Video Analysis System for Behavior and Activity Assessment of Fruit-Flies in High-Throughput Chemical Safety Studies for European Commission PrecisionTox Consortium

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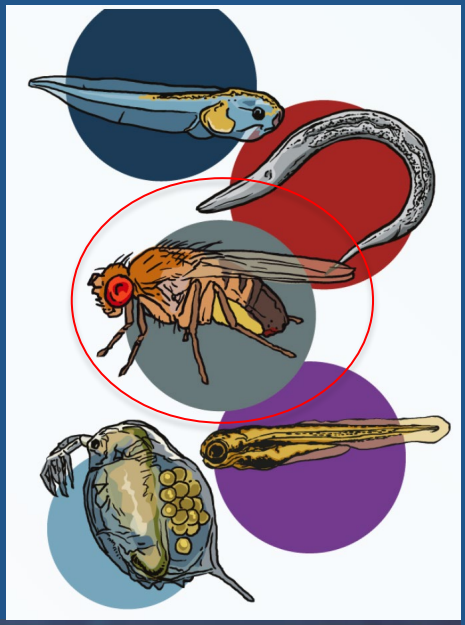
PrecisionTox

Consortium

PrecisionTox gathers 15 European and North American partners led by the University of Birmingham.

-Better protect health of people and environment
-Reduce, refine, replace traditional animal testing
-No perfect whole-organism human surrogate model
-Guesswork in exposure limits
-Scientific findings not translated to real-world

PrecisionTox Project Pillars



Phylotoxicology

Replace traditional animal testing with an Evolutionarily Diverse Model Suite of organisms from multiple branches of the tree of life.

Variation of Susceptibility

Determine safe levels of exposure to chemicals based on genetic variation.

Embedded Translation

Collaborate with regulators and other key stakeholders in project planning, selection of chemicals for investigation, and case studies for applying Precision Toxicology in policy and law.

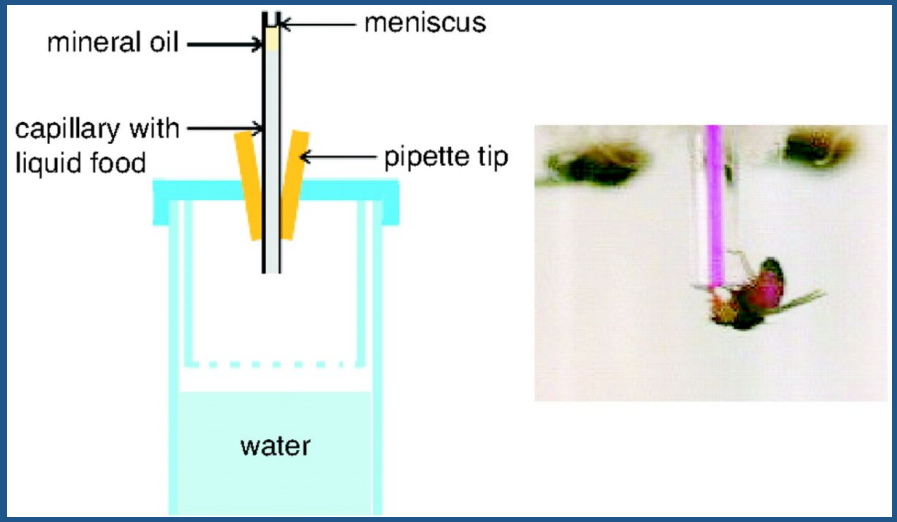
Fruit Fly

Proboscis

-Inexpensive, short life, well-developed tools
-Relevant feeding anatomy
    -~2mm head width
    -~35μm diameter proboscis (tubular sucking organ)

Fruit Fly Studies

-Whole organism: elucidate complex system
-Typically, fly, food, and excrement all in same well
-PrecisionTox:
    -Dose response w/ small consortium molecule libraries
    -Behavior assessment: video analysis

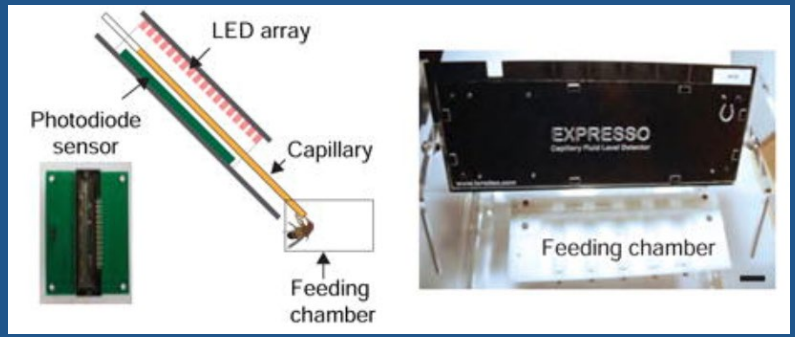


CAFÉ

Existing Solutions

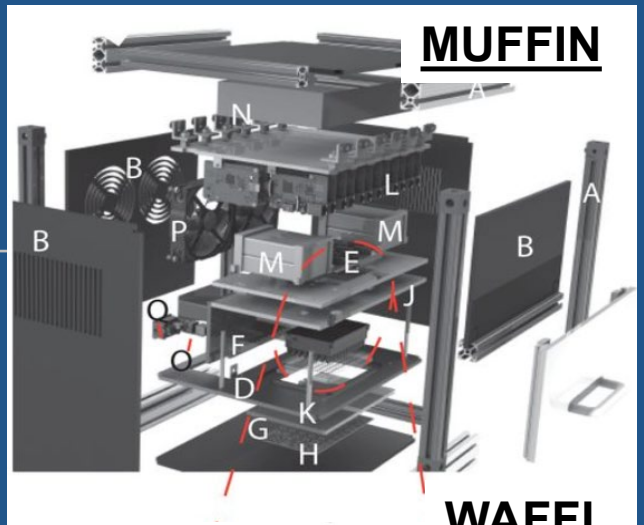
-Capillary feeder (CAFÉ) assay (Ja et al. 2007)
- -Does not scale: one narrow vial
- -Labor intensive: manual measurements of feed
- -Capillary on top occludes video acquisition

