Compensation beads for FACS

- Mix 1 drop of beads + 5 ul of antibodies ➜ 30′ at 4°C
- Wash with 2 ml of facs PBS (**PBS + 3% FBS**) ➜ centri 5′ at 400g
- Remove carefully the supernatant (pellet not visible)
- Add facs buffer to obtain 300-400 ul of solution
- Keep at 4°C in the dark before facs

#facs

Percoll

- Prepare stock isotonic percoll (**SIP**)
  - ➜ mix 9 parts of percoll with 1 part of 10x HBSS (or 10x PBS)
  - ➜ Use 10x HBSS without Ca++ and Mg++
  - ➜ Percoll should be used at room temperature
- Prepare SIP at 70% and 30%
  - ➜ **70%**: mix 7ml of SIP + 3ml of 1x HBSS
  - ➜ **30%**: mix 3 ml of SIP + 7 ml of 1x HBSS

#facs

Tissue dissociation for FACS

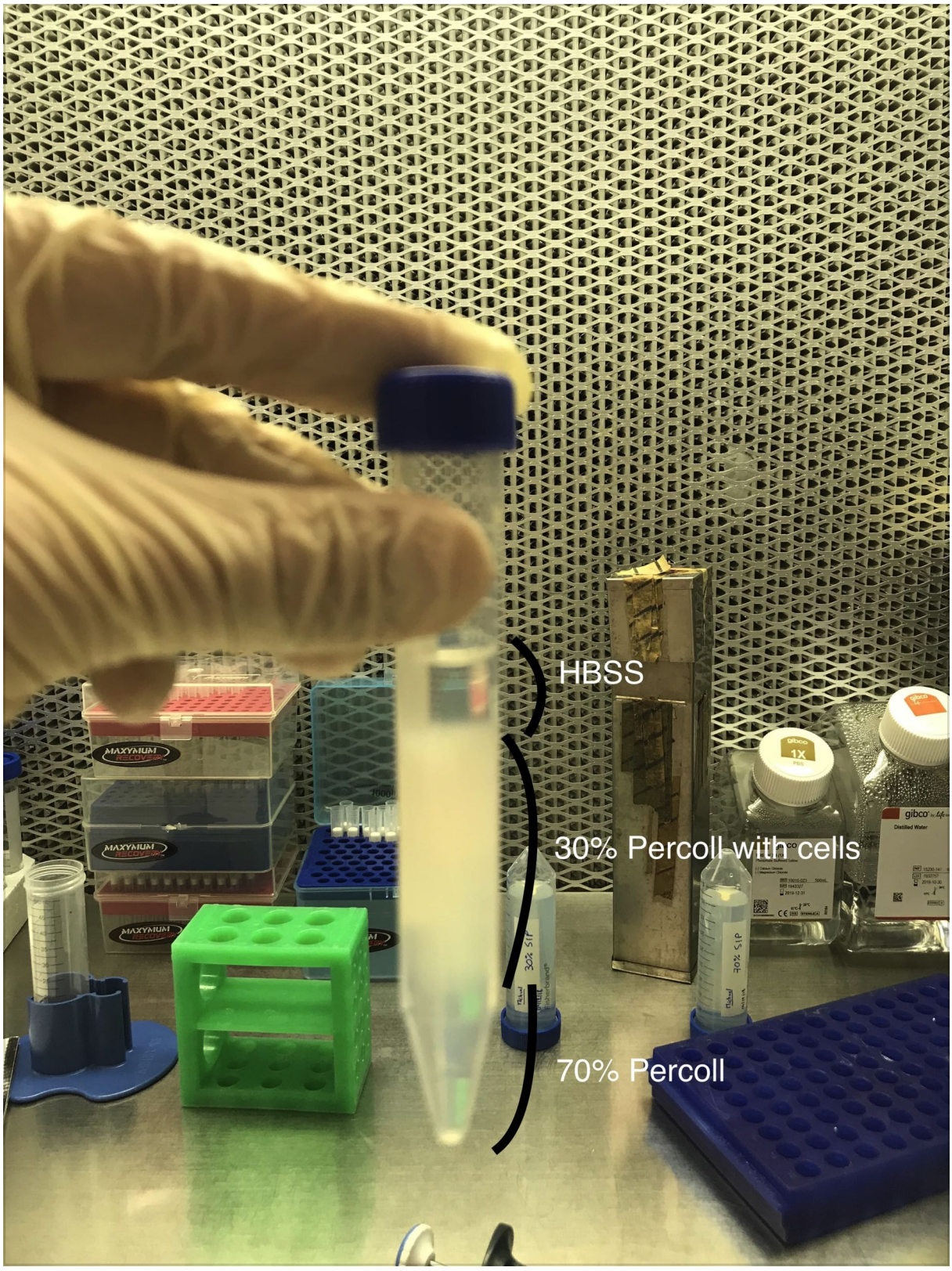
➜ Protocol optimized for neuron sorting

- Remove the brain from the skull
- Cut/dissect the area of interest
- Incubate the tissue with frequent agitation (invert tubes) at 37°C for 20' in **2 ml of 2 mg/ml papain solution in Hibernate-A**
- Perform 2 washes with 5 ml Hibernate-A including 1 mg/ml protease inhibitor
- After the second wash add 2 ml of Hibernate-A and briefly triturate the tissue using a ~~fire polished glass Pasteur pipettes~~ 1 ml pipette until a single-cell suspension is obtained (not too much)
- Pass the suspension onto 70 µm filter previously equilibrated with 2 ml of Hibernate-A. After passing the cells, wash the filter with 1 ml of Hibernate-A
- Centrifuge 5' at 700g
- Resuspend cells in 4 ml of 30% SIP Percoll
- Transfer in a 15 ml tube and underlay 4 ml of 70% SIP
- Add on top of the 30% SIP 2 ml of 1x HBSS
- Centrifuge 30' 400g at RT with **NO BRAKE**
- Remove myelin and debris from the top of the tube and collect the 70%-30% interphase (approximately 2-3 ml but not the pellet) and transfer to a clean 15 ml tube
- Add up to 15 ml 1x HBSS and mix a few times by inversion
- Centrifuge 5' at 700g at RT (18°C)
- Resuspend cells in 1 ml of facs PBS (**count the cells now**) + DAPI in a 15 ml tube and incubate 10'
- Add 10 ml of facs PBS and centrifuge for 5' at 700g
- Resuspend the cells in 0.350-0.400 ul of facs PBS and keep on ice (+aluminium) until facs

