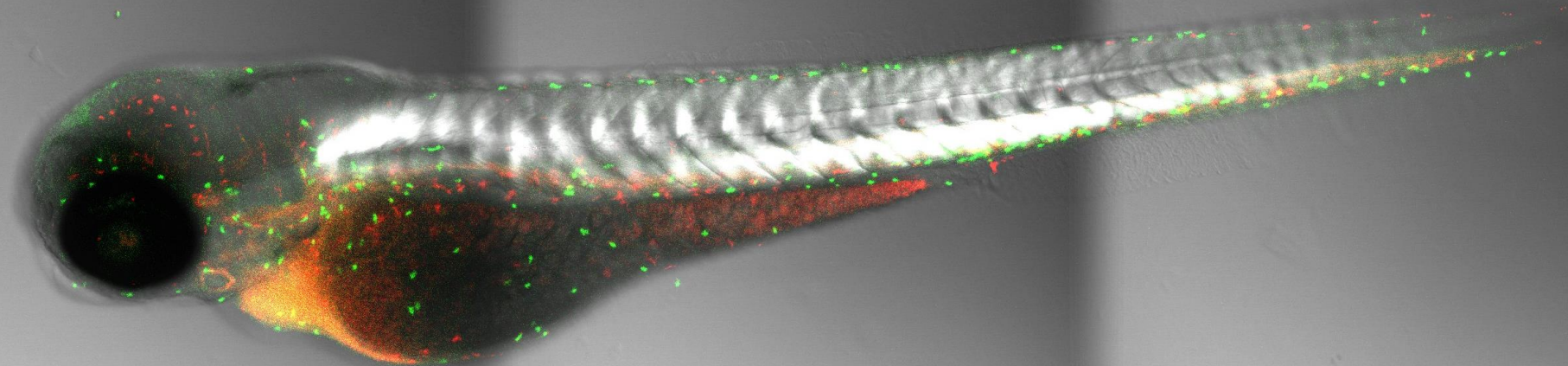


# Zebrafish model for PMM2-CDG

World Conference on CDG

26-07-2019

Paola de Haas





# A cell in a culture flask $\neq$ complex human being

---

We “spoil” the cells with the culture conditions

There is no interaction with other cell types

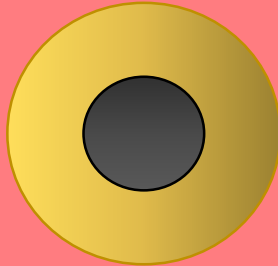
Structural components present in tissues are missing

37 °C

5% CO<sub>2</sub>

25 mM glucose

Etc.....