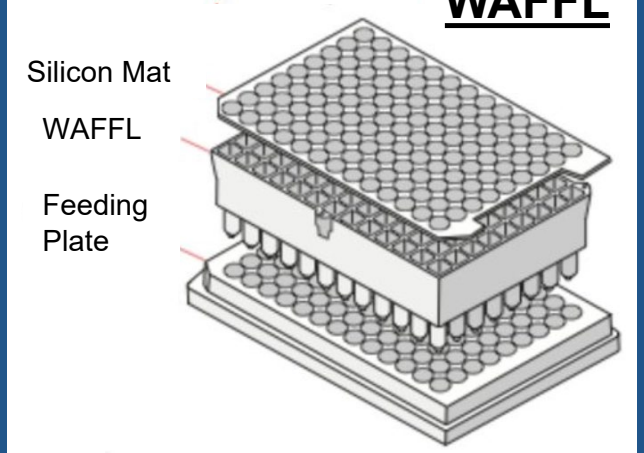
-Expresso assay (automated CAFÉ) (Yapici et al. 2016)
- -Addresses labor
- -Capillary on top still occludes video acquisition



Expresso

Generation 1
Whole Animal Feeding Flat (WAFFL) & Monitoring Unit for Fruit Fly Imaging in Ninety-six-wells (MUFFIN) (NIBIB, NIDDK)

