

## Supplementary information:

### **hFRUIT: An optimized agent for optical clearing of DiI-stained adult human brain tissue**

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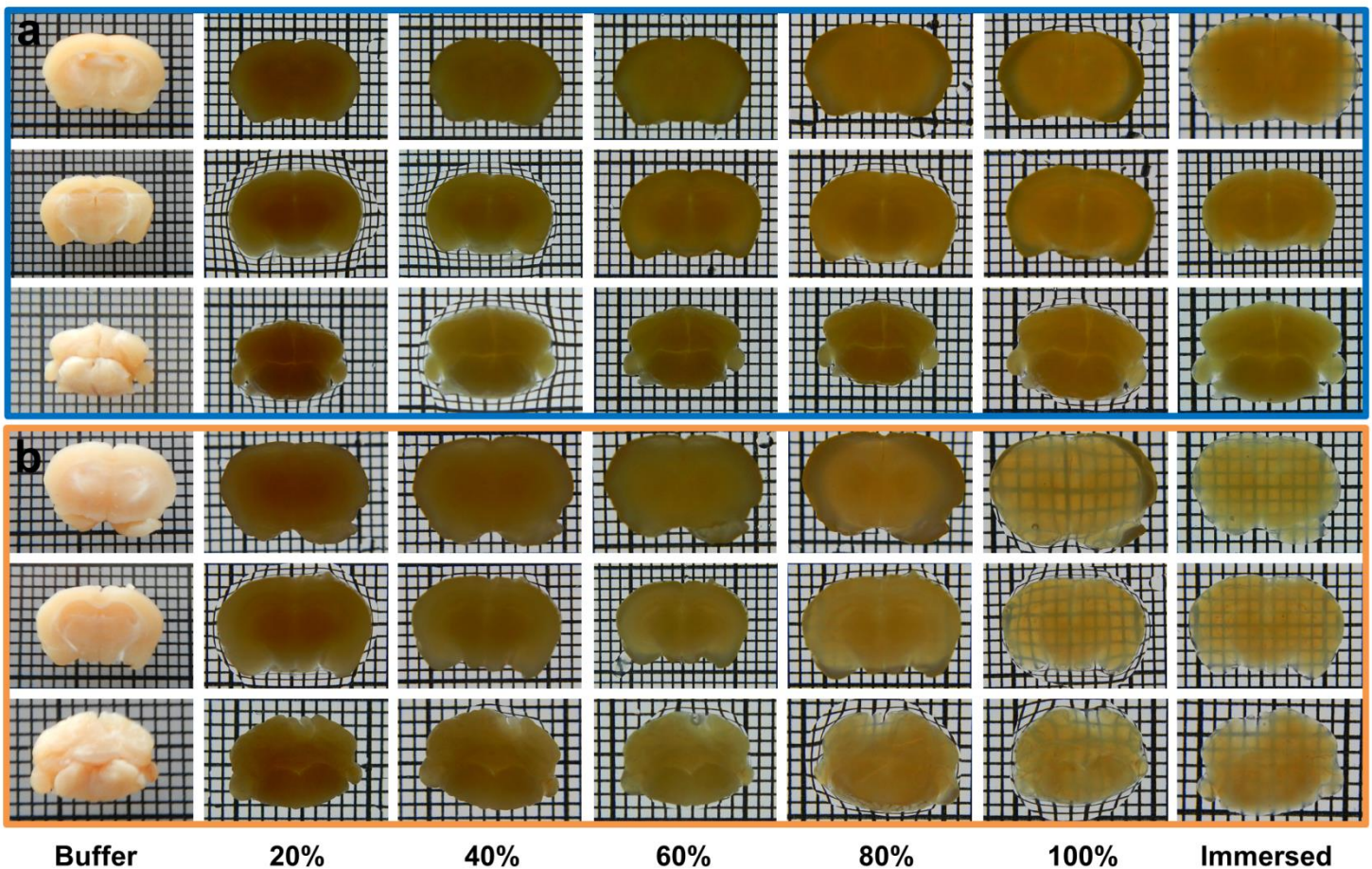
