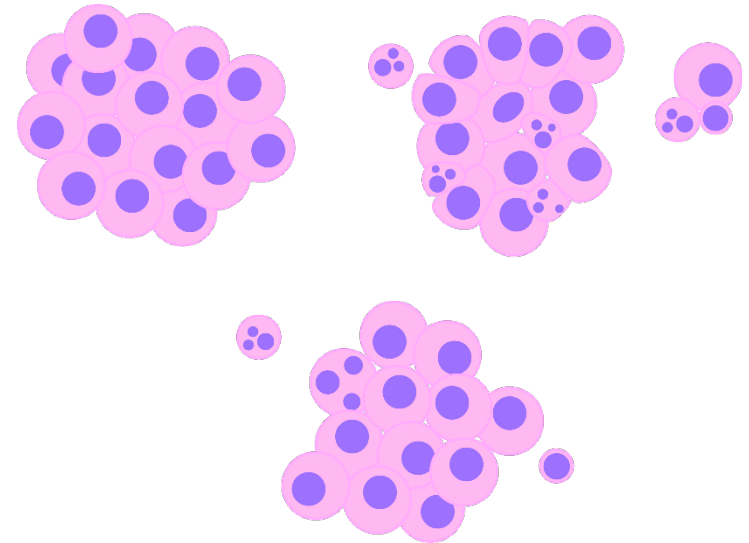
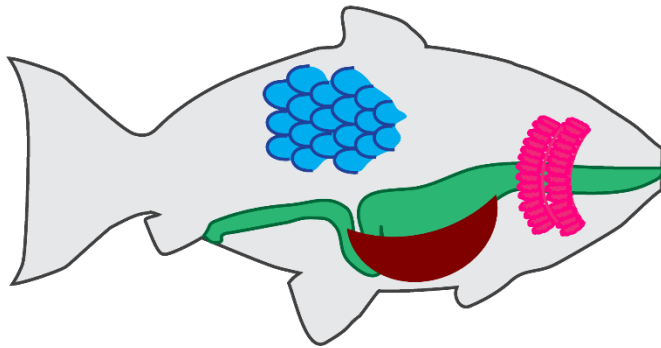


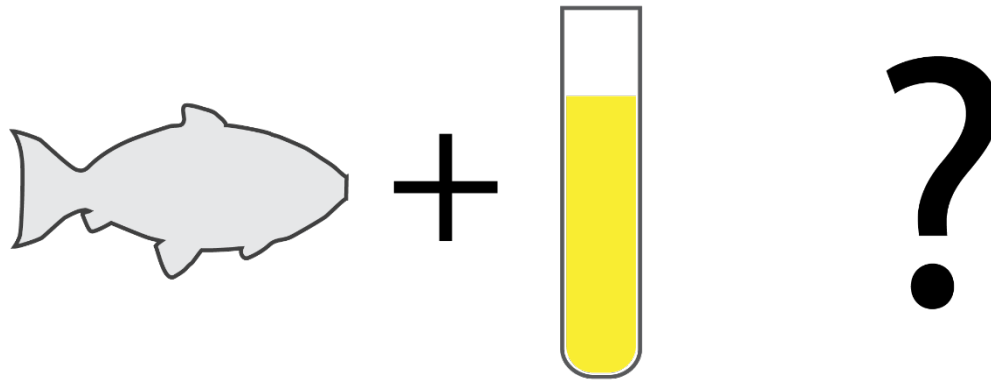
Fish Liver Microtissues for Aquatic Tox: Integrating Morphological & Molecular Responses for *In Vitro* Assessment of Environmental Pollutants



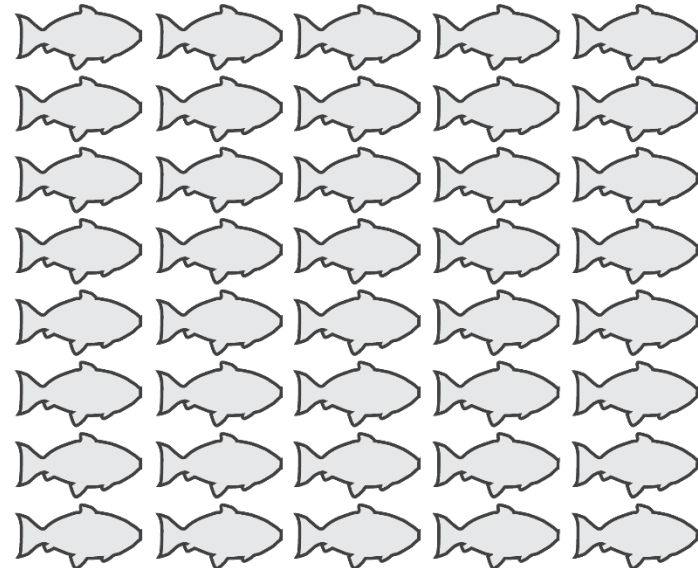
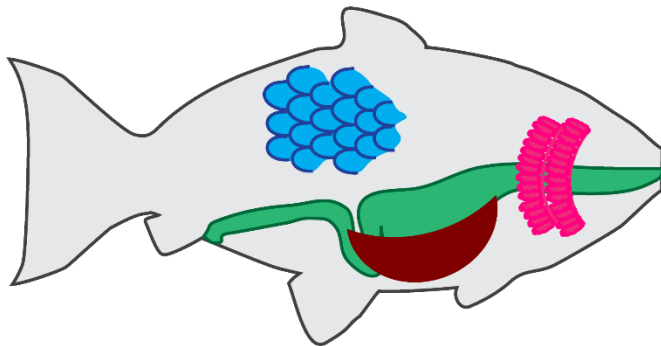
April Rodd

