HR-MALDI-MSI WORKFLOW FOR LIPIDOMIC ANALYSIS OF ZEBRAFISH EMBRYOS

Albert Menéndez-Pedriza[1], Marta Gual-Gimeno[1], Pablo Giménez-Gómez[2], Mercedes Blázquez[3], Joaquim Jaumot[1] and Laia Navarro-Martín[1]

[1]Institute of Environmental Assessment and Water Research, IDAEA (CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain.
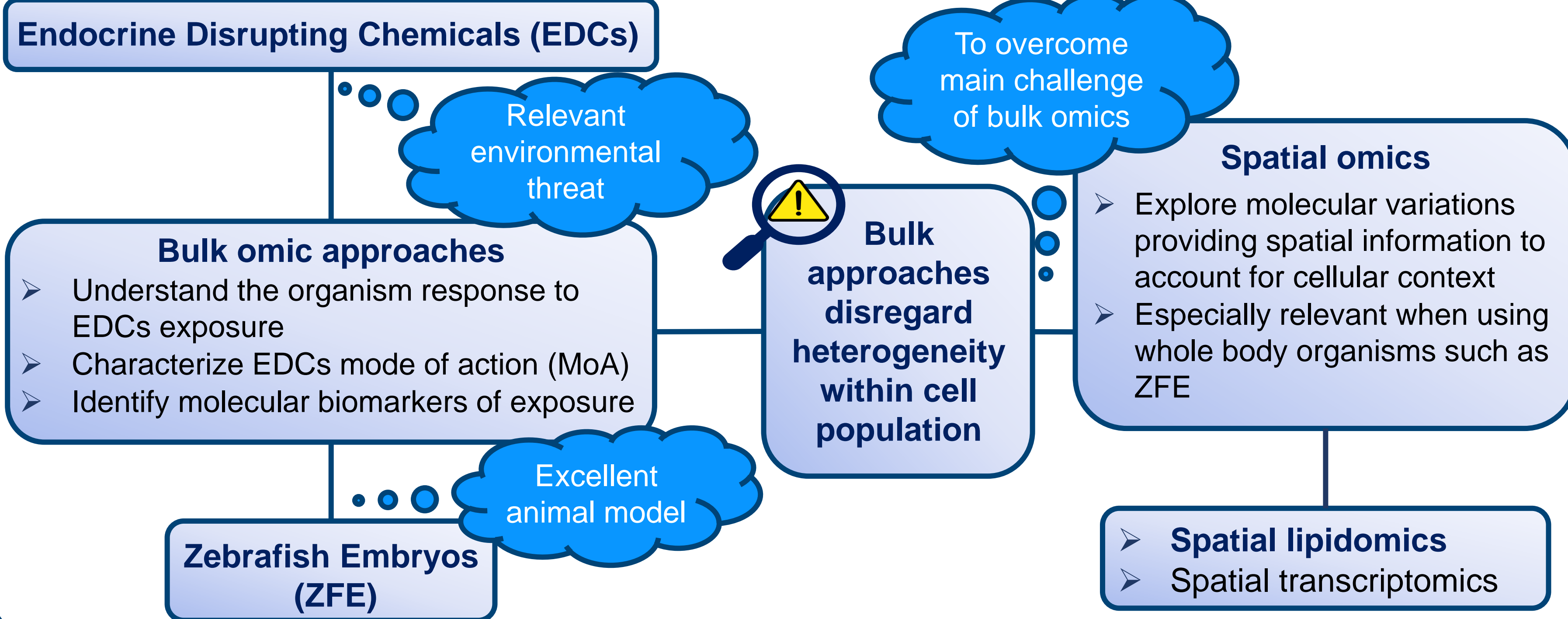[2]Institute of Microelectronics of Barcelona, IMB-CNM (CSIC), Campus of the Autonomus University of Barcelona, 08193, Bellaterra, Spain
[3]Institute of Marine Sciences, ICM (CSIC), Passeig Marítim 37-49, 08003 Barcelona, Spain
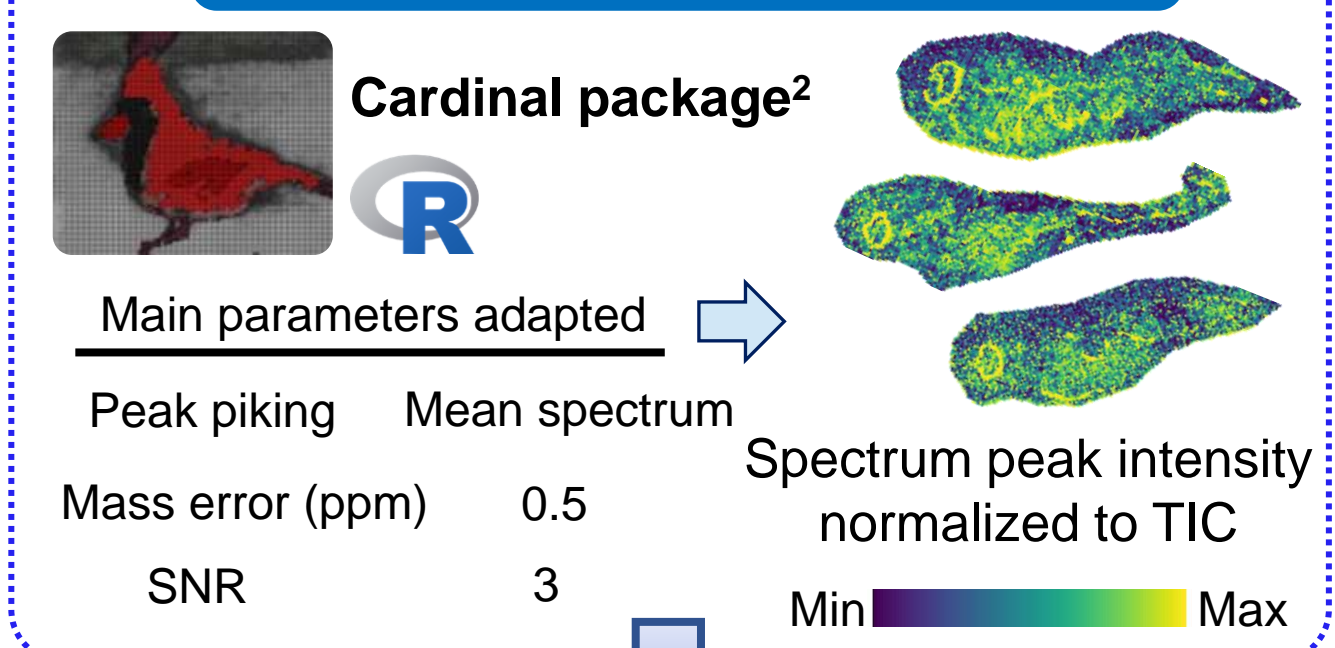Email: ampqam@cid.csic.es

