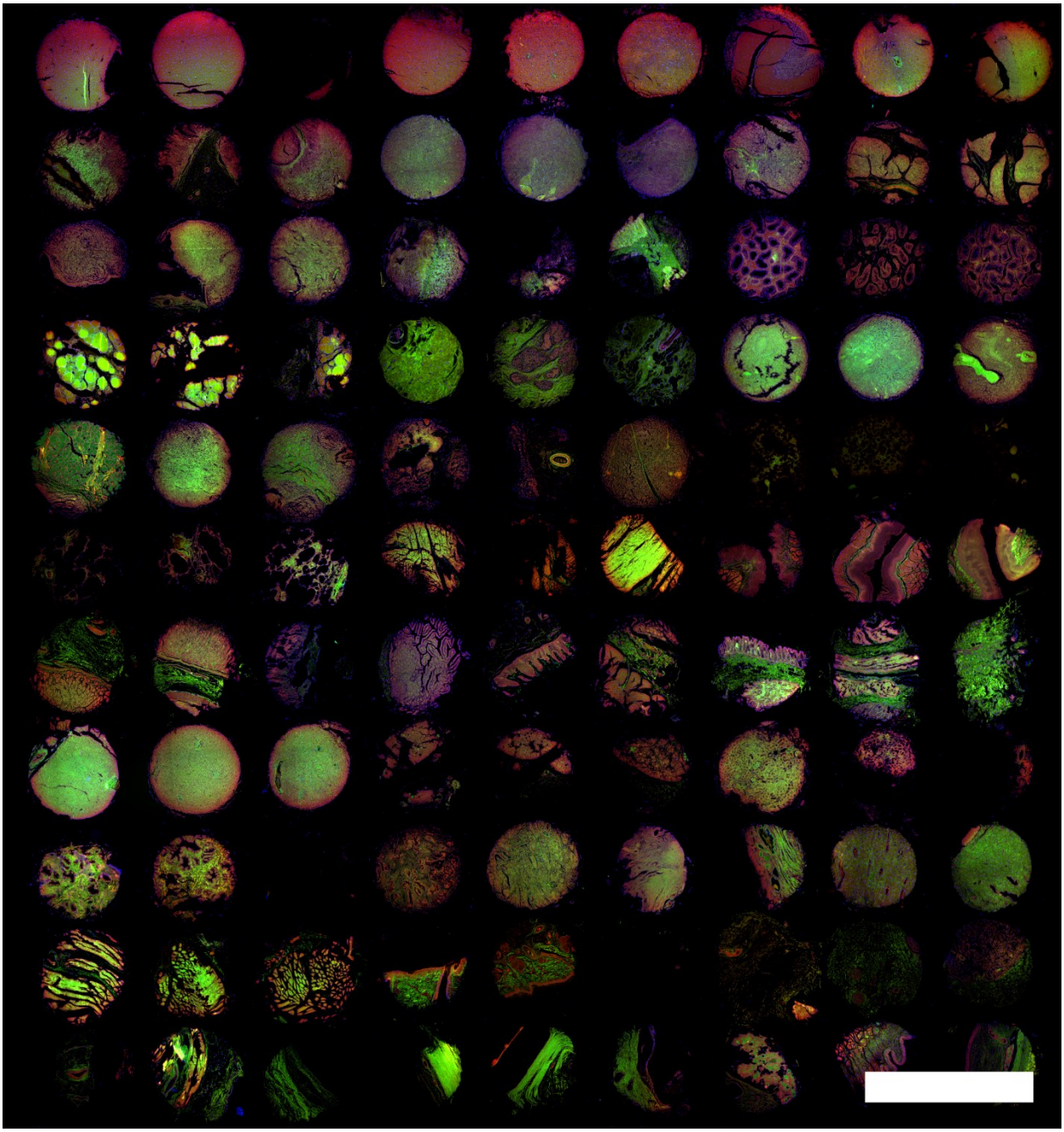
13. Carefully remove hybridization mixture and rinse gels with a pre-heated 2× SSCT **[0.5 mL per tube].**
14. Carefully remove 2× SSCT and incubate in pre-heated 2× SSCT **[0.5 mL per tube]** for 15 min at 60 °C. ▲**Critical** To avoid puncturing or ripping of the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
15. Carefully remove 2× SSCT from the tube and incubate in pre-heated 2× SSCT **[0.5 mL per tube]** for 15 min at 37 °C. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
16. Carefully remove 2× SSCT from the tube and incubate in 2× SSCT **[0.5 mL per tube]** for 15 min at room temperature. ▲**Critical** To avoid puncturing or ripping of

the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.