PostDoc @ Brown University, Dept Pathology & Laboratory Medicine

Alternative Toxicity Testing Approaches

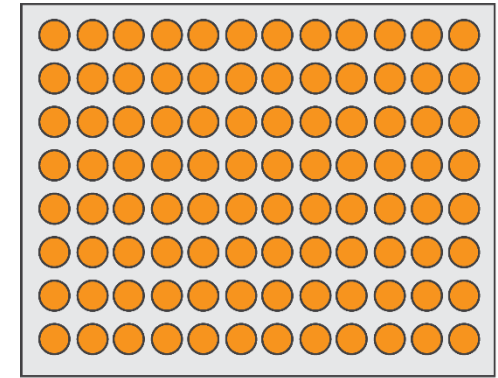
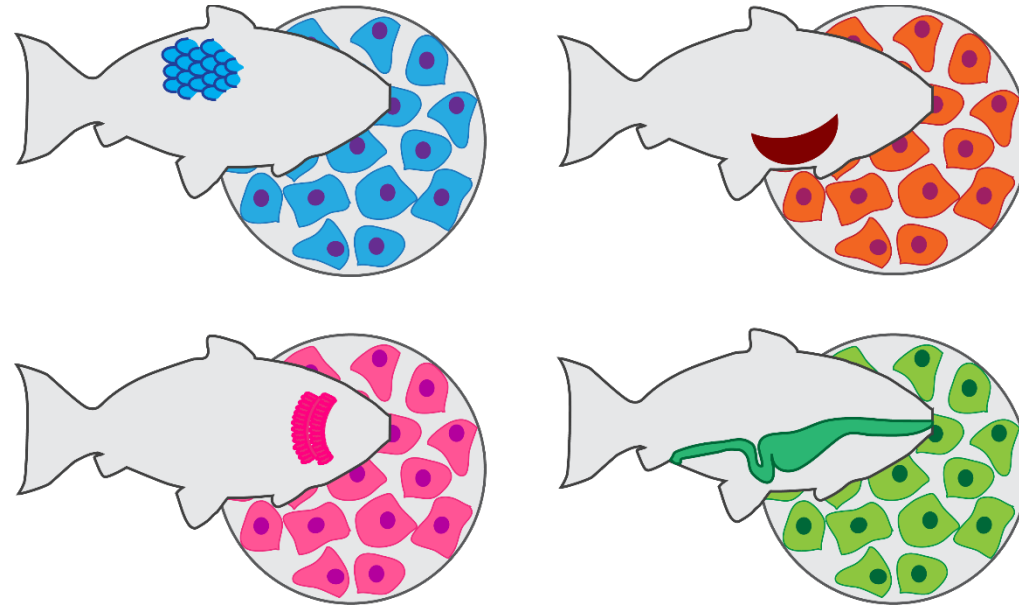


What do we do when a new, potentially toxic material needs to be tested?

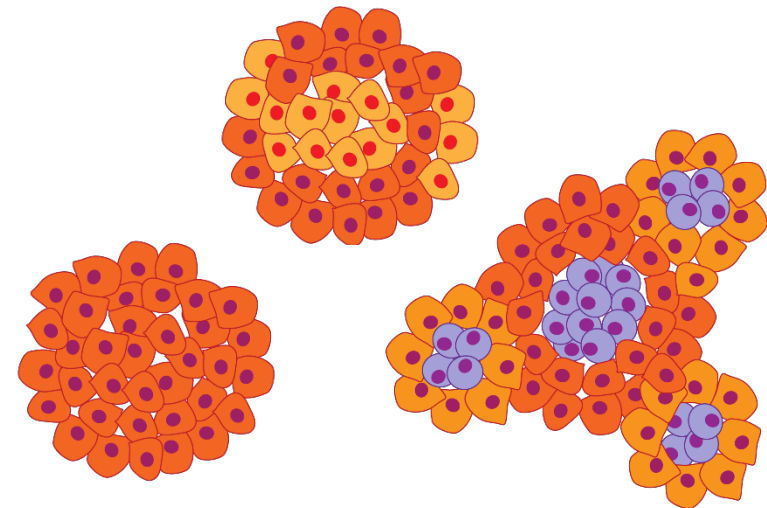


Alternative Toxicity Testing Approaches

Select Target Organs & Cells



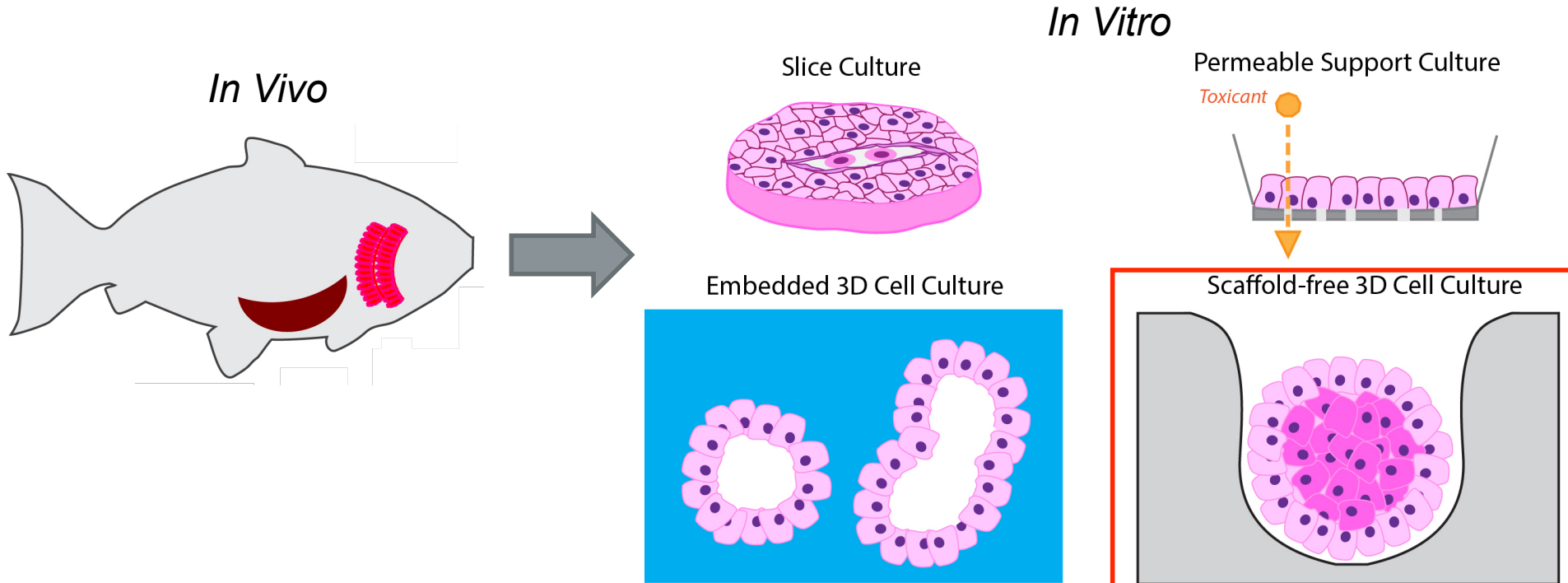
High-throughput Screens



Complex Cell Models

3D Models for Environmental Toxicology

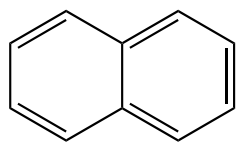
- 2D cells do not accurately reflect the response of *in vivo* tissues
 - Increasing need for advanced screening tools for aquatic toxicology
- 3D cell culture acts as a bridge between monolayer *in vitro* assays and *in vivo* exposures
 - Balancing increased throughput with increased tissue complexity



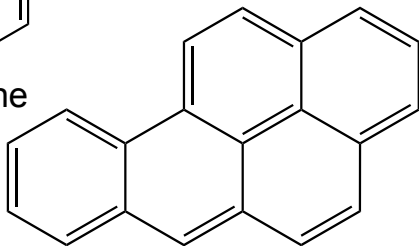
Aromatic Hydrocarbon Toxicity

- Polycyclic aromatic hydrocarbons (PAHs) persist in sediment and can accumulate in lipids
 - Many are carcinogenic and EPA priority pollutants
 - Metabolic activation by Cytochrome P450 enzymes can cause cell death, reactive oxygen species, and DNA adducts