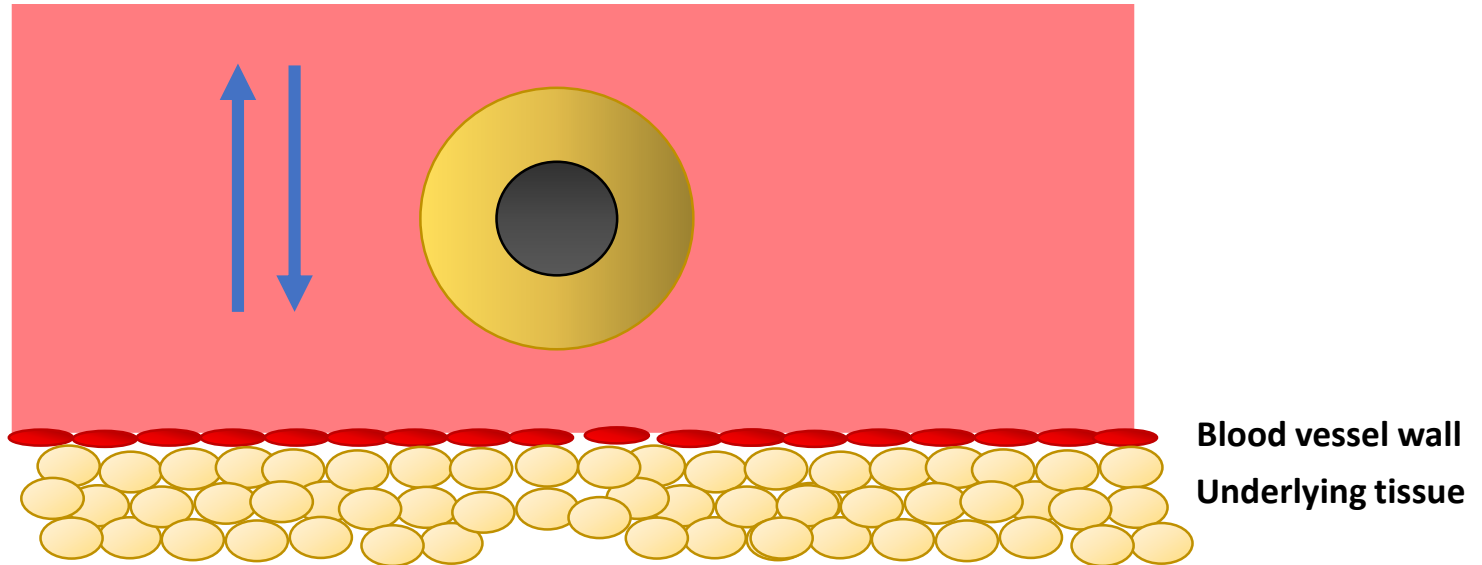
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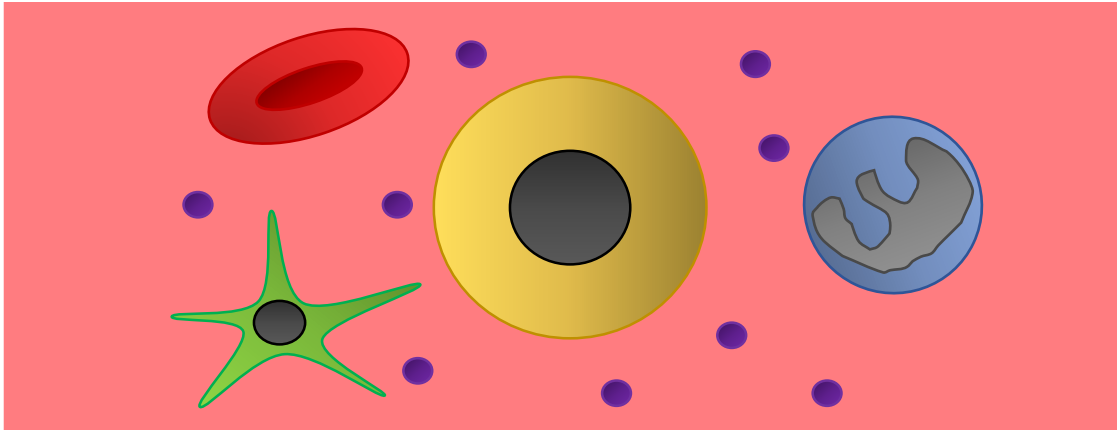
# A cell in a culture flask $\neq$ complex human being

