



## **Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells**

Aurélie Charazac, Célia Decondé Le Butor, Kévin Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, Jérôme Gilleron, Patrick Fenichel, Xavier Descombes, Frédéric Bost, Clavel Stéphan, et al.

### **► To cite this version:**

Aurélie Charazac, Célia Decondé Le Butor, Kévin Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, et al.. Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells. International Congress of Translational Research in Human Nutrition, Jun 2017, Clermont-Ferrand, France. <hal-01548783>

**HAL Id: hal-01548783**

**<https://hal.science/hal-01548783v1>**

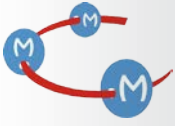
Submitted on 12 Jul 2017

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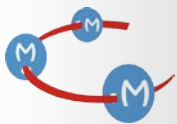
HAL Authorization



# Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.



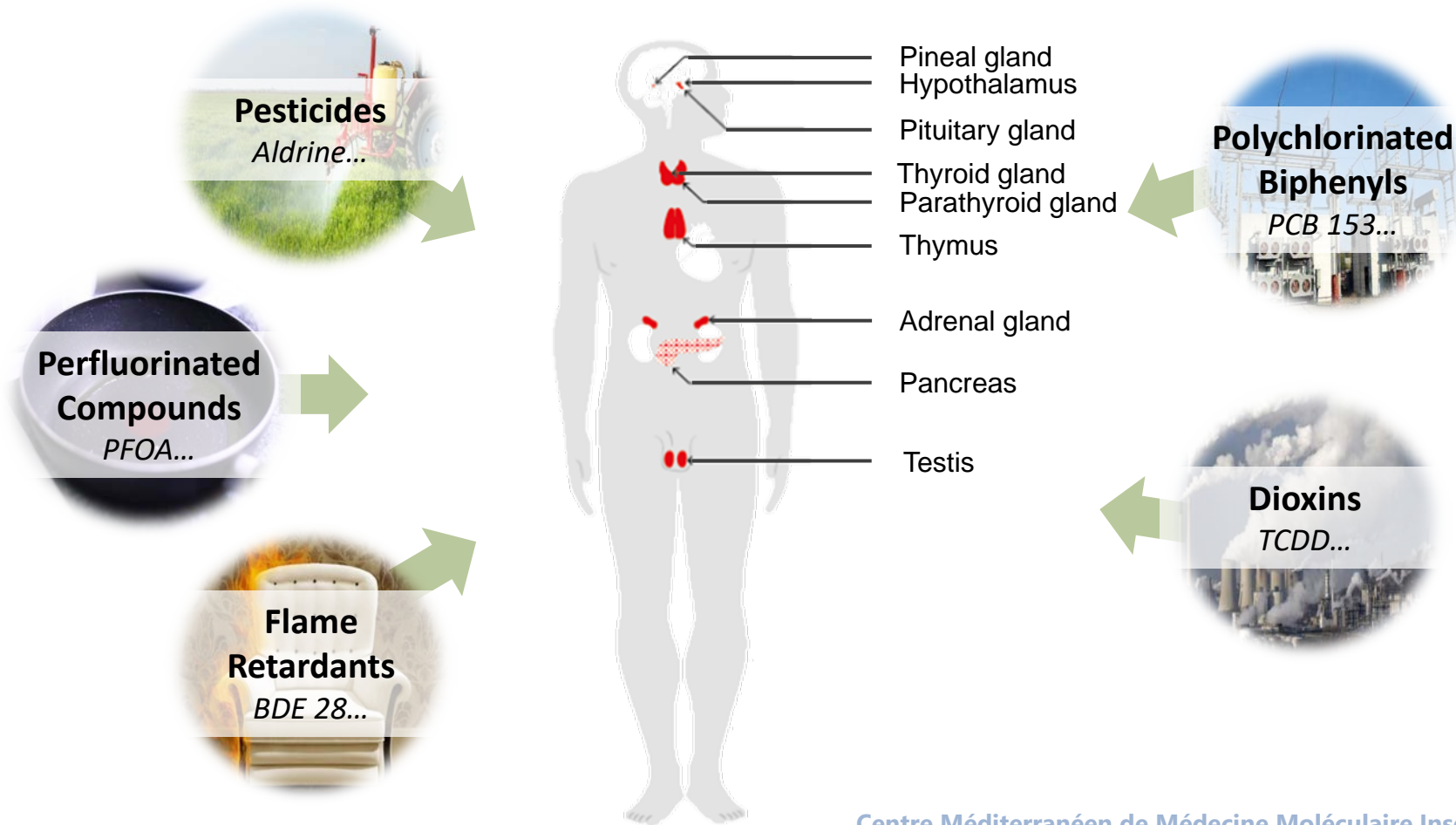
Charazac Aurélie, Decondé le Butor Célia, Giulietti Kévin, Lobaccaro Jean Marc, Baron Silvère, Gilleron Jérôme, Fénichel Patrick, Descombes Xavier, Bost Frédéric, Clavel Stéphan & Chevalier Nicolas.