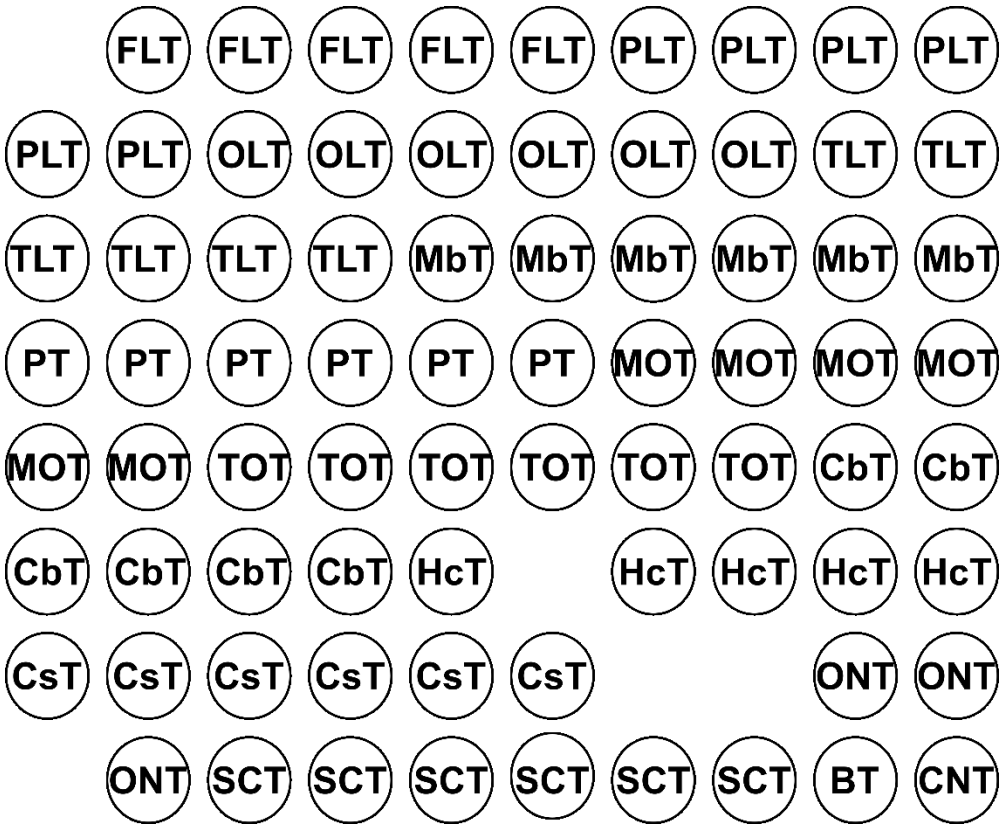
17. Carefully remove 2× SSCT from the tube and incubate in 0.2× SSCT **[0.5 mL per tube]** for 15 min at 4 °C. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid. ■**Pause Point** Able to store gel sample in 0.2× SSCT for up to a week.
18. Carefully remove 0.2× SSCT from the tube and incubate in DI water for 15 min at 4 °C. ▲**Critical** To avoid puncturing or ripping the gel, aspirate liquid with a micropipette by slowly sliding pipette tip down the inner wall of the tube until reaching the bottom, then slowly aspirate liquid.
19. Proceed to **Procedure 1 Step 13** of the main manuscript for sample mounting.