---

We “spoil” the cells with the culture conditions

There is no interaction with other cell types

Structural components present in tissues are missing



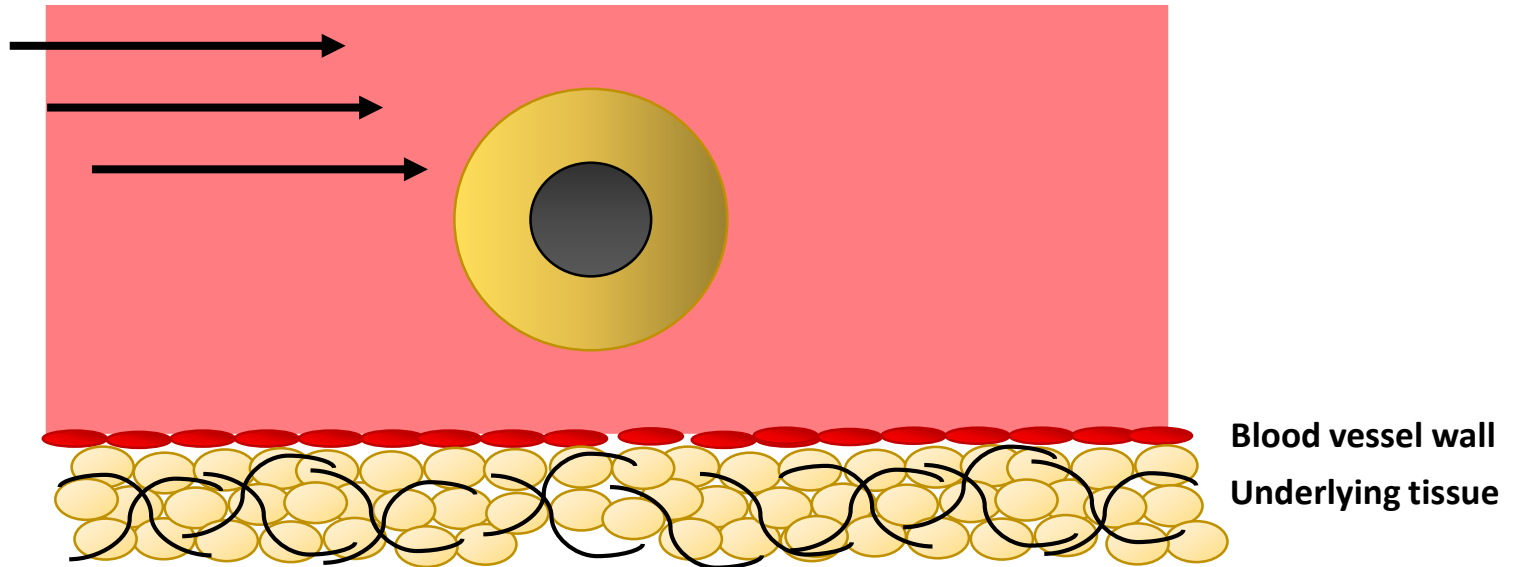
# A cell in a culture flask $\neq$ complex human being

---

We “spoil” the cells with the culture conditions

There is no interaction with other cell types

Structural components present in tissues are missing



# Zebrafish vs human

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70 % of human genes are also found in fish