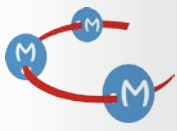


# Endocrine Disruptor Compounds


« An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. »

World Health Organization





# Major challenge

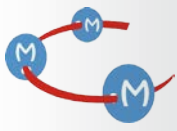
	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
Acceptation of WHO definition	✓	—	✗	✓
Based on risk assessment	✗	✗	✗	✗
List updating	✗	—	✓	✓

Adapted from *EndocrineScience.org*



Registration, **E**valuation and **A**utorization of **C**hemicals

└→ Promotes alternative methods for the hazard assessment of substances.



# Major challenge

	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
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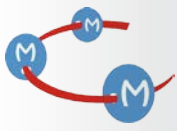


Registration, **E**valuation and **A**utorization of **C**hemicals

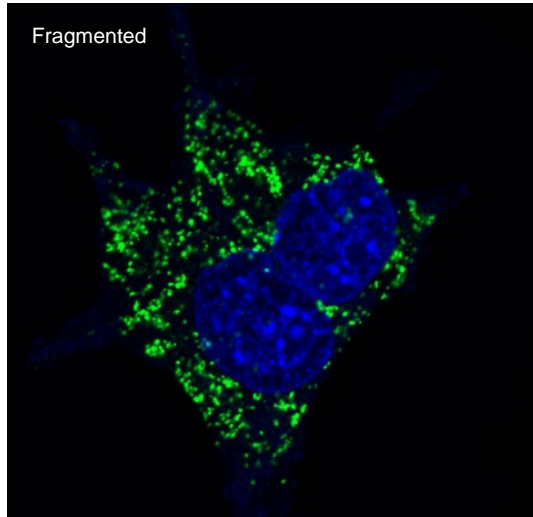
└ Promotes alternative methods for the hazard assessment of substances.

Urgent need for multiparametric, robust and high throughput cell-based assay :

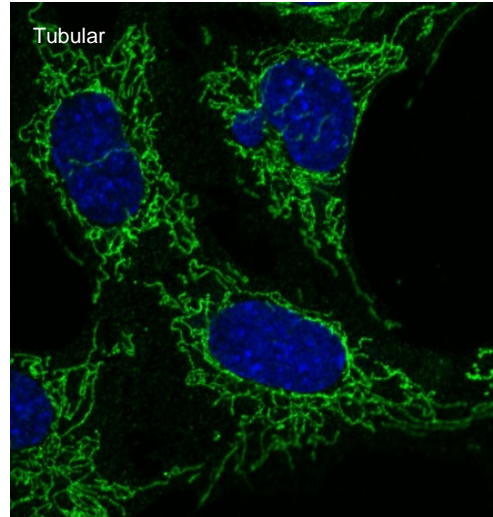
- ➔ To investigate the mechanisms underlying the adverse effects of known EDCs
- ➔ To identify new compounds with endocrine-disrupting potential