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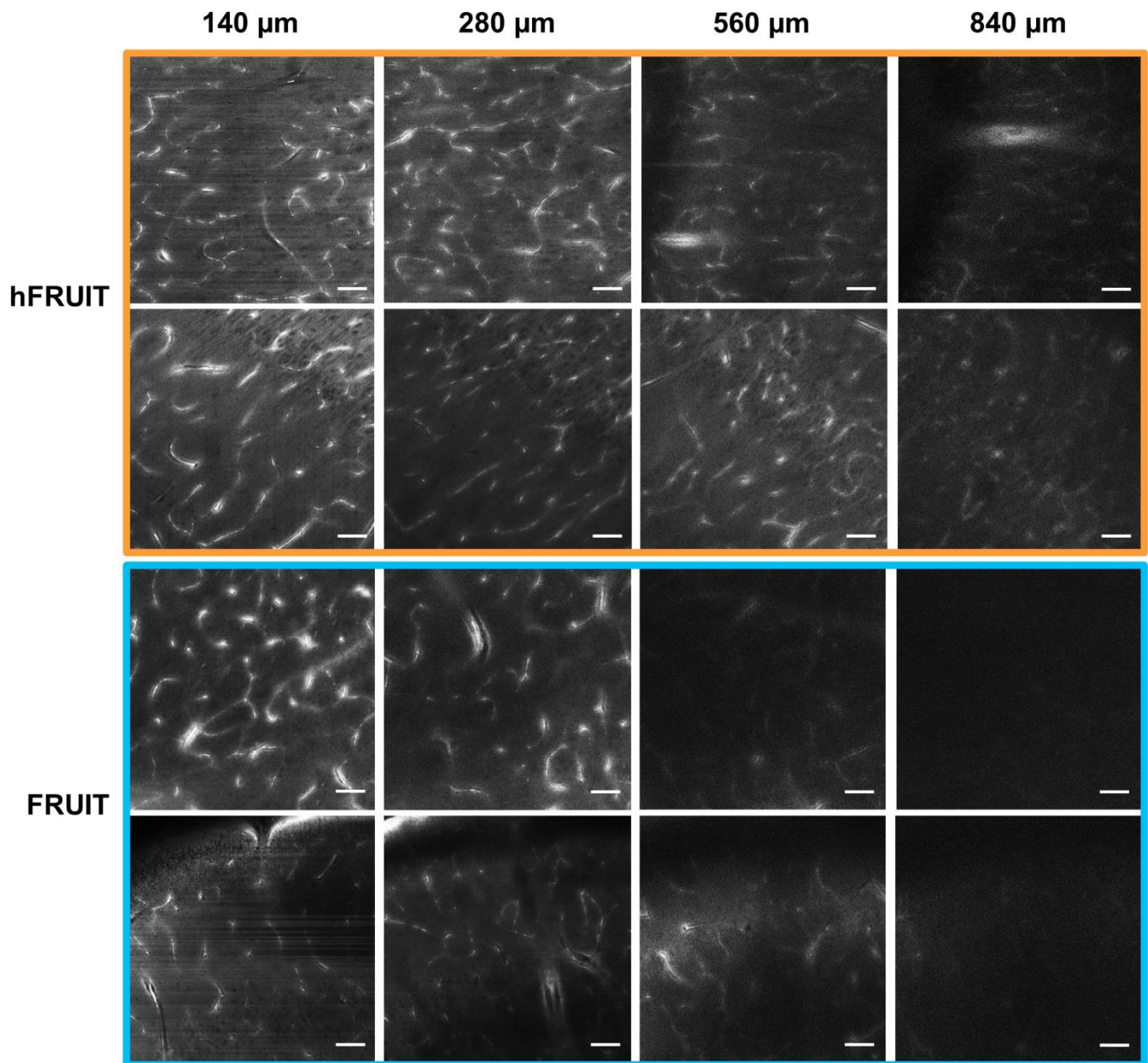
Keywords: human brain, hFRUIT, optical clearing, lipophilic tracer, DiI, PLP fixation