Zebrafish have many tissues also present in humans

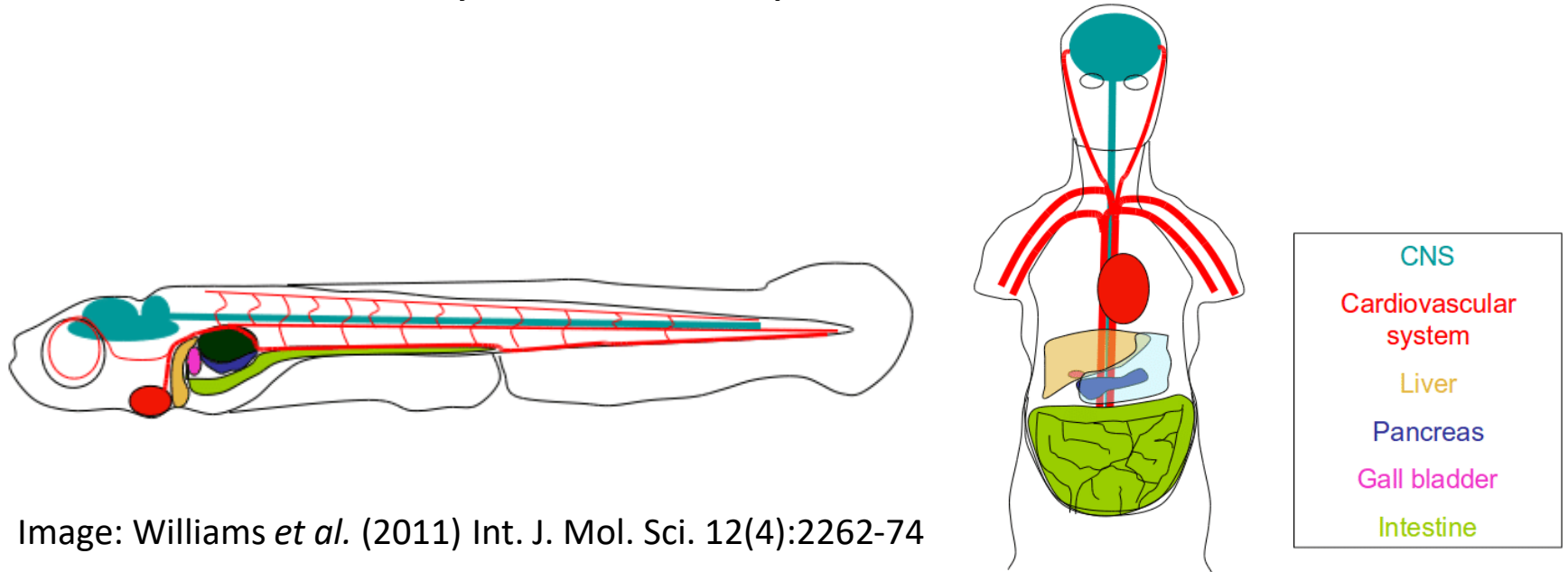


Image: Williams *et al.* (2011) *Int. J. Mol. Sci.* 12(4):2262-74

# Zebrafish to study immunity in CDG

Many CDG patients suffer from:

- recurrent infections
- reduced response to vaccinations

→ We want to study the immune system in CDG by using a zebrafish model

## Zebrafish immune system development

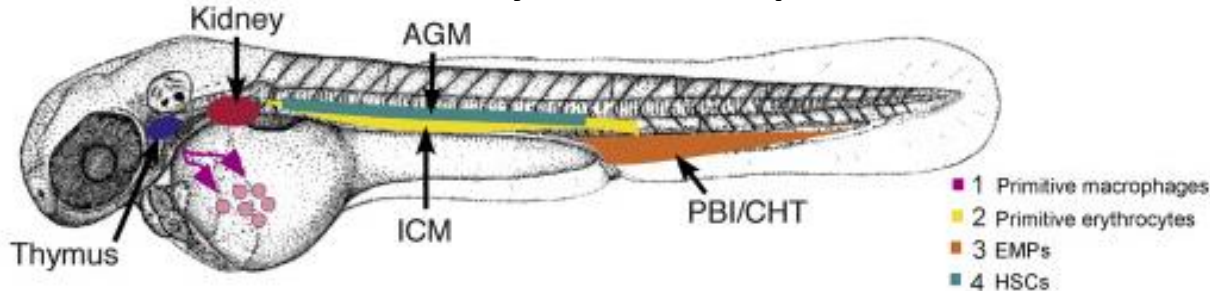