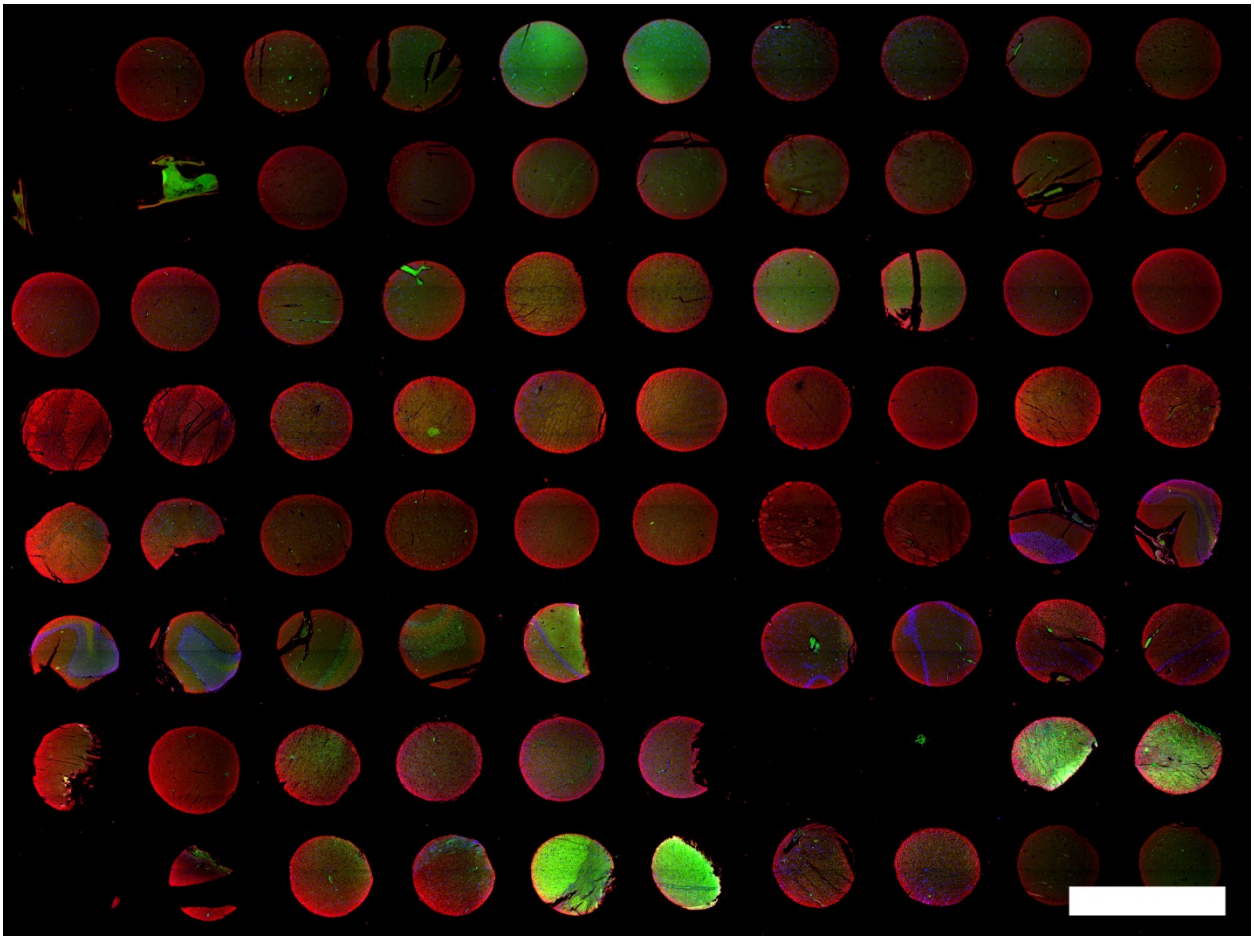

**Supplementary Figure 1. Map and identity of each core of the FFPE tissue microarray (TMA) used for Figure 4a-g.** The slide contains 96 human tissue cores of 30 different organs (US Biomax FDA999rt). Each core is 1.5 mm in diameter and 5 μm in thickness. Bon: bone marrow; Bre: Breast; Ceb: Cerebellum tissue; Cer: Cervix; Col: Colon; Dia: Diaphragm; Eso: esophagus; Eye: Eye; Hea: Heart; Hyp: Hypophsis; Kid: Kidney; Lar: Larynx; Liv: Liver; Lun: Lung; Lym: Lymph node; Ner: Nerve; Ova: ovary; Pan: Pancreas; Per: Pericardium; Pro: Prostate; Sal: Salivary Gland; Ski: Skin; Sma: Small intestine; Spl: Spleen; Sto: Stomach; Str: Striated muscle; Tes: Testis; Thy: Thymus gland; Ton: Tonsil; Ute: Uterus. Note, some cores were missing from the array and their identities are not included in the map.



**Supplementary Figure 2.** A larger view of the FFPE human tissue microarray from Fig. 4a. Scale bar is 3 mm.




**Supplementary Figure 3. Map and identity of each core of the FFPE tissue microarray (TMA) used for Figure 4h-n**. The slide contains 75 human brain cores of 15 different brain regions (US Biomax BNC17011at). Each core is 1.5 mm in diameter and 5 μm in thickness. FLT: Frontal lobe tissue; PLT: Parietal lobe tissue; OLT: Occipital lobe tissue; TLT: Temporal lobe tissue; MbT: Midbrain tissue; PT: Pons tissue; MOT: Medulla oblongata tissue; TOT: Thalamus opticus tissue; CbT: Cerebellum tissue; HcT: Hippocampus tissue; CsT: Callositas tissue; ONT: Optic nerve tissue; SCT: Spinal cord tissue; BT: Brain tissue; CNT: Caudate nucleus tissue. Note, some cores were missing from the array and their identities are not included in the map.