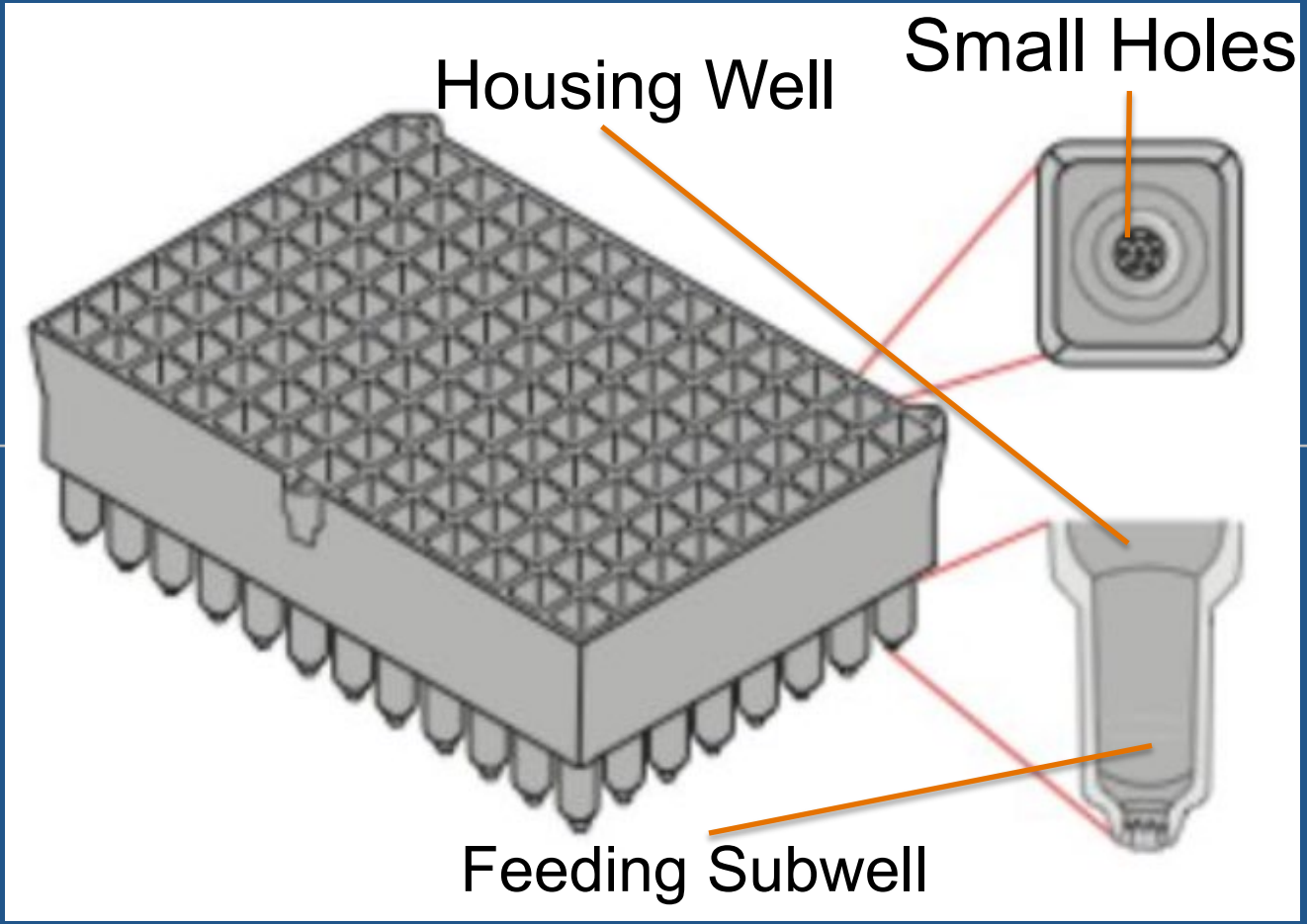




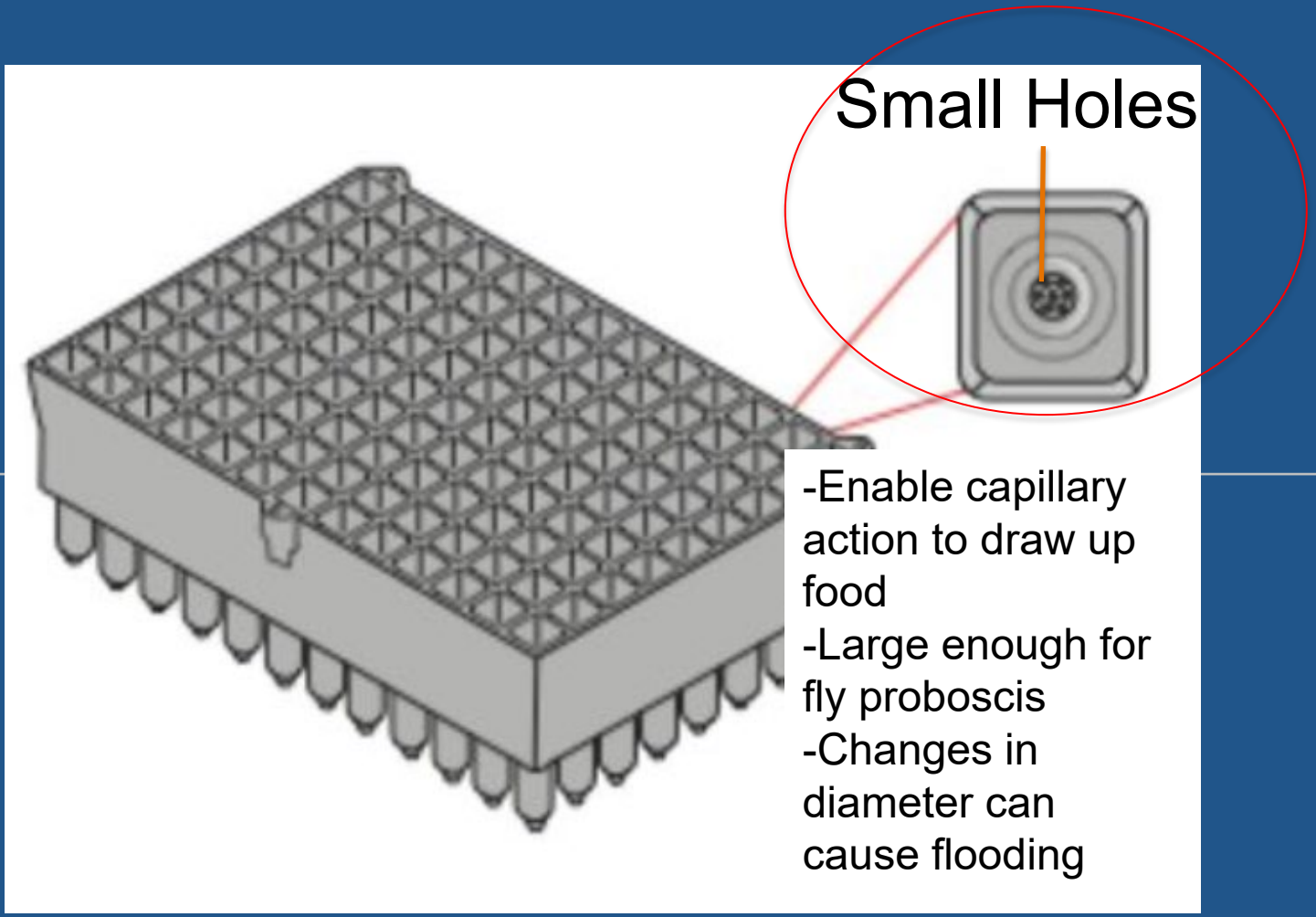
-WAFFL: housing plate & feeding plate for flies
- -Compatible w/ 96-well components
- -20 µL feeding volume: standard 96-well for feeding plate
- -Decreased labor after loading flies
- -WAFFL printed in high resolution (350µm holes)

-MUFFIN: fly video monitoring
- -24 Raspberry Pi single-board computers
- -24 Raspberry Pi v2 Cameras: 8MP
- -4 wells imaged per camera
- -Enough resolution for motion detection