Image: Stachura *et al.* (2016) *Methods in Cell Biology*

## Immune cell types shared between humans and zebrafish

Macrophages

Neutrophils

Granulocytes

Microglia

Monocytes

Mast cells

Dendritic cells

Eosinophils

T cells (CD4+ or CD8+)

B cells (IgM, IgD and IgT)

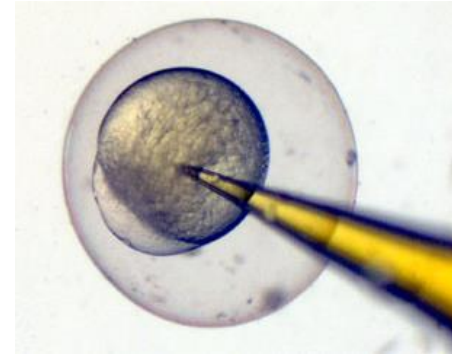
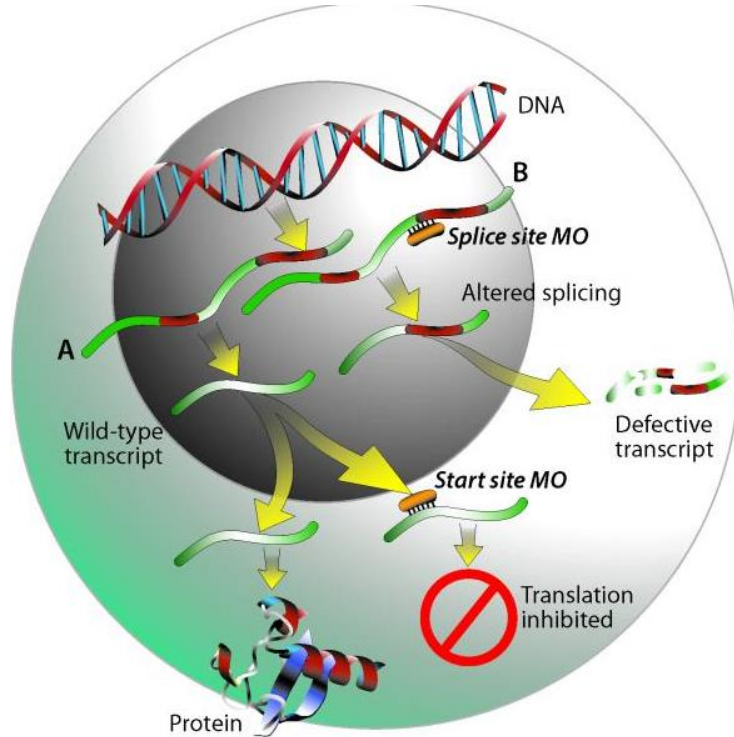
Regulatory T cells?