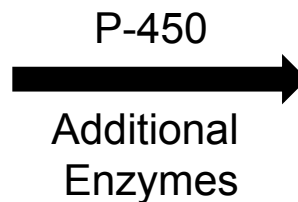
Metabolism of Aromatic Hydrocarbons



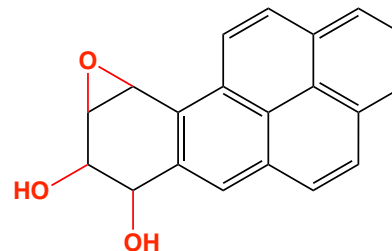
Naphthalene



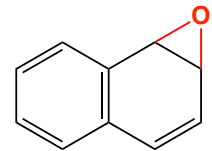
Benzo(a)pyrene



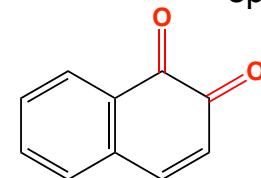
Carcinogenic Metabolites



Benzo(a)pyrene 7,8
 diol-9,10 epoxide



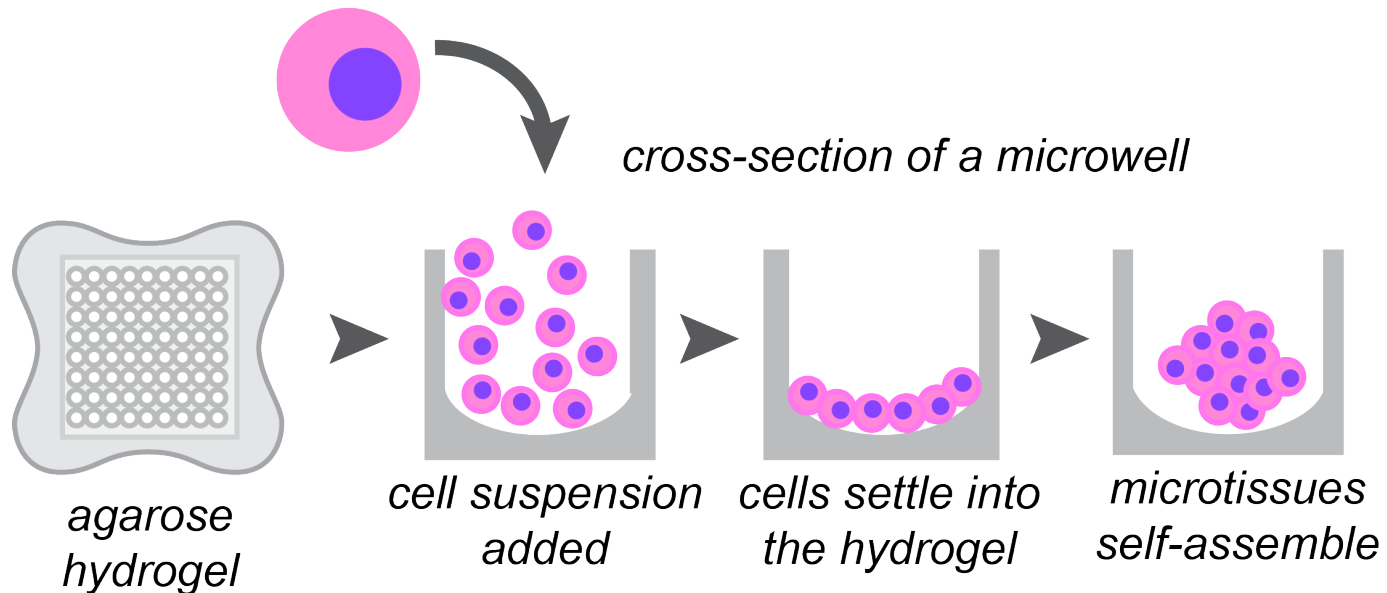
Naphthalene-1,2-
 epoxide



1,2-Naphthoquinone

Fish Liver Microtissue Formation

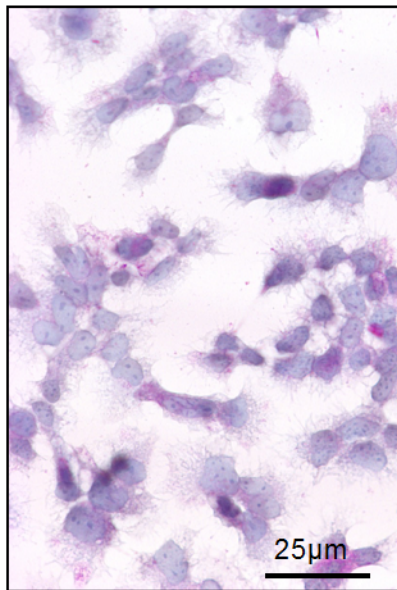
- Microtissues formed with PLHC-1 fish liver cells
 - Self-assemble through cell-cell adhesion and cytoskeletal forces
 - Method applied to many cell types and known to increase hepatocyte differentiation
- Can be assessed using both fluorescent and histological techniques



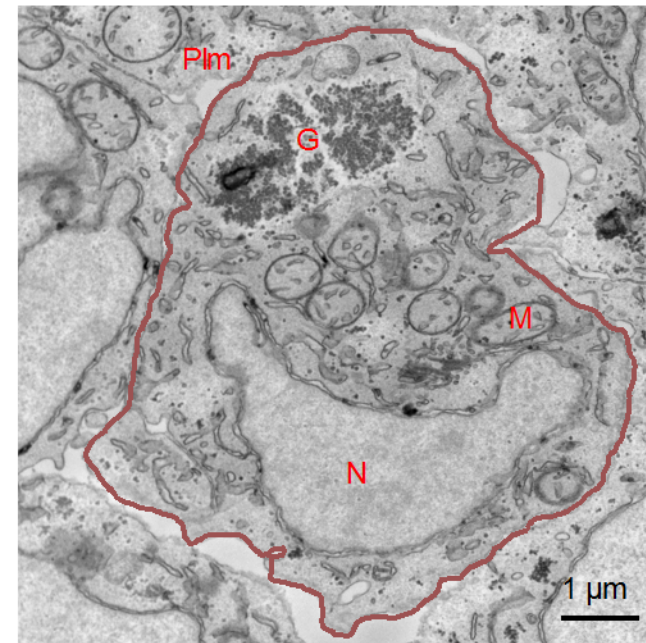
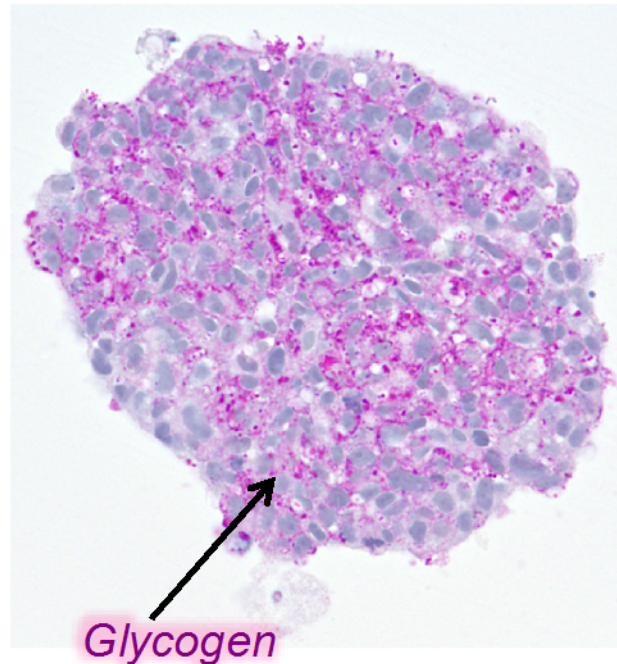
Microtissue Characterization