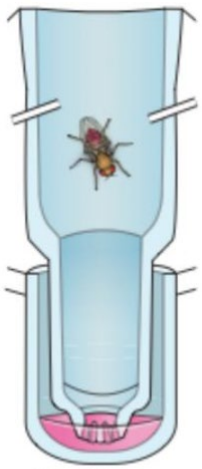
WAFFL Terminology



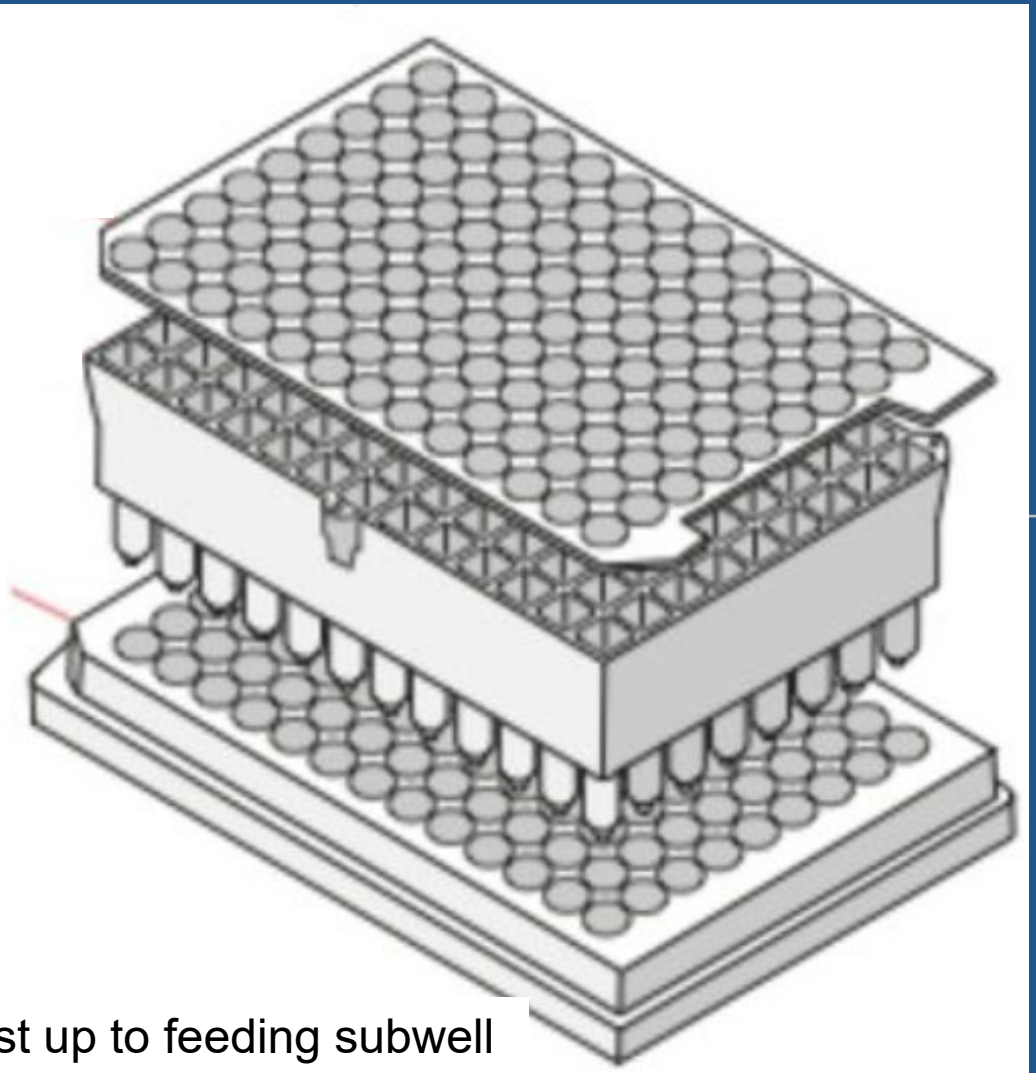
WAFFL Holes


Small Holes
-Enable capillary action to draw up food
-Large enough for fly proboscis
-Changes in diameter can cause flooding

WAFFL Terminology

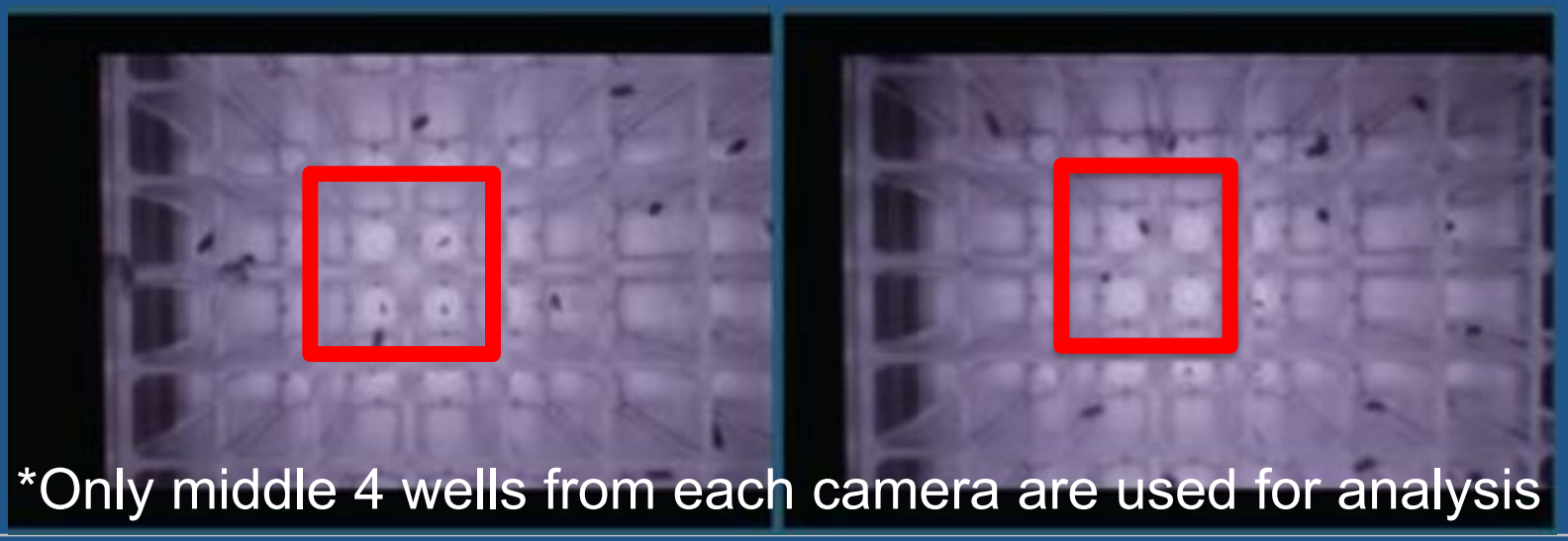


Feeding Plate

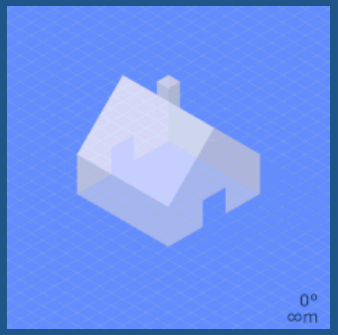


*Must be filled just up to feeding subwell

MUFFIN Video Quality



-Monochromatic image
  -Near IR: sensitive up to 900nm
  -For circadian studies
-Field of View: 4cm x 4cm
-Depth of Field: 1cm
-Blurriness due to depth of field



-Configuration limits perspective distortion (stretching view towards center)
-At the same time, configuration attempts to conserve depth of field
-Cannot move camera closer