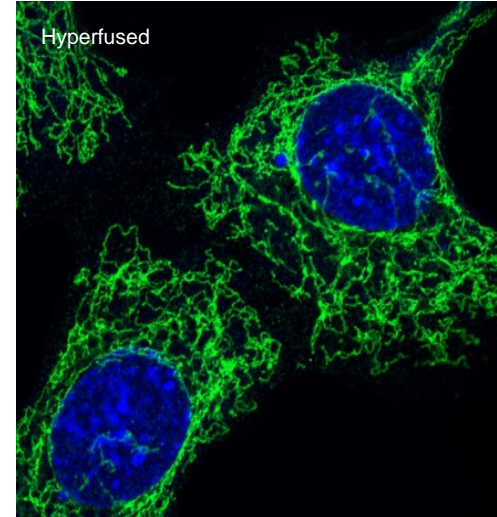
# Mitochondria, a cell sensor



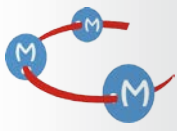
Apoptosis



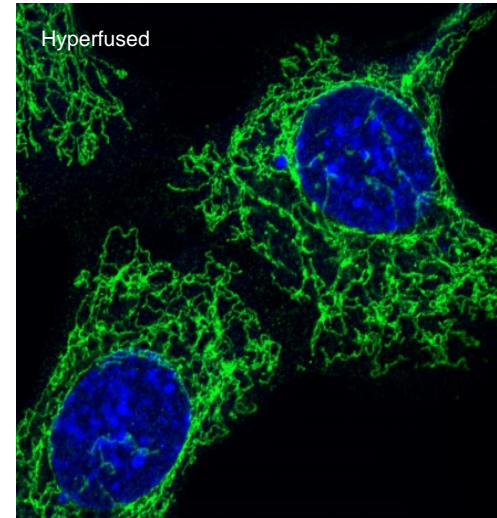
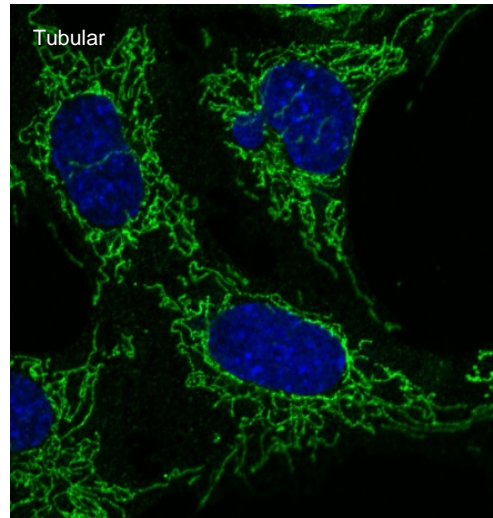
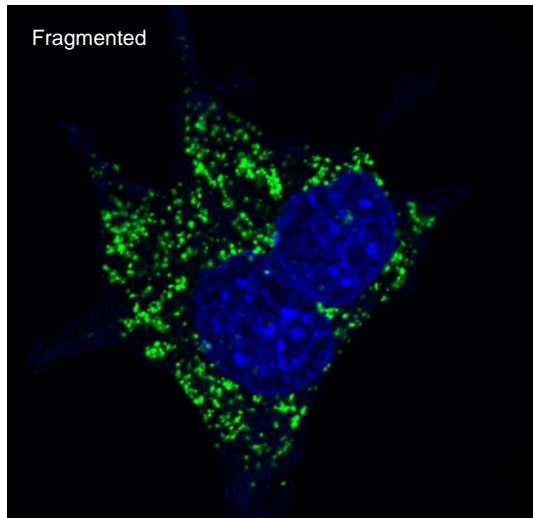
Healthy



Stress



# Mitochondria, a cell sensor



Apoptosis

Healthy

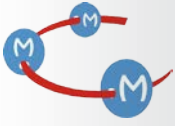
Stress

Calcium signalling

Cell growth and  
differentiation

Cell cycle control

Cell death



# Mitochondria, a cell sensor

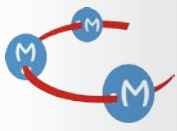
Alteration of **mitochondrial forms and functions** by EDCs ?

1) Quantitative image based analysis of mitochondrial functions

→ High throughput screening

2) Computational image based analysis of mitochondrial morphology

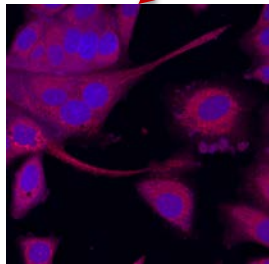
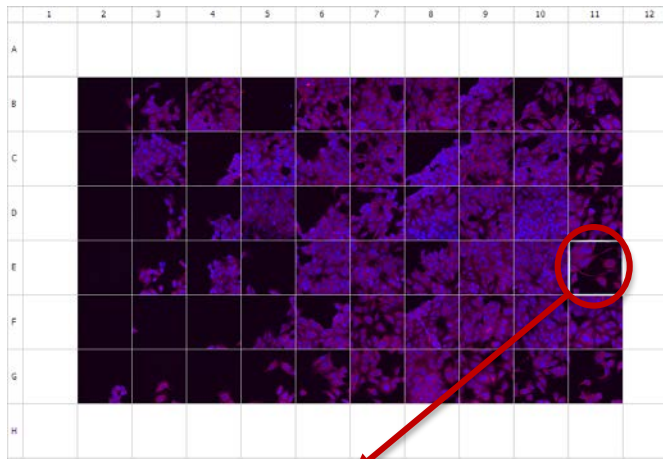
→ Image analysis and classification



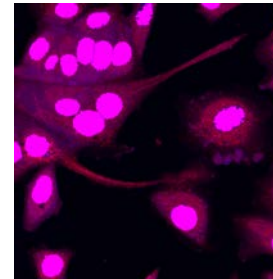
# Multiparametric cell-based assay

## Automated image acquisition

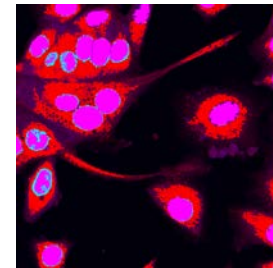
(Nikon A1R-20X)



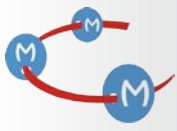
## Image processing



→ 1) **Dapi masking :**  
cell counting



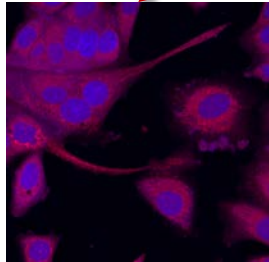
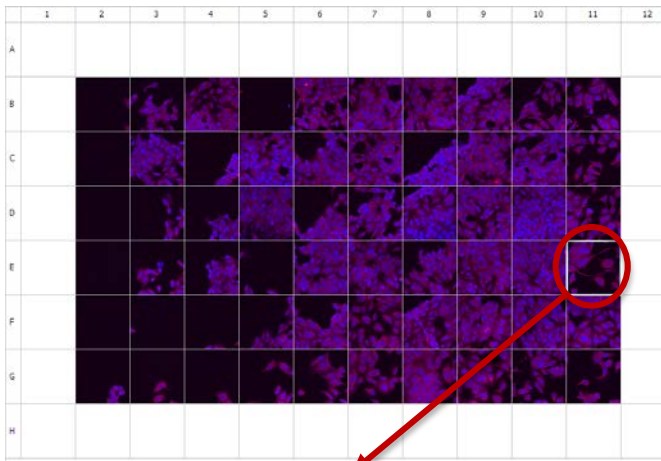
→ 2) **Dye masking :**  
fluorescence intensity