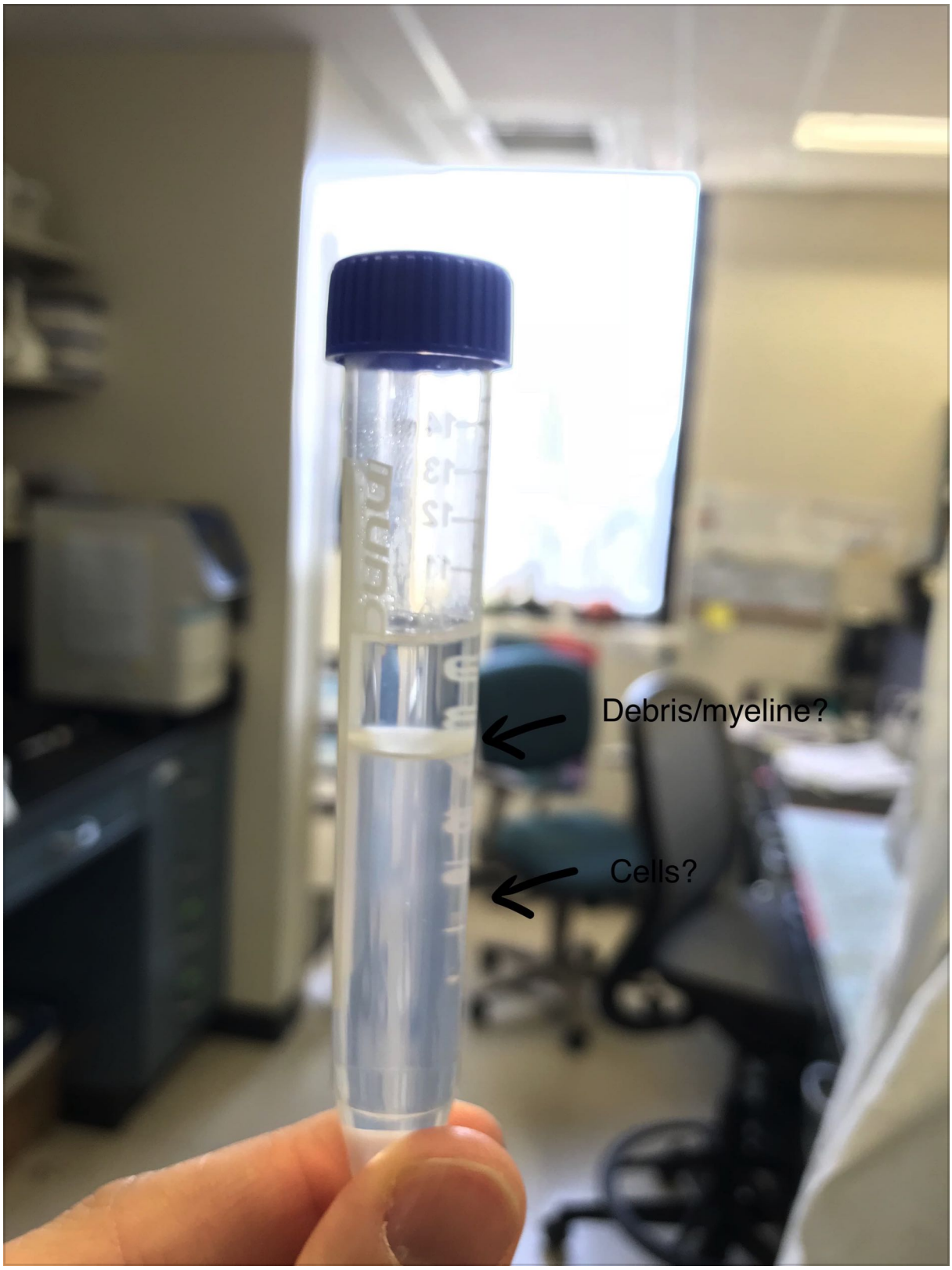
#facs

First test after Percoll

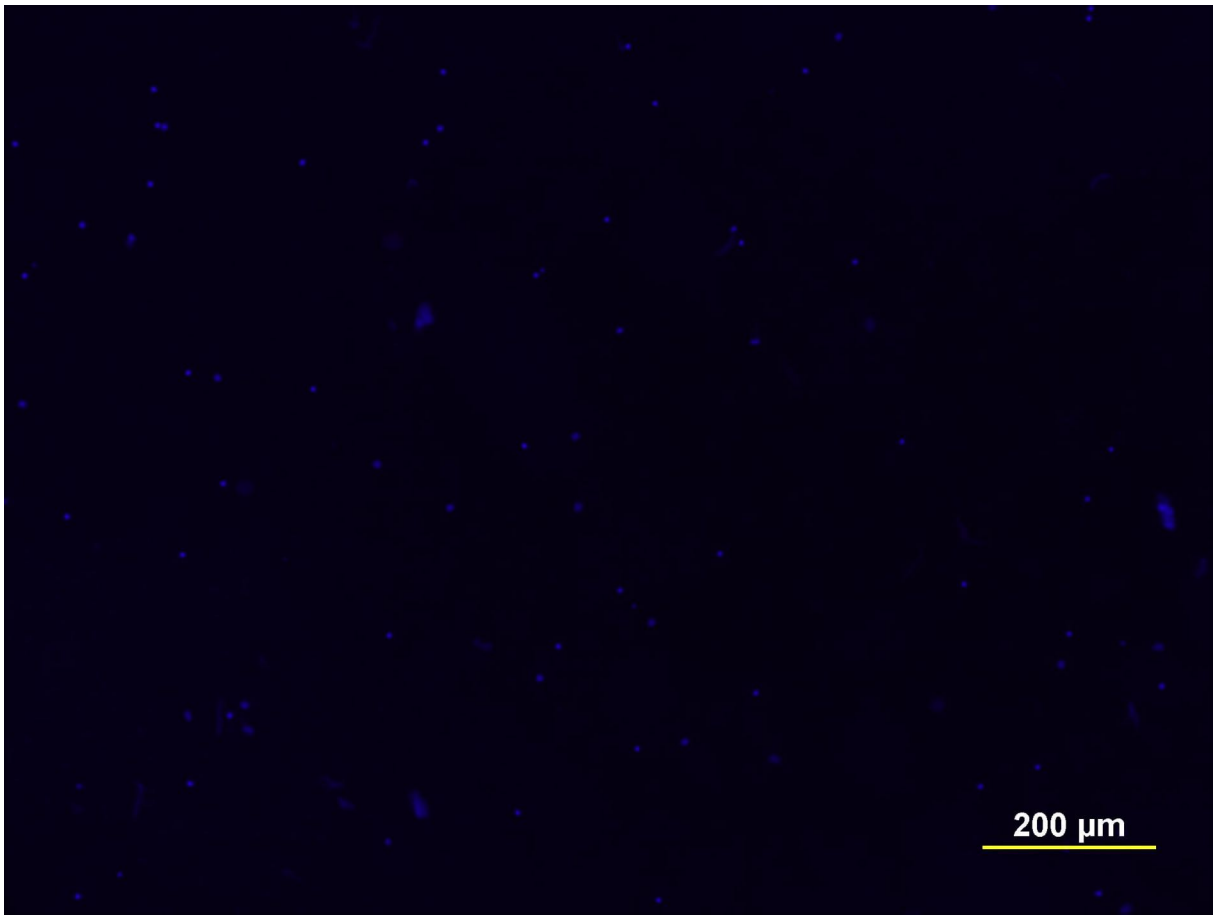
- Percoll before centrifugation



- Percoll after centrifugation



- DAPI after percoll process



- Pictures of the cells placed in well