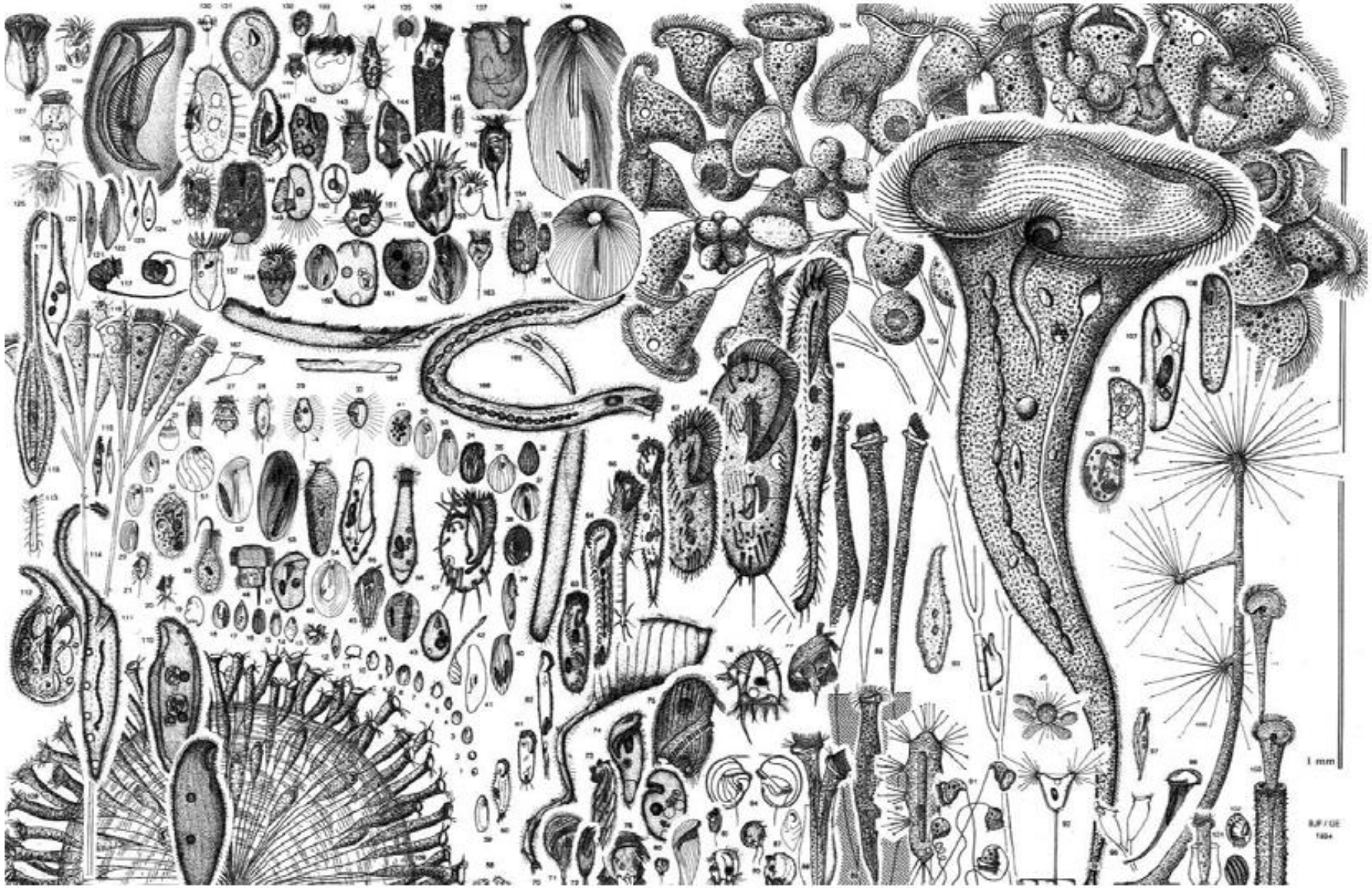
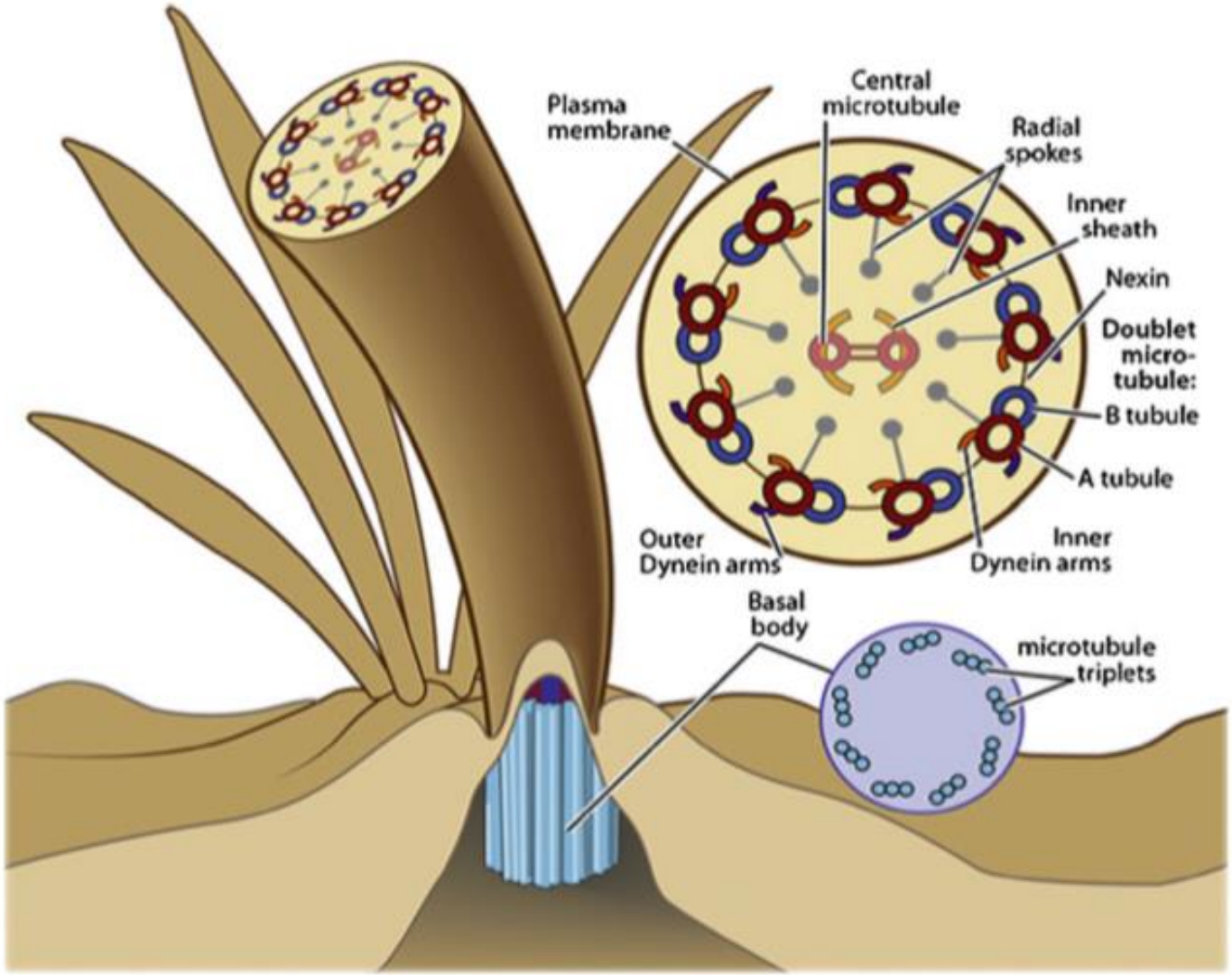
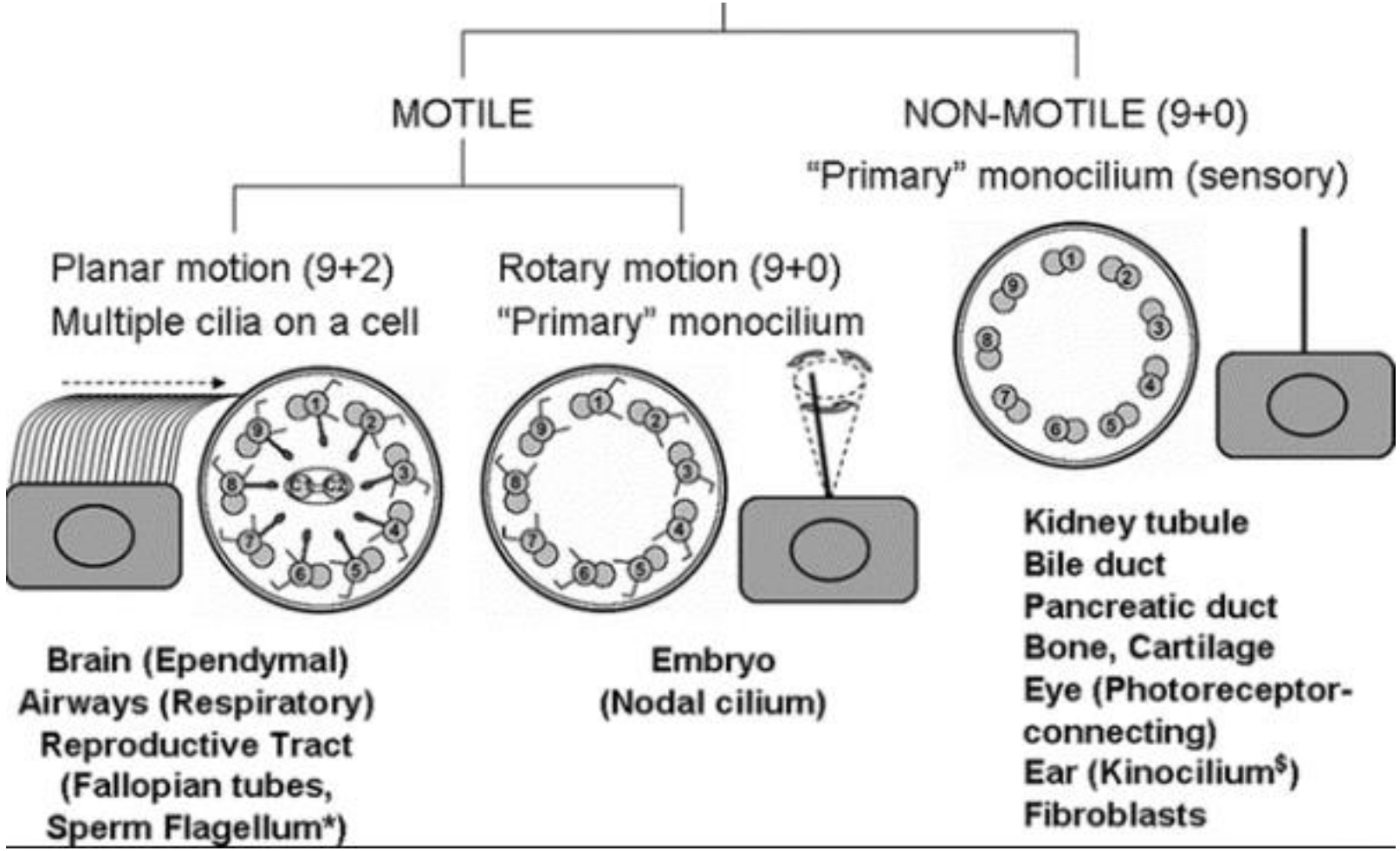
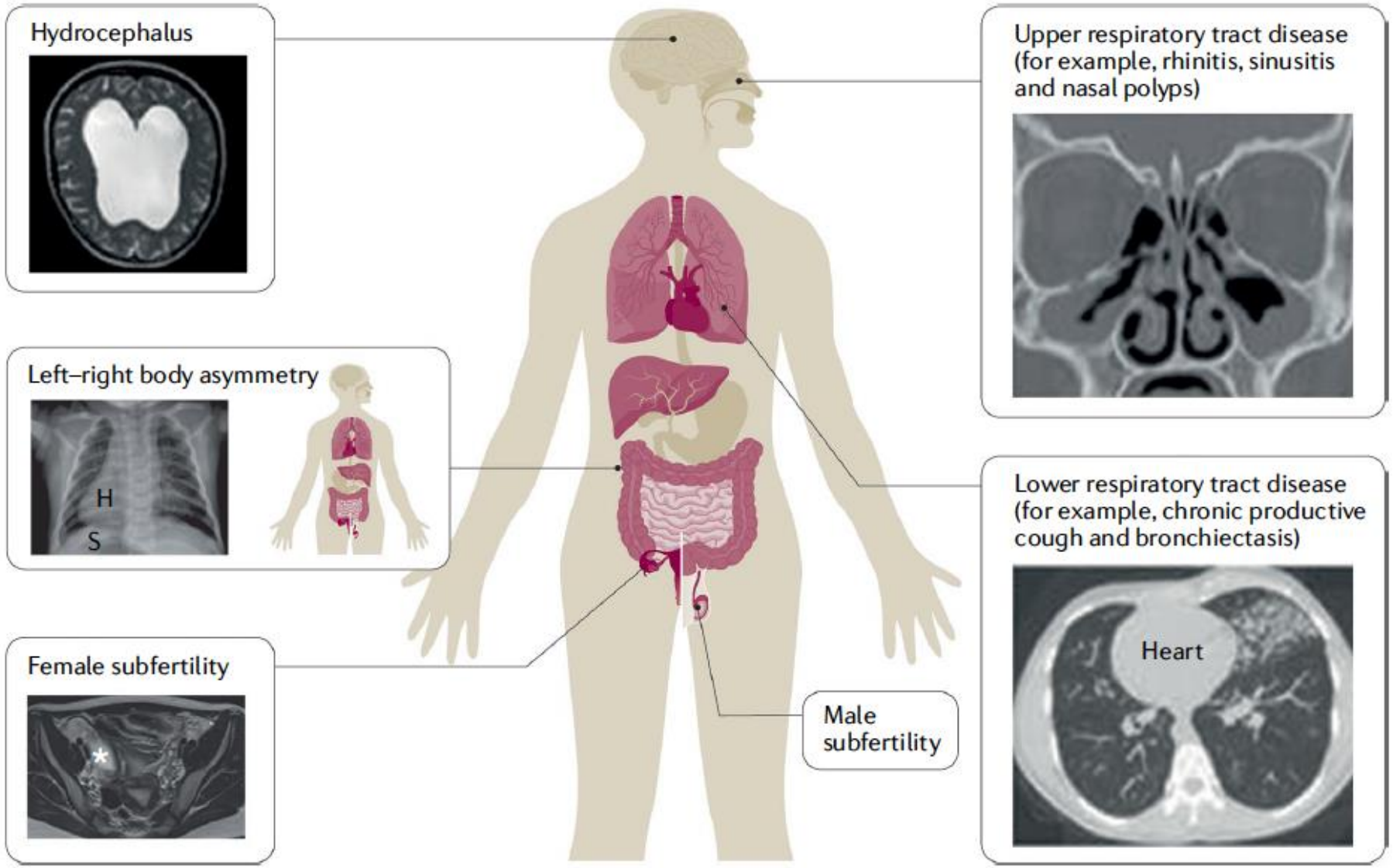