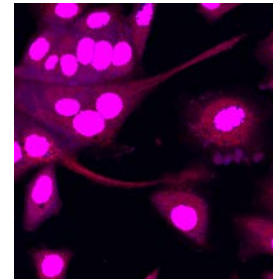
# Multiparametric cell-based assay

## Automated image acquisition

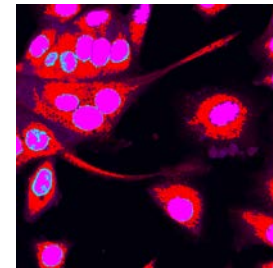
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## Image processing

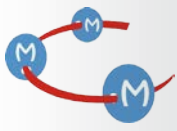


→ 1) **Dapi masking :**  
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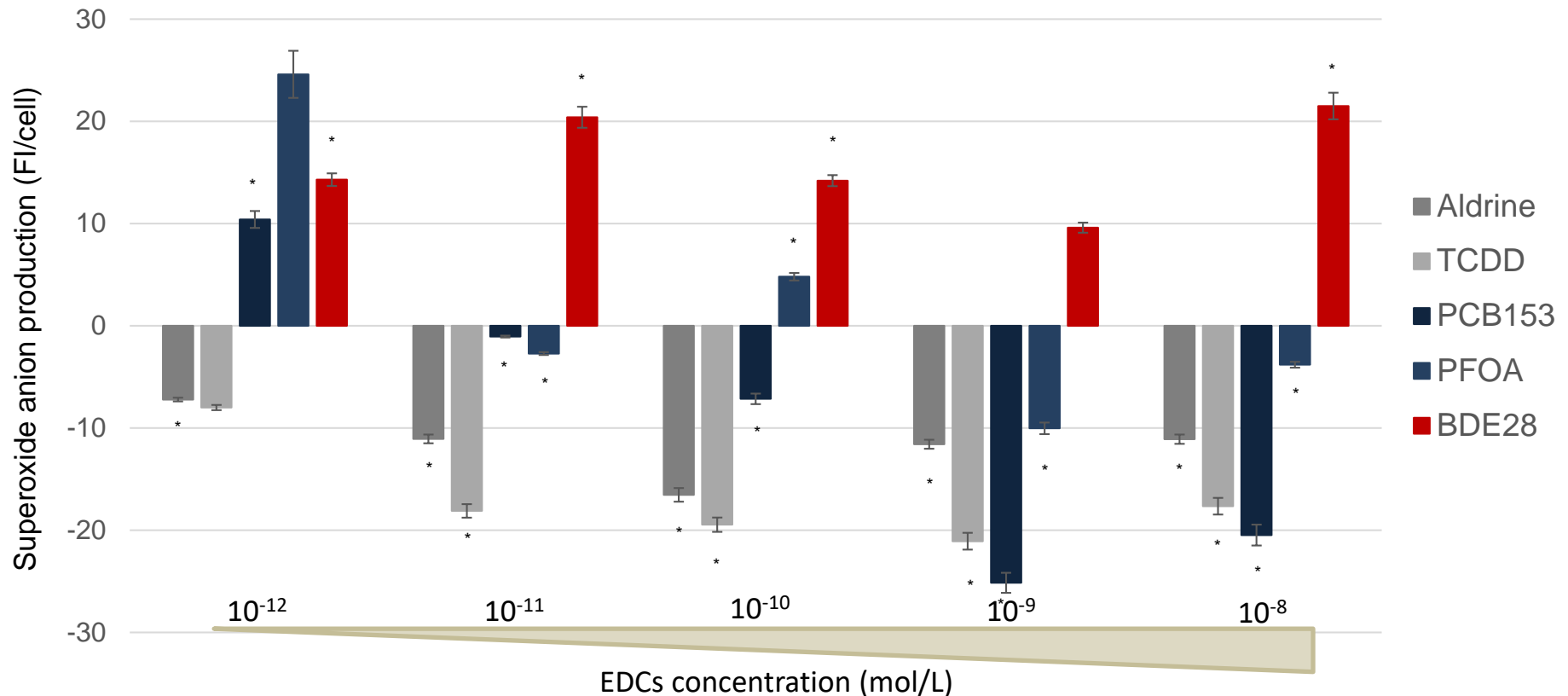
→ 2) **Dye masking :**  
fluorescence intensity