NK cells?

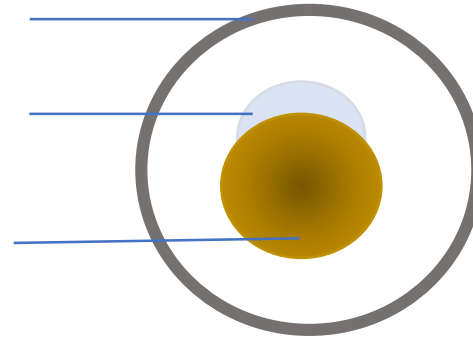


# CDG model in zebrafish

## Morpholino injection



Egg shell  
Zebrafish  
embryo  
Yolk sac



No RNA → no protein

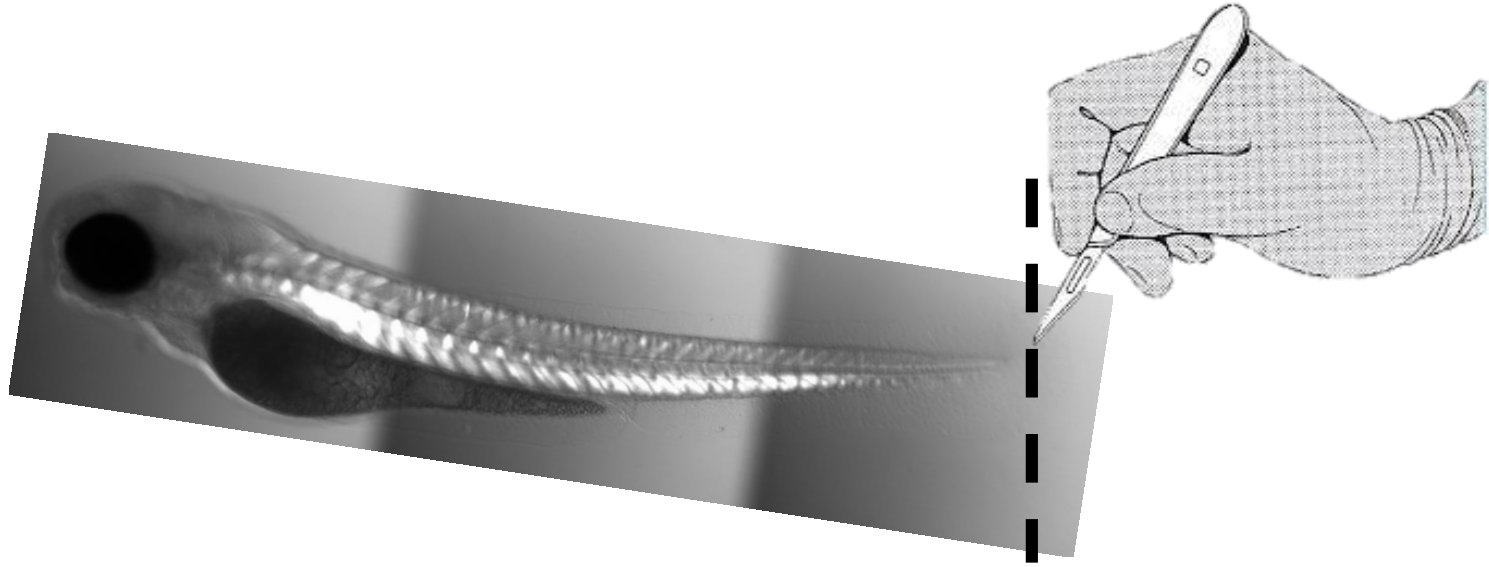
# Morpholino model of PMM2-CDG

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- Morpholino zebrafish model of PMM2-CDG published by Cline and colleagues 2012
- Fish showed developmental abnormalities similar to PMM2-CDG patients:
  - Skeletal deformities
  - Impaired motility
  - Neuronal involvement