- Microtissues are stable and viable for at least 2 weeks
- Markers of liver differentiation stable or increasing over time

2D Fish Liver Cells

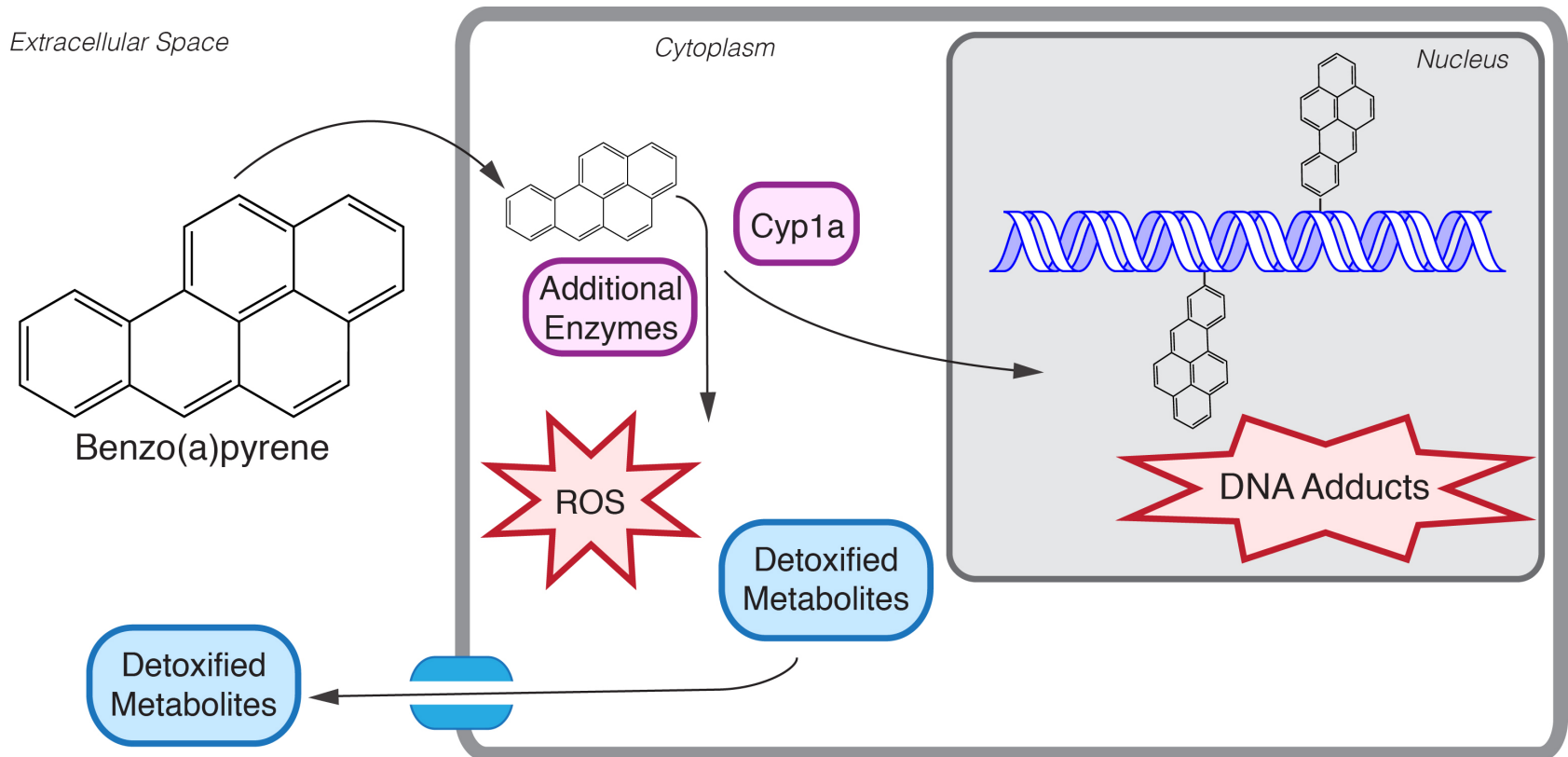


Fish Liver Spheroids



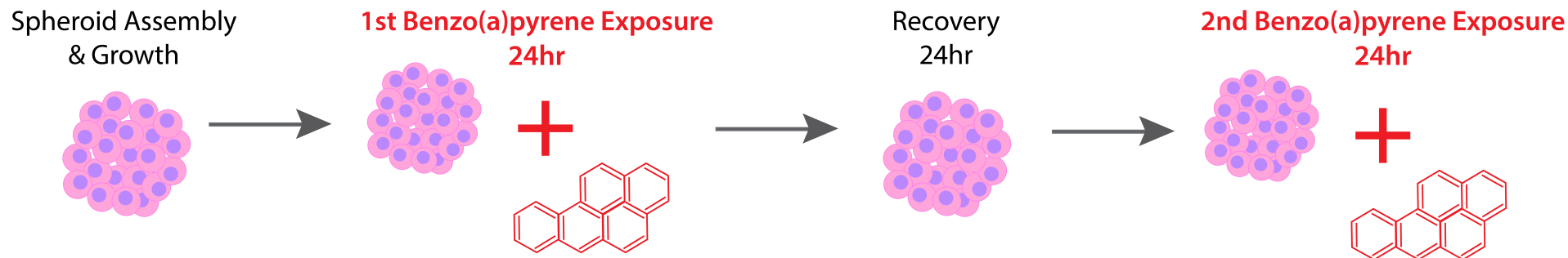
Cytochrome P450 1A (Cyp1a) Expression

- Cyp1a metabolizes polycyclic aromatic hydrocarbons (PAHs)
 - Generates both detoxified and reactive metabolites
- Specific biomarker upregulated in response to PAH exposure



Microtissue Exposure to Benzo(a)pyrene

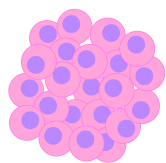
- 3D cell culture allows for more prolonged and complex exposures
 - Microtissues have extended window of exposure
 - Added complexity of multiple exposures
- Metabolic activation of PAHs can cause delayed effects
 - May go undetected following acute, single exposure exposures



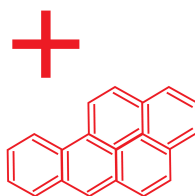
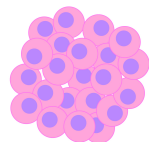
Microtissue Exposure to Benzo(a)pyrene

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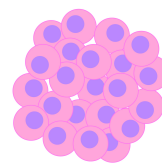
Spheroid Assembly
& Growth