Solution: WAFFL 2.0 & MUFFIN 2.0

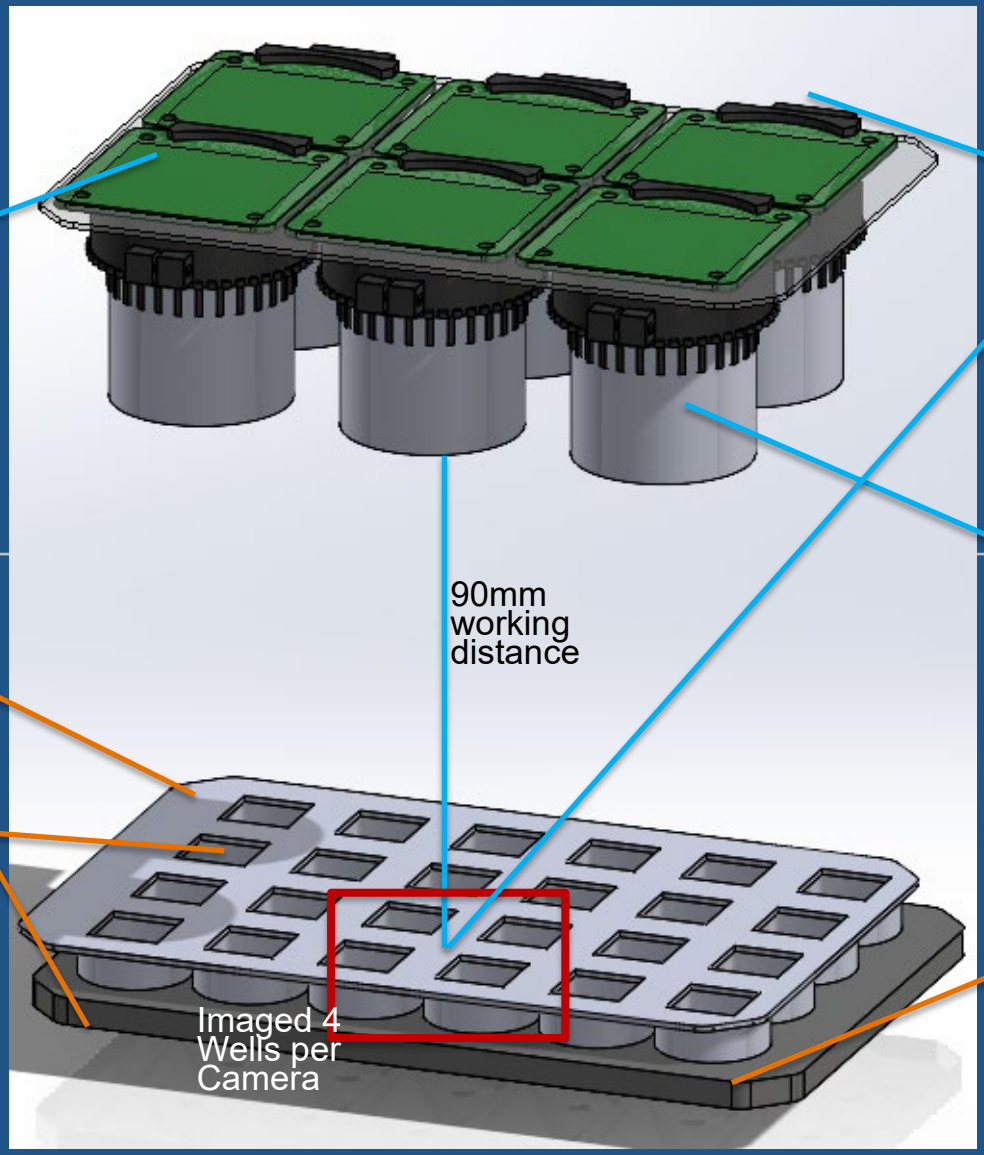
-WAFFL 2.0

- -Scalability with injection molding: PrecisionTox is large-scale study
- -High-throughput: 24-well format
- -Larger housing with custom feeding plate

-MUFFIN 2.0

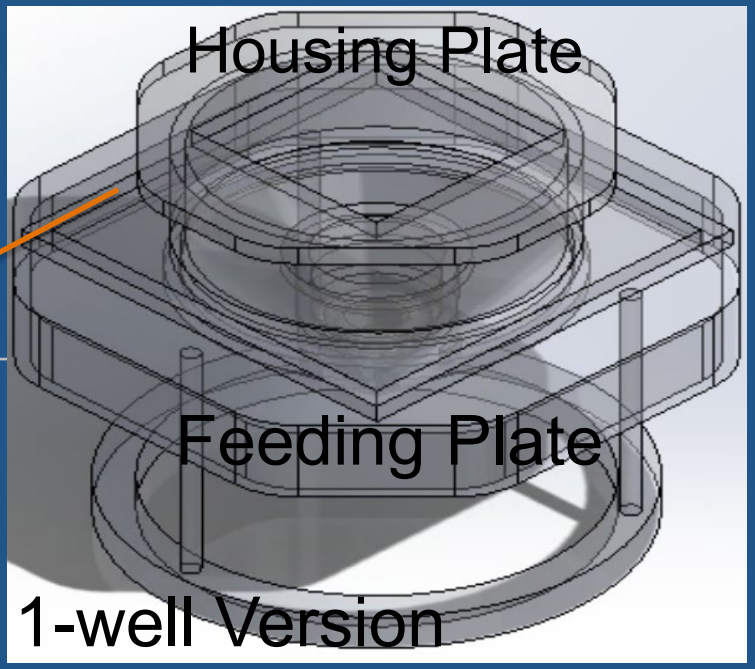
- -Higher image resolution
  - -Resolve 3D position and fine grain behavior
  - -Accurate capture of fly food interaction
- -Higher camera framerate

WAFFL 2.0 and MUFFIN 2.0 Assembly



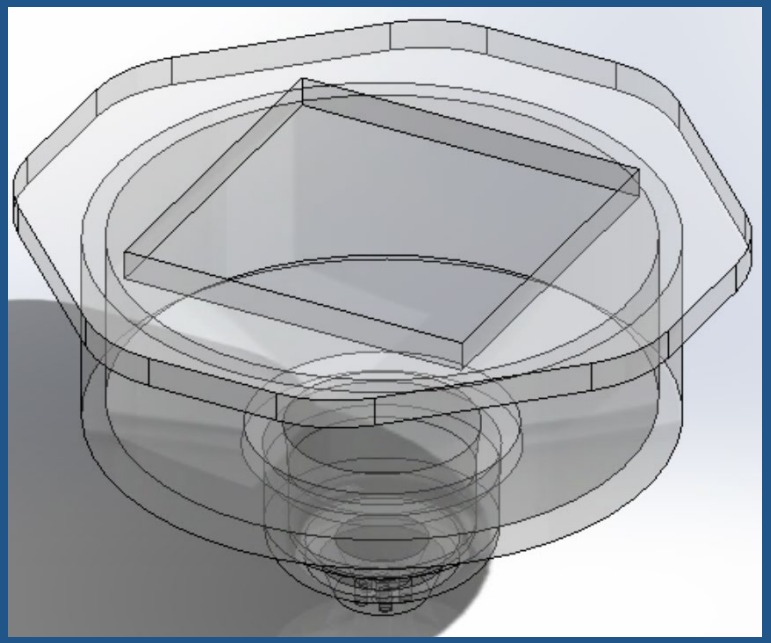
1-well WAFFL 2.0

*Made transparent for clarity

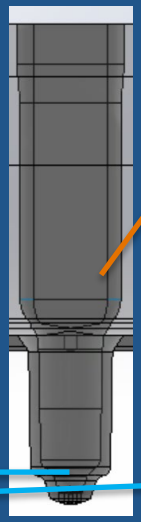


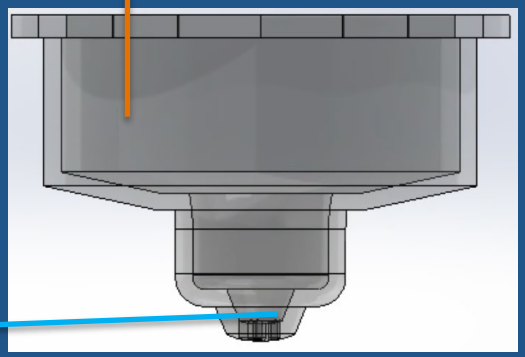
-1-well Version created for testing
-Supports added to 1-well for stability (NOT in 24)

