

# Using image analysis techniques to study the function of the CFTR protein

Catherine Hastings

28<sup>th</sup> June 2017

# Outline

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- ▶ background about cystic fibrosis,

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- ▶ current experimental procedure and how it can be extended,

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- ▶ current experimental procedure and how it can be extended,
- ▶ image analysis technique and possible improvements.

# Cystic fibrosis

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**Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)**

# Cystic fibrosis

## Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

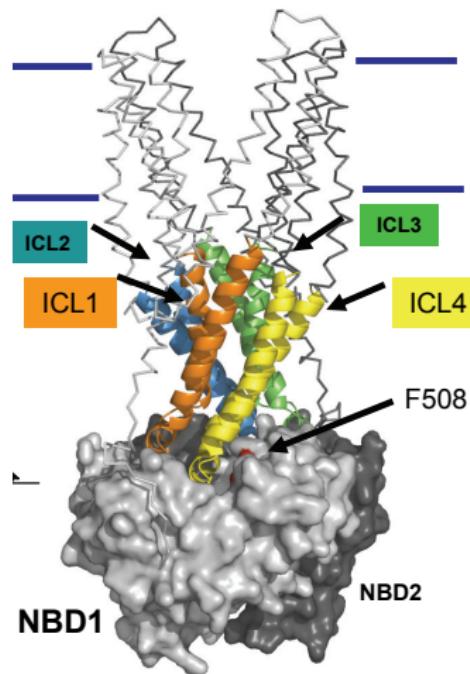


Figure: Representation of the CFTR protein from Millen et al. [2008].

# CFTR malfunction

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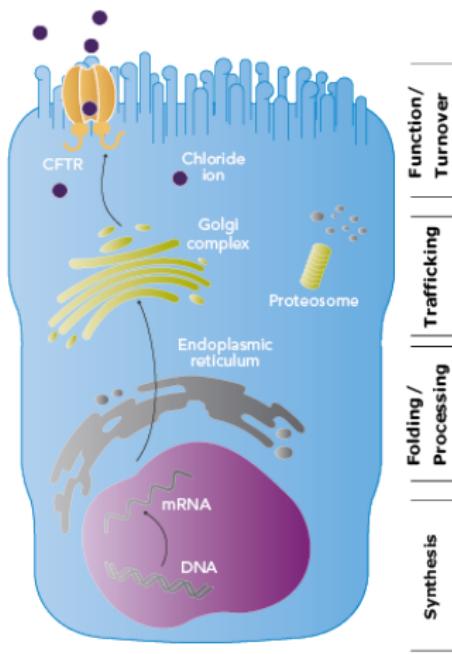


Figure: Image from [www.cftrscience.com](http://www.cftrscience.com).

# Experimental setup

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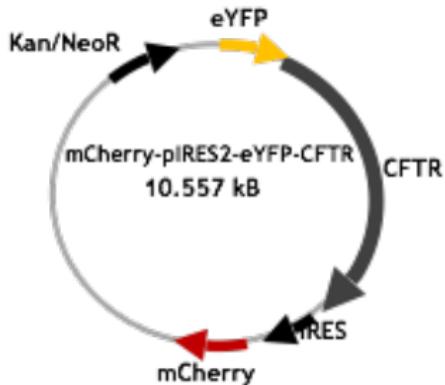
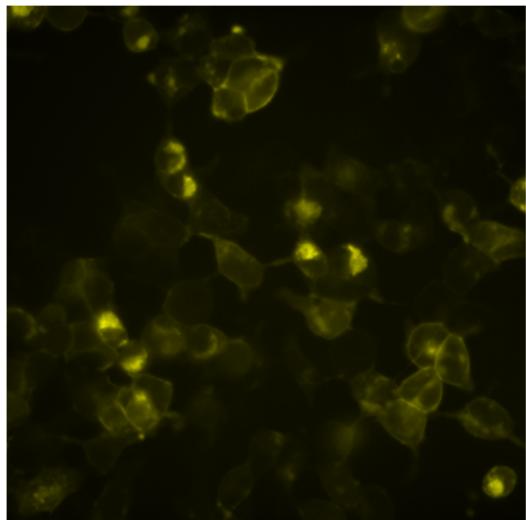
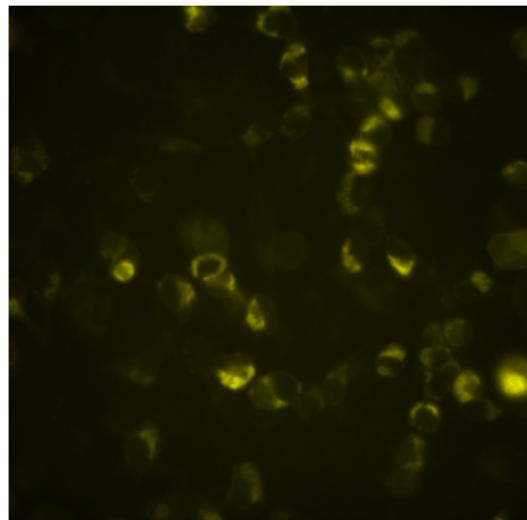