**Supplementary Figure 4.** A larger view of the FFPE human brain tissue microarray from Fig. 4h. Scale bar is 3 mm.

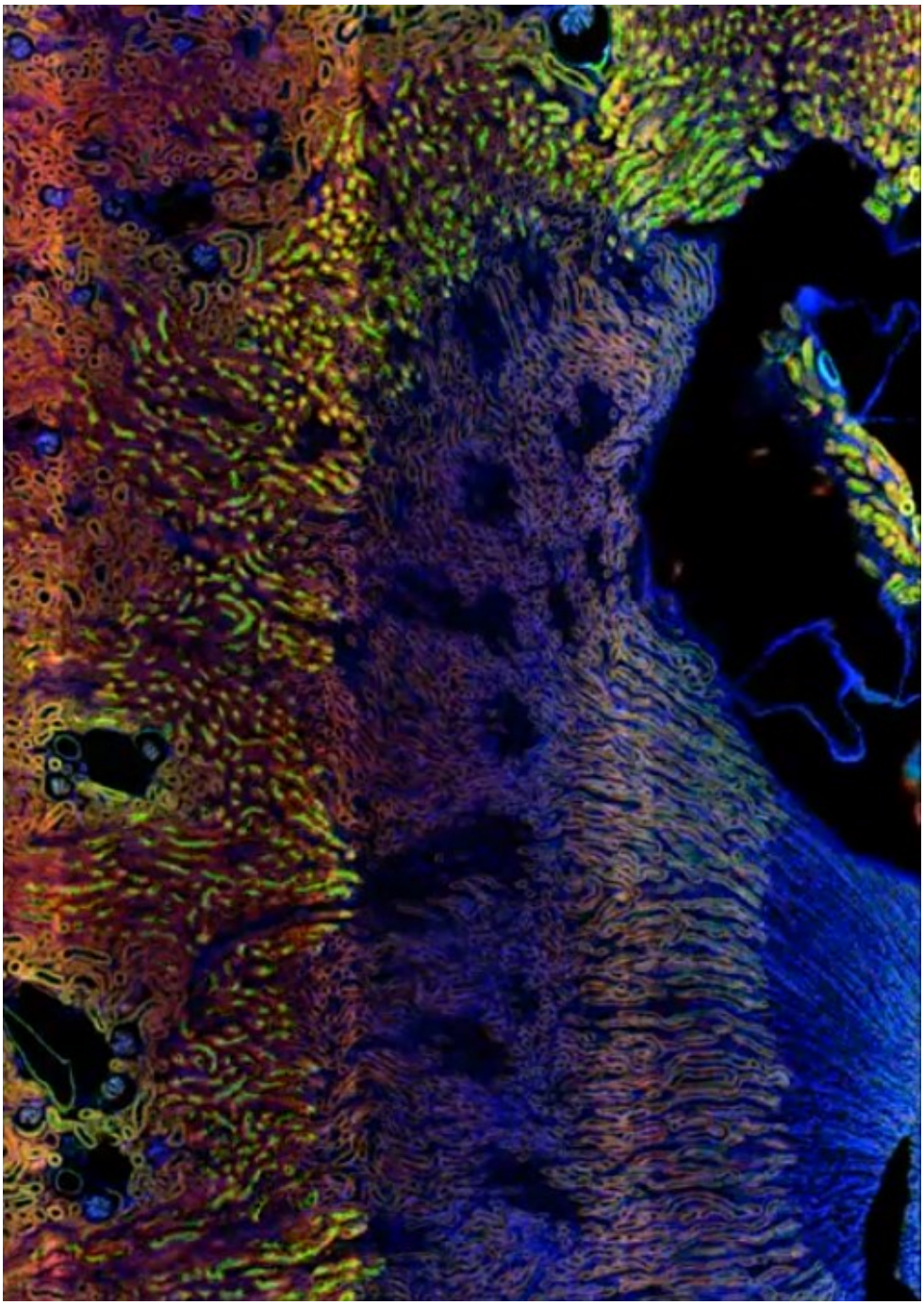
**Supplementary Table 1: summary of sample preparation and imaging conditions**