1 mm

Motile cilia ultrastructure



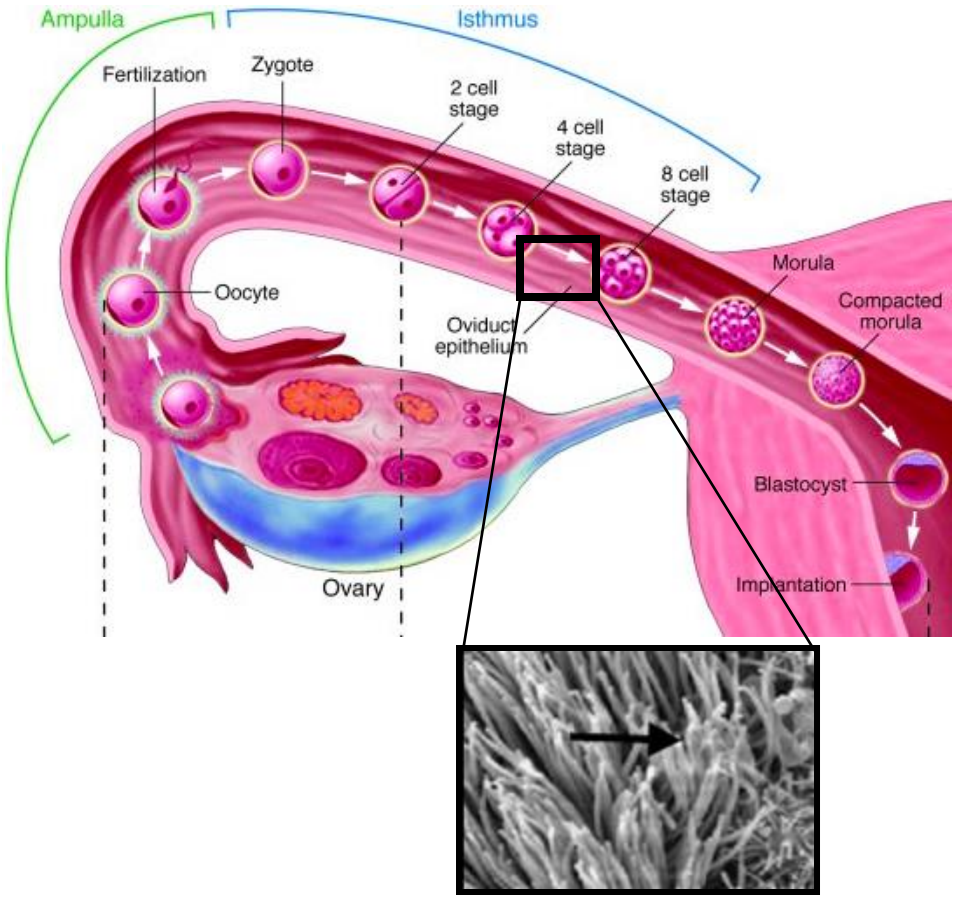

MOTILE
NON-MOTILE (9+0)
"Primary" monocilium (sensory)
Planar motion (9+2)
Multiple cilia on a cell
Rotary motion (9+0)
"Primary" monocilium
Brain (Ependymal)
Airways (Respiratory)
Reproductive Tract
(Fallopian tubes,
Sperm Flagellum*)
Embryo
(Nodal cilium)
Kidney tubule
Bile duct
Pancreatic duct
Bone, Cartilage
Eye (Photoreceptor-connecting)
Ear (Kinocilium$)
Fibroblasts

Primary Ciliary Dyskinesia

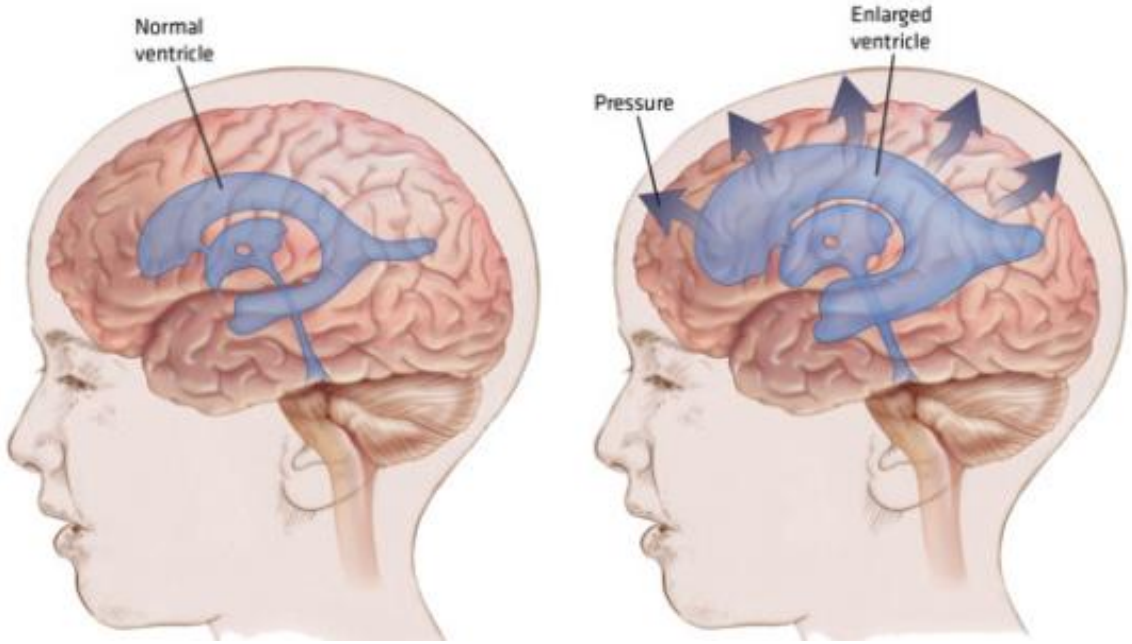


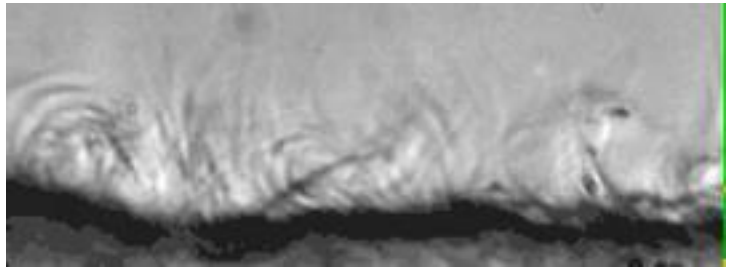
Wallmeier et al. Nature Rev. disease primers, 2020