Figure: A representation of the plasmid used. Diagram by Stella Prins.

## Example images



(a) Wild-type CFTR



(b) CFTR-F508del

Figure: HEK293 cells transfected with CFTR-YFP.

# Extending the experimental setup

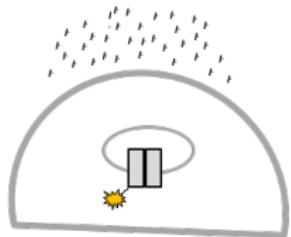


Figure: Diagrams by Stella Prins.

# Extending the experimental setup

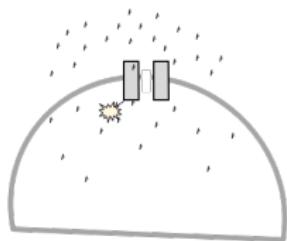
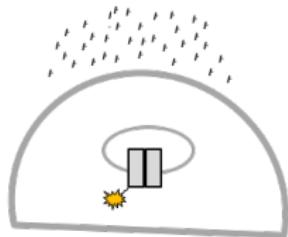


Figure: Diagrams by Stella Prins.

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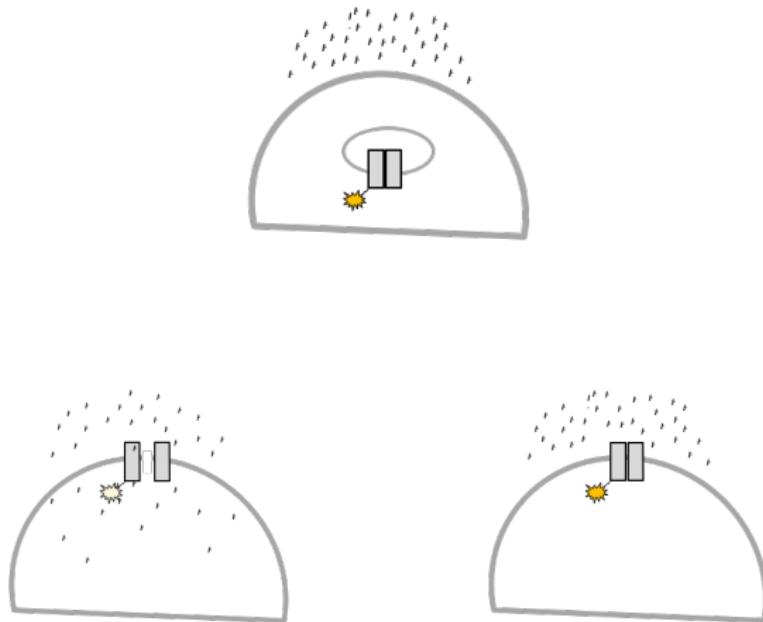
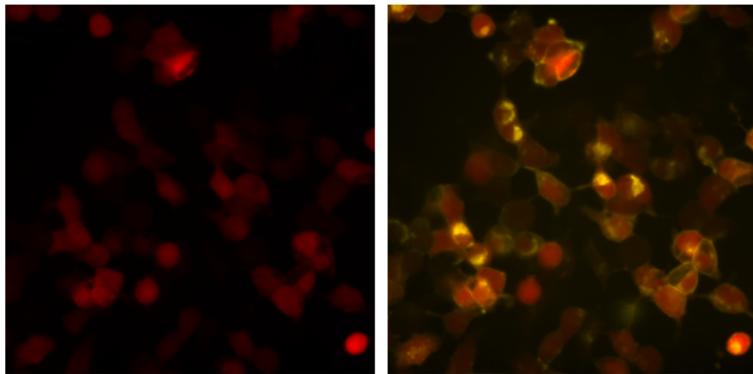


Figure: Diagrams by Stella Prins.