| Figure | Sample | Fixation | Tissue Format | Carbohydrate Stain | Amine Stain | Other Stain(s) and Stain Order | Expansion or Clearing | Imaging |
|---|---|---|---|---|---|---|---|---|
| **Fig. 1c** | Mouse kidney | PFA 1hr | 100µm vibratome sections | 1hr, 20mM $NaIO_4$ in 100mM NaOAc with 1M NaCl, pH5; then 3hr, 6.65µM hydrazide-AT565 in 100mM NaOAc, pH5; then 30 min, 50mM $NaBH_3CN$ in 100mM NaOAc, pH5. | 6hr, 5.9µM NHS-AT647N in 100mM MES, pH6. | DNA: 30min, 1.87µM Hoechst 33258 in PBS, pH7.4. Order: gel, covalent stains, DNA, expand. | Expansion | Confocal, 63× 1.2NA water lens. Image thickness: 92.3nm. Filter: 2. |
| **Fig. 1c** | Mouse intestine | PFA 1hr | 100µm vibratome sections | 30min, 100mM $NaIO_4$ in 100mM NaOAc, pH 5; then 2hr, 3.33µM hydrazide-AT565 in THF:NaOAc (1:1); then 30min, 100mM $NaBH_3CN$ in THF:NaOAc (1:1). | 2hr, 5.9µM NHS-AT647N in THF:MES (1:1). | DNA: 30min, 1.87µM Hoechst 33258 in THF:PBS (1:1). Order: covalent stains, DNA, clear. | Clearing | Confocal, 63× 1.4NA oil lens. Image thickness: 2.26µm Filter: none |
| **Fig. 3a-d** | RPE cell | PFA/GA 10min | --- | Same as Fig 1c mouse kidney. | 1hr, 3.0µM NHS-AT647N in PBS. | DNA: 30min, 1.96µM SYBR Green in DI water Order: gel, covalent stains, expand, DNA. | Expansion | Confocal, 63× 1.2NA water lens. Image thickness: a-d) 738nm. Filter: none. |
| **Fig. 3e-h** | RPE cell | Glyoxal solution mix 10min rt | --- | Same as Fig. 3a-d. | Same as Fig. 3a-d. | Same as Fig. 3a-d. | Expansion | Confocal, 63× 1.2NA water lens. Image thickness: e-h) 3680nm. Filter: none. |
| **Fig. 4** | TMAs | Formalin overnight | 5µm microtome sections | 30min, 100mM $NaIO_4$ in 100mM NaOAc, pH 5; then 2hr, 3.33µM hydrazide-Al568 in NaOAc; then 30min, 100mM $NaBH_3CN$ in NaOAc. | 30min, 3.0µM NHS-AT647N in PBS. | DNA: 30min, 1.87µM SYBR Green in PBS. Order: covalent stains, DNA | Neither | Wide-field epifluorescence, a, h) tiling with 4× 0.2NA air lens. i-k) 4× 0.2NA air lens. Homebuilt spinning-disk confocal, b,e) tiling with 20× 0.45NA air lens, c-d,f-g, l-n) 60× 1.2NA water lens. Image thickness: single plane. Filter: none. |
| **Fig. 5a** | Mouse kidney | PFA 1hr | 100µm vibratome sections | Same as Fig. 4 | 1hr, 3.0µM NHS-AT647N in PBS | Immuno: 2µg/mL G×PODXL, Rb×AQP-1, Gp×CK8+18 18h; 2µg/mL D×G-AFdye405, D×Rb-AF488, D×Gp-AF750 18h; at ~4°C. Order: covalent stains, immuno | Neither | Homebuilt spinning-disk confocal, 20× 0.45NA air lens; imaging cocktail was tris 200mM pH8.0, 10% glucose, 1mM Trolox, 0.4mg/mL glucose oxidase, 0.2% catalase. Image thickness: single plane. Filter: none. |
| **Fig. 5b** | Mouse kidney | PFA 1hr | 100µm vibratome sections | Same as Fig. 1c mouse intestine. | Same as Fig. 1c mouse intestine. | DNA FISH: same as Fig 5b. Order: covalent stains, DNA FISH. | Clearing | Confocal, 63× 1.4NA oil lens. Image thickness: 92.3 nm Filter: none |
| **Fig. 5c** | Mouse kidney | PFA 1hr | 100µm vibratome sections | Same as Fig. 1c mouse kidney. | Same as Fig. 1c mouse kidney. | DNA FISH: denature 10min at 92.5°C; hybridize 18hr at 37°C with 100nM MaSat oligo + 100nM AT647N oligo. Order: gel, covalent stains, DNA FISH, then expand | Expansion | Confocal, 63× 1.2NA water lens. Image thickness: 92nm. Filter: 2. |
| **Fig 5d** | Mouse kidney | PFA 1hr | 100µm vibratome sections | Same as Fig. 5a. | Same as Fig. 5a | Immuno: 2µg/mL G×PODXL, 18h; 2µg/mL D×G-AF488, 18h; at ~4°C. Order: covalent stains, immuno, DNA | Expansion | Confocal, 20× 0.7NA air lens. Image thickness: 776 nm. Filter: none. |
| **Fig. 8** | Mouse kidney block | PFA 1hr | 1mm vibratome section | 5hr, 100mM $NaIO_4$ in 100mM NaOAc, pH5; then 6hr, 3.33µM hydrazide-AT565 in THF:NaOAc (1:1); then 1hr, 100mM $NaBH_3CN$ in THF:NaOAc (1:1). | 8hr, 5.9µM NHS-AT647N in THF:MES (1:1). | DNA: 15hr, 20µM SYBR green in $H_2O$: PBS (1:1). Order: covalent stains, DNA, clear. | Clearing | Light sheet microscope, 20× 0.43NA multi-immersion lens from ASI and Special Optics. Image thickness: f-h) 446nm. Filter: none. |
| **Fig. 10** | Mouse kidney | PFA 1hr | 100µm vibratome sections | --- | --- | DNA: 30min, 1.87µM Hoechst 33258 in DI water, pH7.4. | Expansion | Wide-field epifluorescence, 4× 0.2NA air lens. Image thickness: single plane. Filter: none. |
| **Fig. 12** | Human kidney | PFA 1hr | 500µm vibratome sections | --- | 15hr, 1.2µM NHS-AT647N in THF:MES (1:1). | --- | Neither | Confocal, 10× 0.4NA air lens. Image thickness: single plane Filter: none |