Infertility in both male and female

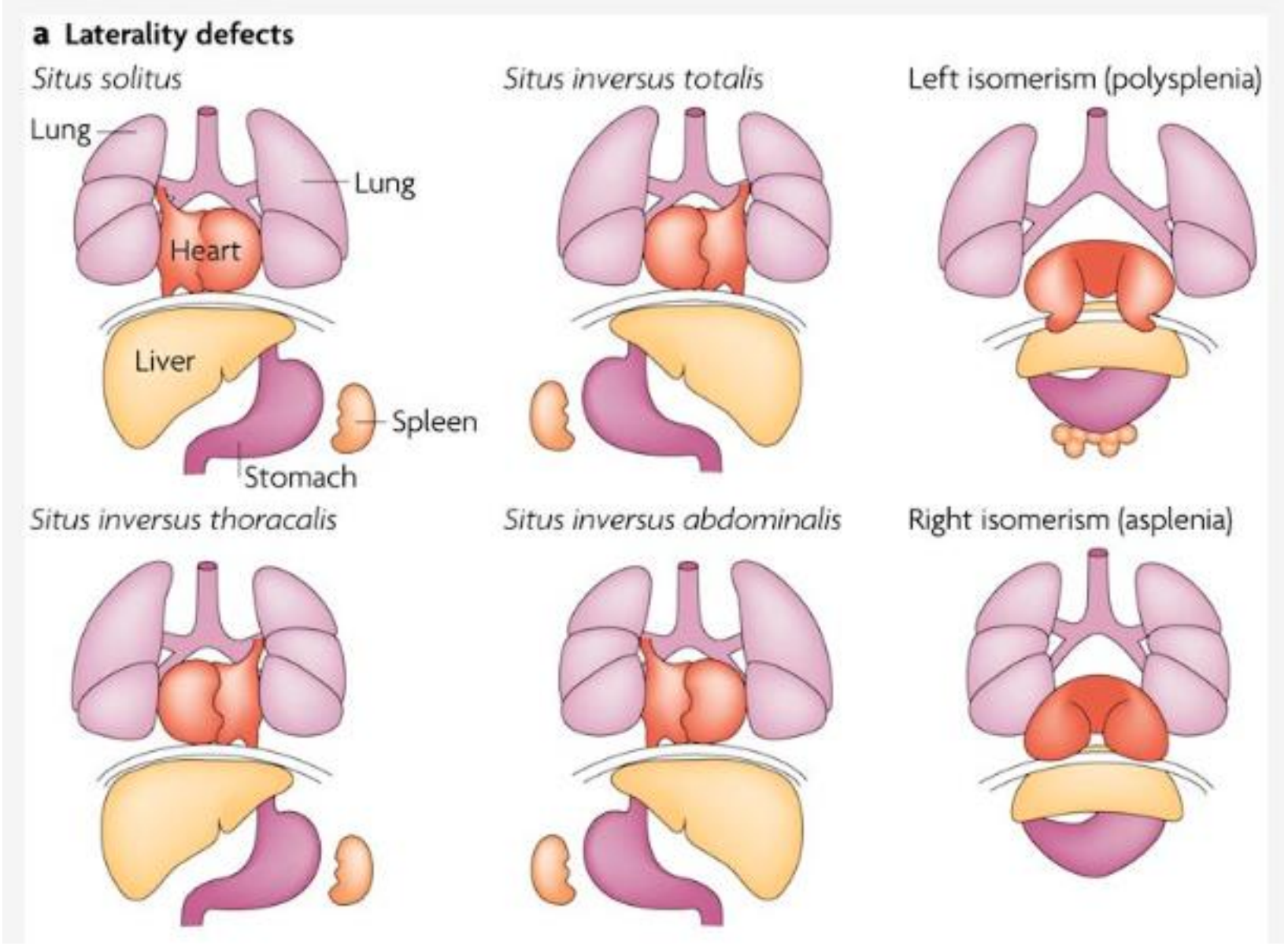


Hydrocephaly

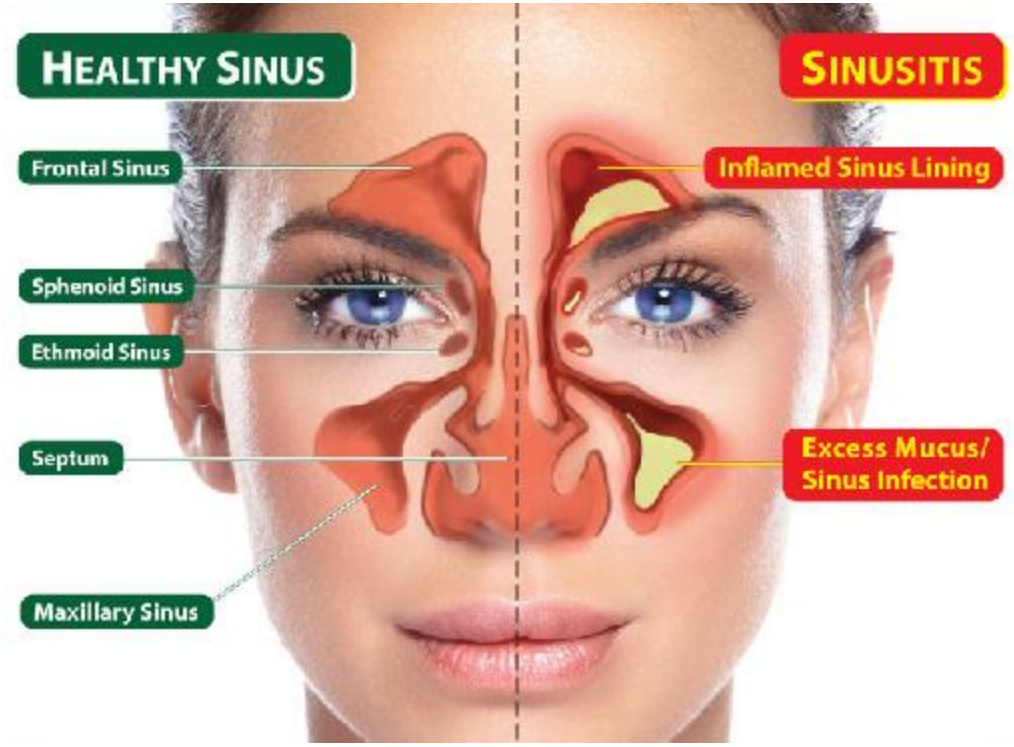


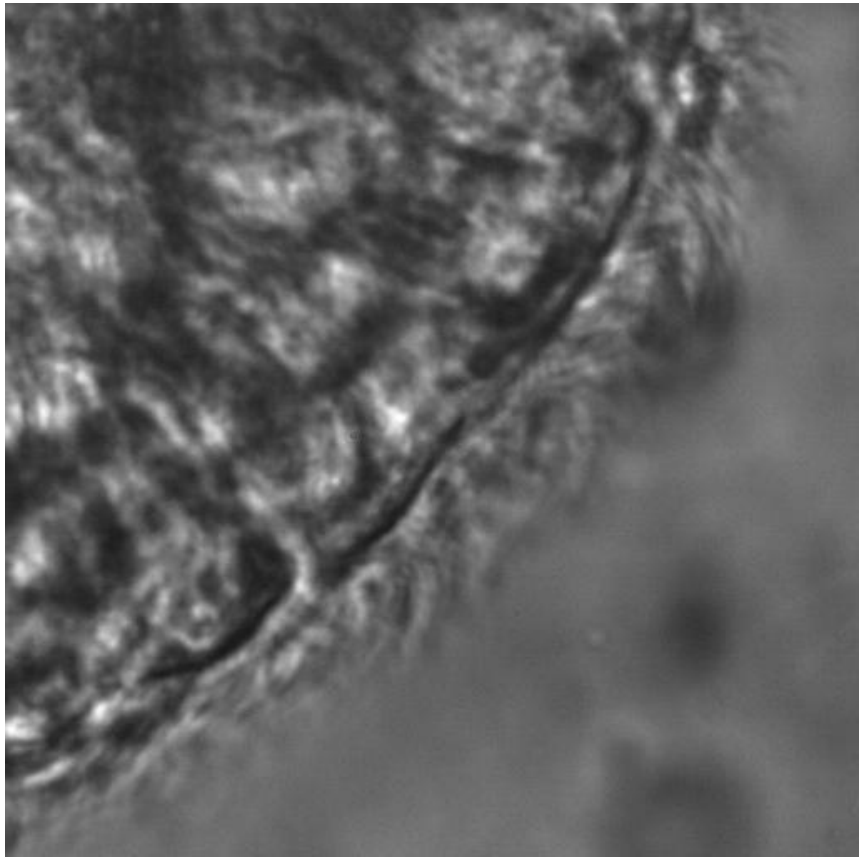


Internal organ laterality

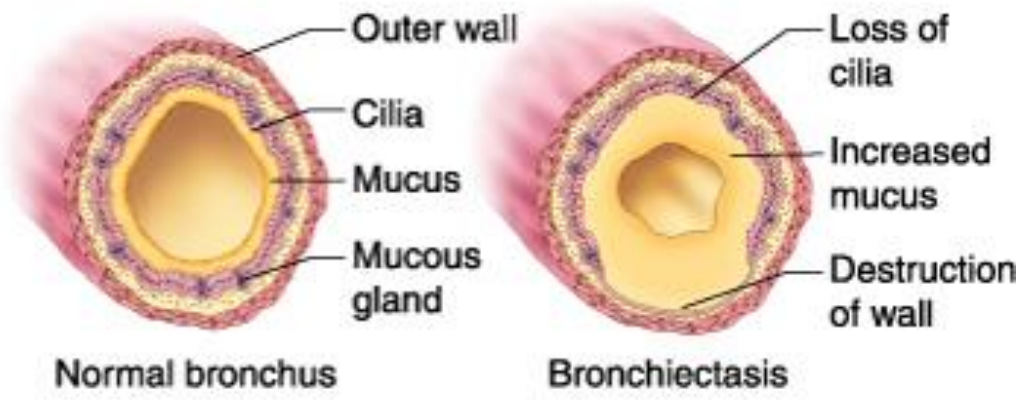


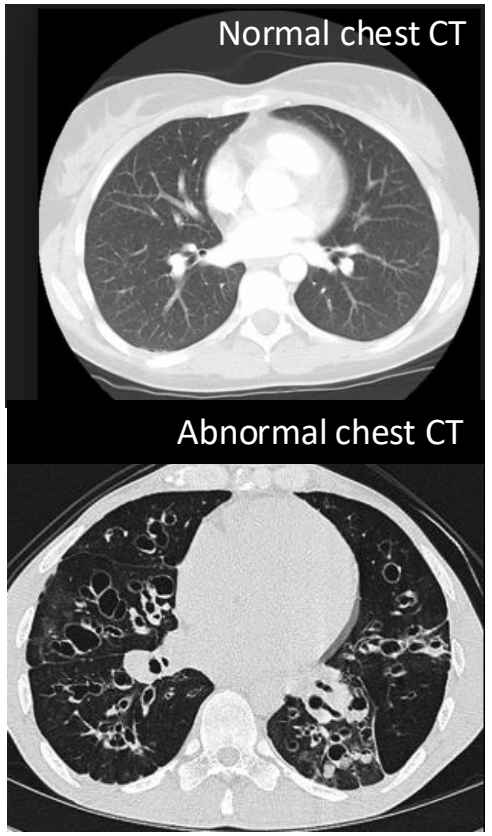
Impaired mucociliary transport in upper respiratory tract