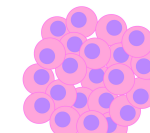
1st Benzo(a)pyrene Exposure
24hr



Recovery
24hr



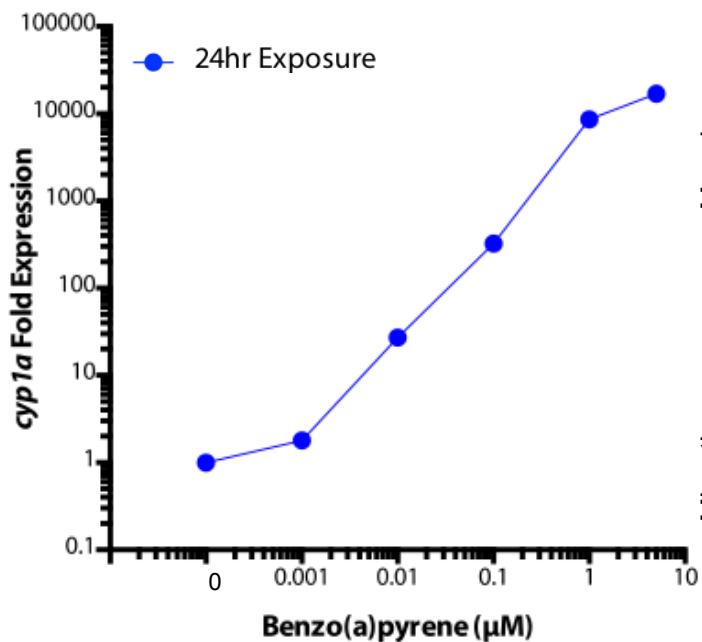
2nd Benzo(a)pyrene Exposure
24hr



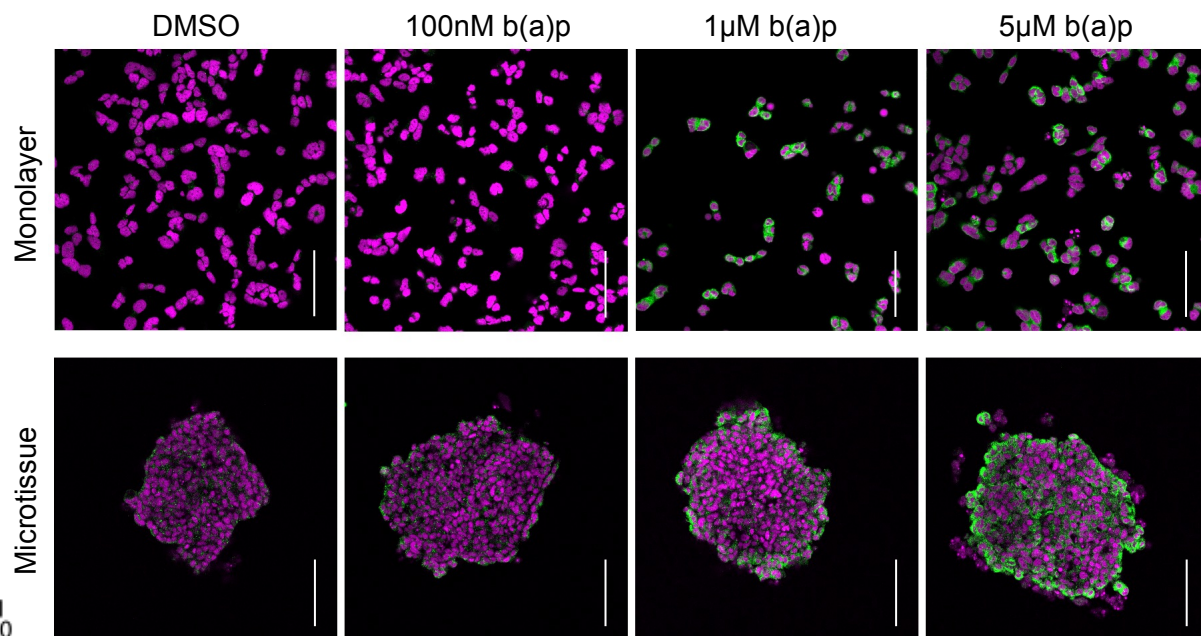
Sensitive Cyp1a Induction After B(a)p Exposure

- Response to a 24hr benzo(a)pyrene exposure
 - Highly sensitive increase in *cyp1a* gene expression
 - Dose dependent increase in Cyp1a protein *in situ* with three-dimensional protein induction

Cyp1a Gene Expression



Cyp1a Protein Expression





Sensitive Cyp1a Induction After B(a)p Exposure

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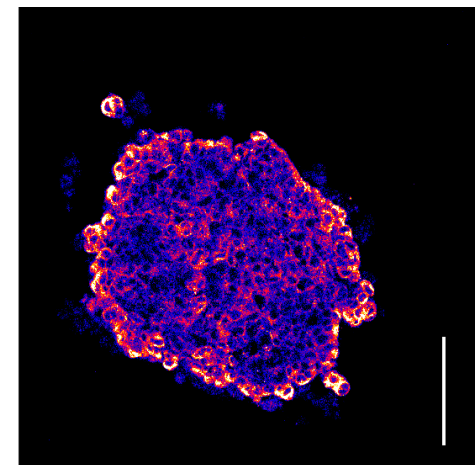
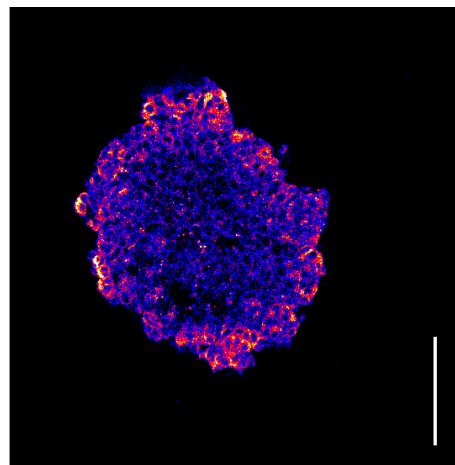
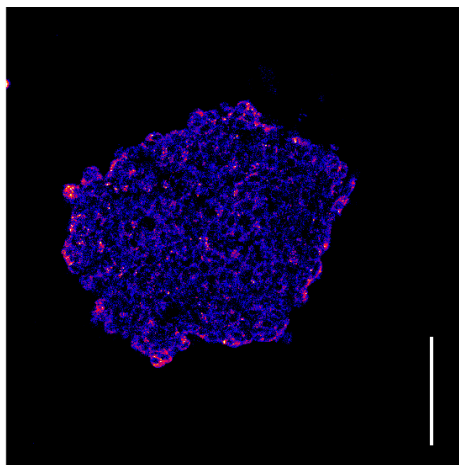
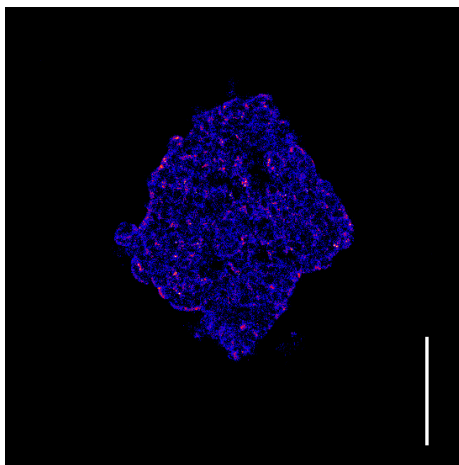
Cyp1a Protein Expression