Acronyms: Ab=antibody; AF=Alexa Fluor; AT=ATTO-TEC; DI=deionized; NA=numerical aperture; rt = room temperature (~22 °C); RPE=retinal pigment epithelium cell line; TMA=tissue microarray.

Additional notes: filter in the Imaging column indicates the number of pixels used for the application of 3D median filter if used on confocal data sets. Image thickness refers to the thickness of the data displayed in terms of the pixel sizes of the data set, where distances are in pre-expansion units for expanded tissues.

| Supplementary Table 2. Summary of Biological Structures Validated with Independent Stains | | | | | | |
|---|---|---|---|---|---|---|
| **Sample** | **Expansion or Clearing** | **Structures** | **Used markers for validation (vendor)** | **Carbohydrate Stain (green)** | **Amine Stain (red)** | **remarks** |
| **RPE cell** | **MAP expanded** | Mitochondria | Anti-TOMM20 (Santa Cruz Biotechnology, sc-11415) | ✓ | ✓ | Red > Green |
| | | Lysosomes | Anti-LAMP1 (Abcam, 24170) | ✓ | ✓ | Green > Red |
| | | Golgi Apparatus | Anti-GM130 (BD Biosciences, 610822) | ✓ | ✓ | Green ≈ Red |
| | | Nuclear pores | SYBR Green II (Invitrogen S7563) | ✓ | ✓ | Green ≈ Red |
| | | | | | | |
| **Mouse kidney** | **Clearing** | Glomerulus | Anti-podocalyxin (R&D Systems INC., AF1556) | ✓ | ✓ | Green ≈ Red |
| | | Proximal tubule | Anti-AQP-1 (Abcam, ab15080) | ✓ | ✓ | Green > Red |
| | | Collecting duct | Anti-CK8+18 (Abcam, ab194130) | ✓ | ✓ | Red > Green |



**Supplementary Video 1**. Open-top light-sheet imaging of a ~1-mm-thick mouse kidney section (slice) labeled with amines (red), carbohydrates (green), and DNA (blue). An animated 2D z-stack through the tissue volume is shown, followed by a 3D volumetric visualization and zoom-in fly-through. Residual vertical seams in the dataset arise from the microscope scanning, stitching, and fusion, and are not due to non-uniform FLARE staining.