Multimodal and multiscale optical imaging of nanomedicine delivery
across the blood-brain barrier upon sonopermeation

Jan-Niklas May¹ⁿ, Susanne K. Golombek¹ⁿ, Maike Baues¹, Anshuman Dasgupta¹, Natascha Drude¹, Anne Rix¹,
Dirk Rommel², Saskia von Stillfried³, Lia Appold¹, Robert Pola⁴, Michal Pechar⁴, Louis van Bloois⁵, Gert Storm⁵,
Alexander J. C. Kuehne¹ⁿ⁻⁶, Felix Gremse¹, Benjamin Theek¹⁻⁷, Fabian Kiessling¹⁻⁷, Twan Lammers¹⁻⁵⁻⁸

1. Institute for Experimental Molecular Imaging (ExMI), University Clinic and Helmholtz Institute for Biomedical
   Engineering, RWTH Aachen University, Aachen, Germany
2. DWI – Leibniz Institute for Interactive Materials, RWTH Aachen University, Aachen, Germany
3. Institute of Pathology, University Clinic RWTH Aachen, Aachen, Germany
4. Czech Academy of Sciences, Institute of Macromolecular Chemistry, Prague, Czech Republic
5. Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht,
   The Netherlands
6. Institute of Organic and Macromolecular Chemistry, Ulm University, Ulm, Germany
7. Fraunhofer MEVIS, Institute for Medical Image Computing, Aachen, Germany
8. Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands
Supplementary Figure 1: Fluorescence microscopy of IgG extravasation. Immunofluorescence staining of endogenous IgG (in purple) and blood vessels (CD31 staining; in green) in the brain showed extravasated IgG only in case of sonopermeation treatment. Images at higher resolution are provided in Figure 3.
Supplementary Figure 2: Fluorescence microscopy of polymer and liposome extravasation. Analysis of nanocarrier (in yellow; fluorophore-labeled) extravasation and penetration out of brain blood vessels (in red; rhodamine-lectin staining) upon sonopermeation treatment. Higher resolution images are provided in Figure 4.
Supplementary Figure 3: STED image processing and 3D rendering. STED images of a vessels in the brain (stained using rhodamine-lectin; in red) and nanomedicine formulations (in green), at different stages of image processing. A: Raw data with background and shifted signal. B: Deconvolved images with background reduction and correction for potential signal shifts, automatically performed by the Huygens Professional software (Scientific Volume Imaging). C: 3D-rendered images based on the gained signals. D: 3D-smoothened final images, with realistic vessel shapes and surfaces.