- **Multiparameter :**
  - mitochondrial membrane potential (MitoTracker Red™)
  - superoxide anion production (MitoSox™)
  - mitophagy (Keima probe)

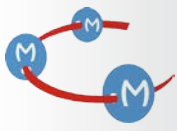


# EDCs affect the mitochondrial function

- ROS production on DU145:

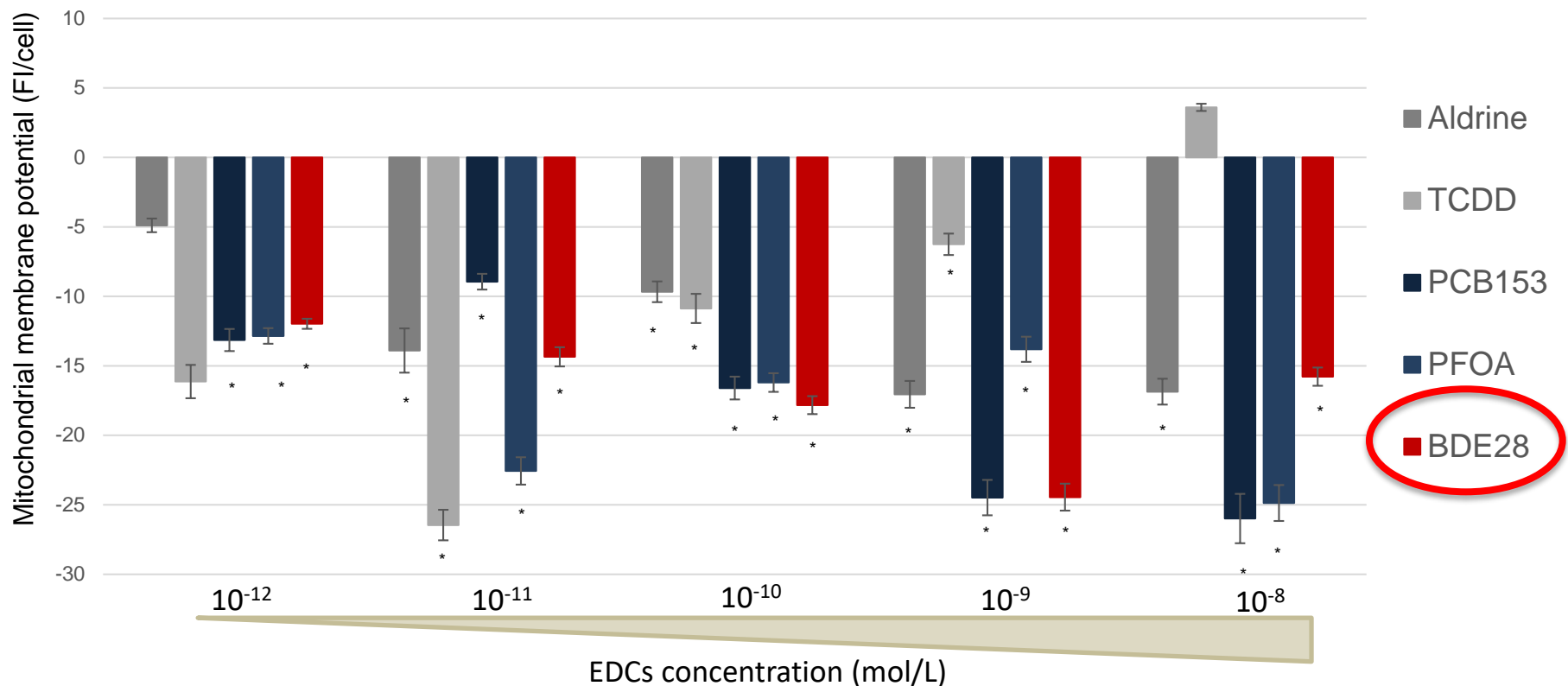


\* significantly different from the control,  $p \leq 0.005$ .

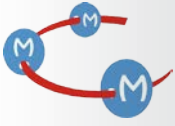


# EDCs affect the mitochondrial function

- Mitochondrial membrane potential on DU145 :

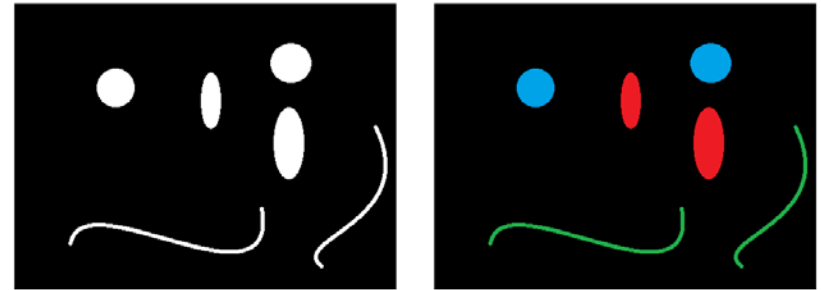
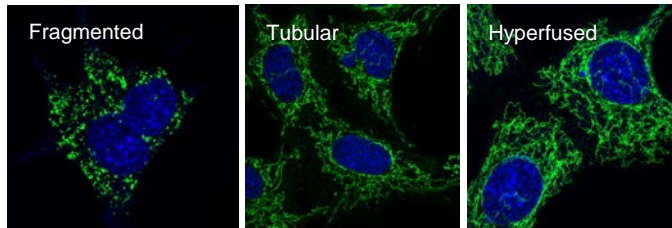


