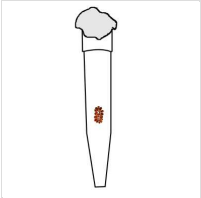

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X-ray Micro Computed Tomography (MicroCT)

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This protocol has been successfully used with colonial tunicates. It has been adapted to our needs based on the following publication: " MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues " by Brian D Metscher.

MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues.

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Colonial tunicates, MicroCT, tomography, ascidians

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4% PFA
PTA
IKI
Ethanol
low melting agarose
fixing paste (e.g. dental wax)
pipette tips 200uL or 1mL
10x PBS, pH 7.2 (store for up to 2 moths at RT)

A	B	C
Na2HPO4*2H2O	0.1M	17.8g
KH2PO4	18mM	2.4g
NaCl	1.4M	80g
KCl	27mM	2g
water		1000mL

10x PBS (amounts calculated for 1L)

Fixation 1h 30m

1 Clean the slide with the colony of your interest. See Cleaning colonial ascidians V.2.

2 Anesthetize the animals following this protocol. See Whole colony fixation V.1

3 Fix the animals in 4% PFA **Overnight** at **Room temperature** . See Whole colony fixation V.1

4 Wash twice in 3.3x PBS for **00:30:00** . 30m

5 Rinse tree times with 1x PBS and leave in 1x PBS for **01:00:00** . 1h

Staining 4d

6 Detach the colony with a razor blade and put in a 2mL tube containing **1 mL** PTA /

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0.6 mL IKI/ **0.4 mL** Ethanol.

7 Incubate for **96:00:00** at **Room temperature** on linear shaker (low speed), protect from light. 4d

8 Rinse quickly 3-4 times with 1x PBS.

Mounting

9 Prepare **10 mL** of **0.5 % volume** low melting agarose.

9.1 When the agarose has cooled down a bit, pipette **125-150 µL** if using a 200ul tip or **750-800 µL** if using a 1mL tip (the tip size depends on the animal size).

9.2 Carefully place your colony vertically inside the tip (it should be as flat as possible, avoid folds).

9.3 Pipette some more agarose in order to cover the animal entirely.

10 Place the tip into a beaker filled with ice-cubes so that the agarose becomes solid.

11 Cover the tip with fixing paste.

12 Store at **4 °C** or proceed to the next step.

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13 Mount the tip on the tomograph support using fixing paste in such way that it is perfectly straight.

14 Place it inside the tomograph.

15 Follow the tomograph's manual and proceed to scan.

16 After scanning, the tip with the sample can be stored at **4 °C** for several weeks and eventually can be reused.

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