hFRUIT clearing protocol

Citation for using this protocol:

Hildebrand, S., Schueth, A., Wangenheim, K. V., Mattheyer, C., Pampaloni, F., Bratzke, H., ... & Galuske, R. A. (2020). hFRUIT: an optimized agent for optical clearing of DiI-stained adult human brain tissue. Scientific reports, 10(1), 9950.

Notes:

Incubation times depend in the sample size. In the lower concentrations samples sink down, once the clearing solution fully diffused through the tissue, but at 40-60% the solution is already so dense that the samples will not sink anymore.
The 80% and 100% solutions are very difficult to dissolve! It's best to prepare them at least one day in advance. If only sugars and thioglycerol are added, the solution can be heated up to enhance the dissolution of the components considerably. Only add urea after the solution has cooled down, since it can disintegrate at too high temperatures! Be careful not to add to much water in the first step, since urea will increase the volume a lot in the end (as a rule of thumb: 10 ml of water added results in a final volume of app. 50 ml).
After incubation in highest concentration, the samples can be immersed in mineral oil (RI = 1,467, M5904, Sigma), silicone oil (RI = 1,495, 175633, Sigma) or a 1:1 mixture of both (the latter suggested by LaVision for ~RI 1,48 clearing protocols). Best measure the RI of the highest hFRUIT solution empirically and mix the two oils in a ratio required to precisely get the measured RI. The better the matching, the less the aberrations. As this clearing approach does not delipidate the samples (to preserve the lipophilic dyes), the clearing capacity of this approach is much less potent as compared to e.g. MASH. Therefore, one will almost inevitably encounter aberrations in the sample and white matter will also not clear. To use this clearing method to the fullest extent, think carefully about sample thickness, orientation and cutting to minimize white matter content and aberrations due to RI inhomogeneities.
All stainings have be done prior to clearing, because the solution gets so viscose, that dyes do not diffuse well into the tissue anymore! Samples are in the original protocol not bleached, because bleaching with hydrogen peroxide would destroy the label. Other bleaching methods such as potassium metabisulfite or amino alcohols (Tainaka et al., 2018) might work while preserving the dye. This should be tested, if bleaching would be necessary e.g. with very bloody samples.

Materials:

Chemicals

- Fructose
- Mineral oil
- Silicone oil
- Sucrose
- 1-Thioglcerol
- Urea

Protocol:

- Incubate the samples in 50 ml of the respective concentration for the indicated time (table 1, larger samples might necessitate empirical adjustment of incubation volumes and/or times)
- Between each change, carefully blot the tissue dry with paper towels
- After final concentration, immerse in imaging medium (see notes)

Table 1: List of ingredients [g/ml] for the different concentration of hFRUIT solutions with approximate incubation times. The incubation times indicated here are for samples up to the size of whole mouse brains with 50 ml clearing solution per sample. Bigger samples and espacially human samples will need longer incubation times, which have to be determined empirically. 100% solution refers to the saturated solution of the mix, not to 100% w/v fructose like in the FRUIT paper (Hou et al., 2015)!

Concentration [%]	Incubation time	Urea	Fructose	Sucrose	Thioglycerol
10	1x	0.24	0.04	0.04	0.02
20	1x	0.24	0.08	0.08	0.04
40	2x	0.24	0.16	0.16	0.08
60	2x	0.24	0.24	0.24	0.12
80	3x	0.24	0.32	0.32	0.16
100	3x	0.24	0.385	0.37	0.2

References:

Hou, B., Zhang, D., Zhao, S., Wei, M., Yang, Z., Wang, S., . . . Jiang, T. (2015). Scalable and DiI-compatible optical clearance of the mammalian brain. *Front Neuroanat*, 9, 19. doi: 10.3389/fnana.2015.00019

Tainaka, K., Murakami, T. C., Susaki, E. A., Shimizu, C., Saito, R., Takahashi, K., . . . Ueda, H. R. (2018). Chemical Landscape for Tissue Clearing Based on Hydrophilic Reagents. *Cell Rep*, 24(8), 2196-2210 e2199. doi: 10.1016/j.celrep.2018.07.056