

1 An updated method and proof-of-concept data for Susaki et al. Nature Communications
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7 8 **Whole-brain NeuN immunostaining by CUBIC-HistoVIsion 1.1**

9 We somewhat modified the original CUBIC-HistoVIsion (HV) 1.0 protocol reported in
10 Susaki et al. (2020) to improve the signal-and-noise ratio. The associated dataset provides
11 a set of z-stack images of the whole ICR mouse brain stained by CUBIC-HV1.1 with
12 anti-NeuN, A555 conjugated antibody (Merck Millipore #MAB377A5).

13 In brief, a fixed and CUBIC-L-delipidated whole ICR brain (8-week-old male)
14 was 3D stained with the dye-conjugated primary antibody in HV1.1 immunostaining
15 buffer (a mixture of HEPES, Triton X-100, NaCl, and casein) at 32°C for 10 days. The
16 staining tube was further incubated at 4°C for 1 day to stabilize the signals (we skipped
17 the hyaluronidase treatment in HV1.0 and instead prolonged the staining period). After
18 the staining, the sample was briefly washed with cold HV1.1 wash buffer (a mixture of
19 HEPES, Triton X-100, and NaCl) at 4°C for 30 min × 2 times. The sample was then
20 postfixed with 1% formaldehyde in 0.1 M PB for 24 h at 4 °C and further incubated for
21 1 h at 37°C. The sample was washed with PBS and used for RI matching with CUBIC-
22 R+.

23 All chemical products and imaging equipment used in the experiment have been
24 documented in Susaki et al. (2020). CUBICStars Co. and Tokyo Chemical Industry have
25 commercialized the CUBIC-HV1.1 reagents.

26 27 **Acknowledgments and COI disclosure**

28 The experiment was performed by Chika Shimizu (RIKEN BDR). RIKEN and
29 CUBICStars Co. has filed a patent regarding CUBIC reagents and CUBIC-HV reagents,
30 respectively, in which Susaki EA and Ueda HR are the co-inventors. They are also
31 involved in the commercialization of CUBIC-HV1.1 reagents at CUBICStars Co.

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