BACKGROUND

Endocrine Disrupting Chemicals (EDCs)

Relevant environmental threat

Bulk omic approaches
- Understand the organism response to EDCs exposure
- Characterize EDCs mode of action (MoA)
- Identify molecular biomarkers of exposure

⚠️ **Bulk approaches disregard heterogeneity within cell population**

To overcome main challenge of bulk omics

Spatial omics
- Explore molecular variations providing spatial information to account for cellular context
- Especially relevant when using whole body organisms such as ZFE

Excellent animal model

Zebrafish Embryos (ZFE)

- Spatial lipidomics
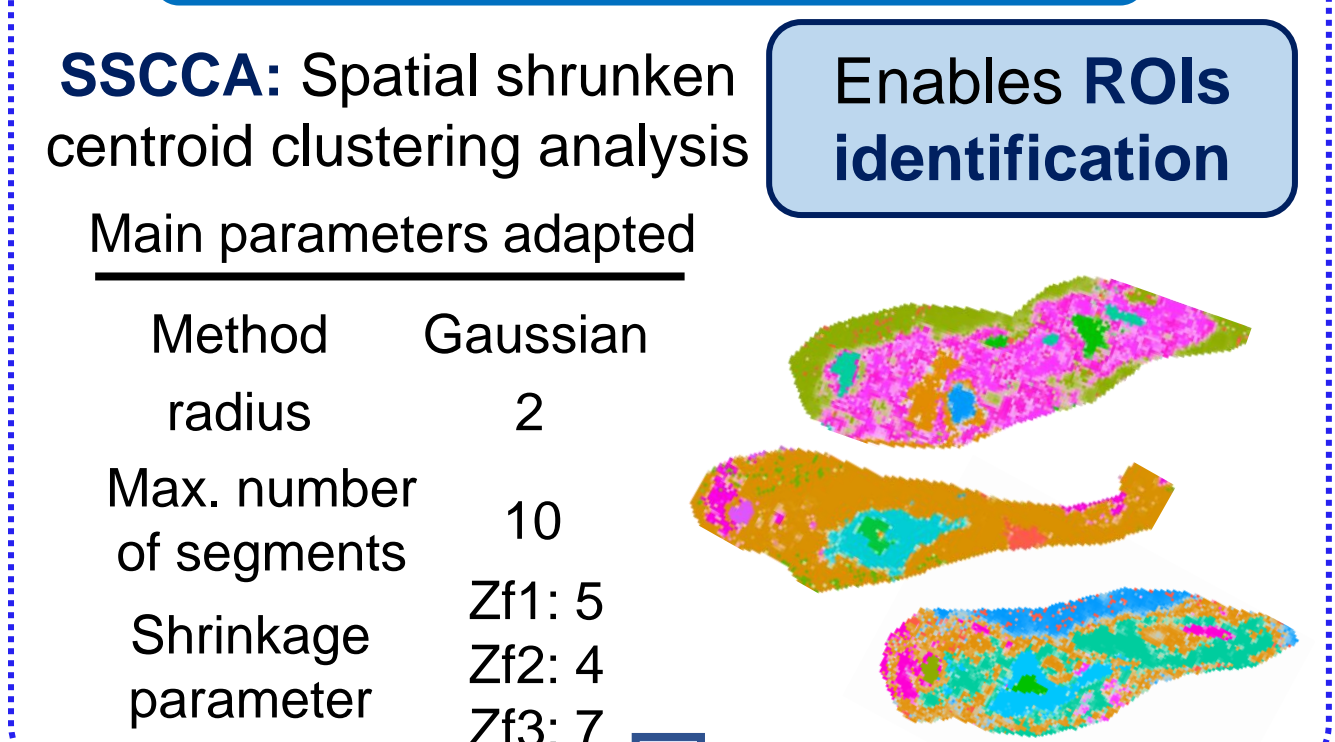- Spatial transcriptomics

RESEARCH GOAL

➤ To **develop** a **spatial lipidomics workflow** using an **HR-MALDI-TOF (Mass-spectrometry imaging (MSI)) approach** to be implemented in ZFE sections

➤ To **identify regions of interest (ROIs)** as a result of the **identification of different lipidic signatures of different cell types**

SPATIAL LIPIDOMICS WORKFLOW

SAMPLE PREPARATION

DESIGN, CONSTRUCTION OF MOLDS AND ZFE EMBEDDING

5.50 mm, 1.00 mm, 0.48 mm, 0.70 mm

- Mold design modified from Copper *et al.*[1]
- Molds built in polydimethylsiloxane (PDMS)
- 6 days post fertilization larvae
- Embedded in Optimum Cutting Temperature (OCT) medium

TISSUE SECTIONING

OCT FREEZING AND CRYOSECTIONING

➤ T (°C): -15
➤ Section thickness: 10 µm

MICROSCOPE VISUALIZATION

MeOH FIXATION AND H&E STAINING

*H&E: Hematoxylin and Eosin

HR-MALDI-TOF ANALYSIS

Matrix deposition, Laser, intensity, pixels, m/z

- Matrix: Dihydrobenzoic acid (DHB)
- UltrafleXtreme MS
- Spatial Resolution: 20 µm/pixel
- m/z range: 420-1300
- Laser power: 18% (base attenuation: 25%)
- Minimum average resolution: 15000 FHWH

CHEMOMETRIC ASSESSMENT

DATA PRE-PROCESSING

Cardinal package[2] R

Main parameters adapted

Main parameters adapted	
Peak piking	Mean spectrum
Mass error (ppm)	0.5
SNR	3

Spectrum peak intensity normalized to TIC
Min — Max

CLUSTERING ANALYSIS

SSCCA: Spatial shrunken centroid clustering analysis — **Enables ROIs identification**

Main parameters adapted

Method radius	Gaussian 2
Max. number of segments	10
Shrinkage parameter	Zf1: 5, Zf2: 4, Zf3: 7

