wMicroTracker
User Frequently Asked Questions and Troubleshooting Guide

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MULTI-WELL PLATES COMPATIBILITY

Would V-bottom plates be better in concentrating the worms than U-bottom of 96-well plates?
The V-bottom can work but using a U-bottom plate actually increases sensitivity, especially when using a low number of worms. We recommend filling the well to 40µl and using at least 25 adult *C. elegans* per well in a 96-well plate. To learn more, down load this Tech Note.

What microplates have been tested on the wMicroTracker System?
Here is a list of the microplates that we have tested:

384-well:

- 384w COSTAR square shape,
- 384w Microtiter round shape by Thermo Scientific

24-well, 96-well:

- Greiner

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SOFTWARE AND EXPERIMENTAL SET-UP

In the set-up window, the blue dots which indicate the active laser points within a well are not evenly distributed between wells. Can I still compare the data between wells?
The wMicroTracker was designed to measure worm locomotion in 96 and 384-well plates. When using other plates, the homogeneity of number of sensors per well is not possible in such plates, due to the sensor/microbeam hardware arrangement.

However, because the activity output is calculated by averaging activity of all sensors within a well, these differences in sensor numbers minimally affect the average activity per well.

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DATA ACQUISITION AND EXPORT

Can I change the measured intervals to be reported?

The wMicroTracker is constantly detecting and recording movement. When you export your data to an excel sheet, you can change the "Bin Size" drop down menu to the desired time interval whether it's 8 minutes or 4 hours. The exported data sheet will then tell you the average activity count at the intervals you specified.

Does the wMicroTracker software recognize the source of activity (i.e. whether in one well there are 50 dead but 1 very active worm. What about 25 dead, but 25 mildly active worms?)

The wMicroTracker utilizes 2 beams per well in a 96-well plate. So 1 very active worm shouldn't be a problem because the activity score is an average per well. However this is when using a sufficient number of replicates becomes very useful to parse out the outliers.

Which analysis method and threshold should I use?

We recommend that you use the standard setting for your data analysis:
(i.e. 1_Threshold Avrg; 1,8). This setting works for most applications.

Is it OK if some of the wells are empty without worms and fluid or do we have to fill in the 100 ul fluid in the empty wells?

It is OK to leave wells dry. However if you wish to use empty wells as negative controls/blanks, then you should fill them with the same buffer used for your experiments.
Note: The software will give you data for all the wells. You can just ignore the results in the dry wells. It is not uncommon for empty wells to return a non-zero, low activity value. It is usually due to the light refraction caused by the uneven well bottom. Adding buffer helps prevent this.

The threshold activity is not linear. What does this mean?

The threshold activity per well is calculated to be approximately 5% of the signal value. Since the signal changes as a function of the worms' position and the OD of the culture medium, the average and therefore the threshold line also moves.
The activity plot shows an approximation of the activity and threshold. The applied threshold is recalculated every scanning round (i.e. every 500 samples).

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WORMS

Should we be using adult worms without eggs?
When measuring activity of gravid adults for a long period of time, it is important to consider the egg laying rate, as activity from a high number of L1s will be detected by the wMicroTracker.

Can we use smaller worms like L4s? If we do, how many worms can we use per U-bottom well?
You can measure movement in populations of larvae as early as L1. Because of the small size of each worm, it is important to place enough worms in the well in order to generate a worm density high enough to disturb the beam.
  • Larvae: For L1 to L3, use round-bottom 96-well plates: 100-150 larvae
  • Older worms: For L4 and older worms, use a flat bottom 96-well plates: 30-70 worms

What should we do if worms are clogging the pipette tip?
We usually use glass pipettes or disposable plastic pipettes. If you notice your worms are getting stuck, you can try cutting off the tip of the pipette to create a slightly larger opening.

How many adult *C. elegans* would you suggest to using when working with 384-well plates?
We suggest you use 15-25 adult N2s per well in a 384-well plate.

Would you recommend using less than 50 adult worms for 96 U-bottom plate?
The U-plate motion detection saturates more quickly than in flat bottom plates. We compared the results of a population of between 10 and 30 worms in this Tech Note (see graph). We suggest you use 25-30 adults per well in a U-bottom 96-well plate.

The worms stick to the plate. How can I fix it?
Some brands of plasticware can create more electrostatic issues. If your worms tend to stick to your plasticware when in M9, we recommend that you add 0.015% BSA, or 5% liquid NGM or 5% OP50 bacteria to the medium.

How do I know which plate adapter to use for my experiments?
When selecting the plate of your choice on the software, you will be prompted to use the appropriate plate adapter. For 12-well plates, use the 384 plate adapter.

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TEMPERATURE

The temperature detected inside the wMicroTracker is different than the outside temperature. Is the wMicroTracker heating up?
Make sure that the wMicroTracker is not exposed to direct sunlight or near a source of heat. And you are using the 9V DC power source adapter that comes with your wMicroTracker.

We recommend that you complete a temperature calibration using the 'Factory Settings' and a mercury thermometer to calibrate against actual temperature.