DMSO

100nM b(a)p

1μM b(a)p

5μM b(a)p



Low Cyp1a

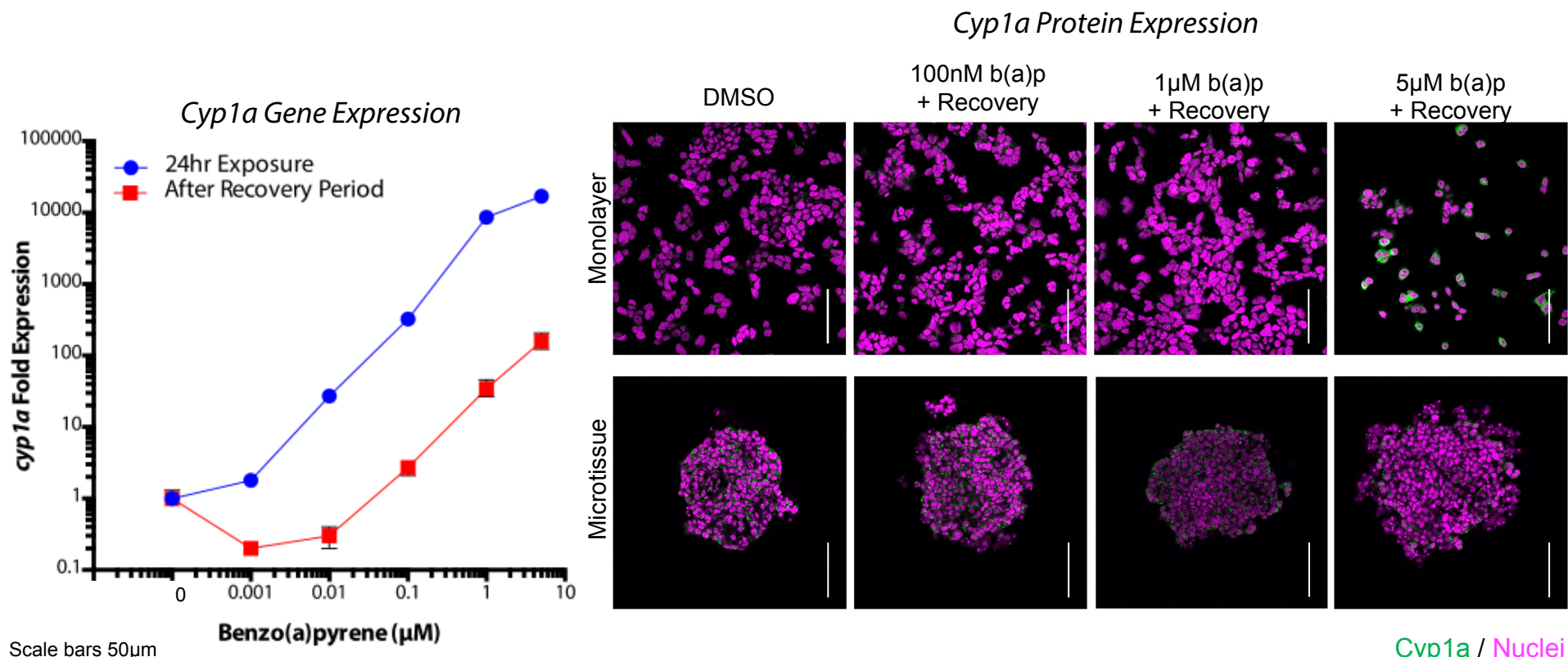


High Cyp1a



Continued Cellular Changes During Recovery

- Continued adverse effects after recovery period
 - 2D monolayer cells proliferate, while 3D microtissues do not
 - Gene expression of *cyp1a* falls following b(a)p removal





Continued Cellular Changes During Recovery

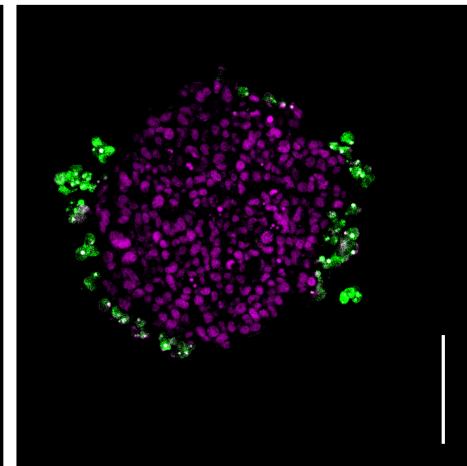
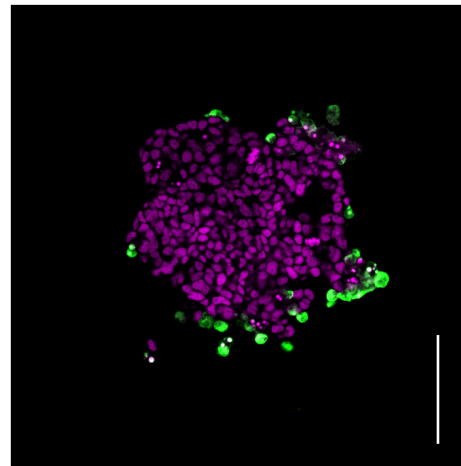
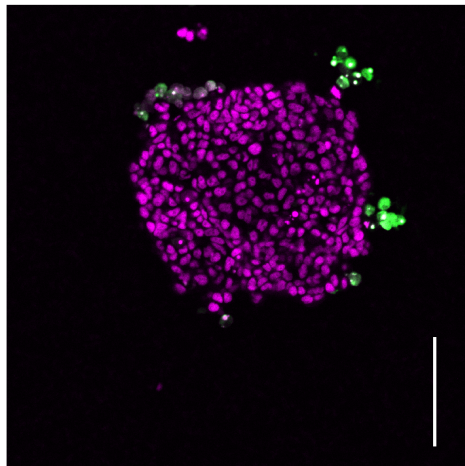
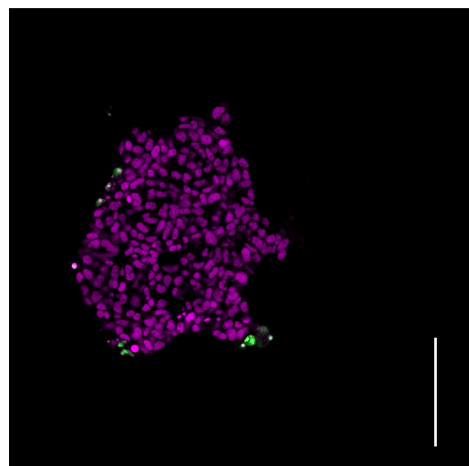
- Continued adverse effects after recovery period
 - Cell death and spheroid morphological change continue to increase