FEATURE SELECTION AND LIPID ANNOTATION

Lipids for each **ROIs** were considered statistically significant if *p*-value < 0.05

m/z value mass error: 25 ppm

LIPID MAPS, hmdb

RESULTS

LIPIDIC PROFILES OF DIFFERENT BODY REGIONS

Assignment of ROIs to putative body regions

- 🟥 Brain/Spinal cord
- 🟧 Eye
- 🟩🟪 Digestive System
- 🟦 Rest of the body

Brain, Digestive system, Spinal Cord, Eye

Zebrafish Histological Atlas[2]
Liver, Intestine, Stomach

⚠️ **Consecutive sections for microscope visualization were not processed**

➤ **Glycerophospholipid classes** (phosphatidyl-cholines (PC), phosphatidylethanolamines (PE), phosphatidylserines, lyso-PC and lyso-PE) were the most **relevant to differentiate among brain, eye and digestive system**

➤ **Glycerolipids** (triglycerides (TG)) **were mainly found in the eyes** and **digestive system.**

➤ **Fatty acyls** and **sphingolipids** (ceramides and gangliosides) **were assigned to the nervous system**

Similar results are obtained for the other ZFE analyzed

LOCALIZATION OF SPECIFIC LIPIDS OF INTEREST

m/z= 834,6013 ±0.05 PC(40:6)
Min — Max

Histological section of ZFE eye[2]

m/z= 924,8020 ±0.05 TG(56:6)

m/z= 556,6248 ±0.05 GM1(40:1)

➤ The **internal retina** is one of the vertebrate tissues with the **greatest concentrations of phospholipids**[3]
➤ **PCs** are an essential **constituent** of the **gastrointestinal tract cells**[4]

➤ Light-sensitive **photoreceptors** in the external part of the retina are **extremely metabolically demanding** (TG are the main **source of energy in ZFE**)[3]

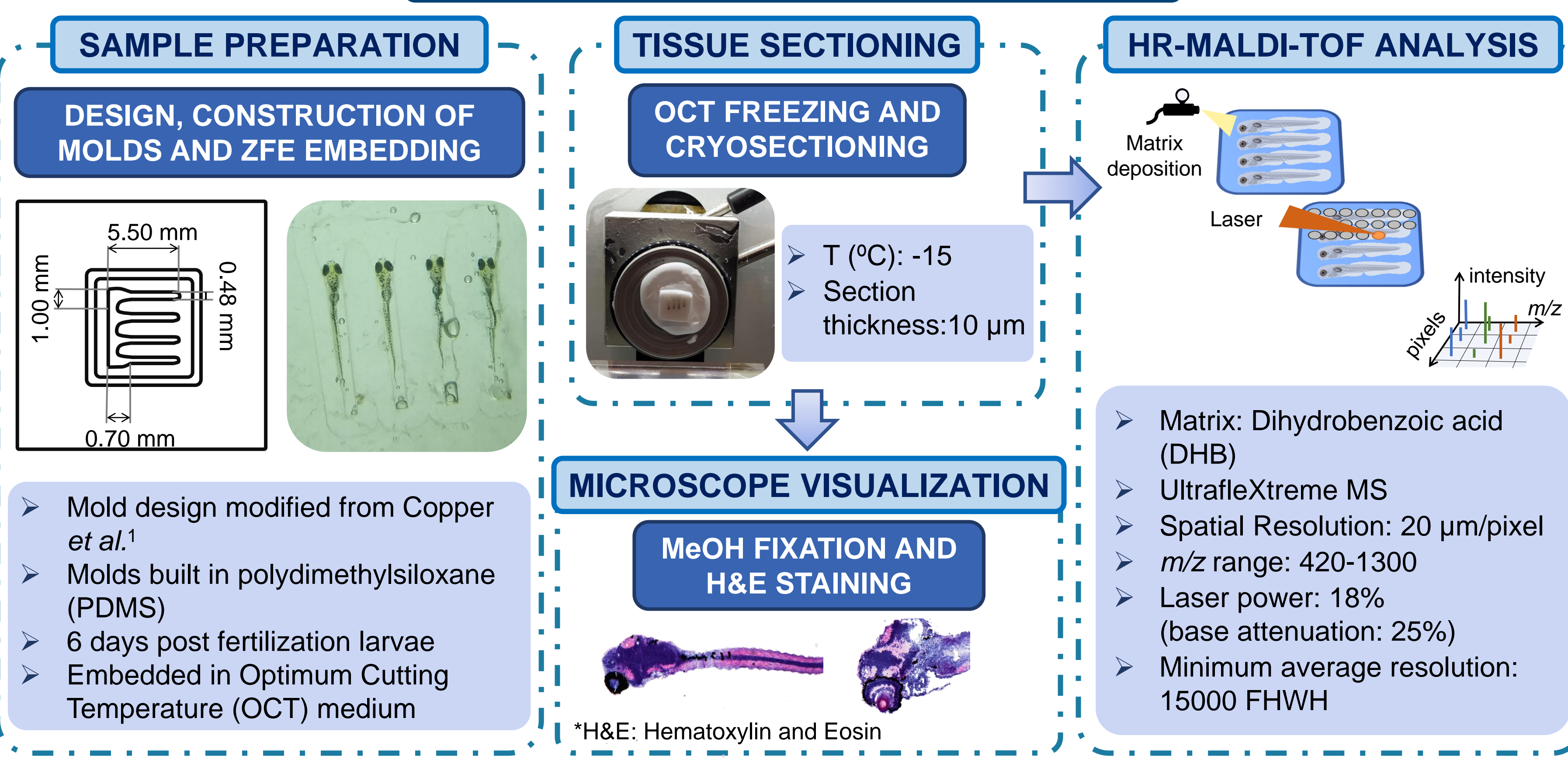➤ **Gangliosides** are the most **abundant lipid class** at the plasma membrane of the **nervous cells**[5]