# Quantifying efficacy of protein trafficking

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(a) mCherry

(b) YFP and mCherry

Figure: HEK293 cells transfected with CFTR-YFP and mCherry.

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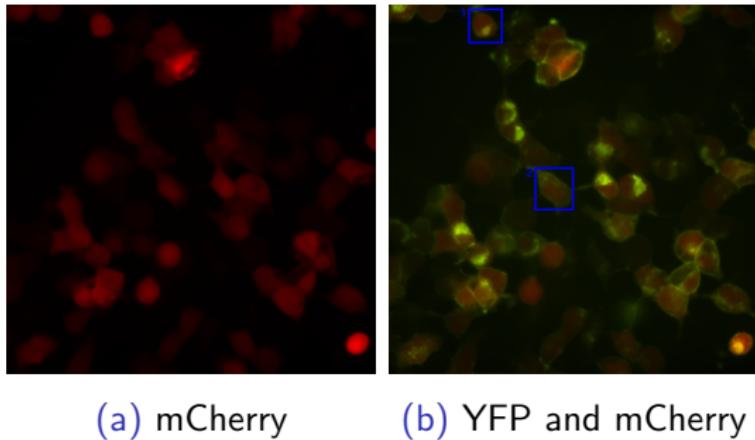


Figure: HEK293 cells transfected with CFTR-YFP and mCherry.

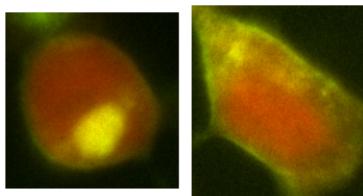
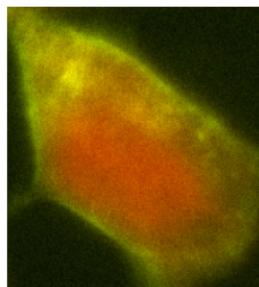
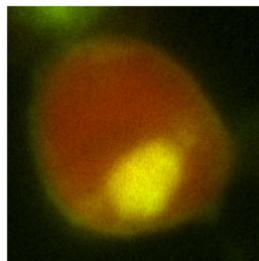
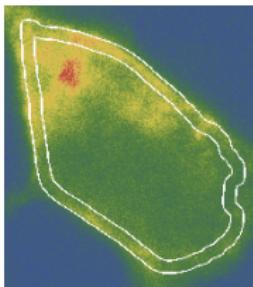
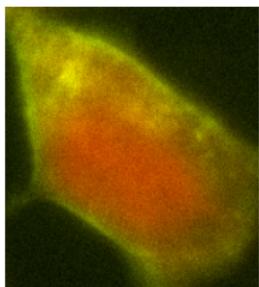
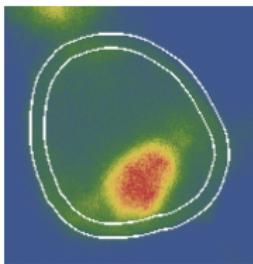
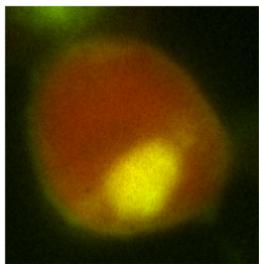


Figure: Example cells

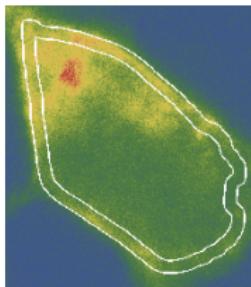
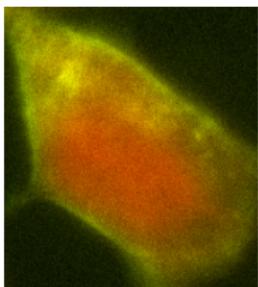
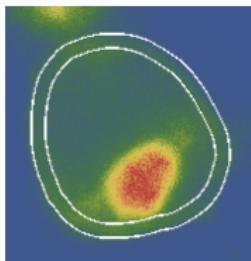
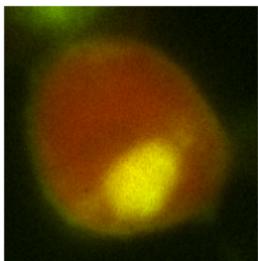
## Current image analysis results