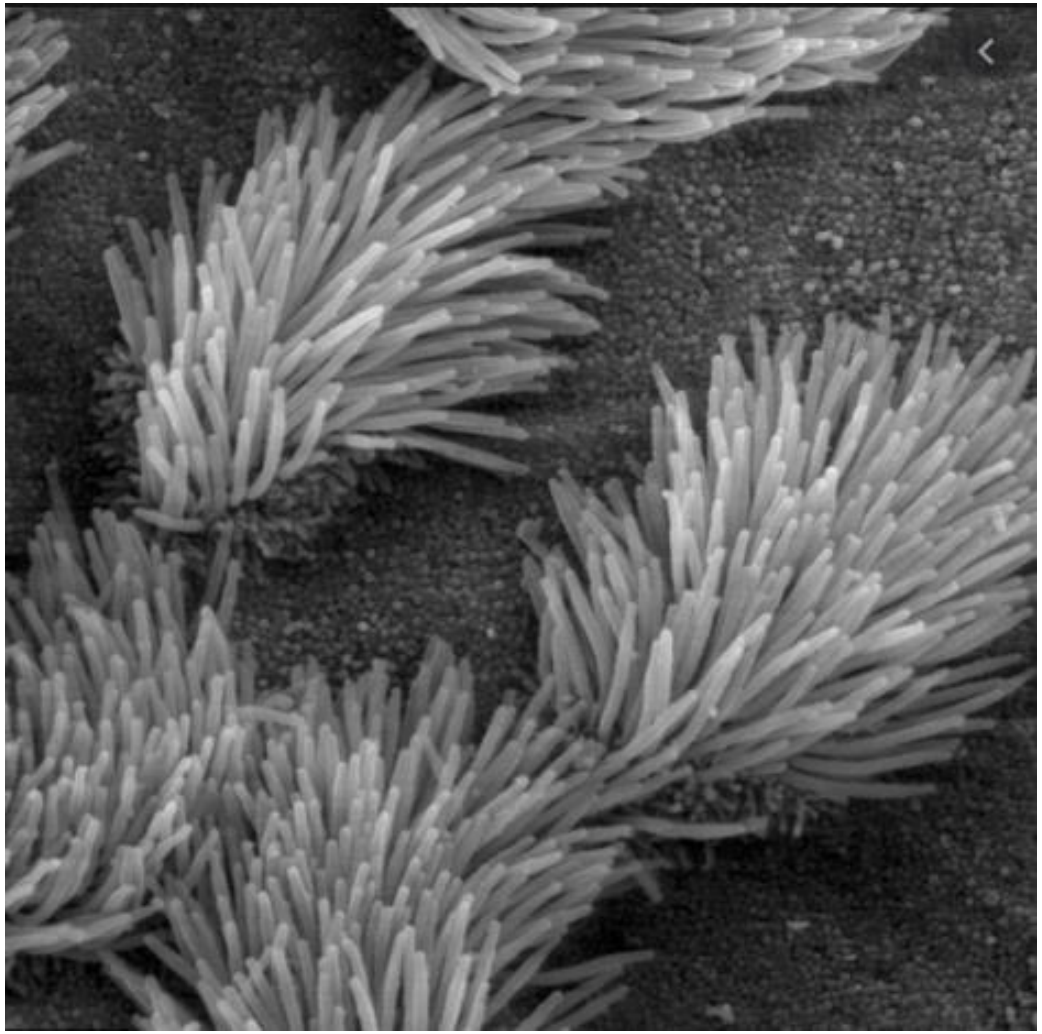
Lower respiratory tract - Bronchiectasis



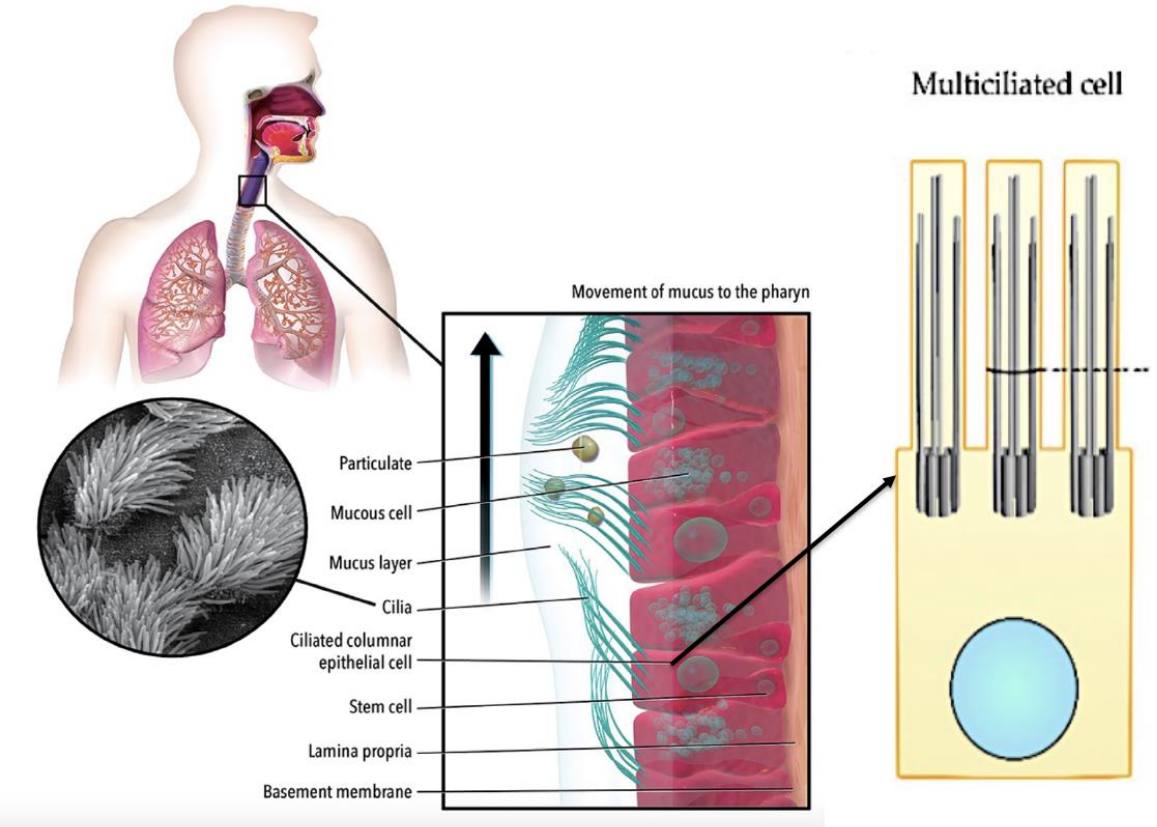


Mucociliary clearance (movie)

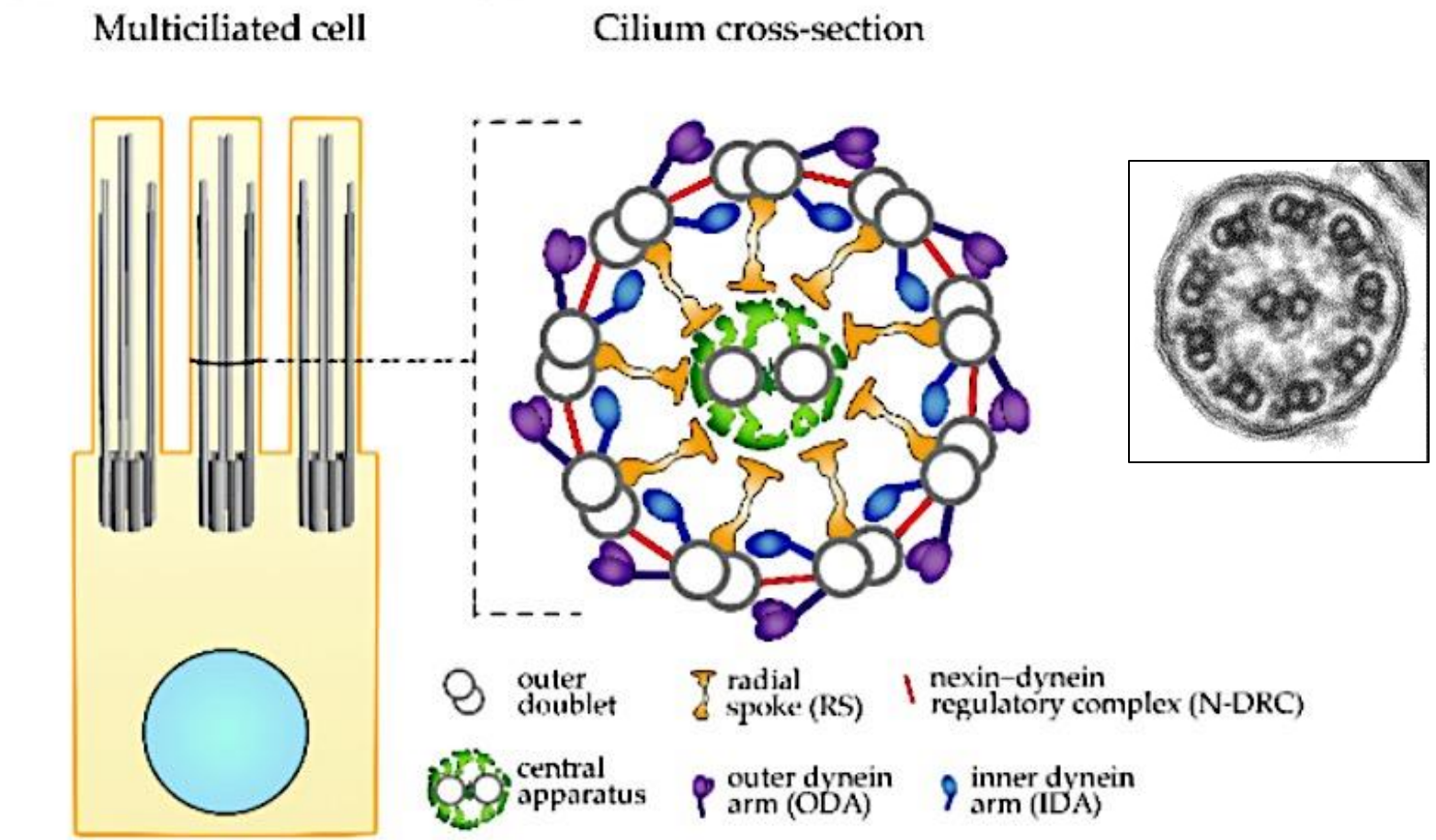
Motile cilia from the respiratory system