CONCLUSIONS

✓ A **spatial lipidomic workflow** to analyze zebrafish embryo sections has been **successfully developed**

✓ ROIs have been **putatively assigned** to different clusters

✓ **Lipidomic signatures** allocated in specific ROIs are **consistent** with **their expected lipidic profile** (e.g., PCs and TGs were located in different cell layers of the ZFE eye)

✓ **MSI** has demonstrated to account for the **cellular heterogeneity** when using whole body embryos
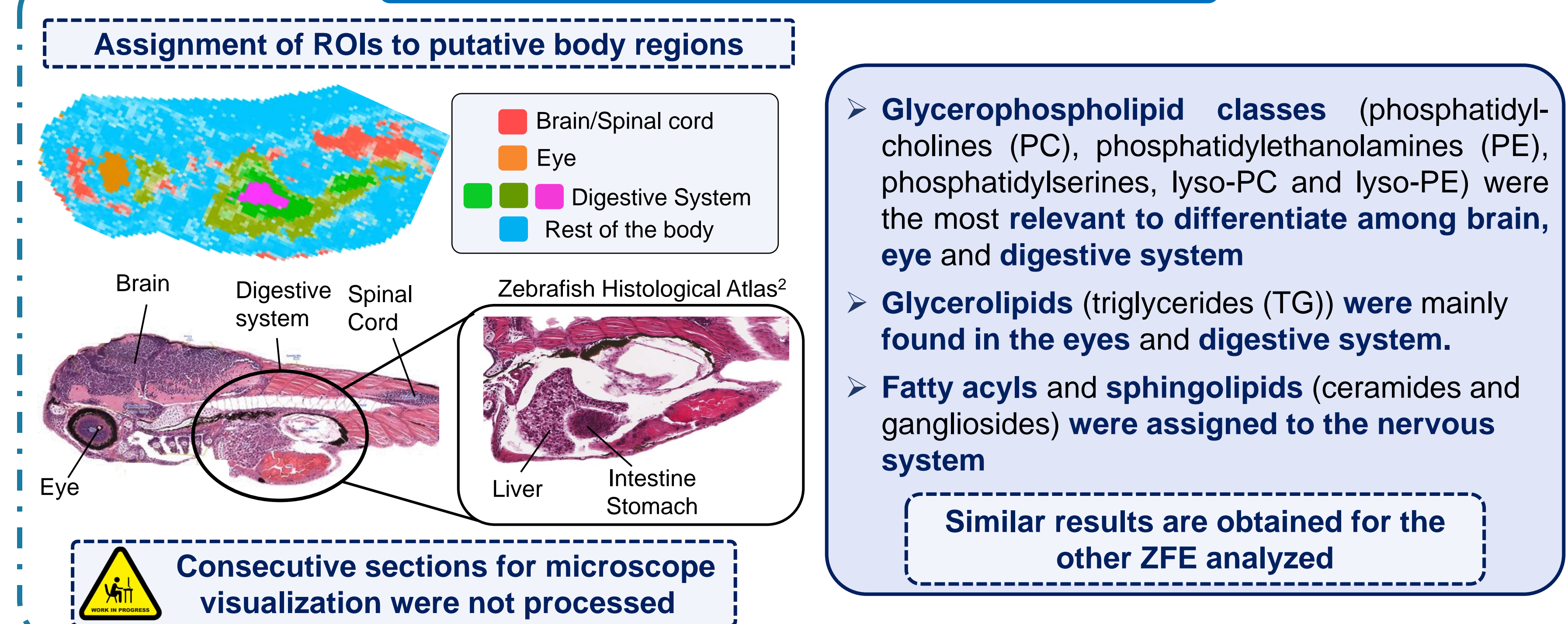
FUTURE PERSPECTIVES