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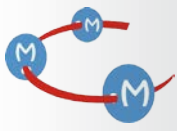


# Forms classification of mitochondrial network

- Collaboration with

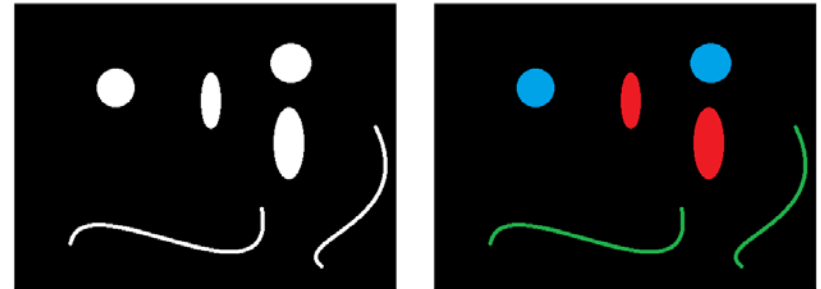
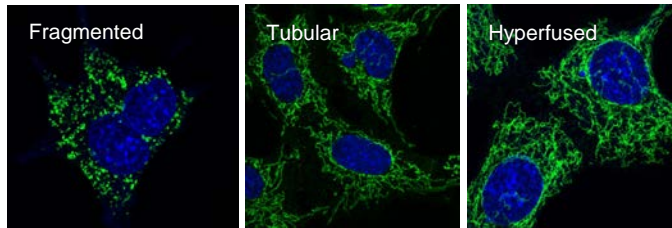


Fragmented, Tubular, Hyperfused



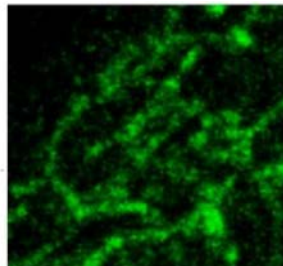
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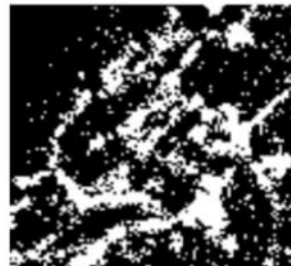


Fragmented, Tubular, Hyperfused

Image

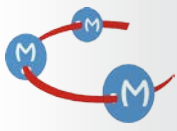


Basic  
thresholding  
method



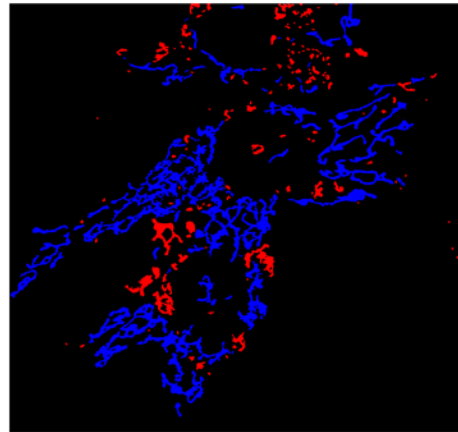
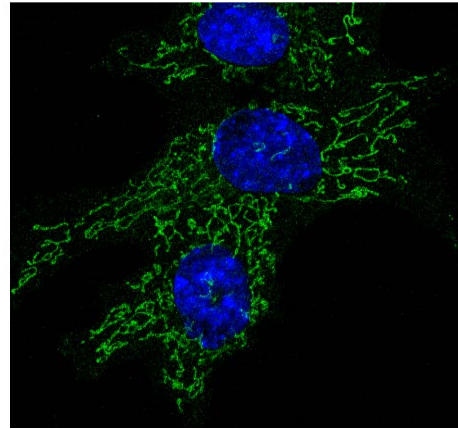
Developed  
method