WAFFL 2.0 Housing Plate



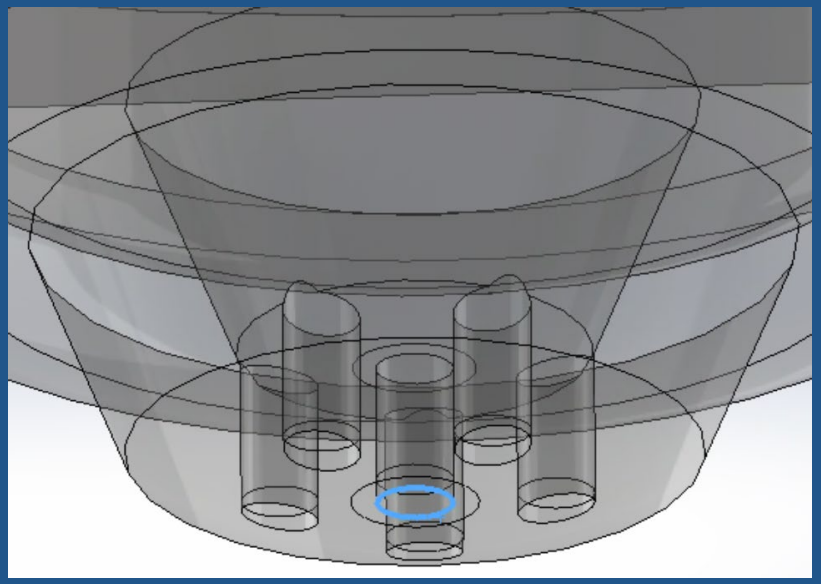
-Reduced well depth
-Larger housing
-Old/New Dia: 5.5mm/17.5mm



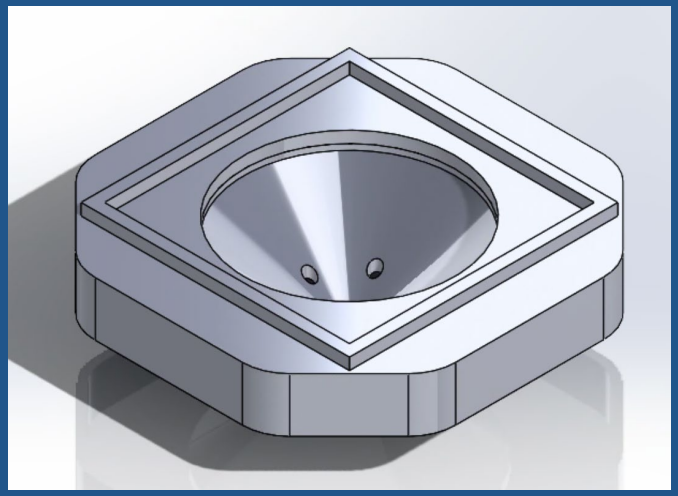


1st Generation 2nd Generation

WAFFL 2.0 Housing Plate Feeding Subwell

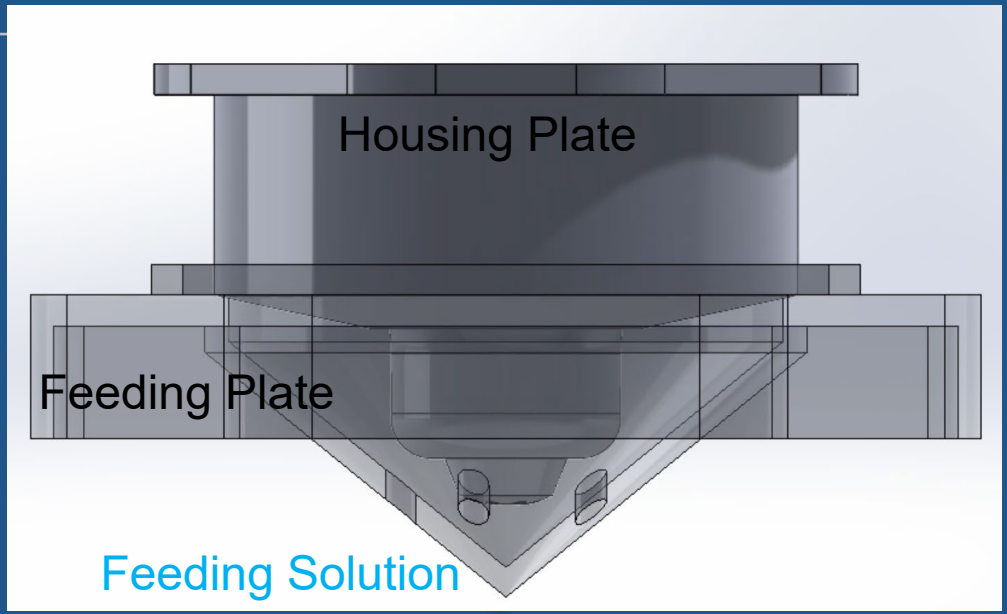


-400 µm hole diameters
-Large enough for injection-molding
-Injection moldable: cupular shape & 2° drafting