Respiratory multiciliated cells perform mucociliary clearance

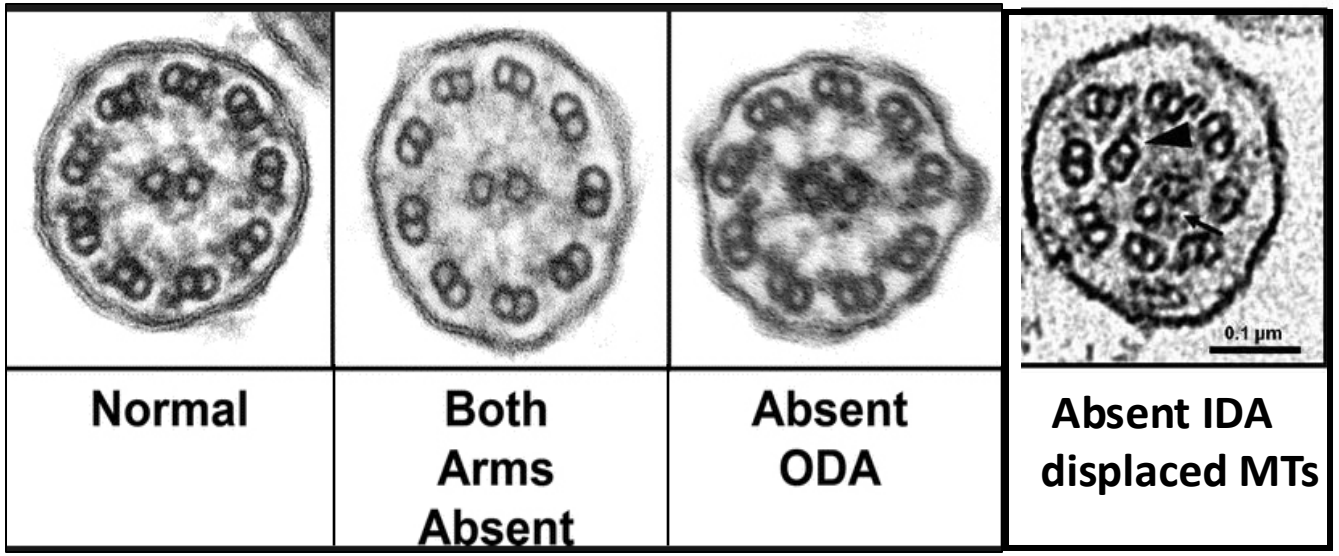


Multiciliated cells



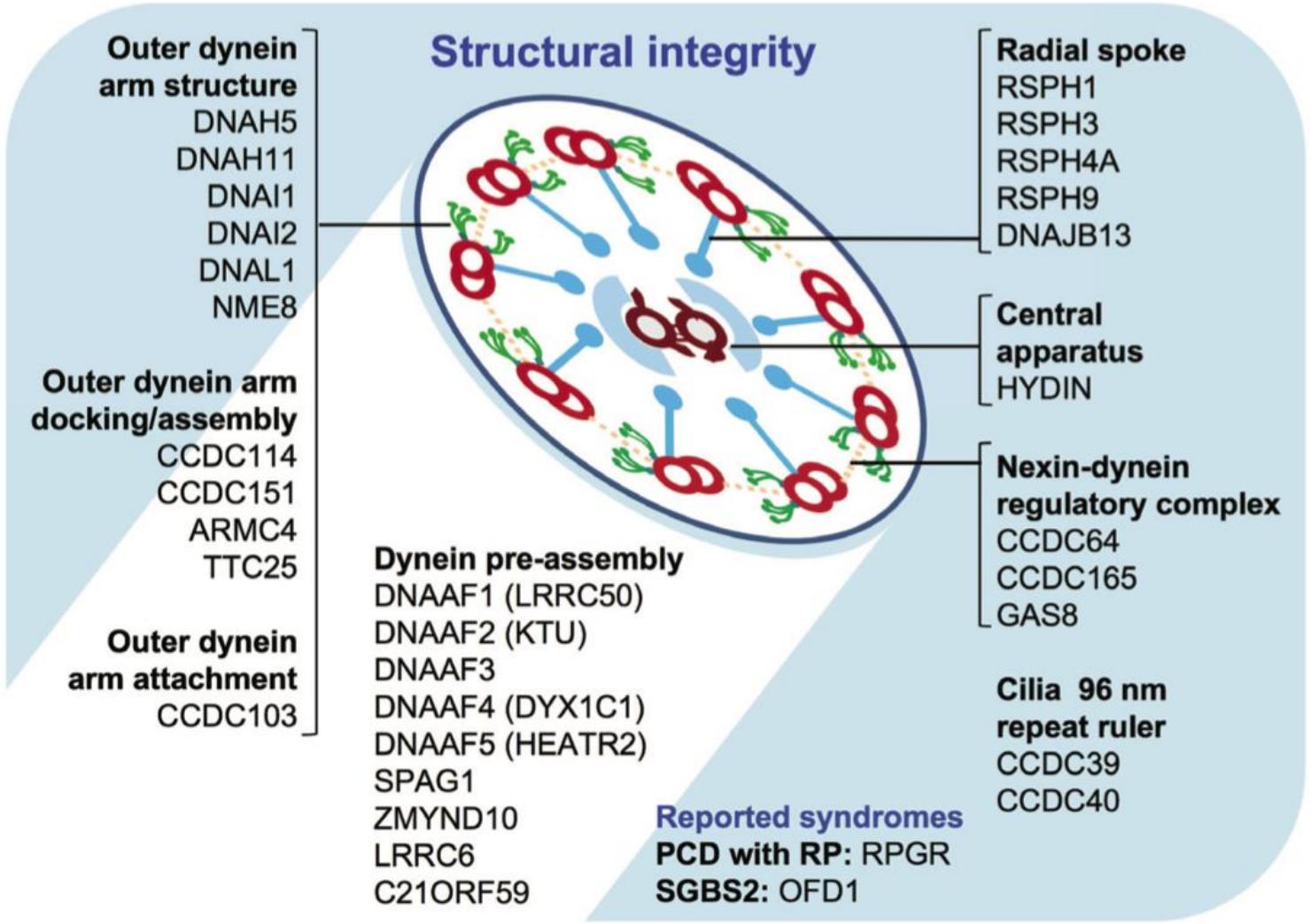
Poprzeczko et al. Cells, 2019

Some structural defects observed in PCD patients



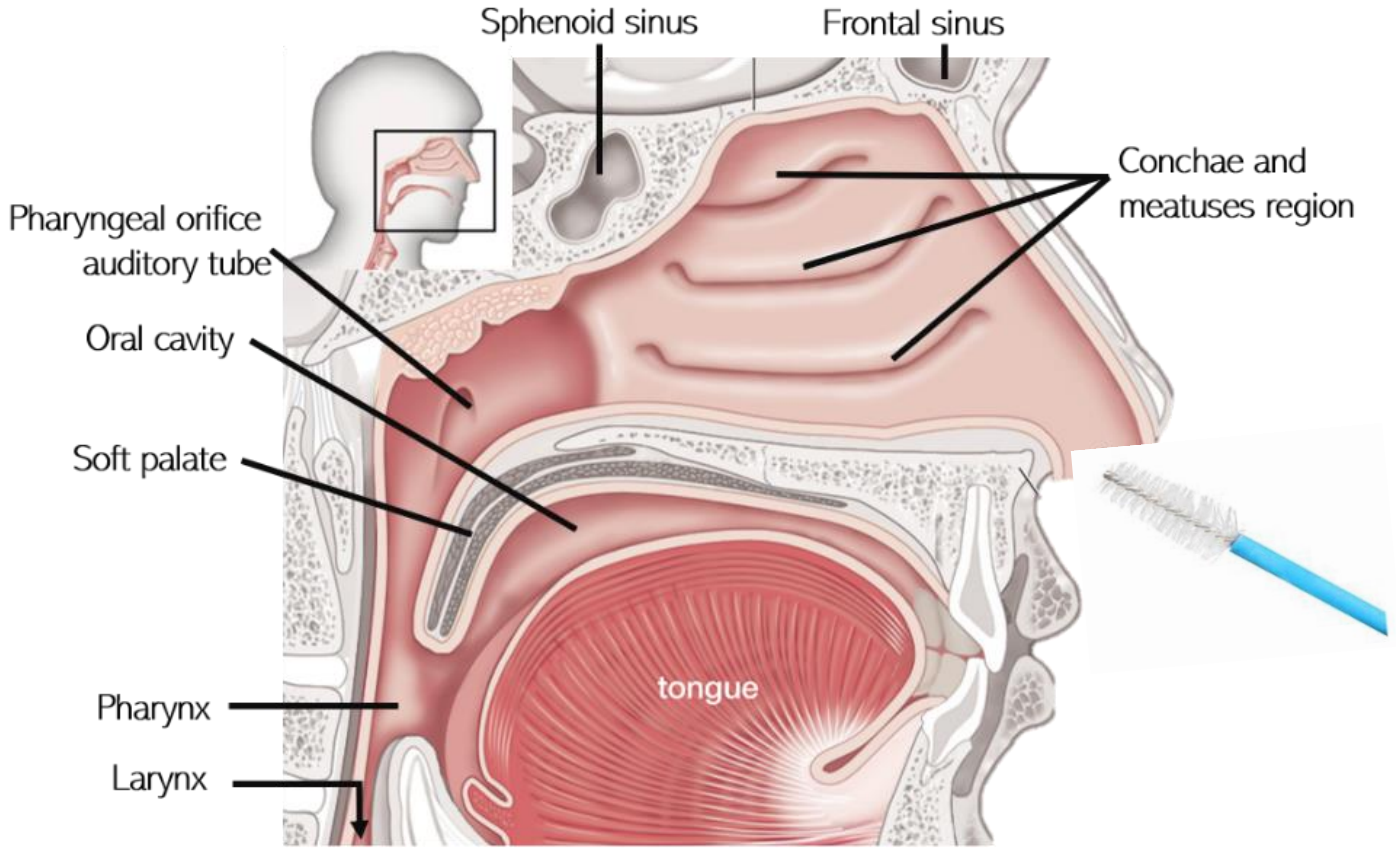
Adapted from Leigh et al. Genetics in Medicine, 2009