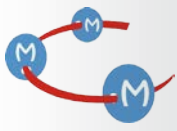




# Exemple of developed method

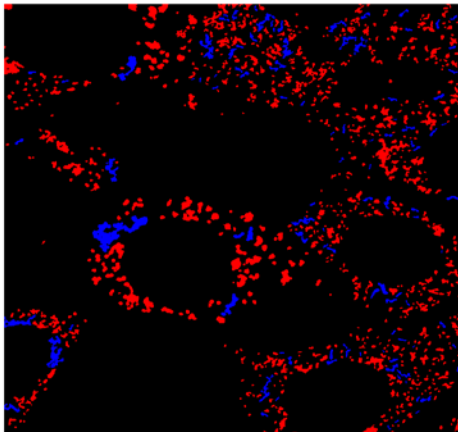
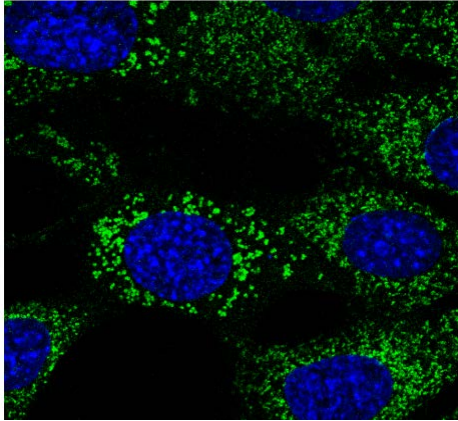
Tubular



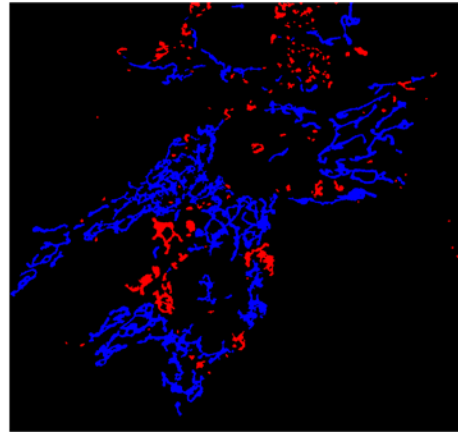
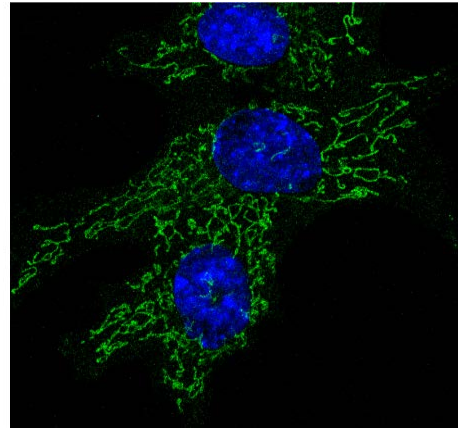


# Exemple of developed method

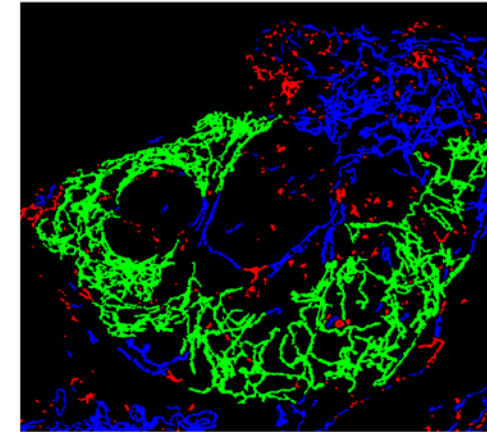
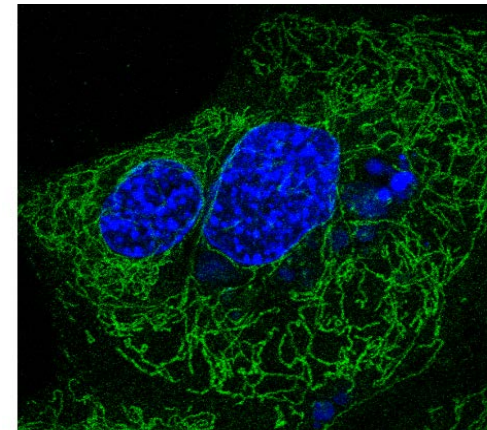
Fragmented  
(Rotenone, 100nM)

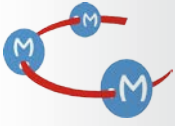


Tubular



Fused  
(Compound C, 20μM)