DMSO

100nM b(a)p
+ Recovery

1µM b(a)p
+ Recovery

5µM b(a)p
+ Recovery

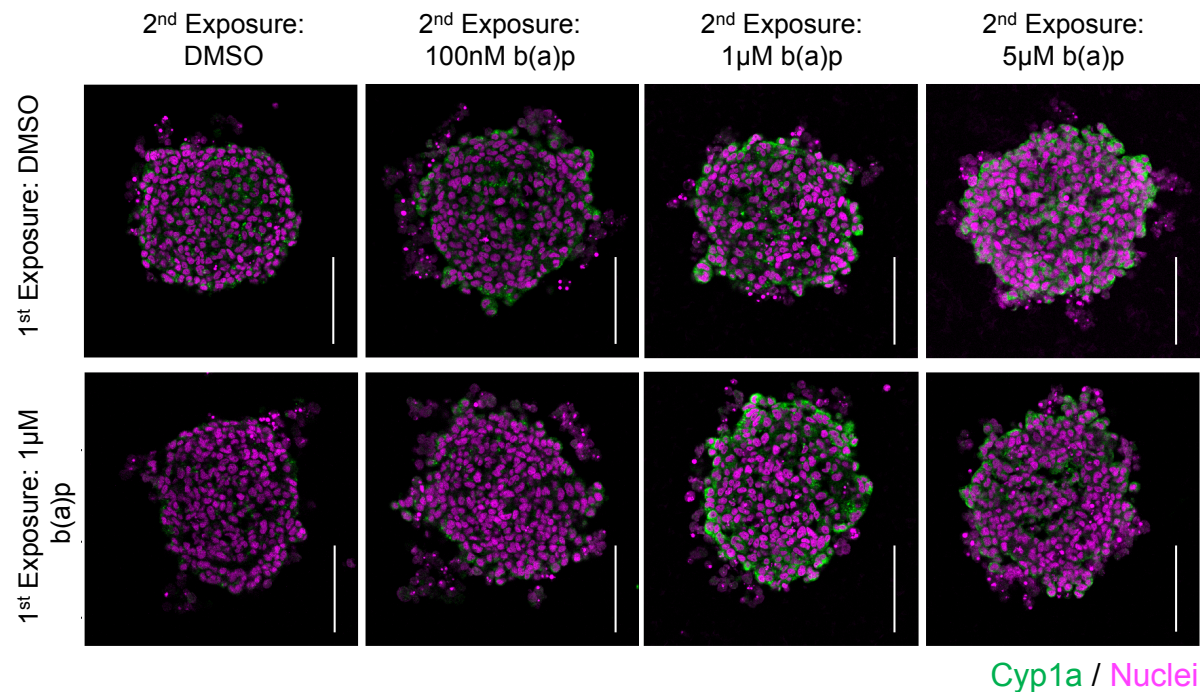


TUNEL / Nuclei

Cyp1a Induction Unaltered By Previous B(a)p Exposure

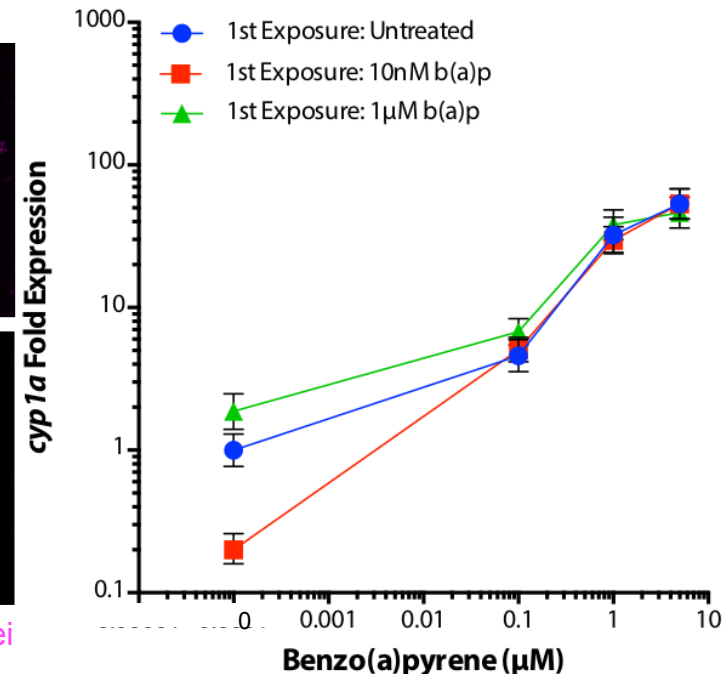
- Cyp1a gene or protein expression equally induced after second 24hr exposure to b(a)p

