ADAPT-3D

ADAPT-3D is a streamlined method that renders tissue specimens optically transparent for light-based imaging without the need for specialized equipment. In its basic form, ADAPT-3D consists of 3 simple steps using tissue fixed with paraformaldehyde: (1) a decolorization buffer to remove pigments (ADAPT:DC), (2) a delipidation buffer to partially remove lipids (ADAPT:PDL); and (3) a refractive index matching solution (ADAPT:RI) that finally makes tissue optically transparent. While refractive indexing matching with ADAPT:RI alone enables imaging of a few hundred microns into a tissue, ADAPT:DC and ADAPT:PDL are necessary pre-requisites for optimal results. Guidelines for buffer incubation times are provided below, though extended durations are well-tolerated, allowing flexible processing of both small samples and larger tissues like whole mouse organs or piglet or human specimens.
Abbreviations: Minutes (min), hour (hr), room temperature (RT)

Prepare Solutions

1. See (Table) and 'Methods' section for how to prepare ADAPT-3D solutions
2. Prepare the working ADAPT:PDL buffer by combining tetrahydrofuran with a stock 20% 1,2-hexanediol solution in a 1:1 mixture.
    a. Note: Prepare this in glass and mix well without introducing air bubbles into the mixture
3. For brain tissue, also add 10X PBS to ADAPT:DC to final concentration of 0.2X PBS

Tissue Fixation (perform before commencing tissue processing)

1. Place freshly harvested tissue or perfused fixed tissue into ADAPT:Fix and leave shaking at 4°C for 12-24 hours (mouse intestine to whole mouse brain)
2. Wash tissue out of fixative for 3 x 30 min or overnight if in excessive large volume (e.g., 50 mL) in PBS containing 10 U/mL heparin with 0.3M glycine.

<u>All following steps performed shaking or rocking at RT such that tissue is visibly moving</u>

Thick Sections or Thin Tissues (<~1 mm thickness)

Thick sections can be prepared directly from fixed tissue on a vibratome.

1. Incubate in 4 mL of ADAPT:DC for 6-12 hr at RT shaking
2. Wash tissue in PBS for 2x30 min + 1 hr wash shaking at RT
3. Incubate in ADAPT:PDL for 3-12 hours at RT shaking
    a. Perform in glass scintillation vial, filled to ≥ 50% of container volume
4. Wash tissue in 0.1x PBS for 30 min
5. Wash tissue in 1x PBS for 30 min + 1 hr wash shaking at RT
6. *~antibody staining if desired~*
7. Shortly before imaging, incubate tissue in 4 mL 50% ADAPT:RI diluted in PBS shaking at RT (0.5-1hr)
8. Incubate tissue in 3mL of 100% ADAPT:RI shaking at RT for (1-3hrs).
9. After imaging tissues can be washed out of RI in PBS and stored at 4°C in PBS (0.05% sodium azide)

Note: During ADAPT:DC and ADAPT:PDL, tissue will turn partially transparent but will return to its normal opacity after washing in PBS. Only in the final 100% ADAPT:RI step will tissue become completely transparent.

Antibody Staining for Thick Sections or Thin Tissues

Primary antibodies directly conjugated to fluorophores for faster single step staining, or use secondary antibodies conjugated to fluorophores for brighter staining.

1. Optional: Block tissue in ADAPT:BS for 3hrs
2. Prepare a cocktail of primary antibodies in ADAPT:BS
3. Incubate tissue floating in primary body antibody solution shaking at RT
    a. Timing: 1 overnight incubation (15 hr) for 500um thick brain sections or for mouse intestine. Increase time accordingly for thicker sections.
    b. Note: Add DAPI, if using primary antibodies directly conjugated to fluorophores
4. Wash away primary antibody for 3x1 hr in wash buffer (1xPBS, 0.2% Tween-20, 10U/mL heparin) shaking RT
5. Prepare an appropriate secondary antibody cocktail, and add DAPI if desired.
6. Optional: filter secondary cocktail through a 0.22 µm filter to remove possible aggregates
7. Incubate tissue in secondary antibody cocktail shaking RT for an equal time to the primary antibody incubation
8. Wash out secondary antibody in wash buffer (1xPBS, 0.2% Tween-20, 10U/mL heparin) for a minimum of 3x1hrs, but if time allows washing overnight in a large volume is optimal

ADAPT-3D Protocol for Whole Organs

Washes and incubations can vary depending on size of tissue.

1. Option: For tissues with bone, incubate in ADAPT:Decal for at least 3 days at RT. Test for tissue softening in stiffest part of bone (ie. nasal sinus in whole mouse head)
    a. Wash out of ADAPT:Decal in excess volume of (50mL) 1X PBS containing 10 U/mL Heparin for 3x30 min
2. Place large tissues into ADAPT:DC until red/brown hue becomes nearly translucent – takes around 48 hours at RT on shaker.
3. Wash in excess volume of (50mL) 1X PBS containing 10 U/mL Heparin for 3x30 min
4. Incubate tissues in ADAPT:PDL for at least 48 hours at RT on shaker. Change out after 24 hours. Use a glass container filled to minimize air exchange and oxidation of tissue.
5. Rinse with 0.1x PBS containing 10 U/mL Heparin for ~30 minutes to minimize any morphological changes.
6. Repeat step 3.
7. Optional immunolabeling: Block tissue overnight in ADAPT:BS, incubate tissue with antibodies in ADAPT:BS for ~3-4 days at 30°C on shaker. Wash with 1xPBS, 0.2% tween-20, 10U/mL heparin) before imaging.
8. Shortly before imaging, incubate tissue in 4 mL 50% ADAPT:RI diluted in PBS shaking at RT (0.5-1hr)
9. Incubate tissue in 3mL of 100% ADAPT:RI shaking at RT until transparent, taking at least 4 hours.

Imaging note: After RI matching the sample should be imaged directly in ADAPT:RI, which depending on tissue size can require an imaging chamber that contains the ADAPT:RI and in the case of confocal has a coverslip separating the tissue from the microscope objective on one side.