**Supplementary Table 1.** List of the different tissue sources and fixation types for every figure and video presented.

| Figure                          | Tissue source/Animal | Fixation |
|---------------------------------|----------------------|----------|
| 1a,b; Supplementary figure 1    | mouse                | 4% PFA   |
| 1c; 2; Supplementary figure 2+3 | pig                  | PLP      |
| 3a; 5                           | body donor           | formalin |
| 3b; 4; Supplementary video 1+2  | autopsy              | PLP      |

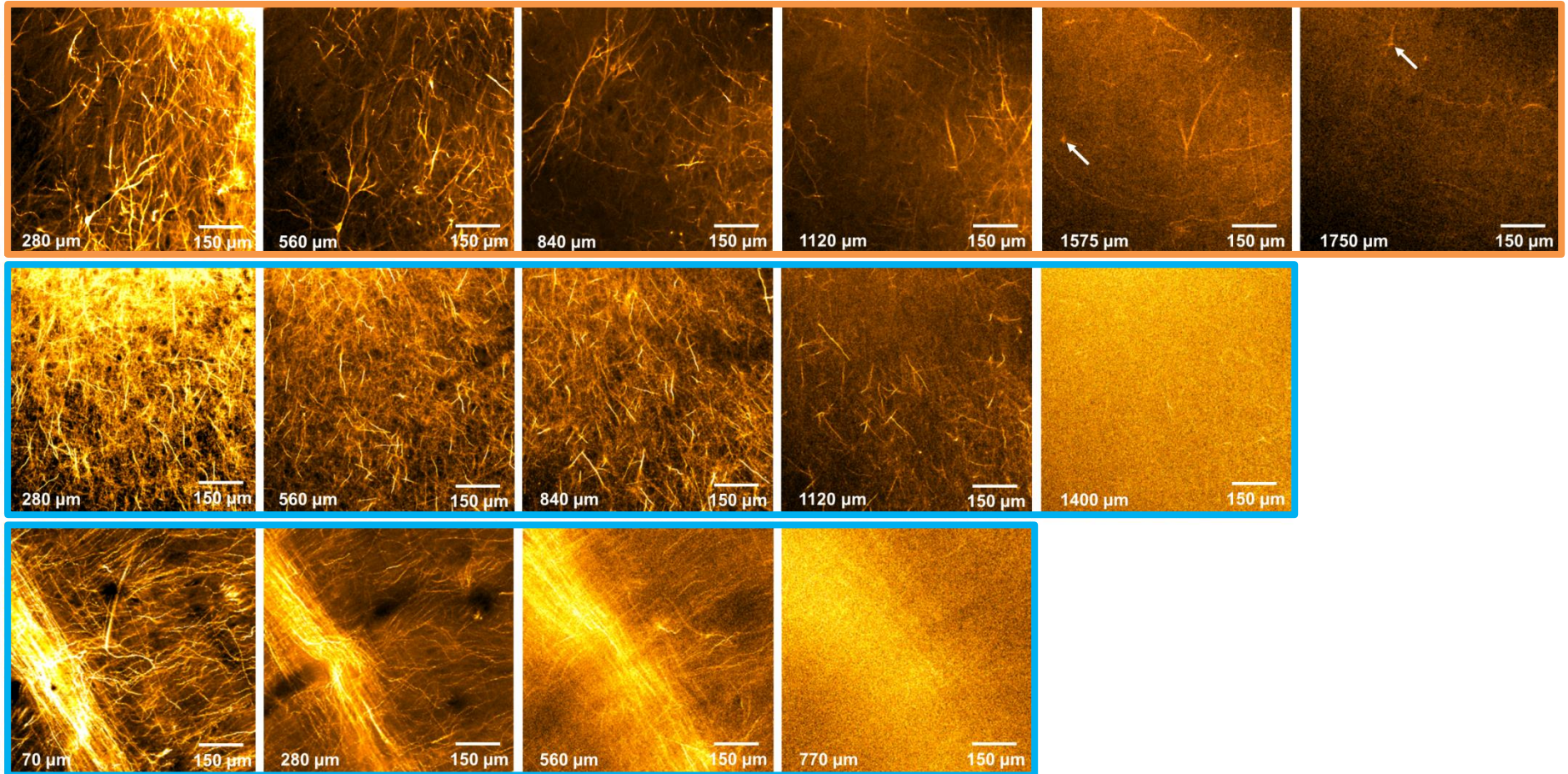


**Supplementary Figure 1.** (a) FRUIT clearing performance on 3 mm thick coronal slices of mouse brain and whole mouse cerebellum (bottom row) over ascending series of concentrations. (b) hFRUIT clearing performance on 3 mm thick coronal mouse brain sections and whole cerebellum (bottom row) for each concentration. Last column shows samples immersed in 1:1 mixture of silicon oil and mineral oil respectively. Grid size 1x1 mm respectively.



**Supplementary Figure 2.** Comparison of four representative porcine brain samples, two cleared with hFRUIT (orange) and two FRUIT (blue) respectively. Shown is a qualitative comparison of the autofluorescent blood vessel signal over increasing depths when excited with 488 nm, with each panel adjusted for contrast and brightness (not the signal drop-off over depth). Scale bars: 100 μm each.





**Supplementary Figure 3.** Comparison of representative DiI-labelled porcine brain samples cleared with hFRUIT (orange box) or FRUIT (blue boxes) and imaged with CLSM. hFRUIT cleared specimen allowed for deeper imaging of structures in general, with cell bodies being visible up to at least 1500 μm.

**Supplementary Video 1.** Single sided x-scan of Dil-labelled human amygdala, cleared with hFRUIT and acquired with the diSPIM set-up. The injection site of Dil crystals is located in the upper left corner. Volume dimensions: 400x772x546  $\mu\text{m}$ .

**Supplementary Video 2.** X-scan from the opposite direction of the same sample. The injection site of Dil crystals is located in the upper right corner. Volume dimensions: 400x772x546  $\mu\text{m}$ .

**Supplementary Video 3.** 3D rendering of a deconvolved sub-volume of the data set shown in Supplementary Video 1 and 2.