Cyp1a Protein Expression



Scale bars 50µm

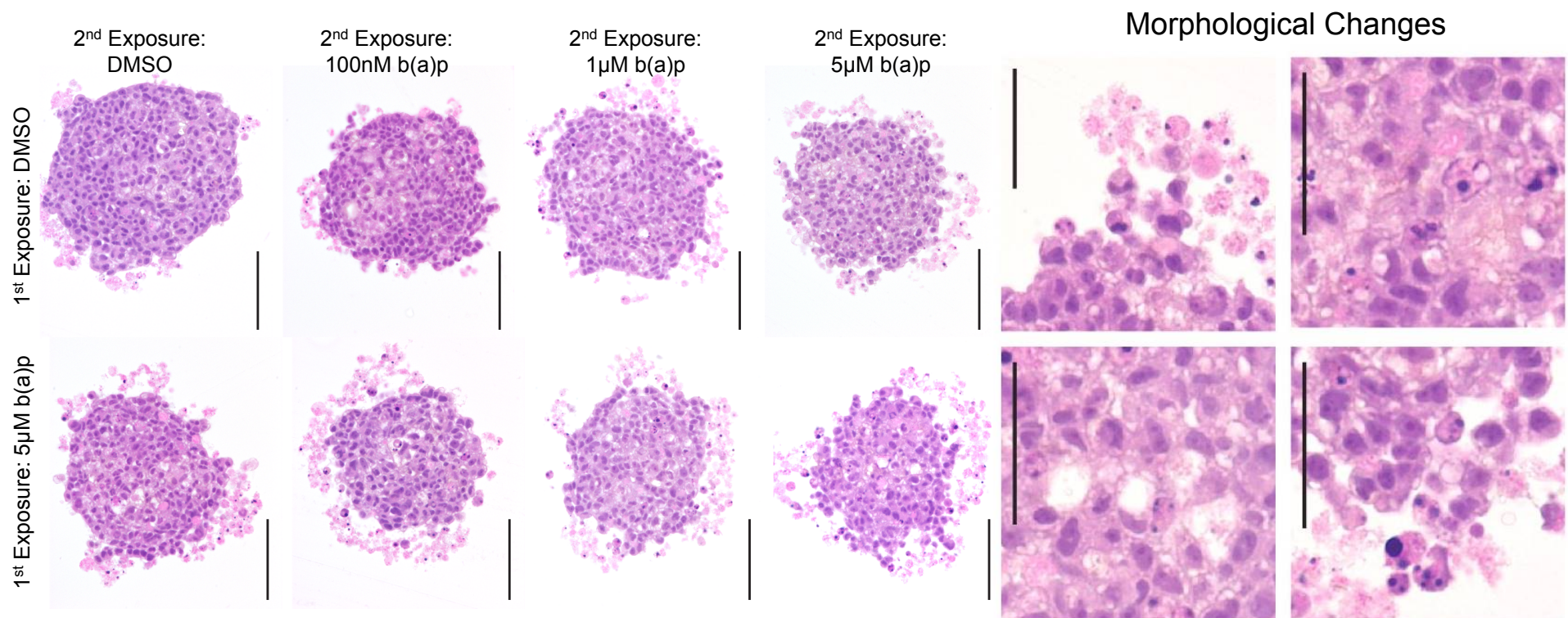
Cyp1a Gene Expression



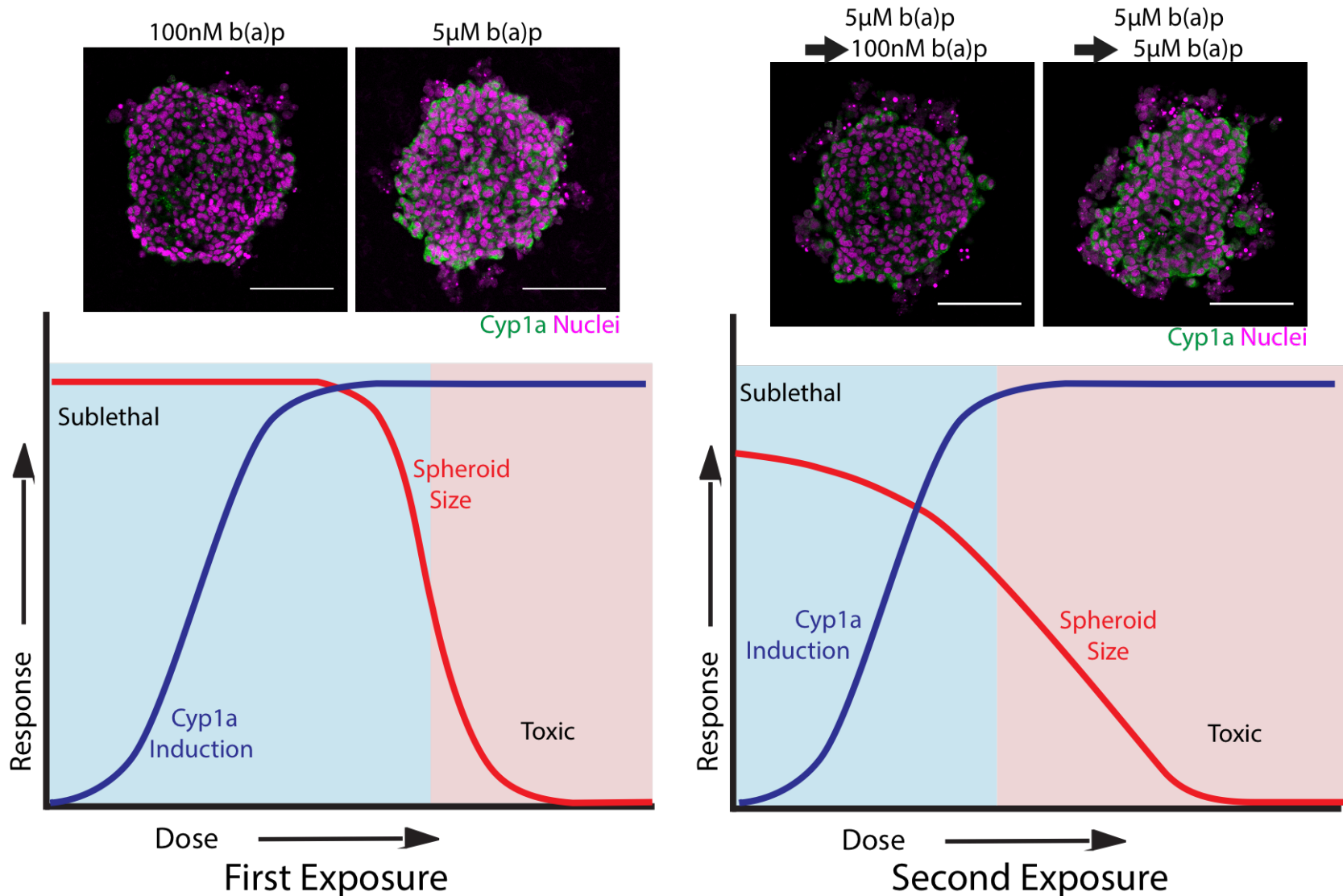


Changes in Microtissue Architecture After Repeated B(a)p Exposure

- Repeated exposure to benzo(a)pyrene shows survivability
 - Survival of spheroid core after high exposures
 - Disrupted spheroid architecture and morphology

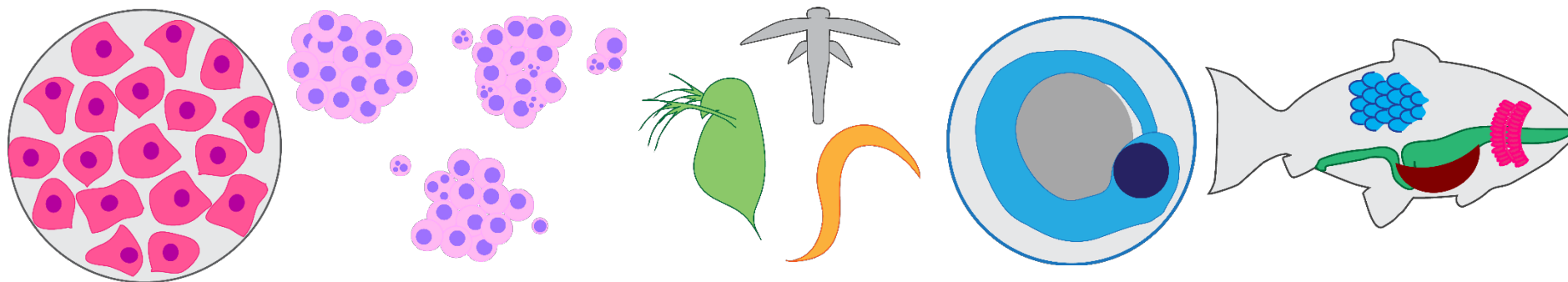


Effect of Multiple B(a)p Exposures



Summary

- Single benzo(a)pyrene exposures elicit sensitive and specific responses in fish liver microtissues
- Repeated exposure results in tissue-level changes to microtissue architecture without altering the induction of Cyp1a
- Microtissues can be used as a sensitive tool to assess environmentally relevant aquatic exposures

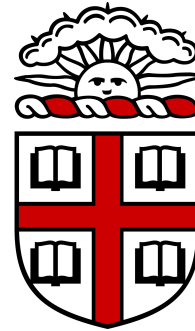


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 - PI: Robert Hurt, PhD



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