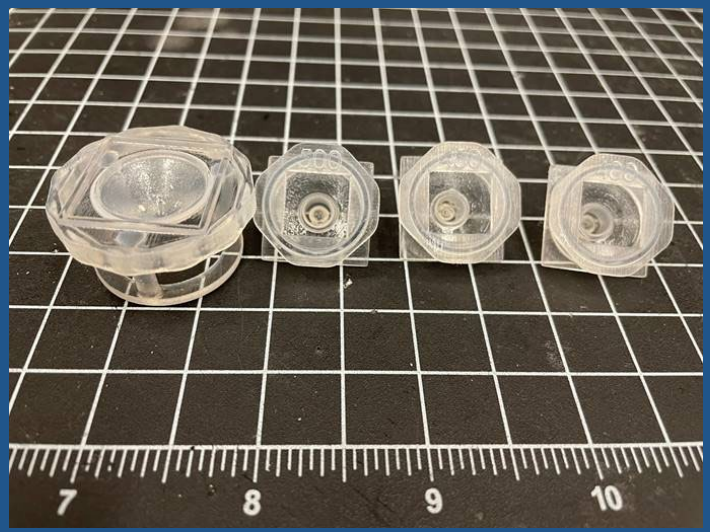
WAFFL 2.0 Feeding Plate

-V-shaped bottom
-20 µL reaches interface
-Conserve small reagent usage
-Five 1mm holes prevent overfilling
-Overfilling causes housing to flood

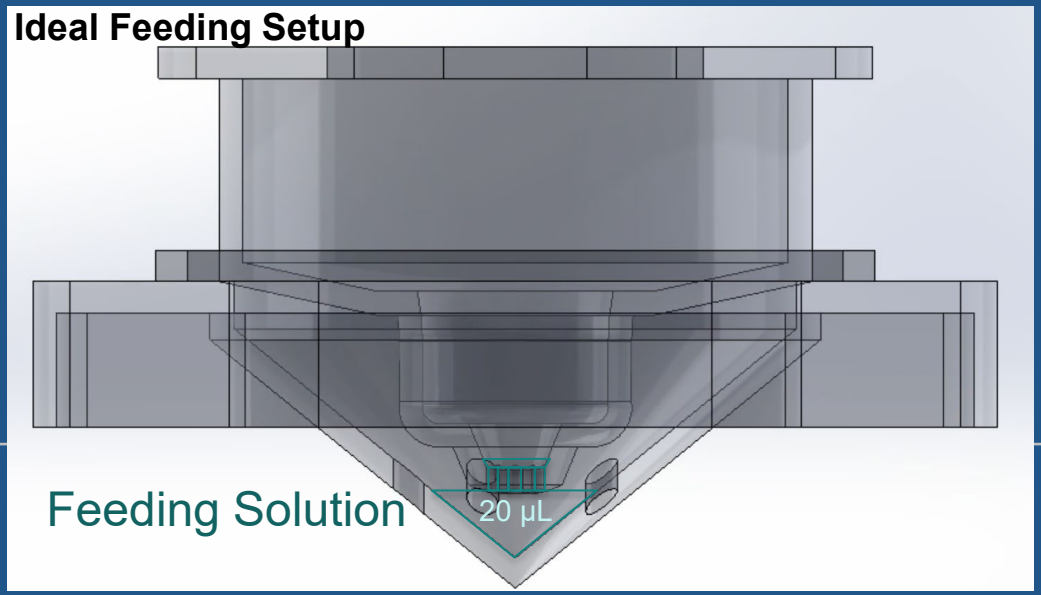


Fabrication and Testing

-Did not injection mold for testing
    -Expensive
    -1-well to test mating between housing and feeding
    -Make sure no flooding
-Material: Veroclear
    -Waterproof
        -Feed not absorbed by plate
        -Easy cleaning
    -Non-reactive & biosafe
-Printer: Eden 260VS
    -High Resolution: 16µm layer height
    -Finishing: Matte