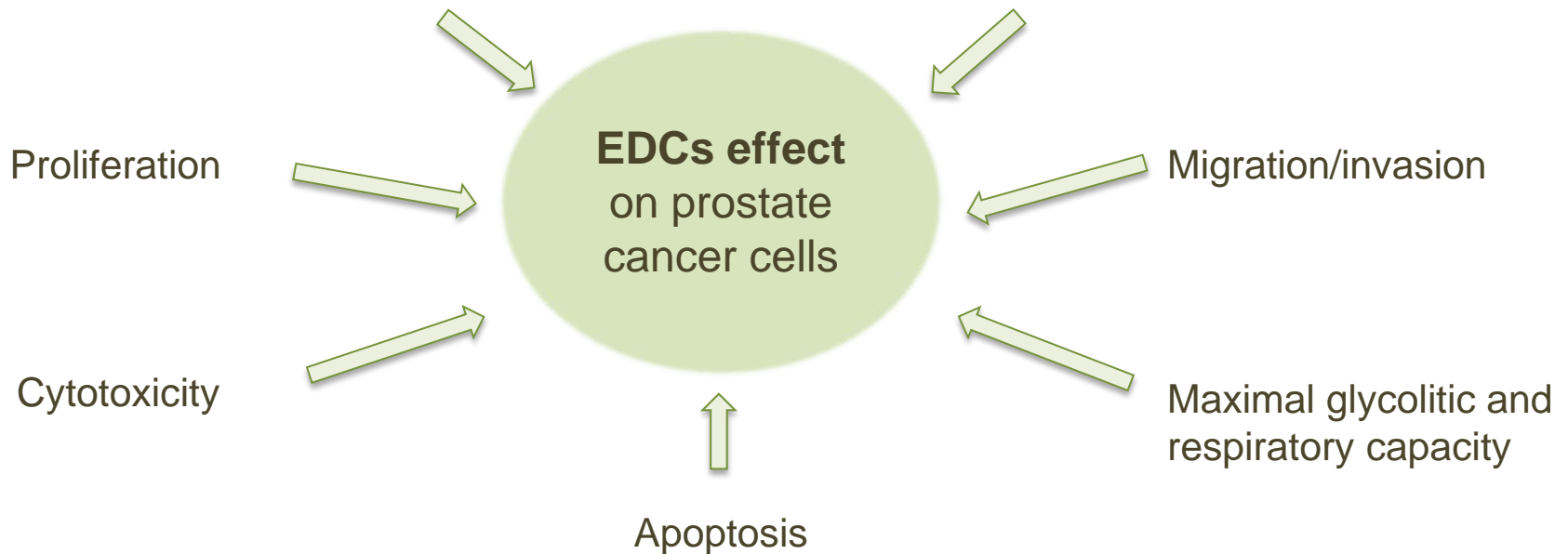


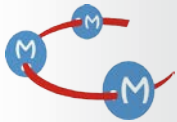
# Final Goal

- Development of a multiparametric high throughput cell based screening :

## Mitochondrial functional parameters

## Mitochondrial morphological parameters



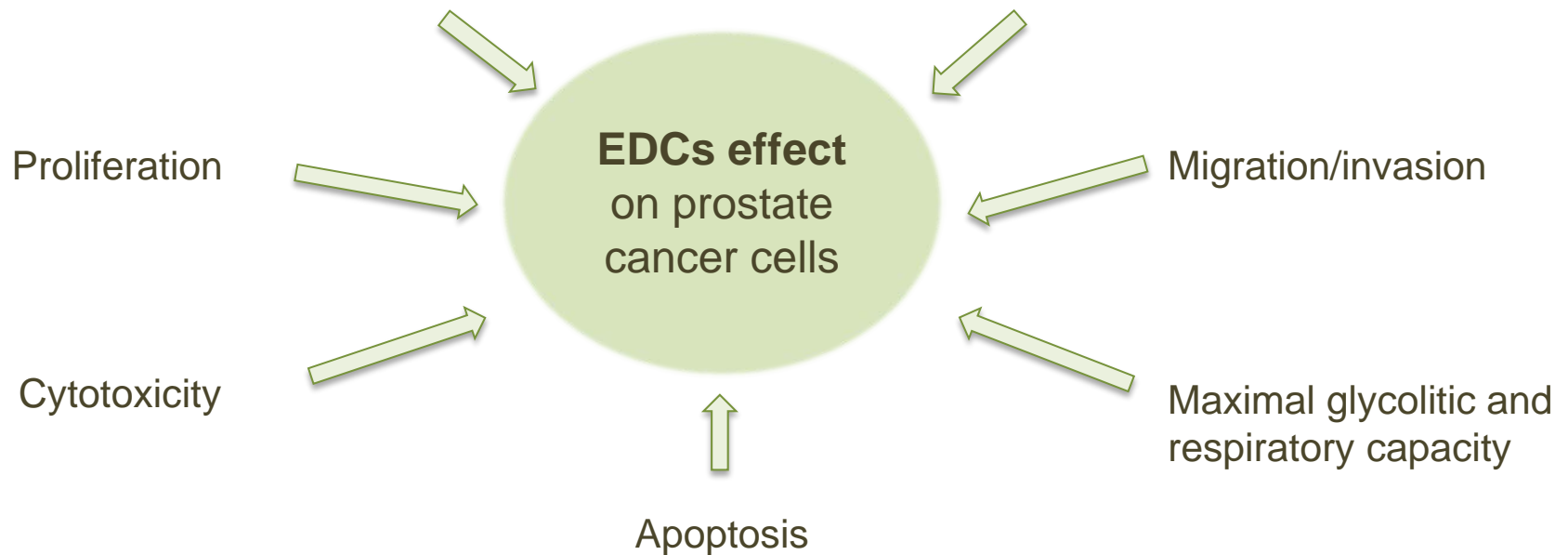


# Final Goal

- Development of a multiparametric high throughput cell based screening :

## Mitochondrial functional parameters

## Mitochondrial morphological parameters



- ➡ Signature of EDCs based on their effects on cell metabolism
- ➡ New perspective in identification and characterization of EDCs



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Faustine Pastor