Mutated Proteins identified in PCD patients



Mitchison & Valente, J. Pathol 2017

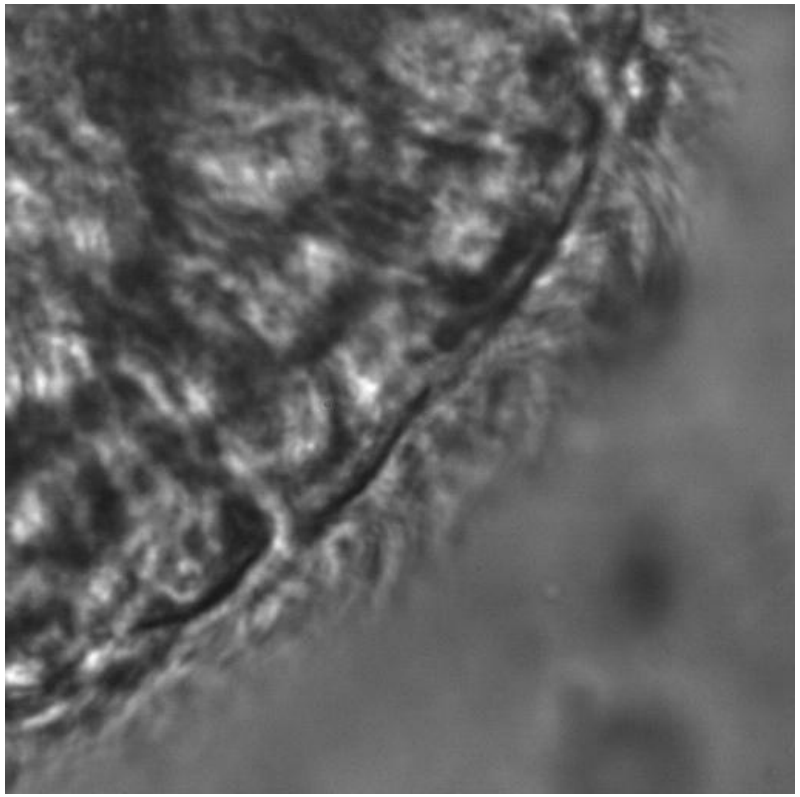
1 - Nasal brushings performed at hospital setting



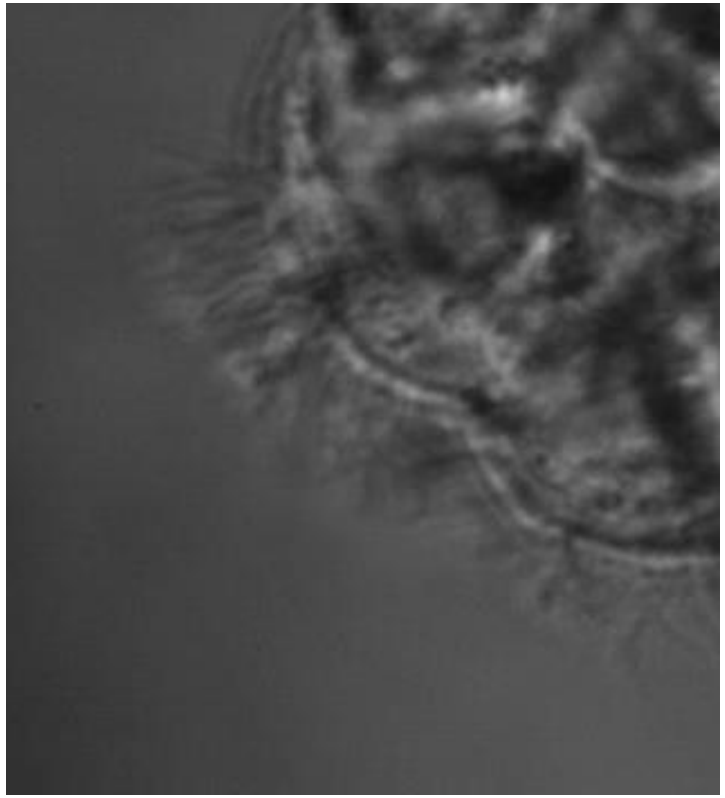
2 - High-speed Video-Microscopy Analysis (HVMA)

- Nikon Eclipse Ti-U
- FASTCAM MC2 camera (Photron)

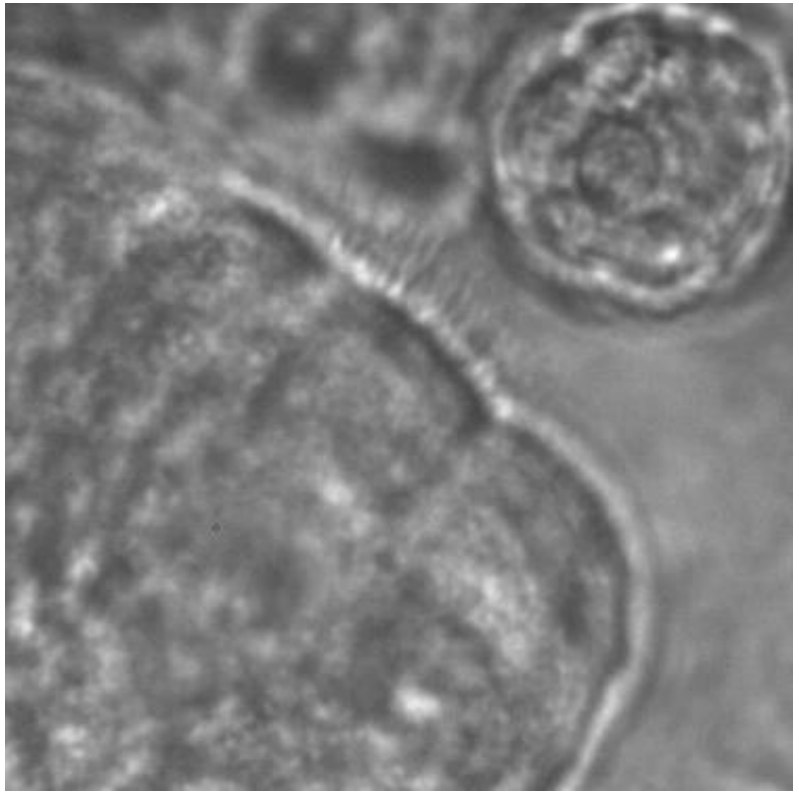
Human nasal samples are used for PCD diagnostic



Healthy control

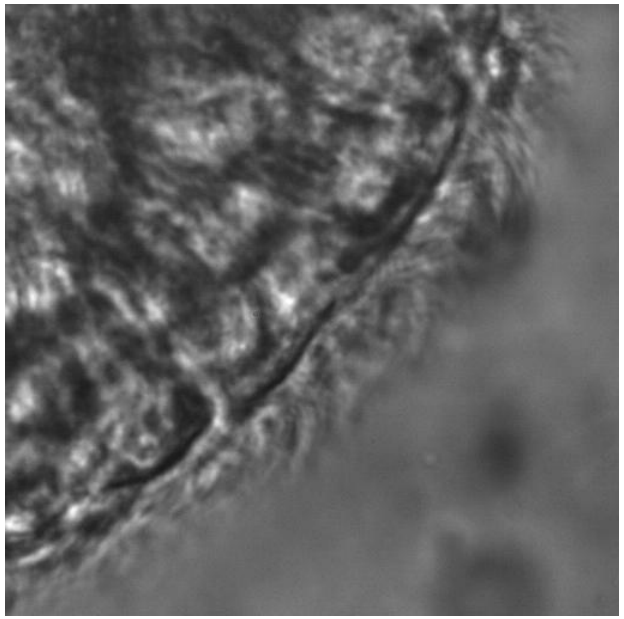


DNAH5 patient

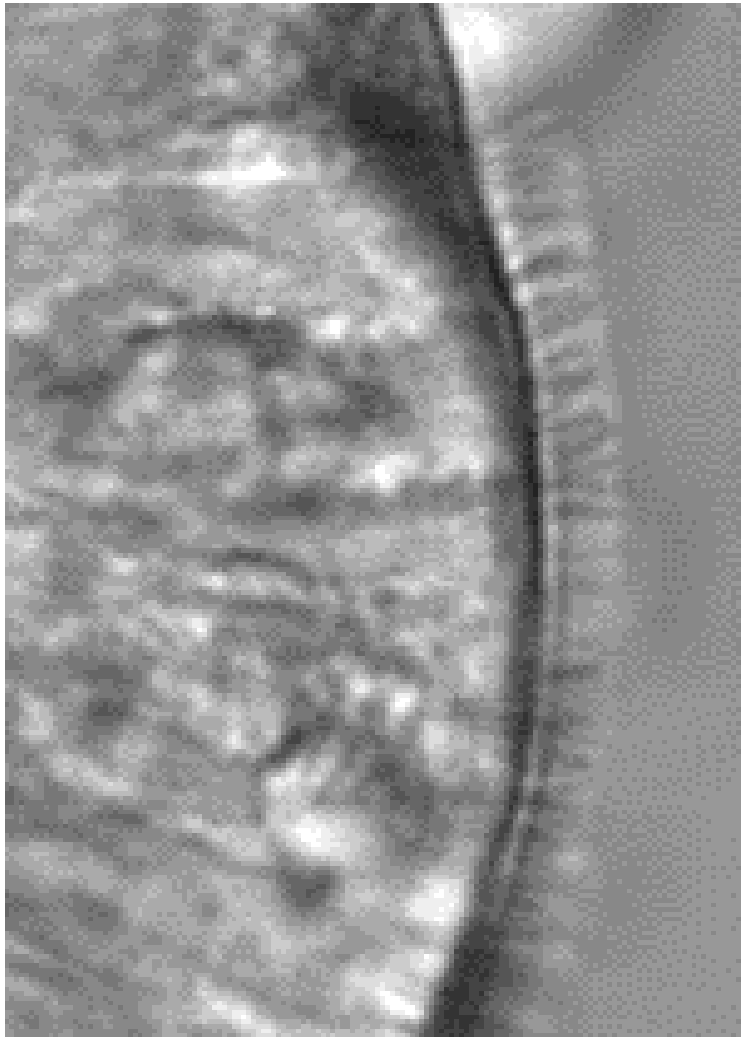


RSPH1 patient

Coiled-Coil Domain-Containing Protein 40 (CCDC40)