Hole-size Determination: Initial Capillary Action Experiments

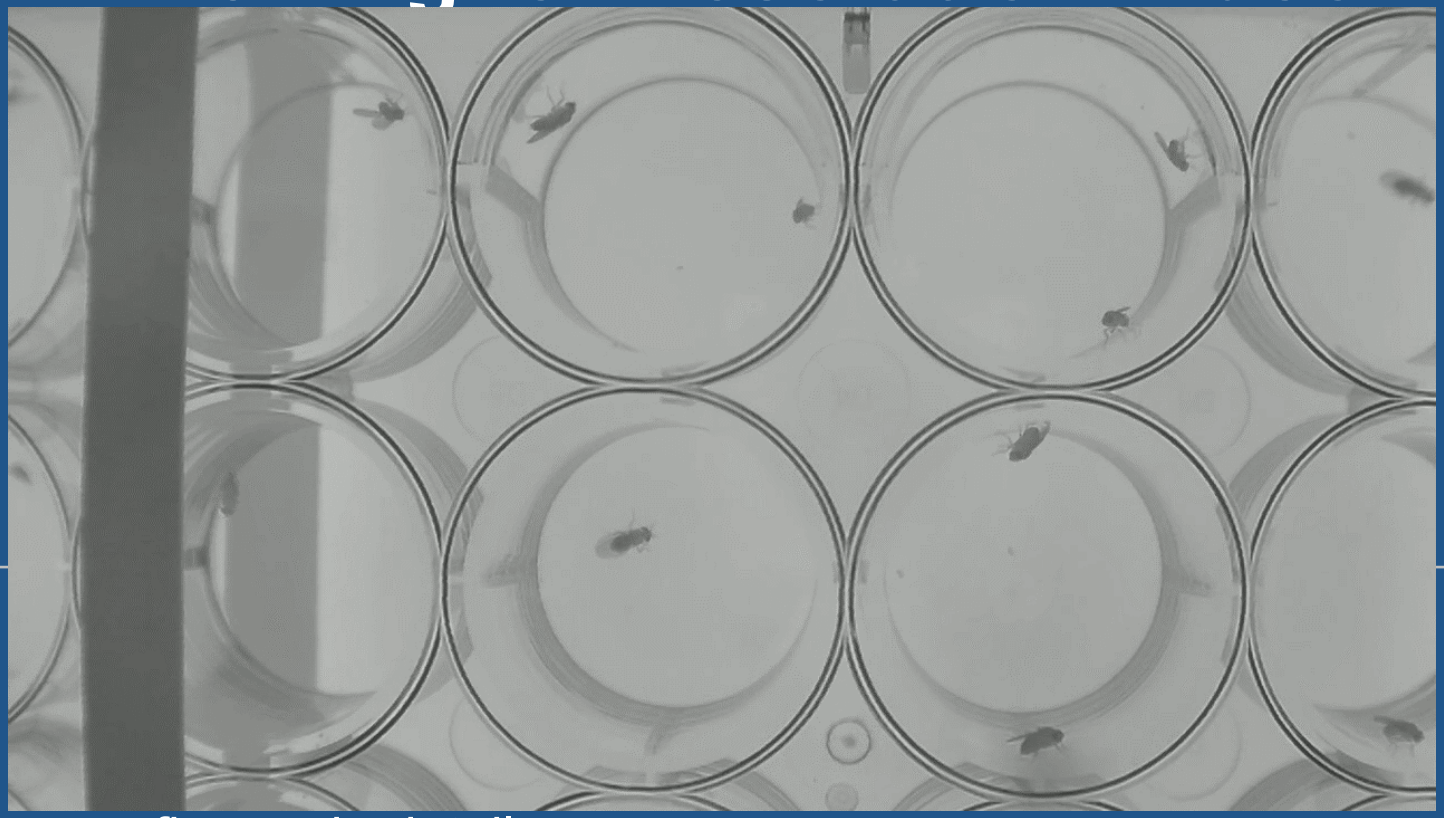


-Jurin's Law/Capillary Rise
-Viscosity & pore size affect chamber flooding
-Goal: Determine appropriate pore size
    -4% sucrose 1.5% yeast extract solution
    -Larger than 350µm for injection molding

Hole-size Determination: Initial Capillary Action Experiments

-20 µL blue dyed 4% sucrose 1.5% yeast extract
-WAFFLs
    -Hole sizes from 500-400µm for injection molding (400µm limit)
    -Diameter decreasing in 25µm increment
-Aligning housing plate and feeding plate properly avoids flooding
    -1-well flooded without secure fit
    -Added rectangular locking mechanism for 1-well
    -Not necessary in 24-well with multiple wells to reference
-400µm flooded least

MUFFIN 2.0: Higher Resolution Video



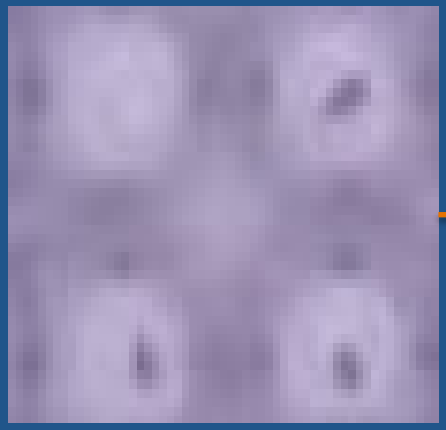
-Resolves more fine grain detail

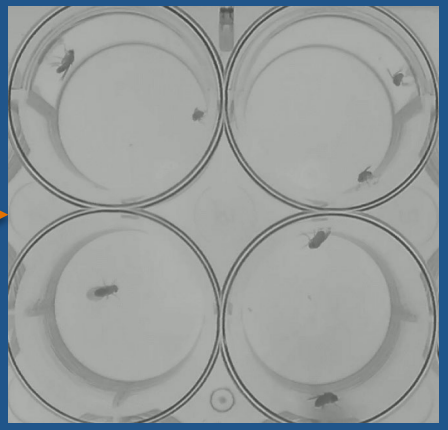
  -Wings

  -Legs

-More behavior measures possible

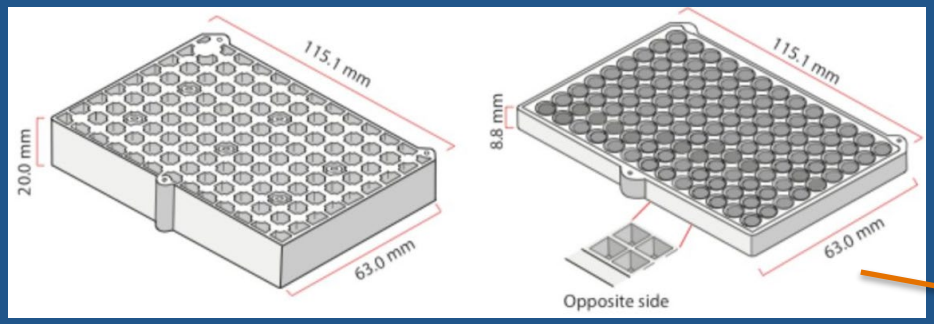
*Sedated flies: little movement



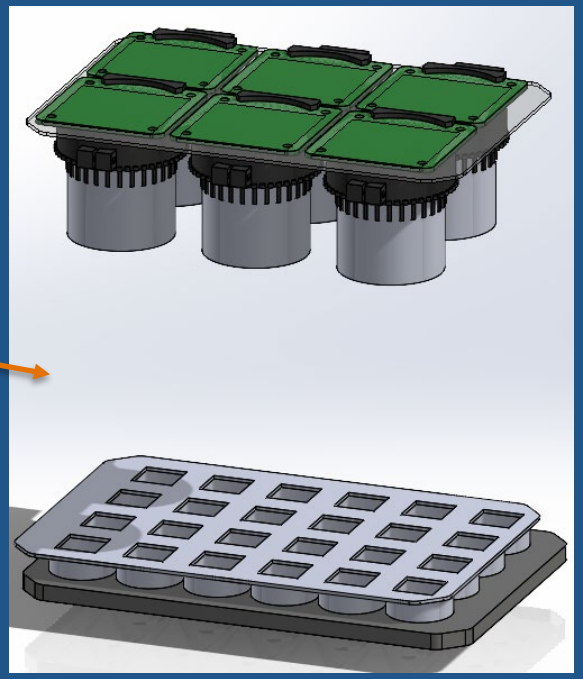


Conclusions

-Feasible current assembly in 1-well setup
-Injection-moldable design
-Higher image resolution



96-well Receiver Plate and Transfer Adapter



Future Directions

-Produce and test full 24-well: interlock components
-In-vivo testing of whole 24-well
-Mass-produced injection-molded parts
-WAFFL: modify tools for harvesting flies and excrement
-MUFFIN: automated detection of motion and behavior algorithms

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References

Jaime, et al. “The High-throughput WAFFL System for Treating and Monitoring Individual Drosophila melanogaster Adults.” *bioRxiv*, (2018)

Salem, et al. “Digital video recorder for Raspberry PI cameras with multi-camera synchronous acquisition.” *HardwareX*, (2020)