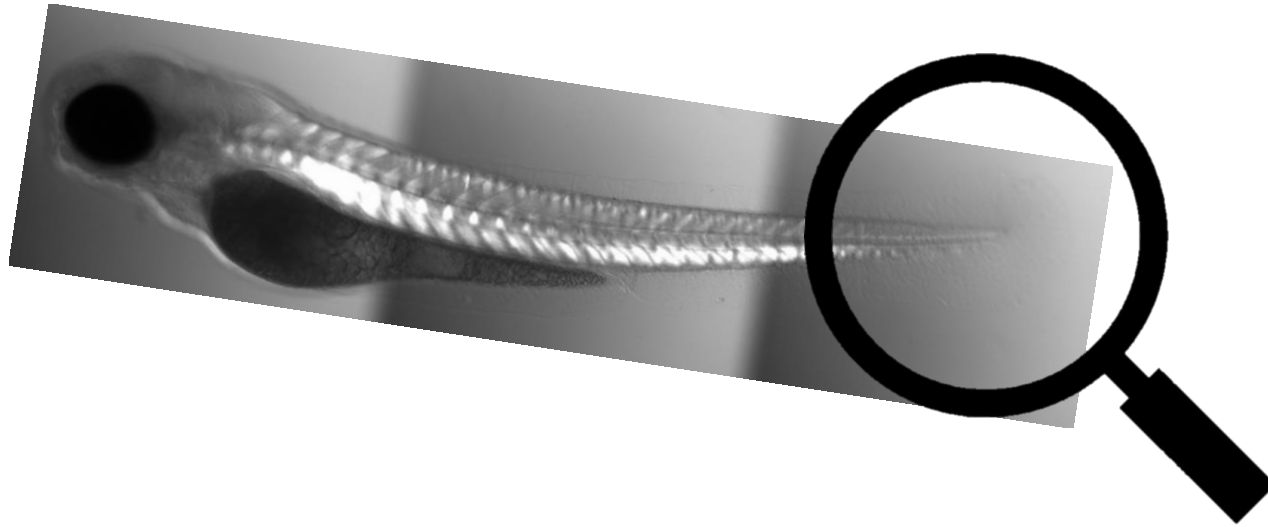
# Immune cell migration

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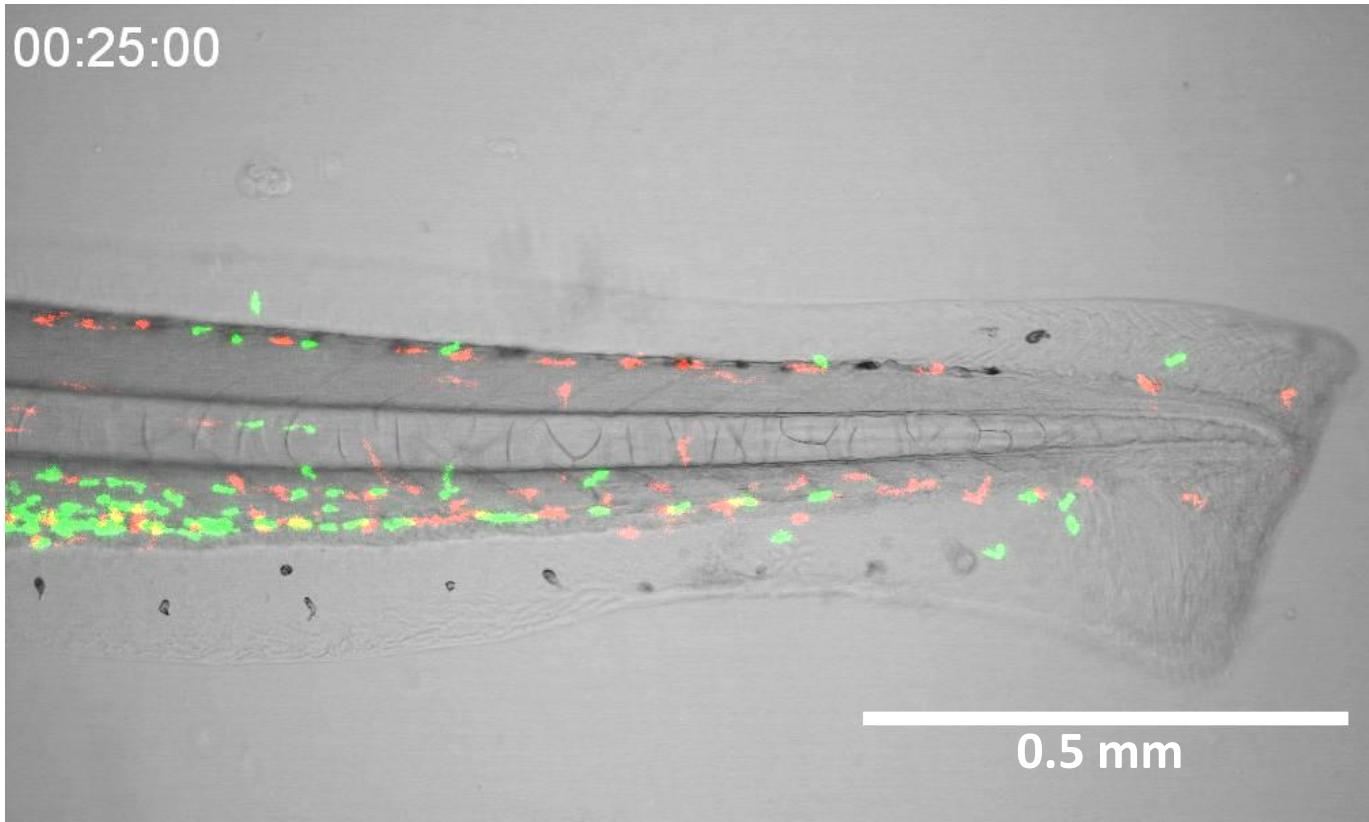
# Immune cell migration

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# Immune cell migration

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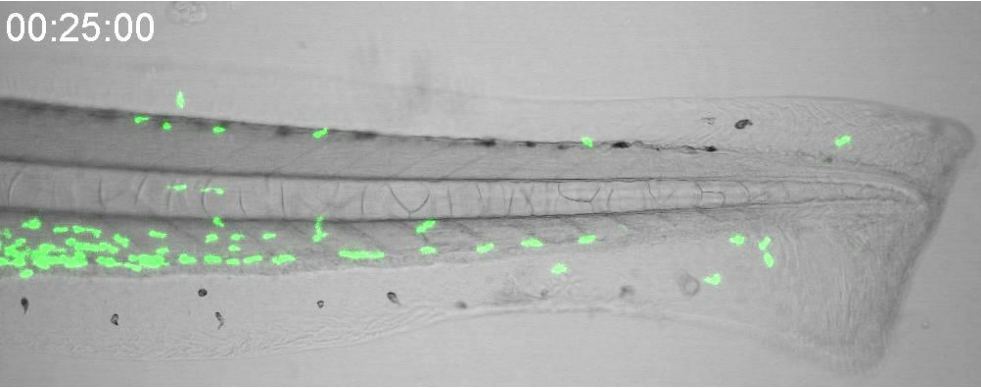