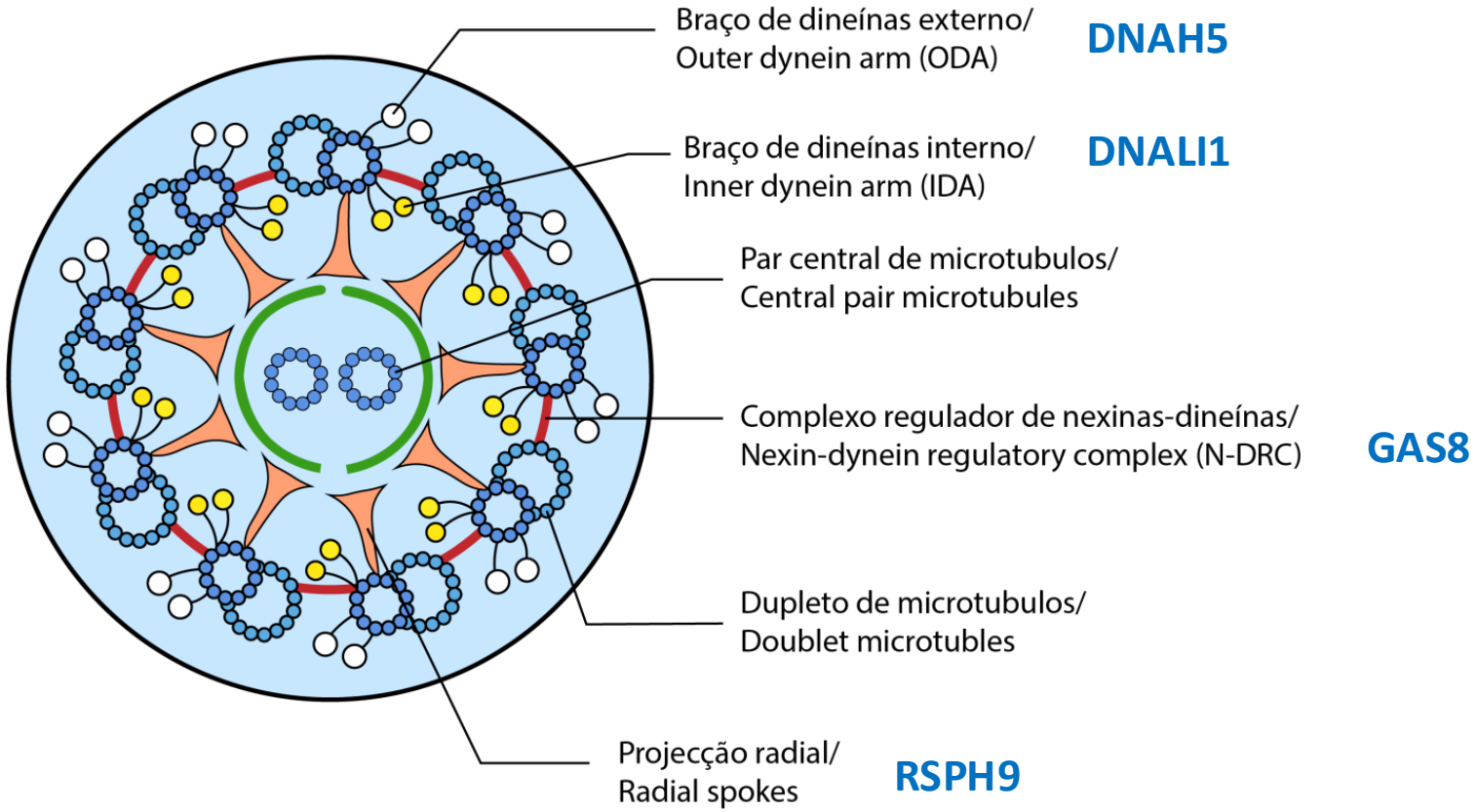


Healthy control

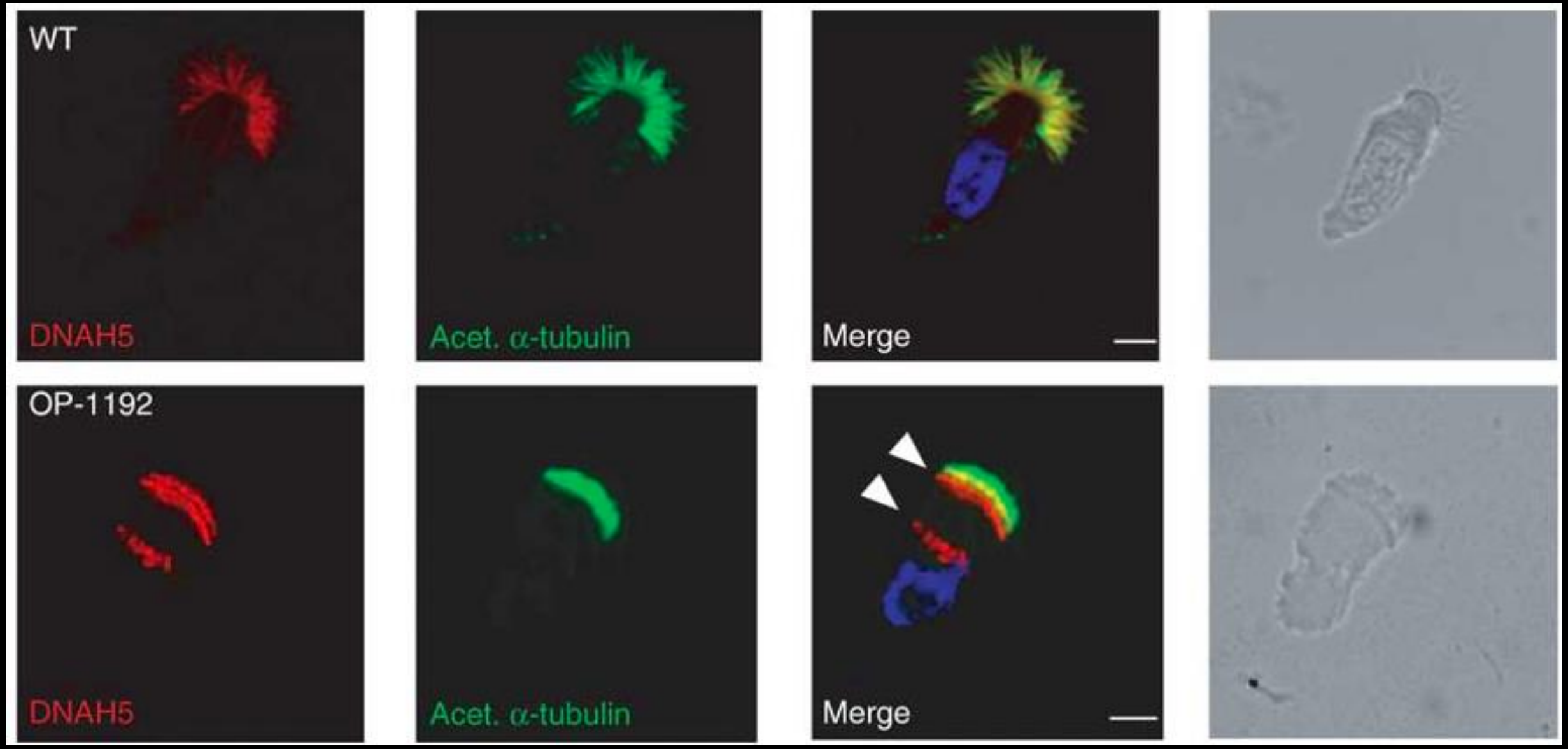


CCDC40 patient

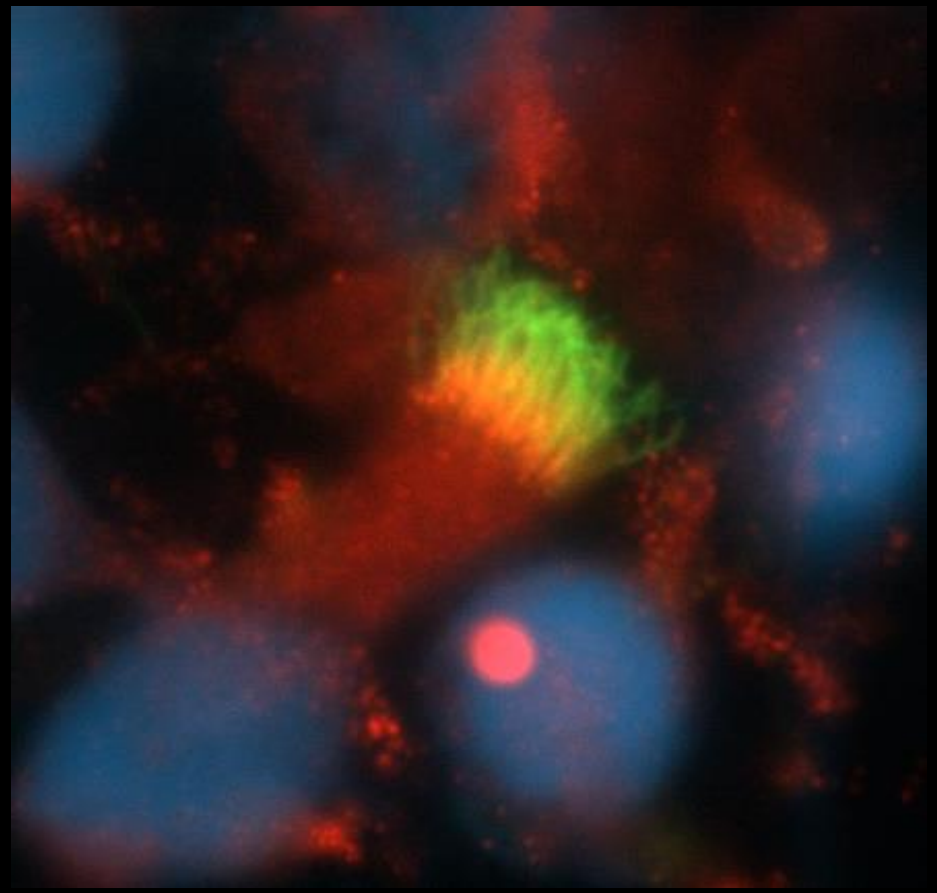
3 - Detection of 4 proteins that will help to diagnose PCD



