Things needed: Tissue, Gatenby subbed slides, 12 well plate, slice baskets (one per section), shaker, and solutions below. (Streptavidin alexafluor, Streptavidin Oregon Green, etc.

1. Store 300 µm brain sections in PFA at least **overnight** 4% PFA at 4°C.
2. Wash sections **3 times for 20 minutes** each wash in PBS with 0.3% Triton X-100 (22.5µl in 7.5ml) on a shaker.
3. Make up Fluorescent reagent Solution.
4. Remove last wash; add fluorescent reagent solution to each well; incubate **overnight at room temperature or 4°C** (leave on shaker for 1 hour or more to distribute solute prior to placing in refrigerator overnight.)
5. Wash section **3 times for 10 minutes** in PBS.
6. Mount sections in PBS onto Gatenby subbed slides.
7. Make sure sections are completely dry and coverslip with fluoromount (*ProLong® Gold Antifade Mountant*).
8. Use a small weight (e.g.: a penny or flat screw) to weigh down coverslip to keep tissue from curling

** Whole slices (600µm or less) can also be visualized by letting the slice incubate in the fluorescent reagent solution overnight.

Solutions:

To make 10x PBS

1. Combine the following:
    - 80g NaCl
    - 2g KCl
    - 14.4g Na2HPO4 (dibasic anhydrous) OR 18.1g Na2HPO4.2H2O (dibasic dihydrate) OR 27.2g Na2HPO4.7H2O (dibasic heptahydrate)
    - 2.4g KH2PO4 (monobasic anhydrous)
    - 800 mL distilled H2O
2. Adjust pH to 7.4 with HCl
3. Add H2O to 1L
4. Filter and store at RT

For 1X PBS, dilute 100mL to 1L with dH2O, store at 4°C

1X PBS is 137 mM NaCl, 12 mM Phosphate, 2.7 mM Phosphate, 2.7 mM KCl, pH 7.4

Fluorescent Reagent Solution:

7.5ml PBS
0.3% Triton X-100 (22.5µl per 7.5ml of PBS)
1.5µg/ml reagent (1:667 dilution of 1mg/ml stock- 11.25µl per 7.5ml of PBS).

This makes enough Fluorescent solution for two µm sections.