- Neutrophils
- Macrophages

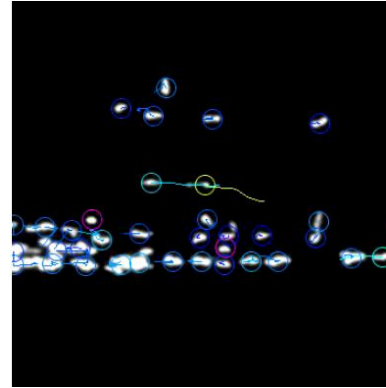
# Quantification neutrophil migration

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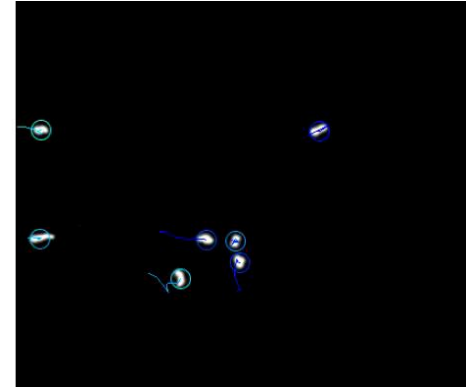
00:25:00



Middle

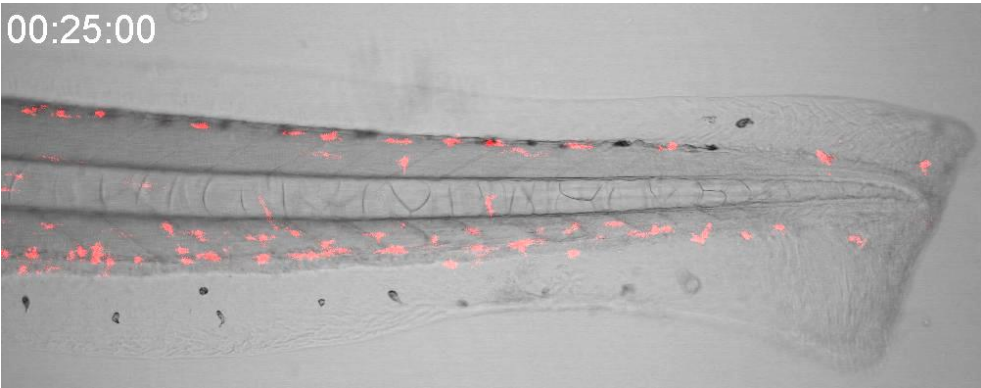


Tail

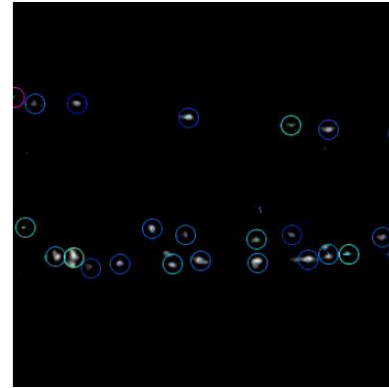


# Quantification macrophage migration

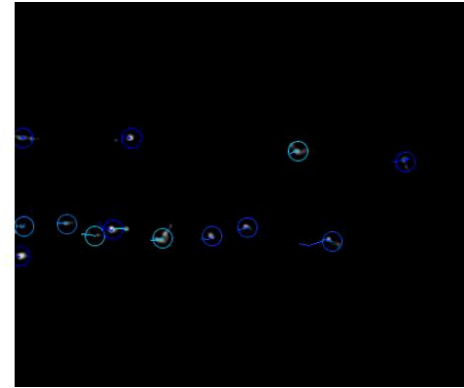
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Middle



Tail



# Conclusion and future perspectives

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Things we are currently working on

- Using this system to compare control fish to CDG model fish
- Setting up a protocol to induce tissue damage with a laser
- Injection of fluorescent pathogens to look at recruitment and phagocytosis



# Conclusion and future perspectives

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Zebrafish model advantages:

- More similar to human than you would think
- Embryos are transparent → Great for microscopy!
- Fast breeding (Once every two weeks, +/- 200 eggs per couple)

Disadvantages:

- Embryos do not have a completely developed immune system
- Morpholino's are only effective for maximum 5 days

# Conclusion and future perspectives

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Zebrafish models could benefit CDG research:

- Better understanding of immune-related symptoms in CDG
- Sugar supplementation used as therapy in CDG can be tested  
by addition of the sugars to the water

# Acknowledgements

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Nijmegen, The Netherlands**

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