Immunofluorescence speeds-up PCD diagnostic



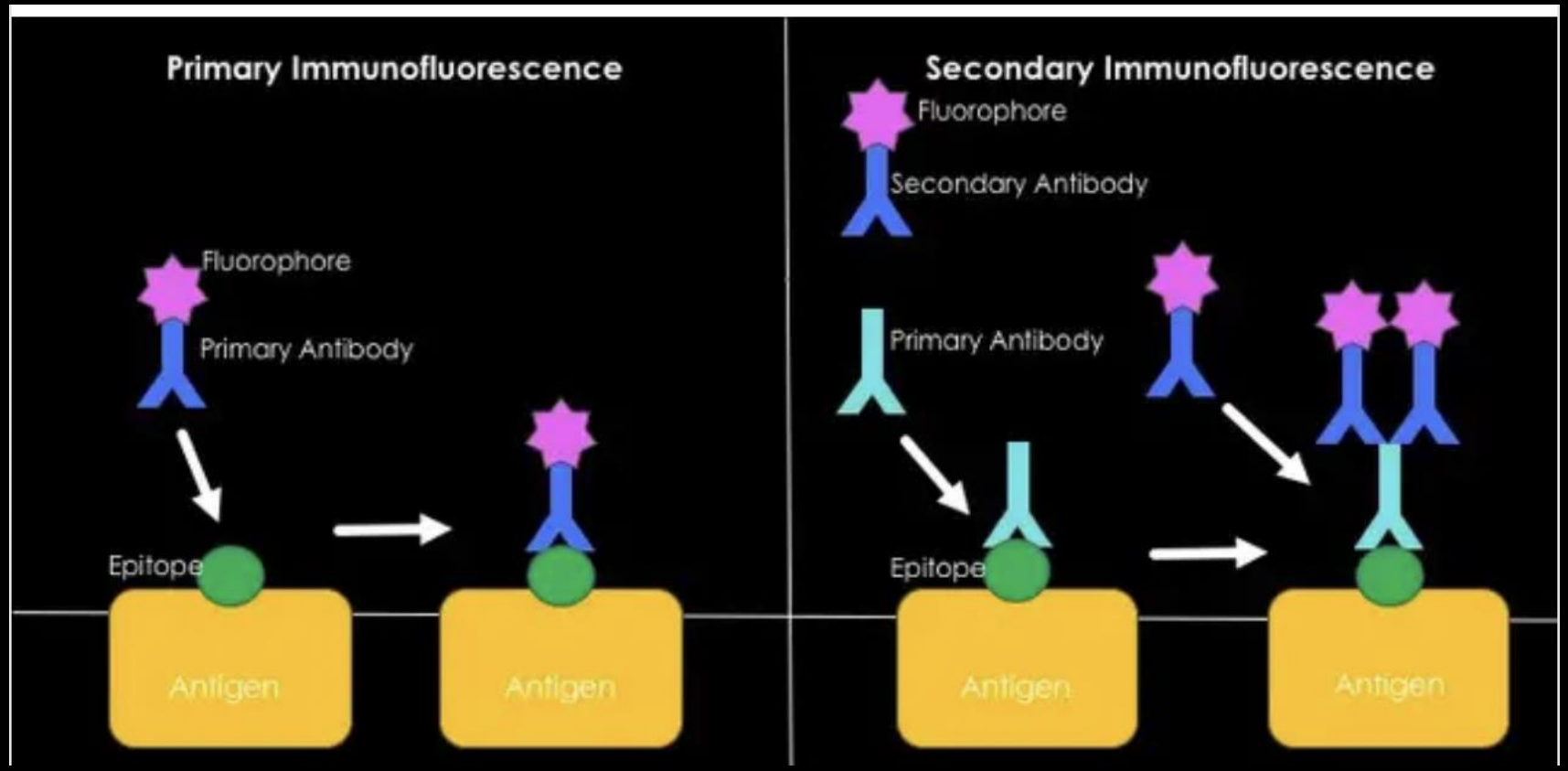
Immunofluorescence in single cells helps PCD diagnostic



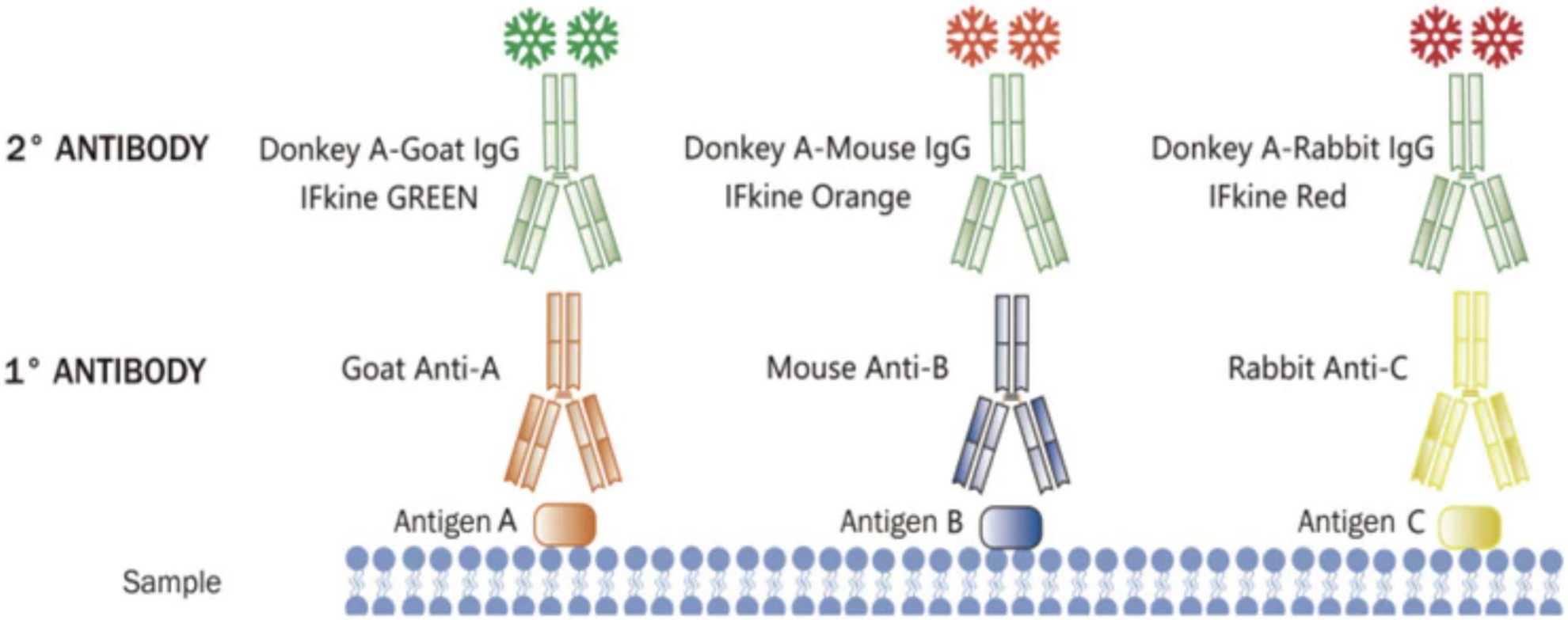
Fluorescent immunostainings

Deteção Direta

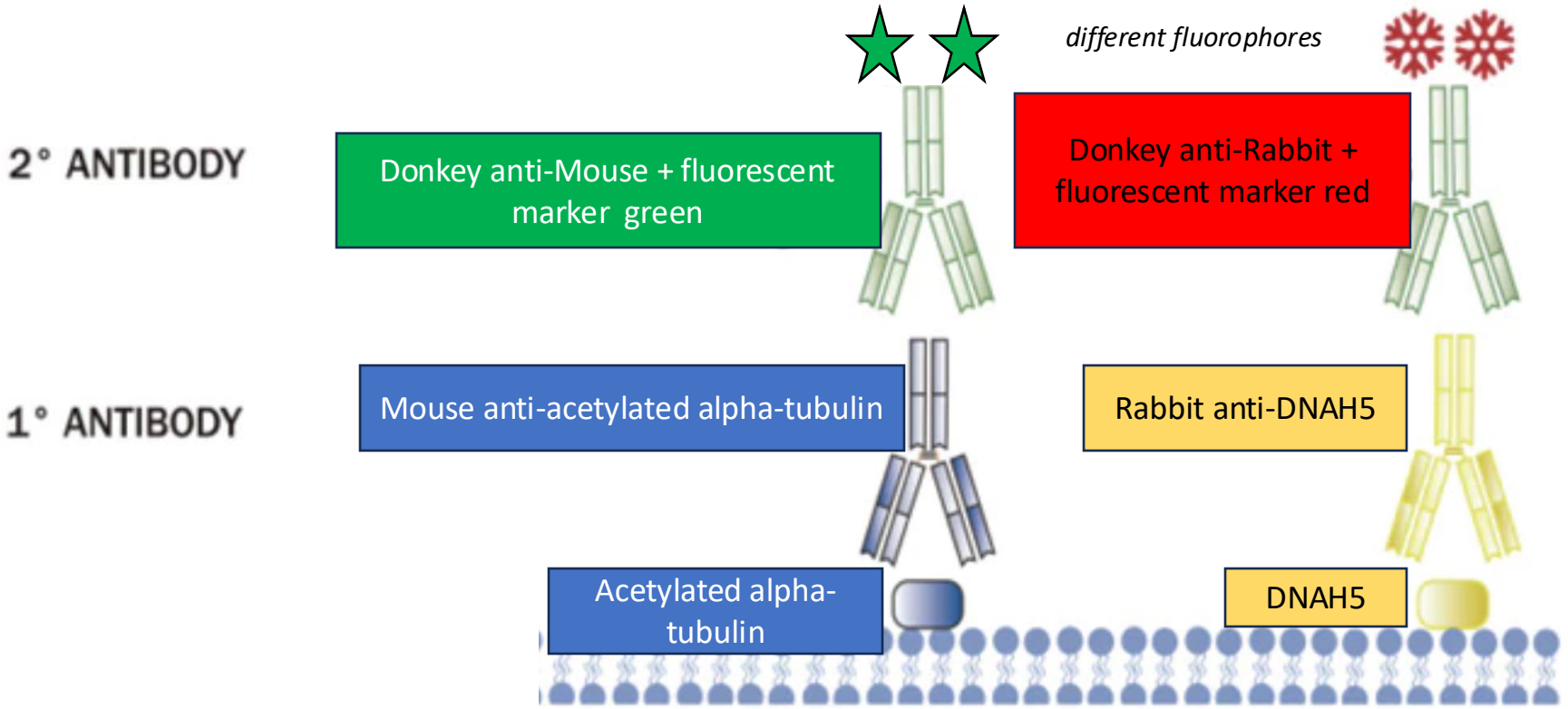
Deteção Indireta



Detection of multiple proteins (antigens)



Our experiment



The basic steps of an immunofluorescent experiment

- Wash in PBS (see protocol for detailed protocol)
- Fix in PFA 4%
- Wash in PBS
- Permeabilyze in Triton-X
- Block in milk
- Incubate with primary antibodies in milk
- Wash extensively in PBS
- Incubate with secondary antibodies in milk
- Wash in PBS
- Mount with coverslip using a fluo shield medium (Dako)

Antibody dilutions

Working dilutions for primary antibodies (tested):

1:1000 Mouse anti-acetylated alpha-tubulin

1:300 Rabbit anti-DNAH5

Each slide needs 200 μl to cover all the cells, so use:

1 μl 1ºAntibody Mouse anti-acetylated alpha-tubulin +

3,3 μl 1ºAntibody Rabbit anti-DNAH5 in a total of 995,7 μl of milk.