➤ Although promising results were achieved, future work is still required

➤ **Consecutive sections** used for histological staining are **required to better characterize** different **body regions** found in the ZFE sections

➤ **Ongoing spatial transcriptomics optimization** will allow identifying ROIs and relevant transcriptomic signatures associated with different cell populations

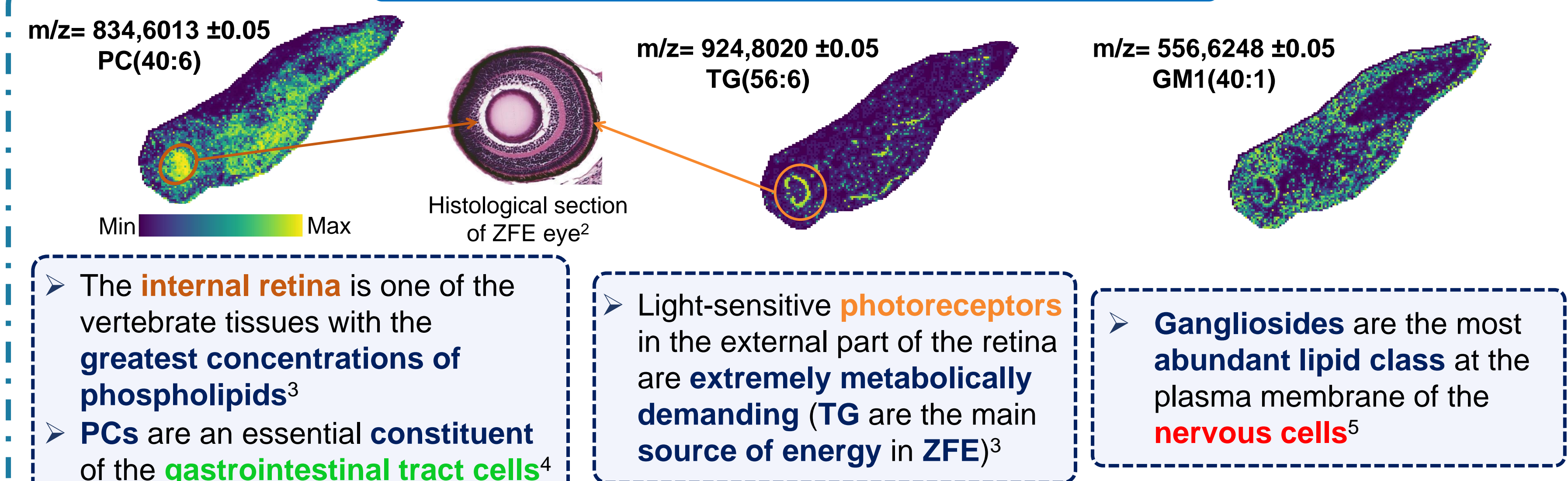➤ **Spatial multi-omic approaches** will be used to **study the effects of EDCs on ZFE.**

ACKNOWLEDGMENTS

REFERENCES

[1] Copper, J.E.; *et al.* Comp. Biochem. Physiol. Part - C. **2018**, *208*, 38–46.
[2] Bemis K.; *et al.* Bioinformatics **2015**, *31 (14)*, 2418–2420.
[3] Dasyani, M.; *et al.* Cells **2020**, *9*, 2583.
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[5] Sipione, S.; *et al.* Front. Neurosci **2020**, *14*, 1004 –1028.
[6] Joyal, J.S.; *et al.* Prog Retin Eye Res **2018**, *64*, 131–156 .