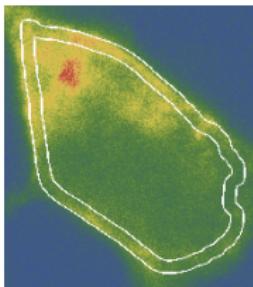
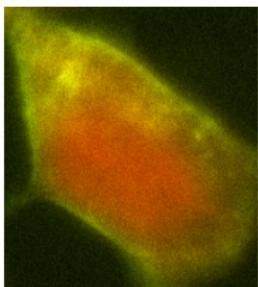
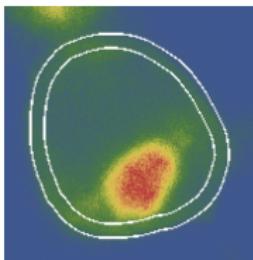
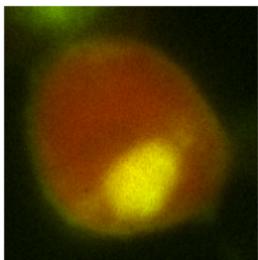
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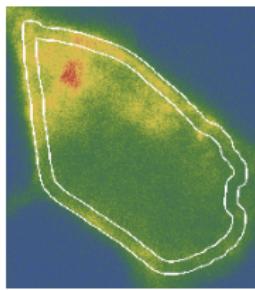
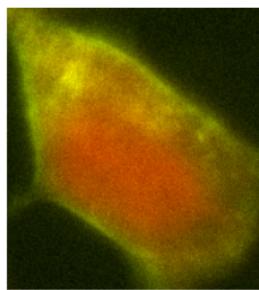
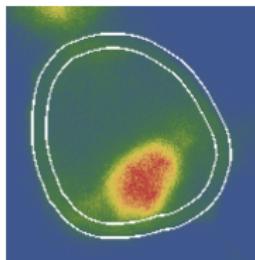
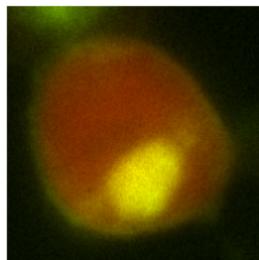
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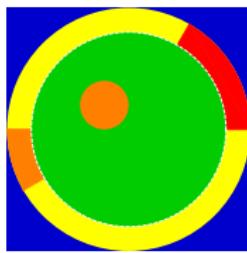
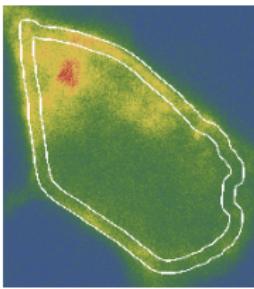
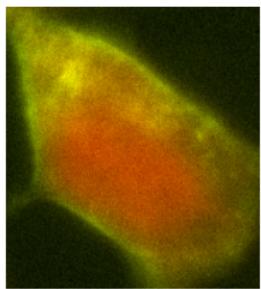
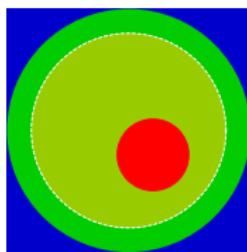
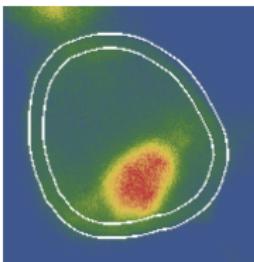
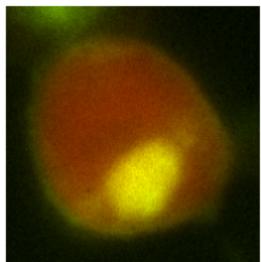
## Current image analysis results



# Improving the image analysis



# Improving the image analysis



## Project aims

1. Collect new data using the extended experimental technique
2. Analyse the results of the YFP quenching
3. Improve current image analysis tool to aid in finding statistically significant differences between CFTR variants

Completing these aims will result in an **image analysis tool** that will be able to study

- ▶ how well the CFTR protein is **trafficked** to the membrane,
- ▶ and the efficacy of CFTR's **gating function**.

# Acknowledgements

- ▶ Lewis Griffin
- ▶ Paola Vergani
- ▶ Stella Prins
- ▶ Emily Langron

## Reference

Linda Millen, Patrick Thibodeau, Juan L. Mendoza, Jon Moody, Chad Brautigam, Sharon Fischman, Hanoch Senderowitz, and Philip J. Thomas. Assessment of domain-domain interactions in cftr. In *ABC 2008*, 2008.

# Using image analysis techniques to study the function of the CFTR protein

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