Protocol for staining motile and primary cilia in formalin-fixed tissue:

2X5 minutes Xylenes
1X3 minutes 100-95-95-95% ethanol
2X5 minutes 1XTBST
10 minutes at RT in 3% aqueous H_2O_2
2X5 minutes 1XTBST

☐ **Antigen retrieval:**

1) Antigen retrieval **buffer** is a 1:20 dilution (in water) of 20X EDTA solution (pH 8; Life Technologies, cat number 005500; 100 ml)
2) Place slides in 250 ml of buffer and subject to heating (I use a pressure cooker, 5 minutes at pressure with slow release). After pressure is released, leave slides in hot buffer (in the pressure cooker) for 10 minutes, then cool to RT in running diH_2O.
3) Wash once in 1XTBST for 5 minutes

☐ **Block** 1 h at RT in **blocking diluent** (1%BSA, 1% milk, 10% normal donkey serum in 1XTBST)

☐ **Primary antibody (1:1000 in blocking diluent)** incubation for 1 hour overnight at 4ºC or at room temp. Do not wash slides after blocking step, just tap off excess blocking solution

- **Antibodies:** Mouse anti-acetylated α-tubulin (Sigma, cat number T6793-.2ML), which stains the axoneme or stalk of the cilia. Rabbit anti-Gamma-tubulin (Sigma, cat number T5192-.2ML) stains the centrosome or basal body of the primary cilia (don't really need gamma tubulin for staining motile cilia, just need acetylated tubulin).
- Acetylated tubulin 1:1000
- Gamma-tubulin 1:800

☐ Wash 2 X 5 minutes 1XTBST

☐ **Incubate with secondary antibodies** for 1 hour at RT in the dark
Donkey anti-mouse-488 or 594 at **1:1000** for acetylated α-tubulin
Donkey anti-rabbit-488 or 594 at 1:800 for gamma-tubulin (if used)
Prepare in blocking diluent

☐ Wash 2X5 minutes in 1XTBST

☐ **Apply $CuSO_4$** solution: 10 mM $CuSO_4$ + 5 mM ammonium acetate, pH 5
Note that this step is not necessary for detection of motile cilia, but it may help suppress autofluorescence. So this step can be skipped if autofluoresence isn't a problem. I'm still working this out, it doesn't hurt anything and helps with primary cilia detection).

☐ Leave solution on for 30 minutes (upto 1 h) but use 30-min protocol

☐ Wash 2 X 10 minutes in 1XPBS (washing time may have to increase if precipitates form under the coverslip over time, assuming that the problem is that $CuSO_4$ reacts with the mounting media in some way). Could also wash with 1XTBS, but I've switched to PBS for this step with no problem.

☐ Coverslip with Prolong Gold + DAPI + Seal with Nail polished around cover slip