

Resource Summary Report

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622.2 URMHC HTC Rapid Clearing of Thick Human Lung Tissue Sections

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Type: Protocol

Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.7d7hi9n>

Authors: Cory Poole, Gloria Pryhuber

Group: Optical Clearing of Tissue

Summary: Purpose and Scope of the Procedure: Provide a relatively rapid clearing of lung tissue sections up to 1 millimeter thick for immunofluorescence staining and imaging with confocal or multiphoton microscopy. Current protocol is limited to tissue sections that are free from severe hemorrhage/thrombosis, containing mostly parenchyma, vessels, and smaller bronchioles. Times and solution volumes may need to be altered slightly if one wishes to clear sections containing a lot of cartilage and/or blood. Scientific Principles: One of the major challenges in conventional brightfield, epifluorescence, and even confocal microscopy is the limitation in imaging depth. This is primarily due to the scattering and absorption of light within the sample of both the excitation light and the emission light leading to a drastic reduction in the image brightness and contrast when deeper than a 100µm. Clearing offers a way around this by both reducing the components that negatively impact the light and by matching the refractive index of the mounting medium to that of the components which make up the tissue, leading to an almost transparent appearance. This protocol is a slight modification of a Clear Unobstructed Brain/Body Imaging Cocktail (CUBIC) protocol described by Kubota et al. developed originally to allow the analysis of cancer metastasis within the bodies of entire mice. By taking advantage of the fact lung tissue has plenty of open spaces and a very large surface area we can reduce the time in each of the solutions to allow complete clearing of the tissue sections to within just a few days. References: •Light Microscopy Methods and Protocols (2017). p. 74 •Kubota et al., (2017). Whole-Body Profiling of Cancer Metastasis with Single-Cell Resolution. Cell Reports 20, 236–250

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